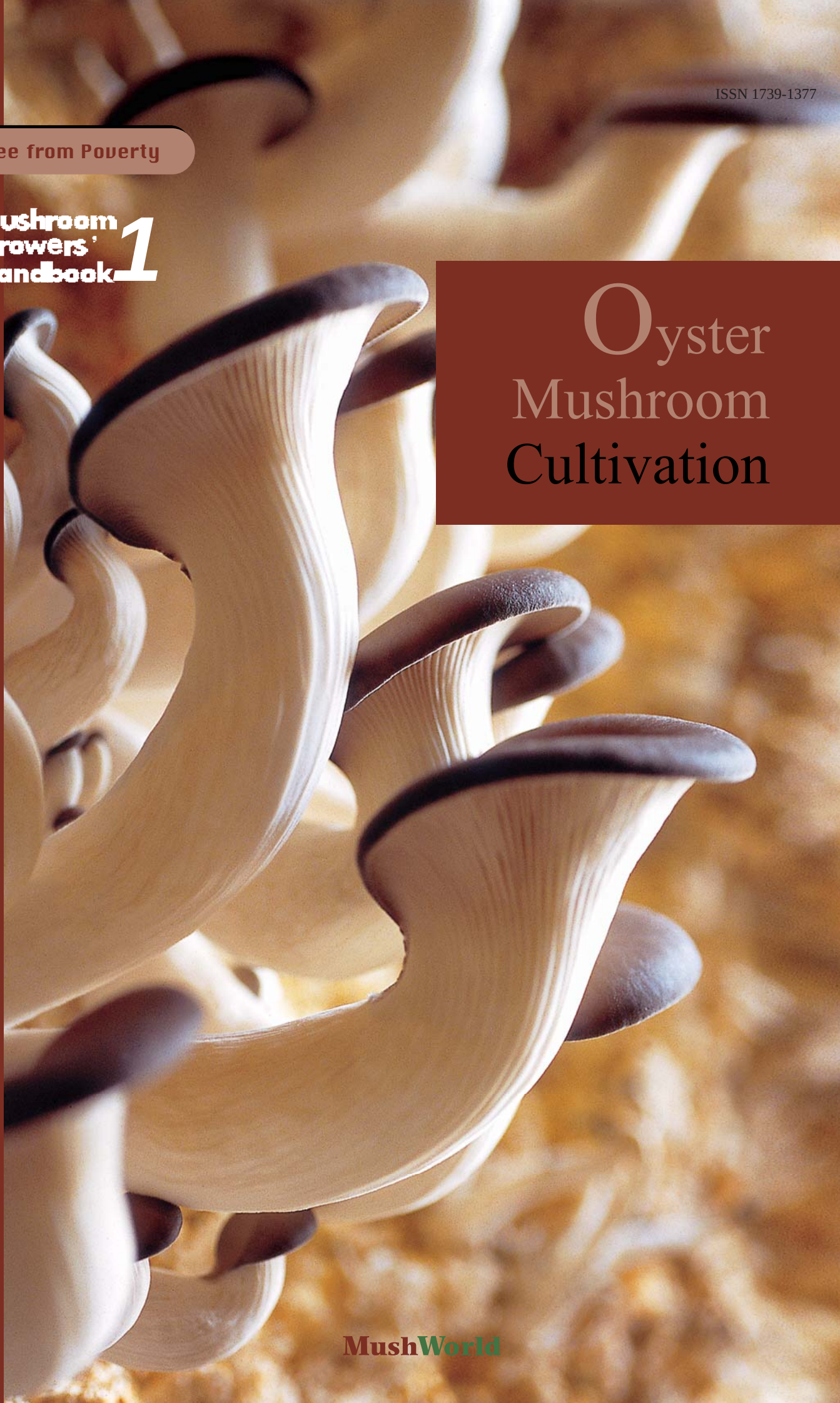
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Free from Poverty

Mushroom Growers' Handbook 1

Oyster Mushroom Cultivation



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Oyster Mushroom Cultivation

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Mushroom Growers' Handbook 1

Oyster Mushroom Cultivation

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Oyster Mushroom Cultivation

PREFACE

Mushroom cultivation has been evaluated as an effective means for poverty alleviation in developing countries due to its possibility of low cost production, high profit and quick return. As a non-profit organization, MushWorld has devoted itself to distributing valuable and abundant information on mushroom science and cultivation via its website, www.MushWorld.com, for free since established in 1998. Though the access to MushWorld is free and unlimited, Internet is not readily available for people in developing countries who sincerely need information sources for mushroom growing.

Mushroom Growers' Handbook is published to provide more accessible information on mushroom cultivation for people in developing countries. It will be distributed to growers, scientists, extension workers and governmental officials in developing countries free of charge. Following Oyster Mushroom Cultivation, the topic of this first book, cultivation of other mushrooms will be explored one by one in the following books to be published each year. Through this **Mushroom Growers' Handbook**, MushWorld hopes to reach more mushroom growers in developing countries and offer practical guides to mushroom cultivation for poverty alleviation.

Mushroom Growers' Handbook 1: Oyster Mushroom Cultivation is consisted of four parts and twelve chapters.

Part I provides overall information on mushroom and its cultivation.
Chapter 1 is an introduction to mushroom cultivation. Brief explanation on mushroom and principles of mushroom cultivation are provided. Why mushroom growing is a good way for poverty alleviation is discussed with specific examples.
Chapter 2 illustrates mushroom growing and project for a living in Nepal, Zimbabwe, India, Thailand and Swaziland.

Part II focuses on various aspects of oyster mushroom cultivation.
Chapter 3 is an introduction to oyster mushroom cultivation. It presents principles of oyster mushroom growing and illustrated guides to oyster mushroom bag and shelf cultivation.
Chapter 4 is about spawn, one of the key elements for high yields. The chapter begins with descriptions of commercially important *Pleurotus* species, followed by grain spawn making in a simply-made clean bench.
Chapter 5 is about another crucial factor- substrate. The chapter lists possible substrate materials for oyster mushroom based on worldwide survey and presents nine examples of substrate materials: cereal straw, coco lumber sawdust, sunflower seed hulls, grass, cottonseed hulls, sugarcane bagasse, rubber tree sawdust, groundnut shells, and non-pasteurized wheat straw.
Chapter 6 reviews the major three factors that should be considered when a farmer builds a mushroom growing house: site selection, construction materials and functions. Various examples of growing house from many countries are provided ranging from simple shade to structural insulated panel house with automatic control.
Chapter 7 introduces different growing methods: log, bag, shelf and bottle cultivation with images of each step. Readers are expected to select appropriate cultivation method for themselves and adopt tips and know-hows from each method.

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Chapter 8 is on pest and disease management. Pathogens, symptoms and control measures of each disease are well summarized. Pests and abnormalities are also well described with informative supporting images.
Chapter 9, the last chapter of this part, covers post-harvest management. Various ways of recycling of spent oyster mushroom substrate is illustrated. Effective ways to extend shelf life of mushroom are introduced with detailed images.

Part III offers case studies on mushroom growing worldwide.
Chapter 10 provides in-depth researches on mushroom industry of three African countries: Kenya, Zimbabwe and Uganda.
Chapter 11 introduces other mushrooms that can be grown in the Tropics. Detailed information on low-cost cultivation methods are illustrated about *Ganoderma* mushroom, shiitake, paddy straw mushroom, and *Pleurotus tuberregium*.

Part IV lists useful information sources.
Chapter 12 lists recommended books and websites on mushroom for further reading.

Acknowledgement
This growing manual cannot be made by MushWorld alone, but together with those devoted scientists and growers with different educational, professional, cultural and national backgrounds. More writers are invited to join in this growers' handbook.

MushWorld takes this opportunity to express our special thanks to Mr. Rick Gush for his great work, who volunteered to copyedit all the manuscripts of this growers' handbook. As a professional copy editor, he devoted his valuable time to editing 320 pages of manuscripts from a score of writers. MushWorld also acknowledges Prof. Jozef Poppe supervised Chapter 5. Substrate. He gave us helpful feedbacks on ten articles in the chapter from his forty-year research and experience.

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TABLE OF CONTENTS

Preface

Photo Gallery

Part I. Mushrooms

Chapter 1. Introduction to Mushroom

What is Mushroom 1

Why Grow Mushrooms 4

Chapter 2. Mushroom Growing for a Living Worldwide

Mushroom Cultivation to Make Living in NEPAL 13

Poverty Alleviation by Mushroom Growing in ZIMBABWE 19

Mushroom Growing in INDIA 24

Mushroom Growing in NORTHERN THAILAND 30

Mushroom Project in SWAZILAND 38

Part II. Oyster Mushrooms

Chapter 3. Introduction to Oyster Mushroom

What is Oyster Mushroom 48

Illustrated Guide to Oyster Mushroom Cultivation 52

Chapter 4. Spawn

Descriptions of Commercially Important *Pleurotus* Species 54

How to Make Oyster Mushroom Grain Spawn in a Simple Way 62

Chapter 5. Substrate

Agricultural Wastes as Substrates for Oyster Mushroom 75

Cereal Straw and Corncobs 86

Coco Lumber Sawdust 91

Sunflower Seed Hulls 95

Grass (Juncao) 101

Cottonseed Hulls 108

Sugarcane Bagasse 112
Rubber Tree Sawdust 116
Groundnut Shells 120
Non-Sterilized Wheat Straw 123
Chapter 6. Growing Houses
Mushroom Growing Houses 129

Chapter 7. Cultivation Modes
Log Cultivation 135
Bag Cultivation 139
Shelf Cultivation 153
Bottle Cultivation 166
Chapter 8. Pest and Disease Management
Green Mold and *Hypocrea* Disease 172
Brown Blotch Diseases 175
Viral Disease 178
Pests 180
Abnormalities in Fruiting Body 183
Chapter 9. Post-harvest Management
Recycling of Spent Oyster Mushroom Substrate 187
Mushroom Storage and Processing 192

Part III. Mushrooms Worldwide

Chapter 10. Regional Research
Mushroom Cultivation in KENYA 197
Mushroom Industry in ZIMBABWE 204
Mushroom Cultivation in ZIMBABWE 212
Mushroom Cultivation in UGANDA 220
Chapter 11. Mushrooms for the Tropics
Growing *Ganoderma* Mushrooms 224
Growing Shiitake Mushrooms 235
Growing Paddy Straw Mushrooms 248
Growing *Pleurotus tuberregium* 256

Part IV. Information Sources

Chapter 12. Mushroom Cultivation Information Sources
Recommended Books 267
Recommended Websites 269

MushWorld's Profile 275

Oyster Mushroom Cultivation

PHOTO GALLERY

Wild Mushrooms



Pleurotus **sp.**



Xylaria **sp.**



Microstoma fluccosa



Marasmiellus **sp.**



Pleurotus **sp.**



Lycoperdon echinatum

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Mushroom Products



Dried shiitake slices, U.S.A



Fresh eryngii, Japan

Photo Gallery



Fresh *Agaricus* imported from the Republic of South Africa / Swaziland



Mushroom pickles and wines, Thailand



Phellinus baumii instant noodle, Korea



Canned mushrooms, China

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Mushroom Dishes



Straw mushroom and eryngii, china



Various mushroom snacks, Thailand



***Agaricus* mushroom cream soup, France**



Mushroom shabu-shabu, Korea



Sandwich with shiitake, China



***Agrocybe* sp., Thailand**

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Mushroom Markets



Dried shiitake (Huagu), China



Wild mushroom market, U.S.A.

Photo Gallery



Wild mushrooms, Thailand (by Tawat)



Matsutake on display, Japan

Photo Gallery



Local market, Mexico (by Armando Lopez)



Local market, Switzerland

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Growing Methods



Bags hung in a wall formation



Horizontal shelf with bags



Inoculated logs of *Lentinula edodes*



Bottles of king oyster mushroom



Shelf cultivation of *Coprinus comatus*



Bags of shiitake mushrooms in a green house

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Growing Methods



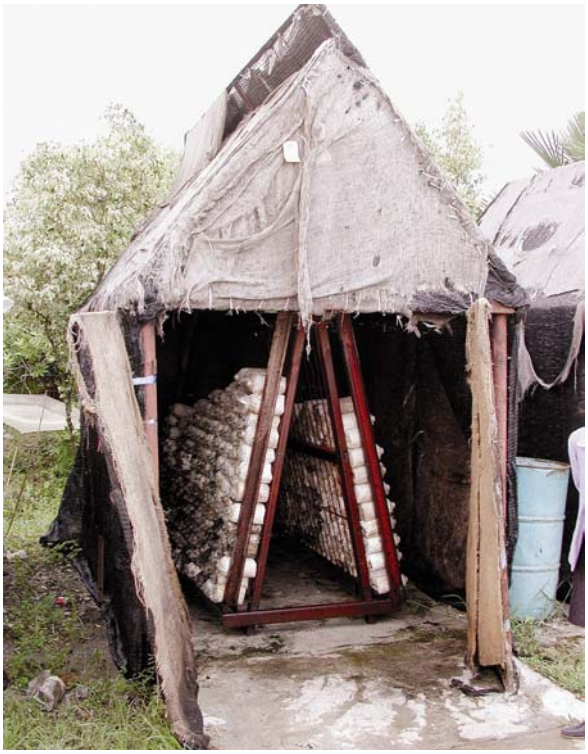
Bag cultivation of *Coprinus comatus*



Shelf cultivation of *Pleurotus* sp.



Tray cultivation of *Agaricus* mushroom



A-frame shelf with bags



Sawdust blocks of *Lentinula edodes*

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Cultivated Mushrooms



***Lentinula edodes*, Block, Japan**



***Pleurotus ostreatus*, Bag, Korea**



***Volvariella volvacea*, Shelf, Thailand**



***Agaricus bisporus*, Shelf, Australia**



***Flammulina velutipes*, Bottle, Korea**



***Coprinus comatus*, Shelf, China**

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Oyster Mushroom Cultivation

Part I. Mushrooms

Chapter 1

Introduction to Mushroom

WHAT IS MUSHROOM

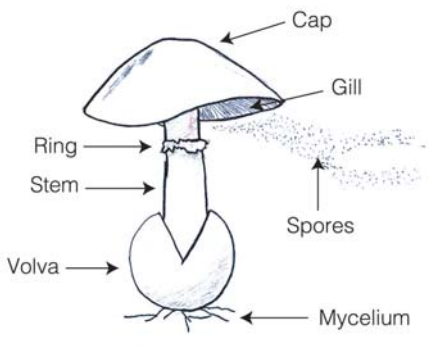
Song Baek Cho
MushWorld
Translated by Seung Woo Kang

What is Mushroom?

A mushroom is defined as "a macrofungus with a distinctive fruiting body which can be either epigeous or hypogeous. The macrofungi have fruiting bodies large enough to be seen with the naked eye and to be picked up by hand" (Chang and Miles, 1992). In a narrow sense, the word mushroom also refers only to the fruitbody.
Mushrooms used to be classified into the Kingdom Plantae, but now they belong to the Kingdom Fungi due to unique fungal characteristics which draw a clear line from animals or plants. Unlike green plants, mushrooms are heterotrophs. Not having chlorophyll, they cannot generate nutrients by photosynthesis, but take nutrients from outer sources. Most mushroom species are under the Basidiomycota and Ascomycota, the two phyla under the Kingdom Fungi (Table 1).

Table 1. Kingdom Fungi

Ascomycota	sac fungi (yeast to large cup fungi)
Basidiomycota	higher fungi (toadstool, puffball, bracket fungi)
Zygomycota	molds, mycorrhizal fungi and soil decomposers
Chytridiomycota	primitive fungi, chytrids
Deuteromycota	asexually reproducing fungi



Mushrooms breed by spores (seeds for plants). Under the proper conditions, spores germinate into hyphae (collectively, mycelia). Mycelia are filamentous and generally unseen with the naked eye. Germinated hyphae form primary mycelia, and then secondary mycelia through plasmogamy (hyphal fusion). They accumulate nutrients from the substrate (soil for plants) and colonize substrate. When stimulated by temperature, humidity, etc., the mycelial colony forms pins under certain conditions and grow to fruitbodies (fruits for plants). Young fruitbodies are called pins (buds for plants). Pins differentiate into a cap and stem forming fruitbodies. Under the cap, spores are produced in the gills (Fig.

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1). Fruitbodies release spores in order to produce the next generation.

This life cycle of mushroom is divided into two phases: vegetative and reproductive growth. Vegetative growth indicates linear growth of fungal mycelia dissolving complex substrate components into simpler molecules and absorbing them as nutrients. When low temperature, high humidity, much oxygen, and sometimes light are offered, the mycelia cease vegetative growth and begin to produce fruitbodies, which we call 'mushroom'. This is reproductive growth. Mushroom cultivation can be said the practice of obtaining fruitbodies by artificially repeating these two growing stages.

Mushroom cultivation requires enough understanding on the optimal growing conditions of each mushroom species and how to make favorable environment for both vegetative and reproductive growth of mushrooms.

Three Factors of Mushroom Cultivation

Spawn



A. Grain spawn



B. Sawdust spawn



C. Plug spawn



D. Liquid spawn

Figure2. Various types of spawn

What spawn is to mushroom is like seed is to crop. Unlike spore, spawn is already at its mycelial stage growing on its own substrate such as sorghum, barley or sawdust. The life cycle of mushroom starts from spores, but growers inoculate mycelial origin spawn rather than spore origin spawn because of possible variations and mutations. The quality of spawn is one of the most decisive factors for successful crop. Therefore, growers need to use qualified spawn for commercial production. Spawn should maintain the strain characteristics and is propagated by subcultures. New strains are developed with genetic methods such as variation and mating. The various types of mushroom spawn include grain, sawdust, plug and liquid.

Substrate

Mushrooms can be classified as 3 categories by their tropic pattern; saprophytes, parasites or mycorrhizae. The most commonly grown mushrooms are saprophytes, decomposers in an ecosystem growing on organic matters like wood, leaves and straw in nature. Raw materials can be used as substrate for primary decomposers such as oyster mushroom and enokitake which have lignocellulosic enzymes. On the other hand, secondary decomposers like button mushroom or straw mushroom require substrate degraded by bacteria or other fungi. Mushroom requires carbon, nitrogen and inorganic compounds as its nutritional sources and the main nutrients are carbon sources such as cellulose, hemicellulose and lignin. Thus, most organic matters containing cellulose,

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hemicellulose or lignin can be used as mushroom substrate. Examples are cotton, cottonseed hull, corncob, sugarcane waste, sawdust, and so on. However, demanded amount of each nutritional sources differs according to mushroom species. For example, button mushroom (*Agaricus bisporus*) requires relatively high nitrogen source, so the optimal C/N ratio of button mushroom compost is 17. On the other hand, oyster mushroom and shiitake require less nitrogen and more carbon source. Mushroom mycelia secrete digestive enzymes into the substrate and absorb the dissolved nutrients. Cellulose, the main nutritional source of mushroom is one of the most abundant organic matters on earth, but its digestive enzyme, cellulase is owned by several microorganisms including fungi. Here comes the reason mushroom is considered an important food source. Mushroom is the only one by which cellulose is dissolved and absorbed and transformed into food for mankind. Mushroom is also influenced by acidity of substrate. The optimal pH value of substrate ranges from 6 to 8, varying with mushroom species.

Environment

The last important factor for mushroom growing is providing an appropriate environment both for vegetative and reproductive growth. Not being protected by a skin layer, fungi are easily affected by their growing conditions. So it can be said that the success or failure of mushroom cultivation depends on the control of growing conditions. Environmental factors affecting mushroom cultivation include temperature, humidity, light and ventilation. Optimal levels of them at vegetative stage differ from those at reproductive stage.

Mushroom mycelia can survive between 5 and 40℃ depending on the species. Mushroom mycelia grow well with the temperature range between 20 and 30℃. Pins form at 10-20℃, lower than that of mycelial growth by 10℃. Over 80% of the fruitbody is water. Substrate moisture content should be 60-75% and log moisture content, 35-45%. During fruiting, different relative humidity levels, ranging from 80-95%, are needed at the early, mid and latter stage. Though mycelia can grow without light, some species require light for fruitbody formation. Being aerobic fungi, mushrooms need fresh air during growing, but ventilation is more required for reproductive stage. No matter how well the substrate is colonized, it is useless if it fails in fructification. Therefore creating the optimal conditions for transition from vegetative stage to reproductive stage is crucial to successful mushroom cultivation.

In conclusion, among the three factors, the most important is environmental control. By maintaining optimal conditions at each growing stage and for each species, growers can produce the desired yield of quality mushrooms.

Glossary

- **Epigeous** Growing on (or close to) the ground.
- **Hypogeous** Growing under ground.
- **Plasmogamy** Fusion of cells or protoplasts without fusion of the nuclei, as occurs in higher terrestrial fungi. Nucleus fusion is called karyogamy.
- **Heterotroph** An organism that cannot synthesize its own food and that is dependent on complex organic substances for nutrition. Most organisms except green plants (autotrophs) are heterotrophs.
- **Saprophyte** An organism which grows on and derives its nutrient from dead or decaying organic matter.
- **Parasite** An organism that grows, feeds, and is sheltered on or in a different organism while contributing nothing to the survival of its host.
- **Mycorrhiza** The symbiotic association of the mycelium of a fungus with the roots of certain plants, such as conifers, beeches, or orchids.
- **Aerobe** Organism that is living or occurring only in the presence of oxygen.

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Oyster Mushroom Cultivation

Part I. Mushrooms

Chapter 1

Introduction to Mushroom

WHY GROW MUSHROOMS

Tricita H. Quimio
University of the Philippines at Los Banos, the Philippines

Mushrooms in History and Different Regions


Views & Comments

the one billion Chinese favour the Straw Mushroom, which plays a large role in Chinese cuisine the world over. It is simple to grow, likes the hot, humid conditions of the tropics and even flourishes in the open air, all of which promise it a major role in future mushroom development schemes. Mushrooms are even being tried as an alternative crop to the opium poppy in the heroin producing regions of Southeast Asia.

The Oyster Mushroom is even more suited for expansion throughout the Third World, as it

Mushrooms for development

In the West, mushrooms are regarded as a luxury food. But the work of various aid agencies in promoting them

Figure 1. Mushrooms for development

Mushrooms have been part of our human diet since time immemorial. They were used as food even before man understood the use of other organisms. Undoubtedly, mushrooms were one of man's earliest foods, and they were often considered an exotic and luxurious food reserved for the rich. Today mushrooms are food for both the rich and the poor. They can be grown anywhere as long as the conditions for their growth and cultivation are provided. Available mushroom technologies range in complexity from very high to amazingly low.


Figure 2. Fruiting bodies of *V volvacea*


Figure 3. Straw mushroom beds

Mushrooms have been variously considered as a hedge against famine or a possible cancer cure. They do

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certainly have enormous potential for feeding third world peoples. In the West, mushrooms are regarded as a luxury food. But in many developing countries of the world, mushrooms can mean cash for the poor (Fig. 1) and a new source of nutrition. Even landless peasants can grow mushrooms as a valuable crop as long as they have the proper technology, the proper substrates, and the planting material called spawn. In some villages of India, it has been reported that farmers are growing mushrooms right in their own homes or immediate surroundings. Villagers growing mushrooms can rapidly begin to bring in more cash than some local landowners.



Figure 4. Harvesting of straw mushroom



Figure 5. Fruiting bodies of oyster mushroom

In some poor countries of Asia, the tropical Chinese straw mushroom (*Volvariella volvacea*) (Fig. 2) is grown in very simple traditional ways. This mushroom likes the hot humid conditions of the tropics and can be cultivated on beds (Fig. 3) made up of agricultural wastes such as straw or banana leaves. Within 2 weeks, fruitbodies are ready to be harvested (Fig. 4).



Figure 6. Oyster Mushroom houses made of grasses



Figure 7. Oyster mushroom growing on sawdust beds outdoor

Oyster mushrooms (*Pleurotus* spp.) (Fig. 5) are even more suited throughout the third world areas that are rich in plant wastes such as sawdust, sugarcane bagasse and others. Moreover, composting–the difficult preliminary step for button and straw mushroom–is not required for oyster mushroom cultivation.

The oyster mushroom growing houses can be constructed of mud as in some villages in India, or made of bamboo and dried leaves as in most of Asia (Fig. 6). In cooler areas, oyster mushrooms may even be grown outdoors if they are shielded from excessive sun (Fig. 7). Oyster mushrooms are easily dried to provide for a longer shelf life and export possibilities (Fig. 8).

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Figure 8. Dried oyster mushrooms ready for export

Benefits Derived from Mushrooms and Growing Mushrooms

Nutrition of the mushrooms

The popularity of mushrooms is still based not on the nutrients that they contain but mostly on their exotic taste and their culinary properties, whether eaten alone or in combination with other foods. It is not well known that mushrooms are full of nutrients and can therefore make a very important contribution to human nutrition. Table 1 shows the food value obtained from cultivated mushrooms compared with other common foods.

Table 1. Food value of the different cultivated mushrooms (% fresh weight)

Mushrooms/ food item	Protein	CHO	Fat	Calcium (Ca)	Thiamine (Vit. B_1)	Riboflavin (Vit. B_2)	Iron (Fe)	Niacin (Vit. B_3)
Pleurotus Pulmonarius	2.9 (26-35)*	5.66	1.79	3.14	0.20	0.22	3.40	7.72
Volvariella volvacea	3.8 (25-29)*	6.00	0.60	3.00	0.10	0.17	1.7	8.30
Agaricus Brunnescens	3.5 (24-34)*	11.4	0.40	2.40	0.10	-	trace	5.85
Lentinula edodes	7.5 (13-17)*	6.50	0.93	3.00	-	-	1.90	7.60
Auricularia polytricha	4.8 (4-8)*	7.16	0.50	3.15	0.08	0.19	3.60	4.00
Potato	2.0	9.10	0	11	0.10	0.04	0.70	0.04
Milk	3.5	4.90	3.9	118	0.04	0.17	0.10	0.17
Fish	14-20	2-3	1-2	15	60	1.20	1.50	1.20
Egg	13	2.0	13.3	68	18	0.27	1-15	0.27
Meat	21	-	3.6	8.3	0.10	0.29	2.52	29.00
Carrot	1.2	9.3	0.3	39	0.06	0.06	0.8	0.06

Compiled from various sources. *Numbers in parenthesis are dry weight data.

(Source: *Tropical Mushroom Cultivation* by T.H.Quimio, 2002)

Protein is one of the most important nutrients in food, being particularly important for building body tissues. Mushrooms with protein content ranging from 3-7% when fresh to 25-40% when dry can play an important role in

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enriching human diets when meat sources are limited. The protein content is almost equal to that of corn, milk, and legumes, although still lower than meat, fish and eggs. As a dietary source of protein, mushrooms are superior to most fruits and vegetables with the exception of beans and peas. Mushrooms can be eaten fresh or cooked, unlike other protein sources such as soya and yeast that have to be processed or disguised in some manner before they are acceptable on the table.

Mushrooms also contain all the essential amino acids as well as the commonly occurring non-essential amino acids and amides. Lysine, which is low in most cereals, is the most important amino acid in mushrooms. Mushroom protein is indeed a valuable addition to the human diet.

Mushrooms also rank quite high in their vitamin content, which includes significant amounts of Vitamin C. Although devoid of Vitamin A, mushrooms make up for that with their high riboflavin, thiamin and cyanocobalamin (Vit. B_{12}) content, the latter usually being found only in animal products. Their content of the anti-pellagra vitamin–niacin–is nearly equivalent to the levels found in pork or beef, which are considered to be the richest sources of this vitamin. Mushrooms are also good sources of minerals such as calcium, potassium, sodium and phosphorous in addition to folic acid, an ingredient known for enriching the bloodstream and preventing deficiencies. Iron is also present in an appreciable amount in mushrooms and together with phosphorous, can provide a good proportion of the recommended daily dietary needs. Mushrooms are low in sodium, making them ideal for persons with certain types of heart and kidney ailments.

As health food and medicinal

For the past 20 years, interest in the medicinal aspects of mushrooms has greatly been stimulated by the large number of scientific studies conducted on mushrooms. Folklores have provided clues for potential sources of medicine from mushrooms as well as from herbal plants. Using modern approaches, scientists have isolated and identified specific components that can either destroy or at least debilitate three of mankinds' killer diseases: cancer, heart disease and AIDS. As a result, a vast body of scientific literature concerning mushrooms has been published since the 1970s, mostly in hospitals and research institutions in Europe, Japan, China and the United States.



Figure 9. Fruiting bodies of ***Ganoderma lucidum***



Figure 10. ***Ganoderma*** tea

The most recent introduction of a medicinal mushroom is *Ganoderma* spp. The fruiting bodies (Fig. 9) have traditionally been used for medicinal purposes and for thousand of years, have been regarded by the Chinese to be a high quality herbal medicine. It has been used clinically since ancient times in China for the treatment of fatigue, coughing, asthma, indigestion, neurosis and a variety of other diseases. Early reports indicated the ability of *Ganoderma* to improve body functions, increasing its healing ability while maintaining a healthy and long life. It is now well established from *in vitro* and *in vivo* studies that *Ganoderma* can help fight viral diseases, and modern research has proven its anti-tumor and interferon-inducing actions. Considerable data now indicates that

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Ganoderma basidiocarps have several components responsible for the inhibition of HIV multiplication. Today, *Ganoderma* is available in many countries in the form of dried fruiting bodies, capsules, tonic and instant teas (Fig. 10), and is grown in culture all over Asia. In California, *Ganoderma* is sold in Chinese stores in dry forms, without the need for pre-processing into teas. Being a tropical fungus, this mushroom can be widely cultivated using sawdust and other tropical agricultural wastes such as palm fibers, coconut wastes and rice straw.

Pleurotus spp., oyster mushrooms, are also good sources of beta-1,3/1,6-glucan. These molecules (called pleuran) stimulate the immune system of the body to help fight abnormal cells as well as boost the system against the damaging effects of chemo and radiation therapies used to kill tumor cells. *Pleurotus* also contains mevinolin and related compounds which inhibit reductase, an enzyme used in cholesterol biosynthesis. The consumption of oyster mushrooms can lower the cholesterol levels in the body. Vita-Glucan tablets and elixirs, formulated from purified glucan extracted from *P. ostreatus*, are now available for strengthening the immune system and lowering serum cholesterol levels to prevent heart disease. According to much folklore, *Pleurotus* can also prevent high blood pressure, impart long life and vigor and assist people in recovering from fatigue. It can also prevent hangovers, constipation and is an aphrodisiac.

Other edible and cultivated mushrooms with reported scientific medical evidence are *Lentinula edodes* (shiitake), *Agaricus blazei* (himematsutake), *Agaricus brunnescens* (champignon) and *Grifola frondosa* (maitake). Those are known to induce the formation of interferon, a defense mechanism against some virus infections, and have displayed hypocholesteromic activity.

Use of agricultural wastes as substrates

Mushrooms are grown on some organic substrates, mostly waste materials from farms, plantations or factories.



Figure 11. Rice straw



Figure 12. Sawdust

These otherwise useless by-products can therefore be recycled to produce value-added mushrooms. Currently, millions of tons of agricultural wastes are discarded, burned and neglected. In the process of mushroom growing, however, environmental pollution from such practices may be reduced. Examples of such agro-wastes in abundance in the tropics are straw (Fig. 11), corncobs, grass, sawdust (Fig. 12), sugarcane bagasse, cotton waste (Fig. 13), oil palm waste, coffee pulp, water hyacinth plants (Fig. 14), coconut husks, tree leaves, branches and logs (Fig. 15). These all can be used alone or in combination to create mushroom growing substrate. With moderate effort and careful management, the very people hungry for food can have within their grasp a new food source in the form of cultivated mushrooms.

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Figure 13. Cotton waste



Figure 14. Water hyacinth plants

Income and job generation



Figure 15. Logs for growing mushrooms

Mushroom growing is labor-intensive, and for countries where jobs are scarce, mushroom growing can create jobs both in semi-urban and rural areas. In fact, some technologies can use family labor thus providing all members of the family with employment.

The labor of out-of-school youths (Fig. 16) and even school children (Fig. 17) can also be utilized, especially as the bagging of substrate and related operations can be easily done by children. A big factory in Indonesia hires some 50 teen-age girls (Fig. 18), who trim the mushrooms ready for canning and for export.
Mushroom growing is also recommended as a project in a cooperative where division of labor is practiced. One group may be engaged in spawn production, another group will do the substrate preparation, and still another group can take charge of growing condition management.



Figure 16. Out-of-school youths filling mushroom bags



Figure 17. School children helping in bagging sawdust for *Pleurotus* cultivation



Figure 18. Girls trimming straw mushroom

Resulting compost used for soil conditioner and animal feed

The used compost that remains after harvesting mushrooms may still be recycled for use as animal feeds and soil conditioner. Earlier studies of the author have demonstrated that spent compost of both *Volvariella* and *Pleurotus* had increased crude protein content compared with raw straw. Poultry feeding trials showed that spent compost

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fed to broilers resulted in greater weight gains compared with commercially used feeds. Low intake and low digestibility however were observed in trials with sheep using *Volvariella*-cropped rice straw/banana leaf compost. Research at the Hebrew University in Jerusalem included the production of a highly digestible nutritious feed for cattle and sheep from *Pleurotus* cotton waste/straw compost.

Numerous studies have indicated that mushroom composts made from wheat straw and other supplements gave comparable or higher yields of such selected vegetables as cabbage, beans, celery and cauliflower when compared with those grown using poultry manure. In Puerto Rico, *Pleurotus* spent-compost made from sugarcane bagasse, has been used by local nursery growers as a good substitute for the expensive commercial fertilizers used in soil conditioning. The spent compost is further composted in the open air, covered with plastic for 4-8 more weeks before it is dried, bagged and distributed to nursery owners.

Case Studies Showing Economic Aspect of Mushroom Cultivation in Rural Areas

Case 1. Village contract growing of *Pleurotus* sawdust bags in the Philippines

The objective of this project was to pilot contract oyster mushroom growing and use the revenues for further expansion of mushroom growing in the rural area. This activity was successfully demonstrated in several small villages near the University of the Philippines which provided appropriate funding support to the participants and where a central laboratory prepared the seeded mushroom bags for fruiting. The support was mainly for the building of small (5 × 5m) mushroom houses made up of nipa and sawali (Fig. 19) or styrofoam.

Each month, 2,000 bags (Fig. 20) were delivered to each of the participants (one from each village in the community). Resulting harvest was individually sold, to provide installment payments for the bags and the house. After 4 months when the project cost of PHP*20,000 (USD400) was recovered, the growers were taught to prepare their own bags, with the spawn provided by the central laboratory.



Figure 19. Mushroom house made up of bamboos, sawali, and nipa roof



Figure 20. Sawdust bags ready for delivery to contractual growers

* PHP (Philippine Peso, PHP1≒USD0.02 in May 2003

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Table 2. Financial aspect of the project for each village

1. Total cost (loan)	
Total amount of loan	PHP20,000 (USD400)
(for house and 2 deliveries of 2,000 bags)	PHP8,000+ (2×PHP6,000)
- Growing house	PHP8,000 (USD160)
(made up of nipa roof and sawali walls, wooden shelves, screened door, floor area of 10m^2)	
- Price for 2,000 bags (PHP3 per bag)	PHP6,000 (USD120)

2. Estimated income per month	
Volume of production per month	350kg
(total production - loss during storage, handling and delivery)	(410kg - 60kg)
Net sales per month	PHP10,500 (USD210)
(total production × price per kg)	(350kg×PHP30)

3. Pay back of loan (for 4 months)	
Collection per month to pay back loan (50% of income)	PHP5,250 (USD105)
	(PHP10,500 / 2)

* The loan can be fully paid back for 4 months

(Source: *Manual on Mushroom Cultivation* by Peter Oei, 1991)

One of the problems encountered was the difficulty of providing the proper temperature (lower than 30℃) for mushrooms to fruit abundantly. This was not a problem during the cool season from December to February when growers would enjoy abundant harvests. There were also some initial problems of bag delivery which made cost and expenses relatively high. The problem in marketing was not due to lack of buyers of mushrooms but the lack of production. Supply does not meet the high demand from traders and restaurants.

Case 2. Bed production of the straw mushroom in the Philippines

This project was done in a small farming community in the province of Cebu where a foundation provided funds to the contract participants, mostly family-based groups. The spawn and supervision was provided by a laboratory, which was also funded by the foundation. Bedding materials consisted of 45cm long, 10cm wide bundled rice straw (Fig. 21) or dried and bundled banana leaves (Fig. 22). Rice straw that was hard to bundle was chopped and molded into a bed (Fig. 23). Each 2m-bed would need at least 60 bundles and each family had to prepare 20-40 beds per month. The laboratory bought the harvested mushrooms back for marketing so the grower had no problem marketing their own produce.



Figure 21. Preparation of beds and spawning of straw mushroom



Figure 22. Dried and bundled leaves

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Growers are expected to harvest at least 2.5kg of fresh mushrooms per growing cycle of 23 days. First flush would bring 2kg, and second flush, 0.5kg at the interval of 7-10 days. Each family therefore would produce at least 95kg of fresh buttons (Fig. 24, 25) and would have a net income of at least PHP2,000 (USD40) after removing the cost of spawn, production overhead/pesticides, monthly payback of loan and interest, as well as the monthly depreciation of their fixed investment in the form of water drum, sprayer and plastic sheets which have an expected 3-year life span.



Figure 23. Small beds for *Volvariella*



Figure 24. Harvested *Volvariella*



Figure 25. Packed straw mushroom for sale

Table 3. Monthly financial state of case 2

Volume of production per month	95kg
Gross Income per month	PHP2,850 (USD57)
(volume of production × price per kg)	(95kg×PHP30)
Total expenses per month	PHP696.43 (USD13.93)
- Spawn (5,500mL bottle/bed)	PHP440 (USD8.80)
- Production overhead/pesticides	PHP200 (USD4)
- Depreciation of investment	PHP37.77 (USD0.75)
- Monthly pay-back of loan and interest	PHP18.66 (USD0.37)
Net Income per month	PHP2,153.57 (USD43)*

* The usual farmer's income in the rural Cebu area is PHP1,500 (USD30)

(Source: *Manual on Mushroom Cultivation* by Peter Oei, 1991)

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Oyster Mushroom Cultivation

Part I. Mushrooms

Chapter 2

Mushroom Growing for a Living Worldwide

MUSHROOM CULTIVATION TO MAKE LIVING IN NEPAL

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A Short History of Mushroom Cultivation in Nepal



Figure 1. Nepal

Mushroom cultivation was initiated by the Division of Plant Pathology, Nepal Agricultural Research Council (NARC) in 1974. The growing technology for white button mushroom was developed during that early period and extended to general farmers starting in 1977. It utilized the synthetic media of paddy straw, which is harvested twice a year in Kathmandu. Of course, a few farmers grew mushrooms before the introduction of the technology but the number of button mushroom growers has increased year after year thanks to the spread of the technology.

The growing technology to grow oyster mushroom using chopped straw packets was introduced to the farmers in 1984, and since then mushroom cultivation has become more popular among farmers. These two kinds of mushroom cultivation systems have been employed by farmers in about 25 districts within Nepal. The Centre for Agricultural Technology (CAT) has recently introduced straw mushroom (*Volvoriella volvacea*) cultivation in the Terai districts and shiitake in the hill districts and has been instructing farmers how to grow them since 2001.

Oyster Mushroom Cultivation in Nepal

Oyster mushroom cultivation was introduced to Nepalese scientists in 1981. Research on the proper substrate and climatic conditions for oyster mushroom growing was carried out by the Division of Plant Pathology. Growing *Pleurotus sajor-caju* on stump and chopped paddy straw packets was successful in Kathmandu in 1982. The technology, which was distributed to farmers in 1984, was so simple, easy to adopt and suitable to the climate of Kathmandu valley that farmers could adopt it quickly. The cultivation practices, which produced quick returns, spread like wildfire. Poor farmers were willing to try mushroom growing on a small scale in order to augment their incomes. The growing of the species *P. ostreatus* was introduced later in 1998. These days farmers prefer *P. ostreatus* because it has higher productivity and can be grown during the winter in Kathmandu. Thanks to this

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durability, local consumers can now obtain oyster mushrooms all the year round. These mushrooms have been grown recently in the Terai districts (a tropic area) during winter and also transported to markets in Kathmandu.

Oyster mushrooms are often grown without any environmental control. *P. sajor-caju* is cultivated for the summer crop at Kathmandu (25-30℃ and 80%) and in the hills of Nepal while it is cultivated in the Terai regions during the winter season (22-26℃ and 70%). *P. ostreatus* is grown during the winter season in Kathmandu and other cool places (5-20℃ and 70%). Some mushroom growers try to grow these two species together. Of course, oyster mushrooms cannot be grown in Terai during the summer (30-40℃ and 70%). The mid hills of Nepal are the most appropriate areas for oyster mushroom production and therefore the mushroom technology has been expanded widely in those villages.

Cultivation Method Practiced in Nepal

The cultivation method for oyster mushroom production using paddy straw in Nepal is as follows. Paddy straw is selected from the field by choosing fresh, not old, clean and straight pieces, of good quality. These straws are manually chopped into small pieces (2-3 inches long) using the locally hand-made chopper (Fig. 2). Chopped straw is then soaked in water for 2-4 hours, or sometimes overnight, in a container or a small ditch specially made for this purpose (Fig. 3).



Figure 2. Straw chopping



Figure 3. Soaking the chopped straw



Figure 4. Cleaning the straw in clean water



Figure 5. Draining water on a wooden framed net

The soaked straw is cleansed in water (Fig. 4) 1-2 times in a plastic bucket or some other container. The water from the straw is drained off in sieve (Fig. 5). Most farmers drain the water off slowly by placing the cleansed straw on a sloped place, a procedure that takes 2-4 hours.

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The drained straw is then steamed in a steamer. The local steamers are clay pots with a number of holes on the bottom. These steamers are put on top of a metallic vessel containing water (Fig. 6). The water is boiled using a kerosene stove. The mouth of the straw steamer is covered with thick plastic sheet (Fig. 7) and tied up by a string so as to make it tight. It takes about half an hour for the steam to reach the top of the steamer. Once the steam reaches to the top, steaming should be continued for about half an hour or more in order to sterilize the straw. The temperature in this process usually goes beyond 90℃.



Figure 6. Local steamer (earthen pot) on a metallic vessel containing water



Figure 7. Covering the steamer with plastic to pasteurize



Figure 8. Steaming the straw in a metallic drum

Instead of the clay pot steamer, a metallic drum (Fig. 8) can be used. In such cases the metallic drum is filled with water to about 6 inches from the bottom and a tripod stand is used to support the grate. The drum is then filled with straw and covered with a plastic sheet. The steaming method is then the same as with the clay pot steamer. The steamed straw is cooled down in the same container or transferred into a plastic sack to prevent contamination from outside.

The plastic bags used for making packets are of different sizes: 12×16” (small) and 18×26” (large). These bags are punched to make holes at a distance of 4 inches apart. Cooled straw is packed in the bags in layers up to 4 inches deep and grain spawn is sprinkled in layer by layer (Fig. 9). Once the bag is filled, the bag mouth is closed with a rubber band. Incubation proceeds at room temperature for 20-21 days (Fig. 10), until the mycelium spreads completely throughout inside the packets.



Figure 9. Making packets and spawning



Figure 10. Incubation of packets at farmer’s house

When the spawn run is completed, the bag is removed by cutting the plastic (Fig. 11). The packets are arranged in a row on the floor using a brick or two underneath (Fig. 12). The spacing between the packets is 6 inches, with 2 feets between the rows. Watering is done every morning and evening using a sprayer. In the dry season, one

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more spraying of water should be done. Primordia appear after 4-5 days (Fig. 13) and develop into a full size mushroom within an additional 2-3 days (Fig. 14).



Figure 11. Packets after opening the plastic bags



Figure 12. Packets are arranged in a row with bricks underneath



Figure 13. Primordia formation



Figure 14. Fruiting bodies

Infrastructure and Investment on Oyster Mushroom Production

Nepalese farmers grow mushrooms in a thatched house (Fig. 15) or a plastic tunnel. The thatched house is made up of wheat straw, bamboo and wooden support (Fig. 16). Plastic cover is used whenever it is necessary.

The plastic tunnels (Fig. 17, 18, 19) are constructed of thick plastic sheets with bamboo support. The size of tunnel is 40 feet long, 15 feet wide and 8 feet high.

The investment cost for oyster mushroom production is quite low. Most of the total cost is for the construction of a mushroom house, which is made of local and easily available materials. Skilled construction labor is available in most villages. The raw materials for mushroom cultivation are agricultural wastes and are usually available at the farmer's door.

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Figure 15. Thatched house



Figure 16. Inside a thatched house



Figure 17. Plastic house (tunnel)



Figure 18. Inside the tunnel



Figure 19. Plastic tunnel covered with straw

Cost and Benefit of Oyster Mushroom Production in Average (for 2 months)*

• Total Production Cost = NPR5,150.00 (USD69.26)**

Item	Quantity	Cost in NPR
Straw for 100 packets	300kg	1,200.00 (USD16.13)
Plastic bags (18 × 26")	100 pcs	400.00 (USD5.37)
Spawn (250g/bottle)	50 bottles	1,200.00 (24 per bottle)
Rent	2 months	1,000.00 (500/month)
Chemicals		150.00 (USD2.02)
Labor		1,200.00 (USD16.13)

• Total Income

= NPR18,000.00-27,000.00 (USD242.09-363.14)

Price	Volume	Value in NPR
90.00 per kg	200-300jg (2-3kg/pack)	18,000.00-27,000.00

* It takes one month growing and another month for harvest.

** NPR (Nepalese Rupee, NPR1≒USD0.0134 in Feb 2004

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• **NET PROFIT = Total Income - Total Production Cost**

► Maximum NPR21,850.00 (USD270.00)

► Minimum NPR12,850.00 (USD160.00)

One oyster mushroom grower produces 5 tons a year on average. The estimated productivity is 800-900kg of oyster mushrooms from 1,000kg of paddy straw. A farmer can grow about 4-5 crops per year and produce the income of NPR200,000-300,000 (USD2,689.9-4,034.99) per year.

Mushroom Growers, Spawn Supply and Product Marketing

There are about 5,000 mushroom growers within Kathmandu valley and 6,000 growers in other districts in total, including growers of other mushrooms. Balambu, with a long history of mushroom growing, has approximately 100 commercial growers and some 100 seasonal growers. They produce 2,000-3,000kg per day during the summer season and about 300-400kg per day during winter season. There are four or five distributors who collect the product from farmers to supply the markets. These same distributors also deliver spawn to the farmers. Some growers purchase directly from the spawn suppliers and sell their products to the market by themselves, and at present this is the system adopted in most of the villages for mushroom marketing and production.

There are about five spawn producers at Kathmandu. They supply spawn to farmers as well as the distributors. Some suppliers deliver spawn to remote places through courier transport services. In most of the other districts, mushroom growing is initiated by the Agriculture Department offices of HMG (His Majesty's Governments of Nepal). There are extension programs of mushroom production of HMG in the country, but most spawn production and mushroom marketing is done by private agencies.

There are no good marketing systems and no stable market price for mushrooms in Nepal. The market price fluctuates according to the demand and supply. The growers in Kathmandu get NPR40.00-60.00 (USD0.54-0.80) per kg during pick production season. However the price ranges from NPR80.00-90.00 (USD1.07-1.20) per kg most of the other times. During the off-season growers get NPR150-200 (USD2.01-2.68) per kg.

Conclusion



Figure 20. Training women farmers

Oyster mushroom production is a most appropriate technology for the poor landless farmers and women farmers in Nepal. Mushrooms can be grown in the small space of a farmers' own house for small scale production and generate income that aids in the family support. Mushroom cultivation is a most popular activity for development programs targeting income generation among women in Nepal because it is suitable for the women's life style.

As the women's responsibility is mainly to take care of the household work and children, they can accommodate mushroom cultivation in between their main work. The product is highly nutritive and a good food for their children and old parents, and because of its high value they can also derive some income from the production. The farmers of many districts of Nepal have grown oyster mushrooms in a small scale and have benefited highly. They have managed to adopt the technology in a simple way whereby they can afford to invest on a small scale. They are mainly utilizing the agricultural waste of wheat and paddy straw, and thus mushroom cultivation has improved the living conditions of many poor farmers in Nepal.

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Oyster Mushroom Cultivation

Part I. Mushrooms

Chapter 2

Mushroom Growing for a Living Worldwide

POVERTY ALLEVIATION BY MUSHROOM GROWING IN ZIMBABWE

A case study : The Chakowa Orphanage Group

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Figure 1. Zimbabwe in Africa

Although knowledge and production levels are still limited in Zimbabwe, no other agricultural crop has generated as much interest in the past three years as the mushroom. One might say that the mushroom industry has literally mushroomed here recently. The white button mushroom (*Agaricus bisporus*) and the oyster mushroom (*Pleurotus ostreatus* and *P. sajor-caju*) are the most commonly cultivated varieties, with the latter being the most popular among the economically vulnerable sector of our society. It is hoped that the new cottage industry of mushroom cultivation in Zimbabwe will soon provide an important tool for the generation of income and the creation of food security for hundreds of households.

Zimbabwe is a country with over 70% of its population of about 1.4 million people living with HIV-AIDS and a much higher proportion suffering from protein malnutrition. Reduced income coupled with increased expenditure on healthcare in a country already facing stiff economic challenges has worsened the poverty situation. Due to the frequency of drought and livestock diseases in this part of the world as well as the high cost of conventional agricultural production, the people of Zimbabwe are anxious to develop an alternative source of protein with a high income generation potential. Mushroom cultivation could possibly offer the solution for poverty alleviation in this situation. Unlike other agronomic crops, the set-up costs for mushroom production are low. Fertilizers, machinery, and pesticides are not used, the market price is relatively high, and profit margins for mushroom crops can be considerably higher than traditional crops. In general the enterprise takes very little space and can produce returns within a short period of time.

Zimbabwean farmers who are using local varieties of seed can grow maize and wheat crops that take an average of four months to reach harvest maturity. This time period is equivalent to at least two crops of mushroom cultivation. Considering this scenario, the relative profitability of these three crops can be compared as shown in Table 1.

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Even if the input costs in the mushroom enterprise were to be doubled, the enterprise would still remain more profitable than that of either maize or wheat. Considering that about five crops of oyster mushroom can be produced per year, the poverty alleviation potential of mushroom cultivation cannot be overemphasized.

Table 1. Compared profitability of maize, wheat, and oyster mushroom in ZWD*

	Maize	Wheat	Oyster Mushroom	
GROSS INCOME	ZWD1,050,000	ZWD2,000,000	ZWD2,400,000	
Expected yield	3 ton /ha	5 ton /ha	240kg /20m^2	
Average price	ZWD350,000 /ton	ZWD400,000 /ton	ZWD10,000 /kg	
TOTAL COSTS	ZWD531,500	ZWD860,000	ZWD697,000	
Labor	ZWD60,000	ZWD25,000	Labor	ZWD50,000
Land preparation	ZWD26,000	ZWD25,000	Firewood	ZWD20,000
Seed	ZWD35,000	ZWD10,000	Spawn	ZWD180,000
Fertilizer/Lime	ZWD285,000	ZWD580,000	Plastic bags	ZWD12,000
Insecticides	ZWD40,500	ZWD45,000	Straw	ZWD120,000
Transport	ZWD40,000	ZWD55,000	Antiseptics	ZWD15,000
Levy	ZWD12,000	ZWD10,000	Construction	ZWD300,000
Miscellaneous	ZWD33,000	ZWD110,000		
NET INCOME	ZWD518,500	ZWD1,140,000	ZWD1,703,000	

Cultivation Method for Mushroom in Zimbabwe

Being an agro-based country with more than 70% of the population employed directly in that sector, Zimbabwe produces vast quantities of crop residues that may be used in mushroom production. Wheat straw, grass, banana leaves, sawdust and water hyacinth are some of the fibrous residues that have been tried as mushroom cultivation substrates. Wheat straw and grass are the most commonly used substrates among current mushroom operations.

The pasteurized substrate is usually spawned and packed into polythene bags of about 30cm wide and 90cm long for the bag culture of the oyster mushroom. The growing rooms are maintained at between 18℃ and 25℃, with a relative humidity of about 75%. Although up to 6 flushes may be obtained from each bag, the first three are the most important in commercial production. For every 10kg of dry substrate used, as much as 20kg of mushroom can be harvested from the first 3-4 flushes. On average, Zimbabwean oyster mushroom growers obtain about 60kg of mushrooms per month. At least 2kg are usually harvested per bag. During the cooler winter season, P. ostreatus is cultivated while the more heat tolerant *P. sajor-caju* is produced in summer. Summers in Zimbabwe may be quite oppressively hot and maintaining the optimum growing room conditions is often a challenge.

The button mushroom, most often grown by well-financed growers, is the main export mushroom. For button mushroom cultivation, wheat straw and horse manure are mixed and used as substrate. Some farmers add inorganic fertilizers and/or peat. Cultivation is carried out in trays. Lower temperatures of about 18℃ need be maintained and diseases and pests must be closely monitored. The expenses and requirements for strict management of the growing room have restricted the number of newcomers going into button mushroom production in Zimbabwe.

Unfortunately, funding to promote the consumption and production of mushrooms has been limited in Zimbabwe. In spite of this, the potential of mushroom cultivation for poverty alleviation among such vulnerable groups as women and orphans has been noticed by some organizations. The Chakowa Orphanage Group

* ZWD (Zimbabwean Dollar, ZWD1≒USD0.0012 in Jan. 20043

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mushroom project is a prime example of the successful results mushroom cultivation can produce among typically vulnerable Zimbabwe citizens.

A Case Study : The Chakowa Orphanage Group



Figure 2. Trainees of Chakowa Orphanage Group

Technology is finally reaching out to some of the resource disadvantaged communities with a tremendous impact, thanks to the effort of the Intermediate Technology Development Group (ITDG) and a team of trainers who are currently running a mushroom production training course in Chakowa. As a result, the locals do not need to wait for the rainy season to enjoy the delicacy of the wild mushrooms collected from the forests and anthills. They can now grow oyster mushrooms throughout the year and get paid for their efforts as well. At Chakowa, ITDG is not only concerned with technology transfer, but also the human factor. In this situation ITDG is impacting positively on the lives of 56 households, including many orphans and those looking after children orphaned due to HIV-AIDS related deaths.

Background



Figure 3. Margaret Tagwira in oyster mushroom growing room (Photo courtesy of Mike Dubose)

Chakowa, a Shona word that means "that which has been harvested." is an appropriate name for this farming community located about 50km from the picturesque Birchenough Bridge in Zimbabwe's lowveld region. In addition to the recently introduced mushroom cultivation, the locals are involved in the production of field crops like maize, millet, sugar, beans, tomatoes and okra. These crops are grown here under supplementary irrigation due to the unreliability of rainfall in this part of the country.

The Chakowa Orphanage Group, initiated a few years ago to assist orphaned children is the brainchild of Mrs. Margaret Tagwira (Fig. 3). With only a few children involved initially, the project supplied spawned bags to trainees and taught them the basics of mushroom growing room management. Today a large project has grown from those humble beginnings. The group now comprises a wide range of participants including youths, women, and men, from sixteen to over sixty years of age. The primary goal is still to improve the livelihood of orphans, and the program is working wonders in that area. The group has not only paid the school fees and bought uniforms and books for the orphans, it has also significantly reduced protein malnutrition among the beneficiaries and the community at large. Although still in its infancy, the potential and success of the mushroom project cannot be underestimated. Their success can be attributed to the commitment by members of the group and the prior training in other technologies also facilitated by ITDG. The group has also previously been trained in embroidery and tie 'n' dying of fabric with pleasing results.

The training

The emphasis of the training has been to impart hands-on experience to the trainees and, at the same time, enough theory has been included to bring about an understanding and appreciation of the mushroom and its production. To achieve this, the farmer's field school concept of training was adopted. This training method has a participatory

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approach that has the advantage of including the trainees in every decision-making step all the way through the course. The trainees or growers feel they are the owners of the project, and this is a vital element that ensures that the project succeeds since all the members are aware of the goals and modus operandi of the project.



Figure 4. The training programme emphasizes hands-on experience (Photo courtesy of Mswake)

Although the trainer to trainee ratio is high, the trainers believe that the training has been effective, and the growers can now produce the mushrooms with minimum input from the trainers. According to Mrs. Musariri, one of the trainers, the success of the project will not only be measured by the yields attained in this cooperative farming, but also in the establishment of individual enterprises by members of the group and community, thus demonstrating their interest and knowledge of this industry.

The growing room

Chakowa lies in the lowveld region of Zimbabwe, an area characterized by high temperatures that sometimes exceed 35℃ in summer. Winters are generally mild here. The feasibility of any mushroom cultivation project in this region hinges on the grower's ability to control temperature and humidity inside the growing rooms.

For this project, a single growing room was constructed. This was made from thatch grass (*Hyperrhenia* spp.), a traditional material that keeps the internal environment cooler during hot weather. To counter the effect of low relative humidity in this drought prone region, plastic tubes filled with water were suspended from the roof adjacent to each bag. The plastic tubes were punctured to allow water to drip out and produce a fine spray that provided a cooling effect in the growing room. The plastic tubes are then re-filled with water once a day. This technique was introduced after it was realized that the initial technique of wetting the floor was labor intensive during hot weather as such watering had to be done three or more times a day. The advantage of using the suspended plastic tube structures is that it has a low input cost. The materials used are available locally and thus easier to reproduce by those wishing to go into this business. The group, with the financial backing from ITDG, is currently constructing a larger growing room. This will be made from brick walls and is intended as a more commercialized production system.

Spawn

The spawn used in this project is being donated by ITDG. This means that input costs are relatively low and the group has a more competitive edge on the market. The mushrooms, often sold within the village, are therefore as cheap as ZWD1,500/kg (USD1.8/kg) compared with about ZWD5,000/kg (USD6/kg) charged by other producers. ITDG is planning to venture into spawn production. As the group matures and starts realizing sustainable incomes, it is expected that most of spawn produced will go to the group itself first to be self-sufficient. In fact, some of the group members will have to undergo training on spawn production to reduce operational costs.



Figure 5. Mushrooms grown on grass substrate

Substrate

Although a variety of crops are grown in Chakowa, the availability of substrates remains a constraint. Grass is the major substrate preferred (Fig. 5), so mushroom

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production therefore competes for grass with livestock feeding programs. The substrate use potential of other locally available crop residues needs to be investigated in order to encourage the possible adoption of other substrates with less supply competition from other sectors.

Feasibility

Yields attained at the Chakowa project are fairly good, and average about 1kg of fresh mushroom harvested per bag. Although more specific yield levels could not be obtained, the trainers estimate that the growers are currently obtaining about 60% of the yield obtained by other producers and they are catching up fast. The project now emphasizes the need for continuous production, and at any given time, at least 30 bags should be bearing some fruit. Although a lucrative tourist spot known as the Hot Springs is located near Chakowa, the growers in this group are still less than confident about tapping the potential in this market. Their main concern is whether they will be able to meet given orders if they obtain a contract to supply mushrooms on a more regular basis.

Competitive advantages

- Spawn is donated. The project can therefore sustain lower market prices and earn higher incomes from increased sales.
- The mushroom project is primarily focused on helping orphans. This adds an emotional appeal to the product and improves sales.
- There is a potential for both fresh and dried mushrooms. Fresh mushrooms can be sold or consumed by the locals while dried mushrooms, because of reduced perishability, can be transported to other markets.
- Farmers have the experience in growing other crops. This allows for product diversification.

Competitive disadvantages

- The growers cannot produce a reliable supply to meet regular orders. The group lacks experience in order fulfillment.
- Adequate substrate is not available due to competition for grass with livestock.
- Temperatures are often very high and humidity quite low. This results in a high labor input in growing room management.
- The group does not have the organic certification that could improve the market base, especially the export market, leading to higher returns.

Conclusion

The Chakowa Orphanage Group is a viable project with a bright future. More technical and financial support could further raise the incomes attained and greatly improve the lives of the Chakowa community. Mushroom is the right crop which can much contribute poverty alleviation thanks to its low set-up cost, high price margin and quick returns. The fact that it requires agricultural waste cheaply acquired and relatively small space makes mushroom growing more accessible for the destitute and landless farmers. In addition, mushroom presents growers high nutritional value as well as income. Mushroom boom in Zimbabwe during last three years proves much of it.

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Oyster Mushroom Cultivation

Part I. Mushrooms

Chapter 2

Mushroom Growing for a Living Worldwide

MUSHROOM GROWING IN INDIA

Nilesh Pakale
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The Potential of the Mushroom Industry in India



Figure 1. Button mushroom grown in India
(Photo courtesy f Meera Pandey)

India is not a major producer of any of the mushroom varieties, but it does cultivate mushrooms and has great potential as an important producer in the future. From a production standpoint, the white button mushroom has the highest growth rate and potential for production. However, the cultivation of oyster mushrooms has been more common since the end of the last century, when the infrastructure of oyster mushroom growing was much improved and therefore capital requirements went way down in comparison with the requirements for white button mushroom cultivation.

Though India's present share in the world production and trade of oyster mushroom is meager, being only an estimated 2,000 tons, the potential for the future is rated as high for a variety of reasons. India has a very large availability of various types of raw substrate material such as wheat straw, paddy straw, bagasse, chicken manure, gypsum, tea waste, de-oiled cakes and so forth in almost all the regions and these materials are relatively inexpensive when compared with international prices. In 2001-2002, the production of wheat and paddy in India was estimated to be 73.53-90.75 million tons respectively. Although the residue straws are commonly used as fodder, almost 50% of the crop residues are still potentially available for the growing of mushrooms.



Figure 2. Oyster mushroom grown in India (Photo courtesy of Meera Pandey)

India has large number of agro-climatic regions that offer congenial climatic conditions for mushroom cultivation. India also has a good combination of both the technical and non-technical manpower needed to operate and manage the mushroom growing operations. The supply and demand gap in the world trade of mushrooms and the shrinkage of production in countries like Taiwan and South Korea due to high labor costs would result in better market prices for Indian mushroom producers. The costs of building materials and other inputs related to construction costs are much lower in India than in many other countries. This keeps the investment cost per unit weight of mushroom produced more advantageous in India.

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Figure 3. India in Asia

India is also developing its infrastructure rapidly and therefore enjoys a large and well-organized distribution network that facilitates the marketing of products in order to meet domestic consumer demands. From a dietary standpoint mushrooms are a particularly favorable food in vegetarian-predominant India. With a domestic population of more than one billion, India itself is a large market for mushrooms. The per capita consumption of mushrooms in India is currently only about 25g per year although there has been a steady increase in the consumption of exotic mushrooms including oyster mushrooms in addition to the use of regular button mushrooms. This increase can be seen as a highly encouraging sign coming from the potential mushroom consumers in India. Cultivated mushrooms are available today in all common vegetable shops, grocery stores, department stores in both small and big towns in India. One final reason for optimism concerning India's potential as a major mushroom producer is its strategic geographical location with respect to exportation, making it convenient to export oyster mushrooms to the Middle East, Europe, the United States, Africa, and Southeast Asia.

Benefits of Oyster Mushroom Growing

There are many remarkable ecological advantages in the cultivation of edible fungi. One major advantage is the efficient re-integration of agricultural residues such as horse and chicken manure, cereal straw, bagasse and others. The spent mushroom substrate can then be used either as animal feed or as compost for application in farm fields.

The cost of oyster mushroom cultivation varies according to regions and the specific type of cultivation, but generally, the growing of oyster mushrooms is less expensive than that of other cash crops. The major reason for this is it requires little space and inexpensive raw materials. Oyster mushroom cultivation is economically efficient for the farmers of other crops, who do not have to buy the raw materials for substrate and can use low cost structures for mushroom cultivation on seasonal basis. Table 1 would provide a view on the cost-benefit relationships of oyster mushroom cultivation in India.

Table 1. Cost-benefit relationship of oyster mushroom cultivation in India

Growing methods	Production cost (USD)	Yearly Production (tons)	Price Per kg* (USD)	Value of sales (USD)	Earning rate
Traditional hut growing with purchased raw materials	16,383.06	27.37	1.1	30,107	45.58%
Traditional hut growing with their own raw materials	12,364.78	27.37	1.1	30,107	58.94%
Seasonal growing with purchased raw materials	6,832.34	11.4	0.95	10,830	36.91%
Seasonal growing with their own raw materials	5,156.81	11.4	0.95	10,830	52.38%
Growing in their own constructed houses	16,392.23	27.37	1.1	30,107	45.55%

*Average yearly price realization for kg of Fresh Mushrooms

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As the table depicts, there are two most likely situations. Some growers are growing mushrooms with purchased raw materials, while others are growing mushrooms with their own raw materials. If the substrate materials are from the owner's own fields, this produces maximum profits. To obtain the maximum benefit mushroom growers should be farmers of other crops or young farmers in rural areas. The Indian agencies involved in the promotion of oyster mushroom growing are using this information to promote self-employment among rural youth. This particular aspect holds good for all developing nations in which rural youth are migrating to industrialized cities in search of employment.

The oyster mushroom has various species and each has its own characteristics. Therefore, each geographic region in India chooses the appropriate species for its climate and environment. In addition, the substrate materials used and growing methods are also different according to species and regions. Table 2 shows the cultivation aspects of various species of oyster mushroom.

Table 2. The cultivation aspects of various species of oyster mushroom

Species grown or collected	Substrate materials used	Regions(States)	System of cultivation	cultivation period	Growing temperature
Pleurotus ostreatus	Paddy Straw paddy Husk Wheat bran	Southern India particularly, Niligiri Hills	Poly bags cylindrical block	The whole year	12-25℃
Pleurotus sojar-caju	Paddy Bagasse, wheat bran	Southern India	Poly bags, Pressed blocks	June to February	22-35℃
Pleurotus Florida	Paddy straw, Wheat straw, Bagasse, Wheat bran and *Cassia hirsute* (a leguminous weed) at 20% combination with bagasse	Off-season in Northern India (Goa, Maharastra karnatake, parts of Gujarat and Andhra Pradesh)	Poly bags and poly blocks of cylindrical shape	June to February	20-32℃
Pleurotus spp.	Paddy straw, wheat straw, wheat bran, and sawdust supple-mented with wheat or rice bran	Goa, Maharastra, Karnataka, parts of Gujarat and Andhra Pradesh	Poly bags and poly blocks	June to March	22-35℃
Pleurotus spp.	From nature	Himalayan regions, Western Ghat, Niligiri Hills and Other forest areas		In rainy season	

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How to Grow Oyster Mushroom in India

Substrate preparation and treatment



Figure 4. Soaking wheat straw in water

The wheat or paddy straw is chopped in 3-5cm long by hand or mechanically. The chopped wheat straw is filled into gunny bags for 12-24 hours of soaking (Fig. 4), while paddy straw is treated in boiled water for 15-25 minutes. The wheat straw is also treated with boiled water. This decision is purely based on the capacity of straw to absorb and retain the moisture. In some cases, bavistin (carbendasim) is used instead of the boiled water treatment. The hours of treatment vary according to the substrate or substrate composition.

Spawn preparation

10kg of wheat grains are boiled for 15 minutes in 15L of water and then allowed to soak for another 15 minutes without heating. The excess water is drained off and the grains are cooled in sieves. The grains should be turned several times with a spoon for quick cooling. The cooled grains are mixed with the gypsum ($CaSO_4 \cdot 2H_2O$) and 30g of calcium carbonate ($CaCO_3$). The gypsum prevents the grains from sticking together and the calcium carbonate is necessary to correct the pH. The prepared grains are filled into half-liter milk bottles or polypropylene bags (150-200g per bottle or bag) and autoclaved for 2 hours at 121℃. After sterilization, the material should have a pH value of 7. The bottles are inoculated with grains or bits of agar medium colonized with mycelium, and then incubated at 22-24℃ in a dark place. The mycelium completely spreads through the grains in about 2 weeks.

Substrate inoculation

The cooled substrate is inoculated with spawn by layers at a rate of 2% on a wet basis to make the blocks. The procedure of block making is as follows.

1. The wooden frame of 60×45cm is placed on a smooth floor (Fig. 5).
2. The jute ropes and poly sheet are placed on the frame (Fig. 6, 7).
3. The frame is filled with approximately 5cm of cooled pre-treated straw and compressed by the wooden lid (Fig. 8).
4. The spawn is sprinkled over the whole surface (Fig. 9).
5. The same procedure is repeated five times to achieve a depth of 25-30cm (Fig. 10).
6. The plastic sheet is folded over the top of the frame and tied down with help of ropes previously placed below the plastic. The frame is removed from the block.
7. Small holes of approximately 2mm in diameter are punched in the block for breathing. The blocks are later placed on the shelves in single layer for incubation.

Spawn run and pin initiation

The block temperature is maintained at 25℃ for 12-15 days. Once the blocks are fully colonized, they are hung, after removing the polythene, in a room where the relative humidity is maintained above 85%. The humidity is normally maintained by frequent spraying of water on the blocks and on the floor. The pins are visible 9 days after the opening of the blocks.

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Figure 5. The wooden frame

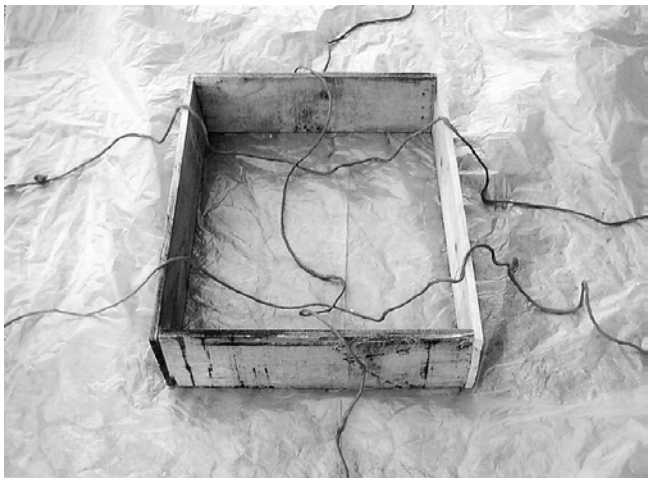


Figure 6. Jute ropes on the wooden frame



Figure 7. Poly sheet on the jute ropes



Figure 8. Filling cereal straws



Figure 9. Sprinkling spawn over the surface



Figure 10. Repeating filling and spawning 5 times

Fruiting and picking

A high relative humidity and proper ventilation is maintained in the growing room during pinning and fruitbody development. The mushrooms are usually picked for fresh market sales. Most of the growers take 3 flushes. Mushrooms picked in the third flush are mostly used for sun drying, where maximum dry matter is achieved.

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For Better Mushroom Industry in India

Most growers in India are self-employed and operating small-scale farms. They have different backgrounds with low or no knowledge of running small biological enterprises. Many short-sighted and non-committed growers are getting out of mushroom growing enterprises due to small setbacks they encounter before they accumulate enough experience in mushroom cultivation management. This situation creates fluctuations in the total number of oyster mushrooms growing units and causes an inconsistent supply-demand curve in the marketplace. This in turn causes the market price for oyster mushroom producing growers to fluctuate. As such, the market of oyster mushroom is highly localized with individual traders having great control on prices. The retail price of fresh oyster mushroom varies in India from INR*30-120 (USD0.66-2.65) per kg.

To make the oyster mushroom growing business more profitable, the following efforts should be made:

- The present growers must join hands to form co-operative societies in order to share the technical information on day to day growing and spawn production, and in order to control the price of mushrooms in the market place. The idea of co-operative formation among growers is already proposed among the grower communities but no leader has emerged to date.
- The costs of production should be maintained as low as possible by utilizing the local agricultural residue.
- Introduction of value-added products like oyster mushroom powder for soups, and oyster mushroom pizza should be made.
- Mature and committed entrepreneurs should be encouraged to become involved in the mushroom industry.
- The Indian government sectors must take the initiative in assisting in the marketing of fresh and processed oyster mushrooms for export by purchasing crops from the small scale mushroom farms. The revenue from this process can later be utilized in improving the rural infrastructure.

* INR (India Rupee, INR1≒USD0.0221 in Feb 2004)

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Oyster Mushroom Cultivation

Part I. Mushrooms

Chapter 2

Mushroom Growing for a Living Worldwide

MUSHROOM GROWING IN NORTHERN THAILAND

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Introduction



Figure 1. Rural community people taking mushroom growing lessons

Thailand is a particularly good place for future growers to learn how to grow tropical mushrooms. Thailand has ideal environmental conditions for mushroom cultivation and a long history of mushroom growing. Thai people, who knew that mushroom growing required low cost materials and technologies while offering a high and quick return on their money, have long grown a variety of mushrooms. Up to date, young rural people are eager to learn how to grow mushrooms using materials readily available to them to improve their living (Fig. 1, 2).

In addition the warm climate favorable for mushroom growing, well-established growing practices and their will to pave their way for a better life, the long & successful mushroom production in Thailand are owed to the sincere efforts and considerable support made by the Kingdom of Thailand and Thai government to enhance Thai people's life by encouraging mushroom growing. The kingdom initiated Royal Mushroom Projects aimed at promoting rural development in Thailand (Fig. 3, 4, 5, 6, 7). The government runs loan programs for rural communities, some of which adopt a mushroom production cooperative. More mushroom production at the community level is expected, enriching rural people.



Figure 2. Rural community people taking mushroom growing lessons

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Figure 3. Where Royal Mushroom Project is being Implemented



Figure 4. A mushroom growing house for Royal Mushroom Project







Figure 5, 6. 7. *Auricula Auricularia*, *Ganoderma lucidum* and *Hericium erinaceus* grown by Royal Mushroom Project

Table 1. Commercially cultivated mushrooms in Thailand

Common name	Latin name	Thai name	Market price (THB*/ kg)
Button mushroom	*Agaricus bisporus*	Hed Kradum	80-120
Black poplar mushroom	*Agrocybe cylindracea*	Hed Yanagi	250-300
Wood ear	*Auricularia auricula*	Hed Hu-noo	30-50
Inky cap	*Coprinus atramentarius*	Hed Muerk	120-160
Enokitake	*Flammulina velutipes*	Hed Khemthong	
		Hed KhaemNgern	150-200
Reishi	*Ganoderma lucidum*	Hed Lin Juer	1,000-1,500
Lion's mane	*Hericium erinaceus*	Hed Hua ling	1,000 (dry)
Shitake	*Lentinula edodes*	Hed Hom	160-180
Parasol mushroom	*Macrolepiota gracilenta*	Hed Nok Yoong	400-500
Golden oyster mushroom	*Pleurotus citrinopoleatus*		150-200
Abalone mushroom	*Pleurotus cystidiosus*	Hed Pao-hue	70-80
King oyster mushroom	*Pleurotus eryngii*	Hed Nanglom Luang	200-250
Oyster mushroom	*Pleurotus ostreatus*	Hed Nanglom Khao	30-40
Silver ear	*Tremella fuciformis*	Hed Hu-nu-Khao	300-350
Straw mushroom	*Volvariella volvacea*	Hed Fang	90-120

* THB (Thai Baht, THB1≒USD0.0258 in Feb 2004

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Growing House

Mushroom growing houses can be classified into two types: those built for temporary use or those erected for long term use. A typical mushroom house (Fig. 8) is made of thatch and bamboo or other kinds of wood poles and shading net. Growers use different dried grasses and leaves which are most readily available or thought to be the best available. Depending on the durability of the roof and wall material (1-5 years), growers should replace with a new roof and walls on a regular schedule. Some button and straw mushroom growers have brick houses with two rows of shelves inside. These houses are highly tolerant to subsequent, severe heat treatment during *in situ* pasteurization. Being secondary decomposers, button and straw mushrooms need compost and it should be pasteurized to be the selective medium for mushroom. See the steam pipes on the wall (Fig. 9).



Figure 8. Typical mushroom growing houses made of thatch and wooden poles





Figure 9. Steam pipes on the brick wall

Mushrooms Cultivated in Shelf

Straw mushroom

Straw mushroom (*Voluariella volvacea*) is a high temperature mushroom and the most popularly grown in Thailand. Thai farmers have grown the mushroom since the 1940s. Thailand has a temperature range that is very favorable for the mushroom's growth (30-37℃). Straw mushroom spawn is easily available for local farmers and that mushroom is cultivated on shelves, unlike other mushrooms, which are grown in bags except button mushrooms, whose spawn is not that easy to obtain here.

Here is a simple way of growing straw mushroom: A bale of straw inoculated with straw mushroom spawn is left in the bag and days later, mushrooms come out (Fig. 10). Traditionally, rice growers also grew straw mushrooms in their rice fields after harvest. They made rows of mushroom mounds with rice straw and other agro wastes in the harvested fields, using a wooden frame. Today, rice growers still produce straw mushrooms in their fields from December to April, using the growing method as described above. But the yield is as low as 20% and so is the price, THB20 (USD0.52)/kg, with comparison with modern growing methods.



Figure 10. Straw mushroom growing in a simple way

Indoor cultivation of straw mushroom is performed all through the year. Being a secondary saprophyte like button mushroom (*Agaricus bisporus*), straw mushrooms grow well in organic compost, where the ingredients are partially decomposed or highly degraded. When the compost is completed, it is placed on the shelf and steam is blown into the house with the temperature maintained at 60℃ for 4-6 hours. When the room temperature cools gradually down to 35℃ with the door closed, spawn is laid about 2cm deep into or on top of the compost. Indoor cultivation using compost achieves much the higher yield of 50-60%. Produced mushrooms are sold fresh in retail markets at THB30 (USD0.77) or to canneries at THB12-16 (USD0.31-0.41)/kg.

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Straw mushroom growing is a handsome income source for rice growers as well as commercial straw mushroom growers. A grower makes a net profit of about THB5,000 (USD129) permonth, greater than from other produce. Even better, the mushroom has a short production cycle, which means a fast return on investment. Further, the mushroom is rich in protein and can be grown with agro wastes, even on the spent mushroom substrate of inky cap, but the protein content of straw mushroom is much higher than that of inky cap.



Figure 11. Composting



Figure 12. Straw mushroom on the shelf

Button mushroom

Button mushroom (*A. bisporus*) is not the second most grown mushroom, nor is it a tropical mushroom. Thus, production of button mushroom is seasonal, usually in the cooler season from November to January. As mentioned above, it requires organic compost and is grown on shelves indoors. The brick house (Fig. 9) is a typical button or straw mushroom growing room. Button mushroom growing methods employed in Thailand are similar to traditional button mushroom growing in other parts of the world and include outdoor composting (Phase I) (Fig. 13), *in situ* pasteurization (Phase II), spawning, spawn-run (Fig. 15), and fruiting. Wood logs or other fuel resources are used as fuel for the boiler to steam the growing room with compost inside.

This farm produces 1 ton of mushroom per year in the 2 rows of 4 tier shelves with a total growing area of 144 (2 rows × 4 tiers × 2m wide × 9m long) square meters. Another farm we visited uses two 5-tier shelves 9m long, 1.7m wide with a yield of 13kg/m^2. The farm produces 200kg per crop. Yields have not been “satisfactory” yet. Local mushroom demand from fast food restaurant chains like Pizza Hut and McDonald's are met by imported mushrooms from Holland and Australia. And it sells at around THB52 (USD1.3) per kg. Farm sanitation practices are required for high yields of quality mushroom production.



Figure 13. Outdoor composting



Figure 14. Filling



Figure 15. Spawn run
(8 days after spawning)

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Mushrooms Cultivated in Bags

Oyster mushroom, abalone mushroom, yanagi and shiitake are commonly cultivated in bags in Thailand. Some large farms are equipped with machines and tools like ribbon mixers, bagging machines and compacting machines (Fig. 16), steam boilers and ventilation fans. They not only produce their mushrooms but also supply ready-to-fruit mushroom bags to neighboring farms. Common bag preparation methods are as follows:

- Mix sawdust (rubber tree) + rice bran (20%) + other additives (gypsum, lime, calcium sulfate ($CaSO_4$) or magnesium sulfate ($MgSO_4$))
- Adjust the water content of the mixture to 60-65%. (A rule of thumb is squeezing the mixture in the palm of your hand. When a droplet or two barely escapes, the mixture has a proper water content.)
- Fill the bags and compact
- Pasteurize the bags in the cooker for 3-4 hours from the time temperature reaches 90-100℃.
- Cool them to 25℃ and inoculate

They commonly use a plastic ring to make a “bottle neck” for easy handling. They put a plastic ring on the bag end, pull out the bag end through the ring, fold down the pulled out part, tie it with a rubber band and plug with cotton, paper or cotton-topped plastic plug (Fig. 17).



Figure 16. Compacting Machine



Figure 17. Plugging



Figure 18. Traditional oil-drum sterilizer



Figure 19. Metal grate for oil drum sterilizer

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Substrate bags are sterilized either in a commercial autoclave at 15-20 psi for 1 hour or in an oil drum sterilizer (Fig. 18, 19) around 100℃ or higher for 3-4 hours. How to use the oil drum sterilizer is as follows. The sterilizer is first filled with water a foot from the bottom, heated and maintained at 90-100℃ for 3-4 hours. Bags for mushrooms with a long cultivation period should be sterilized above 100℃ with pressure. When the bags are cooled to the ambient temperature, inoculate them with spawn in a clean, sterile if possible, room (Fig. 20). Sorghum seed is the most commonly used material for a spawn carrier in Thailand.



Figure 20. Inoculation



Figure 21. Mushroom bags at incubation

Oyster mushroom and abalone mushroom

Being easy to grow, oyster mushroom is favored by more and more growers in the world, especially by those who want simple growing. All the mushroom bags are stacked atop the other bags on the A-frame shelf.

The farm we visited produces oyster mushroom for 6 months per crop and sells them at THB20 (USD0.52)/kg in wholesale markets and THB25 (USD0.65)/kg in retail markets. When the fruiting starts, they harvest mushrooms every day but not from all the bags. Growers can harvest up to 500g from a high quality 1kg bag in a crop. In average, they produce 200-300g from a bag. They also grow oyster mushrooms from Hungary, whose spawn costs THB2-3 (USD0.05-0.08) per kg bag.

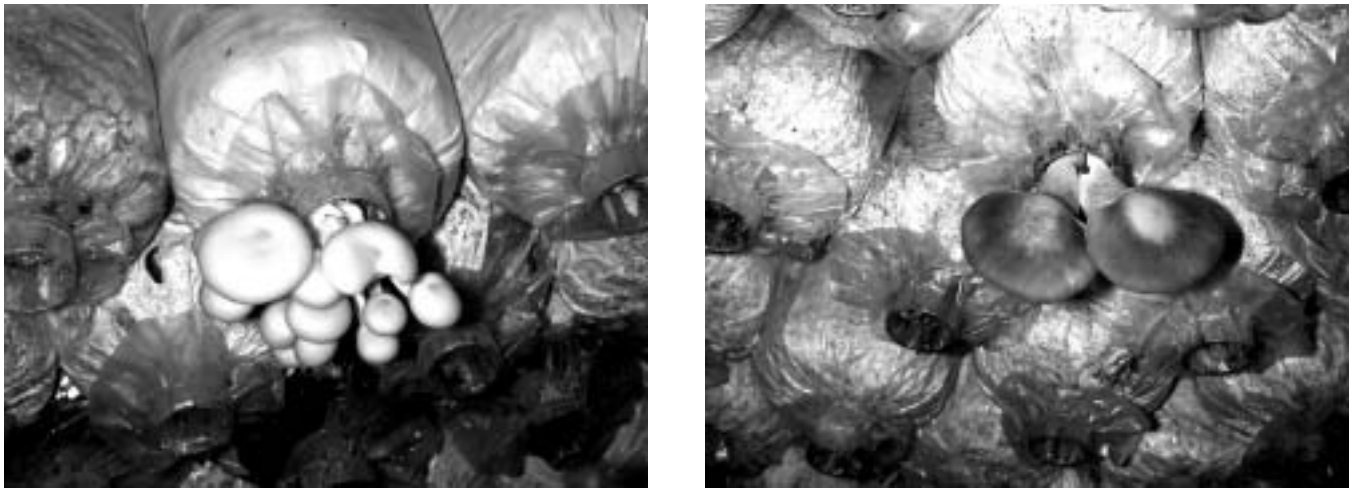


Figure 22, 23. Oyster mushroom and abalone mushroom bags in the shelf

In the meantime, abalone mushrooms are harvested once a week and the production cycle takes a year. The average yield is 500g/kg a year. The mushrooms sell at THB40 (USD1.03)/kg in wholesale markets and THB50 (USD1.29)/kg in retail markets. As a new item, they fetch relatively high prices compared with oyster mushrooms.

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But you should take a note of the productivity. Each mushroom produces 500g from a 1kg bag in one crop. But the crop span of oyster mushroom is half that of Abalone mushroom. That means productivity of the latter is half of that of oyster mushroom, while the price is the other way around. The choice is up to growers.





Figure 24.25. Oyster mushroom in cold storage and in the market



Figure 26. Packaging (Yanagi matsutake)

Yanagi matsutake

Yanagi matsutake (*Agrocybe cylindraceae*) is relatively easy to grow but less easy than oyster mushroom, since the mushroom is said to be more prone to contamination and it requires a longer time before the first harvest (1-1 1/2 months). The mushroom (called Yanagi matsutake in Thailand) is a new item in Thai mushroom markets. Its high demand, thanks to their marketing and promotional efforts, brings a handsome income to growers. The mushroom sells at the price of THB100-120 (USD2.58-3.10)/kg. A mushroom growing bag made of sawdust, rice bran (7.5-10%), $CaCO_3$ (2%), sugar (1%) and gypsum (1%) costs THB6 (USD0.15) per kg bag. A production cycle has ten flushes, lasts one year and produces 150-200g/kg in total. The mushroom can be stored at 7℃ for 7 days.

Shiitake

As grown in relatively low temperatures, shiitake (*Lentinula edodes*) can be cultivated mostly in the highland areas with cooler temperatures or at lower altitudes in the cool season. Shiitake is one of the most expensive edible mushrooms in Thailand because there exist relatively unfavorable conditions for cultivation of those mushrooms. To provide better conditions, mushroom growers cover the roof with a shade net and pour cold water through the roof for evaporative cooling. To induce fruiting, they use icy water. Unlike other mushrooms, shiitake require cooler temperatures and are cultivated on the ground. The floor is limed to prevent fungal contamination, especially from green mould. A 1kg substrate bag costs THB5-7 (USD0.13-0.18). Mushroom growers harvest 3-4 flushes or 7-9 flushes in a crop.



Figure 27. Mushroom growing bags at spawn run



Figure 28. Shiitake grown on the floor

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What Mushroom Growing means to Thai people's Life

Mushroom cultivation in Thailand means much more than growing other commodity crops. Most of the farmers involved with mushroom cultivation recycle agricultural wastes to cultivate mushrooms. Some 70% of rice farmers cultivate straw mushroom by utilizing straw or hay they already have. They don't need to buy basal substrate material. In a few years, straw mushroom cultivation brings them more money than rice. Inspired by the large income from mushroom growing, the Thai government encourages poor rural people to grow mushrooms. Moreover, mushroom growing provides a quick return on investment. Straw mushroom cultivation takes just 3 weeks and other mushrooms like abalone, oyster and ear mushroom 3-4 months to bring money to farmers. And the 10-30% profit is high enough for farmers to continue growing.

Lately medicinal mushrooms such as reishi (*Ganoderma lucidum*) and lion's mane (Hericium erinaceus) were introduced to the country. That brought about a great interest not only in reishi but also other medicinal mushrooms, even in Thai traditional medicines among Thai people. Now Ganoderma mushroom and dried lion's mane fetch the highest prices ever, THB1,000-1,500 (USD25.8-38.7) and THB1,000 (USD25.8)/kg, respectively, 25-50 times the price of oyster mushroom. Growing these medicinal mushrooms is like producing 'golden eggs'.

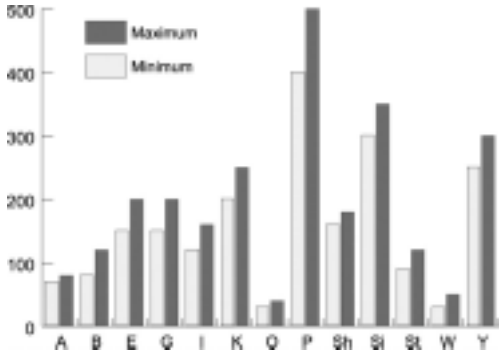


Figure 29. Prices of mushroom cultivated in Thailand

A : Abalone M. (*Pleurotus cystidiosus*)
B : Button M. (*Agaricus bisporus*)
E : Enokitake (*Flammulina velutipes*)
G : Glden Oyster M. (*Pleurotus citrinopoleatus*)
I : Inky Cap (*Coprinus atramentarits*)
K : King Oyster M. (*Pleurotus eryngii*)
O : Oyster M. (*Tremella fuciformis*)
P : Parasol M. (*Macrolepiota gracilenta*)
Sh : Shiitake (*Lentinula edodes*)
Si : Silver Ear M. (*Tremella fuciformis*)
St : Straw M. (*Volvariella volvacea*)
W : Wood Ear M. (*Auricularia auricula*)
Y : Yanagi (Black Poplar)
M. (*Agrocybe cylindracea*)

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Oyster Mushroom Cultivation

Part I. Mushrooms

Chapter 2

Mushroom Growing for a Living Worldwide

MUSHROOM PROJECT IN SWAZILAND

A Government Initiated Mushroom Bag Distribution Center

Kyung Wha Choi
MushWorld

Through the four articles about Nepal, Zimbabwe, India, and Thailand, it has been emphasized how profitable and appropriate mushroom growing is for poverty alleviation. However, its application is another story. Let us have a closer look on the difficulties in performing mushroom project.

Mushroom projects for poverty alleviation have been carried out in many countries with support from various international and local organizations. Each country or region has conducted its own mushroom projects using different methods or systems according to its particular situation. In some countries the government has initiated mushroom projects by inviting consultants from overseas and attracting funds from various organizations (**government-initiated case**). In other countries, several pioneer farmers have produced substantial profits from their mushroom growing and therefore mushroom growing has become popular among other farmers as well, and at this point the government has started to pay attention to mushroom growing and support it (**grower-initiated case**). Both models have their strong points and weaknesses.

Most mushroom projects carried out in African countries can be categorized as government-initiated projects. Nevertheless, they can be also divided into two types. Some programs emphasize training for each grower and financially and technically support them to grow mushrooms by themselves. In other cases government agencies perform key production steps such as spawning and incubation in order to manage the projects. It would be difficult to say which is the better method, but examination of both cases will help project planners when they choose the best methods for their own situations. To help illustrate this point, a mushroom growing project in Swaziland will be described in which the government initiated the project and was in control of the key production systems. Management team of this project performed research to find the most suitable substrate materials, supplied them to growers, produced spawn-impregnated bags, and educated farmers in the management of growing houses.

Kingdom of Swaziland

Swaziland (Fig. 1) might not be a familiar country to some readers. It has a short history as a modern country, as it

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only became independent from England in 1968. In general, Swaziland is a beautiful country with green hills and many cattle. Some call it the 'Switzerland in Africa' (Fig. 2, 3). The climate is moderate, ranging from subtropical to temperate depending on the altitude. As one can see from its formal name, the Kingdom of Swaziland, the king has a firm grip on the country. Though people have the right to vote for one of the cabinets by election, the king appoints the Prime Minister and two-thirds of the country's parliament, and some important government positions are always filled by members of the king's family, known as the Dlamini.



Figure 1. Kingdom of Swaziland

Though Swaziland is not categorized among the least developed countries in the world, the peoples' standard of living is quite low, especially in rural areas (Fig. 5). The GDP per capita was USD4,200 in the year of 2001, and the major industries are sugar, mining (coal and asbestos), wood pulp, agriculture, and the production of soft drink concentrates. It produces a large amount of agricultural wastes such as sugarcane bagasse, corncobs, and other crop refuse. It has several factories in Matsapha and most of the investment capital comes from Taiwan.

Like other African countries, Swaziland has a high percentage of its population living with HIV-AIDS, and even more suffering from chronic malnutrition. Most young adults live in the cities, having left their parents and young siblings in the rural areas where standards of living are far lower than that of cities.



Figure 2. Swaziland-'Switzerland in Africa'



Figure 3. Traditional houses in Swaziland



Figure 4. Students after school



Figure 5. A girl selling fruit on the street

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The Beginning of Mushroom Project

This mushroom growing project in Swaziland was initiated by the king. The king had visited mushroom farms in Thailand and decided in 2000 to start a mushroom project in Swaziland. The king dispatched five persons to a mushroom training center in Thailand, and invited Thai mushroom consultant Anon Auetragul to Swaziland. Anon has had a great deal of consulting experience in the last 20 years with mushroom projects in many Asian and African countries. Anon stayed in Swaziland for six months and taught cultivation technology and production skills to local mushroom growers. He helped perform the research to find appropriate substrate materials, established the distribution center for spawn-impregnated bags, introduced the mushroom growing system used in Thailand, and applied it to the situation in Swaziland. During the course of his stay some local Swaziland people were trained in mushroom workshops that were held for 2 weeks in Shanghai and later in training courses held in Namibia and Malawi. From 2000 to 2002, there were a variety of activities, preparations, and investments made in the local mushroom industry.

Malkerns Research Station was the key facility for this project. It performed research on the preparation of cultures and produced grain spawn from the 3 available oyster mushroom strains. Many mushroom growing research projects were started. The most important were experiments to find the most appropriate substrate materials in this country. They had imported rubber tree sawdust, the main substrate material in Thailand as a control substrate material, and experimented to identify suitable local substrate materials and mixes. Five promising substrate mixes were selected for further observation, for all of which fresh bagasse was the main substrate component. Because bagasse is waste from the sugarcane factories, the growers could get it for free.

Malkerns Research Station-Mushroom Bag Distribution Center

As briefly mentioned above, the bag distribution center is one of the most important features of the mushroom project of Swaziland. Preparation of substrate, bagging, sterilization, and incubation are all done in this distribution center. A mixer (Fig. 7) and a bagging machine were imported from Thailand because it would have required a great deal of time to produce these machines locally. Two sterilizers (Fig. 9) were locally made within 6 months.

Distribution Center of Spawn-impregnated Bags



Figure 6. Place for preparation and mixing



Figure 7. Imported mixer for substrate materials

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Figure 8. Mushroom bags after bagging



Figure 9. Locally made sterilizers for bags



Figure 10. Inside sterilizer



Figure 11. Inoculation room



Figure 12. Incubation room (temp. controlled)-various Sizes of bags are incubated

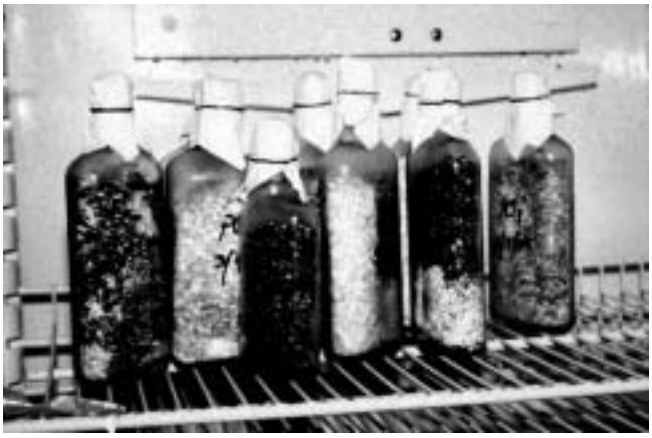


Figure 13. Various cultures in storage inside refrigerator

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Figure 14. Inside experimental growing house

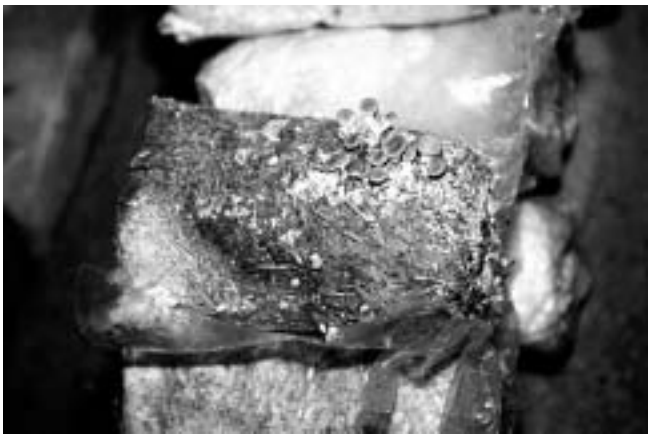


Figure 15. Oyster mushrooms on grass substrate





Figure 16, 17. shiitake and *Ganoderma* mushroom in experimental growing house





Figure 18, 19. Plants cultivated on spent substrate of oyster mushroom

The mushroom bag distribution center established the following oyster mushroom production system; the substrate materials were mixed using the mixer (Fig. 7) and the mixed material was put into bags (Fig. 8) with the bagging machine. The bags were all imported from Thailand, and needed to be heat-resistant as they were sterilized in sterilizers after bagging (Fig. 9, 10). After being sterilized, they were delivered to the inoculation room (Fig. 11). After being cooled down, the bags were carried to the incubation room where the temperature was controlled by an air conditioner (Fig. 12). During incubation, any contaminated bags were destroyed, and finally, well-incubated mushroom bags were distributed to farmers for free. The farmers then watered the bags and controlled the environments of their growing houses and harvested mushrooms.

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The distribution center also had its own growing house for research (Fig. 14) and storage for substrate material. The center distributed only bags of oyster mushrooms (Fig. 15), but they are also growing shiitake (Fig. 16) and *Ganoderma lucidum* (Fig. 17) on an experimental scale. They have also done research on the use of spent mushroom substrate (SMS). Spent substrate of oyster mushroom contains a high amount of nutrition in comparison with other fertilizers (see 'recycling of Spent Oyster Mushroom Substrate' at Chapter 9). In front of the growing house, they were growing cucumbers, spinach, and eggplant on the SMS-fertilized field (Fig. 18, 19).

Two Pilot Export Production Villages (EPVs)

As sites for a pilot project, two export production villages were selected (Fig. 20), each with a different environment and then distributed mushroom bags to the farmers in these regions for free. One village is Siphofaneni in the East where they have somewhat dry weather and the other is Mbangweni (Fig. 21) in the South, where they have a relatively high humidity. The project aimed to find the appropriate growing methods for each region as well as the differences between the fruiting bodies of mushrooms produced in both regions. To begin, the distribution center supplied 300 mushroom bags to about 50 farmers in each village. The center began to distribute spawn-impregnated bags to farmers in March 2001, and it had distributed about 20,000 bags by the end of the year 2002, including 2,000 spawn-impregnated bags imported from Thailand.



Figure 20. Map of Swaziland

The recipients of the bags were expected to keep a complete daily record on numbers of supplied bags, weather, the amount of watering and harvesting, income, and any other observations made within their growing houses. Some of the farmers successfully managed their growing houses until their bags were exhausted after 3 months. Some of the substrate mixes tried did not give good results and produced only minimal harvests. On average, a farmer earned SZL*300 (USD45.57) per month from the 300 bags supplied from the distribution center. Though the number of supplied bags was limited to 300 bags per farmer, they considered this income reasonable because they got the bags for free. Oyster mushroom samples were distributed to NAMBOARD (National Agricultural Marketing Board of the kingdom of Swaziland) and some catering companies in order to establish product market acceptance, and NAMBOARD was impressed by this mushroom product. However, they couldn't create a formal market for mushrooms because of the limited production capacity and erratic supply. Instead the farmers created informal market channels by themselves and sold their mushrooms through those relationships.

What the farmers at Mbangweni accomplished was particularly noteworthy. They established markets at Nhlangano and supplied mushrooms at the price of SZL20 (USD3.04) per kg, which brought them a fair income. They are now in an advanced stage in the construction of a mushroom center with an office, mushroom storage facilities, a packing room and a processing room (Fig. 22). Encouraged by their success, the government provided additional training in business management for the Mbangweni farmers.

Unfortunately, the farmers at Siphofaneni had difficulties in mushroom growing due to their dry climate and unfavorable market conditions. It was quite difficult for them to access clean water for use in their mushroom cultivation activities. Encouragingly, eight of the farmers from the group managed to install piped water systems for watering their mushrooms.

Demonstration mushroom cropping houses were initially constructed and then each of the farmers constructed

* SZL (Swaziland Lilageni, SZL1≒USD0.1519 in Feb. 2004)

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their own houses by themselves (Fig. 23, 25). The growing houses were made of logs and thatched grass (Fig. 26), but these growing houses were not sufficiently durable. They deteriorated quickly and did not allow the farmers to regulate important growing parameters such as temperature and humidity. The use of more efficient and durable materials such as plastic sheeting and bricks were recommended. At Siphofaneni during the dry weather cattle and goats were very hungry due to lack of grasses, so sometimes they ate the grasses of the growing houses! This was the fate of the mushroom farm that we visited in Siphofaneni. The farmer then borrowed a plastic sheet from his friend and surrounded the growing house (Fig. 25). He kept a record of numbers of supplied bags, weather, the amount of watering and harvesting, income, and changes in the growing house everyday (Fig. 27) until he received the last mushroom bags in April of 2003.

Mbangweni EPV in South



Figure 21. Mbangweni EPV



Figure 22. Mushroom center for storage, packing, processing (under construction)



Figure 23. Growing house at Mbangweni



Figure 24. Oyster mushroom bags inside

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Siphofaneni EPV in East



Figure 25. Growing house with plastic sheet -cattle ate grasses (the materials of the house)



Figure 26. Inside growing room made by logs and grass-the roof



Figure 27. Mushroom farmers with records book on her hand



Figure 28. Inside the growing room-mushroom bags

Problems of the Mushroom Project

The activities of the farmers in this mushroom growing project were hindered by unexpected problems: suspension of sugarcane bagasse supply and lack of financial support. Notably, the sugarcane mills stopped supplying sugarcane bagasse in late 2002. Sugarcane bagasse had been previously thrown away by the factory, so the distribution center could get the substrate material for free. However, the factory had stopped disposing of the bagasse after they found that their waste could be utilized by the mushroom industry. This caused a serious problem for the project, and the distribution center was no longer able to produce mushroom bags. The center then asked the factory to sell them bagasse, and the factory replied that it would set the price of bagasse, but in the meanwhile it used all the available bagasse as fuel. The center had to find new substrate material to replace bagasse, and this required additional research.

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Figure 29. Substitute material -grass



Figure 30. Substitute material -corncobs



Figure 31. Farmers at Mbangweni maintain their growing-houses anticipating mushroom bags



Figure 32. Inside growing house in Mbangweni

At this point, the center faced a critical lack of funding. UNDP Swaziland, the custodian of the finances for the project, now blocked funds from the ZERI Regional Project, refusing to support endless research without considerable results. The center was forced to stop the distribution of mushroom bags in April of 2003 and have since produced only a small amount of bags for substrate substitute research. One alternative substrate mix included hay from Bermuda grass obtained from the Highveld, other mixed grasses from the Lowveld (Fig. 29), with added corncobs (Fig. 30). Among other sad news, the center also now needs to establish new facilities to prevent the high contamination rate (20%) of bags. They believe they could lower the contamination rate if they had better facilities and a temperature control system in the inoculation room and all this requires considerable new funding.

Since the center stopped distributing mushroom bags, the farmers have been maintaining their growing houses and waiting for the distribution of new bags (Fig. 31, 32). With extension workers we visited Mbangweni, one of the export production villages. The farmers there were upset that the center is no longer providing mushroom bags. Most of the farmers are very poor and starving. But without additional funding for the distribution center, there seems to be no way to resume its operation.

UNDP Swaziland, which witnessed a considerable amount of money being used up with few satisfactory results, now has another idea in mind, something different from a distribution center controlled by the governmental sector. UNDP Swaziland believes a mushroom project at the farmer's level would provide better results, as projects do in other African countries such as Uganda. Their theory is that they need to educate farmers and let them do the research by themselves to find appropriate and economically feasible substrate materials. The agriculture and environment varies from region to region, so appropriate substrate materials and efficient growing

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methods will also be different according to region. Farmers would learn from others' experience as well as their own. Moreover, they would be more active in finding better growing methods because they would be working for their own poverty alleviation. Now, growers in Swaziland are helpless and can do nothing for themselves when facing difficulties because they have very little knowledge about mushroom growing. Once educated about the fundamentals of mushroom growing however, they would do whatever they could in time of difficulties instead of waiting for mushroom bags helplessly. UNDP Swaziland believes this bottom-up system will make new mushroom projects more powerful and energetic.

Mushroom Project: Top-down vs. Bottom-up

Although it could be a controversial topic for discussion, and we have many unanswered questions, we can learn a lot from this mushroom project in Swaziland. If the bags are well incubated, fruiting and harvesting is not a difficult job. Most techniques and know-how are concentrated in the distribution center while the farmers' jobs in this case don't require much skill. This system is more effective and productive if the government manages the mushroom growing as farmers have little knowledge about mushroom cultivation. Unlike the situation in Asia, which has a long history of mushroom growing and consumption, here in Africa the people have very little knowledge about mushroom cultivation. Most people even don't know what mushrooms are, so it could take a relatively long time for farmers to learn the whole process of mushroom growing. Moreover, Swaziland has enough agricultural extension workers to support farmers in practical ways. Therefore, the top-down system could be the best for Swaziland.

On the other hand, this top down system has a crucial weak point. If the center stops, everything stops, as one can see in the current situation of Swaziland. If they had focused on teaching farmers the whole process of mushroom growing, they could have produced mushroom bags by themselves like mushroom farmers in other countries when faced with these kinds of difficulties.

We all know the government distribution center and UNDP Swaziland have both done their best. Moreover, they have the same goal of facilitating mushroom growing as a means of poverty alleviation. We believe they could cooperate in striving for this goal. If they did, then Swaziland could try to encourage mushroom growing in two levels. The distribution center could be operated by the government, and could sell mushroom bags to growers, and the project could include a new strategy by investing funds in education at the farmers' level. Then both sides could cooperate, which would further promote mushroom growing in Swaziland. Though they resume the mushroom project by focusing education on farmers, distribution center itself and what have been already invested into it including money, time, research, teamwork should not be wasted.

In spite of the current stall in the situation in Swaziland, this mushroom project has appreciable implications for their efforts toward poverty alleviation. It is also expected that their cooperative efforts will hopefully bear fruit even if the future project adopts a different strategy. We still believe that mushroom growing will be an effective strategy for poverty reduction in the near future of Swaziland.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 3

Introduction to Oyster Mushroom

WHAT IS OYSTER MUSHROOM

Seung Woo Kang
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The principles of oyster mushroom growing follow the general characteristics described in 'What is Mushroom' in Chapter 1. In this article three main factors−spawn, substrate and environmental control−will be discussed again focusing on oyster mushroom cultivation. As a fruitbody of an edible white rot fungus, oyster mushroom belongs to *Pleurotus*, Pleurotaceae, Agaricales, Basidiomycota. In nature, oyster mushrooms appear in cluster on dead trees from late fall to spring, and are distributed almost all around the world (Jiyul Lee, 1993). Oyster mushrooms share all the fundamental characters of cultivated mushrooms.

Spawn

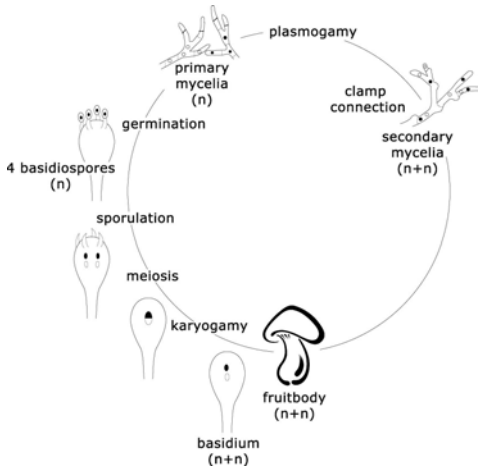


Figure 1. Lifecycle of oyster mushroom

Four basidiospores form at the end of each basidum on the gill of a fruitbody (Fig. 1). Each spore has one nucleus. Spores germinate to become primary mycelia, and then form secondary mycelia by plasmogamy. Chances are 25% that a primary mycelium will meet with a compatible one. Secondary mycelia of oyster mushroom can be distinguished by the clamp connections and each cell has two nuclei. Only secondary mycelia can produce fruitbodies under the proper conditions. In the basidia of a mature fruitbody, the two nuclei fuse into one, then pass through meiosis, and produce 4 haploid nuclei. The four haploid nuclei are then made into four new basidiospores.

Spawn suppliers usually make oyster mushroom spawn from isolated secondary mycelia by tissue culture. Growers can also make their own spawn by incubating the spores or tissue of fruitbody specimen, but highest level of sanitation is required. Tissue culture is recommended for mother culture production because genetic characteristics of the mushroom are preserved to the isolated mycelia. On the other hand, spore culture easily brings about variation of character manifestation due to recombination of genes. During spawn preparation, the first isolated generation (called mother culture) is usually inoculated to substrate and incubated, which is the second generation (called mother spawn). Fully colonized mother spawn is inoculated to another substrate and it incubated to be the third generation (called mushroom spawn). By repeating inoculation and incubation, more spawn can be produced from mother culture, but much repetition will lower the spawn's vitality. Most growers

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utilize the third generation spawn.

During propagation and storage, growers must be faithful to the principles not to decrease the vigorosity or increase the mutation or variations. There are several storage methods for mother culture including subcultures, liquid nitrogen and paraffin sealing. Most common for oyster mushroom is grain and sawdust spawn. Detailed information on characteristics of oyster mushroom strains and spawn production will be provided in chapter 4 (Spawn).

Substrate

Substrate can be understood as soil for plant providing necessary nutrition. Substrate mixture of oyster mushroom should supply specific nutrients required for oyster mushroom cultivation (Table 1).

Table 1. Nutritious materials for oyster mushroom

	Nutrients		**Materials**
organic	C-source	cellulose	humus materials such as wood, straw, leaf, etc.
		hemicellulose	″
	N-source	protein	″
		amino nitrogen	″
inorganic			K, P, Si, Fe, Mg, etc.

(Source: *Oyster mushroom-cultivation technology and management* by Cha *et al.,* 1997)

The main nutritional sources for oyster mushroom are cellulose, hemicellulose and lignin. C/N ratio is important factor for optimal substrate composition for oyster mushroom. Oyster mushroom requires much carbon and less nitrogen source than button mushroom (*Agaricus bisporus*) but most of main substrate materials such as cereal straw, cotton waste, sawdust need supplementation of nitrogen source such as wheat and rice bran to reach optimal C/N ratio for oyster mushroom. Inorganic materials are usually included in substrate materials and need not additional supplement. And amino nitrogen is used during spawn run, but it is not fit for fruiting, therefore, growers commonly do not need additional apply of amino nitrogen during mixing (Cha *et al.*, 1997).

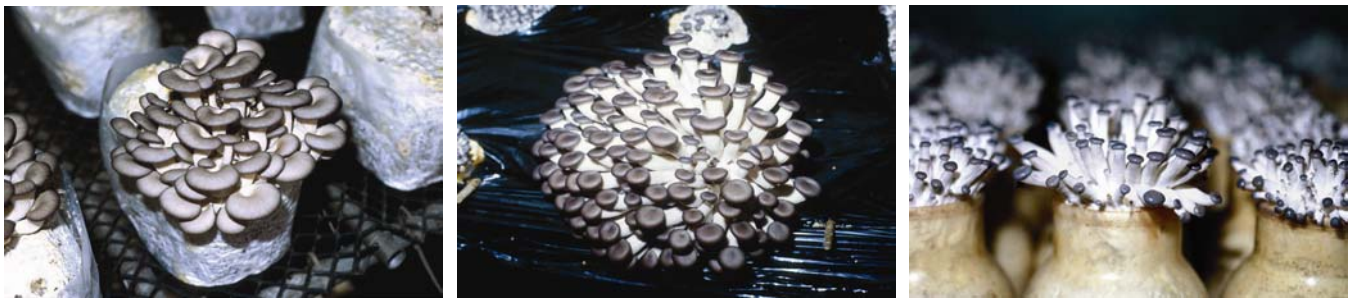
Oyster mushroom growers have wide range of substrate materials as oyster mushroom can utilize various agro-wastes with its enzyme. That is to say, oyster mushroom is a white rot fungus that uses lignin and cellulose together as its carbon source and turns the host into white. Therefore, any type of organic matters containing lignin and cellulose can be used for oyster mushroom substrates, and this includes almost all agricultural wastes. Possible substrate materials are sunflower seed hulls, rice/wheat straw, bean, sugarcane bagasse, rubber tree sawdust, groundnut shells, cotton waste, cottonseed hulls, coco lumber sawdust, coffee pulp, corncobs, paper, water hyacinth, water lily, cocoa shell waste, coir and others. Various utilizations of substrate materials for oyster mushroom are introduced in Chapter 5 (Substrate).

It would be impossible to say the single best mixing formula of substrate that will perfectly satisfy all growers since different materials are available at different prices in different regions. The same supplementation could increase yield in temperate area but contamination in tropical area. Growers' tastes also affect the selection of substrate materials. Above all, it is strongly recommended that each grower find his own best substrate mixing formula by trial and error based on standard substrate mixing formulae.

In choosing a growing method, growers should consider labor availability and the provision of substrate materials. Mushrooms from log cultivation are commonly assumed to be of the best quality. But the recently developed skills of shelf, bottle and bag cultivation seem to have bridged the quality gap. Log cultivation takes a long time for one flush and shows a low rate of productivity in spite of its intensive labor demand. As the most

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widely performed method, bag cultivation provides stable yield with relatively few failures. Shelf cultivation seems to be more risky than either bottle or bag cultivation because contamination once occurs, can rapidly spread through the whole substrate mass on the shelf. Bottle cultivation can be automated and requires a high investment. In choosing the ideal substrate material, growers should consider the long-term availability, expense and productivity of the materials.



A. Bag cultivation B. Shelf cultivation C. Bottle cultivation

Figure 2. Fruitbodies of oyster mushroom from various cultivation modes

Environment

Environmental factors include temperature, relative humidity, light, carbon dioxide and acidity of substrate, which alter together in their co-dependant relationships. As the growing room temperature is raised, relative humidity decreases. A higher temperature promotes fruitbody metabolism, which in turn, increases their respiration rate and results in high carbon dioxide production. Oyster mushrooms also need different environmental conditions at each growing stage. During incubation, appropriate relative humidity is 65-70% and water content of substrate is 65%. Optimal temperature for mycelial growth is 20-25℃, but some thermophilic strains reach optimal growth at 25-35℃. Mushroom mycelia are quite durable to high concentration of carbon dioxide during incubation.

Upon the completion of incubation, pinning induction follows. Pinning induction is made by worsening the environment in order that the mycelia cannot keep on with their vegetative growth and will therefore convert to a reproductive growth mode, which initiates fruitbody formation. Pinning induction includes cold shock, watering and lighting. Once the pins come out, growers stop pinning induction and maintain environmental conditions that are favorable to fruiting. Carbon dioxide concentration should be less than 800 ppm in its reproductive growth though the number differs according to strains. Fruitbody formation also requires high relative humidity up to 80-95% and lower temperature than optimal mycelial growth by 10℃. In addition, some strains also need light of 50-500 lux for primordial formation. Growers then harvest the resultant fruitbodies.

Growers can choose a suitable strain for their own natural environment. Each *Pleurotus* species needs different environmental conditions for fruitbody development (Table 2).

Table 2. Environmental parameters for fruiting of oyster mushrooms

Species	Temperature (℃)	Relative humidity (%)	CO_2 (ppm)	Light (lux)
P. citrinopileatus (Golden Oyster Mushroom)	21-29	90-95	< 1,000	500-1,000
P. cystidiosus (Abalone Mushroom)	21-27	85-90	< 2,000	500-1,000
P. djamor (Pink Oyster Mushroom)	20-30	85-90	500-1,500	750-1,500
P. eryngii (King Oyster Mushroom)	15-21	85-90	< 2,000	500-1,000
P. euosmus (Tarragon Oyster Mushroom)	21-27	90-95	< 1,000	750-1,500
P. ostreatus (Tree Oyster Mushroom)	10-21	85-90	< 1,000	1,000-1,500 (2,000)
P. pulmonarius(Phoenix or Indian Oyster Mushroom)	18-24	85-90	400-800	1,000-1,500 (2,000)
P. tuberregium (King Tuber Oyster Mushroom)	30-35	85-90	< 2,000	

(Source: Stamets, 1993)

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The other key component in environmental control is pest and disease management. The routines of pathogenic invasion are evident. It is obvious that virus infected or bacteria contaminated spawn will cause problems to the whole inoculated crop. Insufficient sterilization cannot remove all pathogenic organisms in the substrate. During inoculation, pathogenic fungi and bacteria can invade via dirty tools, shoes, clothes and hands. During incubation, they also come into the room through the door opening and windows.

It is not easy for even skillful growers to completely prevent contamination. But considering that mushroom marketing emphasizes the nutritional and medicinal effects of mushrooms as a natural food, even when mushroom diseases occur, growers should avoid using chemicals. This is of course totally up to the choice of the growers. The best means of pest management are good preventative practices. Environmental control and pest & disease are discussed further in Chapter 6 (Growing Houses) and Chapter 8 (Pest and Disease Management).

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

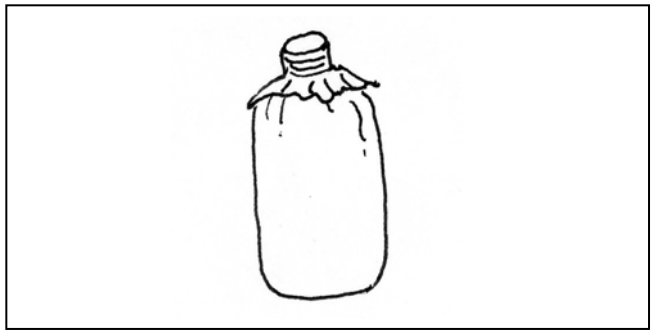
ILLUSTRATED GUIDE TO OYSTER MUSHROOM CULTIVATION

Seung Woo Kang, Hyunjong Kwon, Byung Sik Kim
MushWorld

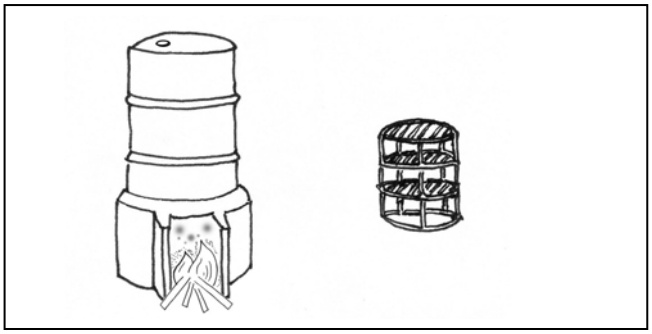
Part I. Bag Cultivation



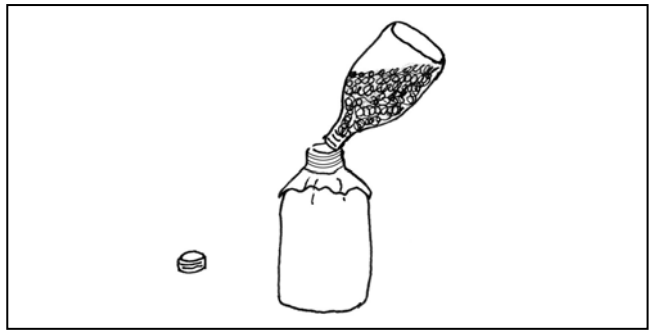
[Bagging] Fill the bags with substrate mixture and compress them with a stick. Some growers bore a hole substrate.



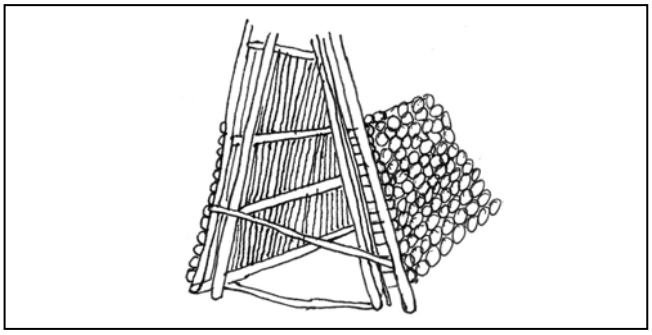
[Neck-making & Plugging] Put a plastic ring and pull the bag top out through the ring. Plug the mouth with a cotton ball or paper and a rubber band.



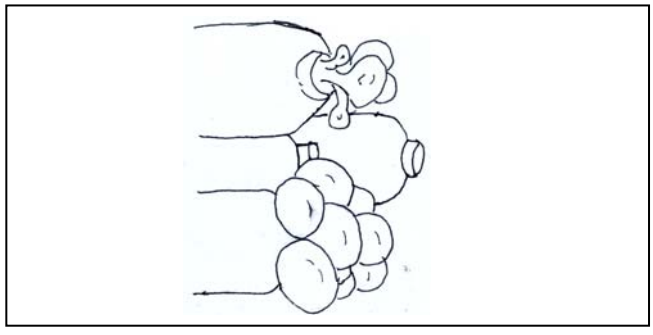
[Sterilization] Put a metal rack in the oil-drum sterilizer. Stack the filled bags on the linen-lined metal rack. Linen prevents bags burning from the searing heat in the sterilizer.



[Pasteurization in bulk] Substrate mixture is pasteurized with live steam at 60℃ for at least 6 hours. During this process, possible pathogenic fungi and bacteria in the substrate mixture are killed.



[Incubation] Inoculated bags are stacked and incubated. When colonization is completed, remove



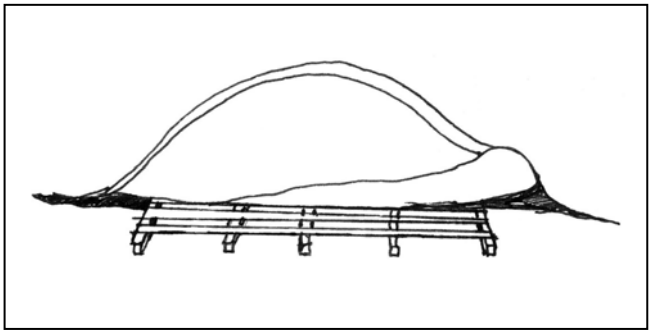
[Fruiting] Fruiting bodies ready to harvest. Frequent, light watering is recommended to produce high

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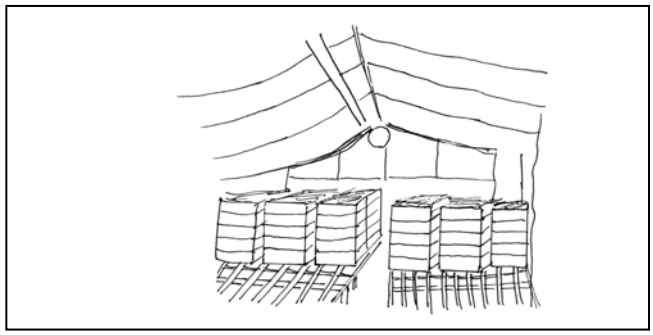
the cap for fruiting induction.

Quality mushrooms.

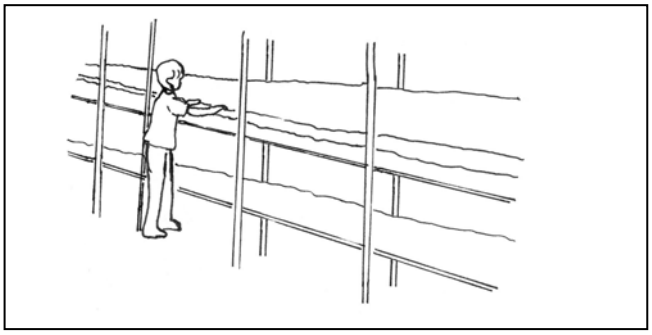
Part II. Shelf Cultivation



[Outdoor fermentation] Substrate materials are mixed and piled outdoors. During this process, organic compounds are degraded into simple substances, more absorbable by mushroom mycelia.



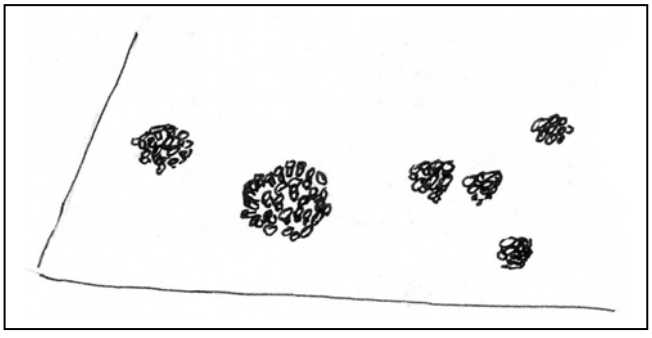
[Pasteurization in bulk] Substrate mixture is pasteurized with live steam at 60℃ for at least 6 hours. During this process, possible pathogenic fungi and bacteria in the substrate mixture are killed.



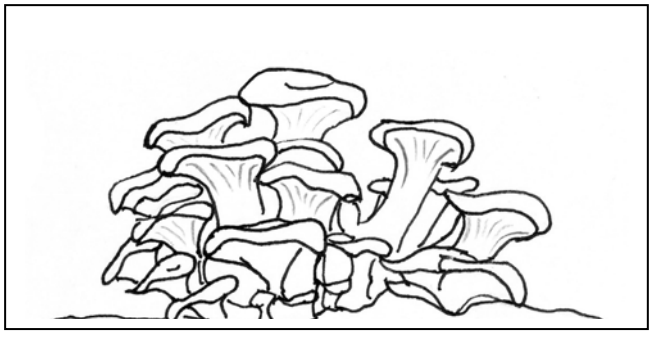
[Filling] When the mixture is cooled, fill the plastic sheeted shelves with the pasteurized substrate. After spawning, the entire shelf is covered by the plastic sheet.



[Spawning] Spawn can be distributed only on the surface or 70% of spawn can be mixed with substrate and the remaining 30% can be scattered on the for other disease and competitor fungi to take root.



[Pinning] Remove plastic cover upon full colonization, and small pins will occur. They are so delicate that heavy watering should be avoided.



[Fruiting] Fruiting bodies produced from the shelf system have high quality because of high spawning rate and quantity of available nutrients.

* Details of these growing methods will be further discussed in Chapter 7. Cultivation Modes.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 4

Spawn

DESCRIPTIONS OF COMMERCIALLY IMPORTANT *PLEUROTUS* SPECIES

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Rural Development Administration, Korea

Introduction

Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family Pleurotaceae. Like oyster mushroom (*Pleurotus ostreatus*), many of *Pleurotus* mushrooms are primary decomposers of hardwood trees and are found worldwide. The type species of the genus *Pleurotus* (Fr.) Quel. is *P. ostreatus* (Jacq. et Fr.) Kummer. This mushroom has basidia with four basidiospores and a tetrapolar mating system. Its hyphae have clamp connections and most members of the genus, excepting a small minority, have a monomitic hyphal system.

To date approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical with previously recognized species. Determination of a species is difficult because of the morphological similarities and possible environmental effects. Mating compatibility studies have demonstrated the existence of eleven discrete intersterility groups in *Pleurotus* (Table 1) to distinguish one species from the others. Some reports indicate partial compatibility between them, implying the possibility for the creation of another species.

Table 1. Established biological species within *Pleurotus*, their corresponding synonyms and/or taxa at a subspecies level, and the respective intersterility groups.

Species	Synonyms-subspecies taxa	Intersterility groups
P. ostreatus	*P. columbinus, P. florida, P. salignus, P. spodoleucus*	Ⅰ
P. pulmonarius	*P. sajor-caju, P. sapidus*	Ⅱ
P. populinus		Ⅲ
P. cornucopiae	*P. citrinopileatus*	Ⅳ
P. djamor	*P. flabellatus, P. ostreatoroseus, P. salmoneostramineus, P. euosmus*	Ⅴ
P. eryngii	*P. ferulae, P. nebrodensis, P. hadamardii, P. fossulatus*	Ⅵ
P. cystidiosus	*P. abalonus*	Ⅶ
P. calyptratus		Ⅷ
P. dryinus		Ⅸ
P. purpureo-olivaceus		Ⅹ
P. tuber-regium		XI

(Source: A pluralistic approach in the study of *Pleurotus* species with emphasis on compatibility and physiology of the European morphotaxa by Georgios Zervakis and Constantinos Balis, 1996)

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Wild *Pleurotus* mushrooms are distributed through out the world as shown in Table 2. *P. pulmonarius* and *P. cystidiosus* are known to be distributed in the tropical and subtropical region, while *P. eryngii* are collected in Europe, Africa and most of Asia except Korea and Japan, where the mushroom is commercially cultivated. *P. ostreatus*, the most important commercial mushroom within the genus *Pleurotus* is widespread in temperate areas. The species is quite adaptable to a range of climates and substrate materials, making itself the second most common mushroom produced worldwide following button mushroom.

Table 2. Established biological species of *Pleurotus* and their known world-distribution

	Europe	Asia	N. America	S. America	Africa	Australasia
P. ostreatus	o	o	o	o	o	o
P. pulmonarius	o	o	o	-	-	o
P. populinus	o	-	o	-	-	-
P. cornucopiae	o	o	-	-	-	-
P. djamor	-	o	o	o	o	o
P. eryngii	o	o	-	-	o	-
P. cystidiosus	o	o	o	-	o	-
P. calyptratus	o	o	-	-	-	-
P. dryinus	o	o	o	-	o	o
P. purpureo-olivaceus	-	-	-	-	-	o
P. tuber-regium	-	o	-	-	o	o

(Source : A pluralistic approach in the study of *Pleurotus* species with emphasis on compatibility and physiology of the European morphotaxa by Georgios Zervakis and Constantinos Balis, 1996)

Characteristics of Commercially Important *Pleurotus* Mushrooms

P. ostreatus is the most important species in which many commercial strains are developed and cultivated. *P. florida* must be generally regarded as a subspecies of *P. ostreatus* but it will be discussed separately because its morphology and physiology are very different. It is known that cultivators and mycologists have mistakenly described a variety of *P. pulmonarius* as *P. sajor-caju*. But this taxon will be used for easy understanding to growers. Other cultivated oyster mushrooms, including *P. eryngii*, *P. cystidiosus* (=*P. abalonus*), *P. cornucopiae* will be explained briefly. Although *P. tuber-regium* is being studied for use of sclerotia and basidiomata, it will be excluded from this discussion due to the lack of pertinent data.

Optimal growth temperatures and characteristics of the important species are presented in Table 3. These characteristics vary with the growth stages of the species and even the strains, but generally remain within the limits of the species. With the progress of breeding studies and other efforts to overcome the limits, the gaps between some species are getting smaller.

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Table 3. Optimal growing conditions for different *Pleurotus* mushrooms

Species Conditions	*P. ostreatus*	*P. florida*	*P. sajor-caju*	*P. eryngii*	*P. cornucopiae*	*P. cystidiosus*
Spawn run (℃)	25	25	25	25	25-30	25-35
Primordia formation (℃)	10-15	10-25	10-25	10-15	20-25	20-25
Fruiting body production (℃)	10-17	15-25	18-25	13-18	20-30	25-30
CO_2 conc. (ppm)	< 1,000	< 800	400-800	< 2,000	< 1,000	< 1,000
Optimum season	Autumn	Spring, Summer	Spring,	Autumn	Summer	Summer
Applied cultivation methods	Log, Shelf, Box, Bottle, Bag	Shelf, Box	Shelf, Box	Bottle, Bag	Shelf, Box	Bottle, Bag

Temperature during spawn run

Even though there are some variations in growth of the mycelium according to the strains in a species, *P. ostreatus*, *P. florida*, *P. sajor-caju* and *P. eryngii* reach their optimum growth at 25℃, while *P. cornucopiae* and *P. cystidiosus* reach their optimum growth at 25-35℃, which suggests that they are a good choice for cultivation in both temperate and tropical regions (Fig. 1). But during the mycelium mass incubation prior to cultivation, the incubation room must be maintained at a temperature 3-5℃ lower than normal optimum temperatures because of their respiration heat.

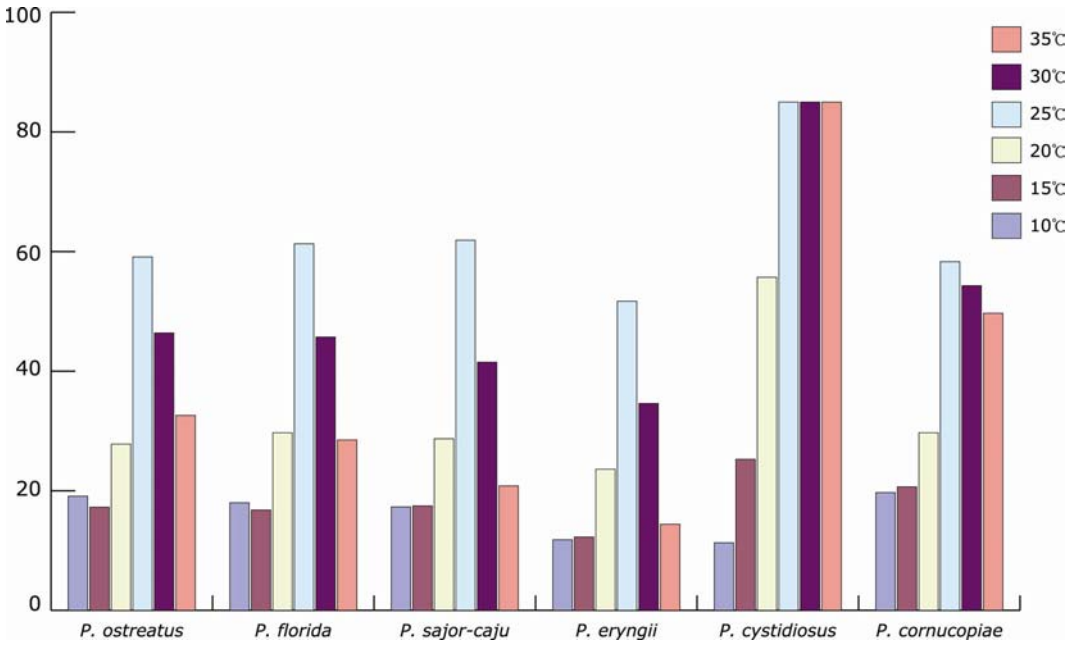


Figure 1. Effect of temperature on mycelial growth of different *Pleurotus* species

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Temperature during primordia formation

In the life cycle of *Pleurotus* mushrooms there are two stages: the vegetative stage and the reproductive growth stage. Generally, some kinds of stimuli are needed for the shift from mycelial (vegetative) growth to the fruitbody formation (reproduction) phase. These stimuli include abrupt changes in temperature, humidity, gas concentration, light and nutrient reserves, and physical stimuli. Among them, a sharp temperature drop is the most effective in fruiting induction for most mushrooms. Fruiting is induced by low temperatures ranging from 10 to 15℃ in *P. ostreatus* and *P. eryngii*. However, the fruiting of *P. florida*, *P. sajor-caju*, *P. cornucopiae* and *P. cystidiosus* is less affected by temperature (Table 3).

Temperature during fruiting body development

Optimal temperature for the production of best quality oyster mushrooms stands between 10 and 18℃ while *P. eryngii* produce best from 13 to 18℃, and *P. florida* and *P. sajor-caju* produce best at 15-25℃, a wider temperature range. *P. cornucopiae* and *P. cystidiosus* can produce good mushrooms even at 30℃. Temperature during fruiting body development affects the color of caps. In order to produce dark colored mushrooms, growers might want to lower temperature within the recommended growing temperature range.

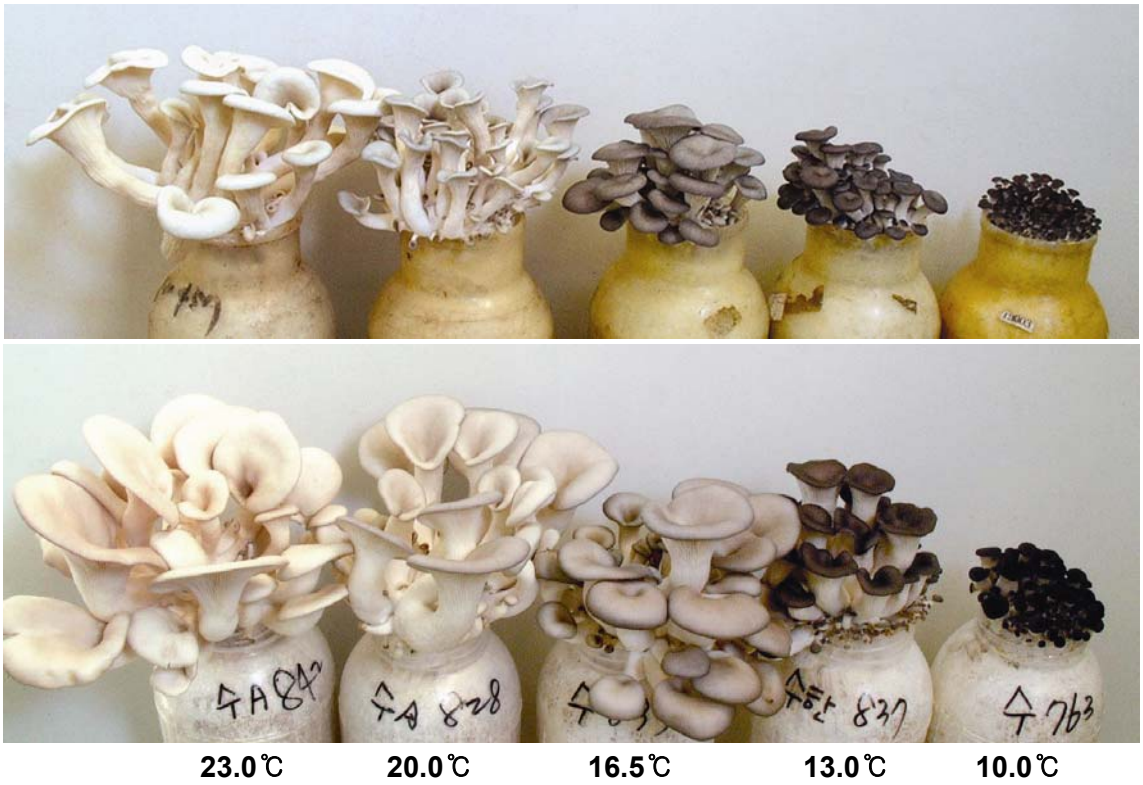


Figure 2. Effect of temperature on mushroom cap color of *P. ostreatus* strains
(Photo courtesy of Chang-Sung Jhune)

CO_2 concentration

Growers must consider the CO_2 gas concentration in the substrate containers during spawn run and the ambient CO_2 concentration during fruitbody development. During mycelial growth, CO_2 concentrations in the containers could rise up to 40%. Mycelial growth of *P. ostreatus* and *P. florida* are stimulated in the high CO_2 concentrations up to 28% and 22%, respectively. The ambient CO_2 concentration in the growing room, however, should be controlled by ventilation, especially during fruitbody formation and development. Under high CO_2 levels or with

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less frequent ventilation, mushrooms produce long stipes with tiny caps, while they produce short stipes with broad caps under low CO_2 levels or frequent ventilation. In *P. ostreatus*, a CO_2 concentration higher than 1,000 ppm will produce stipes that are too long and result in mushrooms of lower quality (Fig. 3).



A. Optimum CO_2 concentration



B. A little high CO_2 concentration



C. High CO_2 concentration



D. Malformed fruit bodies under high CO_2 concentration

Figure 3. Effect of CO_2 concentration on mushroom shape of ***P. ostreatus***

Cultivation methods

Proper cultivation methods vary by variety. A diversity of cultivation methods utilizing log, shelf, box, bag and bottle have been developed and sawdust, log and agro-waste including straw and cotton waste serve as a good source for mushroom substrate. Shelf and box cultivation methods are mainly applied to cultivate *P. florida*, *P. sajor-caju* and *P. cornucopiae*, while bag and bottle cultivations are used for *P. eryngii* and *P. cystidiosus*. Selection of the right cultivation methods is based on the mushroom variety, market demands and farmers' preferences.

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A. Log culture



B. Shelf culture



C. Box culture



D. Bag culture



E. Bottle culture

Figure 4. Various growing methods for oyster mushroom (Photo courtesy of Chang-Hyun You and Young-Bok Yoo)

Commercial *Pleurotus* species

Pleurotus ostreatus (Jacq.: Fr.) Kummer

P. ostreatus, a wood-destroying fungus, is widespread in the temperate zones and forms fruitbodies in relatively cool temperatures in comparison with other *Pleurotus* species. This is the most frequently cultivated species among the genus *Pleurotus*. One of the features of this species is it requires a low temperature treatment called "cold shock" to initiate primordia formation.

Growing temperatures for the production of fruiting bodies is rather low at 10-2℃. As of December 2003, 66 commercial strains are available in Korea. Different strains have different degrees of heat or cold tolerance. Some strains are less affected by unfavorable temperature conditions at the latter flush stages. It is important to select proper strains for the cultivation method of a particular grower's choice. Currently, commercial strains are mainly developed by introduction, mating and selection plus protoplast fusion and mutagenesis.



Figure 5. Protoplast fusion



figure 6. Mutagenesis

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***Pleurotus florida* Eger**



Figure 7. *P. florida*

P. florida is widespread in temperate, subtropical and tropical zones. It is similar in appearance to and was considered as subspecies of *P. ostreatus*. Some modern mycologists are inclined to regard it as another species with different color and different temperature requirements. Actually, there are two groups in *P. florida* at the subspecies level. One group is sexually compatible with *P. ostreatus* and the other with *P. pulmonarius*.

At low temperatures, the color of the caps is light brown, but they turn pale with increasing temperature. It could be harvested in warmer temperatures as its fruiting temperature range is wider than other *Pleurotus* mushroom and it does not require fruiting induction (cold shock). Moreover, it shows the highest yield among the *Pleurotus* species.

***Pleurotus eryngii* (DC.: Fr.) Quel**



Figure 9. *P. eryngii*

Wild *P. eryngii* are usually collected in southern Europe, North Africa and central Asia. It has many subspecies and similar taxa such as *P. fuscus* var. *ferulae* from China. This "King Oyster" mushroom is increasing in popularity due to its unique favor.

However, when one grows this mushroom, closer attention should be paid to room humidity and ventilation during fruiting and mushroom development, and spore load from other mushrooms and other disease or weed fungi in the growing room after harvest. Since *P. eryngii* is more prone to diseases and more sensitive to growing conditions but grows slower than *P. ostreatus*, most growers opt for bottles or bags filled with sawdust. It requires cold shock for primordia formation and forms fruiting bodies at 13-18℃ The cap is cream to grey-brown colored and the stipe is whitish and 10-14cm long.

***Pleurotus sajor-caju* Fr.: Fr.**



Figure 9. *P. sajor-caju*

It is known that *P. pulmonarius* was once mistaken for *P. sajor-caju*. The mushroom grows wild in subtropical and tropical regions like India. It is known to be compatible with *P. sapidus* but they are different in appearance. With its optimal temperature range for fruitbody development relatively high, it is suitable for growing in subtropical and tropical areas.

***Pleurotus cystidiosus* O.K. Mill.**
(*P. abalonus* Han, Chen & Cheng)



Figure 10. *P. cystidiosus*

P. cystidiosus is widely distributed in subtropical and tropical regions. Although it is mainly grown in subtropical region, its productivity is relatively low. The unique characteristic that distinguishes this mushroom from other *Pleurotus* species is the presence of conidia on the mycelium. Conidia are asexual spores composed of white coremia surmounted by black heads of arthroconidia, which occur on the mycelia

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under light. The black spots make them look as if contaminated but do not affect the mycelial growth and fruitbody formation. Bottle and bag cultivation methods are favored for mature mycelial growth.

***P. cornucopiae* (Paulet) Rolland**

P. citrinopileatus is considered as a subspecies of *P. cornucopiae* by reason of compatibility even though it was regarded as a separate species and occurred only the eastern part of Asia until recently. It is distributed in Asia and throughout Europe and occurs on the stumps of broad-leaf trees from summer through fall. The cap is yellowish, 4-12cm and the stipe is white. It has a wheat flour odor. Because it tastes good and has a pretty color, its cultivation is expected to increase.



Figure 11. *P. cornucopiae*

Breeding of *Pleurotus* spp.

It is difficult to accurately describe all the species and commercial strains of *Pleurotus*. Different countries have different weather and growing conditions, cultivation histories, methods of utilizing native agricultural wastes, and different consumer demands. Therefore the species descriptions above may not be definitely true and applicable to all parts of the world. But it is necessary to understand the basic species-dependant characteristics in order to grow mushrooms successfully. The development of commercial strains that are suitable to the growing environment of each region and satisfy the various consumer demands must be accomplished. To this end, genetic resources for breeding have been collected, preserved and exchanged.

The following factors must be considered in breeding:

- Morphological characteristics
- Physiological characteristics
- Cultivation methods and techniques
- Yield
- Resistance to disease
- Consumer & processor demands
- Culinary value
- Storage life

Some species are well studied and have been used to develop many commercial strains, while others are not. The creation of new strains is always required to preserve genetic diversity and meet the ever-changing consumer demands.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 4

Spawn

HOW TO MAKE OYSTER MUSHROOM GRAIN SPAWN IN A SIMPLE WAY

Adrian Ogden , Katherine Prowse
Gourmet Woodland Mushrooms Ltd., U.K.

What did you begin with when you started mushroom growing? Some raw beginners might have started with mushroom growing kits or so-called ready-to-fruit bags. From these bags they can harvest mushrooms by just providing the proper conditions for mushroom growing. Others might have started with the organic materials for mushroom growing, i.e. substrate and mushroom seed purchased from their local provider. The former could see only fruiting but the latter could witness the two phases of mushroom life cycle, mycelial or vegetative growth and fruiting, or reproductive growth.

Mushroom seed, commonly called "spawn" in the mushroom industry is a result of mycelial expansion. Using the following guide, any of you familiar with sterile techniques or having specialized knowledge of mushroom culture can make your own "mushroom starters" under sterile conditions, thereby reducing production costs and even getting spawn of higher quality. The first part discusses the construction of a clean room and the latter investigates spawn production. An understanding of the process will also provide common growers with advanced knowledge of mushroom growing.

How to Build a Clean Room in a Simple Way

A practical description of how to construct temporary building panels from polythene sheets and sawn timber, which can be fixed together to form the basis of a sterile clean room. The clean room utilizes a sterile airflow from a basic bench mounted HEPA filter/fan assembly, or laminar flow bench. The size of the clean room is directly correlated to the cubic airflow capacity of the laminar flow bench.

Introduction

What is a clean room?

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A hygienically clean, sterile enclosed air space where mushroom mycelium can be isolated from its normal environment in which it must compete with a host of other organisms to survive.

How does it work?

All air in the clean room is 'cleaned' by passing it through a High Efficiency Particulate (HEPA) filter to remove the vast majority of airborne particles (living and dead), including: skin, pollen, dust, mould, bacteria, and fungal spores.

Why do mushroom growers need a clean room?

Mushroom spawn is expensive and can be difficult to find. It also does not travel over long distances well. A grower who produces his/her mushroom spawn gains a greater knowledge and understanding of the mushroom growing process.

Overview

This simple clean room design owes its success due it being constructed with minimal mould growing, organic biodegradable materials. High sterile airflow is achieved by placing a large cubic fan capacity in a small area; any airborne contaminants that enter the clean room are quickly removed by the laminar flow bench.

The clean room can be used to inoculate agar plates with mushroom mycelium from culture slants or with tissue samples taken from wild specimens. It can be used to conduct agar-to-agar, agar to grain, grain-to-grain, sawdust to grain, and sawdust-to-sawdust transfers, using a standard domestic pressure cooker to sterilize the various mediums.

The design is intended as a place to teach, learn and experiment with mushroom sterile culture. It is not capable of withstanding the demands of commercial mushroom spawn production.

Constructing the clean room

Materials required

- 50 × 2.5m roll clear polythene sheeting (120 gauge, 8 mil, 0.5mm thickness)
- 2 × 1" (50 × 25mm) sawn timber-preferably treated
- 2.5" (60mm) length counter sunk wood screws, tacks or staples
- Silicone caulking sealant

The basic premise is that clear polythene lined wooden frames are made to a standard size. The frames are then joined together to make an enclosed space that is entirely lined by the mould resistant, sterile polythene. Four frames in total, one containing a door constructed in a similar manner. The room is best sited on a concrete base. Other materials that can be used for a sterile floor are linoleum (lino), or butyl rubber sheet.

Constructing the frames

Construct two side frames using 50× 25mm sawn timber to the following dimensions: 2m long × 2m high with a bracing bar positioned at 1m (Fig. 1). The two upright lengths of timber should be positioned with the narrow edge facing outwards.

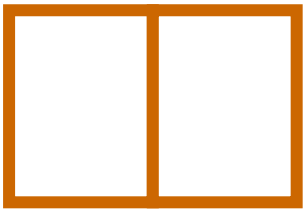


Figure 1. Side frames of clean room

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The polythene is cut to length 2.5 × length of the frame. Then folded back and tacked round the rear of one side, thus creating a clear panel. A bead of sealant can be run down the timber to prevent the polythene from 'flapping'. The two end frames are made to 2 × 1m in size. An entry must be made in one of the end frames (Fig. 2, 3, 4). The door is best constructed along the same design as the frames. Make sure the door fits well with as few gaps as possible. Household window insulation foam makes a good seal round the doorway.





Figure 2, 3, 4. End frames of clean room

Joining the frames together

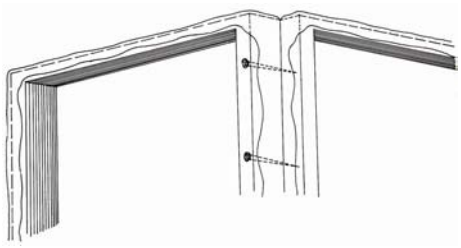


Figure 5. Frame connection

To make an airtight seal between the frames, first run a 10mm wide bead of silicone sealant: 10mm from the edge of each frame, all the way round the edge of each frame. The frames should then be positioned at right angles to each other and secured with screws or nails (Fig. 5).

After securing the four frames, apply another bead of silicon along all the joins, including the floor. Cut a 2.5 × 1.5m piece of sheeting and secure this over the top of the structure in the same way as the frames were made (a bracing bar may be necessary to add strength).

Equipping the clean room

When creating a sterile environment in the clean room a basic laminar flow bench is required. To be effective at stopping contaminants to mushroom culture the air in the clean room must be passed over a HEPA filter (Fig. 6, 7) once every 60-80 seconds. This is achieved by matching a fan with an output range of 400-500 cubic feet per minute (CFM) with a HEPA filter that is no more than 6 inches (15cm) in depth.

Off the face of the filter, an airflow range of 125-150 CFM will give the desired filtering capacity. The cubic air capacity of the clean room is 141 cubic feet.

Work surfaces should be made from scratch resistant non-degradable materials such as steel, and positioned at waist height.

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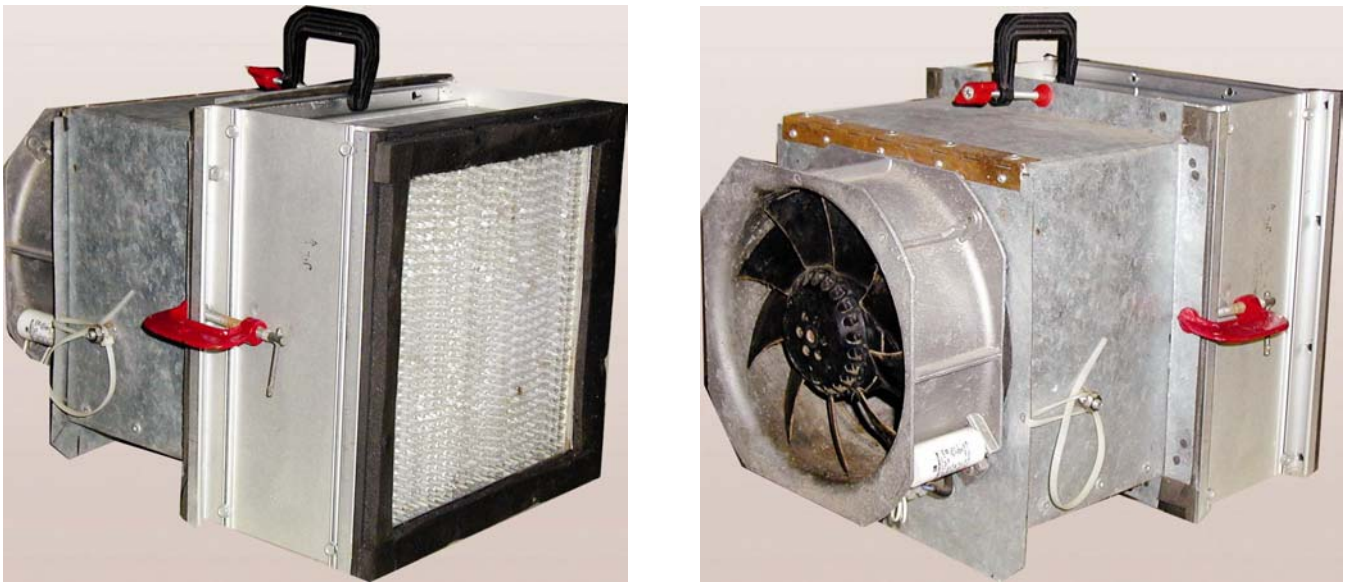


Figure 6, 7. Simply made HEPA filter

Operation, cleaning and maintenance

The laminar flow bench should be left in continual operation. This will keep the room dry and free from mould growth and other contamination. Cleaning is best carried out one hour before use. A 5% solution of domestic bleach (sodium hydroxide) is used to clean all surfaces. All operatives should wear newly laundered clothes when entering the clean room.

General maintenance should be carried out as wear and tear occurs. Any holes in the polythene should be immediately sealed with adhesive tape, and the silicon sealant should be checked regularly (monthly) and remove and replace as necessary. It is recommended that the HEPA filter be changed after 12 months of operation.

Production of Oyster Mushroom Grain Spawn

Introduction

Mushroom growing using home grown spawn is a process of cellular expansion. Mushroom mycelium is initially grown on a nutrified agar media. This is then used to make grain spawn. The grain spawn is subsequently used to make the final fruiting substrate.

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Step 1.



200g prtatoes washed.



Dice into 1/2cm cubes.



Boil in 1.2L distilled water for 15 minutes and strain.



Into extracted liquid add 15–18g agar and boil for 10–15 minutes. Add 15g glucose and 5g yeast extract. Allow to dissolve and remove from heat.



Pour 50–60mL into flat bottles and plug mouth with cotton wool. Secure with paper and rubber band.



Sterilize in autoclave at 15–18kg/cm^2 (pressure) for 30 minutes.



Slant bottles until agar becomes solid.

Step 2.



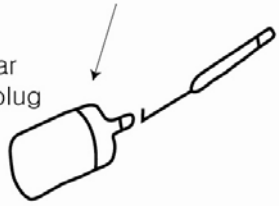
Fresh and tender mushroom



Break mushroom into half.



Use sterile needle to take small piece of mushroom tissue.



Insert tissue into agar medium bottle and plug with cotton wool and paper.



Incubate impregnated bottle at 20–24℃ preferrably with less light for 2–3 weeks.

Step 3.



Soak grain in water overnight.



Wash grain thoroughly with water 2–3 times.



Steam grain in pressure cooker for 1 hour and cool.



Add 1% dolomite to prevent grain from sticking together.



Fill flat bottles with grain up to 3/4 and plug with cotton wool. Sterilize in autoclave for 45 minutes.

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Preparing agar media

The starting point is a mushroom culture (usually in a test tube). This will either be from a clone that you have taken yourself from a mushroom specimen, or purchased from a culture laboratory. Until you have gained experience in cloning and assessing wild mushroom specimens, it is best to use a proven, productive strain from a laboratory.

Growing out your culture

Mushroom mycelium needs nutrition to grow. In other words, it needs something to feed on. Agar agar (a seaweed) contains almost no nutrition, but acts as a gelling agent when mixed with water, so that the mycelium has a flat, solid surface to grow across. A combination of agar agar, water and one or more nutritional substances gives a satisfactory method for growing out healthy mycelium.



Figure 8, 9 Tissue culture from a mushroom specimen and transfer to agar culture

There are various grades of agar agar: food-grade for cooking and higher grades for the purpose of culturing. If possible, it is best to use one of the higher grades, as these will contain fewer impurities and probably have better gelling properties than the food-grade. It comes in dry powder form.

There are many different sources of nutrition that can be used in agar media, but probably the most commonly used formula is Malt Extract Agar (MEA). The MEA formula that we use is as follows:

1 liter water
20 grams agar agar
30 grams barley malt extract*
2 grams nutritional yeast
It is actually called MEYA because of the addition of the yeast.

In addition, commercially prepared nutrified agar media can be purchased from mushroom cultivation suppliers; this needs only water adding to it.

Preparing and pouring media

You will need scales and a liter flask that can be used in a pressure cooker. A domestic pressure cooker is ok, but will not form a vacuum when cooled (i.e. it will draw in contaminated air), and therefore should be placed in the clean room environment for cooling. If possible, a proper pressure sterilizer with a pressure gauge is best to use. For pouring the sterilized media, you will also need a supply of isopropyl alcohol and disposable paper towels.

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1. Weigh out your ingredients, add the correct amount of water (preferably non-chlorinated, sterile) and mix together in the flask (Fig. 10).
2. Use non-absorbent cotton or aluminium foil to stop up the flask. Do not plug it tightly with a stopper, because pressure built up inside the flask while sterilizing will cause it to fly off and the media boil too furiously (Fig. 11).



Figure 10. Add distilled water so that the potato starch extract reaches 1L



Figure 11. Aluminum foil-stopped jar



Figure 12. Put the jars in the autoclave and sterilize them at 121℃ for 45 minutes.

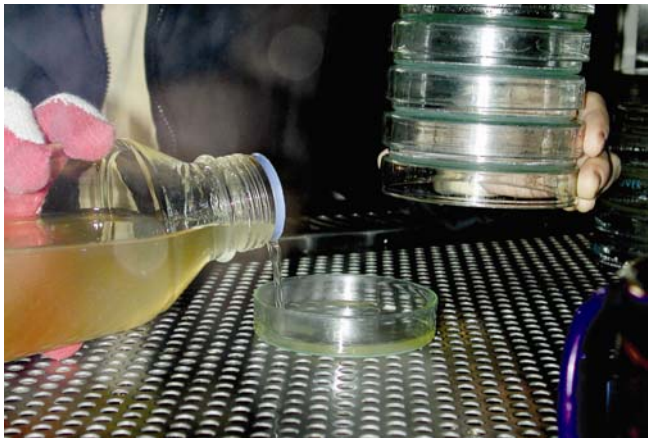


Figure 13. Pour the solution cooled at 50℃ into sterile petri dishes in clean bench.



Figure 14. Early signs of contamination

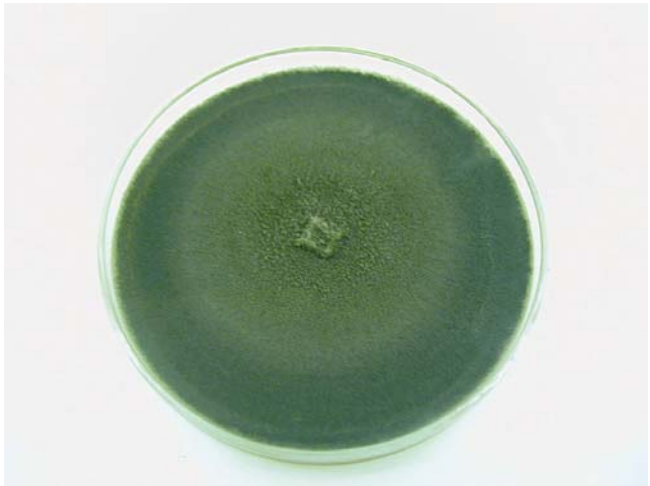


Figure 15. Petri-dish contaminated with green mold

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3. Fill your pressure cooker/sterilizer with the correct amount of water (this will vary according to what model you are using-refer to manufacturer's instructions).
4. Place the flask in the cooker. If you are using autoclavable glass petri dishes, these should be sterilized in the cooker at the same time. If you are using disposable dishes, these are usually sterilized already, and the packet should only be opened in the clean room.
5. Place cooker on heat source, bring it up to the correct temperature/pressure-250°F /15 psi and sterilize for 45 minutes. Try to keep the temperature/pressure constant, otherwise the media will not work as well (Fig. 12).
6. Let the pressure reduce to zero-If using a domestic pressure cooker, place in the clean room for this time period.
7. In preparation for pouring your media, shower and put on freshly washed clothes. The aim is to reduce as much as possible the quantities of contaminants, particularly bacteria and lower fungi, that stick to you and your clothes and which could contaminate your dishes. It is impossible to remove them all-after all, humans are naturally 10% bacteria! If you can put on clothes that have not been exposed to the outside air since being washed, so much the better. Hair carries a lot of contamination-either wash it and tie it back (if long), or use a hair net/clean hair covering.
8. As agar media solidifies before pouring, clean the work surface in front of your laminar flow cabinet with isopropyl alcohol. Also, clean your own hands with the alcohol and continue to do this at intervals, until you have finished. Pour the dishes that are closest to the filter surface first. The clean air coming off the filter will pick up bacteria, skin fragments etc that are on you, and blow them away from the dishes. Do not put your hands between the filter surface and the open dish, or the air will blow any contaminants from your hands on to the surface of the media.
9. Lift the dish lid with one hand and pour the media with the other. Do not let your fingers hang over the rim of the dish or its lid, because skin fragments will cling and cause contamination. While the lid is raised, angle its underside towards the filter so that it only has clean air blowing on to it. At all stages, try not to breathe over the open dishes, as breath is laden with bacteria (Fig. 13).
10. When each dish has been poured, leave to cool and solidify. Don't hang around in the clean room for longer than you have to, as you are just potentially spreading contamination (Fig. 14, 15)!

Inoculating petri dishes

You will need an alcohol lamp for sterilizing the scalpel. If laboratory film (e.g. parafilm) is available for sealing the dishes, there is less chance of contamination. However, if the dishes are left in front of the laminar flow filters until they are colonized with mycelium, film is not essential.

1. Heat the scalpel tip in the alcohol lamp until it is glowing red (Fig. 16). Cool it by dipping it into the centre of the dish that you are about to inoculate.
2. Take your culture (having already loosened the lid) and open it without letting your fingers hang inside (Fig. 17). With the scalpel, tease out a wedge of mycelium, lift the lid of the dish and quickly transfer the wedge to the agar, immediately replacing the lid. This is more difficult than it sounds-the mycelial wedges have a huge tendency to cling desperately to the sides of the test tube, while refusing to be picked up by your scalpel! So don't worry if it's a bit of a mess to begin with, it just takes a bit of practice. The mycelial wedge is placed in the centre of the dish so that growth can radiate away from it.

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Figure 16. Sterilizing transfer tools and the mouth of test tube by alcohol lamp.



Figure 17. Remove a loopful of agar culture from the test tube

3. If you are using laboratory film, immediately seal up the dish that you have just inoculated. If not, place it away from the immediate area where you are working, but still in front of the filter.
4. Repeat the above steps until there is no mycelium left in the test tube. A standard test tube culture can usually inoculate 2-3 dishes.
5. The dishes should be incubated at 24℃, or as close as possible. Oyster mushroom mycelium is usually quick to start growing; you should see fuzziness, the first sign of growth, within 2-3 days. The mycelium is usually white, and most strains should colonize the dish within 10 days (Fig. 18). If the uncolonized parts of the dish develop areas of different coloring, this most likely indicates some form of contamination, perhaps blue-green mould or yellow slime stuff. In this event, the affected dish should be discarded. If throwing it away is not an option, you can try to leave the contaminant behind, by transferring squares of healthy mushroom mycelium from the affected dish to a new, clean dish. But be warned that moulds in particular produce millions of spores, and any disturbance can cause these to become air-borne and contaminate your healthy mycelium and clean dish, or in a worst-case scenario, your whole clean-room. If this last happens, the only option is to empty the whole room and clean every inch. Clean rooms with a high air flow are less susceptible to this, as any spores are quickly drawn into the filters before they have a chance to settle.

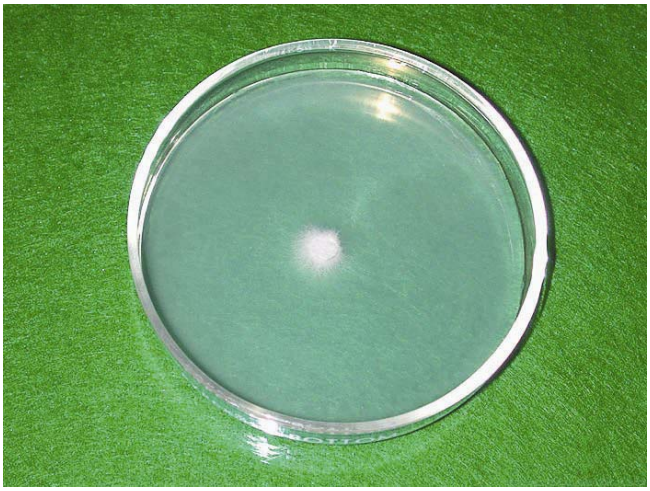


Figure 18. Fuzzy growth on agar culture



Figure 19. Fully colonized pertri dish

When your dishes are fully colonized (Fig. 19), they can be used for 3 purposes :

- To inoculate further dishes of agar media. Follow the above procedures for making the media and pouring the

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dishes, but instead of using a test tube culture, cut squares of roughly 1cm out of a healthy colonized dish and transfer one square to each new dish. 1 petri dish can easily inoculate at least 15 more dishes.

- To inoculate test tubes of agar media so that you have back ups of your strain
- To inoculate sterilized grain, resulting in grain spawn

Preparing Grain Spawn

Grain

Many different types of grain can be used. We use rye grain, which is a relatively soft grain that cooks easily without becoming too sticky and clump forming.

Spawn containers

Containers can be jars (glass or plastic) or plastic bags. It is essential that they are capable of withstanding pressure sterilization. It is easier to thoroughly mix mycelial wedges from your dishes through jars. Jars should be fairly wide-mouthed, for ease of inoculation, and have tight-fitting lids with a 8mm hole drilled through them. If filter discs are available, these should be fitted over the holes, on the underside of the lids. If not, you can cut out a layer of cardboard the same diameter as the lid, and fit this into the lid. Filter discs should be soaked for at least 1 hour in a 5% household bleach solution; cardboard discs should also be soaked, but for not as long - otherwise they will disintegrate! Soaking helps to dislodge hidden contaminants, which are then killed by the sterilization process.



Figure 20. Fully colonized grain spawn in the bag

Bags, if used, should be custom-made with filter patches (Fig. 20). If these are not available, it is best to use jars of some sort. Containers without a filter do not work very well, for reasons explained below, and it is difficult to improvise an effective filter for a plastic bag. Also, bags need to be sealed in some way after inoculation - if you have access to a proper heat sealer this is no problem, otherwise something else such as packing tape will have to be used, which is less effective.

The purpose of the filter discs/patches is to allow a low level of gaseous exchange. Mushroom mycelium needs a supply of fresh oxygen while it is growing, otherwise it will quickly become anaerobic (without oxygen) and contaminate, regardless of how clean the environment and materials. The filters allow oxygen to enter, without contaminants being drawn in. If filtration is a big problem for you, the only other option (that we can think of!) is to put a small hole in the top of whichever container you are using, and place it over a heat source. Hopefully, convection will keep gases moving upwards and carry potential contaminants away, whilst giving the mycelium access to oxygen.

Preparing the grain

1. Weigh out the amount of grain you require. The optimum moisture content of rye grain is roughly 50%, i.e. it will double in weight when cooked. 500g or less of dry grain per container is a good quantity to use when you are transferring mycelium from petri dishes to grain. Any more than this and it becomes difficult to mix the mycelial wedges through properly.

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Figure 21. Sorghum seed



Figure 22. Grain pasteurization in a rice cooker

2. Grain will always contain hidden contaminants, no matter how fresh it is. Therefore, it needs to be pre-cooked, to release these contaminants that are then destroyed by sterilization (Fig. 22). Bring water to the boil in a large pan, and then add the dry grain. Simmer for around 30 minutes. It is properly cooked when still firm, but soft enough to squash. Any longer than this and the grain kernels will swell so much that they will 'explode', which makes spawn more susceptible to contamination. Once you have done this a couple of times, it becomes very easy to determine the correct consistency.
3. Drain the grain in colanders or something similar. If you are making large quantities of grain spawn, it is fairly easy to improvise a draining container-we have used plastic dustbins with holes drilled in the bottom and sides, and also, sheets of metal mesh rolled into a bin shape and attached on to a base. As long as the water can drain off to some extent rather than collecting at the bottom of the container and forming a big grain mush, there should not be a problem, as a lot of excess water is lost quickly to evaporation. Mixing the grain around at intervals helps this evaporation process. If the grain starts to shrivel, it is drying out too much!
4. If you have access to calcium sulfate (gypsum), the addition of this will help to stop grain kernels clumping together after sterilization. Mix through at a rate of approximately 4g of gypsum to 1kg of dry grain.
5. Fill your chosen containers with the grain. If using jars, only fill to 3/4 of their capacity. If using bags, fill to no more than 2/3 of their capacity (Fig. 23). Animal feed scoops are useful for filling containers. You can either use scales to make sure each container is receiving the same amount of grain, or you can see volumetric scoops.
6. Close each container. If using bags fold the excess plastic over the bag. Load the containers into the pressure cookers. If you can space them apart to some extent, sterilization will be more even and efficient. If you have to pack them tightly, remember that it is more difficult for the steam to penetrate the centre of a cooker full of densely-packed containers, and sterilization time may have to be increased.



Figure 23. Jar filled with the sorghum seed

7. Fill the pressure cooker(s) with the correct amount of water (refer to manufacturer's instructions) and bring up to sterilization point−250°F/15 psi. We use jars or bags that are filled with 1kg each of cooked grain, and sterilize these for 105 minutes. Smaller quantities of grain in jars will require shorter sterilization times, e.g. 1L jars with 400g of cooked grain will probably only need 1 hour. If you are using bags and they are very tightly packed, they may need 3 hours. As this depends to some extent on your pressure cooker, you will have to experiment to find the optimum sterilization time.
8. When sterilization is complete, let the cooker return to zero pressure (if it does not form a vacuum on cooling,

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place in the clean room for this). Leave for at least 2 hours so that the jars/bags are cool enough to handle, then unload and place in front of the laminar flow cabinet. It is very important to make sure that they are sufficiently cool, as overly-hot grain will kill the mycelium you introduce. If in doubt, wait until they are cold.

Inoculating the grain

Each colonized petri dish can inoculate up to 20 cups of grain. The more you add to each container, the greater the speed of colonization. We like to use 1/2 to 1 whole dish to 1 jar containing 1kg grain, as this results in very fast colonization. The quicker the grain becomes fully colonized, the less chance there is of it becoming contaminated.

1. Clean the work surface in front of the laminar flow cabinet with alcohol. As always when working in the clean room, you should have showered and be wearing clean clothes.
2. Take a petri dish and remove sealing film (if used). Heat scalpel tip in the alcohol lamp until it is glowing red. Lift the dish's lid and cut a criss-cross pattern of 9 or more wedges of mycelium. If you are not using laboratory film to seal the dishes, it is a good idea to leave a 5mm strip of mycelium running round the edge of the dish-this is in case contaminants have entered the dish from round the edge and fallen on to the outermost mycelium.
3. Replace the dish's lid and reheat your scalpel.
4. Get your container(s) ready for inoculation-if jars then loosen the lids, if bags then open out the mouth. Be careful at all times not to let your fingers hang inside the container.
5. Remove the dish's lid again, and, with the hot scalpel, pick up 1 or 2 wedges of mycelium. Quickly drop into the waiting container and repeat until the required amount of mycelium has been transferred.
6. Reheat the scalpel between each dish-if one of your dishes is contaminated, this will prevent the contamination from spreading across all your containers.
7. If you are using jars, then they need to be thoroughly shaken, so that the mycelium travels through the grain, resulting in even colonization. We find that a rolling and 'see-sawing' motion works well. Try not to let too many wedges stick to the sides of the jar; this again improves with practice.
8. If you are using bags, try to trap some clean air in each bag before sealing. Do this by holding the bag open with its mouth pointing toward the laminar filter, and then sealing. The trapped air helps the mycelium to colonize the bags quickly and healthily. Once sealed, each bag should be agitated so that the mycelial wedges move through the grain.
9. Incubate the containers at 24℃/75°F, or as close as possible. You should see some signs of growth within 3 days. After 7 days, shake the containers to distribute the colonized grain kernels evenly. The grain is fully colonized when it is completely white or off-white. This is the time when it is at its most vigorous, so use it as soon as possible! If it is left to grow further, it will quickly over-incubate, forming a solid lump that is incredibly hard to break up (Fig. 24).



Figure 24. Sorghum seed-based spawn

Your grain spawn can now be used for 2 purposes.

- To inoculate further containers of grain.

  1 jar of grain can inoculate 10 times its own mass, so it is very easy to quickly turn small amounts of spawn into large amounts. As always, the more spawn you add, the faster the grain will colonize. (However, when inoculating a container of grain, beware of adding more than 20% of its mass, as growing mycelium produces heat and this will cause the grain to heat up too much and contaminate). Note that when using grain spawn

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from a jar, it must be broken up first. If it is a glass jar, do not bang it against your hand in case of breakage and injury-use something springy or semi-hard such as a thick padding of towels on a solid surface. Spawn in bags must also be broken up, but this is easily done by squeezing and shaking.

- To inoculate the final bulk substrate, from which mushrooms will eventually be fruited.



Figure 25. Grain spawn colonizing sawdust



Figure 26. Grain spawn ready to colonize wheat straw



Figure 27. Oyster mushroom on heat straw column



Figure 28. Harvested oyster mushroom

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

Mushroom is an attractive crop to cultivate in developing countries for many reasons. One of the most charming points would be that they are grown on agricultural wastes. It enables us to acquire substrate materials at low prices or even for free and to conserve our environment by recycling wastes. Most of all, oyster mushrooms (*Pleurotus* spp.) can utilize various kinds of substrate materials than any other mushrooms. The first article in this chapter, a worldwide survey on possible substrate for oyster mushroom, will illustrate oyster mushroom can be grown on "almost all types of available wastes." Then, nine examples of substrate materials for oyster mushroom are introduced in the rest part of the chapter with analyses on each material and detailed growing methods. Considering the conclusion of the following worldwide survey that about 200 different wastes are available as oyster mushroom substrate, nine materials are very much limited examples. However, more emphasis and pages than other chapters are provided to this chapter in this 300-paged handbook. Much effort is also made to offer practical experience of mushroom growers as well as scholars' experimental study on substrate materials. Therefore, the authors of this chapter have various educational, professional, cultural and national backgrounds and the quality of each article could be varying. Some scientists might emphasize academic point of view while some growers practical point of view. However, readers will find that any of them cannot be neglected and each article has its own value.

AGRICULTURAL WASTES AS SUBSTRATES FOR OYSTER MUSHROOM

Jozef Poppe
University of Gent, Belgium

Since 1999, our overcrowded world can count over 6 billion souls. Almost half of them are poor, hungry, sick or at war. They fight for a parcel of bean soil, coffee field, or rice terrace, while in the same village one can smell burning straw and forest fires or the rotting heaps of organic field waste or other agricultural by-products.

There is an enormous amount of waste in the agro-industry and the wood industry. Only using 25% of the yearly volume of burned cereal straws in the world could result in a mushroom yield of 317 million metric tons (317 billion kg) of fresh mushrooms per year, (Chang & Miles, 1989). But at this moment, the yearly world mushroom production total is only 6 billion kg. For 6 billion persons that equals 1kg per year or 3g per day

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(Courvoisier, 1999).

In fact, considering the yearly available world waste in agriculture (500 billion kg) and forestry (100 billion kg), we could easily grow 360 billion kg of fresh mushrooms on the total of 600 billion kg of dry waste!! This would produce an annual mushroom crop of 60kg per head per year, all containing the 4% protein of fresh mushrooms. We know that the diet of 30% of the world population is protein deficient, and recent analysis has proved that 200g of mushrooms can efficiently replace 100g of meat as a protein source. (Souci *et al*., 1975-1989) Among mushrooms, *Pleurotus* (oyster mushroom) can make use of the largest variety of waste substrates with its fast mycelial growth and its multilateral enzyme system that can biodegrades nearly all types of available wastes.

Listed below are the results of a worldwide survey on agro-forestry waste that can be used as a substrate in the cultivation of oyster mushrooms. All of the following wastes have been used in the past or recently for small or large-scale oyster mushroom cultivation. Most of these wastes have a C/N ratio between 32 and 600 and a pH between 5.0 and 7.5 (Poppe, 2000).

Worldwide Survey on Oyster Mushroom Substrate

- Alang-alang grass, *Imperata cylindrica* - abundant herb in Asia, especially in Indonesia, used for *Pleurotus* (Poppe *et al*., 1997).
- Artichoke waste, useful after drying for different mushroom substrates (Stamets, 1993)
- Azolla, a fast growing fern in Asia, close to tropical rivers used for *Agaricus*, *Pleurotus* and *Collybia* (Poppe, 1995).
- Banana leaves, dried 1.45% N, very productive in bulk for *Pleurotus* or in combination for *Volvariella*. (Chang-Ho 1979; Bhavani *et al*., 1989) (author).
- Banana pseudostems, chopped, gave better results for *Pleurotus* compared to sawdust or rice straw. (Jandaik *et al*., 1976). Jandaik was the first mycologist to use this substrate for *Pleurotus sajor-caju*.
- Barley straw, *Hordeum vulgare*, has a biological efficiency of 96% for *Pleurotus* (Martinez-Carrera, 1989), Chang & Miles (1989): 0.64% N, 0.19% P, 1.07% K, 47% C, C/N = 72. According to Delmas (1989) : 1% protein, 14% lignin, 36% hemicellulose, 43% cellulose, suitable for *Agaricus*, *Pleurotus*, *Volvariella*, and *Stropharia*.
- Bean pods, a substrate component or in bulk for *Pleurotus* (Poppe *et al*., 1995).
- Bean straw, different genera, for *Agaricus* and as a substrate component, for *Pleurotus*, it can also be used as a basic substrate (Poppe *et al*., 1995).
- Brassica-haulms, for *Pleurotus* (Sohi *et al*., 1989), straw of *Brassica napus*, rape, contains 22.7% lignin, C/N = 70, used for *Agrocybe aegerita* (Zadrazil, 1989). On *Brassica* crop residues like rape and mustard, in India, the highest yields were obtained with 50% *Brassica* + 50% rice straw for *Pleurotus sajor-caju* (Pani *et al*., 1998).
- Buckwheat straw, *Polygonum fagopyrum*, for *Pleurotus* (author).
- Cactus, Agave and Yucca : dry-resistant plants useful as a component of mushroom substrates (Stamets, 1993).
- Cardamon pulp, *Elettaria cardamomum*, has a biological efficiency of 113% for *Pleurotus* (Martinez-Carrera, 1989).
- Cinnamon leaves, *Cinnamon zeylanicum*, biological efficiency of 82% for *Pleurotus* (Martinez-Carrera, 1989).



Figure 1. Banana and its leaves



Figure 2. Bean pods

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- Citrus fruit peels, *Citrus unshiu*, dried, reasonable *Pleurotus* production (Yoshikawa *et al*., 1979; Khan *et al*., 1981).
- Coconut fiber pith and coir : can be composted and then used for cultivation of *Pleurotus* or *Volvariella* in India (Theradi Mani, 1992).
- Coconut husks, used for *Pleurotus cystidiosus* in India (Beig *et al*., 1989), used also for *Volvariella* in India (Bhavani, 1989; Gurjar *et al*., 1995).
- Coffee parchment, parche de café, suitable with or without pasteurization for *Pleurotus* (Poppe, 1995).
- Coffee pulp, sundried, stored, later rehydrated for *Pleurotus* (Martinez-Carrera 1989). Good production in Mexico for *Auricularia* when mixed with sugarcane pulp and corn-cobs (Sanchez *et al*., 1995).
- Coffee sawdust: efficient for *Pleurotus* when mixed with ipil-ipil powder (Sanchez *et al*., 1995).
- Coleseed, *Brassica napus*, in combination with straw or hay, it is a useful substrate for different mushrooms (Steineck, 1981). Contents: 2% protein, 11% lignin, 28% hemicellulose, 47% cellulose.
- Corn fiber: In Japan, this waste product of cornstarch manufacture increased the yield very noticeably when added to sawdust + rice bran, for *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pholiota nameko* and *Hypsizygus marmoreus* (Terashita *et al*., 1997).
- Corncobs, hammer milled or crushed, tested first in Hungary in 1956, gave variable results for *Agaricus*. Generally used for *Pleurotus* and shiitake. Contains 40% cellulose, 15% lignin, 0.4% total N, 0.1% P_2O_5, 0.25% K_2O, 0.5% SiO_2, pH 7, C/N 129 (Heltay 1957; Heltay *et al*., 1960) (At least 40 author references).
- Corn stipes, corncobs, corn leaves, corn stover, *Zea mays*: 5% protein, 19% lignin, 31% hemicellulose, 18% cellulose (Delmas, 1989), chopped use for *Pleurotus* and shiitake.



Figure 3. Corn

- Corn stalks, *Zea mays*, chopped as a component of *Agaricus* substrate (Chapuis *et al*., 1951). Also useful for *Pleurotus*. It contains: 48% cellulose, 16% lignin, 0.8% total N, 0.35% P_2O_5, 0.4% K_2O, 1.8% SiO_2, pH 7.2, C/N = 63 (Heltay *et al*., 1960 MS 4). In Chang & Miles (1989), the total N is 0.5%, 0.3% P2O5, 1.7% K, C/N = 97.
- Corn stover: containing 65% polysaccharides + 30% lignin, used for *Pleurotus* (Bassous *et al*., 1989).
- Corn waste: not only corncobs but also the post-shelling dust, the cleaning fiber and the broken pith are useful for *Pleurotus* cultivation with satisfactory yields (Khan *et al*., 1989).
- Cottonseed hulls, *Gossypium hirsitum*: 1% N; were the best substrate for cultivation of *Pleurotus* without any thermic treatment (Sun Pei-Ji, 1989).
- Cotton straw silage, chopped into particles of 3cm and stored in silos of 450 tons stock for *Pleurotus* cultivation in Israel (Danai *et al*., 1989).



Figure 4. Mixture of corn waste



Figure 5. Watered mixture of corn waste

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Figure 6. Dried cottonseed hulls



Figure 7. Cottonseed hulls and fiber

- Cotton wastes: cotton mill droppings, cotton ball locules, cotton husks, gin waste, cottonseed hulls: best substrate for *Volvariella*, used single or composted in combination with rice straw (Hu, 1976). Cotton also gives a productive waste for *Pleurotus*, even simply humidified without any thermic treatment (Poppe personal).
  Cotton waste contains widely variable amounts of total nitrogen from 0.25-1.45% (Chang-Ho *et al*., 1979). The gin waste is a by-product of the cotton purification machine (Khan *et al*., 1981; Cho *et al*., 1981) (more than 50 authors). Cotton waste has a biological efficiency of 56-86% for *Pleurotus*.
- Elephant grass, *Pennisetum purpureum*, for *Pleurotus*, tested in Cameroon by Poppe in 1987 with satisfactory results. In Zambia, it is used for *Agaricus* compost (author).
- *Euphorbia rayleana*, chopped branches could be successfully used for *Pleurotus florida* in India (Khanna *et al*., 1981).
- Grasses, wild grasses, ±3% protein, ±12% lignin, ±23% hemicellulose, ±18% cellulose. It should be dried for hay before using, see also hay. Up to now, not enough research has been done in order to make useful the endless amounts of lawn grass. Used in India, dried and cut, for *Pleurotus* sapidus (Kiran *et al*., 1989).
- Fern high, fern low, fern kukot: have been tried in Asia for *Pleurotus* with semi-satisfactory results (Poppe *et al*., 1997).
- Flax straw, *Linum usitatissimum*, single or in combination with flax tow, for *Volvariella* & *Pleurotus* and *Auricularia* (Chang, 1976; Chang & Hayes, 1978).
- French bean-haulms, *Pleurotus* (Sohi *et al*., 1989).
- Groundnut shells: successfully used for *Pleurotus sajor-caju* in Africa, additive of 10% water hyacinth increased the production by 22% (Tagwira *et al*., 1999).
- Gum-wood sawdust was first used by Block *et al*.,(1960) for the cultivation of *Pleurotus ostreatus*.
- Lemon grass leaves, *Cymbopogon citratus*, biological efficiency of 113% for *Pleurotus* (Martinez-Carrera, 1989).



Figure 8. Commercial waste cotton piles



Figure 9. Various wild grasses



Figure 10. Groundnut

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- Legume straws, mostly rich in N, suitable as *Pleurotus* substrates (Poppe, 1995).
- Maize straw: resulted in India in a biological efficiency of 52% for *Pleurotus sajor- caju* (Pani *et al*., 1997).
- Manioc stipes and leaves, *Cassava manihotis*, chopped for *Pleurotus*, or for *Agaricus* if fermented (Delcaire, 1981).
- *Melilotus haulms*, for *Pleurotus* (Sohi *et al*., 1989).
- *Mentha* stalks: after extraction of the oil it can be used for *Pleurotus*, *Agaricus* and *Volvariella* in combination with cereal straw (Garcha *et al*., 1981).
- Mustard, yellow mustard straw, useful for *Pleurotus* (author).
- Newspapers, shredded, when combined with rice bran or with sawdust for *Pleurotus* (Hashimoto, 1976). Also useful for *Stropharia*. Oak sawdust, supplemented with 10% millet was in Canada the best pasteurized or sterilized substrate for shiitake (Rinker, 1991).
- Oat straw, *Avena sativa*, 2% protein, 17% lignin, 32% hemicellulose, 40% cellulose, *Agaricus*, *Pleurotus*, *Stropharia* (Delmas, 1989).
- Paper pulp by-product: used in South Africa as substrate component for several mushrooms (Eicker *et al*., 1981).
- Paper waste: shredded paper, used for *Pleurotus*, *Stropharia* (Poppe, 1995).
- Papyrus plants, aquatic abundant, to be dried for *Pleurotus* (Poppe, 1995).
- Pea haulms, *Pleurotus* (Sohi *et al*., 1989).
- Pea straw, *Pisum* sp.: a substrate component for *Agaricus* and a basic substrate for *Pleurotus*. Contents 43% cellulose, 15% lignine, 0.9% N, 0.15 P_2O_5, 0.3 K_2O, 1.1% SiO_2, pH 6.8, C/N 45 (Heltay *et al*., 1960).
- Pepper leaves, *Piper nigrum*, biological efficiency for *Pleurotus* is 57% (Martinez-Carrera, 1989).
- Populus wood logs: gave a little bit lower *Pleurotus ostreatus* production compared to Salix wood logs (Anselmi , 1979).
- Potato foliage: useful for *Stropharia* and *Pleurotus* (author).
- Quinoa plant, dried, in Bolivia used as substrate for *Pleurotus* (Poppe, 1995).
- Ragi straw, *Eleucena coracana*, enriched with cottonseed meal, for *Pleurotus flabellatus* in India (Bano, 1979).
- Reed, *Phragmites communis*, chopped, 20% lignin, C/N = 50, as a component of substrate for *Agaricus* (Chapuis *et al*., 1951). Also useful for *Pleurotus* and *Agrocybe aegerita*.
- Rice straw, *Oryza sativa*, immense masses are burned every year or left rotting in the moistened fields, intensively used for *Volvariella* and *Pleurotus*, but also used as a chief component of synthetic *Agaricus* compost. It contains 41% cellulose, 13% lignin, 0.8% total N, 0.25% P_2O_5, 0.3% K_2O, 6% SiO_2, pH 6.9, C/N = 58 (Heltay *et al*., 1960). Rice straw crushed, is also used for *Pleurotus* in Asia (Han *et al*., 1976). Some Indian authors note 14% lignin, 37% cellulose, 0.4% P_2O_5, 0.55% total N 1.6% K_2O, 12% SiO_2 and C/N = 70 (Kaul *et al*., 1981) (also numerous other authors).
- Roadside grasses: different genera and species, should be used for *Pleurotus*, *Agaricus* and *Stropharia* (author).
- Salix wood logs = willow stumps, gave a little bit higher *Pleurotus ostreatus* production compared to poplar stumps (Anselmi *et al*., 1979).



Figure 11. Potato field

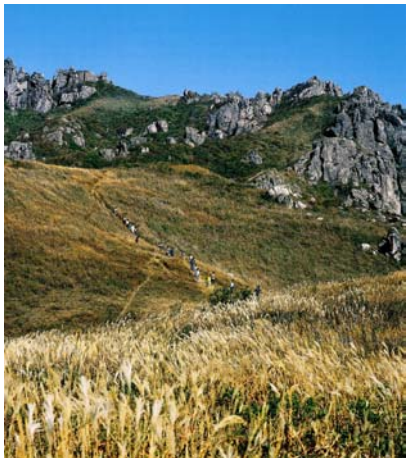


Figure 12. Reed

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Figure 13. Rice straw



Figure 14. Oyster mushroom cultivated on rice straw (Photo courtesy of Chang-Hyun You)

- Sawdust, general, can be used in *Agaricus* compost as a component (more than 5%) or as an additive (less than 5%) with straw, but it can be used also as single substrate for *Pleurotus*, *Auricularia*, *Flammulina*, *Tremella*, *Pholiota*, *Hericium*, mostly sterilized. The sawdust of beech and oak: 44% cellulose, 26% lignin, 0.2% total N, 0.01 P_2O_5, 0.03 K_2O, 0.9 SiO_2, pH 6.8, C/N = 244. (Heltay *et al*., 1960; Gramss, 1979).



Figure 15. Rubber tree sawdust



Figure 16. Oyster Mushroom cultivated on sawdust substrate

- Scrubs in India: disturbing animal grazing, different assortments of these prolific scrubs were cut at the base so that grass can grow for animals. The scrubs were dried and later pasteurized for *Pleurotus sajor-caju* (Singh *et al*., 1989).
- Sesame stems: were in India a satisfactory substrate for *Pleurotus sajor-caju* with biological efficiency of 60% (Pani *et al*., 1997).
- Sorghum stover: a selected substrate for *Pleurotus sajor-caju* in Africa used alone or in combination with cotton waste (Tagwira *et al*., 1999).
- Soybean stems: were in India the best substrate for *Pleurotus sajor-caju* with 77% biological efficiency (Pani *et al*., 1997). Soybean husks and soybean straw were a good substrate for *Pleurotus ostreatus* in Yugoslavia (Bugarski *et al*., 1997).
- Spent *Pleurotus* substrate, suggested by some authors as a substrate for the King *Stropharia* (Poppe, 1995).
- Spent substrate can be used to grow successive crops of mushrooms, like spent *Agaricus* compost amended with cotton waste for satisfactory cultivation of *Volvariella*. Oei (1991) refers to Quimio who made efficient

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Pleurotus substrate mixing half spent *Volvariella* substrate with 20% rice bran.

- Spent *Volvariella* compost: dried and re-used for *Pleurotus sajor-caju* with biological efficiency of 80% (Chang & Miles, 1989).
- Straw = cereal straw, 0.5% total N, 38% cellulose, 15% lignin, C/N = 90 (Kaul *et al*., 1981), basic substrate for nearly all cultivated mushrooms, can be enriched with at least 30 different supplement wastes. Straw is especially useful for *Agaricus*. Compost, chopped for *Pleurotus* and *Stropharia* (hundred author references).
- Subtropical forest dead leaves, *Platanus* spp. has a biological efficiency of 35% for *Pleurotus* (Martinez-Carrera, 1989).



Figure 17. Dry sugarcane bagasse (Photo courtesy of Dewraj Taurachand)

- Sugarcane bagasse, *Saccharum officinarum*, sugar cane rubbish, cane trash, 0.7% N, as bulk ingredient in mushroom compost resulted in normal *Agaricus* yield as well as horse manure (Kneebone & Mason, 1972). Good production was also obtained for *Pleurotus*. For *Pleurotus*, the biological efficiency of the pure bagasse is 15%. This is relatively low compared to many other substrates (Martinez-Carrera, 1989). Alum and Khan (1989) obtained good results with *Pleurotus sajor-caju* (Derks, 1993).
- Sunflower husks = Sunflower peels: the sunflower seeds are peeled before the internal seed parts can be pressed for oil. Up to now, all the precious waste was burned, millions of kilos per year. Very useful for *Pleurotus* without pasteurization and also moderate production for *Stropharia* in open field (Poppe *et al*., 1995). Cultivating *Pleurotus ostreatus* in Yugoslavia, the sunflower husks as a supplement on straw or maize stalk resulted in 8% higher yields (Bugarski *et al*., 1997).
- Sunflower stipes and heads, chopped, very suitable for *Pleurotus* and for synthetic *Agaricus* compost component (Poppe *et al*., 1995).
- Tea leaves: partial or integral substrate for different Asian mushrooms (Stamets, 1993; Poppe & Höfte, 1995).
- Tequila bagasse, *Agave tequilana*: has a biological efficiency of 60% for *Pleurotus* (Martinez-Carrera, 1989).
- Textile industry waste: card sweeping, card drops, blow gutter, chimney, testing-hard waste, reeling-hard waste, spooling-hard waste, weaving-hard waste for *Pleurotus* (Khan *et al*., 1989).



Figure 18. Tea plantation

- Treebark, chopped: can be used alone or in combination with wheat straw, corncobs and feather meal for *Pleurotus* (Imbernon *et al*., 1976) or as a fermented bulk substrate for *Agaricus* (Poppe *et al*., 1974). The origin of most tree bark is from the cellulose paper manufacturers where trees are debarked before chopping and pulp preparation. Delmas (1989) used it as a substrate for *Pholiota*, *Flammulina* and *Schizophyllum*.
- Uncrumpled rice straw: was in India the ideal substrate for *Pleurotus sajor-caju* with a biological efficiency of 85% (Pani *et al*., 1997).
- Used tea leaves: low biological efficiency for *Pleurotus sajor-caju* in India (Pani *et al*., 1997).
- Vegetable biomass: from bitter gourd, chili, cowpea, French beans, winged bean, pumpkin, tomato and okra gave good results with *Pleurotus sajor-caju* in India (Ganeshan *et al*., 1989).
- Water hyacinth, *Eichhornia crassipes*, feared boat propeller disturber; this prolific aquatic weed has gained prominence as a food source through cultivation of edible mushrooms like *Pleurotus* and *Volvariella*. Abundant in the Philippines, Indonesia, Africa and Bangladesh, should



Figure 19. Water hyacinth (Photo courtesy of Keto E. Mshigeni)

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be dried before use. In India the biological efficiency of water hyacinth for *Pleurotus sajor-caju* was 50% (Gujral *et al*., 1989).

- Water spinach, *Ipomoea aquatica*, used in India for *Pleurotus sajor-caju* (Gujral *et al*., 1989).
- Wheat straw, *Triticum aestivum*, main basic component of fermented *Agaricus* compost, in different percentages, up to 90%; wheat straw contains 1% protein, 13% lignin, 39% hemicellulose, 40% cellulose. It was burned in voluminous amounts until 1963 in France. Since it can be used for *Pleurotus*, the price is USD0.1-0.2 per kg. Straw for *Pleurotus* is only pasteurized and rarely fermented (Delmas, 1989). Wheat straw can also be used for *Volvariella*, it contains 48% cellulose, 20% lignin, 0.5% total N, 0.04 P_2O_5, 0.1% K_2O, 4.1% SiO_2, pH 6.9, C/N = ratio 104 (Heltay *et al*., 1960). (Numerous other authors)
- Wood logs: very productive for *Pleurotus quebeca* (Olah *et al*., 1979), but wood logs of at least 75 hard wood species can be used for *Pleurotus*, and at least ten species are a suitable substrate for shiitake. In the book of Stamets & Chilton (1983), we find analysis of wood compared to wheat straw in average percentages.
  Pine and spruce: 0.08% N, 0.02% P_2O_5, 0.1% K_2O, 11% hemicellulose, 56% cellulose, 27% lignin, resin ±3%.
  Beech: 0.13% N, 0.02% P_2O_5, 0.2% K_2O, 11% hemicellulose, 53% cellulose, 22% lignin, 1.7% resin (birch is nearly the same). (Numerous author references)
- Wood shavings, 0.3% N, useful for *Pleurotus*, *Pholiota*, *Flammulina*, *Auricularia*, *Hericium* (Poppe, 1995).
- Wood wastes: a list of ±140 tree species is given in Stamets (1993).

Conclusion

According to this worldwide survey, about 90 kinds of wastes have been proven to be useful for oyster mushroom growing, but some listed wastes such as cereal straw, sawdust, and wood logs can be re-divided into at least 100 individual types of waste linked to different plant species. It means that in fact a range of about 200 different wastes is available as oyster mushroom substrates. So, every grower producing oyster mushrooms can make their own best substrate choice from among all those genera or species having been cited in the substrate list.

We must not be at all surprised that the evaluation of all these kinds of different wastes leads us to a renewed appreciation for what is called a waste. Growing mushrooms gives so much satisfaction and produces so much food and income that further use of this practice can result in a great complete contentment of families and villages!

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Oyster Mushroom Cultivation

Part II. Mushrooms

Chapter 5

Substrate

CEREAL STRAW AND CORNCOBS

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Oyster mushrooms have the ability to utilize cellulose, hemicelluloses and a large or small quantity of lignin thanks to their enzymes. Oyster mushrooms need substrates abundant in polysaccharides (cellulose and hemicelluloses) and lignin for their growth. The mycelial growth of oyster mushrooms makes use of soluble carbohydrates, glucose, molasses, organic nitrogen sources like wheat bran, barley, oat, maize, soybean crust and sunflowers, as well as mineral sources such as ammonium sulphate.

Nutritious substances for oyster mushroom can be categorized into two categories: staples, which are the base nutritional materials, and additives, which are protein and nitrogen sources.

A. Staples that are rich in cellulose and hemicelluloses: wheat straw (Fig. 1), barley, hardwood chips (Fig. 2) or corncobs (Fig. 3).



Figure 1. Wheat straw



Figure 2. Hardwood chips

These can be utilized alone, as is done in 90-98% of the cases, or mixed with other materials, as in the example of 55% wheat straw and 38% corn cobs. Table 1 shows the nutritional values of each staple substrate material.

B. Additives as protein and nitrogen sources: wheat bran, barley, oats, maize; soybean crust, and sunflowers.

These materials are only used in 2-10% of the cases. However, it is recommended to add only a small quantity of them (max. 5%) because they can cause an increase of temperature in substrates during incubation that may

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cause the death of mycelium as a result. According to my personal experience, supplementation with additives didn't increase productivity significantly, but did accelerate mycelial growth by increasing substrate temperature. Table 2 shows the chemical analysis of the substrate in different phases of growth.



Figure 3. Corncobs and rice bran

Table 1. Nutritious substance contents of staples

Material	Nitrogen(%)		Hemicellulose	Cellulose	Lignin
	Total	Assimilable	(%)	(%)	(%)
Wheat straw	0.36	0.07	30.0	41.0	15.0
Barley straw	0.52	0.10	31.3	44.4	5.8
Hardwood chips (of poplar, beech, ashen)	0.57	0.04	15.4	16.7	26.0
Corncobs	0.49	0.06	38.0	28.0	11.0

Table 2. Physio-chemical trend of substrate in each stage

Substance	After soakage with water	After thermal disinfection	After second flush
Nitrogen	0.80	0.72	0.79
Hemicelluloses	24.3	20.1	14.6
Cellulose	33.1	33.5	22.8
Lignin	5.8	7.0	6.5
Phosphor (total)	0.06	0.05	0.04
Calcium (total)	6.64	7.40	8.95
Potassium (total)	0.46	0.49	0.27
pH	6.6	7.5	4.8
Water (fresh material)	73.8	72.4	71.3

According to Table 2, oyster mushrooms consume significant amounts of cellulose and hemicelluloses as their main nourishment source. On the other hand, lignin is rarely used, so any fermentation process, which causes an accumulation of lignin, should be avoided. Calcium serves the function of a catalytic buffer and nitrogen is consumed only during the incubation process. If the pH of the substrate falls to an acidic value, this indicates that it is time to end the harvest.

pH of Substrate (Supplementation with Gypsum)

The optimal substrate pH value for mycelial growth is 5-6.5, though mycelium can survive between pH 4.2 and 7.5. The mycelium grows slowly as the pH lowers and stops growing at pH 4. If the pH is higher than the optimal value, mycelial growth accelerates but produces an abnormal structure. Optimal pH for primordial induction and fruiting is 5-5.5 though it is possible at 5.5-7.8. The pH of the substrate can be adjusted by the addition of gypsum or lime.

Preparation of the Substrate Material

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Staples such as straw of wheat, barley, or hardwood chips must be crushed or chopped into fragments of 1-2.5cm. Corncobs must be ground into fragments of 0.5-1cm. These staples should be stored in a dry condition. If green, black, or white mildew is found, growers should never use the material. Using substrate materials infected by mould, bacteria or insects will considerably reduce crop yield, and will sometimes spoil the whole mushroom crop. To prevent contamination, make sure not to allow contact between substrate materials and remnants from previous crops. Considering that oyster mushrooms can utilize cellulose, hemicelluloses, lignin and soluble carbohydrates such as glucose and saccharine, it is recommended to add about 2-3% of soybeans, barley or oat meal to accelerate mycelial growth and obtain higher yields.

Watering Substrate Material

Water is one of the very important factors in mushroom growing. Suitable amounts of water should be maintained in the substrate during the whole process of cultivation. During preparation the substrate materials are soaked in a pool or a large container for 12-24 hours in order to achieve a water content of 65-70 percent. The soaking time varies according to the season. In summer 12 hours is required. Generally, 100kg of dry straw turns to 300kg of wet straw after soaking. After removing the excess water, the water content must be verified by squeezing strongly with the hand. The palm has to be wet with water drops at the base of the fingers. Appropriate water content for substrates is between 65 and 75 percent. If the water content is too low, mycelia won't grow in the substrate. If the water content is over 78 percent, the substrate becomes anaerobic and mycelia within the substrate die.

Thermal Disinfections - Pasteurization

The harmful bacteria or microorganisms in the substrate can be destroyed through thermal disinfection. After pasteurization, the substrate should have a nice scent and be of a similar color to the initial material. The substrate is usually pasteurized at 60℃ with hot water or steam.

Pasteurization with hot water: Submerge the substrate in a metallic basin and heat it up to 60-65℃. Keep the water temperature at 60-65℃ for 2 hours. Then leave the substrate overnight to cool down to 25℃.

Disinfections with steam: After soaking into the water for 12 hours, move the substrate to the pasteurization room. Heat up the room temperature to 60℃ and maintain the temperature for 2-3 hours by steam injection. Cool down the substrate temperature with the same method above. Cooling can be accomplished by injecting cool air if faster cooling is required.

Mixing of Substrate Materials

After cooling the temperature of substrate to 25℃ and removing the excess water, the pasteurized substrate is put in a pool or on a concrete floor that has been disinfected with a 10% lime solution and 3-5% blue vitriol. Generally, several materials are added, including 5-10% pulverized fungicide, 2% lime powder, 4-6% fodder chalk, or 3-4% gypsum. The additives should be thoroughly mixed with the whole substrate.

Before entering the mixing area, all workers have to step in a powder lime solution outside the area. It is recommended that all workers wear clean clothes and rubber boots and they should not leave the area until they finish bagging. All the necessary materials should be accessible within the mixing area.

Bagging and Spawning

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Figure 4. The holes punched in bag

The bags are made of polyethylene with a thickness of 0.05-0.1cm and have a 25cm diameter and are 80-100cm in length.

At first, the bags are perforated with an arch punch that makes holes 1.0-1.2cm in diameter at intervals of 10cm both horizontally and vertically (Fig. 4). The bottom of the bag should also have 2-3 smaller perforations in order to easily drain excess water.



Figure 5. A bag after filling with substrate

The quality of spawn is a key element in the production of high yields of mushroom. The grain spawn should have a nice scent and the grain should be thoroughly colonized. In Europe, the most popular strains of oyster mushrooms are from Italy, France, Holland and Hungary.

After mixing substrate materials, bagging and spawning is done simultaneously. Growers put a layer of substrate into a bag and sprinkle the grain spawn over the layer. They then put in another layer of substrate and again sprinkle spawn. They repeat this until the bag is full. The last layer spawn is covered with very shallow layer of substrate. They pack the bag well without empty space and tie the bag's opening tightly (Fig. 5). The weight of a bag is about 18-20kg, so the amount of spawn inoculated is about 550-600g per bag.

Spawn Run (Incubation)

After inoculation, the bags are incubated in specially arranged rooms where the microclimate factors such as light, temperature, humidity, and ventilation are strictly controlled. Light should be absent, the temperature should be maintained between 20 and 22℃, humidity should be between 75 and 85%, and ventilation should be done through air filters in such a way as to exchange all of the air in the incubation room 1-2 times a day. The bags should be placed with a distance of 5-8cm between them (Fig. 7).



Figure 6. Bags in incubation



Figure 7. The arranged bags in incubation room

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Figure 8. Pinning of oyster mushrooms



Figure 9. Oyster mushrooms from cereal straw

If procedures are followed carefully, the mycelia will colonize the whole substrate within 20-21 days, but this time can be shorter or longer according to the species and strains cultivated. If the temperature inside the incubation room is lower than optimal temperature, the incubation process can be extended up to 40-50 days. If the temperature is over 25-26℃, the bags can become overheated and the mycelia will die as a result. When the room temperature is over 26℃, the temperature measured inside the bags in the first 10-12 days can be higher than the room temperature by 4-5℃ and sometimes by 7-8℃ The bags should be arranged on the floor or on shelves, with some space between them to prevent overheating. These parameters should be checked daily along with rigorous pest control.



Figure 10. An oyster mushroom bag

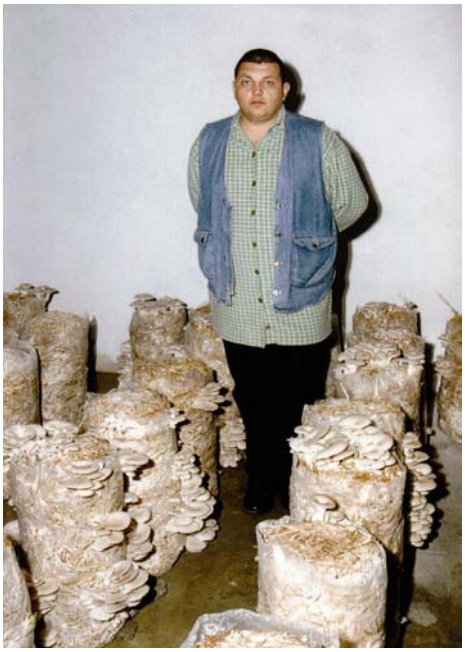


Figure 11. The author and oyster mushrooms

When the mycelia colonize the whole substrate thoroughly, the bags are carried to the fruiting room for fruiting induction, or they can be left in the same place, depending on the farm's arrangement.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

COCO LUMBER SAWDUST

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Oyster Mushrooms (*Pleurotus* spp.) are saprophytic as they obtain there nutrients by decomposing various agricultural by-products. This mushroom has been cultivated worldwide because of its taste and low maintenance technology. There are different substrates that have already been identified that can be utilized for the cultivation of oyster mushroom. The possible substrates include rice straw, coffee pulps, sawdust, and even paper. Most of these are types of low-value lignocellulosic wastes that are primarily derived from agricultural practices or the agro-industry. (J.A. Buswell *et. al.*, 1996) The bioconversion of these wastes is one reason why the cultivation of edible mushrooms is an appropriate practice for a society that depends on its agriculture.



Figure 1. Coco lumber sawdust

In the early 1990s, 'coco lumber' was given a great attention in the province as a substitute for hardwood. Sawmills producing lumber from coconut trees bloomed in reaction to the increasing demand for this low cost constructional material. Though beginners in mushroom cultivation are usually persuaded not to use sawdust from softwoods, sawdust from coco lumber (Fig. 1) is another possible substrate for *P. ostreatus* and has shown great results. Growers living near a coco lumber sawmill can make use of this waste product in order to start their own cultivation of oyster mushroom species.

Coco Lumber Sawdust as a Substrate of Oyster Mushroom

Oyster mushroom is one example of edible mushrooms that can utilize lignocellulosic materials as a substrate. This capability of the oyster mushroom. is due to the presence of its lignocellulolytic enzymes, which help it convert cellulose and lignin into useful carbohydrates such as glucose, that can be used as an energy source for the fungi. Any agricultural waste that contains cellulose and lignin is a possible substrate for growing this fungi.

Cellulose and lignin are both structural carbohydrates that give rigidity to a plant. Lignin gives the plant its wood characteristic while cellulose is the basic component or structure of the cell wall. Once sugar is associated with cellulose or with any structural carbohydrates in a plant body, the plant can no longer utilize the sugar as a

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source of energy. Therefore, the potential energy source is not used by the plant and is preserved for the future use by mushrooms. Hardwoods like mahogany or narra contain much higher amounts of these structural carbohydrates than softwood trees like coconuts. This means that hardwoods have more nutrients that can be used by mushrooms than softwoods do. This is why beginners in mushroom growing technology classes in the Philippines are told to make use of sawdust from hardwood or good lumber rather than sawdust from coco lumber.

Organic supplements are usually added to substrates to provide organic sources of nitrogen (R.C. Upadhyay 2003). Some frequently used organic supplements are rice straw and rice bran. Rice bran is commonly used to provide nitrogen, especially during the formation of fruiting bodies.

Oyster Mushroom Growing with Coco Lumber Sawdust



Figure 2. Removing impurities from sawdust.



Figure 3. Sawdust : rice bran=3:1



Figure 4. Stirring until a change in color is observed

We have tried coco lumber sawdust for cultivation of *P. ostreatus* by bag cultivation. The system followed the normal basic method of all substrate preparation using sawdust. The preparation of the substrate starts with the physical removal of impurities like chips of wood, plastics, leaves of plants, and other organic substances that might cause contamination (Fig. 2).

Afterwards, rice bran will be added to the sawdust. The total volume of the rice bran is 25% of the whole substrate (sawdust : rice bran = 3 : 1)(Fig. 3). The materials will be mixed until a light change in color of substrate materials is observed, but stirring should continue until no lumps of rice bran are found (Fig. 4).

Once the sawdust and rice bran is thoroughly mixed, lime will be added equal to 1% of the total volume of rice bran and sawdust mixture (Fig. 5). Lime neutralizes the acidity of the substrate. The mixture is again stirred until no lime is visible. Then, sugar is added equal to 1% of the mixture. Sugar can temporarily provide glucose to the mycelia while the cellulose and lignin are being converted into useful forms of carbohydrates. It is more practical to separately dissolve both the sugar and the lime in water before adding them to the mixture (Fig. 6).



Figure 5. Lime is added for pH control



Figure 6. Sugar is dissolved before being added to the mixture.

After the addition of sugar, water will then be added to the mixture of sawdust and rice bran. A simple way to determine the water content of the mixture is to get a handful of the substrate and squeeze it. A drop of water indicates that the amount of water added is enough while more than a drop shows that too much water was added

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to the mixture. Before doing so, make sure that the substrate is thoroughly mixed. Another way to add an appropriate amount of water is simply adding 1.5 parts of water to 3 parts of sawdust. But sometimes this amount is not enough due to the dryness of the sawdust.



Figure 7. The substrate is then transferred to pp bags.

When the substrate is evenly mixed, it is then transferred to polypropylene bags that are 6cm wide and 12cm long (Fig. 7). When too much water is added to the substrate it is better to squeeze the excess water away before it is packed into the polypropylene bags. The height of the substrate inside each polypropylene bag is 8-9 inches. The rest part of the plastic bag is fitted with a pvc neck which will serve as the opening of the bags (Fig. 8). After the pvc neck is placed, the opening is covered with a cotton plug and wrapped with paper to prevent the entry of insects. After they are filled with the prepared substrate, the bags are sterilized at 20 psi for about one hour (Fig. 9). After sterilization, the bags are left for cooling and then inoculated with the prepared planting spawn of *P. ostreatus* (Fig. 10). When inoculation is done, the bags are then marked with the date and species used. 10-20g of spawn is inoculated to 1.3kg bag, so spawning rate reaches 0.8-1.5% of wet weight of substrate.



Figure 8. PVC neckplacing



Figure 9. Sterilization at 20 psi for 1 hour



Figure 10. Spawn inoculation



Figure 11. Marking bags

Feasibility of Coco Lumber Sawdust as an Alternative Substrate

After the inoculation, the bags are then stored in a stock room with indirect sunlight and with a temperature of 26℃ Mycelial growth (Fig. 12) is observed after one week. The bags are completely colonized after 4-6 weeks of incubation.



Figure 12. Three weeks of incubation

After mycelia have completely colonized the bags, the bags are then opened to trigger fructification (Fig. 13). A single bag may have 4-6 flushes and the maximum yield is up to 800g of fresh oyster mushrooms per bag (1.3kg). Flushes will occur for 6-8 weeks depending on the humidity and temperature of the room (Fig. 14).

Compared to other substrates, the yield of mushrooms grown on coco lumber sawdust is lower. (A.Y. Gibriel *et al.*, 1996) A trial was also performed to identify the growth rate of mycelia and fruit bodies in the absence of rice bran in the substrate. The result was poor performance of the growing fungi in terms of mycelial growth and

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fructification of *P. ostreatus*. Poor sterilization processes also resulted in a higher rate of contamination. Thus, the one-hour sterilization at 20 psi should be strictly followed.

Infestation of Sciarid flies and mites has also been observed on growing mushrooms. Green molds are also visible especially after its second flush. The infected bags are removed to prevent second contamination to other bags. The chemical or biological control against infection was not performed because the trial only aimed to observe the efficacy of coco lumber sawdust as a substrate for *Pleurotus ostreatus* cultivation.

During or in between flushes, basic maintenance of stock was followed (e.g. watering of the incubation room to lower temperature and increase humidity). Flypapers were also placed to reduce sciarid numbers.



Figure 13. Open bags for fruiting induction



Figure 14. Oyster mushrooms on coco lumber sawdust

Conclusion and Recommendation

Sawdust from coco lumber is a possible substrate for the cultivation of *P. ostreatus*. Although the amount of yield is lower than with hardwood sawdust, growers with no available material other than coco lumber sawdust can make use of this agro-industry waste for oyster mushroom growing.

Further study should also be done to determine the amount of lignin and cellulose on coco lumber together with the effect of other organic nitrogen supplemented with the sawdust on the oyster mushroom cultivation.

The effect of biological and chemical pest control should also be noted. The newly introduced chemical called "Dimilin" (dflubenzuron) is now being used to reduce Sciarid infestation, but it was noted that when used at a normal rate it causes a reduction in yield of 7-8%. (L. Staunton *et al.*, 2002)

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

SUNFLOWER SEED HULLS

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What is There in Sunflower Seed Hulls?



Figure 1. Sunflower whole seeds and hulls (Average size of hulls is 12mm.)

An important portion of the energy invested in producing plant seeds is directed to the seed coatings. These are highly stable in nature as would be expected from their function of protecting seeds against water, providing thermal isolation, serving as a line of defense against pathogens. The coating or hull of the sunflower seed (Fig. 1) is an abundant and cheap lignocellulosic residue of the local (Argentine) edible oil-seed industry. Though the sunflower seed hull (SSH) is not used in human nutrition, SSH composition in organic and mineral substances could theoretically supply a source for other nutritional purposes, but the presence of a high content of lignin renders hulls unmarketable as a dietary supplement for animal feeding or other valuable uses. So, SSH remains an abundant side product of scarce economical value that is usually burned or left in the fields, either of practices that pose an environmental pollution problem.

During the oil extraction procedure raw sunflower seeds are transformed into oil and flour, and seed hulls are produced as a by-product. SSH constitute about 18-20% of the raw seeds. The main organic macro-nutrients of SSH are lipids, carbohydrates and proteins, with the highest percentage of the content being in the lignin and cellulose-hemicellulose portion, with lignin comprising about 20-25% of the total weight (Dorrel and Vick, 1997). Reduced sugars are also an important part of the seed coating, amounting to about 25%. Lipids and protein content are around 5% and 4% respectively, and almost 3% of the lipids are waxes (Cancalon, 1971). This chemical composition makes SSH an attractive material for growing microorganisms.

The high lignin content, however, limits the possibility of rapid biodegradation. The white rot fungi, basidiomycetes, are considered as the primary agents in nature for lignin degradation (Buswell and Oider, 1987; Zadrazil and Reinger, 1988).



Figure 2. Sunflower field

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Could Sunflower Seed Hulls be Used for Cultivation of *Pleurotus ostreatus*?

Sunflower seed hulls composition in organic macronutrients (4% protein, 5% lipids and 50% carbohydrates) is as appropriate as that of other substrates commonly used for the cultivation of oyster mushrooms, such as cereal straws, corn husks, used tea leaves and cotton wastes (2-5% proteins, 0.4-2.2% lipids and 32-37% carbohydrates). Oyster mushrooms (*Pleutorus* spp.) possess an extracellular enzymes system and a strategy via free radicals that make them able to degrade lignocellulosic material of SSH as well as others, thus exhibiting a great adaptability to different kind of lignocellulosic materials.

We initially tested whether SSH contain water extractable compounds which could affect mycelial growth of *P. ostreatus*, and found that aqueous sunflower seed hull extract did not affect its mycelial growth in culture medium. On the contrary, when the inoculated mycelium has been previously grown in a medium with a moiety of hull aqueous extract, mycelial growth significantly increased its rate. We concluded that under adequate conditions sunflower seed hulls could be used for mycelial growth, and that the phenomenon of mycelial growth stimulation, in response to the presence of some substances coming from the substrate in agar medium, needs to be further examined (Darjania *et al.*, 1997) since it could provide a useful tool for an advantageous adaptation of mushroom to a particular substrate (Chang, 1978).



Figure 3. Oyster mushroom cultivated on sunflower seed hull substrate

For cultivation of *P. ostreatus*, the most common supplement added to the end-substrate is cereal bran, a protein-rich substance, which is known to be an additive that stimulates mycelial growth and mushroom yield (Siddiqui and Khan, 1989; Kinugawa *et al.*, 1994). On the SSH substrate supplemented with cereal bran, the mycelial growth of *P. ostreatus* was not increased. Increased percentages of wheat bran to the end-substrate did not markedly influence the rate of substrate colonization. Moreover, substrate colonization was suppressed by the presence of 50% wheat bran or more. At this time, results suggested that non-supplemented sunflower seed hulls could be considered as a complete nutritive substrate to be colonized by *P. ostreatus* (Darjania *et al.*, 1997).

What about the Size of the Hull?

Mycelial growth did not show any significant differences when the sunflower seed hulls were used in three particle sizes averaging 7, 10 and 12mm, with the higher being the waste size from oil-seed factories. Complete substrate colonization by *P. ostreatus* was observed after 18 days of spawn running in all bags. However, there were marked differences in fruiting and crop yield. In these tests mushrooms grew better on substrates of the highest hull size, giving rise to about 65% biological efficiency (B.E., kg fresh mushroom weight/kg dry substrate weight x 100) at the 1st flush, and represented about 85% of the total accumulated B.E. at the 3rd flush. It was concluded that particle size did not affect the colonization rate of this mushroom and that additional hull chopping, which implies an extra cost, is unnecessary (Darjania *et al.*, 1997).

Are Sunflower Seed Hulls an Adequate Substrate?

These first approaches to introduce this new substrate indicated that SSH, as coming from oil-seed factories and without any nutritional supplementation, could be used as an adequate substrate for cultivation of oyster mushrooms, as biological efficiency for the first flush was within the commercially acceptable range. An increase of about 15% in B.E. was accumulated following the second and the third flushes. Thus, a prolongation of cropping did not produce a remarkable increase in the B.E., thus lowering the yield production cycle of *P.*

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ostreatus. Therefore, for this kind of very low cost substrate, it does not seem economically reasonable to keep *P. ostreatus* cultivation for more than 1 flush, mainly due to the high cost of electric energy resulting from the heating and cooling equipment needed to maintain control of the environmental conditions. However, the production cycle can be extended to 2 flushes in 40-50 days, by using optimized formulas containing growth limiting mineral nutrients such as Nitrogen (as ammonia sulfate) and Manganese (II) (manganese (II) sulfate, a co-factor for the lignolitic activity of some peroxidase enzymes), which resulted, for each *P. ostreatus* strain used in this study, in a marked yield increase–up to 100%–over the corresponding control (Curvetto, *et al.*, 2002). In practical terms and for these *P. ostreatus* strains the production was in the range of 1-1.8kg (60-112% B.E.) mushrooms per 4kg substrate bags.

In summary

1) Sunflower seed hulls can be used as a substrate for the cultivation of *Pleurotus ostreatus*; using a simple formula containing 37.5% SSH, 2% calcium sulfate ($CaSO_4$), 0.5% calcium carbonate ($CaCO_3$), 60% water (H_2O) and pH 6.
2) Under favorable conditions for mycelium growth as described here, addition of wheat bran is not needed.
3) The largest particle size corresponding to sunflower seed coatings as they come from the local edible oil-seed industry produced maximum B.E. in fructification, in comparison with smaller particle sizes.
4) First flush produces about 85% of the total B.E. accumulated through 3 flushes.

Other Mushrooms

We found that SSH-based substrate is also adequate to grow other fungi. For *Lentinula edodes*, the basal formula (37.5% SSH, 0.5% $CaCO_3$, 2% $CaSO_4$, 60% water) produced 2kg shiitake/100kg dry substrate per day for a 55 days production cycle with an accumulated biological efficiency of 108% (Curvetto *et al.*, 2002b), a higher yield of shiitake in a shorter cycle of production than is reported with other substrates. Good results were also obtained for *Ganoderma lucidum* on SSH-based substrates supplemented with 2.5 or 5.0% wheat bran or 5.0% malt, and the productivity was similar or even higher than the one reported in literature (Gonzalez Matute *et al.*, 2002). At present, we are developing protocols to use SSH for the production of *Trametes versicolor*, *Hericium erinaceus*, *Stropharia rugoso-annulata*, *Coprinus comatus*, *Flammulina velutipes*, and brown *Agaricus bisporus*.

A Simple Production Protocol for *Pleurotus ostreatus* on SSH-based Substrate

- This low-cost method is suggested by the authors. Detailed explanation on each equipment and each step is provided on Bag Cultivation in Chapter 7.

Spawn production

We prepare grain spawn in thermoresistant plastic bags or in 1L bottles using wheat (*Triticum durum*) grain mixed with 0.1% (w/w) $CaCO_3$, 0.8% (w/w) $CaSO_4$, and 40% water (1kg wheat, 2g $CaCO_3$, 16g $CaSO_4$ and 0.7L water). Calcium salts are used to adjust and buffer the pH of the substrate near 6. Additionally, these salts avoid the formation of clumps either of the grains used for spawning or of the sunflower seed coatings used for the mushroom substrate. Otherwise, those salts provide sulfur and calcium which are essential mineral macronutients. The mixture is sterilized at 15 psi for 1.5 hours. Each bag or bottle is then inoculated under aseptic conditions with oyster mushroom mycelium (two wedges per bag or bottle) (Fig. 4), and



Figure 4. ***P. ostreatus*** spawn prepared on wheat grain in 1L bottle or small plastic bag

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incubated at 25±1℃ in darkness for 15-20 days, with periodical shaking of the bags or bottles to maximize colonization and minimize grain clumping.

Substrate preparation and decontamination

For a 36kg substrate mass with a final composition of 37.5% SSH, 2% $CaSO_4$, 0.5% $CaCO_3$ and 60% tap water, these components are introduced into the drum as follows : 13.5kg SSH, then 10L water containing 720g $CaSO_4$ and 180g $CaCO_3$, and finally 11.6L water (Fig. 5). The decontamination process is always initiated with the gas heater on and the drum in a stationary position, during the first 15 minutes. Heating is provided for 2.5 hours with the drum alternately rotating for fifteen minutes and then stopping for fifteen minutes.



Figure 5. Introduction of sunflower seed hull into drum



Figure 6. Spawning the decontaminated substrate after cooling



Figure 7. Filling the bags

Spawning and spawn running



Figure 8. Puncture of bags for an adequate gas exchange



Figure 9. Colonization of substrate bags 3, 8, and14 days after spawning

The temperature of the substrate is allowed to fall to 35-40℃, with the drum rotating for about 2 hours. The spawn is then added to the substrate at a rate of 5-8% (w/w) (Fig. 6), and keeping the open end of the drum covered, rotation is continued during 15-20 minutes until homogeneous mixing of spawned substrate is obtained. With care

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and decontaminated rubber gloves on, the operator fills plastic bags of 0.25m diameter with substrate to make blocks of 4-10kg (Fig. 7). The substrate is compressed by repeatedly tapping the bag on the floor thus obtaining a density of about 0.5kg/L and then, the bag is tightly closed. To assure an adequate O_2 and CO_2 concentrations and gas exchange, they are aseptically punctured on the whole surface using a rod with sharp pointed nails (we recommend an ad hoc device with pins (Fig. 8) that finally gives approximately 7,000 micro-holes per square meter, each one separated by 1.2cm from the surrounding mini-holes). The bags are placed in a growing room at 24±1℃, and after 15-18 days the substrate becomes completely colonized by the mycelium (Fig. 9). During this stage, the bags are daily observed for possible contamination.

Fruiting

Once the substrate in the bags is completely colonized by mycelia, the blocks are transferred to a fruiting room and the plastic covers are evenly punctured using a device with attached archery broadheads (Fig. 10) (Stamets, 1993) to expose a fructification surface of ca. 1% of the total bag surface to the following environmental conditions: 20±1℃, 80-90% R.H., and 12-hour photoperiod (150-200 lux). Observations for possible contamination are also done. Pinning (Fig. 11) and subsequent growth of fruiting bodies in a first flush (Fig. 12) occurred 15-20 days after spawning. A second crop is obtained between 10-15 days after the first one, and usually for this second flush a new set of punctures on the surface of the bags is needed.



Figure 10. A device with attached arrowhead to expose substrate surface for fruiting



Figure 11. Pinning of oyster mushroom



Figure 12. ***P. ostreatus*** on sunflower seed hull

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

GRASS (JUNCAO)

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JUNCAO (菌草) - Jun(菌) means fungi and Cao(草) means grass in Chinese.

Juncao techniques were invented in China in 1983 by Professor Zhanxi Lin, director of Fujian Agriculture & Forestry University and JUNCAO Research Institute. He used herbaceous plants such as *Musa nana*, *Miscanthus sinensis*, *Dicranopteris dichotoma*, *Miscanthus floridulus* as new cultivation substrates to replace such traditional substrates as sawdust, wheat bran and rice bran. Before Juncao techniques were invented, many species of edible fungi and medicinal fungi, such as shiitake and wood's ear, were mainly cultivated on sawdust or logs. The recent expansion of mushroom production, however, resulted in the over-exploitation of the broad-leaf trees resource and caused a shortage of raw substrate materials. Major mushroom growing countries like Japan and China encountered such problems, which had restricted the further development of large-scale mushroom production. The invention of Juncao techniques has solved the conflict between the increase of fungi production and the protection of ecological environment, and enabled fast and sustainable development of mushroom production.

Definition of Juncao Technology

Juncao is defined as the herbaceous plants that are suitable for cultivating edible and medicinal fungi. Juncao Technology is defined as a series of comprehensive techniques to cultivate edible and medicinal fungi and produce mycelium protein forage with Juncao. The Juncao Industry is defined as the industry utilizing Juncao technology and other relative techniques.

The conversion rate of solar energy into Juncao grasses is 6-8 times higher than that for broad-leaf trees. With Juncao techniques, 1kg of dry grass can be converted into about 1kg of fresh mushrooms. This technology combines ecological, economical and social benefits. Therefore, the Juncao Industry is a new ecological industry that possesses the advantages of a high utilization rate of natural resources and great potential for continual development.

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Juncao Species as Substrate Material for Mushrooms

From 1983 to 2003, 37 Juncao species have been selected which are suitable for mushroom cultivation using the three-stage selection method (Table 1).

The test results of the Fujian Agriculture University Central Laboratory show that the nutrients contents of most Juncao species are richer than those of sawdust (Table 2). The contents of protein, nitrogen, fat, phosphorus, potassium, and magnesium in Juncao are higher than those in sawdust of broad-leaf trees. Among them, the protein content of wild *Dicranopteris dichotoma, Neyraudia reynaudiana, Saccharum arundinaceum, Phragmites communis, Miscanthus floridulus* and *Themeda gigantea* is 2-4 times as much as that of sawdust. Their fat, nitrogen, phosphorus, potassium and magnesium contents are respectively 101-216%, 232-353%, 225-685%, 346-908%, and 191-303% of those of sawdust.

Table 1. Juncao species suitable for mushroom cultivation

Pennisetum pupureum Schum.	*Arundo donax* L.
Pennisetum sinense Mez	*Triticum aestivum* L.
Pennisetum alopecuroides Steud.	*Oryza sativa* L.
Phragmites communis Trin.=P. australis (Cav.) Steudel	*Achnatherum splendens* (Trin.) Nevski
Neyraudia reynaudiana (Kunth) Keng ex Hitchcock	*Arundinella hirta* Tanaka
Miscanthus floridulus Warb. Ex K. Schum. & Lauterb.	*Arundinella nepalensis* Trin.
Miscanthus sacchaiflorus Hack.	(=*A. brasiliensis* Raddi)
Miscanthus sinensis Anderss.	*Spartina anglica* C.E.Hubb.
Saccharum arundinaceum Retz.	*Musa nana* Lour.
Saccharum sinense Roxb.	*Setaria anceps* Stapf ex Massey
Saccharum robustum Brandes & Jiesw. ex Grassl	*Eichhornia crassipes* Solms
Themeda gigantea (Cav.) Hack. ex Duthie var. villosa	(=*E. speciosa* Kunth)
Themeda gigantea Hack. ex Duthie var. caudata	Gossypium hirsutum L.
Paspalum wettsteinii Hack.	(=*G.. herbaceum* L.)
Paspalum dilatatum Trin.	*Medicago sativa* L.
(=P. dasypleurum Kunze ex Desv.)	*Helianthus annuus* L.
Vetiveria zizanioides Stapf	*Ferula sinkiangensis* K.M,Shen
Sorghum propinquum (Kunth) Hitchcock	*Dicranopteris dichotoma* (L.)Farw.
Sorghum sudanense Stapf	*Dryopteris ampla* Kuntze
Cymbopogon citrates Stapf	

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Table 2. Nutrients contents of Juncao (%)

Nutrients Contents (%)	Protein	Fiber	Fat	N	P	K	Ca	Mg
Sawdust	1.19	84.82	0.93	0.19	0.02	0.11	0.22	0.03
Dicranopteris dicnotoma	3.75	72.10	2.01	0.60	0.09	0.37	0.22	0.08
Neyraudia reynaudiana	4.42	58.80	1.72	0.67	0.14	0.96	/	0.09
Saccharum arundinaceum	2.75	62.50	0.99	/	/	0.76	0.17	0.09
Phragmitas communis	3.19	72.50	0.94	0.51	0.08	0.85	0.14	0.06
Miscanthus floridulus	3.56	55.10	1.44	0.57	0.08	0.90	0.30	0.10
Themeda gigantea	3.85	51.1	1.38	0.61	0.05	0.72	0.19	0.08
Pennisetum purpureum	5.91	68.88	/	/	0.18	0.78	0.40	0.24
Spartina atierniflora	9.90	23.58	2.96	/	/	/	/	/
Sorghum propinquum	4.17	49.47	/	/	0.08	0.46	0.44	0.17

Medicinal and Edible Fungi Species Cultivated with Juncao

45 fungi species have been selected which are suitable to be cultivated with Juncao as the cultivation substrates (Table 3).

Table 3. Mushroom species suitable to be cultivated with Juncao

Agaricus bisporus (J.E.Lang) Pilát
Agaricus blazei Murill
Coprinus comatus (O.F.M&unml;ll.)Gray
Dictyophora rubrovolvata M.Zang, D.G.Ji & X.X Liu
Dictyphora duplicate (Bosc)E. Fisch.
Pleurotus ostreatus (Jacq.) Quél.
Pleurotus sapidus (Schulzer) Sacc.
Pleurotus rhodophyllus Bres.
Pleurotus sajor-caju(Fr.) Singer
Pleurotus citrinopileatus Singers
Pleurotus cystidiosus O.K.Mill.
Pleurotus abalones Y.H.Han, K.M.Chen & S.Cheng
Pleurotus eryngii (D.C.) Gillet
Pleurotus tuber-regium (Rumph.ex Fr.) Singer
(=Lentinus tuber-regium (Fr.)Fr.)
Armillariella mellea (Vahl) P. Karst.
(=Armillaria mellea (Vahl) P.Kumm.)
Armillariella tabescens (Scop.) Singer
(=Armillaria tabescens (Scop.) Emel.)
Lentinus edodes (Berk.) Singer
(=Lentinula edodes (Berk.) Pegler)
Collybia velutipes (Curtis) P.Kumm.
(=Flammulina velutipes (Curtis) Singer)
Tricholoma giganteum Massee
(=Macrocybe gigantea (Massee) Pegler & Lodeg)
Hypsizygus mamoreus (Peck) H.E. Bigelow
Oudemansiella radicata (Relhan) Singer
(=Xerula radicata (Relhan)Fr.)
Stropharia rugosoannulata Farl. ex Murrill
Pholiota nameko (T. Itô)S.Ito & S. Imai
Volvariella volvacea (Bull.) Singer
Agrocybe cylindracea (DC.) Gillet
Pholiota aegerita (V. Brig.) Quél.
(=Agrocybe aegerita (V. Brig.) Singer)
Hericium erinaceus (Bull) Pers.
*Poria cocos (*Schwein.*)* F.A. Wolf
(=Wolfporia extensa (Peck) Ginns)
Ganoderma lucidum (Curtis) P Karst.
Ganoderma sinense J.D.Zao, L.Y.Hsu&X.Q.Zhang
Coriolus versicolor (L.) Quél
(=Trametes versicolor (L.) LIoyd)
Grifola frondosa (Dicks.) Gary
Grifola albicans Imazeki
Auricularia auricula (Hook. f.)Underw.
(=Auricularia auricula-judea (Fr)Schröt)
Auricularia cornea Ehrenb.
Auricularia polytricha (Mont.) Sacc.
Auricularia peltata LIoyd
Auricularia delicate (Fr.) Henn.
Auricularia mesenterica (Dick.) Pers.
Tremella fuciformis Berk.
Tremella aurantia Schwein.
Tremella cinnabarina Bull.

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Oyster Mushroom (*Pleurotus ostreatus*) Cultivation with Juncao

Oyster Mushroom (*P. ostreatus*) can utilize a wide range of the available culture materials due to its great adaptability. In addition, it is easy to cultivate with simple technology and has a short growth cycle. A large variety of Juncao and crop stalks are suitable for the culture medium for mushrooms. Under certain suitable conditions, 4-5 weeks are enough from spawning to harvesting. Due to these advantages, *P. ostreatus* will be one of the most important mushroom species and will make a great contribution towards solving the problem of the lack of protein in developing countries.

Nutrition

P. ostreatus is classified as a wood-saprophytic fungus. In nature, it grows on the dead branches of broad-leaf trees, such as poplar, willow, elm, maple, beech and Chinese ilex. In artificial cultivation, either logs or sawdust can be used as the culture substrate. A proper amount of rice bran and sugar can be added in order to promote the mycelia growth and fruit body formation while cultivating with sawdust. In Japan, the amount of rice bran added is up to 36-40% in *P. ostreatus* cultivation with sawdust.

The research done in 1986 discovered that JUNCAO, such as *Neyraudia reynaudiana*, *Miscanthus floridulus*, *Saccharum arundinaceum*, *Themeda gigantea*, *Miscanthus sinensis*, *Spartina anglica*, *Pennisetum purpureum* and *Sorghum propinquum*, are high quality culture materials for *P. ostreatus* cultivation. They can be used as substitutes for sawdust and partially as substitutes for rice bran. Moreover, *P. ostreatus* can also be cultivated with corncob, wheat straw, bagasse, *Musa nana*, *Pistia strationtes*, rice straw and other crop stalks.

Temperature

The appropriate temperature range for spore germination is 24-28℃. Mycelia can grow properly at 7-35℃, while the best range is 20-25℃. The suitable temperature range for fruit body growth is 10-28℃ varying among the different strains. In accordance with the suitable fruiting temperature, strains are classified into psychrophilic (12-15℃), mesophilic (16-22℃) and thermophilic (20-26℃) types. *P. ostreatus* growing in the lower portion of the suitable temperature range generally are of a higher quality.

Humidity

The water content of substrate suitable for mycelial growth is about 65%. Mycelial growth is inhibited if the water content is less than 50%. In bottle cultivation of *P. ostreatus*, mycelia can grow properly under conditions where the air humidity is 65-70%. During the fruiting period, the suitable air relative humidity can be as high as 85-90%. If the relative humidity is less than 85%, the growth of fruit bodies will be slowed down. The mushroom quality will also be negatively affected if the relative humidity is higher than 95%.

Ventilation

P. ostreatus is an aerobic fungus. Its fruitbodies cannot grow normally without fresh air. Although fairly high concentrations of carbon dioxide will not affect the growth of mycelia, such is not the case for that of the fruitbodies. When the concentration of carbon dioxide is higher than 600 ppm, the stipes elongate and cap growth is inhibited. Due to the lack of oxygen, fruitbodies cannot form, or they become malformed.

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Illumination

Although mycelia can grow properly even in total darkness, the primordia formation and fruit-body growth require a certain amount of light. Primordia formation is only possible after 12- hour illumination with an intensity of 200 lux. For the proper growth of fruitbodies, the light intensity should be within 50-500 lux. The color of the caps is also related to the light intensity, so insufficient amounts of illumination will lead to pale colored caps.

pH value

Mycelia can grow properly when the pH value is between 4 and 7.5, whereas the most suitable range is pH 5.5-6.0.

Cultivation Steps of Oyster Mushroom with Juncao

Juncao pretreatment

Because of their different biological characters, the harvesting, processing and storage of Juncao is different from that of sawdust. Successfully undertaking the three steps below will help growers realize the full potential of Juncao's nutritional value.

Juncao harvesting

Due to the high nitrogen content of *Dicranopteris dichotoma*, *Neyraudia reynaudiana* and other Juncao, the harvesting season and weather must be carefully chosen. If harvesting takes place during rainy days, drying and processing will become more difficult and this will result in mildew and lower utilization rates of the Juncao. Therefore, harvesting must be arranged in 5-7 days that are sunny. Harvesting time depends on the different species of Juncao and cultivated fungi. For example, *Dicranopteris dichotoma* can be cropped in the whole year, but is best harvested from May to July. *Neyraudia reynaudiana*, *Miscanthus floridulus* and other Juncao of grass family are best cropped in flowering and heading stages. *Neyraudia reynaudiana* used for cultivation of shiitake (*Lentinus edodes*), *Auricularia peltata* and *Auricularia polytricha* should be cropped after heading and aging, whereas those used for cultivation of enokitake (*Flammulina velutipes*), Straw mushroom (*Volvariella volvacea*), *Pholiota nameko* and *Pleurotus sajor-caju* should be cropped just before heading.

Juncao drying

After cropping, grasses must be placed in the sunlight to dry thoroughly, a process that is always affected by the weather. Thus, growers should try to store Juncao before the rainy season. Two storage methods are commonly employed: indoor storage in dry rooms and outdoor haystack storage. For outdoor storage, waterproof coverings are important. For both methods, great care must be taken for fire-prevention. Loose grasses normally occupy large spaces indoors, and they are easily dampened outdoors, so it is necessary to process them into powder immediately after drying. Juncao powder with a small volume is convenient for both storage and long distance transport.

Juncao processing

Processing of Juncao is very different from that of sawdust and rice straw because of the physical structure and nutritional differences. Special Juncao grinders are necessary. The size of the grinder sieve also depends upon the different species of Juncao. For example, a sieve whose holes are of diameter about 2.5mm is used for *Dicranopteris dichotoma* while a sieve with holes of a diameter of 3.0-3.5mm is usually suitable for *Neyraudia reynaudiana*.

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Juncao powder storage

Juncao powder must be stored in dry rooms. Otherwise, it will become mildewed or blocked, which will exhaust the nutrients and lower the nutritional value of Juncao.

Substrate formula

Some substrate formulas for *P. ostreatus* cultivation are listed below.

1. *Miscanthus floridulus* 60%, *Sorghum propinquum* 10%, *Pennisetum purpureum* 6%, wheat bran 17%, rice bran 5%, gypsum powder 1.5%, sugar 0.5%.
2. *Miscanthus floridulus* 72%, *Pennisetum purpureum* 5%, wheat bran 15%, rice bran 5%, calcium carbonate 2%, sugar 1%.
3. *Miscanthus floridulus* 49.5%, *Sorghum propinquum* 20%, wheat bran 20%, rice bran 8%, calcium carbonate 2%, sugar 0.5%.
4. *Miscanthus floridulus* 43%, *Neyraudia reynaudiana* 33%, wheat bran 16%, rice bran 6%, calcium carbonate 1.5%, sugar 0.5%.
5. *Pennisetum purpureum* 75%, Phragmites communis 6%, wheat bran 10%, rice bran 6%, calcium carbonate 2%, sugar 1%.

Mixing substrate materials

Growers should weigh all the raw materials according to the substrate formulas, stir the Juncao powder, wheat bran and rice bran evenly, and then pour them into the mixer. They should first add additional nutrients into the water, followed by calcium carbonate or gypsum powder. They should put the mixture into a mixer after it has been stirred thoroughly for 30-40 minutes. Note that the water content of substrate should be 62-65% and the pH value should be 5.5-6.5.

Filling substrate into container

In the case of bottle cultivation, growers should use 850mL plastic bottles and put 500-550g of wet materials in the bottles. In case of bag cultivation, they should use 24 x 44cm plastic bags and put 1.8-2.2kg of wet materials in each bag. Mixed materials should be packed tight in containers as soon as mixing is done. Growers should make sure to clean the surface of bottles or bags.

Sterilization

In the case of high-temperature sterilization, growers need to keep the temperature of the sterilization room at 121℃ for 2 hours while they need to maintain the compost at a temperature of 100℃ for 6 hours for normal-temperature sterilization. Growers need to make sure to record the temperature of both the room and compost at scheduled times. When sterilization is finished, they should lower the temperature slowly. They should open the exhaust valve only after the temperature is lower than 80℃ or the pressure falls to 0.5kg/cm^2.

Inoculation

The temperature of the substrate should be cooled down to 18-25℃ before inoculation. Growers should keep the room temperature between 8 and 15℃ and the humidity less than 60%. Growers need to make sure to inoculate according to the micro-organism and bacteria-free processing rules. They should not use old spawn and should get rid of old spawn on the surface of spawn bottles before inoculation.

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Mycelia culture

Bottles and bags should be sterilized by using ultraviolet light and medical liquids before being moved into the culture room. The appropriate room temperature for mycelial growth is 20-24℃ and the appropriate relative humidity is 65-70%. Growers are advised to ventilate with fresh air but no light is required. Growers should observe the temperature variations and mycelia growth situation frequently. It takes about 18-24 days until the substrate is fully colonized.

Primordia formation

In the case of bottle cultivation, growers are advised to scratch the surface of the bottle for primordial induction. To induce fruiting, the water content of substrate should be increased. Growers can add 10-15mL water into each 850mL bottle, pour out the water and place the bottles upside down on the culture shelves. After 20-30 minutes or when no more water drips, they should then turn them right side up again. An alternative way is just to let the compost dry naturally after adding water. An appropriate room temperature is 13-14℃ and an appropriate room moisture is 85-95%. Adequate ventilation is required. Buttons appear in 6-8 days.

Fruiting and cropping

According to the strain type used, appropriate room temperatures vary: 12-15℃ (psychrophilic), 16-22℃ (mesophilic) or 20-26℃ (thermophilic). Growers need to keep room moisture at 90-95%. They should increase the air flow by turning on the exhaust for 30-50 seconds per half hour. A certain amount of scattered light is required. It takes about one week for full fruiting. If the crop is harvested when the caps are nearly open and flat, a high yield will be gained. On the other hand, mushrooms can be preserved for a long period if they are harvested before the cap spreads out.

Culture of second mycelia generation

After harvesting the first flush of mushrooms, growers should keep the room temperature at 20-24℃ and the room moisture at 60-70% in order to induce a second flush. They should make sure to clean the racks of old spawn after cropping and level the surface of the bottles. At this point no light is required, but fresh air is necessary. The second flush should start 8-10 days later. Primordia induction and fruiting are same as with the first flush.

Artificial Planting of Juncao

From the past years' practice, we strongly suggest that mushroom growers who want to adapt Juncao as the culture substrate should plant Juncao too. This is because wild Juncao normally does not grow in fields, a fact that always causes inconvenience and low yields. The yield of planted Juncao can reach 30,000kg per Mu (about 667m^2).

We recommend two methods for Juncao cultivation: artificial reproduction and closing hillsides to facilitate grassing. Wild *Dicranopteris dichotoma* has a wide distribution and always grows in tracks. So growers can get high yield by just closing hills to facilitate grassing, an action that makes transplanting unnecessary. If possible, growers should apply fertilizer before the rainy days in spring, as this will which result in higher yields.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 1

Substrate

COTTONSEED HULLS

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Figure 1. Cottonseed hull

Cottonseed hull is one of the most efficient substrate materials for oyster mushroom cultivation. Cottonseed hull contains hot water extracts and alcohol extracts that mushrooms can easily utilize. Cottonseed hull is easy to dry, so can be stored for relatively long period of time. If the substrate is to be sterilized or pasteurized, cotton waste is suitable because it emits extra heat by itself.

However, cottonseed hull cannot absorb water thoroughly and it is difficult to remove extra water content if it is overwetted. During fermentation, concentrated gas cannot escape to the outer surface of the cottonseed hull. The control of gas and water is essential for successful cultivation using cottonseed hull.

Cottonseed hull (Fig. 1) is the coating of cotton seeds, the substance that remains after the cotton has been peeled off. As a waste material of the oil industry, it is about 32-40% of the total weight of cottonseed. In China the annual output of cottonseed hull is about 12 million tons. It generally contains 9.1% water and 90.9% organic matter that consist of 4% crude protein, 1.4% crude fat, 40.9% crude fiber, 34.9% soluble carbohydrate, and 2.6% ash. Its C : N ratio is about 59 : 1. This chemical composition shows how nutritious a raw material cottonseed hull can be when cultivating oyster mushrooms. Thanks to its soft texture, high water-holding capacity, and good physical structure, cottonseed hull is used worldwide as a good substrate for cultivated oyster mushrooms.

Treatment of cottonseed hull

Pre-wetting

Being fresh and contamination-free are the basic requirements for cottonseed hull that is to be used as substrate material. In the pre-wetting step, the amount of water should be carefully calculated rather than measured by eye. The water usually sinks to the bottom of the pile, so the cottonseed hull pile's surface still looks dry, which encourages growers to overwater. A graduated container is recommended to calculate the necessary water according to the amount of dry cottonseed hull. After being mixed well, the cottonseed hull should remain soaking

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overnight. It is helpful to add 1% $Ca(OH)_2$ during the overnight soaking.

Supplementation

The nutrient content of cottonseed hull is enough for cultivating oyster mushrooms due to its high C/N ratio. However, 5-10% of rice or wheat bran is usually added as a supplement before sterilization, as this may produce higher yields. Many Chinese growers use non-sterilized substrates in autumn when the temperature drops.

Bagging and Sterilization

The plastic bag used here for growing P. ostreatus is 40-50cm in length and 25cm in diameter. A specially designed plastic ring is wrapped at the top end of the bag to form a bottleneck with a cotton plug in it (Fig. 2). After bagging, the bags are usually sterilized to reduce the risk of contamination. If the cottonseed hull is sterilized at normal pressure, it is necessary to add 10% bran or other kinds of nitrogen supplements to raise yields. 2% calcium carbonate and 1% $Ca(OH)_2$ is also added to adjust the pH of the substrate to 8-9. The cottonseed hull is sterilized at 100℃ for 10 hours (Fig. 3). Then, the pH changes to reach the optimum level of 6.5-7.0.

Some growers skip the sterilization of substrates to reduce their costs for heating and autoclaving. This method is frequently chosen by relatively poor Chinese growers in autumn and winter. If not sterilized, the substrate materials must be fresh and should be pasteurized for 2-4 days by sunlight before pre-wetting. In addition, no nitrogen should be supplemented in order to lower the risk of contamination, but 1% of calcium hydroxide ($Ca(OH)_2$) needs to be added.



Figure 2. Bagging of cottonseed hull mixed with corncobs



Figure 3. Boiler and autoclave

Spawning

During spawning, the spawn should cover the surface of the substrate to reduce the possibility of contamination during spawn run. 50-60g of spawn is inoculated to 1kg of substrate, so spawning rate reaches 5-6% of wet weight of substrate. Then the bags should be incubated at 25℃ until the mycelia grow fully. When sterilization is not available, an increased amount of spawn needs to be well mixed with substrate. After spawned substrate is put into each bag, a layer of spawn is placed on the substrate surface in the bag. The bags should be incubated at a relatively low temperature, about 15-20℃, in order to decrease the amount of contamination.

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Laying the Growing Bags

After the mycelia have fully grown, the bags are then moved to the growing house for fruiting. We use a unique method of making walls with mushroom bags and soil. 1-meter wide ridges are made at 0.8-meter intervals and the space between the ridges is used for draining. Fully incubated bags are cut open at the bottom and laid on one side of the ridge with another bag on the other side of the same ridge (Fig. 4). The length of the ridge varies according to the width of the house. It is necessary to leave the 0.8-meter wide aisles for both drainage and pickers passing through.



Figure 4. The walls shown from above



Figure 5. The walls of bags Adhered by fertilized soil



Figure 6. Mushroom bag walls without soil

The unique characteristic of our method is the use of fertilized soil with urea as both cement for building the wall and a source for nutrients and humidity (Fig. 5). The fertilized and moistened soil is filled into the 20cm space between the two tiers of bags and is also layered 3cm thick on top of the growing bags. The proper quantities of urea or other kinds of inorganic nitrogen supplements are spread on this soil if necessary. Then, another layer of bags are put on top of the previous layers until the ridge height reaches 1.5m with the wall length and width gradually tapering (Fig. 5, 6) to prevent the wall from falling down. A layer of soil is placed to cover the top of the wall of bags (Fig. 4). The number of ridges is dependent on the dimension of the growing house.

By using this method, we can manage the bags easily and raise the yields. The mycelia of the mushrooms stretch into the fertilized and moistened soil and absorb the nutrients from it. Watering is necessary during the fruiting periods. Water is sprayed on the soil, not on the substrate or fruit bodies, in order to keep the mushroom pins from dying.

Cropping Management

After the wall of bags are finished, the plastic rings and the cotton plugs are usually removed and the mouths of the bags are pulled straight to let fresh air enter in order to stimulate pinning. Some growers don't cut off the plastic at the top end of the bags because this part can generate a small micro-climate where the relative humidity is high, and water is not directly sprayed onto the pins thanks to this part. However, the methods vary from province to province according to local climate conditions. Some growers leave the plastic rings on during fruiting (Fig. 7), while others get rid of the excess plastic (Fig. 8).

During fruiting, growers maintain proper temperature and 85% relative humidity by spraying water on the ground and soil. Usually, 5-6 flushes are harvested before the weather is no longer suitable for oyster mushroom growing. Biological efficiency has reached 150-200%.

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Figure 7,8. Oyster mushrooms growing from the bag wall

Harvest

It is necessary to stop watering two days before picking. The mature fruitbodies are recognizable by their shape. The process of picking is very easy as growers simply pull out the fruitbodies by hand. It is not advisable to use knives to cut the fruitbodies at the base because some stumps will be left and these can cause the crops to suffer from infections.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

SUGARCANE BAGASSE

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Mauritius is a small island in the Indian Ocean with a population of about 1.2 million. Mushroom industry is in its infant stage and oyster mushroom is mainly cultivated. Other species such as shiitake and *Auricularia* are cultivated on experimental scale at several governmental institutions. Oyster mushroom is rich in protein as well as contains less fat, less carbohydrate and salts. It also has rich fibre, high Vitamin B_{12} and folic acid contents uncommon in vegetables. Therefore, oyster mushroom is ideal food for patients suffering from hypertension, diabetes and obesity.

Table 1. Characteristics of sugarcane bagasse

	Moisture	Fibre	Soluble Solid (mostly sugar)
Composition (%)	49	48	2.3

Table 2. The Composition of Fibre

Cellulose	48%*
Pentosan	28.7%
Lignin	14.3%
Ash	2.4%
pH	6.1%
Total nitrogen	1.23%
Carbon	29.36%
Available phosphorus	2,399ppm
Available potassium	21.63ppm

* out of which 26.6% is alpha cellulose(a)

Sugarcane bagasse is the only substrate material available in large amount in Mauritius, and no other substitute has been proved as a good mushroom substrate till now. Sugarcane bagasse requires no chipping, cutting, or grinding to be utilized as substrate material, unlike corn cobs, grasses and banana leaves. It can be collected directly from factory, so we don't need extra labour for collecting and can preserve environment by using this agricultural waste. Previously, sugarcane bagasse was distributed by sugar factories free of charge. However, mushroom growers have to pay for it nowadays because the sugar factories see the waste as a fuel for electricity generation if ever earned. Moreover, the factories can sell the excessive power to the Central Electricity Board, so they have no reason to throw away the bagasse. Sugarcane bagasse is not available in out of season, so most mushroom growers suffer from substrate shortage. The sugarcane bagasse cannot be stored for a long time.

Sugarcane bagasse mainly consists of moisture, fibre, and soluble solid (Table 1). The main constituents of fibre are cellulose, pentosan, and lignin. Table 2 shows the composition of fibre. Sugarcane bagasse contains

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cellulose which is easily degraded by oyster mushroom, a cellulolytic fungus. It also contains cellulo sugars especially sucrose which provides energy for mushroom. The total nitrogen content indicates that bagasse is not poor in nitrogen. The nitrogen is mostly in the organic form especially protein which is required for growth of mushroom. We use this waste from sugarcane industry to grow highly value added product, oyster mushroom.

Spawn Preparation

Tissue culture of oyster mushroom is inoculated to Potato Dextrose Agar (PDA) media (Fig.1) and incubated. After colonized by mycelium, the PDA media is cut into pieces and inoculated to sterilized millet grains, which will be mother spawn. After fully incubated, this mother spawn is inoculated to spawn bags filled with maize seeds. After fully colonized, this bag is used as spawn (Fig. 2). The spawn bag can be purchased from governmental organization in Mauritius.



Figure 1. Transfer of mycelium



Figure 2. Spawn bags in Incubation

Preparation of Mushroom Bags



Figure 3. Mixing substrate materials



Figure 4. Mixed substrate (sugarcane bagasse : crushed maize seed:lime=8:1:1)



Figure 5. Bagging

To prepare substrate for oyster mushroom, we mix 80% of sugarcane bagasse, 10% of lime, and 10% of crushed maize seed thoroughly (Fig. 3). Then, tap water is added to the dry mixture to keep the water content 60% (Fig. 4). The sugarcane bagasse is slightly acidic, so lime is added to adjust the pH. And crushed maize is a supplement to provide nitrogen source. The prepared substrate is filled into polypropylene bags (Fig. 5, 6) and the open end of the bag is tied. The size and weight vary ranging from 0.75 to 3kg according to growers. We have experimented with 25kg bags.



Figure 6. Weighing bags

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Pasteurization and Inoculation

The bags are pasteurized at 60-70℃ for three hours by steam in pasteurizer (Fig. 7). The prepared bags are placed in three layers of the pasteurizer (Fig. 8). Enough space is left between bags so that steam can circulate and heat the bags evenly. Water is poured into the bottom of pasteurizer and boiled for 3 hours to keep the inside temperature of pasteurizer at 60-70℃. A mercury thermometer is used to monitor the temperature inside pasteurizer. The pasteurizer should be airtight to prevent any loss of steam and hence avoid a drop in temperature. It can be operated by electricity, gas or wood burning. As the temperature inside the pasteurizer rises to 70℃, it should be maintained for 3 hours to make substrate pasteurized. When pasteurization is done, the bags are cooled down in room temperature. Then, oyster mushroom spawn grown on maize seed is inoculated into the bags and PVC pipe of 53mm in diameter is placed in the open end of the bag and plugged by a piece of foam. The spawning rate reaches 2.5% of the wet weight of substrate.



Figure 7. Pasteurizer (outside)



Figure 8. Pasteurizer (inside)

Incubation and Fruiting

After inoculation, the bags are incubated in a dark room for 3-4 weeks. After the substrate bags are fully colonized by mycelium, the foam is removed and PVC pipe is replaced by PVC ring. To induce fruiting, the bags are watered 2-3 times daily at the opened ends, which should always remain moist.

5-10 days later, pinheads of oyster mushroom appear and grow into mature fruit bodies in 3-5 days (Fig. 9, 10). The mushrooms are harvested when the caps are flat and the gills are open (Fig. 11). Frills are observed at the edges of mushroom cap. The whole cluster should be harvested at one time. Mushrooms are packed for domestic and overseas market (Fig. 12).





Figure 9, 10. Oyster mushrooms growing from sugarcane bagasse substrate

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Figure 11. Harvested oyster mushrooms



Figure 12. Ready for delivery and export

After harvest, remnants of fruit bodies on substrate should be removed from the bags to prevent contamination. Watering is done to induce second flush. Second flush can be harvested in 2 weeks. We usually harvest three flushes from each bag and the yield is approximately one quarter of the dry weight of substrate used in bag. We harvest 250g of oyster mushroom from 1000g of dry substrate (bagasse 800g + lime 100g + maize seed 100g), so biological efficiency is 25%. After completion of the final harvest, the spent substrate can be used as compost that can eventually be returned to the field.



Figure 13. Shiitake cultivated on sugarcane bagasse bagasse



Figure 14. ***Auricularia*** cultivated on sugarcane

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

RUBBER TREE SAWDUST

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Rubber tree (*Hevea brasiliensis* (Willd ex. A. Juss.) Muell.- Arn.) is an industrial tree cultivated in some parts of the Highland and South-East Provinces of Vietnam such as Daklak, Gialai, Kontum, and Dongnai. Every year, a large number of rubber-exhausted trees are cut for new planting and for producing furniture from rubber wood. This results in a lot of sawdust waste that may cause serious environmental problems. For many years, that sawdust was either burned or naturally discharged, and now it is recognized to be very suitable for mushroom cultivation in Vietnam. Many mushroom species, such as *Pleurotus* spp., *Auricula* spp., *Lentinula* spp. and *Ganoderma* spp., are popularly cultivated on rubber tree sawdust. In this article we are going to deal with special methods for preparing rubber tree sawdust as a substrate material for oyster mushroom (*Pleurotus* spp.) cultivation.

The Rubber Tree Sawdust

Rubber tree sawdust has a uniform size structure so it is not only suitable for utilization in plastic bag cultivation, but the structure also facilitates enrichment of the substrate. In addition, the levels of nutritional elements in rubber tree sawdust are a little bit higher than those of mixed broad-leaf tree sawdust.

Table 1. Comparison of rubber tree sawdust and mixed broadleaf tree sawdust

Elements	Rubber tree Sawdust (%)	Mixed broad-leaf tree Sawdust (%)
N	1.68-0.20	1.27-0.20
P	0.48-0.04	0.43-0.06
K	1.18-0.05	0.77-0.05
Ca	0.12-0.03	0.23-0.06
Mg	0.04-0.01	0.03-0.01

(Source : *Studies on Biotransformation abilities of oyster mushroom* - Master thesis by Tran Huu Do, 1999)

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Table 2. Comparison of rubber tree sawdust and mixed broadleaf tree sawdust

Elements	Rubber tree Sawdust (ppm)	Mixed broad-leaf tree Sawdust (ppm)	Error (%)
As	0.03	0.22	5
Cd	0.05	< 0.14	-
Cs	1.1	1.66	10
Cu	23.83	13.29	5
Fe	113.76	167.85	5
Hg	0.01	0.07	25
Mn	31.26	41.13	5
Pb	2.08	1.75	10
V	0.22	0.16	10
Zn	31.28	28.79	3

(Source : *Studies on Biotransformation abilities of oyster mushroom* - Master thesisby Tran Huu Do, 1999)

Substrate Formulation

Although the nutrients of the substrate always affect mushroom production, we still propose three substrate formulae for growing oyster mushrooms in Vietnam. Growers may choose the most convenient and appropriate formulae for each specific case. For example, the substrate formula number one is not recommended for any poorly equipped mushroom farm because of its high risk of contamination and formula number three is used only for fresh sawdust.

Formula 1 (T.H. Do, 1999)		**Formula 2 (L. T. Chau, *et al.*, 2003)**		**Formula 3 (L.D. Thang, 1993)**
sawdust :	75%	sawdust :	85%	Sawdust : 99%
rice bran :	10%	rice bran :	10%	Lime : 1%
corn bran :	5%	lime :	1%	
lime :	2%	ammonium sulphate :	0.5%	(Only used for fresh sawdust)
peanut waste	5%	sugar :	1%	
super phosphate :	1%	gypsum :	2%	
ammonium sulphate :	0.5%			
magnesium sulphate :	0.05%			

Treatment of Substrate



Figure 1. Sifting of sawdust



Figure 2. Rubber tree sawdust mixed with additives

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Figure 3. Bags after bagging

The sawdust must be pre-wetted for 2-3 days in advance, and then shavings and pieces of wood that are too big or too sharp are removed by a sift (Fig. 1), because these pieces absorb water poorly and easily pierce plastic bags during handling. Rice and corn bran and peanut waste are supplemented as organic nitrogen sources while urea and ammonium sulphate as inorganic nitrogen sources. Lime should be diluted with water, and then showered onto the sawdust to adjust pH. Mix the components carefully and adjust the moisture content to 60-65% and the pH to 5.5-6.5 before filling the bags (Fig. 2).

Many kinds of plastic bags are used in Vietnam, and growers can choose the sizes of plastic bags according to the hygienic conditions and equipment of their mushroom farms.

After filling, the bags are closed by putting on a ring made of plastic or thick paper forming a 'mouth' (Fig. 3) inside which a cotton plug is put as a stopper, and the whole ring is then wrapped with a piece of old newspaper.

Sterilization

Sterilization of substrate bags is done under high pressure and high temperature using an autoclave, usually at 1 atm, 121℃, for 60-90 minutes, depending on the volume of the bags.

Normal pressure sterilization is done in 95℃ for 5 hours in a drum (Fig. 4). As an alternative method for sterilization, bags can be sterilized twice at 95℃, for 3 hours each time, with a 24-hour interval between treatments.



Figure 4. Drum for normal pressure sterilization



Figure 5. Bags after sterilization

Inoculation

Spawning is conducted in a clean room and some inoculation rooms are made of a plastic sheet in Vietnam. Growers buy mushroom spawn from the Institute of Biology or the Agriculture Center near their mushroom farms. Spawn is inoculated into substrate bags manually after disinfecting the gloves and bottlenecks with an alcohol flame. Spawn in 750g bottle is inoculated to 70-80 bags of 1kg each, so spawning rate reaches 1% of wet weight of substrate.

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Incubation

Inoculated bags are hung by chains in a dark room at 25-30℃ for incubation. Incubation periods vary according to the species: 20-25 days for *Pleurotus sajor- caju*, *Pleurotus pulmonarius*, and *Pleurotus ostreatus*, 30-40 days for *Pleurotus cystidiosus*, and 50-55 days for *Pleurotus eryngii*.

Fruiting

When the mycelium has covered the substrate completely, the bags are transported to the fruiting room, and then some slits are made around the plastic bags to accommodate fruit body formation. All the windows of the growing room are open for ventilation and light, and humidity is maintained by spraying water 3-4 times per day. In most developing countries including Vietnam, growing rooms don't have ventilation systems or temperature controls, so cultivation is absolutely dependent on natural conditions. Therefore, the temperature and humidity of a growing area should be thoroughly examined when choosing suitable species or strains.





Figure 6, 7. ***Pleurotus pulmonarius*** growing from rubber tree sawdust bags



Figure 8. ***Pleurotus abalonus***



Figure 9. ***Pleurotus cystidiosus***



Figure 10. Harvested oyster mushrooms

Harvest

In Vietnam, 4-5 flushes of oyster mushroom are harvested. The yield of *Pleurotus eryngii* is around 23-30% the weight of the dried substrate while the yield of *Pleurotus pulmonarius*, *Pleurotus sajor-caju* and *Pleurotus ostreatus* are higher, at about 40-50% of the weight of the dried substrate.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

GROUNDNUT SHELLS

Emilia Masenda
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Though the cultivation of oyster mushrooms by smallholder farmers has only recently been introduced in Zimbabwe, it has become a very popular activity among many farmers. It has also caused direct competition for substrate material with the livestock industry. Zimbabwe citizens raise cattle and goats in many areas. During the dry season from May to November the cattle graze on dry veld grass and their diets are supplemented with various agricultural wastes including primarily maize and groundnut stover. Millet straw is also used for this purpose as is any other cereals that are grown. Farmers value their livestock more than the mushrooms so agricultural wastes are usually given to livestock.

Although groundnut shells (Fig. 2) are sometimes used for stock feed, they are not as popular as cereal straws and legume stovers. Therefore, they can be used as substrate for growing oyster mushrooms without much competition from the livestock industry. In some towns and centers, large quantities of groundnut shells can be obtained very cheaply from the companies that are involved in shelling groundnuts.



Figure 1. Groundnut



Figure 2. Groundnut shell

Nutritional Composition

According to analysis by the Animal Science department, groundnuts shells contain an average of 68% organic matter, 6.8% crude protein, 18.2% crude fiber, and 7.1% ash. Another nutritional composition analysis of

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groundnut shells indicates that the shells contain 65.7% cellulose, 21.2% carbohydrates, 7.3% protein, 4.5% minerals and 1.2% lipids. Since the processed shells from shelling machines contain bits and skins of nuts, the actual protein and lipid contents of this waste material are probably much higher.

Substrate Preparation

Crushing shells

Shells from machine-shelled groundnuts do not need to be crushed further but those from hand-shelled nuts should be ground in a mill or by hand mortar. Before crushing, growers should pick out rotten or blackened shells.

Washing shells

Shells have to be washed to remove soil. Two methods are used for washing the shells. In the first method, the shells are placed on a plastic sheet and water is poured over them with a hose while they are being turned (Fig. 3). Then, the soil moves to the bottom and is carried away by the run-off water. In the second method, the shells are put in a plastic container and water is poured over them. The soil will fall to the bottom after agitation (Fig. 4). When the shells are clean, they are placed on a plastic sheet and left overnight to absorb moisture.



Figure 3. Washing with water



Fighre 4. Removing soil by agitation



Figure 5. Deeper color of groundnut shell after soaking

The substrate starts heating up and turns a deeper color after soaking (Fig. 5). Growers should observe the pile carefully for soil which might remain stuck on the shells even after washing. Growers should never use moldy shells and should collect shells before rainfalls and store them in a dry place.

Addition of supplements

The use of supplements is optional, but wheat bran (1%) and cottonseed hulls are added as supplements in the ratio 1 : 4 to make substrate more compact and increase yield. With cottonseed hulls supplemented, biological efficiency can be increased up to 200%. In winter, cottonseed hulls have additional advantage of keeping the bags warm and accelerate spawn run.

However, the bags can be overheated by cottonseed hulls in warm season, so addition of cottonseed hull is not recommended in those seasons. Lime (1%) and gypsum (3%) are also added. Lime is added to improve and maintain a favorable pH. Rapid drop of pH has been experienced after substrate fermentation if no lime is added. A high pH also discourages competitor molds. Gypsum is added to prevent stickiness and absorb excess moisture. Lime and gypsum can be added at spawning to avoid them being lost during steaming.

Heat Treatment of Substrate

The substrate is steamed using a drum for 2 hours and the temperature is maintained at 70 ℃ by controlling the

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flow of steam. If the groundnut shells were not very clean, the time of steaming can be prolonged to 4-6 hours. Good indicators of proper steaming are the nut shells that become tender and cooked by the steam. If steaming time is too short, the substrate quickly develops contaminants after spawning. Another popular heat treatment method is to heat the substrate using the sun. This method is commonly used in some regions where firewood is very scarce and expensive. Some local white button mushroom growers also use this method to heat-treat their composts. The substrate is pre-wetted and then wrapped tightly with black plastic sheets and left in the sun for 4-6 hours. Condensation on the plastic keeps the substrate from drying and so does turning the bundle from time to time. After heat treatment, the substrate should retain 70% of its moisture.

Spawning

After the substrate cools down, it is packed into black plastic bags weighing 5kg each and spawned simultaneously. 100g of spawn is inoculated to 5kg bag, so spawning rate is 2% of wet weight of substrate. Roughly crushed shells should be tightly packed to avoid air spaces. Growers should make small holes in the bags using a pen through which fermentation gases can escape. The bags are then left for incubation in a dark room. It is recommended not to place the bags too closely together because that can cause overheating, and as a result, contamination.

Fruiting and Yield

When spawn run is complete, the bags are hung in the fruiting room and holes are made on the bags. Humidity in the room is kept at 85-90% by watering the floor and periodic spraying of water in the air with a hand spray. Usually 6-8 flushes are harvested.

When growing *P. salmoneo-straminieus*, 1kg of fresh mushrooms are harvested from a bag (5kg with 70% of water content) on average. In calculating biological efficiency (B.E. = fresh weight of mushroom / dry weight of substrate x 100%), the biological efficiency of this method is 67% (1kg / 1.5kg x 100% = 67%). With *P. pulmonarius*, B.E. is 120% and with *P. ostreatus* it is 150%. When the substrate is supplemented with cottonseed hulls, biological efficiency for *P. ostreatus* is on average 180%.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

NON-STERILIZED WHEAT STRAW

Ahklaq Khan
Pakistan

Oyster mushroom growing requires several essential steps including pasteurization or sterilization. Pasteurization or sterilization is often the most expensive step because it requires fuel-consumption for steaming or boiling. Though this is important for obtaining high yields, it is a very tricky and labor-intensive process. If mushrooms could be cultivated without pasteurization or sterilization, the whole process would be much easier, faster, and less expensive. I have used natural sources such as lime stone and pulse powder to successfully dispense with the pasteurization or sterilization steps.

I have been made many experiments with growing oyster mushrooms on different kinds of substrate materials such as cotton waste, rice straw, sawdust with poultry manure, dried grass, waste cloth, oak and others. Nevertheless, all the materials required sterilization at high temperature. Without sterilization high yields couldn't be produced. For example, cotton waste is one of the best substrate materials, but it requires a long time for sterilization. However, wheat straw showed great results (almost 100% success) without pasteurization or sterilization. Moreover, it is very simple to grow oyster mushroom in large quantities.

Since I have no idea about the scientific analysis of wheat straw itself, I cannot explain why wheat straw is so appropriate for oyster mushroom cultivation. However, wheat straw was the best of all the non-sterilized substrates in my experiments. I would like to share my experience with you step by step.

Subject matter : Growth of oyster mushrooms using wheat straw by adding the cheapest possible material such as lime stone ($CaCO_3$, Calcium Carbonate) and yellow pulse in ground powder form.

Ingredients and material

Wheat Straw (Chopped): 40kg
Lime Stone ($CaCO_3$): 20kg
Pulse Powder: 1kg
Wheat Bran or Rice Bran: 4kg
Water: 100L + more
Polythene Sheet (If necessary)
Bricks (for grounds shelving)

Note: The above ratio is for the example showing a simple production. Increase materials in the same ratio, if necessary.

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Step 1

Pour water into a large container or drum and add 40kg of wheat straw and leave until the wheat straw is soaked (Fig. 2, 3). Fill 100L of water into another container (Fig. 1) and add 5kg of lime stone to another container and let it dissolve (Fig. 4, 5, 6). The dissolving lime emits heat and gases. Add the soaked wheat straw to this solution (Fig. 8, 9). Lime stone acts as an anti bacterial agent and kills all the viruses harmful to the initial growth of the mycelium. It also lowers the acidity of the wheat straw which is not good for the growth of the mycelium.



Figure 1. Fill two containers with 100L water respectively



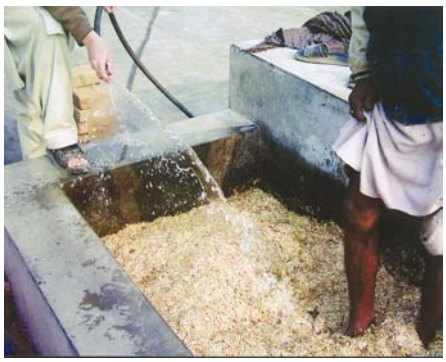


Figure 2, 3. Pour the wheat straw on one container and soak it







Figure 4, 5, 6. Add lime stones to the other container and they dissolve emitting heat and gas



Figure 7. Reaction to water added





Figure 8, 9. Add soaked wheat straw to the lime stone solution

Step 2

Take the soaked wheat straw out of the water (Fig. 10) to mix with 4kg of wheat bran or rice bran (Fig. 11). Mix them thoroughly on a clean floor with a polythene sheet on it (Fig. 12).

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Figure 10. Take the straw out of the water





Figure 11, 12. Add 4kg of rice or wheat bran and mix them thoroughly

Step 3

Make a closed tray pattern on the clean floor with bricks in a circular shape. Spread the remaining 10kg of dry lime stone inside the circle of bricks (Fig. 13). Pour the soaked wheat straw mixed with wheat bran on the lime stone layer (Fig. 14, 15). Pour in water until the base gets wet to speed up the chemical reaction of the dry lime stone (Fig. 16). By this chemical reaction a lot of heat and gases are produced which are helpful in pasteurizing the material.



Figure 13. Spread lime stone



Figure 14. Take the mixture of wheat straw and rice bran





Figure 15, 16. Pour the mixture inside the brick circle and pour water

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Step 4

Quickly cover the substrate with a plastic sheet to keep in the heat and humidity for 24 hours (Fig17, 18). The substrate should be covered no longer than 24 hours. The substrate is ready for spawning the next day. You can save energy costs by using this step.




Figure 17, 18. Cover the substrate with a plastic sheet to keep the heat, gas and humidity

Step 5

After 24 hours, move the substrate to a shelf, tray, or bags for spawning. In my case, a brick shelf was used inside an available growing room (Fig. 19). Spread the ready substrate on the shelf bricks equally (Fig. 20) and pour on more water. Sprinkle the spawn on the substrate evenly (Fig. 21). The last ingredient to add is yellow pulse powder (Fig. 23). This powder is also equally spread on the shelf over the spawned substrate (Fig. 24).



Figure 19. Make shelf bricks on floor




Figure 20, 21. Pour the ready substrate and sprinkle spawn on the substrate



Figure 22. Yellow pulse



Figure 23. Yellow pulse powder



Figure 24. Spread yellow pulse powder on the substrate

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Step 6

Cover the all the shelves tightly with a plastic sheet in order that no air can enter and so the required humidity level is maintained (Fig25, 26).




Figure 25, 26. Cover the shelf with a plastic sheet

Step 7

After 7-8 days, the mycelium starts growing and spreads all over the shelf. During mycelial growth, keep the temperature of the room at 10-15℃ for the best results and to provide the most favorable environmental conditions.

Step 8

About 50 days later, the whole shelf will be colonized with a white milky color by the mycelium. The wheat straw mixture is no longer seen. Remove the polythene sheet from the top of the substrate and induce pinning.

Step 9

It is time to start spraying water 3-4 times each day on the substrate. Maintain proper ventilation to control the flow of air because mushrooms are more nourished and grow better with air that contains good oxygen content. If the amount of carbon dioxide increases in the room, the size of the mushrooms starts decreasing and yields lessen.

Step 10

In 8 -10 days, the mushrooms will appear on the shelf. This is a delicate stage for the growth. Continue water spraying daily. Make sure to spray water to keep the humidity at 90-100% and the temperature at 15-20℃. The mushrooms will be ready to be picked after 2-3 days. You can harvest mushrooms for up to 3-4 months continuously. It can be extended to 1-2 years if good climatic conditions are maintained. Mushrooms are harvested whenever they are fully grown. Usually 1-2kg of mushroom is picked from 1kg bag.

The above steps have proven to give the best results on an even larger production scale with a healthy and fruitful harvest.

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Figure 27, 28. Oyster mushroom grown on non-sterilized substrate in bag cultivation.

Foot note

The experiences of the editor Jozef Poppe have shown since 40 years the proof that a pasteurization of wheat straw during 2 minutes between 65 and 70 degrees Celcium gives a perfect incubation in 3 weeks and a fast harvest 3 weeks later.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 6

Growing Houses

MUSHROOM GROWING HOUSES

Hyunjong Kwon, Seung Woo Kang, Song Baek Cho
MushWorld

In the early days of mushroom growing and in most parts of the world, mushrooms were grown outdoors. Early growers depended on natural environment for the control of mushroom growing conditions. Growers today in the regions with favorable conditions for mushroom growing still grow their mushrooms as if they were wild mushrooms growing in natural situations. But commercial mushroom growers or those who want to produce as many mushrooms as they cannot rely on the natural environment. Most modern mushroom farmers build mushroom growing houses, simple temporary structures, or retrofit an existing structure for mushroom production. Providing good conditions for mushroom growing can lead to a higher yield of mushrooms. A farmer who plans to build a mushroom growing house will have to consider where, with what and how to build it. These topics will be discussed in the following sections.



Figure 1. Outdoor straw mushroom cultivation house
(Photo courtesy of Tricita H. Quimio)



Figure 2. A warehouse-turned mushroom growing-

Where to Build - Site Selection

The determination of where to build a house is important, especially when building a simple, makeshift structure where the crop yields will be dependant on the environmental conditions of the growing site. The major factors that should be considered when selecting a mushroom production site are described below.

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Climate conditions

Although the optimal growing conditions for different mushroom strains vary, mushroom growth is generally favored by warm and humid conditions. In temperate regions, growers who want all-year-round production need to build their growing houses in places that are warmer, sunnier and less windy in the winter. Their counterparts in subtropical regions, however, might want to grow their mushrooms in highland areas that are cooler than other lowland areas during summer. Where environmental conditions are unfavorable for mushroom growth, one could insulate or equip the structure or house with appropriate structures in order to minimize the influences of outside conditions on the microclimate inside the growing room. Polyethylene sheet is one of the most commonly used materials and is often applied to maintain the proper temperature and humidity in the growing house and to shelter thatch houses or makeshift structures from such adverse conditions as heavy rain and strong winds.



Figure 3. Thatch mushroom growing house covered by a plastic sheet, with the floor lined with plastic sheet



Figure 4. Thatch mushroom growing house draped by shade cloth and surrounded by a stone fence



Figure 5. SIP (Structural insulated Panel) house

Some large-scale commercial growers build well-insulated growing houses, in which all the room conditions including temperature, humidity and CO_2 concentration are controlled automatically. By selecting an ideal mushroom growing site, growers with these types of growing houses can also minimize the significant costs for maintaining desired room conditions.

Access to water

It is widely known that mushrooms are 90% water and are best grown at high humidities around 80-90% R.H. (relative humidity). Growing mushroom requires a great deal of water and ensuring a sustainable water supply, especially during dry spell, is crucial to successful mushroom growing. Growers need a large amount of water when they prepare substrate pasteurize or sterilize the mixed substrate, water the floor to maintain the constant high humidity, water the mushroom bags, or clean the rooms.

Groundwater is widely used, especially for cooling and moistening the room air and for cleaning the room. Selecting a site with a secure access to water source is a must for sustainable mushroom production.

Environmental integrity

Most farmers grow their mushrooms organically, which is one of the main reasons for the increasing popularity of mushrooms. Air-borne pollutants and chemicals could be detrimental to the organic production of mushrooms and the health of farmers as well. Locations near industrial complexes, waste incineration facilities, or sewage treatment plants should be avoided.

Proximity to markets

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Mushrooms are highly perishable produce. The price of mushrooms depends on their quality, especially their freshness. Once mushrooms lose their freshness, their marketability and price will drop drastically. To earn the most money available from selling mushrooms, growers need to shorten the time from growing room to store shelf. Selecting the growing site which is not far from mushroom markets is quite helpful to getting more money by selling mushrooms as fresh as possible and reducing the transportation costs. They are also advised to locate potential customers and make contact with supermarket produce buyers, restaurant supply persons and produce wholesalers well before their mushrooms are harvested.

With What and How to Build - Examples

Once the growing site is determined, growers have to consider construction materials and methods with which to build their growing houses. Usual construction materials are those easily available to growers, such as wood poles, steel pipes, bricks, plastic, blankets, leaves, straw, thatch and hay. Some growers can build simple houses with readily available materials. When and where environmental conditions are within acceptable temperature and humidity ranges, a simple, open-style structure built of any available materials will do its job well enough. Other growers will need to construct a closed-style growing house in which room conditions are less affected by outdoor weather conditions. The following examples, ranging from simple to sophisticated, will provide basic ideas about good growing houses and structures.



Figure 6. Steel frame structure with a roof

Simple structures with a roof

The structure shown in Figure 6 is one of the simplest structures for mushroom growing. The open system and the pitched-roof permit good ventilation. However, the control of humidity in this system is not easy when the weather is too dry or too wet. The system requires more water than other closed-style structure because of its greater evaporational water loss.



Figure 7. Steel structural tubing covered with tarpaulin

Simple structures draped by a proper covering

These draped structures are more insulated from outside weather conditions, but are still simple structures. The proper covering provides a good insulation and a high humidity holding capacity for the structure but growers will need to pay a close attention to temperature, ventilation - removal of excess carbon dioxide and supply of oxygen.

Thatch houses



Figure 8. Bamboo woven matting



Figure 9. Bamboo pole rafter and



Figure 10. Thatch house with

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leaf-woven roof shade cloth



Figure 11. Thatch house with insect screening



Figure 12. Rodent/snake barrier in the bottom part
(Photo courtesy of Audrey R.S Mabveni)



Figure 13. Rodent repellent

Thatch houses are the most widely found simple growing houses. Straw, leaves and wood poles are readily available and serve as good construction materials. They are air permeable, thermo-insulating, lightweight and highly pliable. Possible shortcomings, including the easy entry of contaminants, can be improved with proper application and usage of sheltering, insulation, screening, barriers and other materials that could be readily placed on the house.

Pests, diseases and other possible contaminants entering from outside can deteriorate mushroom quality, which translates into a significant drop in income. Some insects spread mushroom diseases. Other pests including snails and rats and their predators like snakes gnaw and eat away mushrooms, substrate bags and even growing houses. Installing proper physical and chemical protective barriers is recommended for these thatch houses. Protective barriers include stone fences (Fig. 4), screening (Fig. 11), plastic sheeting (Fig. 12) and rodent repellent (Fig. 13).

Brick and clay houses



Figure 14. Brick house with a thatch roof and a vent house
(Photo courtesy of Audrey)



Figure 15. Kitchen-turned mushroom growing

(Photo courtesy of Audrey R.S. Mabveni)

Thatch wears out within a few years. Once it begins to leak, the thatch structure should be renewed. Commercial scale growers might want a more durable growing house that is suitable for all seasons. Clay and earthen bricks are good choices that allow for good insulation, ventilation and prevention from pests. Depending upon material availability and their preferences, growers can choose from a variety of materials for the roof material of their growing house. They are well advised to make ventilation openings in order to ensure frequent air-exchanges.

Greenhouses sheathed in insulation and SIP (Structural Insulated Panel) houses

Sustainable production and a constant supply of harvestable mushrooms are important to successful marketing that can provide growers with a sustainable income. In some parts of the world with adverse climate conditions and

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varying seasons, well or totally insulated growing houses are needed for all-season production. Growers in these regions invest a considerable amount of money to set up their growing houses and provide an ideal microclimate for the growth of mushrooms to produce the highest yields possible.

In these closed-style growing houses (Fig. 5, 16, 17), growers need to monitor and control temperature, humidity and CO2 concentration at all times. They partly or fully depend on sensors and controllers for the growing room control. These sheathed houses are durable. Simple insulation houses (Fig. 16, 17) last 5-7 years and the SIP houses (Fig. 5) can last for more than 15 years.





Figure 16, 17. Green house clad with insulation (glass wool) and roof vents

Why to Build - Functions of a Mushroom Growing House

The major value of a mushroom growing house is to provide favorable conditions for mushrooms and protect them from adverse environmental factors such as harsh weather, pests, pathogens and pollutants. Good mushroom growing houses perform these tasks effectively. Growers might want to have a mushroom growing house well-insulated and at the same time, well-ventilated. Insulation materials mentioned above such as polyethylene, tarpaulin, shade cloth, thatch, clay, glass wool and SIP (Structural Insulated Panel) will work well to provide temperature and humidity control. For easy ventilation however, a pitched-roof and ventilation openings are recommended, especially in closed-style growing houses, as are shown in most figures in this discussion. A pitched-roof requires more money, time and higher technology to set up but provides better drainage, ventilation and temperature control.

Protection against pests and pollutants is also one of the major roles of a mushroom growing house. Among the materials mentioned above, thatch is not good for protection against them. A thatch house should be lined or covered with screening (Fig. 11), plastic or other comparable covering, and surrounded by some barriers or a fence (Fig. 4, 11, 12). Growing houses made of the other materials are free from pests and pollution but some screenings or filters should be installed on the vents to further block access.



Figure 18. EPS (Expanded Polystyrene) growing house with polyethylene insulation and a bottom barrier along the ground (Photo courtesy of Tricita H. Quimio)

The high-tech, insulated panels are an effective means of preventing possible pathogens from entering the room. But fungal and bacterial pathogens can come from the ground. Paving (Fig. 2, 5, 6, 7, 14) or the application of gravels or plastic sheeting (Fig. 3) on the floor is highly recommended for disease prevention, especially where the ground is muddy. Mud on the footwear is a potential

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contamination source in mushroom growing houses.

An ideal mushroom growing house does not necessarily need to be a high-tech, high-cost structure with all automatic controls. Some growers ruin their crops even in these state-of- the-art growing houses and other growers reap a rich harvest in humble sheds or garages. The most important consideration is keeping an eye on preventing possible pests and pathogens and understanding the relation between temperature, humidity and air-exchanges. Room conditions including temperature, humidity and air movement are correlated. In a closed environment, when the room temperature rises, relative humidity of air falls. When outside air flows in, temperature and relative humidity of the room air change according to the condition of the outside air. Further, close observation of different growing houses and different practices for room condition control could be helpful to mushroom growers who seek to create optimal growing room conditions for their own operations.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 7

Cultivation Modes

LOG CULTIVATION

In the Temperate Regions

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MushWorld

Depending on the length of logs, there are two basic methods for log cultivation of oyster mushrooms. In the long log method, growers cut trees into one-meter long sections and drill a series of inoculation holes in the logs into which spawn is inoculated. This technique is somewhat similar to traditional shiitake log cultivation. In the short log method, growers cut the tree trunks into pieces 20cm in length and inoculate those sections. Though the short log method is more labor intensive, it shows a relatively higher production than long log cultivation.

For that reason, we will narrow down the topic and discuss only the short log method in this article.

Log Preparation

Species

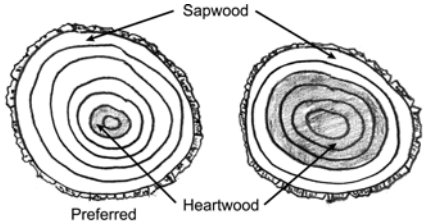


Figure 1. Sapwood/heartwood ratio in wood



Figure 2. Tree felling in winter



Figure 3. Fallen tree is cut to size

One can see wild oyster mushrooms after a rainfall on the dead broad-leaf trees (hardwoods) such as poplars. This indicates that those tree species are suitable for oyster mushroom cultivation. On the other hand, most needle-leaf trees (softwoods) contain phenolic resin compounds and show low productivity when used for oyster mushroom cultivation. Sawdust from conifers can be used after the phenolic compounds are gone. Hardwoods such as poplar, willow, beech, elm and alder are the most commonly used tree species. Unlike shiitakes, oyster mushrooms do not grow well on oak tree logs.

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Since mushrooms feed primarily on sapwood, any tree trunks selected for inoculation should have a large sapwood area. The lighter or outermost wood of a log is the sapwood and the darker or inner wood is the heartwood. A log with a small amount of sapwood will probably produce mushrooms for fewer years than another log with a greater amount of sapwood.

Tree felling

Logs should be felled during the dormant season when the bark adheres tightly and the tree trunks are full of sap and nutrients. A bit later the season, these nutrients are likely to have been consumed during the germination of the buds.

When recently cut logs are used, living wood cells interfere with mycelial growth. When too old logs are used, older and drier logs with less water content also retard mycelial growth. In order to prevent water loss and contamination, felled trees should remain uncut in the shade for several days before inoculation. In order to avoid contamination from the ground, cut trees should not contact the ground during storage. Experienced growers cut their logs into 20cm long pieces a few days before inoculation to allow the logs to achieve the proper log moisture at inoculation. As fungi secrete digestive enzymes and absorb dissolved nutrition, mycelial growth requires the proper amount of water content (38-42%) in logs for the smooth transfer of enzymes and nutrition. In practice, when the logs have a coin-thick crack in the cut section, one can assume that the logs have the proper water content. Logs of 15-20cm diameter are an ideal size for handling, therefore efficient.

Spawn Preparation

Sawdust spawn is the usual inoculation medium for oyster mushroom log cultivation. Growers employ low-temperature strains that form bunches easily and produce high yields. High-temperature strains make fast flushes and produce high yield, but are not fit for the production of high quality fruitbodies.

It takes at least 1-2 months for spawn suppliers to make spawn from a mother culture after they receive an order. Therefore, growers should order their spawn from a reliable dealer sufficiently ahead of schedule before the spawning. Growers should give the spawn dealer their desired shipping date so their spawn will be as fresh as possible.

Inoculation

Inoculation season begins when the temperature outside is close to the best growing temperature for mycelia. The mycelia of oyster mushroom are viable at a wide temperature range, and can survive from 5 to 35℃, but they grow best at 25-27 ℃. Beyond the proper temperature range, the mycelia lose vitality and may die. Therefore, the right time for inoculation is when the lowest temperature is 5 ℃ and the highest is 20℃ in spring.

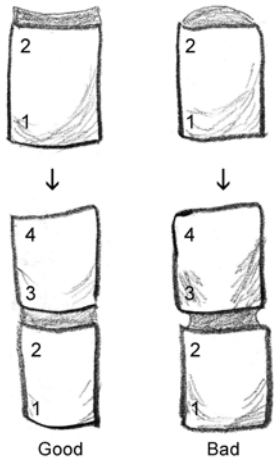


Figure 4. Inoculation of the logs

Method

Growers can either inoculate with original spawn only, or original spawn with supplements that increase the quantity of total spawn. With the supplement, growers can use less original spawn, but when it is hot, contamination may increase with poor management because the supplement usually contains much nutrients. To begin with, growers should sort the logs by width. Next, they should make the inoculum by mixing 20-50% spawn with the supplement, which is composed of sawdust and rice bran at a ratio of 4:1 on a volume basis with a moisture content of 60-65%. Too much

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supplement can increase contamination. Next, they should apply the inoculum 5-10mm thick to the log ends with more on the margin of each log (Fig. 4). And last, they should stack five or six logs on top of another like a sandwich. Stacking logs in the order of cutting minimizes the contamination risk by reducing the exposure of spawn to contaminants (Fig. 5). In order to prevent damage by respiration heat, growers should arrange the stacked logs in three or four rows and make walking aisles between the rows.

Incubation

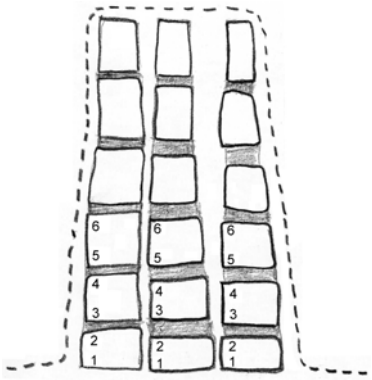


Figure 5. Incubation of the inoculated logs

Inoculated logs should be covered with plastic and the mushroom mycelia allowed to colonize the logs in a shady moist area. When it is dry, growers should water the surrounding ground. It takes 3-4 days for mycelia to recover and begin regrowth. Early in the incubation, growers should keep the room temperature as steady as possible, somewhere between 15 and 20℃ being best. A wide variation in temperature will result in a high risk of fungal infestation. When growers notice green mold, they should apply fungicides such as Benlate to the spot. With summer comes, growers may need to use appropriate temperature controls to keep the area around 20℃.

Burying

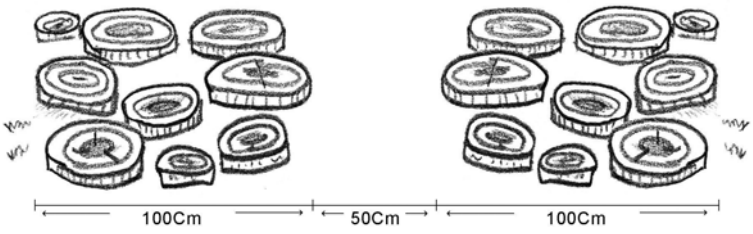


Figure 6. Burying after incubation. The embedded logs will be covered with shade cloth

During the summer, the well-colonized logs will adhere to each other. At this point they are ready to fruit. Growers should move them to the desired place for mushroom fruiting. Growers may grow mushrooms either by standing the logs or by burying the logs. Burying is recommended because sand can prevent drying. Growers should separate each log unit and bury them all vertically in the mound with the inoculated sides up and with about 10-20% of the length above ground level. Each log should be spaced 10-15cm apart on a 1m wide ridge. Growers should direct free water to a 50cm furrow for good drainage (Fig. 6). After burying, growers pitch a tent over the buried logs to prevent direct exposure to the sun and moisture loss. The tent must not disturb watering or harvesting.

Pinning Induction

During mid-autumn when the temperature goes down below 20℃, it is time to induce pinning. The usual pinning stimulation methods for mushroom are from light, cold-shock, soaking or physical impact. About ten days before pinning, growers should apply enough water to promote fruitbody pinning. Fruitbodies appear mainly on the cambium.

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Fruiting and Harvesting

During fruiting, Growers need to water enough to keep the logs and soil moist. They can expect to see mushrooms form on the top surface first around the boundary between bark and wood. They should then remove the covering sheet from the logs and increase irrigation. Growers should stop irrigation 1-2 days before harvest. Fruiting takes nearly 5-6 days. When is the right harvest time? Growers should pick mushrooms when the caps reach 5cm in diameter. They must reap the pleasure of harvest along with clusters of fresh mushrooms from the logs (Fig. 7).



Figure 7. Mature fruitbodies on logs

When they finish the first cycle harvest, they should clean the mushroom remains and maintain the logs in a moist condition. Fifteen days later or so, they may see a second flush. If the outside temperatures go down in winter, they should cover the logs with a plastic sheet. When it gets warm again, they can harvest again.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 7

Cultivation Modes

BAG CULTIVATION

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Bag cultivation is the most commonly used method for mushroom cultivation in many locations around the world. Its advantages are as follows:
1) Much smaller risk of crop failure compared with other cultivation methods
2) Possible inside houses or unused structures
3) Possible with a small initial investment
5) Easy to control pests and diseases
6) Quick return of capital
7) Production is possible all year round

Substrate Preparation

Substrate materials and additives

The substrate is to mushrooms like soil is to plants. Mushroom mycelia grow on the nutritious media, taking from it the nutrients necessary for their growth. Commonly used substrate materials are sawdust, cottonseed hull, cereal straw, corncob, sugar cane bagasse, and other plant fibers with high cellulose contents. One of the merits of mushroom growing is that the agro-wastes used as substrates are very inexpensive and sometimes free. Moreover, oyster mushrooms can be grown on a wider variety of agricultural wastes than any other cultivated mushrooms thanks to their multilateral enzyme system.

At the beginning, growers must decide what kind of materials to use for the substrate. One of the best choices is using the substrate materials that a grower can secure around their neighborhood. A secure supply of readily available materials allows for sustainable mushroom production. The substrate materials are supplemented if necessary with additional nitrogen sources such as wheat bran, rice bran, sorghum, or millet. Other additives include gypsum, limestone, and sugar. Gypsum, limestone, and chalk function as buffers to control the pH value in the substrate. The proper use of additives that supply nutrients is recommended in order to achieve the best quality mushrooms.

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Sawdust



Figure 1. The sawdust ready to mix on the cement floor

There are various trees available in each region that can be used as sources for sawdust. The frequently used tree species for mushroom cultivation include oak, poplar, alder, maple, birch, wild cherry, mango, and elm. It is important to select tree species that are both favorable for the growth of mushrooms and easily available. Tree species that should be avoided or that requiring significant additional treatments before their use include pine, cedar and redwood. Pine tree sawdust has resins that inhibit mycelial growth, and cedar and redwood sawdusts are also resistant to mycelial colonization.

Sawdust is mixed with water and other supplements either outdoors or indoors, either manually or mechanically. Close attention should be paid during mixing to the even distribution of water throughout the entire substrate. The substrate moisture content is usually adjusted to 65%. A palm test method is a simple way to check whether the mixture has the proper water content or not.

=Palm Test Method=



Figure 2. Optimum (left) and high substrate moisture contents

First, take a fistful of sawdust mixture and squeeze tightly. If just a few drops of water are released with pressure, the substrate mixture has the proper water content. If the sawdust is too wet, it could impede the free flow of air in the substrate. Too low a water content prohibits mycelial growth.

[Example 1. The use of sawdust as a substrate material in different countries]

Thailand - Sawdust made from rubber trees is used as a substrate material.
Bangladesh - Mango sawdust mixed with wheat bran in a ratio of 4:1 is used.
America - Oak, poplar, alder, maple, birch, and wild cherry are used as sawdust sources. Wood chips, cereal grain straw, corncob, hay, and sugar cane bagasse are used as well as sawdust. They are supplemented with such nitrogen sources as rice, wheat bran, pelt, and other grains, corn meal, and cottonseed meal at a ratio of 4 to 1. Other additives include sugar, molasses, gypsum, and limestone. All the ingredients of choice are mixed dry, and then water is added to obtain a 60-65% moisture content.
Korea - Sawdust from oak, poplar, and other broad-leaf trees are most commonly used. Some growers opt for

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sawdust-only substrates and others use sawdust substrates supplemented with rice bran or other nitrogen sources. Supplements in powder form are mixed together but some supplements with tough textures need to be soaked for about 12 hours before mixing. Mixing is usually done in the ribbon mixer (Fig. 1).

Straw



Figure 3. Rice straw pile

Straw has long been favored because it is easy to get in most regions and rich in lignin and cellulose. Dirt, pest and mold-free straw should be chosen. Growers might want to secure a sustainable supply of high quality straw from one region, for this will allow for easy preparation with minimum efforts.

To make a substrate, straw is cut into one-two inch-long pieces. Different tools are used to chop the straw in different countries. Straw choppers, shredders, garden chippers and hand straw choppers are used. Extra precaution should be made not to get one's fingers into the blades. Chopped straw is soaked in water for 1-2 hours, then rinsed 2 or 3 times in clean water and left for 3 or 4 hours to drain excess water off.

The "Palm method" is also used to check whether this substrate mixture has the proper moisture content. If the moisture content is too high, the substrate is more vulnerable to infection. If the moisture content is too low, the spawn could grow poorly and the harvest quantity would decline.

[Example 2. The use of straws as a substrate material in different countries]

India - Paddy straw is used as a substrate. It is chopped manually or mechanically into bits of 3-5cm in length.
Nepal - Rice straw is cut into one or two inch pieces and soaked into water for about 1-2 hours. Then the soaked straw is washed 2 or 3 times in clean water and put aside for 3-4 hours to allow excess water to drain. Growers here prefer to supplement the substrate with protein-rich substances such as wheat or rice bran.
Vietnam - Straw is soaked in a container of limewater. The limewater contains 2kg of slacked lime per 100kg of dry straw, with as much water added as is needed to just cover the straw. The straw is left in the limewater for half an hour, so that it is thoroughly soaked. The straw is then piled up on a cement floor and covered with plastic or sacking. The top of the pile is left uncovered. The straw is left to ferment for 7-10 days. During this time, it will begin to ferment and become hot. The straw is turned once every three days, first from the top downwards, then from the bottom upwards, then from the inside outwards and finally from the outside inwards.
S. Africa - The main raw materials used as substrates here are chopped wheat straw and other available agricultural by-products such as sugarcane bagasse. These basic substrate materials are mixed with lime, gypsum and water. Wheat straw is available in large round bales (250kg), large square bales (350kg) and smaller 12kg square bales.
Some growers add organic nitrogen supplements to the substrate in the form of alfalfa meal, soybean meal, canola meal, and commercial delayed-release supplements. However, supplements are used only if sufficient cooling is available to control substrate temperatures.
Zimbabwe - The most common substrate is wheat straw and grass. Banana leaves, although higher yielding and producing higher quality mushrooms, are not usually preferred because they give a delayed break and this substrate is not as abundant as the other two.

Cotton waste or Cottonseed hulls

Cotton wastes are also a good substrate material for mushroom growing. Many growers choose cotton waste due

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to their experiences in which cotton wastes give higher yields than sawdust.



Figure 4. Cottonseed hull

However, using cottonseed hull alone is not desirable due to its low moisture retention capacity. The maximum water holding capacity of cottonseed hulls is about 55-58 %; therefore growers need to mix it with other substrate materials to effect a higher water content in the substrate.

[Example 3. The use of cottonseed hulls as a substrate material in different countries]

Korea - 150kg of cottonseed pellets are mixed with 30kg of beet pulp, 15kg of cottonseed residue, and 2kg of charcoal. 350kg of water is then added.
Zimbabwe - Cottonseed hulls are supplemented with lime and gypsum and then very slightly wetted overnight.

Filling and compressing



Figure 5. Bags filling and compressing



Figure 6. Bag plugging

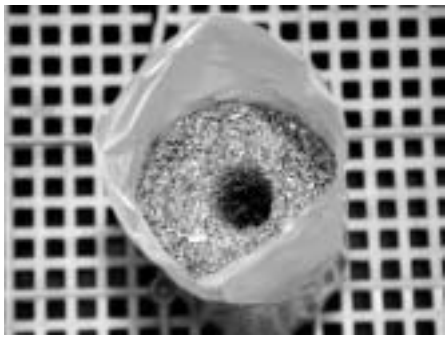


Figure 7. Inoculation hole

The prepared substrate mixture is usually filled into bags before heat-treatment, but some growers pasteurize or sterilize the substrate in bulk and then fill the bags. The first method is recommended to minimize contamination risk. The filled bags are then properly compressed for fast mycelial colonization. After filling and compressing, a 2-3cm diameter hole in the center is made that allows for inoculation down at the bottom of the bag. This permits deeper inoculation and a better oxygen supply, thus encouraging faster colonization. Growers who don't compact the bags don't always need to make an inoculation hole.

Closing the bags



Figure 8. Sealing the bags by rubber band



Figure 9. The plastic cap

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The proper ventilated sealing of the bags is very important to good colonization. Mycelia need oxygen to breathe, therefore breathable plugs or stoppers are recommended for free air exchange. Plugs with cotton balls or breathable micro-filters will provide free air exchange and at the same time filter out possible contaminants.

[Example 4. Bagging in different countries]

Bangladesh - About 500g of mango sawdust is mixed with wheat bran in a ratio of 4 to 1, then watered and sterilized and then put into polypropylene bags measuring 25 × 20cm.

Nepal - Before filling, the polyethylene bags measuring 14 × 24 inches, or 18 × 26 inches are punched every two inches in rows all over the surface with a punching machine. The conditioned straw is filled about 4 inches thick at a time, pressed slightly, and then another layer of straw is added. This is called the "layer method."

Vietnam - Plastic bags measuring 20 × 30cm or 18 × 25cm are used as mushroom beds. Growers open the bags and put a handful of straw inside. They press the straw down tightly, to make a layer 3-5cm thick at the bottom of the bag. They put additional layers in the bags, and then they put a final layer of straw on top in such a way that the top of this final layer is 5-7cm below the mouth of the bag. They put a clean piece of cotton in the mouth of the bag, seal it, and then tie the bags together with a nylon rope (3-5 bags per rope) and hang them.

India - They use a wooden mold to make a bag. It is a wooden frame of 45 × 30 × 15cm size, having no top or bottom but having a separate wooden cover 44 x 29cm in size. They take the wooden frame and place it on a smooth floor, placing jute ropes underneath, two vertically and one horizontally. They then line the frame with a plastic sheet that has been previously sterilized by dipping in boiled water. They fill the frame with about 5cm of boiled straw and compress it with the help of the wooden lid, and then continue to add 4 or 6 layers.

S. Africa - Cropping containers are made of large clear or black polyethylene ducting cut into 2-meter columns (50cm in diameter) with a metal support. These hold approximately 50kg of substrate. Other growers use small plastic bags containing about 20kg of substrate. Holes are punched into the bags for aeration and mushroom maturation.

Heat treatment



Figure 10. Commercial Sterilizer

Substrate needs to be treated before spawning. Heat treatment is most frequently used to kill or reduce pests and microorganisms, and there are two categories: sterilization and pasteurization. Sterilization kills all microorganisms whether they are harmful or not to mycelial growth, while pasteurization reduces the number of microorganisms. The right pasteurization time and temperature depend on the possible pathogens in a given substrate material.

Normal pressure sterilization

Growers put the substrate bags in the autoclave or sterilizer. The desired sterilization temperature is maintained for 5-8 hours at 90-95℃, or 4 hours at 100℃ measured from the time the inside temperature has reached the target temperature. The substrates are then taken out and moved to a cooler room.

High pressure sterilization

Under pressure of 1.5kg/cm^2 or 20 psi (lb per square inch), the inside sterilizer temperature rises up to 121℃. When it reaches 121℃, the temperature is maintained for 60-90 minutes. Upon completion of sterilization, the pressure is released first. After steam comes out, the substrate bags are moved from the sterilizer to a cooler place.

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Table 1. Advantages and disadvantages of high and low pressure sterilization

	Advantages	Disadvantages
Hight pressure	- time saving	- considerable loss in substrate moisture
	- fuel saving	- nutrient loss possible
	- complete sterilization possible	- high set-up cost
Normal	- little loss in substrate moisture	- insufficient sterilization effect
Pressure	- conditioning of nutrients for	- time consuming
	Increased absorption by mycelia	- fuel consuming
	- easy and low-cost to set-up	

Oil drum pasteurization

Oil drums are cheap and easy to get and install in one's yard. They are widely used to pasteurize substrate bags. Usually they are mounted on bricks, rocks or any other heat-resistant material. This drum pasteurizer is an assembly of oil drum(s), metal or wooden grates, lids with steam escape hole(s) and kiln.

Oil drum pasteurization

- The first grate is fit into a notch in the drum (Fig. 13).
- Water is filled about 15cm below the grate.
- The grate surface and the drum wall are lined with linen to avoid bag burning (Fig. 14).
- The prepared bags are stacked in a layer and then on the next grates.
- The lid is placed and the lid rim is sealed.
- Water is boiled and the heating is maintained for 4-6 hours from the time when the vapor starts to rise. The pasteurization time depends on the bag size and substrate material.



Figure 13. Inside the drum sterilizer



Figure 14. Bags ready for sterilization

Outdoor, simple pasteurization

Bag pasteurization in bulk

- Substrate bags in stack and nest containers are placed on the shelf (Fig. 15).
- They are covered with plastic sheet, insulation and tarpaulin (Fig. 16).
- Steam fed into the tent-like structure by a steam boiler is passed through all the containers.

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- After about 10-hour steaming, bags are left to cool to 25℃.



Figure 15. Bags ready to pasteurize



Figure 16. Bags under pasteurization. They are covered with a plastic sheet, insulation and a tarpaulin.



Figure 17. Electric boiler to feed steam into the bags in bulk.
Here is a simply way of bag pasteurization in bulk.

[Examples: substrate heat treatment in different countries]

India - Growers place a gunny bag of straw into the boiling water for 15-25 minutes. They then remove the gunny bag from the drum and let it sit for 8-10 hours to drain off the excess water and allow the straw to cool. Care is taken that the bag is not opened until the time of block making as this would possibly contaminate the boiled straw. The desirable moisture content of the straw can be tested by the "palm method."
Another method of pasteurization of straw is by steaming. This method requires little modification of the drum. Growers punch a small hole in the lid of the drum, and while boiling the straw, seal the lid with a rubber tube. They place a few stones in the drum and pour in water only to the level of the stones. They steam the wetted straw by keeping it in a bamboo basket that is placed over the stones inside the drum. They close the lid of the drum and seal the rim by means of a rubber tube. The steam generated from the boiled water passes through the straw and pasteurizes it. After boiling, they transfer the straw into a previously sterilized gunny bag and leave it sit for eight to ten hours to cool.
Nepal - The wetted substrate is steamed in a 200L drum for 1-2 hours at a temperature of 90℃. The steamed straw is then allowed to cool down. At this time some growers supplement the substrate with a protein-rich substance such as wheat or rice bran.
Vietnam - There is no special pasteurization except the fermentation is performed outdoors.
S. Africa - Substrate preparation varies from grower to grower ranging from hot water treatment to pasteurization, depending on the available equipment. Chopped wheat straw is mixed with lime, gypsum and water in rotatable pasteurization vessels. The substrate is pasteurized with live steam for 2 hours at 70-75℃ and cooled overnight.
Bangladesh Substrate is sterilized at 121℃ for 15 minutes in an autoclave and then cooled at room temperature for 24 hours.

Spawning

Mushroom spawn is a medium that carries mushroom mycelia. Most growers use spawn produced by cultivators or commercial spawn providers. Here is how to inoculate substrate bags.

Inoculation

- The work surface, inoculation room and gloves should be clean and disinfected with 70% alcohol solution. To

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make a 70% alcohol solution, some growers dilute methanol with water. It should be avoided since the prolonged use of methanol might cause a serious injury in the brain and eyes.

- A spoonful of spawn is put into substrate bags as quickly as possible for secure sterile operation. The spawning rate is about 2-2.5% of the dry weight of the substrate.



Figure 18. Spawning room, Swaziland



Figure 19. Additives, spawn bottles and spawned-bags at different stage

[Examples: Spawning methods in different countries]

India - Fill approximately 5cm of boiled straw into a wooden frame and compress it with a wooden lid and sprinkle spawn over the whole surface. After the first layer of spawning, put another 5cm of straw and again sprinkle spawn over the surface, compress it as in the first layer. In this way, continue to sprinkle spawn over the layers of straw for 4-6 layers until the straw is level with the top of the frame. Only one (1) packet of spawn should be used for 1 cube or block. Growers should inoculate in the dark at an optimum temperature of 24℃ until the straw is fully prepared.

Once inoculated, the plastic sheet is folded over the top of the frame and tied down with help of jute ropes previously placed below the plastic. The frame is then removed to access the block. Small holes of approximately 2mm are punched in the block for breathing. The blocks are later placed on the shelves in a single layer for incubation.

Vietnam - Mushroom spawn must be purchased commercially, unless it is provided by an extension center. Around 2.5-3.0kg of spawn is needed for 100kg of straw.

S. Africa - Spawn is inoculated at 3-4% of wet weight.

Bangladesh - Substrate is inoculated by one or two spoonfuls of spawn per packet.

Nepal - Initially conditioned straw is put into the bags in a layer about 4 inches thick and the spawn is spread uniformly all along the periphery of the bag. Then another layer of straw is added. In this method, two bottles (250g/bottle) of spawn are sufficient for three polyethylene bags (14 x 24 inches size) containing 3kg of rice straw (dry weight) each. In the same way, one bottle of spawn will inoculate an 18 x 26 inch plastic bag containing 4kg rice straw.

Hungary - After treatment the substrate is watered with a benomyl solution and spawned at 4-5% of wet weight.

A Low-cost Technological Proposal: from Mixing & Spawning to Bagging

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Figure 20. The equipment to pasteurize substrate

Growers here use a low-cost method to decontaminate the substrates. Decontamination of substrate is one of the first and most important procedures in mushroom production and can be accomplished in several ways. For small farms, autoclaving and steam pasteurization are expensive due to the high cost of the equipment. Pasteurization by immersion in hot water has some disadvantages, as it is time consuming and it is not easy to control the water content, and it makes amendment with additives difficult.

Thermal treatment in ovens usually takes 48 hours at 95℃ to decontaminate the substrate. Chemical sterilization / decontamination using formaldehyde in the range of 25-1,000 ppm was also studied (Earanna and Shetty, 1994) but this method could harm the operator, the environment, and possibly the consumer.

Some growers now propose a different and low-cost decontamination method using a piece of equipment which we constructed with the following characteristics: a metallic rotary 180L drum of 0.60m body diameter and 0.38m open end diameter (see Fig. 20, three fixed metallic bars inside to improve the mixing action in order to avoid formation of substrate lumps, the angle of the drum main axis was 11℃ with respect to the horizontal, helping to minimize substrate losses through the open end during operation. The open end of the drum was covered with a close mesh fabric to isolate the substrate during the decontamination process. The rotation speed was 32 rpm. A gas heater with 2 ring-shaped burners of 0.21m and 0.08m diameter was placed at 0.04m distance from the lower base of the drum. An electric motor of 0.75 HP rotated the drum and rotation was automatically interrupted every 15 minutes with the help of an automatic on/off device.

The decontamination process always began with the gas heater on and the drum (with 35-40kg wet substrate mass inside), in a stationary position, during the first 15 minutes. Heating is continued for 2.5 hours with the drum alternately rotating for 15 minutes and stopping for 15 minutes.

During this period, a threshold of 65℃ was reached at 45 minutes and the beginning of the plateau at 80℃ and 90℃ occurred after 50 minutes and 60 minutes, at the surface and at 0.30m depth, respectively. An additional heating period of 90 minutes after reaching 80℃ was considered appropriate for total decontamination. Thus, approximately 2.5 hours were enough to prevent contamination during the mycelium colonization and fruiting stages.

It is interesting to note that heating the drum during the periods without rotation greatly increased the temperature of the substrate near the heat source, and this, in turn, produced a vapor blow (or stroke) immediately following each activation of rotation. There was a more effective heat transfer when the overheated portion was mixed with the rest of the substrate.

In addition, an effective decrease in energy cost could be obtained by using a lower HP motor or placing a tandem system of 4-5 drums driven by a single motor. These considerations apply to the low-cost equipment already found on the market (USD130-150, in Argentina).

With this decontamination method, an estimated 5.5 hours are required for one skilled person to prepare 35-40kg of substrate ready to put in bags for mycelium colonization. This time includes 0.5 hour for weighing the components and filling in the drum, 2.5 hours for the decontamination process, 2 hours for allowing the temperature of the substrate to fall to 35-40℃ previous to spawning, and 0.5 hour (or less) for homogeneous mixing of spawned substrate. It is important to point out that this method has the very convenient advantage of

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effecting both the decontamination and the inoculation of substrate in the same recipient, saving spawn running time because of the better spreading of mycelium compared to top- or layer-inoculated bags that are time consuming to produce manually.

Finally, we consider that with three pieces of equipment, composed of 4 drums each, it is possible for two workers to decontaminate 430kg substrate in twelve hours. Of course, the proposal of the gyratory drum could be scaled up to higher volume. Growers should remember that in order to effectively decontaminate the substrate it is necessary to treat the materials for a minimum of 90 minutes following the reaching of the 80℃ threshold temperature. A similar protocol could be effectively applied for decontamination of other agricultural waste-based substrates.

Incubation

The inoculated bags are moved to the growing house, or if one is available, to the incubation room. Growers can have a separate incubation room or they may use the growing house as an incubation room by providing higher temperature and higher humidity, which are proper room conditions for mycelial growth.

Bags are incubated at an optimum temperature of 20-25℃ in darkness since spawn run does not require light. Full colonization takes 15-25 days depending on the bag size and substrate material and condition.



Figure 21. Partial colonization



Figure 22. Full colonization

[Examples: incubation in different countries]

Nepal - The inoculated bags are incubated at 20-25℃ for 10-15 days for spawn run. As soon as the substrate is fully covered with a whitish mycelial growth, the polyethylene cover is removed.

India - Spawned bags are placed slightly apart from each other on shelves in an incubation room, lest they should generate excess heat. The temperature of the substrate bag is maintained at 25℃. Temperature is measured by inserting a thermometer in the bags. If the bag temperature rises above 25℃, it is advisable to lower the room temperature. If the bag temperature is low, the room should be warmed slowly. The completion of spawn run, when the entire bag turns white in color, takes about 12-15 days.

Vietnam - Bags are tied one atop the other with a nylon rope (3-5 bags per rope) and hung from a perch. The mouth of each bag points upwards. Bags are a few centimeters apart in order not to touch each other. After 25-30 days, mycelium will develop throughout the bags. Growers use a sharp knife to cut 4-6 slits in the sides of the bag. Each slit should be 3-5cm long, and an equal distance from the other slits. The cuts should not be in a line around the bag, as this will weaken the bag.

Bangladesh - After inoculation, the bags are incubated for 15-25 days at room temperature. When the mycelium

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is growing, the incubated poly bags containing the substrates are punched on the top sides of the packets for facilitating the luxuriant growth of mushrooms.

Hungary - Bags containing the spawned substrate are either placed in incubation rooms or directly in production rooms for spawn run. During this period, substrate temperature is maintained at about 25℃. Care is taken to avoid overheating of spawned substrate. There are two critical points when overheating may be a particular problem. The first is shortly after spawning (accelerating growth of microorganisms), and the more intensive second peak is 7-15 days after spawning (caused by metabolism of *Pleurotus* sp. mycelium growth). The relative humidity is maintained between 90-95%, and no light provided during the spawn run.

S. Africa - Cropping containers used for *Pleurotus* production typically are made of large clear or black polyethylene ducting cut into 2-meter columns (50cm in diameter) with a metal support and holding approximately 50kg of substrate. Other growers may use smaller plastic bags containing about 20kg of substrates. Regardless of the sizes of the bags used, holes are punched into the bags for aeration and mushroom maturation. Upon completion of spawn-run, growers usually move the bags into the production room or the growing room.

U.S.A. - The pasteurized and supplemented straw or hulls are spawned and filled (12-15kg) into clear or black perforated polyethylene bags and then incubated at 23-25℃ for 12-14 days. Some growers use bags with pre-punched holes while other growers cut holes in the bags after spawning.

Fruiting and harvesting

Unlike other crops, mushrooms - fruit bodies of fungi are quite sensitive to the growing conditions including temperature, humidity, light and ventilation. The correct temperature enables them to grow well in growing house. The light and ventilation influence the color, size and texture of the mushrooms.

Temperature

Mycelia of oyster mushroom grow best at a temperature range between 20 and 25℃. In order to induce fruiting after full colonization, the room temperature must be lowered to below 15℃ or colonized bags must be moved to a growing room. Otherwise, growers cannot expect good primordial formation despite good colonization.



Figure 23. Simple structure for mushroom growing



Figure 24. Thatch growing house covered with a shading net

Light

Unlike mycelia, which do not require light, primordia are formed under light. Mushroom formation and growth stages require 80-210 lux of light. Without light, fruiting bodies of oyster mushroom would abort or malformed. Light influences on the fruitbody color and stipe length. Under poor light, mushrooms with an elongated stipe and light-colored cap are produced while under excessive light, they will be short and dark-colored.

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Ventilation

Oyster mushroom growth is stunted under high CO_2 concentrations. Young mushrooms suffocate due to lack of oxygen. Mushroom stipes become enlongated and twisted and cap growth is very poor. Excessive ventilation, however, causes heavy water loss, resulting in lower room humidity and drier substrate.

Humidity and substrate moisture content

After ventilation, the room humidity drops. Spraying water onto corridors and into the air is recommended. The proper room humidities for pinning and fruitbody development are 90% and 80-85%, respectively. Once pinheads appear on the substrate, indoor humidity must be lowered to 85%.

Excessive substrate moisture content could cause the lack of oxygen in the substrate, which, in turn, could keep mycelia growing vigorously. In this situation, the growth of fruitbodies in the substrate bags is delayed and stunted. When both the substrate moisture content and room humidity are low, mushroom growth will also be stunted due to the lack of water. In this situation young mushroom caps may upturn earlier and release more spores.

Watering

Because most edible mushrooms are 90% water, humidity is critical during the fruiting stage. Growers should water the growing room frequently in order to raise the relative humidity during this reproductive growth phase. Watering frequently, using small amounts of water is desirable. For example, 2 or 3 buckets of water 5 or 6 times a day is better than 10 to 15 buckets of water twice a day. Applying water directly to the mushroom bags should be avoided because drops of water are harmful to pinheads and standing water attracts a variety of pathogens. Growers should water the floor of the growing room and around the bags. Some growers utilize humidifiers to increase the relative humidity inside the growing rooms. Watering is especially important during the dry season when the ambient humidity is very low.



Figure 25. Bags laid on their side in row on the wall.



Figure 26. A-frame rack



Figure 27. Bags bound and hung with wire

Bag arrangements

Various practices of bag arrangement are found worldwide. Some growers arrange bags so that they are not touching each other to avoid overheating of the bags. Mushroom mycelia emit heat during incubation, so the bags can be easily overheated if they touch each other. Air can easily flow through the spaces between the bags, preventing the temperature of the bags from increasing.

However, many growers still arrange bags stacked against each other in order to grow more mushrooms in a small growing room. Shelves and A-frame racks (Fig. 26) are used for efficient utilization of space inside growing rooms. In some countries growers bind bags with wire and hang them (Fig. 27).

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[Examples: Fruiting and harvesting in different countries]

Bangladesh - Growers cultivate mushrooms at room temperature.

Nepal - When the substrate is fully covered with a whitish mycelial growth, the polyethylene cover is removed. The open bags are transferred into a new room with good ventilation. The bags are kept about 15cm apart from each other on a wooden block or brick. Watering is done 3-4 times daily. Mushroom primordia start appearing 2-3 days after the removal of polyethylene and they reach maturity 5 or more days later. Oyster mushrooms have a shorter growing cycle and a total of 3-4 flushes could be harvested during this period.

Mushrooms are picked carefully without disturbing other developing mycelia. Then harvested fresh mushrooms are packed into plastic bags for the fresh market.

Under commercial cultivation, there is a great variation in biological efficiency from farmer to farmer. Usually they produce mushrooms equaling from 40-100% of the initial dry weight of substrate.

Vietnam - After the bags have been cut with 4-6 slits in the sides of each bag, they are sprayed with water 2 or 3 times a day to keep the mushrooms moist, and the growers are careful not to give them too much water. No water should collect inside the bag. Growers take the cotton out of the mouth of the bag and suspend the bags on a wire or rope, with the mouth of the bag pointing downwards.

The mushrooms will begin to appear in the slits, looking like small round buttons. As soon as they begin to appear, growers should move the bags to the growing or harvesting area. The bags should be placed 7-10cm apart.

The first oyster mushrooms can be harvested 7-10 days after the bag is cut. After the mushrooms are harvested, growers stop spraying water for several days. When the young fruits begin to appear again, they begin to spray the water again. This cycle can be repeated three or four times, giving a total harvest of 50-80kg of oyster mushrooms from 100kg of straw.

Hungary - Air temperature is maintained at 15-20℃ and the CO_2 level is lowered to 600-1,000 ppm by ventilating with fresh air. The spawn run usually takes 2-3 weeks after spawning. During this period, the humidity is maintained at 90-95% to provide for optimum condition. Subsequently, relative humidity may be lowered to 80-90% in order to minimize development of bacterial blotch. 8-10 hours of 50-150 lux light is provided daily to allow for normal fruit body development.

India - Once the blocks are fully colonized during the spawn run, they are hung after removing the polythene in a room where the relative humidity is maintained above 85%. The humidity is normally maintained by frequent spraying of water on the blocks and room environment. The pins are visible on the ninth day after the opening of the blocks. Proper relative humidity and proper ventilation is maintained in the room during pin growth and picking. The mushrooms are picked generally for the fresh market. Most of the growers take three flushes. Mushrooms picked in the third flush are mostly used for sun drying, where maximum dry matter is achieved.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 7

Cultivation Modes

SHELF CULTIVATION OF OYSTER MUSHROOM

With Emphasis on Substrate Fermentation

Kyung Wha Choi
MushWorld

Shelf cultivation of oyster mushrooms is a unique Korean production method. This method was adapted by Korean scientists in the early 1980's from button mushroom (*Agaricus bisporous*) growing on shelves. Button mushroom cultivation can be summarized as follows: Phase I (outdoor fermentation), Phase II (pasteurization and conditioning), Phase III (spawning and incubation), fruiting, harvesting and emptying. The unique characteristics of shelf cultivation of oyster mushroom in Korea include the fact that the substrate is fermented in three steps (pre-fermentation, pasteurization, and post-fermentation) rather than on the shelf itself. Unlike the pasteurization process that lasts for 2-6 hours in most countries, shelf cultivation requires the substrate materials to be pre-fermented outdoors for 2-3 days, then pasteurized at 60-65℃ for 8-10 hours, and finally post-fermented at 45-55℃ for 3-4 days. This process requires considerable time and generates significant expenses.

In Korea, the main methods of oyster mushroom growing are shelf cultivation, bag cultivation and bottle cultivation. Korean oyster mushroom growers ferment the substrate materials in shelf cultivation while they sterilize substrate materials in the cases of bag and bottle cultivation. Button mushroom (*A. bisporus*) is a secondary decomposer requiring the previous degradation of substrates by bacteria or other fungi in order to be able to absorb nutrients from the substrate. On the other hand, oyster mushrooms (*Pleurotus* spp.) are primary decomposers, so they have ability to break down and absorb the components of substrate materials that have not been composted or degraded. Korean growers have normally fermented substrate materials in spite of the high fuel costs generated during the fermentation process because fermentation is definitely helpful in producing high yields and high quality oyster mushrooms. This article will discuss the process of shelf cultivation and the events relative to the substrate materials during each fermentation step.

Shelf Cultivation at a Glance

The process of shelf cultivation is summarized as follows, with images

Pre-fermentation ▷ Filling ▷ Pasteurization and Post-fermentation ▷ Spawning ▷ Incubation ▷ Pinning and Fruiting ▷ Harvesting ▷ Emptying

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Pre-fermentation



Figure 1. Pre-fermentation and turning of cotton waste

As the first step of fermentation, pre-fermentation is a part of Phase I of button mushroom (*A. bisporus*) cultivation. Most Korean farmers utilize rice straw or cottonseed hull as substrate materials for shelf cultivation. Substrate material is piled up outdoors and then watered. The temperature of the heap gradually increases as microorganisms activated by the water begin to propagate themselves, eventually resolving the high molecular carbon sources into simpler molecules and absorbing them (Shim, 2001). The pile is turned (Fig. 1) to provide fresh air and prevent overheating. The temperature drops initially after turning but it increases again as the activities of the microorganisms continue (Shim, 2001). This step usually takes 2-3 days and the duration differs depending on the substrate materials. When this method was first adapted from button mushroom cultivation methods by Korean scientists, farmers had always gone through this step. As time went by however, farmers came to understand that outdoor fermentation could be simplified in order to save labor costs. Nowadays some growers just water the substrate materials outdoors and keep them overnight, and then ferment them at 45-50℃ for about two days before pasteurization. Though this is a simplified version, many growers, especially the most successful growers, still go through this or a somewhat modified pre-fermentation process.

Pasteurization and post-fermentation

The substrate is pasteurized usually at 60℃ for 6-10 hours and then goes through the post-fermentation process at 50-55℃ for 3-4 days. The temperatures and times of pasteurization and post-fermentation vary slightly according to growers' experience.

Some growers in other countries pasteurize substrate materials. However, pasteurization and post-fermentation for shelf cultivation are very technology-intensive activities, and unlike bag cultivation, they require many years' experience to effect high productivity. Pasteurization and post-fermentation are the key factors for producing high yields in shelf cultivation. Through this process, the substrate becomes more of an appropriate food source for mushrooms, and microorganisms that can be possible competitors for nutrients are eliminated from the substrate (Shim, 2001).





Figure 2, 3. Room for pasteurization and post-fermentation (Farm A and B)

Previously, pre-fermented substrate was filled into the shelves of the growing room and then went through pasteurization and post-fermentation in the growing room. However, the substrate wasn't thoroughly fermented and the extent of fermentation differed according to the specific layer of the shelf because temperature differences in the growing room were too large to evenly ferment substrate materials. In addition, this practice consumed a large amount of fuel. As a result, many growers nowadays have built a special room that is heated by steam for pasteurization and post-fermentation (Fig. 2). Baskets filled with pre-fermented substrate materials are stacked (Fig. 3) and pasteurized and post-fermented. Thanks to this room, growers can ferment substrate materials evenly in a relatively small space and save money by using this system.

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Figures 2, 3. Room for pasteurization and post-fermentation (Farm A and B)

If the substrate is pre-fermented sufficiently outdoors, its temperature can be quite high when moved into the room. The temperature inside the room is increased by steaming and kept at 60℃ for 8-10 hours in order to accomplish the pasteurization. Though the room temperature is kept as 60℃, the internal temperature of the substrate rises up to 65℃. After 8-10 hours the temperature is lowered and maintained at 48-53℃ for 4-5 days. Some growers say five-day is too long for post-fermentation and the substrate is like to be too wet after five days of post-fermentation.

If the substrate is just watered outdoors without pre-fermenting it, it can be fermented in the room before pasteurization. The room temperature should be slowly increased by steaming up to 45℃ and then gradually raised up to 46℃ and then 48℃ and finally up to 53℃ for two days to accomplish pre-fermentation.

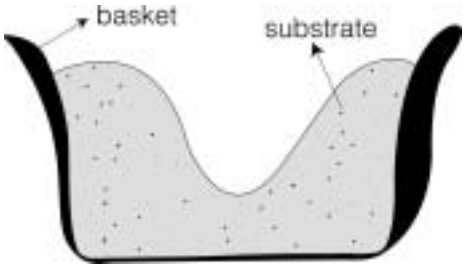


Figure 4. Cross-sectional view of basket

Although the principles are same, each expert grower has his own know-how in this process. Some growers make an additional effort to keep each part of the substrate at a similar temperature for thorough fermentation. Figure 4 shows a cross-sectional view of a basket filled with substrate materials. The central part of the substrate is removed, and by doing this, the temperature difference among each part of the substrate in the same basket can be reduced down to 4℃. Otherwise, the temperature difference between the hottest part and the coolest part in the same basket reaches as far as 8-10℃, which hinders thorough fermentation.

When pasteurization and post-fermentation is completed, a white color is visible on the substrate. These are actinomycetes, one of the thermophiles produced in the last stage of post-fermentation. If actinomycetes exist in sufficient quantities on the substrate, the substrate can be said to be well fermented and suitable for mushroom growing. Actinomycete will be discussed further later in this article.

Filling and spawning

After pasteurization and post-fermentation is completed, the substrate is filled into shelves in the growing room. Filling and spawning is one of the most labor-intensive processes in shelf cultivation if it is done manually. The post-fermented substrate is poured from the box onto the shelf and this is repeated until each shelf has the allotted amount of substrate (Fig. 5). Though differences exist, growers usually fill 15kg of dry substrate per square meter of shelf, as is the usual case with cotton waste. As the moisture content of the substrate is about 70%, filling the proper amount of the fermented substrate can be calculated as 50kg per square meter. However, the filling weight varies depending on growers, substrate materials, and seasons. If rice straw is selected as the substrate, greater amounts are filled than if the substrate is cotton waste. More substrate is used in winter than in summer because the substrate is more likely to be overheated in summer.

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After filling, the substrate is covered with a plastic sheet (Fig. 6) to keep in humidity, and stays overnight (Fig. 7) for cooling. The next day, when the substrate has cooled down to 20-25℃, about 60-70% of the spawn is inoculated (Fig. 8) and thoroughly mixed with the substrate on the shelves. The substrate is then spread evenly on the shelves and the remaining 30-40% of the spawn is sprinkled onto the substrate surface (Fig. 9). The spawning rate is generally higher in shelf cultivation and can be as much as 14% of the wet weight of the substrate, namely 7kg of spawn is inoculated to 50kg of substrate per square meter. The shaped substrate is mulched with a plastic sheet that has very small holes for ventilation (Fig. 10).

<Manual Filling and Spawning>



Figure 5. Filled substrate on shelf



Figure 6. Covering filled substrate with plastic sheet



Figure 7. Cooling substrate overnight



Figure 8. The first spawning

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Figure 9. The second spawning after flattening the surface



Figure 10. Covering with a plastic sheet after spawning

These processes are very labor intensive, so some growers utilize equipment such as filling machine and spawn mixing machine. Farm A saves 50% of their labor costs of the spawning operation by using a spawn mixing machine. Filling is done manually one day before spawning, and 60% of the spawn is inoculated by sprinkling (Fig. 11). Then the substrate and spawn are mixed (Fig. 12) by the wires of the machine (Fig. 14), which rides on the rails along the both sides of shelf (Fig. 13). The rail for this machine is attached to both edges of the shelves (Fig. 13). The substrate is then flattened (Fig. 15) and the rest of spawn is sprinkled on the surface (Fig. 16).

<Farm A: Spawning with spawn mixing machine>



Figure 11. The first spawning by sprinkling

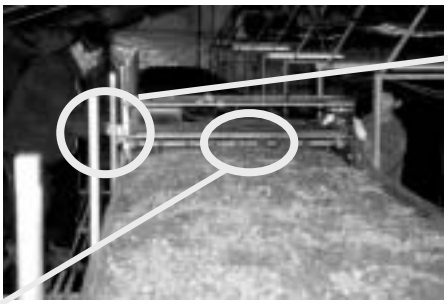


Figure 12. Mixing substrate and spawn



Figure 13. Rail for the machine at the edge of shelf

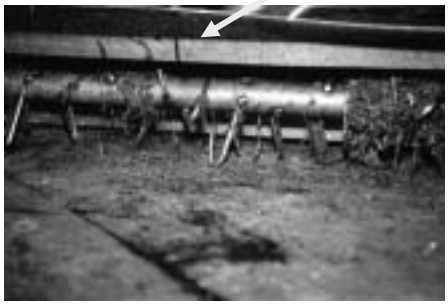


Figure 14. The wires for mixing in the machine



Figure 15. Flattening substrate after mixing



Figure 16. The second spawning on the surface

Farm B saves much more labor by using a filling machine (Fig. 17). In this case, filling and spawning are done simultaneously. The post-fermented substrate stays overnight for cooling down to the 20-25℃ temperature

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appropriate for spawning, and then the substrate in baskets is poured into the filling machine (Fig. 18) from where it moves onto the shelves via the conveyer of the machine. As soon as the substrate falls onto the woven textile mat on the shelf, two workers sprinkle spawn, mix it with the substrate, and flatten the substrate by hand on both sides of shelf (Fig. 19). This procedure takes some time, so another worker controls the speed of the filling machine (Fig. 20). When the substrate is flattened, the woven textile under the substrate is winched toward the other end of the shelf (Fig. 21, 22). The rest of the spawn is then sprinkled on the surface of the substrate.

<Farm B: Filling with filling machine>



Figure 7. Filling machine



Figure 18. Filling substrate



Figure 19. Substrate on shelf is Spawned and mixed.



Figure 20. Controller of filling machine

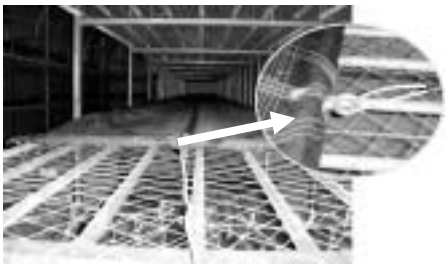


Figure 21. Substrate winched toward the end of the shelf



Figure 2. Winch machine

Incubation

Mushroom mycelia are incubated for 17-23 days while covered with a plastic sheet. The temperature should be maintained at 20-22℃ during the first stage, and then increased gradually up to 25℃. As the incubation progresses, the substrate emits heat by itself due to mycelial growth. Therefore, the temperature should be set at 22-23℃ though the optimal temperature for growing of the oyster mushroom mycelia is 25℃ (Cha *et al.*, 1997). The mycelia don't require much ventilation during vegetative growth, but it should be kept in mind that they do require sufficient oxygen during this stage.

Pinning and fruiting

When the spawn has fully colonized the whole substrate (Fig. 23), the environment in the growing room is adjusted and made appropriate for reproductive growth and fruiting. To convert to the reproductive growth stage, the factors such as adding light, performing a cold shock, maintaining a high relative humidity, and providing enough oxygen are implemented. Light levels are raised to 80-120 lux, sufficient for newspaper to be readable, for 3-4 days before the plastic sheet is removed for ventilation. The temperature of the growing room is lowered to

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15-18℃, but the optimal temperature for pinning varies from 10-24℃, depending on species and strains (Cha *et al*., 1997). Growers should be aware of the characteristics of the species and strains they are growing. Relative humility inside the growing room should be kept as high as 85-95% by watering once or twice a day. Pinheads (Fig. 24) should be observed within several days and grow to full size soon thereafter.



Figure 23. Fully colonized shelf

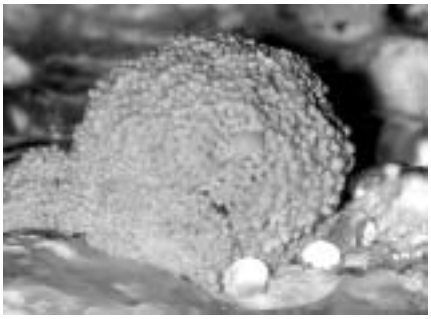


Figure 24. Pinheads of oyster mushroom

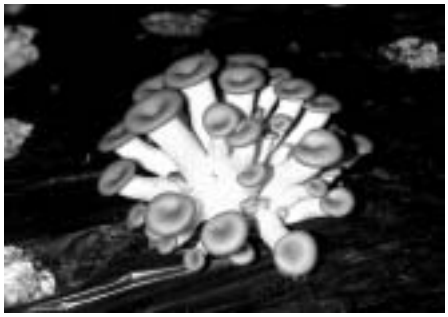


Figure 25. Fruitbodies growing from perforated parts

Some growers cover the whole shelves with black perforated plastic sheets with 5-10cm holes every 10-15cm. At spawning, 60-70% of the spawn is mixed with the whole shelf and then the shelf is covered with the perforated plastic sheet and the rest of the spawn is inoculated into the holes. Then, the shelf has 80% of mulched area and 20% of perforated area. The fruitbodies grow only from the perforated parts (Fig. 25) in clusters. The mulching with a plastic sheet provides many benefits. It reduces the labor input during harvest and the abortion of small pins, and produces quality mushrooms with favorable color and long stipes, which are more marketable in Korea, and most importantly, it produces greater yields (Oh *et al*., 2003b). In addition, mulching with a plastic sheet is effective for in preventing diseases such as bacterial brown blotch and various fungal diseases by preventing invasion of the pathogens and reducing waterlogged areas on the shelves (Oh *et al*., 2003a).

Harvest



Figure 26. Oyster mushroom on conventional shelf



Figure 27. Oyster mushroom on vinyl mulching

Oyster mushrooms are harvested when they grow to full size. Several flushes are harvested because the abundant quantity of substrate on the shelves still has plenty of nutrition for oyster mushrooms even after several harvests. Most growers harvest 3-4 flushes from the shelf, and about 50% of the yield is from the first flush. Accumulated biological efficiency reaches 100%, but it fluctuates according to seasons.

After 3-4 flushes, the shelf can still produce more mushrooms due to the existence of sufficient nutrients on the shelves. However, harvesting more than 3-4 flushes is not economically reasonable in Korea where land and labor costs are very high and high fuel expenses for growing room control are required in summer for cooling and in winter for heating. More flushes could be harvested in countries with low land costs and labor costs and tropical or sub-tropical climates, and in those situations the biological efficiency could reach much higher rates.

Oyster mushrooms from shelf cultivation are considered to be of a higher quality than those from bag or bottle cultivation thanks to the rich nutrients available for mushrooms grown on shelves. This high quality produce also earns more money. In the Korean mushroom market, high quality oyster mushrooms are usually three times more

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expensive than low quality ones.

Emptying

When the substrate has produced an economically reasonable amount of mushrooms, the shelves are emptied. Emptying is also very labor-intensive, but many growers have discovered more convenient methods that save labor. In principle, the substrate on the shelves is steamed and then removed. However, many farms have a steaming facility only in the room used for pasteurization and post-fermentation, so growing rooms are disinfected by fungicides or insecticides such as diluted formalin solution, Benlate, or Panmashi. The spent substrate is moved away from the farm to prevent the infection of new crops. Sometimes the spent substrates are utilized as pig's fodder.

Fermentation, the Art of Microorganism

> The following section is excerpted and translated from 'The essence of mushroom cultivation - Fermentation of Substrate' (in Korean), written by Dr. Moon-soo Shim and excerpted here with the permission of the author.

Fermentation can be defined as the process of converting or decomposing organic matters into unique final products by the function of microorganisms' enzymes. However, fermentation in mushroom cultivation can be defined as the converting by microorganisms of the nutrients of substrates into proteins.

Selecting substrate material

Button mushrooms naturally grow from materials with a relatively high nitrogen content such as horse manual (1.8% nitrogen) and wheat straw (0.65% nitrogen). The optimal C : N ratio for cultivating button mushrooms is 17 : 1. On the other hand, oyster mushrooms and shiitake grow from wood with a relatively low nitrogen source, of which the C : N ratio is 350 to 500 : 1. The optimal C : N ratio differs according to mushroom species. Therefore, the C : N ratio of substrate material should be first considered in substrate preparation. Table 1 shows composition contents of each substrate material.

Table 1. Comparison of composition contents of substrate material (%)

Material	pH	Cellulose	Lignin	Total Carbon	Total Nitrogen	C:N ratio
Cotton Waste	6.2	73	6	24	0.41	59:1
Rice Straw	6.7	42	13	46	0.63	72:1
Wheat Straw	6.9	48	20	47	0.48	97:1
Corncob	7.2	47	25	47	0.48	97:1
Sawdust	5.5	54	29	49	0.1	491:1

As shown in Table 1, the main substrate material alone sometimes cannot provide enough nitrogen required for optimal growth of mushrooms, so additives such as rice and wheat bran are supplemented as a nitrogen source. The amounts supplemented vary depending on which substrate is chosen. If cotton waste is selected, a smaller amount of nitrogen source is added than when wheat straw is selected.

The C : N ratio is also important because it affects the fermentation process. Through the fermentation process, nitrogen is converted into ammonia nitrogen, restraining the growth of mushroom mycelia as well as making nitrogen available for the mycelia. If available nitrogen increases, ammonia nitrogen also increases, and ammonia

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nitrogen decreases when available nitrogen decreases. Therefore, the amount of ammonia produced by fermentation should be considered in substrate selection. Table 2 shows the result of comparison of oyster mushroom yields after supplementing different amounts of rice bran. As supplemented rice bran increases, total nitrogen and ammonia nitrogen increase and the oyster mushroom yield is affected as a result. When 0.98% of total nitrogen added, the amount of ammonia nitrogen (28 ppm) is too little to influence oyster mushroom yields. When 1.48% of total nitrogen is added however, the yield decreases to 45.2kg because the ammonia nitrogen levels are enough to restrain the oyster mushroom from growing. In conclusion, oyster mushroom yield decreases when the ammonia concentration is higher than 68 ppm as well as when total nitrogen is smaller than the optimal amount.

Table 2. Oyster mushroom yield according to total nitrogen and ammonia nitrogen

Total Nitrogen (%)	Ammonia nitrogen (ppm)	Yield (kg/m^2)
0.98	28	173.9
1.08	68	193.4
1.48	84	149.2

Therefore, C : N ratio and the amount of ammonia nitrogen should be both considered. If cotton waste is chosen as the main substrate material for oyster mushroom, a nitrogen source such as rice bran should be supplemented considering the optimal C : N ratio. The amount of nitrogen source supplemented should be up to the total amount of nitrogen from which the amount of ammonia nitrogen created during fermentation doesn't restrain the mycelial growth of the mushroom.

Cotton waste and sawdust, the major substrate materials, have naturally occurring microorganisms that participate in fermentation of the substrate. Figures 28 and 29 show incubated microorganisms from sawdust and cotton waste. Both substrate materials were soaked in water and then the water was inoculated on nutrient agar and incubated. The first two Petri dishes were incubated at 30℃ and several kinds of mesophiles were propagated on them (Fig. 28). The other two Petri dishes were incubated at 50℃ and some thermophiles were cultivated on them (Fig. 29).



A. from sawdust B. from cotton waste

Figure 28. Mesophiles incubated at 30℃



A. from sawdust B. from cotton waste

Figure 28. Thermophiles incubated at 50℃

Table 3 calculated the number of microorganisms in cotton waste, hardwood sawdust, and rice bran. Cotton waste has the most microorganisms while sawdust has less and rice bran has the lowest amount. Though the results vary a little bit according to how and how long the materials are stored, the result will be same as below if they are stored under similar conditions. Sawdust has as many mesophiles as cotton waste, but cotton waste has 390 times more thermophiles than sawdust. This is because cotton waste is more easily exposed to microorganisms in nature than sawdust. Therefore, materials with more microorganisms are desirable as

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mushroom substrate if the substrate is to be fermented.

Table 3. Numbers of microorganisms according to materials

Substrate materials	Cotton waste	Hardwood sawdust	rice bran	note
Mesophiles (incubated at 30℃)	75×10^4	54×10^4	8×10^2	Cotton waste has 1.3 times more mesophiles than sawdust.
Thermiphiles (incubated at 50℃)	47×10^4	12×10^2	12	Cotton waster has 390 times more themophiles than sawdust.

Pre-fermentation

Fermentation aims at repressing microorganisms that might possibly compete with oyster mushrooms and converting substrate materials into a superior nutritional source for mushroom through the actions of a succession of microorganisms. Outdoor fermentation is the first step. Substrate materials in nature have microorganisms attached on their surfaces and these microorganisms are suppressed on dry material. Once water is applied, microorganisms on substrate material can propagate themselves. If the initial temperature of the substrate is 20℃, microorganisms suitable for this temperature range will increase and begin consuming the water-soluble carbon source that is relatively easy to absorb. Generally, the organism utilizes 35% of the nutrients for energy but the other 65% cannot be utilized and is emitted as heat. As the microorganisms increase by geometric progression, the heat emitted by the microorganisms is accumulated and the temperature of substrate increases to 30℃. Then, growth of the microorganisms that prefer the temperature of 20℃ is suppressed and other microorganisms appropriate for 30℃ begin to increase. In this way, the temperature of substrate increases up to 50℃.

The water-soluble carbon sources in substrate are all consumed by microorganisms as the fermentation proceeds. At that point microorganisms begin to consume high molecular carbon sources that are relatively hard to absorb, such as cellulose, hemi-cellulose and lignin. In theory, the increase of substrate temperature up to 50℃ means that microorganisms have decomposed the high molecular carbon sources that are relatively hard to utilize as well as the water-soluble carbon sources that are easy to utilize, and that the nutritive substances of the substrate are accumulated in the microorganisms in the form of protein. However, the substrate temperature is not uniform within a pile in actual fermentation, so the substrate pile is turned several times to encourage thorough fermentation by repeating the process described above several times. In addition, turning helps aerobic fermentation by providing more air to the pile.

Some growers may wonder what would happen if the substrate materials were fermented at 50℃. Would this make the fermentation process faster and easier? The answer is negative. The initial microorganisms that exist on cotton waste or cereal straw come mainly from the soil and many of them are mesophilic microorganisms best cultivated at about 30℃. Because 50℃ is not a usual temperature in natural environments, there are not many or many varieties of thermophiles that are best incubated at 50℃. There are not many microorganisms available to ferment the substrate if the fermentation starts from 50℃. In addition, various kinds of microorganisms are more able to ferment the whole substrate depending on the diverse nutritional components. However, oyster mushrooms can be still cultivated successfully even though the substrate has been fermented only from 50℃ if the mushrooms are well managed in each step of cultivation. This is because oyster mushrooms grow very well on various substrates without fermentation.

Pasteurization

The substrate is pasteurized at 65℃ for 6-8 hours. Pasteurization is sometimes said to aim at killing insects and

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mold spores, but this is not a sufficient explanation of the process of pasteurization. The spores of molds are generally killed at temperatures over 80℃, and therefore the pasteurizing temperature of 65℃ is not enough for killing most mold spores. Moreover, the spores are more durable in a 65℃ substrate with relative humidity of 60-70% than in 65℃ water. This can be easily understood if you think that people can stay in an 80℃ sauna but not in 80℃ water. Some growers pasteurize substrate at 80℃, but this temperature can kill useful microorganisms as well as mold spores. The substrate is pasteurized in order to soften the substrate materials and kill mesophilic microorganisms, not mold spores.

Post-fermentation

After pasteurization is completed, the substrate is post-fermented at 50-55℃ for 3-4 days. Though pre-fermentation is completed before pasteurization, the whole substrate is not fermented. Post-fermentation aims at a thorough and even fermentation of the whole substrate. During pre-fermentation, mesophiles are converted to thermophiles, but mesophiles are still abundant because considerable parts of the substrate are not fully fermented. These mesophilic microorganisms later compete with mushroom mycelia because both grow well at similar temperatures. Once these mesophiles are converted into thermophiles, however, these thermophilic microorganisms cannot grow at the incubation temperature of mushroom, and so cannot compete with mushroom mycelia.





Figure 30, 31. Actinomycetes on cotton waste(above) and rice straw (below)

White actinomycete grows at the last stage of the fermentation (Fig. 30, 31). The presence of actinomycete indicates that the substrate is well fermented aerobically and has become appropriate for the mycelial growth of mushrooms. The presence of actinomycete on the substrate indicates that the pH of the substrate is more than pH 7, a level that suppresses the growth of green molds.

After post fermentation, the substrate has become a superior nutritional material for mushroom mycelia. Useful nutrients are possessed by microorganisms as proteins and these proteins are not spoiled because they are inside the living organisms. Mushroom mycelia vegetative growth cells are able to secrete a greater variety of digestive enzymes than any other microorganisms. Once mushroom mycelia are inoculated into the substrate in the form of spawn, by using various digestive enzymes the mycelia can digest materials that other microorganisms could not process. Moreover, the mycelia have enzyme-digesting microorganisms, so they can dissolve and absorb the proteins, lipids, minerals, and vitamins of the microorganisms.

Characteristics of microorganisms participating in fermentation

One of thermophiles and one of actinomycete were separated and their optimal pH and temperature were examined. According to Figure 32, the thermophilic microorganism showed optimal growth at pH 7-8 while the optimal pH for the actinomycete was 8-9. Therefore, both grew well on alkaline (over pH 7) substrate. Considering only microorganisms, pH 8 would be the optimal for substrate, but the optimal pH for mushroom growth is 6-8. Therefore, pH 7 is the best level for the growth of both mushrooms and thermophilic microorganisms. Figure 33 shows that the thermophiles and the actinomycete grow best at 50℃. The thermophiles propagate well enough below 50℃, but their growth is much repressed above 50℃. On the other hand, the

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actinomycete prefer 45-55℃. This result indicates that the thermophiles participate in fermentation at the early stage while actinomycete participate actively later, when the temperature is over 50℃.

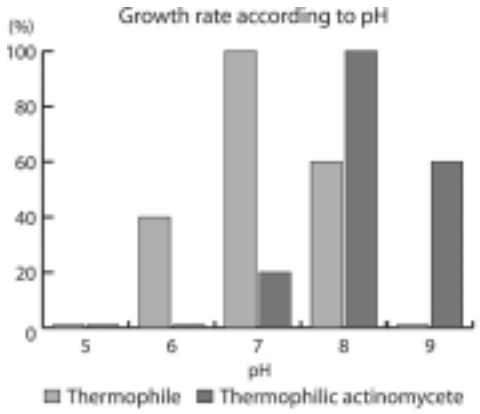


Figure 32. Optimal growth of thermophile and actinomycetes according to pH

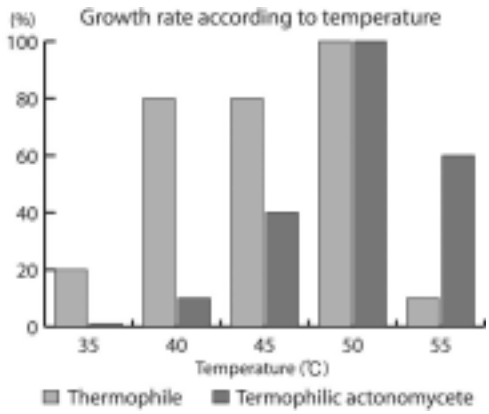
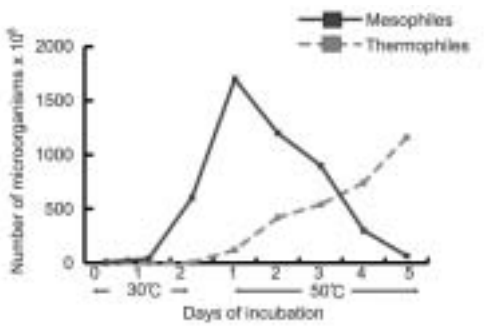
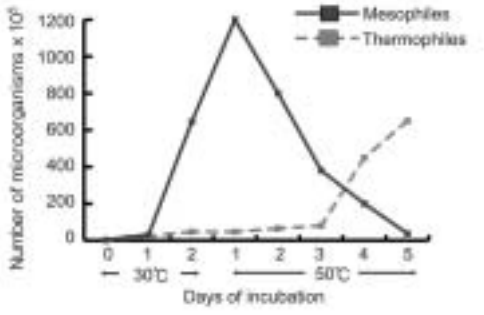


Figure 33. Optimal growth of thermophile and actinomycetes according to temperature





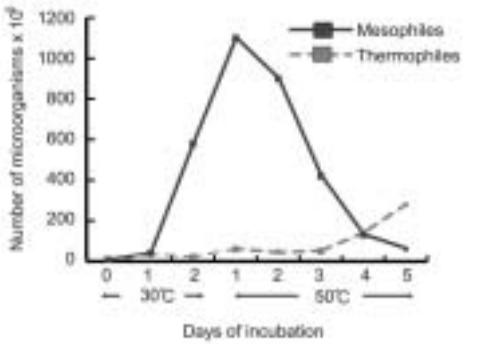


Figure 36. cotton waste:sawdust=2:8

Various microorganisms participate in the fermentation process as dominant species in each step in the succession. To monitor the changes of microorganisms during fermentation according to substrate materials, substrates were prepared by mixing different rates of cotton waste and sawdust and incubated at 30℃ for 2 days and then at 50℃ for 5 days, after which the propagation of mesophiles and thermophiles were examined (Fig. 34, 35, 36).

According to Figure 34, mesophilic microorganisms begin to propagate after the first day and multiplied in number over 1,000 times until the first day of incubation at 50℃ began. On the first day of incubation at 30℃ mesophiles did not grow at all, because this was a preparatory period when appropriate microorganisms for temperature, pH, oxygen and nutritional condition of the substrate adapt themselves and microorganisms participating in fermentation are selected. When ready, the mesophiles increased explosively. However, they begin to decrease rapidly at the beginning of the first day of incubation at 50℃, and their numbers dropped down to the level where fermentation started. On the other hand, thermophiles increased from the first day at 50℃ until the last day. Seeing that mesophiles did not stagnate at 50℃ but decreased and disappeared, it can be inferred that they were utilized as nutrients for the thermophiles.

Figure 33, 34 and 35 shows how important the choice of substrate material in fermentation process is for two reasons. As the rate of sawdust increases among substrate (Fig. 35), thermophiles as well as mesophiles cannot increase because sawdust doesn't contain enough microorganisms that are

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participating in fermentation. However, the number of microorganisms doesn't increase as much as when the rate of cotton waste is higher (Fig. 33, 34) even though the incubation days are extended. The second reason is that sawdust lacks nutrients easily utilized by microorganisms. Sawdust has more hemi-cellulose and lignin, which are relatively hard to utilize, while cotton waste has more cellulose, which is dissolved relatively easily by microorganisms. Therefore, the growth of microorganisms is restrained and fermentation is not increased as the amount of sawdust increases within a substrate. Another important finding is that if mesophiles don't increase, thermophiles also cannot increase. Mesophilic microorganisms affect the growth of thermophilic microorganisms.
Note : End of excerpt.

Conclusion

Shelf cultivation of oyster mushroom adopts the composting technology of button mushroom cultivation. Though not essential, fermentation contributes to the high quality and high yield of oyster mushrooms. Nowadays, many Korean growers have converted from shelf cultivation to bag cultivation due to the high risk of shelf cultivation. The fermentation of substrate requires many years of experience and skill, so many inexperienced oyster mushroom growers fail to produce profitable amounts of mushrooms. Moreover, shelf cultivation entails high expenses due to the large amounts of substrate and spawn, the high fuel cost for fermentation, and so forth. On the other hand, bag cultivation is relatively easy and safe because it produces appropriate yields though not of as high of a quality. Nevertheless, it is expected that the principles of substrate fermentation could be applied to the respective situations of oyster mushroom growers. Fermentation might require far less costs in tropical or subtropical regions because less fuel is required.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 7

Cultivation Modes

BOTTLE CULTIVATION

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MushWorld

The New Substrate Container



Figure 1, 2. Plastic bottles in use for mushroom growing

Oyster mushroom cultivations on logs, on shelves and in bags are discussed earlier in this chapter. Now, the latest container used for oyster mushroom growing is a polypropylene bottle. Propylene bottles were first favored by spawn suppliers since they are heat-resistant and thus, autoclavable, endurable and thus, re-usable, and easy to handle.

Mushroom growers with experience and some specialized knowledge of mushroom growing and sterile techniques can make use of bottle culture systems for production of spawn and mushrooms. The system, however, might be impractical for growers who have just started mushroom growing or those who use pasteurized bulk substrates or composted substrates that are not appropriate for bottling. Favored are the small particle-sized growth media types such as sawdust, spent grains and grain hulls. In addition, the initial set-up cost of the system may be too high for many small-scale growers to adopt. Still, some growers may be able to develop some viable ideas from this up-to-date growing method.

Floor Plan for the Efficient Use of Space

Commercial large-scale mushroom operations usually have two complexes, one for substrate preparation and the other for growing. An ideal floor plan for mushroom bottle preparation complex is shown in Figure 3. One can see the structure is designed to minimize the length of pathways, a design feature that minimizes contamination risks. Sterilized substrate in the bottles harbors neither harmful nor beneficial microorganisms to the mycelial growth. That means, the first comer can occupy the entire substrate in the bottle. That accounts for why sterilized substrate bottles are so vulnerable to diseases when they are exposed to air-borne contaminants before mushroom mycelia colonize them. Growers are advised to maintain the highest levels of hygiene and sanitation in mushroom operations.

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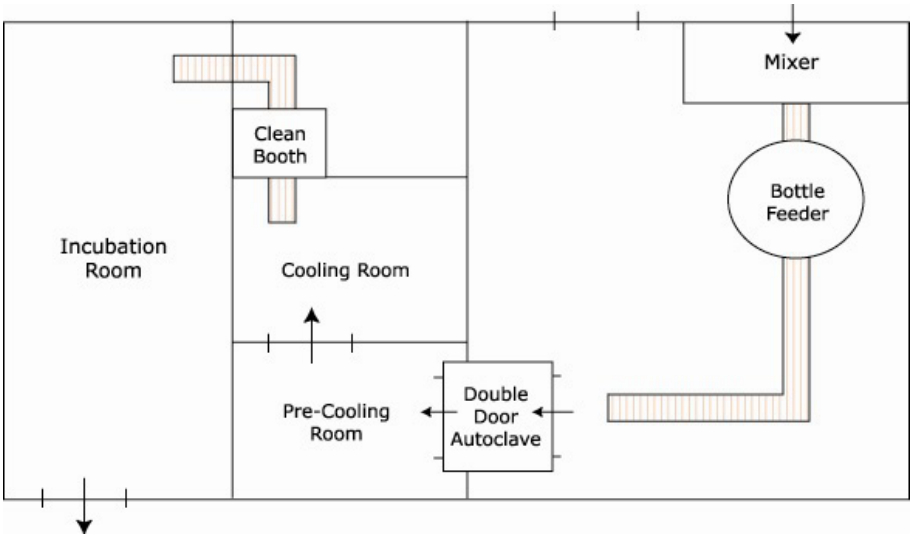


Figure 3. Floor plan for an efficient mushroom operation

Oyster Mushroom Cultivation in Bottles

A bottle cultivation system employing sawdust as the growth medium would be similar to a sawdust-based spawn production system. The differences between the two include the material used as inoculum and the stage that occurs after incubation. In spawn production, inoculum is spawn master ('starter spawn') and mycelia-colonized sawdust become spawn. In bottle culture the inoculum is regular spawn and the colonized substrate is encouraged to produce fruiting bodies.

Substrate preparation



Figure 4. Substrate mixing

The substrate materials should be in particles small enough to run smoothly into the bottle. Sawdust from hardwood or broad-leaf trees such as poplar, alder, and cottonwood are preferred. Sawdust from softwood trees like Douglas fir can be used after a three to four month-outdoor fermentation process during which the phenol compounds are dissipated. Growers are advised to use mature sawdust, but not overly aged material that may contain heat- resistant bacteria and substances unfavorable for mycelial growth.

The same substrate preparation recipes used for mushroom bag culture can be applied to substrate bottle preparation. Rice or wheat bran, corncob or other cellulosic materials can be supplemented to promote mycelial growth. Although optimal substrate formulation varies among strains, generally four parts of basal ingredient and one part supplement are mixed and the final moisture content should be 65%. How can growers know whether the moisture content is appropriate or not? A rule of thumb among mushroom growers is one or two droplets should be released when they squeeze the mixture in the palm of their hand. Some growers add lime (calcium carbonate) to the substrate mixture to improve physical structure and lower acidity.

Bottling

Prepared and moisture-conditioned sawdust mixture is loaded into the bottle feeder. Through the feeder, bottles are filled with the preset quantity of mixture. Once bottles are filled, compactors press the mixture in the bottle

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down to the pre-set height and hole-makers go through the compacted mixture.

Proper compaction gives the substrate high density, which means more nutrients will be available to mycelia and thus produce a higher yield. Vertical holes in the bottle permit even distribution of mushroom spawn to the bottom, which allows for fast, even colonization. Fast depletion of nutrients in the substrate, in turn, leads to an early fruiting.



Figure 5. Mixer to feeder



Figure 6. Bottle feeder



Figure 7. Feeding, compacting and hole making



Figure 8. Inoculation holes

Sterilization



Figure 9. double-door autoclave

Filled bottles are loaded into an autoclave. Commercial scale autoclaves have double doors: one for entry and the other for exit. As seen on the floor plan, post-sterilization contamination risk from exposure to outside air is almost removed since sterilized bottles are removed from the exit-only door in the well-controlled, dust-free cooling room.

Growers are advised to make sure the autoclave has enough water and fuel so that sterilization will not be interrupted. Bottles should be sterilized at 121℃ or 15 psi for 60-90 minutes (from the point the temperature or the pressure reaching 121℃ or 15 psi). More reasonable precautions would include wearing protective gloves and removing sawdust litters from the bottle surface that could act as a contamination vector from the bottles.

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Cooling and inoculation

When the bottles are removed from the autoclave they should be cooled to 20℃ in the cooling room. Slow cooling is advisable as condensation occurs when hot bottles are abruptly exposed to cool air. This is why some mushroom operations have a pre-cooling room that is used before the cooling room. Bottles ready to inoculate are moved from the cooling room through a small window into the clean bench on the skate wheel conveyor. Before inoculation, the inside of the laminar flow hood must be disinfected with an ultraviolet lamp and 70% alcohol. The floor must be mopped with 10% bleach. Some large-scale farms have an air-shower before entry to the inoculation area. The highest sanitation is required since one might grow weed and disease fungi inside contaminated bottles. Visitors and workers should take off their shoes and wear clean clothing when entering this part of the operation.

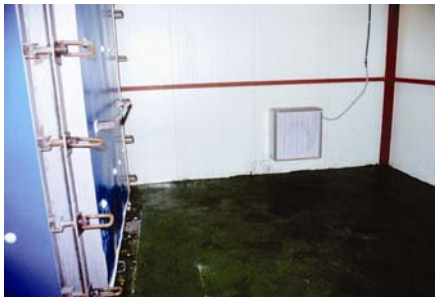


Figure 10. Autoclave door to the pre-cooling room



Figure 11. Wheel conveyer from cooling room to inoculation room

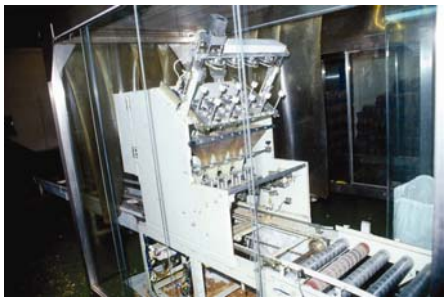


Figure 12. Inoculator

Spawn run

Inoculated bottles are hauled to an incubation room, where temperature and humidity is maintained at 17-18℃ and 65-70%, respectively. The spawn run is strain-dependant, but usually takes 20-25 days. Ventilation time and frequency vary largely depending on room temperature, humidity and the number of bottles. Growers can determine ventilation time and frequency by measuring the CO_2 concentration. The maximum upper limit of CO_2 concentration for mycelial growth is 3,000 ppm. During incubation, it is critical to perform a close examination of the bottles and look for any contamination. When unnoticed, contaminated bottles can ruin all the hard work involved in substrate preparation, inoculation, and incubation. Before fruiting, some growers opt for removing aged mycelia on the top part of the bottle.



Figure 13. spawned bottles at the incubation room



Figure 14. Good colonization

Fruiting

When 90% of the substrate in the bottle is colonized, they are brought to a growing room or exposed to a lower

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temperature (such as when one incubates and grow mushrooms in the same place). Fruiting is induced by low temperature or high humidity as the mycelia shift into reproductive growth from vegetative growth. Growth parameters for mushroom development are the same as with mushroom bag or shelf cultivation.



Figure 15. Pinning

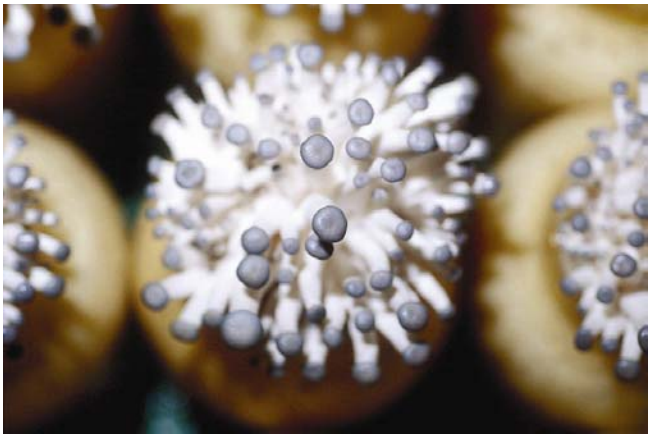


Figure 16. Fruiting



Figure 17. Fruiting bodies in the bottles

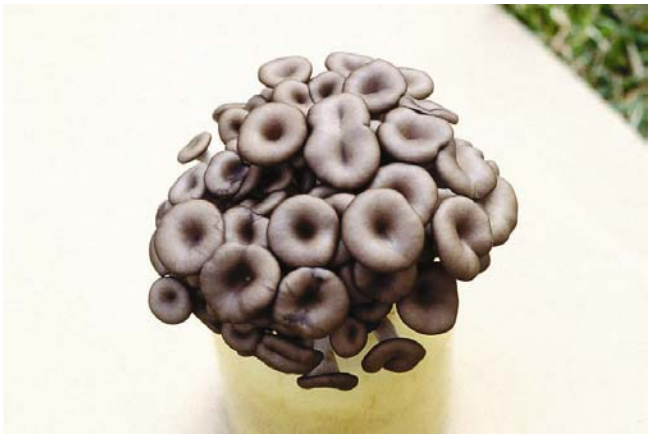
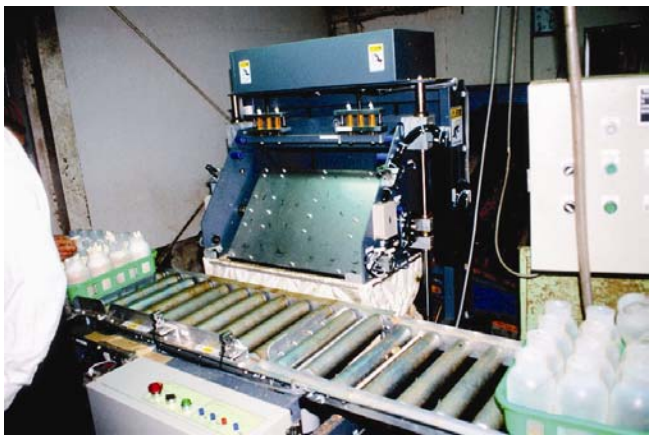


Figure 18. Fruiting bodies ready to harvast

Emptying

After harvest, bottles are loaded into the emptying machine. This de-bottler first removes spent substrate and then washes the emptied bottle with air or water. The emptying area should be far from the growing facilities since the used mushroom substrate might harbor spores of weed or disease mold.





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Figure 19, 20. De-bottling

This new growing method saves much labor by automating the whole production process. Mushrooms can be "manufactured" all through the year in microclimate controlled rooms. This allows for a predictable and stable cash flow. However, as one may imagine, the initial set-up cost is too high for most beginning growers. In addition, as mushrooms are mass-produced and spawn is also self-produced in the system, skilled sterile techniques and strict hygiene practices are required. Wise growers employing different cultivation methods could use their creativity to adopt the good points of a bottle cultivation system. Growers are advised to **"start small and smart but grow big."**

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 8

Pest and Disease Management

Pest and Disease Management

Jae-Soon Cha
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A wide range of diseases and pests can cause serious problems in mushroom cultivation, and management of those diseases and pests is a key factor in successful mushroom production. The main reasons for the existence of many diseases and pests problems in mushroom cultivation can be summarized as

- Mushroom cultivation conditions such as high humidity and warm temperature are favored by many pathogens and pests.
- There is a limit on chemical use for control of diseases or pests in mushroom cultivation.
- Pathogens and pests are readily attracted inside and/or outside mushroom houses involved with continuous cultivation.
- Growing houses are not usually well equipped for environmental control.

Basic Practices for Disease and Pest Management

- Sanitation and strict hygiene are the most important preventive methods for pest and disease control. Without them, effective disease or pest control will never be achieved. Every practice must focus on exclusion and elimination of pathogens or pests.
- Keep doors closed and avoid any practices that expose substrates to pathogens or pests during spawning.
- Keep mushroom flies from entering mushroom houses by installing screens on windows and doors.
- Inspect mushroom bags or beds carefully for early detection of pests and diseases.
- Keep mushroom bags or beds clean by removing any mushroom debris or mushroom stumps shortly after harvest.
- Keep the floors clean. Do not dump any waste near mushroom houses, which can attract mushroom flies.
- Disinfect or pasteurize spent substrate before removing it from mushroom houses after cultivation.
- Clean and disinfect mushroom houses thoroughly before a new crop.
- Clean and disinfect equipment frequently.
- Wear clean clothes and shoes and wash hands before entering mushroom houses.

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GREEN MOLD AND *HYPOCREA* DISEASE

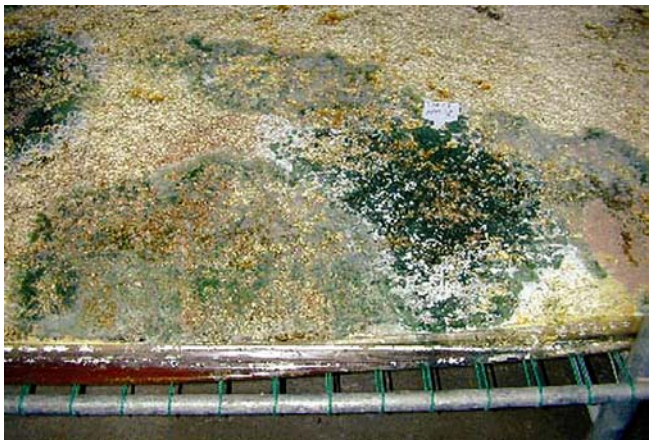
Any disease caused by green colored mold (fungi) on mushroom bags or beds is called "green mold disease." Green color showed by the fungi comes from their spores, not from hyphae. Hyphal color of the fungi is usually white. More than 30 fungi are reported as casual agents of green mold disease on mushrooms. *Trichoderma* spp., one of the major pathogens of green mold disease, reproduce by asexual spores − green conidiospores. However, some of *Trichoderma* spp. have not only asexual cycle but also a sexual stage (*Hypocrea* spp). *Hypocrea* spp. form white or brown stroma in which sexual spores, ascospores are formed. Recently *Hypocrea* spp. that do not produce asexual stage in their life cycle have been shown to cause a severe problem in oyster mushroom cultivation in Korea.

Pathogens

- Major pathogens of green mold disease on oyster mushroom are reported as *Trichoderma virens* (= *Gliocladium virens*), *T. viride, T. harzianum,* and *T. koningii* in Korea.
- *Hypocrea* sp. forms white or brown stromata for *Hypocrea* disease.
- Major pathogens of green mold can vary dependant on region or cultivation method or medium because more than thirty fungi are known to cause green mold on mushrooms, and pathogenicity or proliferation conditions of each fungus are different. For example, *T. harzianum* 2 is a major pathogen of green mold in Europe, while *T. harzianum* 4 is a major pathogen in the USA on button mushrooms.

Symptoms

- Hyphal growth stage of pathogens in mushroom bag or on mushroom bed is difficult to distinguish from mushroom hyphae by color since both are white. However, green mold fungi form denser mycelia and more aerial hyphae than oyster mushroom.
- Green color appears when pathogen produces conidiospores from aerial hyphae. If pathogen was introduced at the spawning stage, green patch usually appears 10-15 days later on cultivation bed.
- It is difficult to early identify *Hypocrea* spp. because they do not turn the infected area green and a white stroma formed by *Hypocrea* spp. is similar to the primordia of oyster mushroom.
- If stroma appears, the pathogen has already occupied the substrate deeply and to a wide extent.
- Mushroom hyphae stop their growth around the green patch and are gradually covered by green mold.



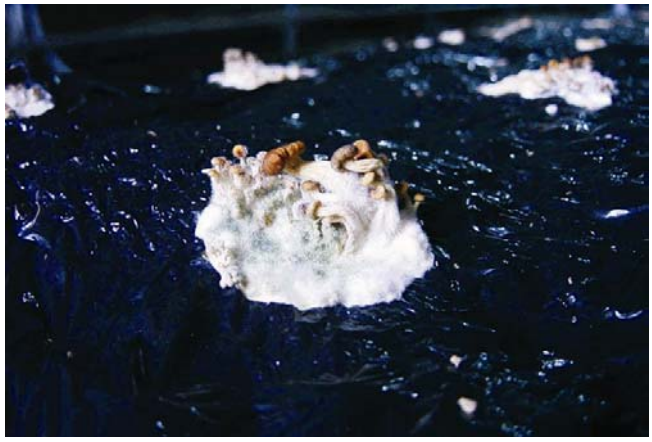


Figure 1, 2. Green mold on cotton waste substrate

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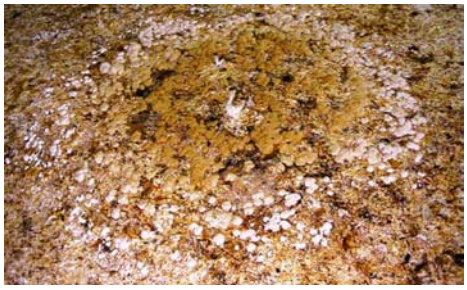


Figure 3. White stroma of ***Hypocrea*** sp. on cotton waste substrate (Photo courtesy of Seung-Hun You)



Figure 4. White stroma of ***Hypocrea*** sp. on rice straw substrate



Figure 5. Brown stoma of ***Hypocrea*** sp.

Control Measures

- Sanitation and hygiene are the most important control methods for green mold disease. Stick to the **"Basic Practices for Disease and Pest Management."**
- Severe infestations with green mold are found in poorly pasteurized substrates with uneven moisture content.
- Do not use green mold-contaminated spawn. Any green patch in or around spawn bottles is a major source of pathogenic spores. Dust from a green patch can provide inoculum for whole mushroom bags or beds at the inoculation stage.
- Observe carefully mushroom substrates during hyphal growth stage, and remove or treat any spot with dense white mycelial growth indicating green mold mycelia. Spray or drench with a 500 ppm solution of Sporgon (prochloraz-manganese complex; 50%) on the spot.
- Spraying the affected parts with 250-500 ppm of Sporgon before pasteurization is reported to prevent green mold and *Hypocrea* disease.
- Benomyl and thiabendazole are also known as control agents for green mold disease. However, resistant strains of the pathogens are more common in Korea, and recent results of experiment showed that prochloraz-manganese complex is by far the most effective.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 8

Pest and Disease Management

BROWN BLOTCH DISEASES

Brown blotch disease by bacterial pathogen causes significant crop loss. The disease is very common in mushroom houses in Korea. Various disease symptoms are observed on cultivation beds. Yellowing of fruiting bodies can be easily caused by environmental factors. A rapid change of humidity caused by too much ventilation is diagnosed as being conducive to bacterial brown blotch.

Bacterial Brown Blotch Disease

Pathogens

- Major pathogen is *Pseudomonas tolaasii*. *Pseudomonas agarici* was also reported as causal agent, but the importance of that bacterium as a pathogen of brown blotch disease is questionable.

Symptoms

- Bacterial brown blotch has various symptoms (Fig. 1). The most typical symptom is a brown spot on the caps and stipes. The brown spots enlarge and coalesce with other spots, and the affected areas are sunken and covered with sticky material. At this stage a rotten fish smell is evident.
- Rarely, the entire fruiting body is discolored with a reddish brown color and appears water logged.
- Young fruiting bodies are covered by a clear, glossy material and stop growing.
- *Pseudomonas tolaasii* is isolated in all these diseased mushrooms, but it is still possible that mixed infections cause these various symptoms.

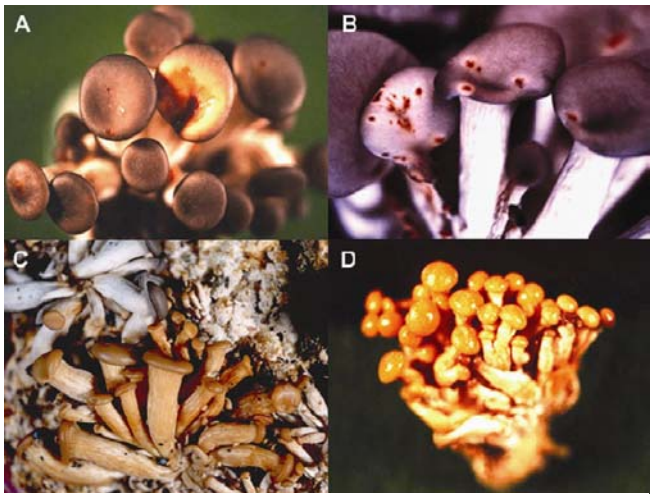


Figure 1. Symptoms of bacterial brown blotch disease on oyster mushroom

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Control measures

- Sanitation is the basic control measure for bacterial brown blotch. Follow the **"Basic Practices for Disease and Pest management."**
- Pasteurize substrates thoroughly and use healthy spawn.
- Control mushroom flies. Mushroom flies are well known vectors of the pathogen.
- Try to maintain constant humidity and temperature in growing houses. Abrupt temperature and humidity changes increase the incidence of brown blotch.
- Free water on fruiting bodies makes the pathogenic bacteria grow rapidly. Try to avoid free water on mushroom surfaces by ventilating after watering.
- Do not water too much. Brown blotch is favored by excessive moisture.
- Chlorinated water is effective to prevent the brown blotch disease. Sodium hypochlorite (NaOCl) and calcium hypochlorite [$Ca(OCl)_2$] are most commonly used. Recently Biospot®, sodium dichloroisocyanurate, has also become available. Active chlorine content varies among the different formulas and chlorine is well known to be vaporized easily. A routine use of 5 ppm chlorinated water (active chlorine concentration) prevents brown blotch incidence. If brown blotch is observed in mushroom bags or on mushroom beds, use 20 ppm chlorinated water.

Fungal Brown Blotch Disease

Pathogen

-*Verticillium fungicola*. This pathogen is suspected, but the actual cause of the disease remains yet to be proved.

Symptoms

- Mushroom cap is partially or entirely discolored yellow to brown (Fig. 2a). The spots are not as clear as the spots caused by bacterial brown blotch.
- The shape of fruiting bodies become abnormal and mushrooms stop growing (Fig. 2b, 2c, 2d).



A. Discoloration



B. Abnormal growth

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C. Stunted growth



D. Malformation

Figure 2. Symptoms of fungal brown blotch

Control measures

-Typical bactericide or chlorine disinfectant is not effective. Fungicides such as Sporgon, benomyl, and thiabendazole, are effective, which indicates the cause of disease is fungi rather than bacteria.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 8

Pest and Disease Management

VIRAL DISEASE

Viral disease in oyster mushroom has not been well documented. However, they occur sporadically and cause huge losses in some mushroom farms.

Causing Agents

- Two isometric viruses, OMIV-I and -II (oyster mushroom isometric virus I and II) were isolated from oyster mushrooms showing viral disease symptoms.
- Virus particle sizes of both viruses are same as 30 nm at diameter. Coat proteins and ds-RNAs that the viruses contain are different.
- Except these two viruses, the same size of cryptic virus was found in healthy oyster mushroom. The third virus is also isometric and 30 nm in size.

Symptoms

- Typical symptoms of viral disease on oyster mushroom are quite similar to 'La France disease' which is a well-known viral disease in button mushrooms (*Agaricus bisporus*).
- Delay in fruiting body formation, shortening in stipe, abnormal shape and thin mushroom caps are the major symptoms (Fig. 1b, 1c, 1d). Fruiting bodies are not formed at all on some infected mushroom beds.
- The viral-infected hyphae grow very slowly on agar and their density is very low (Fig. 1f).



A. Healthy oyster mushrooms

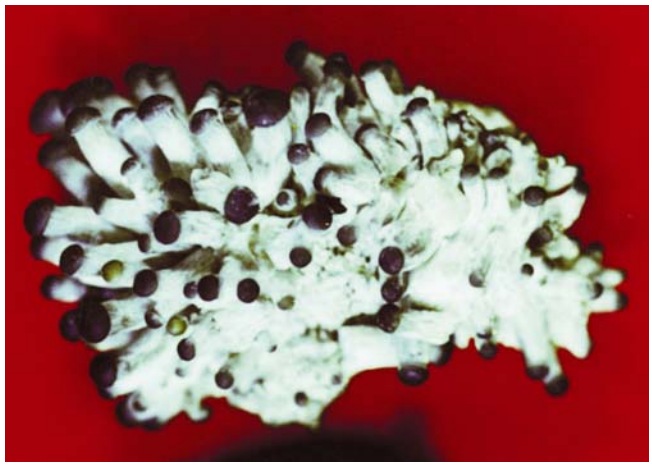


B. Viral-infected mushrooms

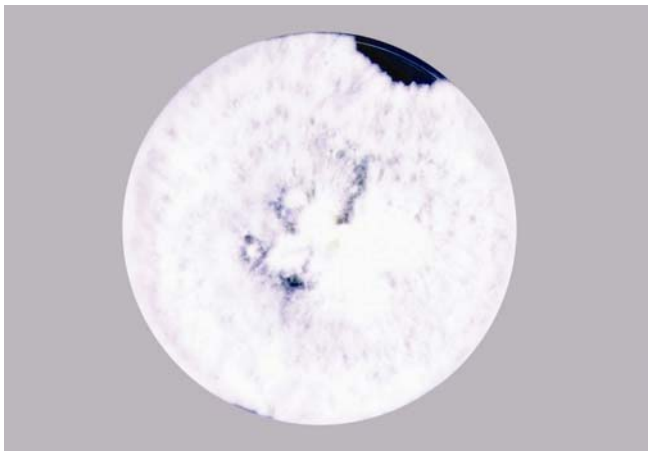
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C. Viral-infected mushrooms



D. Viral-infected mushrooms



E. Healthy culture on agar plate



F. Viral-infected culture

Figure 1. Healthy and viral-infected oyster mushrooms and culture (Photo courtesy of Hyun-Suk Lee)

Ecology

The ecology of viral disease in oyster mushrooms is not known at all so far. For La France disease, a known viral disease of button mushroom, it has been known that basidiospores mediate the spread of the virus. How this virus is spread in oyster mushrooms is not yet known.

Control Measures

- Viral diseases cannot be cured in infected mushrooms by any cultural or chemical treatment. Prevention is only the way of control the viral diseases.
- Use healthy spawn. Prepare spawn using virus-free strain. Do not use any culture containing the viral particles.
- Clean and disinfect thoroughly the growing house in which any viral disease occurred. It has not been proven yet, but spores or mycelium of viral infected mushrooms can transfer the viral disease in a manner similar to the spread of La France disease of the button mushroom.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 8

Pest and Disease Management

PESTS

Oyster mushroom cultivation beds provide very good conditions for pests, plenty of food, warm temperatures, and high humidity. Five kinds of flies and two types of mites are reported as the major pests for oyster mushrooms.

Sciarids (*Lycoriella mali*)

Sciarids are the most important pests of oyster mushroom. Adults are about 2mm with long thread-like antennae (Fig. 1). Larvae are 6-12mm long with a distinct black head capsule (Fig. 2). Larvae feed on mycelia, small pin-heads, and large mushrooms. Such feeding results in cuts in the mycelium, less primodium formation, and cavities in the stipes and caps of large mushrooms. Adults spread diseases and mites. Female adults lay 100-130 eggs at a time on cultivation beds and the eggs hatch after 4-5 days at 20℃. Growth and development of the fly is delayed or poor when temperatures are lower than 15℃ or above 30℃.



Figure 1. Female adult sciarid and eggs

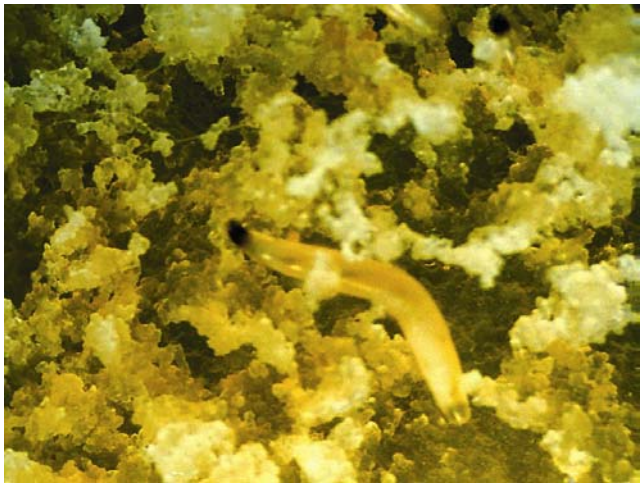


Figure 2. Lava of sciarid

Scaptosids (*Coboldia fuscipes*)

This fly occurs mainly during summer crop cultivation. Larvae feed on the mycelium, causing rotting of substrate which results in yield loss. Both adults and larvae are known to transfer mites and diseases. Larvae grow and develop fast at above 25℃, but it takes much longer for their growth and development when the temperature is below 20℃. This indicates that their growth is favored by high temperature during summer cultivation.

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Figure 3. Male adult ***Coboldia fuscipes***



Figure 4. Larva of ***Coboldia fuscipes***

Cecids (*Mycophila* sp.)

Adults are very small, less than 1 mm, which makes them difficult to see inside the growing room (Fig. 5). Larvae are 1-3mm in length suck the nutrients from hyphae and also attack mushroom stipes and caps. Larvae populations can increase rapidly within a short time because they can reproduce by paedogenesis during which each larva releases 14-20 daughter larvae every 6 days. Mushroom bags or beds become orange in color if huge numbers of orange colored larvae occur. Larvae are well known to transfer various bacteria that cause the breakdown of mushrooms.



Figure 5. Adult cecid



Figure 6. Larva of cecid



Figure 7. Cecid larvae on mushrooms

Phorids (*Megaselia tamiladuensis*)

Adults are 2-4mm and move quickly by hopping on the substrate. Larvae are 4-6mm long with a white and transparent body and they do not have a distinct black head. Larvae feed on mycelia and make cavities in mushroom fruiting bodies. Phorids usually occur during summer cultivation, but they normally cause less damage than other flies.



Figure 8. Adult phorid

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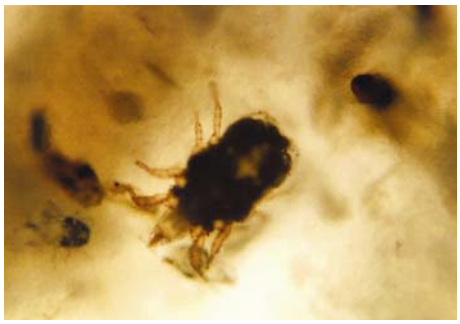


Figure 9. Mite

Mites

Mites belong to the class Arachnida, not Insecta. *Tarsonemus* sp. and *Histiostoma* sp. are major mushroom damaging mites. They are small and invisible to the naked eye. Mites feed on mycelia and fruiting bodies, causing yield loss and a decrease in mushroom quality. Mites carry pathogens and nematodes, sometimes causing itchy rashes among growers.

Mycetophil (*Mycetophila* sp.)

Adults are big and yellowish (Fig. 10). Larvae are 15-20mm long and grayish brown and construct cocoons with threads on the substrates or mushrooms. Young fruiting bodies become brown and stop growing. Larvae also cause large cavities in the stipes (Fig. 11).



Figure 10. Adult mycetophil

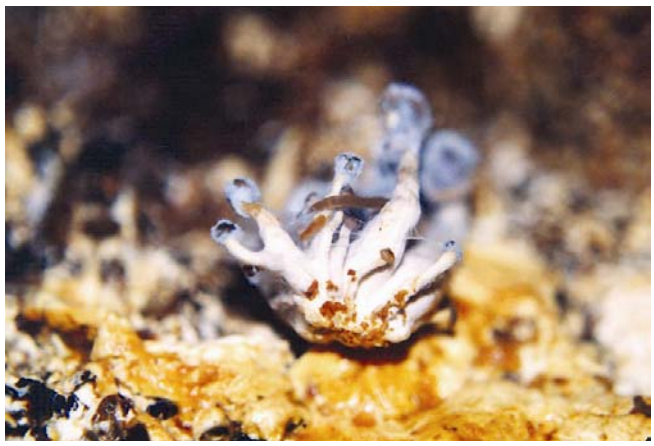


Figure 11. Infection with mycetophil larvae

Control Measures

- Sanitation and hygiene is the most important control method of pests. Keep the **"Basic Practices for Disease and Pest management"**
- Clean and disinfect mushroom houses thoroughly before cultivation.
- Remove any waste, weed, mushroom debris, and water containers inside or outside mushroom houses that attract flies or on which flies can live.
- Exclude flies with a mesh with apertures not greater than 0.5-0.6mm on air inlets. Keep doors closed insofar as possible, particularly during spawning and mycelium growth phase.
- Maintaining a low fly population during spawn run is of major importance as early flies give rise to the initial infestation which culminates in the high populations that appear later in the cropping cycle.
- Pasteurize substrates thoroughly. This is very important, especially for mite control.
- Burning mosquito coils is known as a very effective control method of adult flies inside mushroom houses.

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 8

Pest and Disease Management

ABNORMALITIES IN FRUITING BODY

The formation and growth of fruiting bodies are sensitive to environmental conditions, such as temperature, humidity, carbon dioxide concentration, and moisture content in the mushroom substrate. Improper balance of these factors can induce fruiting body deformations.

Temperature and Relative Humidity

Temperature and humidity affect the fruiting bodies' shape. Optimal cultivation conditions vary with strains. The changes in the fruiting body shapes of an oyster mushroom strain at different conditions are described below. Optimal temperature and humidity for fruiting body formation of this mushroom is known as 13-16℃ and > 80%. High and low temperature indicates > 16℃ and < 12℃, respectively and high and low humidity indicates > 80% and < 60%.

Under high temperature and high humidity
- Cap/stipe ratio smaller (small cap/long stipe)
- Cap color becomes lighter (grey-brownish grey)
- Depression in the center

Under high temperature and low humidity
- Cap margin gets thinner and brittle
- Cap turns into umbrella shape
- Cap color become very light (light grey-white)
- Stipe becomes very thick

Under low temperature and low humidity
- Cap color becomes dark (dark brown)
- Stipe becomes thick or middle of stipe is swollen or barrel-shaped
- Fruiting bodies grows very slowly and produces low yields

Under low temperature and high humidity
- Relatively strong color and strong fruiting bodies formed
- Fruiting bodies grow slowly and the number of fruiting bodies reduced

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Figure 1. Fruiting bodies at 60% (R.H.)



Figure 2. Fruiting bodies at 90% (R.H.)

CO_2 Concentration

High carbon dioxide (CO_2) concentration inside mushroom houses is one of the major causes of abnormality in fruiting bodies. Proper ventilation is needed in order to reduce CO_2 concentration. However, too much air movement caused by excessive ventilation also induces abnormalities in fruiting body shapes. An increase of carbon dioxide concentration can decrease cap sizes and increase length of stipes. However, even stipes are short at CO_2 concentrations of more than 0.5%.

Table 1. Fruiting body shape at different CO_2 concentration

CO_2 conc. (%)	Diameter of cap (mm)	Length of stipe (mm)
0.03	6.5	4.6
0.1	3.4	6.8
0.3	2.4	6.7
0.5	0.6	2.5



A. CO_2 concentration 0.03%



B. CO_2 concentration 0.1%

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C. CO_2 concentration 0.3%



D. CO_2 concentration 0.5%

Figure 3. Effect of CO_2 concentration on mushroom morphology of ***P. ostreatus***
(Photo courtesy of Kap-Yeol Jang)

Watering (Substrate Moisture Content)

Watering after primodium formation to maintain optimal moisture content in substrate is very important for the production of high yields of high quality oyster mushrooms. Disease usually increases with too much watering on cultivation beds (excessive moisture content). Too little watering reduces yields and induces abnormal shapes in fruiting bodies. Substrate blocks shrink and fruiting bodies become brown on dry cultivation beds, and new mycelia grow and many small new fruiting bodies are formed on old mushroom fruiting bodies.



A. Substrate was separated with box by shrinking



B. Browning of fruiting body

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C. Baby fruiting bodies formed on an old fruiting body



D. Baby fruiting bodies formed on an old fruiting body

Figure 4. Oyster mushroom fruiting bodies with low substrate moisture content
(Photo courtesy of Kap-Yeol Jang)

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 9

Post-harvest Management

RECYCLING OF SPENT OYSTER MUSHROOM SUBSTRATE

Danny L. Rinker[1], ZERI[2], Seung Woo Kang[3]
[1] University of Guelph, Canada
[2] ZERI (Zero Emissions Research and Initiatives)
[3] MushWorld



Figure 1. Mushroom bed contaminated by green mold

Spent Mushroom Substrate (SMS) needs heat treatment before being removed from the growing chamber. But it takes extra cost, and thus, some mushroom growers want to throw away the contaminated SMS far from the farm in order to prevent re-contamination (Fig. 1). Without proper treatment, contaminated SMS can cause re-contamination. In opposition, recycle of SMS can increase sustainability and also help farm economy.

This article is excerpted from "Spent mushroom substrate around the world" (Danny Lee Rinker) and "Project report" (ZERI Foundation), and edited by Seung Woo Kang.

A Brief Description of Spent Oyster Mushroom Substrate

At the end of several mushroom harvests, the growing material is considered spent. SMS contains enough digestible nutrition, primarily decomposed by mushroom, to be fed livestock (Table 1, 2). It will increase growers' income and protect environment to recycle SMS for feeding livestock or soil for other plants. As you can see in Table 2, *Pleurotus* compost contains high percentage of three primary nutrients (nitrogen, N; phosphorus, P or P_2O_5 ; potassium, K or K_2O) as a fertilizer.

Table 1. Characteristics of spent oyster mushroom substrate

Ash	TSS*	C (%)	H (%)	N (%)	Mg (mg/L)	Ca	Na	K	Mn	Ni	Zn
72.92	830	23.6	4.06	5.99	7.72	30.13	1.32	4.47	2.2	nil	2.34

* TSS: Total Soluble Solids

(Source: Chiu *et al*., 1998)

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Table 2. Analysis of the fertilizer value of compost from the edible *Pleurotus ostreatus*

	N (%)	P_2O_5 (%)	K_2O (%)
Pleurotus compost	1.70	0.61	1.13
Human manure and urine	0.30	0.16	0.30
Pig manure	0.60	0.60	0.50
Cow manure	0.59	0.28	0.14

(Source: Zheng *et al*., 2002)

For instance, a Thai mushroom grower recycles his spent substrate as a soil for other plants (Fig. 4, 5). He put the spent substrate for over one year under outdoor condition before reuse.



Figure 2. Spent sawdust substrate from bag cultivation



Figure 3. Spent cotton-waste substrate from shelf cultivation



Figure 4. Spent straw substrate during aging before reuse



Figure 5. Tropical plants grown on aged spent straw substrate

SMS Recycling Cases in ZERI Projects

Colombia:spent coffee-substrate for feeding cattle and pigs

The organic wastes from a coffee farm contain biochemicals, which do not permit their reuse as cattle feed. Therefore, they could at best be used for earthworm farming. However, enzymes of the tropical mushrooms are capable of neutralizing these biochemicals. Even better, the mushroom mycelia (roots) are rich in protein (up to 38%). This means that the waste from the coffee farm-after mushroom farming - becomes an excellent additive to cattle and pig feed.

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4kg of vegetable or fungal protein produces 1kg of pig meat. In the case of cattle farming, the ratio is 7 : 1. Many consider this to be a very inefficient way for us to get protein. However, we usually do not consider the volume of energy pig or cow manure can produce in a digester. 100 pigs produce enough manure each day to generate a calorific energy value equivalent to 10L of petroleum. Manure energy (biogas), should be used first and foremost by the coffee farmer for the preparation of the substrate for mushroom farming. The coffee bush waste needs to be pasteurized, and for specific types of mushrooms sterilized, before being used as a mushroom growing substrate. And since this requires a continuous flow of energy, it is best to use a locally available renewable energy source - and pigs always produce waste.

Africa: spent substrate of water hyacinth weed for cattle feeding and vermiculture

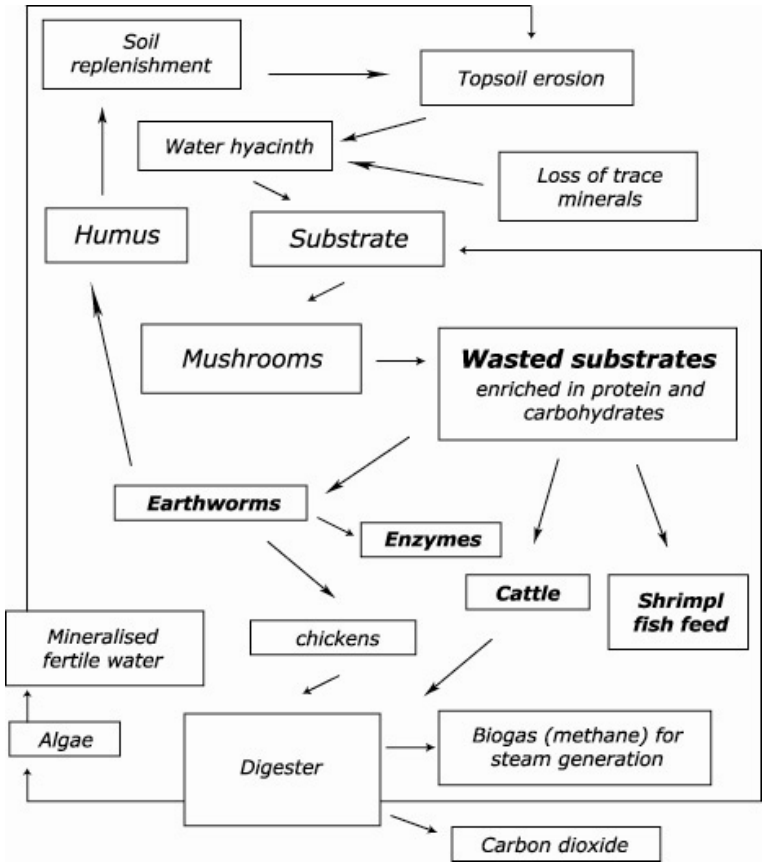


Figure 6. The integrated biosystem for the water hyacinth

The Southern African region has an abundance of the waterweed commonly known as water hyacinth (*Eichhornia crassipes*). This aquatic weed has become a serious problem because it grows very fast and in the process chokes up waterways, blocks navigable waterways, reduces fishing points, and in some cases blocks water pumps. The adverse impact of the excessive growth of the water hyacinth is being felt in the economies of all lake districts of Africa: Zimbabwe, Malawi, Zambia, Tanzania, Kenya, and Uganda. Then, scientific research initiated by the ZERI Foundation demonstrated that dried water hyacinth is the best substrate for farming mushrooms and that the spent substrate after fungi harvesting is rich in protein from the mycelia of the mushrooms and is excellent feed for earthworms, which convert it all into humus and can be fed to chickens, ducks and pigs.

After only 30 days, the dried substrate from water hyacinth produced a variety of mushrooms. Once harvested, it did not take more than 10 days to harvest a second and even a third flush. 1 ton of dried water hyacinth substrate generated 1.1 tons of mushrooms, thus generating more mushrooms than base material and out-performing traditional substrates such as sawdust. The residual substrate of water hyacinth after mushroom farming, is a rich food-base for cattle. Since nearly all the lingo-cellulose has been broken down by the enzymes of the mushroom, the rest of the material can also be used to farm earthworms, which will convert the material into a humus. The humus that is produced in the process would then be reapplied to the soils, recovering and replenishing some of the lost topsoil. Earthworms are also an excellent chicken feed.

Some Studies on SOMS* Recycling

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Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 9

Post-harvest Management

MUSHROOM STORAGE AND PROCESSING

Byung Sik Kim
MushWorld

The following paper is summarized from *Shiitake Growers' Handbook*: *The Art and Science of Mushroom Cultivation* by Paul Przybylowicz and John Donoghue and *Tropical Mushroom Cultivation* by Tricita H. Quimio.



Figure 1. Harvested oyster mushroom

Mushrooms continue to respire after harvest and they have a relatively high respiration rate compared to other fresh produce, the respiration rate of oyster mushroom being three times greater than most fruits for example. Respiration rate is a good indicator of storage life and respiration results in changes in mushroom texture.

Spoilage during storage can be caused by bacteria and fungi within the mushrooms. Bacteria and enzymes continue to increase during cold storage. This results in rapid deterioration when the mushrooms are removed from cold storage. The mushrooms' texture is altered as they lose their firmness and their flesh darkens. The water inside the mushrooms is also favorable for bacterial growth.

Many mushrooms are white to gray in color while they are growing. Under certain storage circumstances, however, the enzymes react with oxygen and form brown pigments. Such discoloration seriously decreases the quality of mushrooms. Mushrooms are 85-95% water. There are no barriers to water loss from their surface. Water loss in the mushrooms after harvesting is influenced by the status of the mushrooms, the humidity, fresh air and atmospheric pressure. When mushrooms wilt and shrivel, the quality of fresh mushrooms is lowered.

Fresh mushrooms have a short shelf life. Therefore it is necessary that they are either marketed soon after harvesting or preserved with special care such as in cold storage or other controlled environment storage.

Short Term Storage

The shelf life of fresh mushrooms may be extended by refrigeration at 1-4℃. Cooling the mushrooms result in lower rates of all the physiological process within the mushrooms. During the initial cooling there is a high cooling load. Once the mushrooms are pre-cooled, however, the cooling load is much reduced. The shelf life of mushrooms

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may vary from 1 day to 2 weeks.



Figure 2. Trimming mushroom





Figure 3, 4. Trimming. Washing, Weighing and packaging

Preservation of mushrooms at cool temperatures generally results in effective short term preservation by retarding the growth of microorganisms, reducing the rate of post harvest metabolic activities of the mushroom tissues, and minimizing moisture loss.

The temperature of the mushrooms at harvest is equal to the temperature in fruiting area. Generally the metabolic temperature of mushrooms is 15-18℃ after harvesting. Heat is generated by processes within the mushroom and is high during fruiting. If mushrooms were not rapidly cooled but were put into the boxes or covered by PVC film storage, their temperature would increase due to the metabolic processes and then spoilage during storage could be caused by bacteria and fungi within the mushrooms.

To stop this metabolic process rapidly, the mushrooms should be cooled to storage temperature of 0-2℃ within five hours of picking.



Figure 5. Low temperature room for the storage of Mushrooms



Figure 6 Fresh mushrooms were packed by PVC film in the commercial refrigerator.

The best method for fresh storage of oyster mushroom is to keep them at 8-10℃ in packed container wrapped in plastic film. It's called 'PVC film storage'. Wrappping mushrooms with such microporous or perforated plastic film can improve their storage life, as this reduces the moisture loss and preserves the quality of mushrooms. Carbon dioxide levels increase and oxygen levels decrease in wrapped containers due to mushroom respiration. The gas composition can be modified by the respiration of mushrooms inside the package.

Long Term Storage

For long-term storage of mushrooms, canning, pickling and drying processes are employed. The quality of the preserved product is rarely comparable with that of fresh mushrooms, and these processes are not always suitable

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for all types of mushrooms.

Drying

Drying is a method of preserving edible mushrooms such as shiitake and wood ear mushrooms. It is not often used for button mushrooms or oyster mushrooms, but oyster mushrooms can also be stored and marketed in dried form. Drying preserves the mushrooms by removing enough water to inactivate the enzymes and microorganisms. Mushrooms preserved by drying have a good flavor and the drying prevents deterioration. Dried mushrooms are convenient for long-term storage and transportation. The moisture content of fresh mushrooms is 70-95% depending upon the harvest time and environmental conditions; that of dried mushrooms is near to 10%. There are several methods commonly employed for mushroom drying.





Figure 7, 8. Dried shiitake and white wood ear mushroom

Sun drying



Figure 9. The mushrooms are put into the bags after drying.

In this drying method, mushrooms are spread on the shelves in such a way that the gills face upward and are directly exposed to sunlight. Drying time required will vary depending on the weather conditions. In general, the quality of sun-dried mushrooms is lower than that of the mushrooms that are dried by the thermal power drying or hot-air drying. The moisture content is also higher and this means higher susceptibility to molds and pests.

Thermal power drying

The process of thermal power drying should begin with mushrooms at a relatively low temperature. Mushrooms should be dried during sunny days at an initial temperature of 35℃ while mushrooms should be dried during damp days at an initial temperature of 30℃. After five hours of heat for mushrooms under sunny conditions and seven hours of heat for those during the rainy season, the temperature can be raised gradually and then kept at 40-60℃ for 12-18 hours. In addition to preserving the product, drying can enhance the flavor and appearance of the mushrooms.

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Figure 10. Ear mushrooms are dried In 60-80℃ for 6 hours in firewood stove



Figure 11. Product of dried *Phellinus baumii*



Figure 12. Dried fruitbodies of *Hericium erinaceus*

Hot-air drying



Figure 13. Hot-air drying of *Agaricus blazei*

In the hot air drying method, hot air is blown into the dryer and mushrooms on the shelves are exposed to hot air. The temperature and humidity of the air can be controlled to optimum conditions by use of heaters and recirculation vents. Mushrooms produced by this method have better quality with higher hygienic conditions and brighter color compared to the sun-dried mushrooms. The size of the drying chamber varies depending on the production scale. Usually 10-12 shelves are installed with the clearance of 15cm between shelves and mushrooms are placed on the shelves stem downward inside the drying chamber. The drying chamber should be heated up to 40-50℃ prior to loading the mushrooms. If the mushrooms are loaded at the beginning, it will take a longer time before the chamber reaches the effective temperature, and this will cause self-digestion of mushrooms by their inherit enzymes and will result in softening of texture and spoilage.

It is desirable to sort mushrooms according to the size before drying. This will ensure uniform drying and results in good quality products. The dried mushrooms are apt to absorb moisture from the air, so they should be properly stored. If the moisture content of the mushrooms reaches about 20%, the mushrooms will easily be infested by insects and molds. Therefore, the dried mushrooms should be put into polyethylene bags, sealed and kept in a dry, cool and dark place. For prolonged storage, the mushrooms should be packed in cartons or wooden boxes and kept at 2-5℃ in a low temperature storage area.

Canning and bottling

Canning is by far the most common process used for preserving mushrooms. The production of mushrooms by canning has become considerably more specialized in recent years. In general terms, canning is divided into seven basic operations: cleaning, blanching, canning, sterilization, cooling, labeling, and packing. This method is widely used by the industry.

Browning and blemishing of mushrooms can be reduced by trimming immediately after harvest. If mushrooms are not canned immediately, they should be refrigerated until processing starts. Color and texture are retained by storage and proper storage can also increase canning yield. At this stage, an appropriate level of sodium metabisulphite or ascorbate is incorporated for color retention. The mushrooms are then rinsed and blanched for two minutes. Blanching is used to reduce the activity of enzymes.

After blanching, the mushrooms are placed in cans containing 2.5% sodium chloride and 0.24-0.5% citric acid. The cans are then sealed and sterilized. Sterilization methods vary according to the type of equipment used. The

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most commonly used method is the batch process in which the cans are placed in an autoclave and sterilized for one hour 120-130℃. The cans are then rapidly cooled in the wash sink.



Figure 14. The mushrooms are put in the bottle with brine



Figure 15. Canned *Pleurotus nebrodensis*

The principle of bottling is the same as canning but requires much less instrumentation, and therefore bottling can be adopted by small-scale growers without difficulty. The procedure for bottling mushrooms can be summarized as follows: Mushrooms must be processed right after harvesting in order to maintain their quality. Spoiled mushrooms must be sorted out from the wholesome mushrooms. The mushrooms should then be sorted in terms of size and quality and then boiled in water containing 0.1% succinic acid and 1% salt for 4-6 minutes of blanching.

A stainless steel knife is recommended when progressing mushrooms in order to minimize browning. During blanching, a weight loss of 35-40% is likely. Brine should be prepared according to the salinity desired by the consumers. The bottles are filled with brine and the blanched mushrooms in a desired proportion. After closing the cap halfway in order to allow air to escape from the bottles the bottles are boiled for 30 minutes or more depending on the size of the bottles. The caps are then closed tight before the bottles are taken out and cooled.

Pickling

Pickled products such as cucumber pickles are popular in many countries throughout the world. Mushrooms can also be successfully picked and produce quite favorable products when the right pickling formula is chosen.

In this process, the mushrooms are sorted and washed. They can be sliced if desired. Then they are blanched with 3% salt water for three to four minutes in boiling water. After the water drained off, they are placed immediately in cold water to cool. They are then transferred to a jar or bottle, and brine (22% salt) is added with a little vinegar, sugar and other spices such as vitamin C or citric acid to give the mushrooms some fresher color. The jars are then loosely closed and steamed for one hour. The lids are tightened when cooled and the contents chilled before eating.



Figure 16. Pickled mushrooms in Chinese market



Figure Pickled mushrooms *Coprinus comatus*



Figure 18. Pickled mushrooms *Volvariella volvacea*

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Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 10

Regional Research

MUSHROOM CULTIVATION IN KENYA

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Introduction



Figure 1. Map of Kenya

Kenya is a medium-sized, East African, tropical country with a total land area of 582,646 sq. km. The equator cuts the country in half so the sun is usually directly overhead. This third world, Sub-Saharan country has a population of 30 million people.

Kenya's economy, like those of its neighbors Uganda and Tanzania, largely depends on agriculture. Kenya has a diverse climate that allows for the growing of many agricultural crops like coffee, tea, maize, wheat, rice, sugarcane and cotton all the year round. We intend to use the residues from many of these agricultural crops as substrates for growing mushroom for both commercial purposes and home consumption.

The Kenyan people are diverse, and the country's population is made up of 42 different indigenous tribes, each of which has different eating habits. Among these, 38 tribes are known to use mushrooms as food. Apart from these indigenous tribes, the population also includes many immigrants and visitors mainly of Asian, European and American origin. It is also immigrants and foreign visitors for whom many hotels here prepare mushroom dishes. But more local people are now trying these mushroom dishes and are discovering that mushrooms are both tasty and full of nutrients.

Kenya is an active tourist destination and offers beautiful beaches along the Indian Ocean and vast savannahs rich in wildlife. These attractions lure many tourists throughout the year, and this creates a great demand for mushrooms in the hotels. Agricultural products provide Kenya's primary income, but tourism is the second major income source. Part of mushroom production in Kenya does make its way to the leading supermarkets in major towns like Nairobi and Mombasa, but the current local Kenyan production does not meet the total demand for mushrooms and must be supplemented by canned mushroom imports.

The mushroom industry in Kenya is still in its infancy and is growing slowly. To many people, mushroom growing is still a myth because there is a lack of communication between the researchers in this field and the farmers, and the exchange of cultural knowledge is rather poor.

The mushroom that is commonly grown here is *Agaricus*. Its cultivation is highly sophisticated and requires a lot of capital, which discourages most potentially interested farmers. There are a few very-small scale producers of

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oyster mushroom and shiitake in Kenya, but the few people with the knowledge of how to grow mushrooms here keep it secret and usually charge a lot of money to educate any interested farmers. This has caused slow development of the industry in a country that has a great potential for producing mushrooms.

Current Kenyan Mushroom Industry

There are several commercial mushroom farms here in Kenya. They include Agridutt Ltd., Rift Valley mushrooms, Olive mushrooms, and Devani and Kanchan mushrooms. There are also other small farms producing mushrooms but only the four major farms have their produce sold in the supermarkets. Small farms usually sell their produce in the hotels and restaurants.

The current producers can hardly meet the demand in the supermarkets and sometimes the supermarkets run out of stock of the mushrooms. It is important to note that the four major producers are just medium-sized farms with limited capacities for production.

Three types of mushrooms, including *Agaricus*, oyster mushrooms and shiitake are grown here, and the button mushrooms account for over 95% of the mushrooms production volume. Only *Agaricus* is sold fresh in the supermarkets. Only on very few occasions have fresh oyster mushrooms been sold in the supermarkets. Shiitake are usually sold directly to the hotels and individuals.

The price of mushrooms is very high compared to that of other vegetables. Due to the low supply the price has remained unnecessarily high. In the supermarkets, *Agaricus* is sold in 250g packs at a price of KES*150 (USD2). The price for the oyster mushrooms is comparable to that of *Agaricus* but shiitake costs as much as KES1,000 (USD13) for one kg of fresh mushrooms. Many poor Kenyans earn less than a dollar a day so they cannot afford a meal that makes use of these expensive mushrooms. For purposes of comparison, 250g of beef costs approximately USD0.5.

Establishment of traditional commercial-scale farms requires a huge initial capital investment, so smaller farmers are hard to grow mushrooms in a commercial scale. The commercial farm's technical expertise comes from personnel who have been trained abroad in countries where mushroom farming is popular. To start a medium-sized farm would require a capital investment of around KES40 million (USD520,000). This includes construction of mushroom houses, purchase of the land, purchase of the machines used for compost preparation, installation of air conditioners, acquisition of spawn, educating staff abroad, and many other costs related to mushroom production. These costs could be lower for a person who had the knowledge concerning mushrooms cultivation because many of the required systems could be improvised to lower the initial investment.

Many farmers interested in growing mushrooms here do not have a lot of capital and cannot afford to hire trained personnel. The greatest problem, though, is the lack of availability of mushroom spawn. There is not even one single spawn manufacturing company here in Kenya. Interested farmers have to either import spawn or use cultures from culture collections to make their own spawn.

Spawn making requires well-trained personnel in order to keep the quality high. Culture preservation is not easy for small farmers and after a few months the quality of spawn diminishes so they need to continually import the cultures in order to remain in the business (Fig. 2, 3). The quality of the spawn they make themselves is also usually low and this translates to poor yields. Mushroom growing technicians who have trained abroad are expensive, a significant portion of the businesses investment.

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Figure 2. Cultures stored in a cabinet at room temperature. Small farmers cannot afford the methods of culture preservation



Figure 3. A collection of cultures stored in a refrigerator. The cultures normally lose their vigour after six months of storage. This may be due to electricity power fluctuations which are very common and prolonged.

This writer knows of several people working in the commercial farms who have received training in Japan, Britain, France and Belgium. Few local farmers are able to go abroad for training and are discouraged from entering the mushroom cultivation business as a result.

Most commercial farmers import expensive spawn from other countries. Mushroom spawn usually costs KES600 (USD7.8)/kg including the airfreight charges.

Mushroom farmers keep their growing procedures highly secretive and access to the farms is highly restricted. As a result, the exchange of information is very poor and it is even difficult to ascertain the total production of mushrooms from these farms. This writer has personally been denied entry many times to the commercial farms. These mushroom establishments will summon mushroom experts only when problems such as diseases threaten their production.

The personnel employed in the farms can be divided into two groups. The first group includes the well-trained, well paid managers and technicians. More than half of the money a farm pays out as wages and salaries goes to this group. A single commercial farm could usually have only two people with such advanced training. The other group involved in the actual production is the workforce that provides the unskilled labour, and their number could be as high as 50 in big commercial farms. In order to increase their profits, most farms pay these people meager wages, and since the farms belong to rich owners who pay low wages to their workers, there is very little that these types of mushroom farming operations can do to change the economic situation of the people in the regions where they operate.

It is estimated that the current production of mushrooms in Kenya is 500 tons per annum, which is very low. Of this production, *Agaricus* mushroom accounts for 476 tons. Other mushroom species are not widely farmed. Some small farms exist that do produce around 20kg of shiitake within a period of one week (Fig. 4, 5, 6, 7). Oyster mushroom cultivation is not yet popular although there are four small farms with an average production of around 120kg each per week. The likely demand for the oyster mushrooms is not high because few people know about them. This explains why many commercial farmers don’t grow oyster mushrooms. From an objective point of view, however, Kenya has the potential to produce over 100,000 tons of mushrooms every year.

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Figure 4. Recently inoculated bags



Figure 5. Bags of sawdust inoculated with shiitake ready for fruiting



Figure 6. A pile of tree logs during spawn run



Figure 7. Logs producing a few mushrooms. The logs are kept inside a green house in order to fruit. The major challenge in this green house is overheating during the day. This problem is solved by shielding it from direct sunlight.

There are a few people in Kenya currently growing oyster mushrooms for home consumption. In August, 2003, a project was initiated involving over one hundred families whereby they grow *Pleurotus sajor-caju* at home in small spaces like their kitchens. The major aim of this initiative is to provide the people with alternative food sources to counter the problem of malnutrition in Kenya. Many people in the rural areas and the urban slums suffer from malnutrition because the prices of foods rich in protein and minerals are generally expensive.

Owing to the ease of growing, high yields, high fruiting temperature and high nutritional content, it was appropriate to introduce small-scale mushroom cultivation in Kenya. Today there are a number of farmers who have expanded because they grow more than what they can consume, they can market their surplus.

Among the 42 tribes in Kenya, some like eating mushrooms very much. Only a few tribes do not value mushrooms as food although they are also changing as nutritional awareness grows. In the rural areas, people collect wild mushrooms and prepare them traditionally with other foods for consumption. The most popular mushrooms collected are the *Termitomyces* and *Pleurotus* species commonly growing in the forests.

A portion of the Kenyan forests is lost each year due to deforestation, so the natural habitat for wild mushrooms

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also decreases every year, and this has led to a decline in the collection of mushrooms from the wild. A large percentage of the Kenyan population, especially those living away from wild mushroom habitats, and those who cannot afford the mushrooms sold in the supermarkets, have never had the chance of eating mushrooms. There are still many delicious and nutritious species of wild mushrooms yet to be identified and possibly domesticated but this will depend on future research efforts.

Several species of *Pleurotus* have been domesticated from Karura, Kakamega and other forests. These species are more adapted to local tropical climate and generally grow fast and fruit readily, but it may take some time before they appear in the markets.

Recommendations for the Fast Growth of Kenyan Mushroom Industry

- There should be a local producer of high quality mushroom spawn that could be sold directly to local farmers. The spawn manufacturer should also advise the farmers on which strains grow well in their particular localities because the country has diverse cold, moderate, and hot regions.

- The Ministry of Agriculture and other supporting institutions should give more emphasis to encouragement of mushroom cultivation. The Ministry does not currently have officers specifically assigned to mushroom extension services. The extension officers should be more accessible and less expensive than the present mushroom experts.

- Donors who usually shun projects involving new technology and huge amounts of money should give support to women's groups or small farmers to initiate small local projects for mushroom cultivation as a family food source. This would go a long way to solving the problem of malnutrition, particularly protein deficiency among children, and food insecurity that is common in Kenya.

- Exchange of information between farmers and researchers should be encouraged. The current mushroom farmers don't trust their competitors, and unfortunately neither do some researchers. This selfish and short-sighted attitude has inhibited the development of Kenya's mushroom industry. A research institution established specifically for mushroom research would greatly support the industry. There are research institutions for other crops like coffee, tea, pyrethrum from which the country earns a great deal of foreign exchange. For example, due to the enormous amount of research that has been performed concerning tea cultivation, Kenya has now risen to be the world's third largest tea producer.

- Promotions for the consumption of mushrooms should be undertaken. The Kenyan people should be taught the nutritional and medicinal attributes of mushrooms in order to encourage them to eat more mushrooms. There are still many people who have never eaten mushrooms. When this writer exhibited mushroom cultivation at the Nairobi International Trade Fair in October, 2003, he realized that some people didn't know that mushrooms are eaten as food. Many people do know about the poisonous mushrooms and this makes them fear eating any mushrooms, even cultivated mushrooms. These promotional campaigns would expand the local market and the growers would therefore be able to sell a large proportion of their produce here even before they exported the surplus.

Feasibility of Growing Mushrooms in Kenya

- It is possible to grow mushrooms in Kenya but the growers will need to find solutions to the problems facing mushroom growers in this country. These problems are more or less similar to those in other third world countries and the approach could be compared to mushroom projects in these countries.

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- Kenya generally has a tropical climate and most of the areas are hot, and these areas are suitable for *Pleurotus*. There are cold regions favorable for *Agaricus bisporus* and other mushrooms growing in cold areas.

- The lowlands are usually hot (21-34℃) and are generally poor in agricultural production. Though some of the lowlands receive adequate rainfall, the soils are poor and this results in small quantities of agricultural residues being available for use as substrates.

- Areas of moderate altitude that receive moderate rainfall (800-1,500mm per annum) are where most of the agricultural activities take place. Temperatures in these areas range between 18 and 27℃. This climate allows for the cultivation of a variety of crops such as wheat, rice, maize and cotton. And the agricultural residues suitable for use as mushroom growing substrates in these areas are plentiful.

- The highlands are generally cold, with temperatures ranging between 14 and 23℃. The highlands produce less agricultural residues but mushroom farmers can get their substrates from nearby moderate areas. The risk of contamination of substrates in the hotter areas is higher than the cool areas and the polythene bag technique is the most appropriate for oyster mushroom and shiitake cultivation here. In oyster mushroom bag cultivation, it is advised that the volume of the bags should not exceed 15L in order to avoid overheating.

- In the hot areas the mushroom houses should be highly insulated to keep the heat out. Mushroom houses made with locally available materials used as insulation and covered with polythene are suitable for farmers with only small capital investments.

- In order to fight temperature fluctuations, the commercial farmers should build mushroom houses using concrete and use air conditioners to regulate the environment inside. In cold areas the temperature at night falls to as low as 4℃ which would inhibit the growth of mushrooms.

- Substrates for growing mushrooms are plentiful in areas where wheat and rice are grown in large scale. Other substrates like sawdust, sugarcane bagasse, corncobs, coffee pulp, cotton wastes and other straws are also available. The country is a big producer of agricultural produce so the availability of any of the substrates is not a problem (Fig. 8).

- Straw burning is a common practice. Wheat straw is sometimes baled and used as animal feeds but in some areas it burned as a means of disposal. Rice straw, like other straw, is also used as animal feed. Rice bran and wheat bran produced in the processing industries are used as animal feed and are sold at inexpensive prices.

- Sawdust is available free of charge from the lumbering yards. Only in a few areas is it used as fuel. This may change very soon because the government has recently banned cutting down of forests for timber. Sugarcane bagasse is available for free from the crushing industries and the locals do not currently have a way of making use of this residues.

- Most farms are located near the markets and they can therefore easily supply the markets with fresh mushrooms. Mushrooms are currently produced only near major towns because people in the rural areas cannot afford them. People in the rural areas collect their mushrooms from the wild. First quality fresh mushrooms are supplied to hotels and supermarkets with second grade mushrooms being sold to lower markets at a cheaper price.

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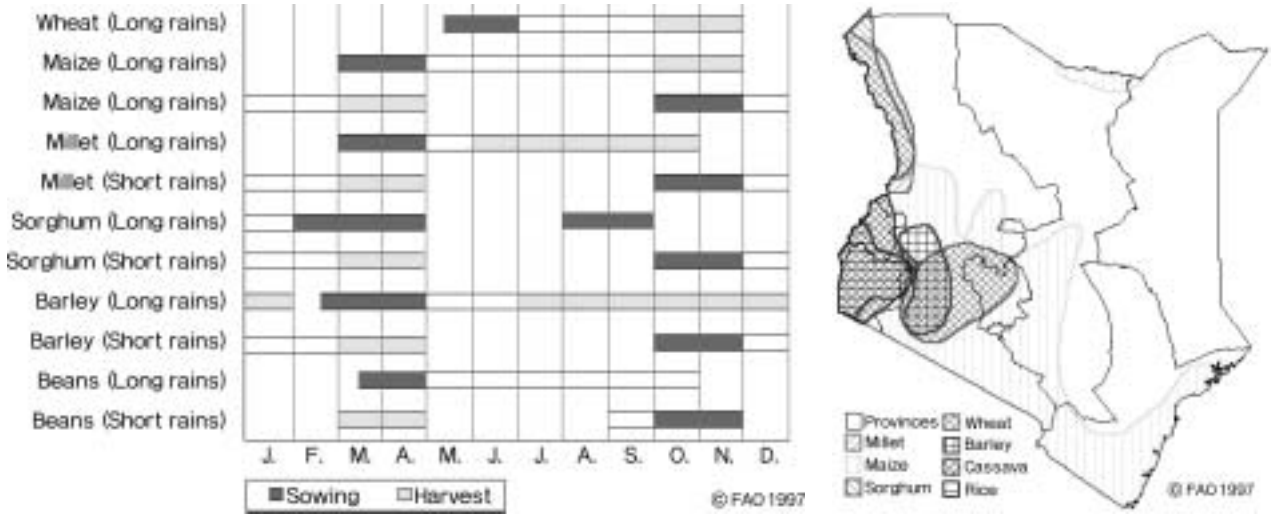


Figure 8. Crop calendar and main crop zones of Kenya

- Some companies do canning of their mushrooms but the canned Kenyan produce is not sold in the supermarkets. There could be other outlets currently being used, but the companies always keep their activities secret. Canned mushrooms imported from Europe are sold in the supermarkets and food stores to supplement the local production. Dried mushrooms are also available here. It is worth noting that the proportion of both dried and canned mushrooms is very small compared to the fresh mushrooms consumed.

- There is a great possibility of producing mushrooms here and exporting to other countries. It is the policy of the government to attract investors into the country and set up industries to ease the problem of unemployment. Companies intending to export produce are greatly encouraged because this earns the country foreign exchange credits.

- Importing of mushrooms does not account for a major proportion of the mushroom consumption. More promotion of mushrooms as a nutritious food item has to be done to increase local consumption before more imports can be made. What would be more beneficial to the country is to encourage more local production to meet the local demand.

- Consumption of mushrooms in Kenya is not high. In the hotels, the mushrooms are cooked by chefs who cook them in combination with other ingredients to suit the taste of visitors and customers. Many local people simply fry and mix them with meat and vegetables. Some people who collect wild mushrooms roast them before eating. Some wild mushrooms are dried and ground together with maize or sorghum for making porridge fed to babies, pregnant women and nursing mothers.

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Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 10

Regional Research

MUSHROOM INDUSTRY IN ZIMBABWE

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The Climate of Zimbabwe



Figure 1. Map of Zimbabew

Zimbabwe is a beautiful country that lies on the great southern African plateau. Being in the subtropical region, the vegetation is predominantly savanna with short scattered bushes and lush grass to feed the livestock and, of course, provides ample substrate for mushroom cultivation. The year is divided into two main seasons the hot and wet summer stretching from October to March and the long dry winter covering the remaining months of the year. Although summer temperatures are often oppressive, in excess of 35℃ in the southeast Lowveld, the northern Highveld region is often cooler with a summer average of 26℃ and receiving more rainfall (about 1,500mm per annum versus 500mm in the Lowveld). The eastern highlands receive the highest annual rainfall amounts (2,500mm+) distributed more or less evenly throughout the year and enjoy temperatures averaging 18℃. The region, naturally, is home to most of the wild mushrooms found in Zimbabwe. As rainfall amounts (and thus relative humidity) and temperatures increase and decrease respectively from south to north and from the west to the east of the country, so does the concentration and importance of mushroom farming. The variability in soils and climate within Zimbabwe allows for the production of various crops that produce a wide range of residues suitable for use as substrates in mushroom cultivation.

Mushroom Industry

The mushroom industry in Zimbabwe is dominated by many small-scale producers and a few well-established companies. The larger companies, accounting for approximately 75% of the white button mushrooms marketed, are mostly family-owned businesses concentrated around the county's major cities of Harare, Bulawayo and Mutare in the eastern highlands. There are approximately ten large-scale producers, of which two are located around Bulawayo, the country's second largest city. Although no statistics are available, this group of producers accounts for about three hundred tons of button mushrooms and an estimated 50 tons of oyster mushrooms produced per year.

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The small-scale producers, concentrating on the most important specialty mushroom, the oyster mushroom, account for about 60% of the total annual production of that species.

Although per capita demand in Zimbabwe for both the button and oyster mushroom has increased dramatically in the last five years, mushrooms still remain a preserve for the high-income class comprised of mostly the white population and an emerging group of rich and trendy indigenous black people. The rapid growth of this industry in recent years, most importantly the oyster mushroom cultivation, could be attributed to the need for alternative sources of protein to complement the traditional sources. This has become necessary because of the falling yields in legumes and pulses due to frequent droughts, and diseases like Anthrax and foot-and-mouth disease, which have detrimentally affected the livestock industry in recent years. In a country with beef priced at ZWD*18,000/kg (USD22.50/kg) and an unemployment rate of well over 75%, mushrooms not only reduce protein malnutrition, but also provide an important avenue for income generation especially among women and orphaned youths. In fact, most mushroom growers are women's co-operative groups, and they are located in both urban and rural areas. Mushroom cultivation has, therefore, to a large extent, been adopted as a tool for poverty alleviation most importantly for families affected by HIV-AIDS. Zimbabwe has about 1.4 million people (35% of the population) living with HIV-AIDS.

Important Mushrooms in Zimbabwe

The wild mushrooms

The diversity in vegetation and climate in this country allows for a wide range of wild mushrooms to flourish. There are over 60 wild edible species in Zimbabwe and these are most commonly found in Miombo woodlands, composed mostly of *Brachystegia, Uupaca* and *Julbernadia* species. The Mackintoshia truffle (*Mackitonshia persica*), although little known and very rare, has recently been added to the list of edible fungi of Zimbabwe. The most important wild mushrooms are listed in the table 1.

Table 1. Wild mushrooms of Zimbabwe

Mushroom group	**Popular varieties (English)**	**Popular varieties (Vernacular)**
Termite fungi	Beefsteak / Taproot mushroom	Huvhe/Nhedzi (Shona)
(Termitomyces)		Amakhowa (Ndebele)
Chantarelles	Apricot fungus	Tsuketsuke (Shona)
(Cantharellus)		Ubushabishabi (Ndebele)
Button mushrooms	Field mushroom	Chikunguwo (Shona)
(Agaricus)		Ubudzugwe (Ndebele)
Boletes	Penny Bun / Cep / Sponge fungus	Dindinde (shona)
(Boletus)	/ King Bole	Kapewpew (Tonga)
Parasols		Nzeveyambuya (Shona)
		Indlebekagogo (Ndebele)

(Source: AREX and Partners Integrated Community Support Programme)

The boletes and the chanterelles are by far the most important mushrooms and they have a ready market for export. A number of South African and Italian companies visit the Eastern Highlands and areas around Domboshawa and Gweru during the rainy season to buy these fresh mushrooms that are picked by the locals from the forests and anthills. Unfortunately the pickers, who are often rural communities with no knowledge of the market potential or value of the mushrooms, do not earn much income from this business. For example, a picker can only get USD1 for a bucketful of fresh mushrooms!

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Although locals enjoy eating these mushrooms, there is still a widespread fear of mushroom poisoning especially in urban areas. Although poisoning is not very common, there is a need to train mushroom lovers on identification of edible mushrooms and to break the misconceptions concerning mushroom poisoning.

The button mushroom

Button mushroom (*A. bisporus*), originally cultivated as a hobby, is Zimbabwe's most important export mushroom. It is mainly produced by the more serious growers with a large capital base and sizable investment in training concerning cultivation and management of the enterprise. Although the wholesale and supermarket prices are higher than in the oyster mushroom, many newcomers are often worried about the higher prevalence of diseases with button mushroom. This mushroom, being of temperate origin, requires a more strict management of temperature while oyster mushroom can tolerate temperatures of up to 27℃ as it is of subtropical origin. Electricity is expensive and few low capital investors tend to grow the button, as heaters or air conditioners are required. The button mushroom is mainly grown in trays. Wheat straw and horse or chicken manure are mixed and used as substrate.

The oyster mushroom

The oyster mushroom bears close resemblance to some wild mushrooms found in Zimbabwe. This, and the fact that it is easier to cultivate using low cost inputs makes oyster mushroom the favorite for smallholder farmers especially those in rural Zimbabwe. Oyster mushroom has been popularized by organizations seeking to alleviate poverty through employment creation. Other objectives include reduction in protein malnutrition and promotion of environmental awareness. The Biotechnology Trust of Zimbabwe (BTZ) has trained some rural farmers in Hwedza and Venice Mine among other sites while the Intermediate Technology Development Group (ITDG) has initiated training of orphans and other members of the community in Chakowa, located in Zimbabwe's Lowveld region. Two varieties of the oyster mushroom are grown: *Pleurotus ostreatus* in winter and *P. sajor-caju* in summer.

Oyster Mushroom Cultivation and R&D

Spawn



Figure 2. Oyster mushroom spawn marketed in bottles(Photo courtesy of Mswaka)

Spawn production is perhaps the most important factor limiting mushroom industry, not only in Zimbabwe, but in other countries in the region as well. Although there are a good number of people trained in microbiology, molecular physiology or basic spawn production techniques, it is unfortunate that setting up these laboratories and purchasing such expensive equipment as autoclaves has been hindered either by lack of capital or interest in this business. Spawn production has, therefore, been historically performed by mushroom enthusiasts as a hobby and only quite recently as a money producing enterprise. Local spawn is widely viewed by investors as inferior in quality. They prefer to import spawn from South African companies. Although the Biotechnology Trust of Zimbabwe (BTZ) has cultured some high quality spawn at the Biological Science Department, University of Zimbabwe, the spawn has been targeted mainly for the farmers who are involved in their programs. Local spawn usually degenerates quickly and contamination levels are also high. Rural farmers usually do not have the facilities to store spawn for a later spawning date. There is a poor network of spawn supply, and customers need to respond to advertisements listed in newspapers.

Spawn is commonly sold in plastic packets, each weighing about 500g. Bottles are not very common nowadays because of the higher cost attached.

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Table 2. Prices of spawn in Zimbabwe

Variety	Price per kg in USD
Oyster mushroom	10-15
Button mushroom (Local)	12-20
Button mushroom (imported)	40-50

There is a growing need to train farmers, especially those in rural areas on low cost spawn production in order to improve the profitability and viability of their enterprises.

Substrate

The most common substrate is wheat straw and grass. Banana leaves, although higher yielding and producing higher quality mushrooms, are not usually favored because they give a delayed break and this substrate is not as abundant as the other two. Water hyacinth, a problem weed on some of Zimbabwe's most important lakes, e.g., Lake Chivero that makes the function as an important domestic and industrial water source for the capital city, Harare, has been used in a project run by Margaret Tagwira in Mutare.

Growing rooms

There is a wide variability in terms of growing room construction. They range from mud and pole thatched huts in the rural areas to modern state-of-the-art growing rooms in peripheral urban areas. One common growing room in both urban and rural areas is the thatched wooden house. Thatch is important because it allows indoor temperatures to remain cooler under hot weather. The emphasis has been on encouraging growers to use any locally available materials to construct their growing rooms while at the same time ensuring that these rooms provide the right environment required for the production of mushrooms.

Processing

75% of the mushrooms marketed in Zimbabwe are fresh. About 40% of the oyster mushroom is sold dried while virtually all the white button mushroom is sold fresh. With the recent trend towards partially processed foods, some suppliers are now slicing these mushrooms and mixing them with such vegetables like broccoli, carrots and peas. Packaging is often done in plastic packets with a net mass of 200 gram. Mushrooms are also sold from baskets in both rural and urban markets.

Marketing

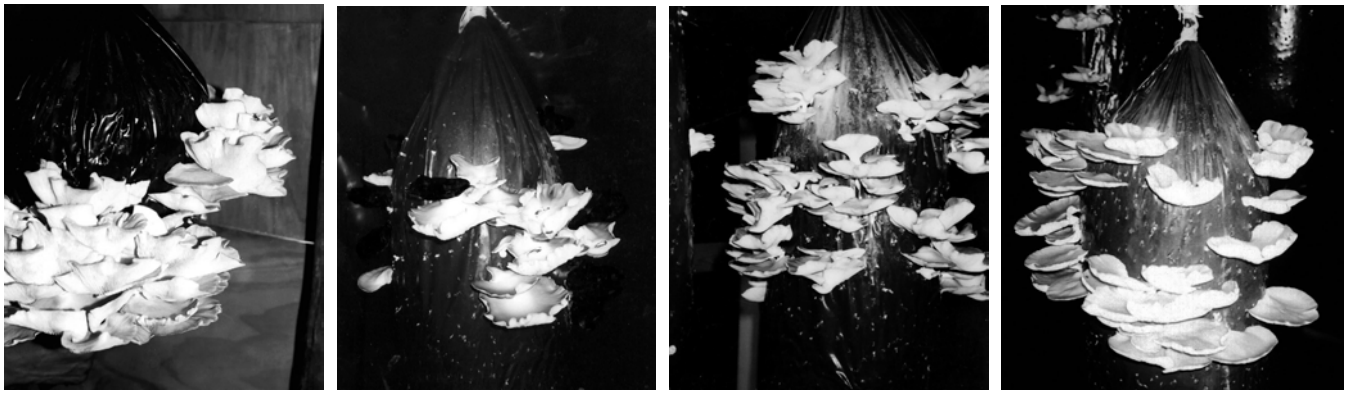
The phrase "whoever has the market is a king" is very relevant in the mushroom business in Zimbabwe. Market information especially on mushroom trade is kept in secret. The most common buyers are fast food chains (for pizza and burgers mainly), hotels (usually served as a soup), restaurants, supermarkets especially in the leafy suburbs and exporting companies. Although prices vary greatly, these are the general price guides:

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A. Banana leaves B. Groundnut shells C. Grass D. Wheat straw

Figure 3. Oyster mushrooms cultivated with substrates



A. Banana leaves B. Groundnut shells C. Grass D. Wheat straw

Figure 4. Oyster mushroom yields from different substrates

Table 3. The prices of mushroom at market

	Price per kg in USD	
	Wholesale	Retail
Oyster mushroom	5-7.50	10-15.00
Button mushroom	7.50-10.00	12.50-17.50

Training

Although many people in both urban and rural Zimbabwe could benefit immensely from mushroom production, there are very few trainers available. Assistance in terms of funding to make this training feasible and affordable to the general populace has been very limited. The Biotechnology Trust of Zimbabwe (BTZ) and the Intermediate Technology Development Group (ITDG) have been very instrumental in facilitating training and promotion of the adoption of mushrooms for poverty alleviation in a number of communities in Zimbabwe.

Consultants often charge high fees, about ZWD160,000 (USD200) per grower



Figure 5. Trainees pasterurizing substrate(Photo courtesy of Mswaka)

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per day. Very few would-be growers can afford these fees, and those who can afford are usually not willing to pass on this knowledge for free to other people interested in going into mushroom projects. Short courses, although sketchy and done over six to eight hours spread over two days, are available in most urban centers. This training costs about USD40-60. Unfortunately, trainees still need a follow-up consultancy to complement this training. In rural areas especially, courses should be run over a number of weeks as this would allow the trainees to master the art of mushroom growing and have enough knowledge to produce mushrooms in an environmentally sustainable and financially profitable manner.

Research and development

Most research done on mushrooms in Zimbabwe has been centered on improving the capacity of farmers with low resource bases to adopt this vegetable as both an income-generating and diet-supplementing crop. Examples of projects undertaken by some undergraduate students at the University of Zimbabwe are:

- Evaluation of mushroom yields under different crop residues.
- Determination of suitable spawn carriers to achieve spawn run. Wheat, millet, crushed maize and sorghum were tested and wheat was determined to be a more suitable spawn carrier.
- Comparison of biological efficiencies of different strains of the oyster mushroom on various substrates.
- Post-harvest storage and handling of mushrooms



Figure 6. A researcher culturing *Termitomyces* spores(Photo courtesy of Mswaka)

Breeding trials are currently underway and local scientists are trying to select suitable strains for low rainfall and high temperature regions, where poverty is more common due to higher risk of crop failure. Trials have also been done with culture spores from wild mushrooms, the *Termitomyces* in particular.

Future studies

Mites, flies, pathogenic fungi and bacteria are all common problems that reduce yields and quality of mushrooms produced in this country. There is a need to develop an integrated pest management programme to enable the small-holder producers of mushrooms in Zimbabwe and neighboring countries to produce with minimum reliance on chemicals. They have detrimental environmental effects and also increase the cost of production. This author seeks funding to carry out this research project at the Masters of Philosophy level.

Constraints

- There are still widespread misconceptions about mushrooms. Many people fear mushroom poisoning even in cultivated mushrooms like the button and oyster.
- Lack of capacity for research due to limited funding from both the corporate and donor sector.
- The Mushroom Growers Association is little known and has not been helping growers to secure fair prices for their produce. The buyers, not the growers, often decide prices.
- Growers lack knowledge of business management.
- Poor spawn quality and limited availability of both spawn and substrate in some areas.

Advice for Prospective Growers in Zimbabwe

Mushroom production may appear to be a lucrative business, but before one ventures into this business there are a number of factors that should be considered to avoid disappointment at mushroom growing business.

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- Is mushroom production a viable business?

Mushroom growing is one of the most viable enterprises when one uses low cost inputs to produce the crops. Harvesting may be done a few weeks after spawning (planting) and it thus offers an earlier income. However, unless one is able to secure a good market he is likely to earn low incomes. Mushrooms are highly perishable and unless they are processed, they should be sold as soon after harvesting as possible. It is advisable that growers are able to locate a suitable market prior to harvest.

- Which mushrooms should I grow?

You may grow any mushroom of your choice, as long as you can access the spawn and the market for that type of mushroom. However, for a new grower it is advisable to start with oyster mushrooms which require low capital investment and low level of management skill. This will afford the grower a chance to learn more about the practices in mushroom production and prepare them for higher capital investments in such mushrooms as the buttons, creminis and portabellas. Although many mushrooms can be cultivated, the market for specialty mushrooms is still limited. The potential mushroom producer would be wise to thoroughly investigate the demand for each species before committing large amounts of time and capital to the production stage of the enterprise development.

- What does it take to grow mushrooms?

Perhaps the most critical input in any enterprise is skill. Prospective growers need to undergo training. This training may be done over a short period of less than a week in some cases.

Another important element is time. Mushrooms are like children in that growing them involves dedication of time, love and discipline. Management of the internal environment may be time consuming, especially where investment in machinery like air conditioners and humidifiers is low or absent. Critical stages like substrate pasteurization, bagging and harvesting may require one to hire extra labor.

- What is the risk of producing poisonous mushrooms?

The risk of mushroom poisoning is lower in cultivated mushrooms than in wild mushroom collecting where similar looking mushrooms can, in fact, be toxic relatives of the edible mushroom. However, if one uses substrates previously treated with chemicals, e.g. sawdust, the residual chemicals may find their way into the mushroom body, accumulate and render the mushroom toxic. Accumulation of gases like ammonia in the growing room as well as spoilage of the mushrooms may lead to stomach upsets when consumed.

- Which is the best substrate to use?

Mushrooms can be grown on a wide range of crop residues. In Zimbabwe, wheat straw, horse manure, and grass are the most commonly used substrates although banana leaves, sawdust, water hyacinth, maize stover, and groundnut shells have also been tried. It should be noted that the profitability of a mushroom enterprise will not only be determined by the final yield attained at the end of the cropping season, but, as well, the cost of the substrates used to produce that yield. Current training emphasis is on using what are readily available within one's locality. When harvesting the substrate, growers need to make sure that this action is in harmony with the environment. In urban areas, women's groups involved in mushroom production may benefit from the roadside grass mowed by the city or town council to improve visibility for motorists, or they could sickle the grass themselves and save their communities' financial resources. Growers may also have to enter into contracts with wheat farmers so that when the wheat crop has been harvested, they may come in and collect the straw.

- Is growing room environment manageable?

Efficiently managing of the growing room is perhaps the most critical stage in ensuring good yields. Growers should be concerned with temperature, humidity, pests and diseases.

Most of the mushrooms cultivated are not adapted to local climates. Button mushroom originated from temperate regions and thus growers need to ensure that temperatures remain below 18℃ to allow for optimum

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productivity. Always keep a thermometer in your growing room and check the temperature up to three or so times a day. Enquire with your trainer or spawn supplier the temperature requirements for the mushroom you intend to cultivate. The oyster mushroom does relatively better in the Zimbabwean climate and the challenge of growing room management is lower. Use *P. ostreatus* in winter and *P. sajor-caju* in summer, as the latter is more adapted to warmer temperatures.

Where mist blowers or humidifiers are not available, keep the floor continuously wet by pouring water on it. Placing a plastic sheet below the sand floor will prevent water loss by infiltration. Keep a hygrometer and use the readings to determine when to raise the humidity. A small difference between the two thermometers means that humidity is very high!

- How do I manage diseases and pests?

Most trainers will emphasize the need to minimise use of chemicals to control pests of mushrooms. Organically produced foods (without chemical application) are healthier to eat and will fetch a higher price. Management of diseases and pests in mushrooms is possible without reliance on chemicals. Ensure thorough pasteurisation of your substrate and practice extreme hygiene at every step of your production line. Too many people entering a growing room should be avoided as they may introduce various insects and pathogens. Prevent entry of insects which may transmit diseases by blocking any possible entry points. A grower friend of mine bought mosquito nets to cover behind the doors and the windows! Mosquito net is a good way to keep out insects.

- How do I get an 'Organic Certificate'?

The demand for organically produced foods is still low in developing countries like Zimbabwe, but a wider market exists in Europe and USA. And early birds are already producing organic foods in Zimbabwe! 'Organic Certificate' may be obtained from your local agriculture department after an inspector has verified a number of factors. Neighbouring farms' use of chemicals might affect one's produce. If so, close attention should be made to preclude this inadvertent chemical exposure.

- How can one best market mushrooms?

Produce high quality mushrooms. This is one of the best and self-evident ways to best market one's products. In Zimbabwe the major buyers of mushrooms are the high-income group who tend to be choosy and trendy. They will not settle for poorly packaged and stale mushrooms. Sell your mushrooms while they are at their freshest and invest enough money in packaging. Always secure a ready market prior to each harvest otherwise you risk running around with your mushrooms trying to find a customer while your mushrooms become stale. Ensure that you honour contracts by delivering the agreed quality and quantity on time. That will give you a good name and more customers to go with it.

- How can I improve my market base?

A successful grower keeps old customers satisfied and goes on to get new customers. Some people only want the slight mushroom flavour- not the whole gourmet. You may pack sliced mushrooms together with minimally processed vegetables like carrots, broccoli and peas and recommend these for soups for mushroom beginners. Try introducing mushrooms to vegetarian clubs or certain religious groups that do not eat flesh. The major challenge facing mushroom industries in developing countries is low demands. Encourage consumption by giving out spoonful of mushroom soups in large supermarkets where you intend to sell your crop. Beware; some people may react to mushrooms! Local growers may also export regionally or to the international market. Visit embassies of countries to which you wish to export get to know the buying companies and contact them. If you have access to the Internet, you may also get your customers there, BUT, beware of con men!!

So, I wish you good luck in your chosen enterprise. You have chosen well. Now you need to work extra hard to reap the mushrooms of your labour. And always ask when you are not sure.

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Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 10

Regional Research

MUSHROOM CULTIVATION IN ZIMBABWE

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Introduction



Figure 1. Map of Zimbabwe

Zimbabwe is located in southern Africa, surrounded by South Africa to the South, Zambia to the north, Mozambique to the East, and Botswana to the West. Its geographic co-ordinates are 20° 00'S and 30° 00'E.

The climate of Zimbabwe is moderated by the high altitude, the proximity to maritime influence from the Mozambique Channel, the influence of the mid-continental high pressure (the Botswana upper high) and the volatile, warm, moist conditions of the intertropical convergence zone (ITCZ). Three distinct seasons are discernible. The hot-dry season, which begins in mid-August and lasts up to mid-November, is followed by a warm-to-hot wet season characterized by thunderstorms from the onset of the rains in November until March and April. The cool-to-warm dry season, marked by warm sunny days, cool nights and high evapotranspiration, lasts from May to August. Humidity changes (20% in October to an uncomfortable 80% in January) depend mainly on the season and the time of day. In most places the air temperature varies within the temperate/sub-tropical range of 10-28℃. The climatic comfort is generally optimum in most parts of the country, except during the month of October, when the whole country is extremely hot excepting the eastern highlands. The general climatic favorableness notwithstanding, the people living in the lowlands of the Zambezi and Limpopo valleys endure prolonged heat stress (Fig. 2).

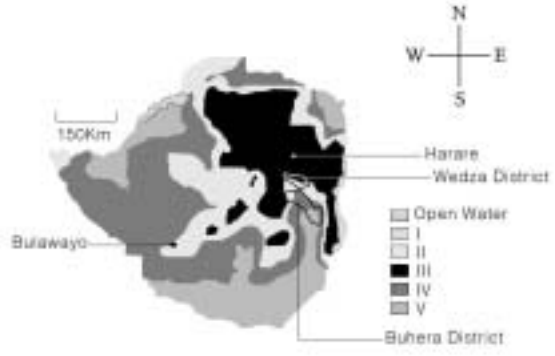


Figure 2. The natural regions of Zimbabwe and the location of Hwedza & Buhera districts

The country is divided into five natural regions as follows:

I. Natural region 1 (5,835km^2)-High rainfall (900 - 1,000mm/year), specialized, diversified farming.
II. Natural region 2 (72,745km^2)
   (a) Moderately high rainfall (750-1,000mm/year),

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confined to summer months. Intensified farming region.
(b) Same as (a) More severe dry spells during rainy season or relatively short rainy seasons.
III. Natural region 3 (67,690km^2)- Moderate rainfall (650-800mm/year), semi-intensive farming.
IV. Natural region 4 (128,370km^2)- Fairly low rainfall (450-600mm/year), semi-intensive farming.
V. Natural region 5 (112,810km^2)- Low and erratic rainfall (less than 650mm/year), extensive farming.

Mushroom Growing in Zimbabwe

Introduction

In the past five years interest in mushrooms greatly increased in Zimbabwe. This is mainly due to the current shortage of mushrooms on the local market, which has caused prices to escalate. Mushroom cultivation has subsequently become a highly profitable activity.

The use of mushrooms as food crosses all cultural boundaries. Mushroom consumption in Africa and especially in Zimbabwe, has deep traditional roots. Mushrooms have been gathered since time immemorial in Zimbabwe and are featured in many local dishes, medicines, and appear in culture and folklore. Many Zimbabwean traditions regard mushrooms as gifts presented to the people by their ancestral spirits in the forests. For this reason the sale of wild mushrooms is strictly forbidden in certain areas of the country.

Identified species / collections

There are over 60 species of edible species of wild mushrooms found in Zimbabwe. The majority are found in the Miombo woodlands, which cover over 60% of the country and are dominated by *Brachystegia* and *Julbernadia* tree species. Mushrooms play a very important in the dietary calendar of most resource-poor farmers. They occur soon after the first rains in November and are available until March. They are eaten fresh and the surplus is sun dried for consumption during the dry season. Mushrooms therefore assist in overcoming malnutrition among the low-income groups in Zimbabwe.

Mushroom Cultivation

Despite their popularity, culinary uses, financial appeal and primary dietary role at the smallholder level, the amount of wild mushroom gathered continues to decline due to deforestation. This realization necessitated the development of suitable small-scale mushroom cultivation. If successful, this small-scale cultivation would make them easy to get mushrooms any time and at more reasonable prices.

For the Zimbabwean smallholder farmers and resource-disadvantaged communities, mushroom cultivation enables them to have a balanced diet at a relatively inexpensive cost (Mswaka, *et al*., 2001). Edible mushrooms rank above all vegetables and legumes (except soybeans) in protein content and have significant levels of Vitamin B and C, and are low in fat (Stamets, 1993). Mushroom cultivation also enables farmers to utilize organic substrates that would otherwise be regarded as waste products (Wood, 1985; Labuschagne, *et al*., 2000).

Justification

A detailed socio-economic study of agriculture and horticulture in Hwedza and Buhera, commissioned by the Zimbabwe Biotechnology Advisory Committee (ZIMBAC), was done by a local non-governmental organization (NGO) called COPIBO-Zimbabwe (now VECO-Zimbabwe) in 1997. The study identified, among other constraints, a general lack of vigor among rural households. This tended to be linked to malnutrition among children. It was recommended then in the report that poverty alleviation projects had to be introduced that would address these major problems and also have an income generation component for the household. Mushroom cultivation was

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identified as one of the projects that could be introduced to address the problems highlighted. It was noted that women traditionally collected mushrooms, would play a central role in mushroom cultivation without significant distraction from other activities. The ultimate goal of the project was to improve the nutrition and income of resource-poor farmers (RPFs) through oyster mushroom cultivation (Mswaka, *et al*., 2001). The specific objectives were: 1. To assist RPFs in establishing functional mushroom growing facilities. 2. To train selected RPFs and agricultural extension officers in mushroom cultivation. 3. To produce and disseminate literature on mushroom cultivation. 4. To carry out research on the cultivation of edible mushrooms.

Hwedza and Buhera were selected as pilot districts in Zimbabwe because these districts span natural farming regions II(b) to V (Fig. 2). Results from these areas could be extrapolated to other areas with similar environmental conditions. Oyster mushrooms (*Pleurotus sajor-caju* and *P. ostreatus*) were selected by smallholder farmers because of the adaptability of the cultivation technology (Wood, 1985), and also their similarity to local indigenous mushrooms (Nzeve and Huvhe) (*Termitomycetes*). Cultivation of oyster mushrooms by the smallholder farmers would also help reduce the incidence of mushroom poisoning by providing known, well-identified edible species (Alexoupoulous, *et al*., 1979).

Oyster mushroom cultivation

Spawn supply, sources

Spawn was produced in research laboratories at the University of Zimbabwe (Department of Biological Sciences, Microbiology section) and at the Scientific and Industrial Research and Development Centre (SIRDC), Biotechnology Research Unit (BRI). Standard procedures of oyster mushroom spawn production were followed. The substrates used for the spawn were wheat (*Triticum* sp.) and sorghum (*Pennisetum* sp.) grains (Fig. 3).



Figure 3. Mushroom spawn incubator at the University of Zimbabwe

Mushroom growing house (MGH)

The recommended MGH was a 8 × 6m brick and mortar structure, with cement floor, gable thatched roof with jute bags ceiling. The building was to have screened (wire gauze) windows on all four sides, with 2 windows on one of the longer sides and a door between the windows on the other longer side. Double doors were recommended, with the outer door being a screen door (Fig. 4). At the selected sites, resources (money) were provided to acquire bricks, cement, window and door frames and pay the builders. The community was to provide all the other resources necessary to complete the mushroom growing houses. A MGH ready for use was to have the floor covered with river sand up to a depth of 2cm.

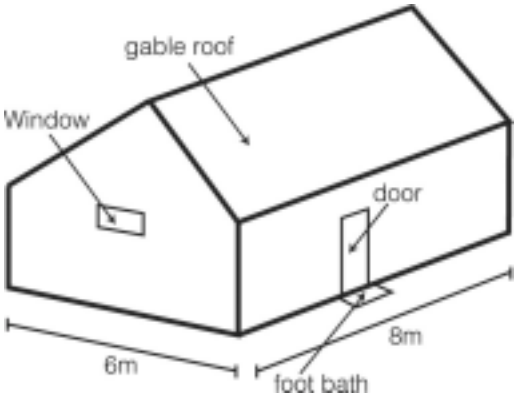


Figure 4. Structure of recommended oyster mushroom growing house

At the eleven sites, the participants modified the gabled roof to a flat roof (Fig. 5), because the recommended roof type was expensive to put up. The flat roof had its own drawbacks. All the roofs collapsed inwards within the first year, and the participants had to repair the roofs. This collapse also tended to damage the walls of the MGH, which had to be periodically repaired. At one of the sites, the size of the MGH was reduced because it was a family unit and there was a limitation on labour to work in.

At all the adopter sites, the MGH was modified in various ways. At some sites, the structure was made of grass, with a lining of plastic sheeting inside (Fig. 6). Generally, the size of

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the MGH was reduced to 4 × 3m, with a flat roof. At another site, the walls of the mushroom house were made of pole and dagga, with a flat thatched roof.



Figure 5. Recommended MGH a flat thatched roof



Figure 6. Mushroom growing house made of grass



A. Traditional kitchen



B. Partitioned structure

Figure 7. Modified structures into MGH

Other adopters modified already existing structures into mushroom growing houses. In some cases, traditional kitchens (round huts) were renovated and modified into mushroom houses (Fig. 7a). In other cases, existing structures in the homestead were partitioned and modified into mushroom houses (Fig. 7b).

Various reasons were given for the modifications observed. Generally, it was noted that the 8 x 6m MGH was too big to be maintained by a family unit. The gabled roof was too expensive to put up, though it was easier to maintain than the flat roof. The flat roof was cheap to put up, difficult to maintain and had to be replaced every year. However, on the smaller MGH, the flat roof was considered ideal.

Labour to work on the mushroom house was family-based. The work involved the construction of the MGH, laying of sand for the floor, collection and processing of the substrate and spawning, and watering of the mushroom house at least once a day. With the family as the labour source, all adopter sites constructed smaller mushroom houses. The source of water and the distance between the water source and the MGH were very important factors that determined the location and the size of the MGH.

Where resources were very limited, the family modified disused structures in the homestead. These included round huts and partitioned structures in the already existing buildings. Resources were then channeled into acquiring the spawn and plastic tubing, which were needed to grow the mushroom.

Uses of naturally available materials were demonstrated in MGHs that were constructed utilizing thatch grass, pole and dagga. These materials are readily available, especially after the rainy season, and are normally free. One just has to have the labour to cut and collect.

Substrates

While the recommended substrates were used, the farmers showed preferences for a particular type of substrate and they also tended to use materials that were readily available and also considered the amount of time and energy

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Figure 8. Chopping substrate (banana leaves)

spent in collecting and processing the substrate (Fig. 8).

Gondya was a favorite substrate when available. This type of grass was easily collected in river valleys, and grows in very large mats. It gets soft when soaked in water, as a result is very easy to manipulate. Banana fronds, when available were also a preferred substrate, because they are soft when soaked in water and also readily available because bananas are normally grown near homesteads. Mowed lawn was also preferred because no further processing was required after collecting. In maize and wheat growing areas, residues from these crops were used as the mushroom substrates. Thatch grass grows quite well during the rainy season, and it was also used as substrate on its own or was mixed with maize stover or groundnut shells.





Figure 9. Women filling mushroom growing bags with chopped and pasteurized banana fronds

Growing conditions



Figure 10. River sand covering the floor of a mushroom growing house Notice the black plastic sheeting covering the grass walls, to prevent water loss

Moisture and temperature are important factors that had to be monitored in the MGH. River-sand on the floor of the MGH was used to maintain high humidity in the MGH (Fig. 10). Clean water was periodically sprayed on the mushroom growing bags, especially during fruiting.

Those farmers who had readily available water flooded the cement floor, without the sand (Fig. 11). In the thatch grass MGH, the walls were lined with plastic sheeting to prevent excessive water loss through the grass walls (Fig. 10). With the soil rammed floors, the floor was also covered with plastic before river sand was added. This prevented excessive seepage into the ground. Where resources were limited, instead of buying a new hand sprayer for the MGH, new traditional hand brooms were recommended. These would be dipped in clean water and used to spray the bags.

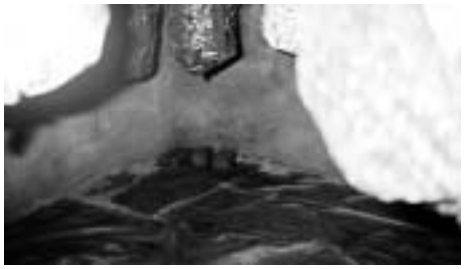


Figure 11. Mushroom house floor without sand. Water flooded onto the floor directly

Black or white plastic tubing could be used as mushroom growing bags (Fig. 12, 13). Some farmers preferred the white tubing as this allowed them to monitor activities in the mushroom bag. Others

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preferred the black pockets because they reduced the need to close up the MGH during the first 4 weeks when no light is required in the MGH.



Figure 12. Mushroom growing bags (black), hanging on wooden beams, with distinct mushroom pinheads showing after 5 weeks of incubation (spawn run)

Harvesting and marketing

Oyster mushroom pinheads normally appear 5-6 weeks through the punched holes (Fig. 14) after the substrate has been fully colonized by the oyster mushroom mycelium (Fig. 13). The bags are periodically sprayed with clean water to avoid the drying of the mushroom pinheads. Within 3-5 days the mushroom fruiting bodies will be ready for harvesting (Fig. 15), and the size at harvesting is dependent on the market demands.



Figure 13. fully colonized clear/transparent mushroom bags. Before holes are punched. This is after the 4 to 5 weeks incubation period



Figure 14. Young fruitbodies on the bag



Figure 15. Oyster mushroom ready for harvesting:size at harvesting is depent on the market demands

Economics of oyster mushroom production

(a) Growing bags: can be obtained from

(i) Farm and City at ZWD*350.00 per metre. These are narrower and not as strong as the ones used in the project.

(ii) Ecoplastics and Proplastics. The Project buys from either. Their prices are the same. The minimum quantity that they sell is 300kg, currently at ZWD7,000.00 per kg (ZWD2.1 million for 12 rolls). Each roll is approximately 175m long. The cost works out at roughly ZWD1,000.00 per metre or ZWD175,000.00 per roll.

(b) Spawn: The actual cost of production is ZWD9,400.00 per kg, but currently sold to project farmers and adopters at ZWD3,000.00 per kg.

1kg of mushroom spawn seeds 5 bags, each 1 metre long. Each bag takes about 10kg of dry substrate. The biological efficiency of oyster mushrooms is about 100%, that is, for every kg dry weight of substrate the yield of

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mushroom is 1kg. Therefore you can expect to get 10kg mushroom from each bag in total (throughout the entire harvest period), provided all production procedures are optimized. With 1kg of spawn and 5×10kg substrate, 50kg of mushroom will be produced in a crop.

Production cost for 5 bags = ZWD32,000

Material: spawn (ZWD3,000) + bag (5m×ZWD1,000 = ZWD5,000) = ZWD8,000

Labour: estimated at ZWD8,000 × 3 = ZWD24,000

Sales income for 5 bags = ZWD5,000.00/kg × 50kg = ZWD250,000.00

Net income for 1 bag = (ZWD250,000.00 - ZWD32,000) /5 = ZWD43,600 (USD53)

Remember that this profit is spread over the mushroom production cycle of 3-4 months. The more bags you have in a production cycle, the more mushrooms they will produce and the higher the profit levels will be.

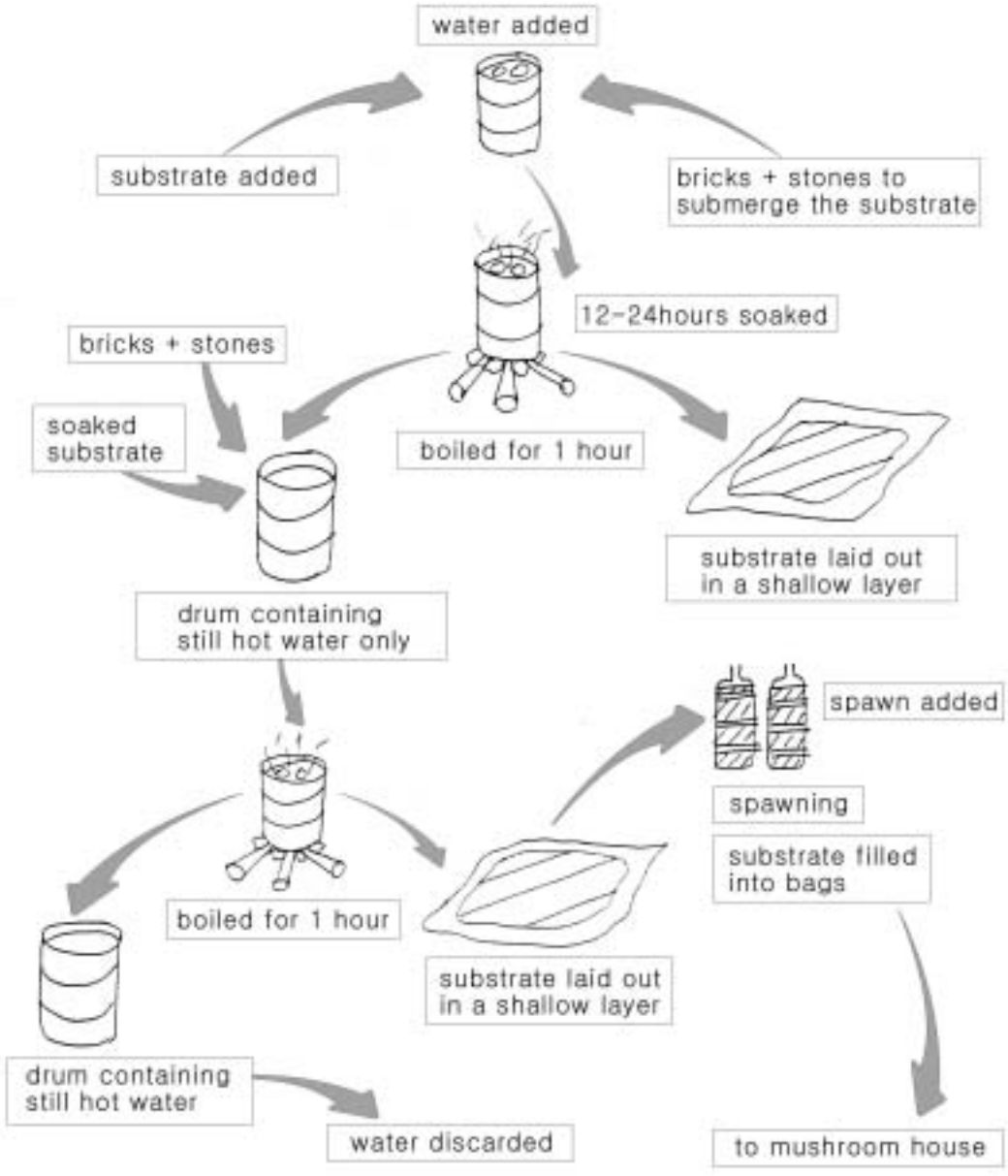


Figure 16. Diagrammatic presentation of oyster mushroom cultivation from substrate preparation to hanging bags in mushroom growing house

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Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 10

Regional Research

MUSHROOM CULTIVATION IN UGANDA

Federica Nshemereirwe
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Introduction



Figure 1. Map of Uganda

The climate of Uganda is mildly tropical. The temperatures range from 17 to 30℃ depending on the region, the northern part generally being warmer and drier than the southern region. There are two rainy seasons (March to June and August to October) in most parts of the country, the exception being the northeastern arid area that gets very little rain and is inhabited by nomadic pastoralists. Uganda is bisected by the equator, but despite this, the average room temperatures are around 25℃ because of the high altitude. There are highlands in the East, West and Southwest, which possess most of the wild mushroom species and are the areas most suitable for mushroom cultivation. In the highlands temperatures are as low as 11℃ and as high as 25℃. All types of tropical crops can be grown most of the year, and some temperate crops can be grown. Potential mushroom growing substrates include all crop residues of cereals and legumes, corncobs, tree leaves, sawdust, coffee hulls, banana leaves, bagasse, cotton waste, cotton seed hulls, brewers waste, papyrus reeds and elephant grass. Uganda has also the largest fresh water lake in the world, Lake Victoria, as well as four other large freshwater lakes. The climate around the shores of these lakes is warm and humid, suitable for cultivation of tropical mushroom species.

The major types of wild mushroom species are the *Termitomyces* species, with the most popular for consumption being the *Termitomyces microcarpus*. Mushrooms are mainly consumed with vegetables, in soups, or mixed in peanut sauce.

Ugandan society is patrilineal, with men mostly dominating women. This results in women doing most of the work, both farming and house chores, but they cannot own the land on which they work. Indeed, land ownership and the heavy workload are currently hot gender issues.

All Ugandans, except the nomadic pastoralists, appreciate mushrooms as a food delicacy and some tribes even use them as medicine and as fertility enhancers. There is a thriving market for local edible wild mushrooms, especially along motorways. There would be no problem at all in introducing any additional useful mushrooms into the diets of people.

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Current Situation of Mushroom Industry

Scale of production: It is not clear how many mushroom growers we have in Uganda, nor the total production amount as no census has been carried out. A national association has been formed to work on these problems. There are, however, about six spawn centers that can serve as mushroom training and collection centers. Ugandan traders are still importing mushrooms from Kenya and South Africa, but the quantities imported are not known.

It is assumed that there are over 400 mushroom growers in Uganda, mainly concentrated in the southern and central districts, where there is a problem of land shortage. Due to the small-scale nature of Ugandan agriculture, substrates are also available on small scale, so the mushroom growers are also doing it on small scale. Most farmers have 20-100 bags of fruiting bags at a time, yielding only an average of 1-2kg per day per farmer, sometimes less depending on the humidity and season.

Species: Only oyster mushrooms (*Pleurotus* spp.) of various strains are cultivated. Some people are now eager for domestication of local wild edible mushrooms as they believe these would be more appreciated by the market.

Substrates: Farmers in different districts use different substrates. In the southwestern areas, where sorghum is the main staple food crop, they use sorghum stover and inflorescence residues. They also use bean trash and sometimes wheat and barley straws. In the midwestern areas, they use mainly millet straws, bean trash and dry banana leaves. In the central areas, they use mainly cottonseed hulls.

Surprisingly, there are plenty of bagasse and brewery residues polluting the environment around the sugar factories and breweries but nobody is using them due to the long travel distance to these factories, and also due to the bureaucratic difficulties associated with entering and leaving factory premises.

Post harvest and marketing: The marketing of mushrooms is mostly informal, by the roadside, or in individual arrangements with those growing the mushrooms. Due to the small-scale nature of Uganda's agriculture and industry, there is an inadequate supply of substrates for mushroom cultivation. The mushroom growers, therefore, operate on a small scale. This can be a hindrance to marketing, as buyers would like reliable, constant supplies of set amounts. This problem can be solved by the formation of co-operatives for the collection of substrates, accessing spawn, and collective marketing. The recent National Seminar on Mushrooms in Uganda has encouraged such activity. Mushrooms are marketed fresh in areas near the capital, Kampala, but mainly in dried form in outlying areas. The mushrooms are air-dried indoors. There has been an attempt to develop inexpensive solar dryers, but still many farmers cannot afford them at their scale of production. Moreover, the mushrooms dried in solar dryers become brittle and easily break into powder during packing.

Fresh mushrooms are sold at UGX*5,000/kg (USD2.5/kg). Dried mushrooms are sold at UGX25,000-30,000/kg (USD12.5-15.0).



Figure 2. Demonstrating how to make PDA medium for mushroom culture

Cultivation

Species: Cultures of different strains of Pleurotus have been multiplied and preserved on Potato Dextrose Agar media.

Cultures: Fruiting cultures are made on PDA in small gin bottles that are re-usable (Fig. 2). PDA is made locally by using potatoes boiled in water that is then extracted and dextrose and agar are added to it, boiled then sterilized.

Spawn: Trials on different spawn substrates have been done. Trials included wheat grain, finger millet, sorghum and corn. We eventually settled for sorghum (Fig. 3).

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We tried adding calcium sulfate and lime to the spawn grain substrate, but this was expensive and yet did not significantly affect the yields.



Figure 3. Mature spawn in the laboratory at Kawanda Resaearch Institute, Uganda



Figure 4. Inside the growing house, mushrooms growing out of black plastic bags on bamboo shelves



Figure 5. Oyster mushrooms growing out of a transparent plastic bag on sorghum stover as substrate

Substrates: Trial results with various substrates showed that cottonseed hulls were the best, followed by cereal straws (wheat straw, rice straw, millet straw, sorghum stover), then legume crop residues (beans and soy bean). Maize cobs, grass and banana leaves had the lowest yields. We tried mixing wheat and maize bran into the poorer substrates but this caused a lot of contamination and was discarded.

Plastic bags: We have tried using black and transparent plastic bags and these gave no significant differences in yields (Fig. 4, 5). We settled for the black bags because when black bags are used, there is no need for a dark incubation room.

Humidity: We tried different ways of keeping high humidity in the cropping rooms, including water filled clay pots, wet sacks and clothes around the walls, wet sand on the floor and direct watering of bags (Fig. 6). We settled for clay pots as these were easily available unlike sand that is available only in certain places. The hanging wet clothes and sacks proved catastrophic as farmers started using all types of unsightly old clothes that grew molds and became even more unsightly.



Figure 6. A farmer using clay pots filled with water to maintain high humidity in her mushroom



Figure 7. A growing house. In the foreground, a wooden rack for draining the substrate (rice straws) after pasteurization

Mushroom houses: We tried different housing units and settled for mud and wattle walls with grass or papyrus roof (Fig. 7). Where there are plenty of termites we recommend brick walls. All-straw structures are too temporary and have to be reconstructed after every rainy season.

Post harvest and marketing: We tried direct sun drying, but the mushrooms changed color drastically and also attracted flies. We tried a solar dryer but the mushrooms became brittle and were not easy to pack. We also tried indoor air-drying, and this is the method that we are currently using (Fig. 8). Marketing is a bit difficult because of inadequate infrastructure. We are now organizing the mushroom growers into associations for collective marketing and accessing spawn and training. We have also set up 5 spawn centres in up-country districts, but these need strengthening. They will in future also act as marketing centres.



Figure 8. Drying oyster mushrooms indoors

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The Next Step for Mushroom Industry in Uganda

This author thinks the next step is to develop, through adoptive research, cultivation techniques for other species of mushrooms, especially medicinal mushrooms (shiitake, *Ganoderma*, *Agaricus blazei*, and *Auricularia*). This is in order to diversify and give people a choice, and also for future development of the nutriceutical industry in Uganda. The diversity of species will also provide a broader base for income generation by farmers as it will widen the market. There is also a need to actively promote the consumption of mushrooms through Television, radio talks, print media and posters, calendars T-shirts and seminars. This will help create and expand the market for the growers. If the University gets involved in Mushroom Science, there is also a possibility of domesticating the saprophytic wild edible mushrooms.



Figure 9. Training the Poor Claires nuns and novices. Background, right, cottonseed hulls on a straining wire mesh draining into a sink. The black plastic bags have been filled with the cottonseed hulls and inoculated with spawn.

Advice to Prospective Growers

Advice is to join the newly formed association in order to access training, spawn supply and collective marketing (Fig. 9). This will also enable them to diversify the cultivated species and increase production. Then the possibility of exporting within the region can become a reality.

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Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 11

Mushrooms for the Tropics

GROWING *GANODERMA* MUSHROOMS

Alice W. Chen
Specialty Mushrooms, U.S.A.

Why Choose to Grow *Ganoderma* Mushrooms?

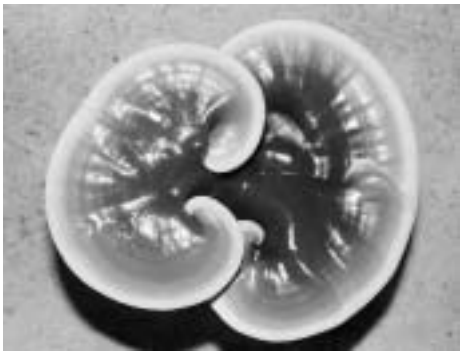


Figure 1. *Ganoderma lucidum* in a kidney shape (Photo courtesy of Henk Voogt)

Ganoderma lucidum, the most famous species in this group is a legendary mushroom in China with a long fascinating history dating back over two thousand years. Not only it is a sparkling beautiful woody mushroom, but more importantly, *G. lucidum* is known as the mushroom of immortality and is the number one medicinal mushroom in China. Dr. Andrew Weil, a most popular authority in the West on Eastern medicine, recently advised readers of his daily health tip to consume Reishi to prevent cancer. Reishi is the Japanese name for *G. lucidum*, while Ling Zhi is the Chinese name. Dr. B.K. Kim, a world leader in research on *Ganoderma* in Korea showed that *G. lucidum* has an anti-AIDS property. AIDS is a worldwide problem, particularly in Africa and Asia.

Best known as an immune system enhancer and modulator with health benefits, *G. lucidum* is generally safe for long-term use. The LD 50 (lethal dose to kill 50% of the study subject) for a single intraperitoneal injection dose of *Ganoderma* extract in rodents was as high as 38g/kg. The LD 50 of a water-soluble polysaccharide fraction of *G. lucidum* in rodent was higher than 5g/kg. Since the toxic/lethal doses in rodent are quite high relative to conventional human dosages, they do not indicate significant limitations for clinical dosages of *Ganoderma* (Chang, 1995).



Figure 2. *Ganoderma* bonsai

The following section will discuss the methods for cultivation of *G. lucidum* although other medicinal species in this group include *G. tsugae*, *G. sinense*, *G. applanatum*, and *G. capense*, whose cultivation methods are similar to those for *G. lucidum*. To bring this much-worshipped mushroom alive to aspiring mushroom growers, the author will build up readers' interest and knowledge as she describes why to choose this mushroom, how many ways there are to grow them, and how *Ganoderma* mushrooms grow. Such questions as what are the crucial stages in *Ganoderma* cultivation, how to speed up the spawn run, how to control the

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environmental factors, and how to produce *Ganoderma* mushrooms with caps will be addressed. Growers will benefit by concentrating on the step-by-step instructions on how to cultivate this widely distributed species.
It is essential for growers to learn from successful cultivation examples of *G. lucidum* first, and then adapt them to their own local needs. *G. lucidum* is fascinating to look at, beneficial to health, and has the possibility to generate income for growers. Based on toxicity studies, it has the reputation of being safe for long-term consumption with a large safety margin.

Selection of *Ganoderma lucidum* Strains

It is crucial for both new and experienced growers to understand the features and qualities of the best strains. This knowledge gives a grower a good head start. The choice of a proper strain can determine success or failure. For growing *G. lucidum* to be used for their medicinal benefits, there are good strains from Japan, China, Korea and North America.



Figure 3. Reddish strain with a distinct white margin on synthetic log



Figure 4. Yellowish brown strain with a growing edge on its cap margin

Table 1. Strain selection for *Ganoderma lucidum*

A. Superior genetic make up.
B. Stability of the strains.
C. Strains producing prime fruiting bodies
- Well formed caps, broad kidney shape, with stalks attached laterally.
- Choose reddish strains or yellowish strains.
- Produce highly glossy lacquered surface.
- Yellow underside at harvest, indicating high triterpenoid content.
- Thick fertile hymenium layer with long spore producing tubes, indicates high yield of triterpenoids and spores.
- Size of basidiocarps (fruiting bodies), 9-12cm (width) or above.
- Weight of basidiocarps, 15-30g or above.
- High contents of bioactive polysaccharides and triterpenoids, etc.

D. Vigorous and fast growth rate.
E. High mushroom yield.
F. Resistance to weed molds (unwanted mold contamination).

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Synthetic Log Cultivation

Preparation of substrates

Substrate formulations

Table 2. Formulation for supplemented sawdust-bran substrate for *Ganoderma* bag cultivation

Oak sawdust	80%	400g
Wheat bran, coarse, unprocessed	18%	90g
Sucrose	1%	5g
$CaCO_3$	1%	5g
Water	approximately 67%	1L

(Source: Chen and Miles, 1966b; Chen, 1999)

Scale up according to your need. This formulation was developed on a laboratory scale first, and successfully duplicated in scale-up operations by mushroom growers in US and Canada. In large-scale cultivation, well water can be used. Be sure that the water is not contaminated with undesirable pollutants.

Table 3. Formulations of commonly used *Ganoderma* fruiting substrates

Sawdust	Bran	Supplement	$CaCO_3$	H_2O	References
80%	18%	Sucrose	1%	1%	67% Chen and Miles, 1996b
80%	20%	-	a little	70%	Hseu, 1993
78%	20%	-	2%	*	Liu *et al*., 1990
75%	25%	-	-	*	Lu and Chang, 1975 Quimio, 1986
87%	10%	-	3%	*	Tong and Chen, 1990
93.5%	5%	Mg SO_4 0.2%	-	*	Triratana *et a*l., 1991
sawdust (alder) : wood chip (oak) = 1 : 1 (5lb /bag wet wt.)					
wood chips soaked and fermented in molasses-enriched water (50mL of molasses/5 gal of water)					Stamets, 2000

*Appropriate amount of water.
(Source: Chen, 1999)

Making the synthetic logs in bag cultivation

Synthetic logs are made by filling the bags (heat resistant polypropylene or polyethylene) with the chosen substrate. For *G. lucidum*, a sawdust-bran substrate with supplements is generally used. Check the formulation of recommended substrates in Table 2 and 3. Using heat-sealed bags with microfilter windows, substrate is usually fills two thirds of the bags to leave air space for ventilation. The substrate is sterilized



Figure 5. Heat-sealed bags with microfilter windows



Figure 6. Cylindrical bags with plugs

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right after bagging, if possible, to avoid contamination. To sterilize the substrate is to ensure that it is germ free for you to grow your chosen mushroom. Cylindrical bags with plugs can also be used.

Substrate sterilization

An autoclave is the standard equipment for sterilization. New growers or small growers with no autoclave may contact the mushroom training center or university to obtain already sterilized synthetic logs in bags.
Substrate media is usually autoclaved by standardized sterilization at 121℃ (15 psi) for 15 minutes. Adjust the time according to the amount of substrate to be sterilized. A greater amount of time is required for sterilization of sawdust-based substrates. Do not sterilize for a longer than necessary time, to avoid a possible breakdown of substrate components.

In a home laboratory, a pressure cooker can be used. For example, Stamets (1990) recommends sterilization of 17.50 × 8.25 × 4.75" bags of heat-resistant polypropylene, filled with 3 lbs wet weight of a sawdust/wood-chip substrate, at 15 psi for two hours. On the other hand, 2-lb polyethylene bags, which are not heat resistant, should be sterilized at a lower temperature of 85℃ for 72 hours. Again, depending on the nature and the bulk of the substrate, sterilization of the woody substrate may need to be adjusted (Stamets, 2000).

The cultivation process

Spawn and spawning



Figure 10. Spawning

Spawn is the seed inoculum used to inoculate the sterilized substrate after cooling. Many growers use grain spawn, while some prefer liquid spawn. There are also sawdust-bran spawn, dowels, skewers and grooved woody plug spawn. Choose spawn from the best strain with the most desirable qualities, including good genetic traits, stability, production of quality fruiting bodies of high health benefits, and high yield. Use vigorous spawn of the right age. Use fresh spawn that has not been stored, or with the least amount of storage time possible.



Figure 11. Spawn run

Methods of spawning (inoculation)

It is smart to follow up inoculation right after sterilization and cooling of the substrate to avoid uninvited contamination (Chen and Moy, 2004). New growers may not be aware that the air is full of contaminants such as uninvited fungal and bacterial spores. Inoculate under a hood in a clean place. There are two ways for spawning: "through spawning" and "localized spawning." Through spawning involves mixing the spawn throughout the entire sterile substrate, by shaking for instance, while localized spawning involves depositing the spawn on top or on the sides of the substrate block. The choice of spawning procedures should be based on the following desirable characteristics. Growers should seek the lowest possibility of contamination by uninvited microorganisms, the highest speed for inoculation, the greatest ease for handling, the least amount of labor, the most cost-effective methods, the grower's preference, and a fast spawn run.

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How should the bags be arranged?

After inoculation, how should the bags be arranged? In some growers in Taiwan, China, and the U.S.A., bags are arranged vertically on shelves. In some growers in Thailand and the U.S.A., bags are arranged horizontally. Use durable, strong and mold-resistant material for making the shelves. The bottom of each shelf should allow air circulation. In North America, open lattice designs are usually chosen. Treated wood, bamboo, stainless steel and high quality synthetic materials have been used by growers for shelves.

How to control the environmental factors?

The environmental factors, such as humidity, light, and oxygen supply, and temperature are usually known as growth parameters. As *Ganoderma* mushrooms grow from the mycelial stage to fully differentiated and mature mushrooms, each stage has a unique set of requirements for the growth parameters. Since *G. lucidum* is a subtropical mushroom that can also be found in temperate climates, a high temperature near 30℃ supports rapid mycelial growth and shortens the time required for spawn run. It has been suggested that spawn run in the absence of light promotes the formation and accumulation of fungal food reserves such as glycogen and lipids. These energy reserves are essential for producing macroscopic mushrooms from microscopic mycelia.

What are the crucial stages in Ganoderma cultivation?

Growers should pay special attention to transitional stages, such as the stage from vegetative phase to reproductive phase when primordia (the initial stage for fruiting body formation) are beginning to form. The most crucial factor during primordia initiation is high relative humidity, preferably 90-95%. Oxygen supply, exposure to diffused dim light and inclusion of calcium in the fruiting substrate are also important.

The most crucial management practice during pileus differentiation (the specialized growth for mushroom cap development) is to increase ventilation to reduce CO_2 concentration, along with high humidity and diffused dim light. When the temperature is too high, the fruiting bodies produced are very thin and of poor quality. Differentiation of *Ganoderma* fruiting is highly sensitive to CO_2 concentrations as these will determine whether antler-shaped fruiting bodies (CO_2 > 0.1%), or fruiting bodies with well-formed caps (CO_2 < 0.1%) will be produced. Fresh air contains 0.03% CO_2. Aim for reducing CO_2 to 0.04-0.05%, as close to fresh air as possible, for the production of mushrooms with caps (Table 5). Humidity is provided by fine mist 1-2 or 3-4 times per day.



Figure 7. Antler-shaped fruit bodies

Table 4. *Ganoderma* growth parameters

Stage	Duration	Humidity (%, R.H.)	Light (lux)	CO_2 (%)	O_2 (Ventilation)	Temperature (℃)
Spawn run	up to 2 months	60 -70%	Nil	Tolerate/ high conc.	0-1 exchange	25-30*or lower (20)
Primordia initiation	50-60 days after spawning	90-95%	100-200	0.1-0.5% or lower	O_2 a plus	25-30*or lower (20)
Stipe (stalk) formation	10-14 days in development	70-80% or higher	150-200	0.1-1% high conc. (branching)	low	25-30*or lower (20): thicker

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Pileus (cap) differen-tiation	25 days or longer from primordia to harvest	85-95% (on/off)	150-200 12hr circulation	< 0.1% thicker	low conc. air	25-30*or lower (20):
For further	7-10 days	85% 50-60%	Additional incubation of after cap maturation			growth

*Set temperature at 28℃, the actual temperature may become 2-3℃ higher (heat generated by massive mycelial respiration).

(Source: Chen, 1999; Stamets, 2000)

Table 5. Control of CO_2 concentration to produce *Ganoderma* with caps

Fresh air	0.03%
Ganoderma with caps	< 0.1% (Optimum: 0.04-0.05%)
Ganoderma with antler fruiting body	> 0.1%

How *Ganoderma* mushroom grow

Vegetative phase (spawn run)

Spawn run simply refers to the growth and propagation of the mushroom mycelia in the colonized substrate bags during the vegetative stage before the formation of mushrooms. *Ganoderma* spawn should be incubated in the absence of light at 25-30℃ or lower, even as low as 20℃. Almost all strains of *G. lucidum*, regardless of the geological location where the strain was isolated, have an optimal spawn run temperature of around 30℃. Avoid drying. When the substrate in the bag is fully covered by the whitish growing mycelia, it is time for fruiting. Liquid droplets and color may occur. Spawn run can be speeded up.

How to speed up the spawn run

- Select a vigorous, fast-growing fruiting strain with a superior genome.
- Choose the best substrate formula. Provide an appropriate level of substrate moisture. Avoid the substrate's being waterlogged. Make sure that the substrate provides sufficient aeration. Check also for good substrate texture and particle contact for excellent nutrient and water transport. Use the right amount of substrate and leave ample air space in the bag.
- Choose a properly functioning bag system of the right size. When heat-sealed bags with microfilter windows are used, bags of large diameters (not long and narrow) with ample air space above the substrate will facilitate oxygen supply and air exchange.
- Use a generous amount of fresh, pure, vigorous and high quality spawn.
  Avoid spawn being too old or too immature. Avoid long spawn storage, if any.
- Use through spawning by distributing the spawn throughout the substrate.
- Light inhibits mycelial growth. No light is necessary for spawn run.
- Set the highest optimal incubation temperature for spawn run (usually 30℃).
- Keep the inoculated bags free from contamination.

Trigger primordia formation

Primordia formation is triggered by exposure to light (100-200 lux), oxygen and high relative humidity (85-95% R.H.). Cold shock is not necessary to trigger the formation of *Ganoderma* primordia. However, when occasionally

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a few colonized blocks fail to respond to the standard triggering treatment, they can be transferred to a freezer over night. The remedial cold shock can be applied to unresponsive vegetative blocks more than once. Brief exposure to low levels of light is sufficient to initiate *Ganoderma* primordia.

Fruiting differentiation and development

Towards the end of the vegetative spawn run, whitish mature mycelia begin to form tighter growth in knots in response to environmental stimulation. These centers of tighter mycelial growth gradually develop into primordia and rise above the surface of the substrate as whitish rounded mounds, much bigger than the pin heads of white Agaricus button mushrooms. Amorphous primordia mass may ooze out first.

These primordia elongate vertically in the air into whitish finger-like young stalks. Growth of the stalks takes place by elongation and an increase in diameter. Under favorable conditions, the tips of mature stalks with color and shine on their lower parts begin to enlarge and give rise to young mushroom caps that are laterally attached to the tips of stalks. The young mushroom caps continue to grow and develop into typically broad kidney-shaped caps, increasing in size at the cap margins. Meanwhile as the cap matures, beautiful yellowish brown or reddish and then reddish brown coloration appears, depending on the strains. Under the diffused dim light, the mushrooms transform into sparkling specimens with lacquer-like shining upper surfaces and glossy dark brownish stalks.

Less visible to our eyes, but more important, is the differentiation of a fertile layer called the hymenium on the underside of the mushroom cap. The hymenium contains long fertile tubes in which basidiospores are produced. With our naked eyes we are only able to see the ends of these tubes as pores when we turn the cap upside down. That is why *Ganoderma* mushrooms are woody polypores, quite different from the fleshy shiitake mushrooms with gills and a centrally attached stalk. This knowledge is important because a biomedically important component called triterpenoid is produced in this region. The thicker the hymenium layer and the longer the tubes, the better, provided the physiologically active triterpenoids are produced. This determines the medicinal value of the *Ganoderma* mushroom strain and whether it is worthwhile to grow it.

Natural Log Cultivation

In the past, natural logs as long as 1m were used without sterilization in growing *Ganoderma* species in China. A long incubation of two to three years was required to obtain mature fruiting bodies on such substrates. Since late 1980s, new trends have been developed that use short logs. Today, almost all *Ganoderma* natural-log growers adopt the time-saving short-log cultivation. This is true in China, Japan, the United States and elsewhere. Here we focuses on growing *G. lucidum* on short natural logs enclosed in air-permeable synthetic bags during spawn run. Such a strategy shortens the production time and ensures mushroom quality. Addressed here are the crucial factors and methodology controlling growth and fruiting.

Preparation of logs

Tree species and log size

Most broad-leaf hardwoods can be used to cultivate *Ganoderma lucidum* and other *Ganoderma* species. Commonly used species include oak, pecan, elder, choke cherry, and plum etc. (Chen, 1999; Stamets, 2000; Chen and Chao, 1997). To be avoided are conifers and hardwoods containing harmful aromatic compounds, such as camphor-producing species, although these tree species can be used after fermentation. The standard log size used in cultivation of *G. lucidum* is 15cm in diameter or thinner, and 15-24cm long (Table 6). Commercial growers in Fujian province in China harvest logs from about 30-year old hardwood trees. Moisture content in the log should be taken into consideration.

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Table 6. Size and moisture content of *Ganoderma* short natural logs

Country	Log size (diameter × length)	Moisture content	Reference
China	15 × 18-24cm	36-38% (tight)	Huang (ed.), 1993, p. 238
	6-15 × 15cm	38-40% (loose)	Chen and Chao, 1997, p. 514
Japan	15 × 15cm		Mayzumi, Okamoto/ Mizuno, 1997, p.365
U.S.A.	12.7 × 20.3cm		Chen, 1999, p. 182

The correct time for harvesting the logs, air-drying and cutting into short logs

Cut logs from chosen hardwood species 15-20 days before spawning (Chen and Chao, 1997). Choose logs with intact bark and a diameter of 15cm unless otherwise specified (Table 6). Harvest the logs during the dormant season of the tree prior to the formation of new buds, when the tree trunks are full of sap and nutrients and before these nutrients are consumed during the germination of buds (Chen, 1999; You, 1987). Lightly air dry the logs for 15-20 days in a clean and well-ventilated place to obtain the desirable moisture content in the logs. For logs with a tight and firm woody texture, a lower level of moisture is required compared to logs with a looser texture (Table 6). Cut into short logs, 15cm or so in length. Retain the bark, but trim the periphery of the logs by removing small side branches, thorns, and any rough spots that may puncture a synthetic bag.

Choice of bag design, bagging and sterilization

Enclose logs singly in each bag, or two logs end to end in a bag having a diameter slightly larger (> 15cm in diameter), and a length of 25-50cm. Sterilize logs in bags at high pressure (1.5kg/cm2) for 1.5 hours or at normal air pressure, or 100℃ for 10 hours. Heat-sealed polypropylene or polyethylene bags with microfilter windows can be used. Permeation of air exchange of such bags is regulated by the size, shape, number, locality and nature of the microfilter on each bag, as well as air space above the colonized substrate in the enclosed bag.



Figure 9. Short log in the bag before sterilization

Preparation of spawns

A variety of spawns, such as pure culture liquid mycelial spawn, grain spawn and sawdust-bran spawn can be used. Pure-culture liquid mycelial spawn can be grown in potato-dextrose broth. For information on the sawdust-bran substrate used for spawn (Table 2 , 3).

The cultivation process

Spawning

Apply spawn evenly on the cut surface, 3-5cm thick, usually 5-10g spawn for each log. When using freshly cut logs, instead of sterilized logs, as in traditional log-cultivation in Japan, inoculation is applied immediately, or soon after log cutting to avoid contamination, based on the fact that the interior of a healthy tree is sterile. Alternatively using an inoculation gun, liquid mycelial spawn can be dispensed into the drilled holes on the periphery of the log. Colonized dowels can also be used.

Spawn run (mycelial penetration)

Special attention should be given to ensure proper mycelial growth in the log. Efforts should be made to encourage

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mycelial growth throughout the interior of the log. Avoid having superficial mycelial growth on the log surface only as a tough leathery mycelial coat (layer). The formation of superficial leathery mycelial coat on the log surface only without mycelial penetration into the center of the log is related to the log oxygen and moisture content. Lack of oxygen or poor aeration, such as water-logged, results in poor and slow growth. This is the opposite to Shiitake synthetic logs cultivation, for Shiitake, a mycelial coat on the surface of the colonized log is desirable. For proper management of the environmental factors during spawn run, refer to growth parameters. Log spawn run also tolerates fairly high CO_2 concentration, and is carried out in the absence of light.

Primordia initiation

Same as Bag synthetic log cultivation, brief exposure to very little light triggers *Ganoderma* primordia. Oxygen is also conducive to primordia formation. In contrast, spawn run is carried out in darkness, and less oxygen is required. *Ganoderma* primordia are usually formed 50-60 days after spawning in natural log cultivation.

Embedding in soil

Embed the colonized logs directly in soil after primordia formation, leaving the primordia above ground. Then cover the soil with chopped straw to retain moisture. During fruiting, at the primordia stage, the colonized logs become resistant to microbial contamination in the non-sterile soil (Chen, 1996b). Embed the short logs vertically, with the cut surface where spawning is applied facing upwards. Soil with good drainage, such as sandy soil, should be used. Following is an example: embed only 16-21cm or 9/10th of the log in soil, leaving well-formed primordia above ground (Chen and Chao, 1997). Log moisture can be better conserved by burying the logs in soil. Embedding logs in soil also enables mushroom mycelia to absorb nutrients, particularly minerals and trace elements from the soil. Soil-buried log cultivation can be done in easily-constructed mushroom houses. Within the mushroom house, low loop frames with covers usually in 2 rows, are routinely set up. Alternatively, soil-buried log cultivation of *Ganoderma* species can also be carried out in the open air in the wild.



Figure 12. Tips of young mushrooms about to form caps



Figure 13. short logs embedded with top soil in nursery pots

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Figure 14. *Ganoderma* mushroom growing on Short logs



Figure 15. *Ganoderma* with further cap growth

Harvest the Mushrooms

From primordia formation to fruiting bodies ready for harvest, takes approximately 25 days under favorable conditions. Disappearance of the white growing margin at the edge of the yellowish brown or reddish brown mushroom is a sign for harvest. Continue cultivation at reduced air humidity of 85% R.H. for an additional 7-10 days to encourage further growth in pileate thickness and firmness (50-60% R.H. in another practice). Harvest by cutting the stipe (stalk). Keep only 2cm of the stipe with the pileus. If so desired, continue cultivation under the optimal growth parameters for second and third flushes, although the subsequent flushes have lower yields, especially the third flush.

Post Harvest

After harvest it is essential to avoid storage of the dried mushrooms under humid, damp, warm and soiled unsanitary conditions. If not properly cared for, you could be shocked to find that the beautiful mushrooms you grew have been deteriorated into powders by the infestation of miniature beetles called ‘cecids.’
Air dry harvested fruiting bodies under the sun or with heat (60℃) immediately. Complete drying within 2-3 days. Place the fruiting bodies with the underside of the mushroom cap facing down. During cloudy or rainy days, apply low heat (60℃). Improper prolonged drying lowers the quality of the product by turning the underside pore surface dark brown or becoming contaminated by molds.

Discussion and Conclusion

Whether to use bag cultivation or natural log cultivation, growers should be thoroughly familiar with how the mushrooms grow and what the proper environmental factors are for each developmental stage. The major focus is high humidity for primordia initiation followed by an increase in ventilation during pileus differentiation to allow an increase in the oxygen supply.

It is advisable to grow *Ganoderma* organically. Unsound practices with the risk of undesirable environmental contamination have been detected in connection with log cultivation (Mushroom Growers’ Newsletter, Sept., 2001). The question arises as whether to use bag synthetic log cultivation or to use natural log cultivation for *G. lucidum*. Successful natural log cultivation produces *Ganoderma* mushrooms with superior quality. Thick and firm fruiting bodies are produced with desirable coloring and luster that command good prices in the markets of Southeast Asia. However, the yield could be lower, and the production time could be a little longer. The major issue is conservation of the natural resource of the forest where the logs come from, which is a significant environmental concern.

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Selection of logging should be carefully done, such as choosing very old forests within which some logging does not have any significant environmental impact. Long-term planning of forestation should be coordinated with log cultivation.

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Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 11

Mushrooms for the Tropics

GROWING SHIITAKE MUSHROOMS

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Specialty Mushrooms, U.S.A.

Why Choose Shiitake Mushroom?

Shiitake is, by far, the most popular and important edible medicinal mushroom in many countries (Chen, *et al*., 2000; Chen, 2001; Humble, 2001; Royse, 2001; Stamets, 2000). Shiitake, a name originated from Japan, is known in China as xiangu and in France as lentin. By using its scientific name, *Lentinula edodes*, you can be sure that you are talking about the same mushroom with people in other parts of the world. First discovered in China, this ancient Chinese mushroom, primarily in temperate climate, has a long-standing history as a culinary delicacy and an immunity booster, and produces lentinan, which is recognized in Japan as an anti-cancer drug. An anti HIV property has also been detected along with other health benefits.



Figure 1. shiitake

It may come as a surprise that shiitake can only be found as a native species in Far Eastern countries such as China, Japan and Korea, but not in North America nor in Europe. Recent sightings of shiitake in the wild in U.S.A. are likely mushroom runaways escaped from cultivation, or cooking preparations. In China, where shiitake was first found, xiangu, the Chinese name, means fragrant mushroom (xian means fragrance, gu means mushroom). Two highly prized forms of xiangu are dongu, the winter shiitake (dong means winter), and huagu, the flower shiitake (hua means flower). Both forms with thick meaty mushroom caps are produced at winter-like temperature. Huagu, the most sought-after shiitake and the most expensive, is a form of dongu with a flower-like cracking pattern on the upper surface of the cap (Wu, 2000).

It is only natural that China was the first to discover how to grow shiitake almost a thousand years ago. Credit was given to Wu, Sang Kwuang in Zhejian Province as the ingenious observer who figured out how to enhance shiitake fruiting on logs found in the wild during Sung Dynasty in 1100 A. D. (Miles and Chang, 1989). During such early days, drifting shiitake spores implanted spontaneously on, perhaps, fallen tree trunks or branches. Scientific methodology evolved much later, when Dr. Shozaburo Minura in Japan developed the technique of inoculating natural logs with pure shiitake mycelial culture in 1914 (Stamets, 2000). During the early twentieth

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century, Prof. Chang-Chich Hu, of Jing-Ling University (now Nanjing University) in Nanjing, China, was one of the pioneers promoting shiitake cultivation in China, after he returned from Tokyo University in Japan. Only about two decades ago, in 1979, after a dozen years or so in research, China succeeded in large-scale shiitake synthetic-log cultivation on substrate blocks in bags, a much faster production compared to cultivation on natural logs (Huang, 1997). Today, China remains to be one of the largest in producing, consuming and exporting shiitake. In the new millennium year of 2000, imports of shiitake to Japan rose 33% to 42,057 tons, with a value of USD93.65 million. Almost all of the shiitake imported to Japan came from China.

In the U.S.A., shiitake cultivation began to take off between 1986 to 1996, following the lifting of a ban on importing life cultures of *L. edodes* by the USDA (United States Department of Agriculture) in 1972 (Royse, 2001). Now, shiitake in fresh produce, cultivated by American growers is the leading specialty mushroom in supermarkets across the country, while dry shiitake has a long-standing history as a mushroom treasure in Oriental grocery stores, particularly in China towns and other Oriental communities.

Shiitake growing is widely practiced not only in Southeast Asia (China, Taiwan, Japan, Korea, Singapore, the Philippines, Sri Lanka and Thailand) but also in North America (U.S.A. and Canada), Europe (with France leading, Germany, the Netherlands, Spain, Italy, England, Switzerland, Belgium, Finland and Sweden), Australia and New Zealand (Oei, 1996; Romanens, 2001). Shiitake cultivation is, indeed, a global-wide industry.

Growing shiitake in bags of synthetic logs is now the number one method used in China (Wu, 2000). It is also the most common way to grow shiitake in the U.S.A. Labor and material costs for using the traditional natural log for shiitake cultivation appear to be less profitable for American growers. Here we focus on shiitake bag cultivation, based on American and Chinese methodology. To encourage consistent success, the author provides a deeper understanding on crucial and basic stages in growing shiitake as a practical guide to the growers, including critical selection of strains, progress in mycelial growth and maturity in preparation of forming mushrooms, how to control the environment at each stage, how to trigger the mushroom formation, and how the mushroom grows. Special techniques for forcing huagu (the flower shiitake), the most expensive form on global markets are also described.

Shiitake Bag Cultivation (Synthetic Log Cultivation)

Strain Selection

To know the importance of shiitake strain selection is crucial. Shiitake strains vary widely, particular in fruiting temperature and mycelial maturation (early or late; shorter or longer production time). Also strain-related are substrate selectivity, growth rate (some fast strains may produce pre-mature fruiting), shiitake mushroom quality (shape, size, thickness, color, flavor and fragrance, etc.), yield and ecological adaptability to extreme temperature, usually cold tolerance. Based primarily on Chinese system, strains are classified into 4 categories according to their fruiting temperatures (Table 1).

Table 1. Shiitake strain classification largely based on Chinese system

Low temperature	Mid temperature	High temperature	Wide-range temperature
10℃	10-18℃	20℃ and above	5-35℃

In awake of intrusion of massive imports, Japanese developed a number of cultivation technique-dependent new shiitake strains with large and thick fruiting bodies known as basidiocarps (Watanabe, 2001). Both performance and

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stability of superior strains are important. Experienced growers are aware of the potential problems of strain attenuation. For example repeated subcultures and prolonged storage of the stock culture may result in smaller fruiting bodies and lower yield (Huub Habets).

Substrate selection

Aged broad-leaf sawdust is the preference for many for growing shiitake. Fresh sawdust without aging by fermentation can be used for shiitake only if it is from high quality tree species. Oak, chinkapin, hornbeam, sweetgum, poplar, alder, ironwood, beech, birch and willow are examples of commonly used non-aromatic broad-leaf hardwoods in the U.S. Sawdust from tree species of lower quality must be aged by fermentation (Oei, 1996). Select locally available and inexpensive resource, for example, fermented eucalyptus sawdust is used in Australia. There are growers who prefer to use aged sawdust regardless of tree species. Both substrate nutrients and physical textural property in aeration are important. Sawdust particles should not be smaller than 0.85mm.

Substrate formulation

For commonly used supplemented hardwood sawdust formulations, see Table 2. Many use a simple substrate with sawdust, bran and 1% $CaCO_3$ (Oei, 1996). 1% sucrose is also frequently added. In addition to hardwoods, the use of pine is a subject of great interest, since pine is a readily available forestry resource. Supplemented pine-hardwood substrate (Table 2-Formula C) was used as partial substitute for basal ingredient by the Forestry Research Institute of New Zealand for shiitake production with satisfactory results. Agricultural wastes, such as cottonseed hulls, corncobs, bagasse and straw can also be used as alternative basal ingredients.

Table 2. Formulation of sawdust-based substrates for shiitake cultivation

A.	Broad-leaf sawdust based (Wu, 2000)	
	sawdust	100kg
	wheat or rice bran	23.25kg
	gypsum	2.5kg
	calcium superphosphate	0.5kg
	sucrose	1-1.5kg
	water	100-140kg
B.	Broad-leaf sawdust based (Stamets, 2000)	
	sawdust	100 lb (or 64 gal)
	woodchips	50 lb (or 32 gal)
	rice or rye bran	40 lb (or 8 gal)
	gypsum (calcium sulfate)	5-7 lb (or 1 gal)
	water	60%
C.	The Forestry Research Institute of New Zealand	
	pine sawdust	6 parts (Monterey pine-*Pinus radiata*)
	hardwood sawdust	3 parts (beech or poplar)
	grain	1 part (barley)
D.	Straw-based substrate (Oei, 1996)	
	rice straw	50kg
	wheat straw	20kg
	sawdust	20kg
	sucrose	1.3kg

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$CaCO_3$	1.5kg
citric acid	0.2kg
$CaSO_4$	0.5kg

Substrate sterilization

Sterilization depends on the nature of bags (polypropylene or polyethylene), bag size, nature and amount of the substrate per bag, and the total bulk. For sawdust-bran substrate, sterilize from 2-3 up to 4-5 hours at 121℃. Growers are advised to test their mixtures and adjust accordingly (Stamets, 2000).

Spawn and spawning

Fresh and vigorous spawn of appropriate age should be used. Select the best strain to match your interest. In general, through spawning (spawn thoroughly mixed with the entire substrate) in larger bags is used in the U.S., while top or localized spawning (spawn is left on the substrate surface or the inoculation hole) in smaller bags is used in China and Australia. Through spawning gives a much faster growth rate. Heat-sealed larger bags with microporous breathing filters, partly filled with the substrates, allow the manipulation of mixing the spawn with the substrate by shaking mechanically or manually. Smaller bags with ring necks and plugs that are fully loaded without leaving any air space in bags do not lend themselves to through spawning.

How to control the environmental factors?

Stamets (2000) summarized the growth parameters for shiitake cultivation in Table 3.

Table 3. How to provide the right environment for growing shiitake

	Spawn run	**Induction of primordial**	**Fruiting development**
Temperature	21-27℃ (70-80˚F) for all strains	10-16℃* 6-21℃** (50-60˚F) (60-70˚F) temperature fluctuation	16-18℃* 21-27℃** (50-70˚F) (60-80˚F)
Humidity	95-100% R.H.	95-100% R.H.	60-80% R.H.
Incubation	ca. 1-2 months strain-dependent	5-7 days	5-8 days
CO2	> 10,000 ppm, tolerant	< 1,000 ppm	< 1,000 ppm
Ventilation (oxygen)	0-1	4-7 /hour oxygen	4-8 /hour oxygen
Lighting	50-100 lux	500-2,000 lux at 370-420nm (green-uv)	500-2,000 lux < 500 lux (long stem)

*cold temperature (Source: Stamets, 2000)

**warm temperature

Be sure to remember that as a primarily temperate species, shiitake mushrooms are best produced at low temperature and little fluctuation of temperature and humidity, although high temperature strains are now available.

How shiitake mushrooms grow in cultivation

Production of shiitake involves both a vegetative phase of mycelial growth and maturation, and a reproductive phase of fruiting body formation. It is imperative for growers to observe closely the spawn run with many stages of

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intricate physiological changes and morphogenesis (change in physical features in growth), focusing on the transition from the vegetative phase to the reproductive phase. Growers should study the cultivation sections for details and check the sequence of shiitake photos.

Spawn run (mycelial growth and maturation)

For spawn run this intricate vegetative phase consists of 5 stages. All shiitake strains show optimal mycelial growth at 25℃. The duration of spawn run is usually 1-4 months, depending on the strains and methodology. No light is necessary during spawn run, however some light in the day/night cycle towards the end of the spawn run is conducive to induction of primordia. Different approaches can be used, such as providing light towards later stage in spawn run, short exposure to light of 4-hour/day-night cycle (Royse, 2001), or use a low level of light 50-100 lux, throughout spawn run (Stamets, 2000). The dramatic change from vegetative mycelial growth to the production of macroscopic fruiting bodies in the reproductive phase requires an enormous amount of energy reserves. A vigorous spawn run is of ultimate importance. It should be noted that strains vary greatly in duration for mycelial maturation. For one strain, 60 days is sufficient to mature, whereas this would be insufficient time for another strain which may produce deformed mushrooms (Miles and Chang, 1989).

Mycelial growth

Immediate following spawning (inoculation), whitish shiitake mycelia begin to grow on the supplemented substrate, until colonization is completed. This is an active assimilation phase with a high fungal metabolic rate. Enzymes are activated to break down complex substrate components (e.g. cellulose, hemicellulose and lignin) into simpler molecules which can be absorbed by the mycelium as nutrients for growth and propagation under favorite growth conditions.



Figure 2. Spawn run

In special cultivation practices, colonized mycelial blocks are subject to higher temperature toward the end of spawn run. In Japan, colonized blocks are often exposed to temperatures of 25-27℃ for a week before fruiting (Watanabe, 2001). In China, colonized mycelial blocks are sometimes exposed to temperatures at the upper limit of 27-30℃ for a period of time (Miles and Chang, 1989). The rationale of these methodologies is based on the conjuncture that while such higher sub-optimal temperature does not promote mycelial growth, it may facilitate the degradation of sawdust. It is not clear whether such claims have been supported by independent studies on decomposition of lignocellulose in sawdust. Also applied is the use of water spraying to colonized mycelial blocks without bags to promote mycelial maturation and browning (Watanabe, 2001). Growers should be aware that these effects may vary depending on the strain.

It helps to keep in mind that some fast-growing strains may produce unexpected pre-mature fruiting before mycelial maturation, which is not desirable. Care should be taken to move the blocks as little as possible during spawn run as moving or any physical shock may trigger pre-mature fruiting. Fast growth and shorter production times may not be the best choices. The resulting mushrooms may not have the meaty texture desired in Asian markets, but they could be acceptable in newer markets elsewhere. Usually, slow growth at winter-like temperature produces high quality dongu, the winter shiitake or huagu, the flower shiitake, the most expensive and sought-after shiitake, which are formed at cold and dry temperatures with diurnal fluctuations.

Mycelial coat formation

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On the outer surface of the colonized substrate block, a layer of thick mycelial coat, initially white in color, is developed 2-4 weeks after spawning - the later stage of spawn run. At high CO_2 concentrations, a very thick mycelial coat could be formed.

Bump formation (blister stage, or popcorn stage)

Clumps of mycelia appear as blister or popcorn-like bumps of various sizes are formed on the surface of the mycelial coat in most strains, usually when the colonization of white mycelia covers the entire substrate in the bag, or sometimes earlier. Primordia are produced at the tips of some of these bumps. However, most bumps abort and never develop into fruiting bodies. The time of bump formation varies with strain, substrate and temperature. Bumps usually form 10 days faster at 25℃ than at 15℃ (Miles and Chang, 1989). Fluctuation of temperature and high CO_2 concentrations encourage bump formation. Growers should lower the CO_2 concentration in the bag by cutting slits on the bagin, when bumps become too numerous. In any case, some aeration should be provided during this time.

Browning and bark-formation (pigmentation and coat-hardening)

There are two different approaches, browning outside of the bag versus browning inside of the bag. Some growers remove the entire bag when browning covers 1/3 to 1/2 of the mycelial coat in the bag (Oei, 1996). Royse (2001) adopts browning outside of the bag. Bags are removed before pigmentation. Timing of bag removal is crucial. Yield can be affected if bag removal is too early or too late. Growers should maintain 60-70% R.H. to avoid contamination after bag removal. Air enhances browning by oxidation. When exposed to air, mycelia turn reddish brown at the surface and eventually form a dark brown protective, dry, and hardened surface which functions like a tree bark. The inner substrate becomes soft and moist as a consequence of fungal metabolism. The inner moisture content can be as high as 80% (Oei, 1996), ideal for fruiting body formation, except for strains that do not turn brown.

Fruiting induction to trigger primordia formation

Growers should remove the bag towards the end of the spawn run before or after browning. They should apply fruiting induction when the spawn reaches physiological maturity and after browning and bark formation. Water soaking is commonly used for fruiting induction after browning and bark forming. In general, the following factors promote fruiting.

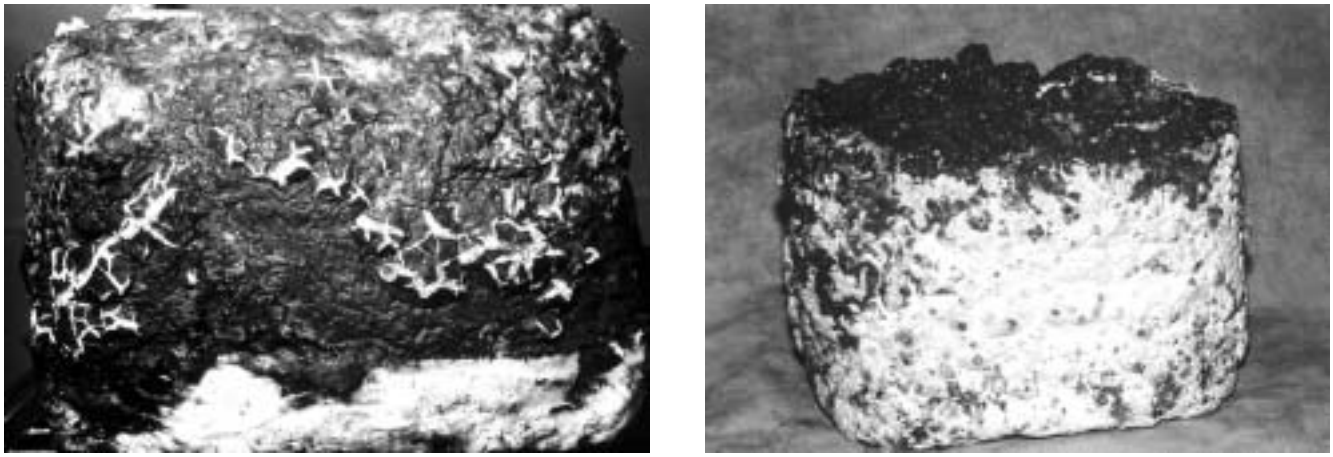


Figure 3. Plastic bag-removed substrate blocks

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Table 4. Fruiting induction: factors promoting primordia initiation in *Lentinula edodes*

water soaking, most common (Royse, 2001 : 2-4 hours at 12℃; Stamets, 2000 : 24-48 hours)	
water spraying (Watanabe, 2001)	
temperature fluctuation	
high humidity; fluctuation of humidity	
removal of CO_2, or increase of oxygen supply	
physical shocks (agitation, disturbance)	stab (with a long metal needle) and inject (with water) turn the blocks upside-down (half-way during spawn run: Watanabe, 2001) beating (e.g. natural logs) electric stimulation

(Source: Oei, 1996; Watanabe, 2001)

Basidiocarp formation

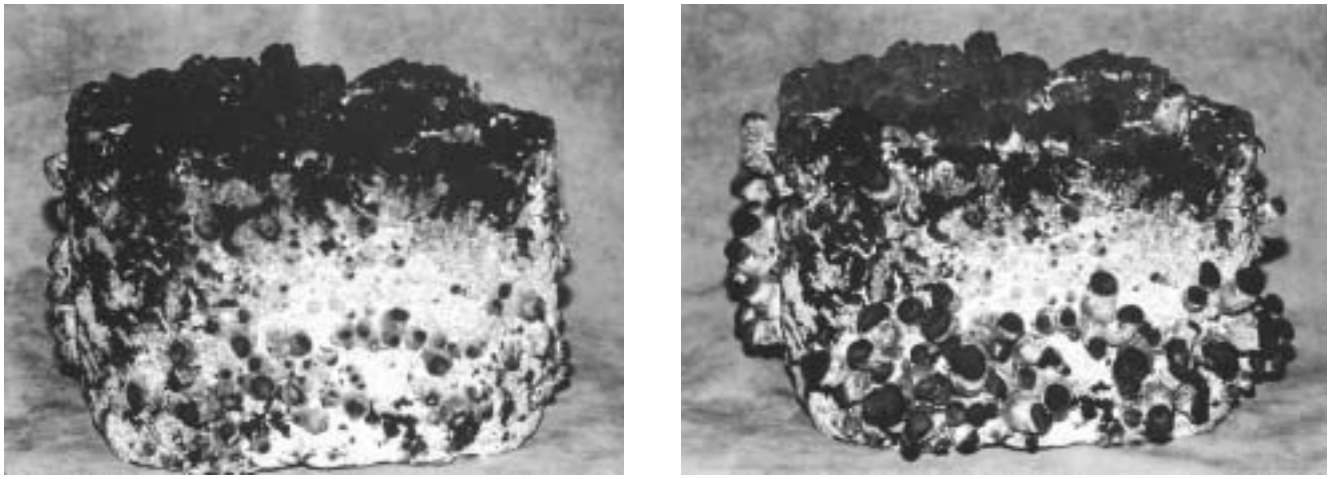


Figure 4. Basidiocarps on the synthetic logs

- primordia formation at the tip of the bump (blister)
- primordia develop into young mushroom button (dark brown)
- elongation of the stipe (stalk) as the button increases in size
- mushroom increases in size and thickness while color becomes lighter
- expanding (opening) of the mushroom cap from younger stage when the margin of the cap in-rolled downward

Harvest, post harvest and subsequent flushes

Growers should lower the humidity to 60% R.H. for 6-12 hours before harvesting for better shelf life. Harvest when the edge of the mushroom cap is still in-rolled, or when the mushroom cap is only partly extended (60-70%). This is the form desired by the Asian markets. Growers should hand-pick the mushrooms by holding the mushroom stalks and gently twisting them from the substrate block. They trim the end of the stalk of the harvest when necessary, and cut off residual stubs of stalks from the substrate. Remnants of residual stubs invite microbial contamination. After mushroom harvest, the humidity is lowered to 30-50% R.H. at 21℃, for 7-10 days of dormancy (Stamets, 2000), then soak the substrate block for up to 12 hours for the second flush, and up to 18 hours for the third flush (Royse, 2001). Larger bags with more substrate used by American growers produce more flushes, up to 5-6 flushes. Harvested mushrooms are dried at 60℃.

In China and Japan, shiitake quality is determined by shape (rounded with downward in-rolled edge before the

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cap is fully extended and central stalk), texture (thick and tight context, the meaty part), size, color, flavor (enhanced by cooking especially the fresh shiitake) and fragrance (enhanced by drying), in addition to freshness and being free from contamination, pests and impurities.





Figure 5. Fruitbodies

Growing shiitake in U.S.A.

In contrast to growers in Southeast Asia, such as China, larger heat-sealed bags with microfilter breathing windows are generally used by growers in North America for shiitake cultivation. These bags, each filled with 2-3kg, or more (5.5kg) of substrate in wet weight, produce more flushes of mushrooms in a shorter production time by using through spawning. These bags are in general less labor-intensive, less time-consuming, and experience less contamination. In methodology, through spawning, browning outside of the bag and inside of the bags, and water soaking for fruiting induction are all commonly practiced here. Mushrooms are produced under indoor controlled growth parameters. The more sophisticated growers use mechanization. There is a tendency for North American growers to use faster growing strains, especially the new growers, in order to gain confidence. The markets of fresh specialty gourmet mushrooms in North America are fairly new and include perhaps some less sophisticated consumers.



Figure 6. Shiitake on log



Figure 7. American style

Shiitake Natural Log Cultivation

Let us examine the traditional natural log cultivation to see how shiitake has been grown for nearly a thousand years. Log cultivation today prefers indoor log cultivation under sheltered or controlled environments, instead of leaving it entirely to nature. Outdoor natural log cultivation is still in practice however, and may be best suited as the first step for growers who have limited resources.

Selection of tree species for logs to match specific strains

Non-aromatic hardwood logs for growing shiitake are strain-specific. Selecting the right tree species to match the chosen shiitake strain is indispensable. Favorite tree species to use in shiitake natural log cultivation in the United

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States are oaks (*Quercus*), Chinkapin (*Castanopsis*), hornbeam (*Carpinus*) and tan oak (*Lithocarpus*). Softwoods such as alder and birch, although they have shorter shiitake production time available can also be used. Growers should check to see what kind of suitable trees are available locally.

Felling of trees and preparation of logs

Felling or chopping down the tree should be done during dormant season when log nutrient content is the highest and the bark tightly attached to the wood. Trees with intact bark are selected. Logs 7-15cm (2.5-7") in diameter are cut into 1m length. Logs thicker than 25cm (10") should be split lengthwise. Growers should drill inoculation holes 2.5cm apart, lengthwise every 15cm, and start the adjacent row midway at the top (placing holes in diamond shapes) as mycelium spreads faster along the wood grain than across. Lime water can be applied to the exposed surfaces at the end of the log to prevent uninvited weed molds.



Figure 8. Tree felling in dormant season



Figure 9. Drilling inoculation holes



Figure 10. Spawning

Spawn and spawning (inoculation)

Growers should select and prepare (or obtain) spawn of the best matching strain with the right temperature range and duration for maturity (see strain selection in bag cultivation). In China supplemented sawdust bran spawn is used for spawn, while wood plug spawn is preferred in the United States. Grain spawn is unsuitable because it could be eaten by rodents and infected by flies. Growers should use fresh spawn to inoculate the log 15-30 days after felling, when log moisture is reduced. Since *Lentinula edodes* is a saprophyte, it can only grow on dead wood, and not on newly felled trees still containing living cells. Spawn-filled inoculation holes should be sealed with hot wax or a mixture of hot wax and resin to prevent contamination and evaporation. In China, caps made of tree barks or plastic foams have been used to seal the inoculation holes.

Temporary laying for spawn run

In a laying yard indoor or outdoors, lined with pebbles on the bottom, inoculated logs should be stacked next to each other. The stack should be loosely covered to encourage spawn run. Growers should keep in mind that 25-28℃ is the optimum temperature for spawn run and avoid excessive moisture or drought. This stage is when the inoculation becomes established and begins to colonize and digest the woody substrate, as *Lentinula edodes* is a white rot fungus. Most mycelial colonization occurs during this period.



Figure 11. Temporary laying for spawn run

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Permanent laying for spawn run

Logs are stacked indoors (in green-houses in the winter) in various arrangements, such as chris-cross vertically with one log space apart, to provide ventilation for continued spawn run. In China, during laying, logs are turned. If too dry, they should be sprayed with water. It takes 6-18 months for logs to be fully colonized by shiitake mycelium. The rate of the spawn run depends on such factors as shiitake strain, tree species, log size, log moisture content and temperature.



Figure 12. Permanent laying for spawn run

Raising for fruiting



Figure 13. Water-spraying on the logs for fruiting induction

When the logs are fully colonized, they are agitated by banging the logs with a hammer or dropping on end before they are moved to the raising yard for fruiting. Raising yards with shade cloth cover to avoid direct sunlight are usually cooler and provide more ambient moisture, which is conducive to fruiting. Small tree branches and straw can also be used for cover. For winter, green houses can be used. In such cases, soaking in water, a common practice, is used for fruiting. Water temperature should be 13-18℃. In the winter, logs are soaked for 16-48 hours, while in the summer, for 6-8 hours (Oei, 1996). A steel pipe on top of the logs prevents floating. During soaking CO_2 in the log is replaced by the water, giving the log enough moisture for the flush of mushrooms. The replaced CO_2 appears as bubbles. When the bubbles are no longer seen, it is an indication that the logs have soaked long enough. In China, logs are agitated within 36 hours before soaking, while in US vibration is applied prior to soaking. Soaking times should not be over 48 hours. In the raising yard, logs are stacked to allow air exchange for fruiting and ease for harvest.



Figure 14. Young fruiting body

Primordia and fruiting body formation

Primordia break through from under the bark usually within a week following soaking. Growers should maintain 80% R.H. for fruiting development (See control of environmental factors and how the mushroom (basidiocarp) grows).

Subsequent flushes

Following harvest of mushrooms, logs should go through a dormant stage, then an incubation period of 3 months to build up the nutrients and replenish with water in soaking for the next flush. This may be repeated 5 times under optimal conditions. The second and third years are the prime production periods when 75% of the total yields are produced.

Production of Huagu, the Flower Shiitake

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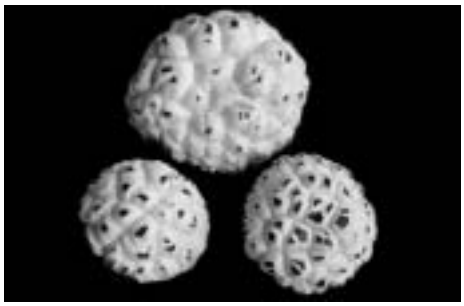


Figure 15. Huagu

Huagu, the flower shiitake, occurs spontaneously in nature during cold and dry winter months when mushroom spores are deposited by chance. Huagu is not a characteristic of a particular genotype, and it is not a genetically inherent trait. On the contrary, huagu, the shiitake mushroom with a unique morphological flower-like cracking pattern on the upper surface of the cap, is produced through manipulation of growth parameters. Success in cultivation of huagu can bring growers considerable extra income when they are growing for domestic and foreign consumption. Model huagu production systems can be found.

The principle of huagu formation

During the formation of shiitake basidiocarps (fruiting bodies), under winter winter-like conditions, or when the young mushroom buttons (not primordia) reach 2-3cm in diameter, dry air and cold temperature force the pilial (cap) surface into dormancy. Under such adverse environmental conditions, with drastic diurnal fluctuation of temperature and humidity, a protective dry surface is formed on the young mushroom cap. However, the inner context continues to grow at a slow pace with water available solely from the substrate. When favorable growth conditions return, the surface grows at a retarded rate, while the inner context develops at a normal pace. Under these conditions, shiitake mushroom buttons grow with the alternation of dormancy and growth, and a considerable differential growth rate between the surface and the inner context. In time, the rapid growth of the inner context ruptures the mushroom surface, producing a flower-like cracking pattern on the cap surface, thus the name, huagu.

Selection of strains

Low temperature, high quality and ecologically adaptable strains with cold tolerance are selected for huagu production. Strains towards the lower temperature margin in mid-temperature range can also be used. Examples of desirable Chinese huagu strains are: L-241-1, Jean-Yin #1, Yee-You #5, 7402, N-06. Strain characteristics should be thoroughly studied before cultivation. For fruiting outdoors, the time of spawning should be coordinated with the maturation characteristics in order to benefit from the winter stimulation. For example, strains 7402, N-06, late maturing strains, should be inoculated early during March and April, while 9018, Le 204, early to mid-maturing strains, should be inoculated in May-June in Bi-Yang, China (Yu, 1998). Growers should adjust for their local weather.

Timely application of forcing of huagu

Huagu forcing is initiated when the mushroom buttons reach 2-3cm diameter. If huagu forcing is applied too early when the buttons are smaller than 1.5cm diameter, these fragile young buttons may die of drought or freezing. If the technique is applied too late when the mushroom has already reached 3.5cm diameter or larger, the mushrooms do not respond readily.

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Figure 16. Huagu, flower shiitake



Figure 17. Growing houses in Biyang, China

Conclusion

Shiitake cultivation on synthetic-log or natural log is a world-wide industry. Recent trends suggest that future shiitake production will be likely on synthetic logs that will shorten production time and provide year-round fresh shiitake for most markets.

In growing shiitake, the correct strains must be used for a given methodology. Close attention should be given to the intricate stages in the vegetative phase, the spawn run, and the transition to the reproductive phase. The importance of tree bark on natural logs and the artificial bark (the browned and hardened coat) on synthetic logs cannot be overemphasized. As shiitake logs age by going through the production of flushes, barks on logs become loose, detached or slough off. Production of shiitake mushrooms stops in areas where the bark is detached from the wood. In cultivation, growers should be keenly aware that *L. edodes* is primarily a temperate species. Quality shiitake mushrooms are produced at low temperature and fluctuations in temperature and in humidity between 70 and 90% R.H. (Stamets, 2000). Constant temperature is not conducive to fruiting.

With these detailed descriptions on crucial stages in growing shiitake and vivid photos, it is hoped that growers in countries with urgent economic needs will be inspired to use agricultural waste to cultivate this worthwhile mushroom which can be a delicious and nutritious supplement to daily food as well as providing medicinal benefits and possible income. Growing shiitake, if successful, may also lead to job creation. Japan alone employs more than 20,000 people in the shiitake industry.

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Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 11

Mushrooms for the Tropics

GROWING PADDY STRAW MUSHROOMS

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Historical Overview



Figure 1. Paddy straw mushrooms at different development stages

Paddy straw mushroom (*Volvariella volvacea*) is the most popular mushroom in the rural areas of the Philippines and was commonly known to Filipinos even before the generation of a technology for its artificial cultivation was launched in the country. This mushroom is called kabuteng dayami or kabuteng saging by Filipinos since it naturally grows on paddy straw and decomposing piles of banana leaves and pseudostems.

Commercial mushroom growers, who were at the same time rice farmers used paddy straw and banana leaves. However, due to the intensification of rice production (i.e. from one cropping season to two) as a result of efficient irrigation practices and the availability of high-yielding but short duration rice varieties, most mushroom growers became full time rice growers.

Unlike the commercial production of paddy straw mushroom in neighboring countries such as China, Vietnam, Indonesia, and Thailand, production in the Philippines is still a backyard undertaking. Attempts to upgrade its production were made in the early part of the 1990's with financial assistance from international donors (Tharun, 1993; Ferchak and Croucher, 1993). Still, paddy straw mushroom production has been out paced by the newly introduced Pleurotus mushrooms, despite the abundance of substrates, availability of technology and high market demand. A rough estimate of paddy straw mushroom growers in the Philippines indicates the existence of approximately five hundred growers in the entire country.

A number of agencies scattered throughout the archipelago are actively involved in the promotion of paddy straw mushroom cultivation. The participation of these agencies in the dissemination of information varies from research,

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training, and extension services to spawn production. State colleges and universities are primarily involved in research, training, and the provision of starter cultures, while government and non-government agencies are active in the spreading of cultural technology, most particularly in the rural areas.

Production of paddy straw mushroom in the Philippines is still a hit and miss practice since growers lack necessary facilities for the maintenance of controlled physical conditions (temperature, light, relative humidity). Though indoor technology had already been introduced, most growers still adopt the traditional method due to its simplicity and low input. Maintenance of environmentally controlled conditions is one of the necessary factors to attain stable and relatively higher production, and would ultimately ensure the regular availability of paddy straw mushroom in the market.

Most of the paddy straw mushroom producers who are small scale growers are not sufficiently trained in business management. These traditional growers posses the technical skills for backyard paddy straw mushroom production which they acquired and developed from the seminars and trainings sponsored by the different agencies involved in the dissemination of the technology. Expansion of their small-scale paddy straw business undertakings is deterred by lack of funds and insufficient business skills. Moreover, unlike their counterparts in developed countries such as Japan, Filipino small-scale mushroom growers do not have a strong cooperative spirit that could assist their marketing and technical needs. Efforts are being made by some rural growers and various academic groups to create a vibrant rural-based mushroom industry. Rural-based mushroom growers have been assembled into groups in order to establish marketing linkages.

In order to increase the scale of production, the fabrication of equipment by local contractors is being encouraged. The local government units and individual entrepreneurs have now started to establish their own rural-based and practical laboratories utilizing this fabricated equipment.

Paddy Straw Mushroom Production Technology

The two production technologies for fruiting body production that are being adopted by the growers include outdoor and indoor techniques.

Outdoor cultivation of paddy straw mushrooms

The traditional outdoor method uses the bed-type approach and utilizes a number of agricultural wastes like dried paddy straw, rice stubbles, water lily, banana leaves, and stalks (Reyes and Abella, 1993). The use of these wastes as mushroom substrates depends on their local availability. The following is a description of the step by step procedure for the preparation of mushroom cultivation beds



Figure 2, 3. Bed-type production of paddy straw mushroom under the mango trees

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Site selection and preparation

Growers should choose an area that is free from potential insect pests such as ants, termites and rodents. The selected site should preferably be under trees with a wide canopy. In order to ensure that the selected site is pest free, growers can spread rice hulls onto the area and burn them until they turn into ashes. This physical method of eliminating pests also reduces the occurrence of soil-borne pathogens.

Collection and preparation of bedding materials

The bedding materials collected from the field should be sun dried. Growers should trim and bundle the substrates into bundles twelve inches long with a diameter of two inches. The bundled substrates should be soaked for twelve hours and washed with clean water.

Layering of bundled substrates into bed and spawning

The bundled substrate should be drained of excess water to attain a 65% moisture content. Growers should pile the bundled substrates one after the other into the bed forms. On top of every layer, spawn should be sprinkled thinly over the bundled substrates. An ideal bed size consists of six layers and has a length of three meters.



Figure 4. Spawning

Incubation and fruiting

A plastic sheet should be used to cover the entire mushroom bed. This sheet maintains the appropriate temperature for the mycelial ramification (30-35℃) and fruiting body formation (28-30℃). It usually takes 10-14 days before the first flush of marketable fruiting bodies (button stage) come out from the edge of the mushroom bed.



Figure 5. Covering the mushroom bed of dried banana leaves



Figure 6. Paddy straw mushrooms in the rice straw bed

Harvesting

With bare hands, growers should harvest the button stages of *V. volvacea* by simply pulling the cluster out from the bed.

Indoor cultivation of paddy straw mushrooms

A more improved technology that is now gaining interest among potential growers is the indoor production technology. This method, which utilizes paddy straw as the main substrate, has three salient features: composting,

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pasteurization, and cultivation inside a mushroom house.

Composting is an important process that allows the microbial decomposers to loosen the tensile strength of paddy straw. This process also prepares the paddy straw to be easily colonized by mycelia of *V. volvacea* (Quimio, 1991). Pasteurization is a critical process that eliminates the undesirable microorganisms that may compete with *V. volvacea* during the production proper. This process also renders the composted paddy straw more easily able to be successfully permeated by the mushroom mycelia. The cultivation of mushrooms inside a growing house allows for the control of the fluctuations of temperature and relative humidity which may be hazardous to the mycelial growth and fruiting body production. In the Philippines, two methods of indoor cultivation have been developed and introduced. The key features of both are similar, but the manner by which spawn is inoculated into the substrates differs. The indoor cultivation of Quimio (1993) is a standard method that is also used abroad, and features the actual spawning on the mushroom beds. The other method promotes the use of wooden shelves or crates which facilitate the easy handling of substrates (Reyes and Abella, 1993). The following section describes the step by step procedures for the indoor cultivation of V. volvacea:

Soaking

Rice straw of any type can be used as substrate for the indoor cultivation of *V. volvacea*. Rice stubbles could also be used. The rice straw should be soaked for 12 hours in clean water. This procedure loosens the substrates as a prelude to composting.

Composting

The previously soaked substrates should be piled up and sprinkled with 1% molasses and 0.5% complete fertilizer. Growers should cover the pile of substrates with plastic sheets and compost the pile for 14 days. On the seventh day, the partially composted substrates should be turned with a spading fork in order to ensure even composting. At this stage, the population of thermophilic decomposers starts to pile up. Growers should now add 1% agricultural lime, replace the plastic sheets and continue the composting process until completing the required fourteen day composting period.



Figure 7. Composting of rice straw

Crating and steaming

Growers should dispense the composted substrates on 12 x 24 x 18 inches wooden crates that are open on all sides. Moisture content of the substrate should be 65% (no drippings of water when squeezed between fingers). Growers should make sure that the substrates are compactly placed inside the wooden crates, and should deliver the crated substrates into the steaming room by piling them one on top of the other. Growers should then start introducing the steam into the mushroom house. Steaming usually lasts from four to six hours, and the temperature should maintained at 60-80℃.



Figure 8. Steaming of rice straw

Spawning

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The next morning after steaming the substrates, growers should check the temperature of the steamed substrates. The temperature should be 30℃ in order not to harm the mycelia of *V. volvacea*.

Incubation and fruiting

In order to encourage mycelial proliferation of *V. volvacea*, the mushroom house should be sealed. During this stage, it is very important to maintain the desirable temperature for mycelial ramification (30-35℃) with no ventilation and light. The spread of mycelia takes from seven to ten days after spawning. After this period, growers should check the status of the substrates. Fruiting initials should start to appear. At this point, the temperature should be lowered from 35 to 28℃. This can be done by sprinkling clean water on the floor of the mushroom growing house. Three to five days after the appearance of these fruiting initials, the first harvest of the button stages of *V. volvacea* can be performed.



Figure 9. Mushroom growing house

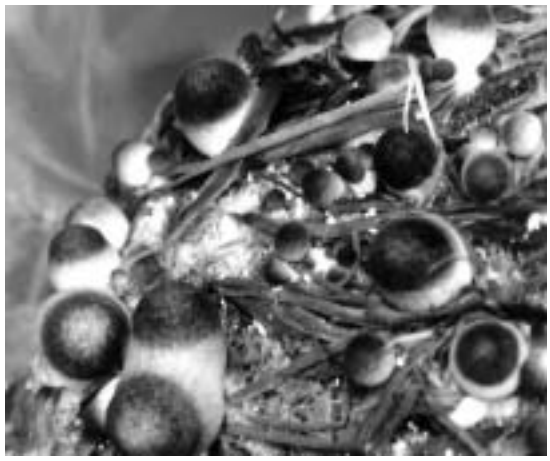


Figure 10. Fruiting bodies

Production of Spawn

A number of locally available substrates are being used as spawning material for paddy straw mushroom. In Northern Luzon for instance, tobacco midrib, a waste product of the cigar industry, is being used by the spawn producer of Pangasinan in Northern Luzon. Tobacco midrib that has been soaked in water for three days and later washed and air dried is mixed with sawdust. The mixed formulation is then placed in empty mayonnaise bottles and sterilized by autoclaving. In Central Luzon, the Center for Tropical Mushroom Research and Development at the Central Luzon State University developed and introduced the use of rice hull, a waste material from rice milling. Rice hull is moistened and mixed with 10% of either corn meal or rice bran and dispensed in heat resistant polypropylene bags and microwaveable plastic trays. In other areas of the country where leaves of leguminous trees like *Gliricidia* and *Leucaena* are abundant, dried leaves of these trees are soaked for three days and later air dried. The leaves are then mixed with sawdust and rice bran at a rate of seven parts leaves, three parts sawdust and one part rice bran. Coffee hulls are also being used in areas where coffee is grown. Moisture content of all the preparations is 65%.

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Figure 11. Tobacco midribs in glass bottles



Figure 12. Tobacco midribs in polypropylene bags



Figure 13. Autoclave for large scale preparation of culture media



Figure 14. Simple autoclave and Mr. Eduardo Matic, a mushroom grower cum innovator and the author

Feasibility of Paddy Straw Mushroom Production

Economic potential

The supply of fresh paddy straw mushroom in the domestic market is still lacking since this commodity is generally preferred by the Filipino consuming public. Its availability in the market is still erratic which makes it a luxury food. A kilogram of fresh mushroom fruits sells at 150-180 pesos (i.e. USD3-3.5).

Technical and environmental feasibility

The Philippines are a tropical country and have a maximum temperature ranging from 30 to 35℃ and a rainfall ranging from 55 to 225mm (Philippine Statistical Yearbook, 1996). The prevailing temperatures and rainfall in its three major islands of Luzon, Visayas and Mindanao are relatively the same, and this makes the management of environmental conditions favorable for paddy straw mushroom country wide. Mushroom substrates such as paddy

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straw, water lilies and banana leaves are abundant throughout the year.

Nutriceutical benefits

Though paddy straw mushroom is known primarily for table consumption due to its nutritional content, its use as a functional food has started to be recognized. A number of studies on its immunobiological activities have been reported (Kishida *et al*., 1992; Kishida *et al*., 1989; Misaki *et al*., 1986 and Sone *et al*., 1994). Thus, its additional use as a nutriceutical could be an additional factor in marketing this type of mushroom.

Zero farm wastes technology

Paddy straw mushroom cultivation utilizes large volumes of paddy straw as substrates for fruiting body production. Hence, tons of mushroom spent are also generated which results in the accumulation of wastes in the form of mushroom spent. If improperly disposed, these wastes might pose environmental hazards. Traditionally, the mushroom spent of paddy straw mushroom is burned in order to get rid of contaminants. The spent from paddy straw mushroom production can further be efficiently utilized to harness its full potential for food production. It has shown promising results as potential substrates for *Pleurotus*, *Auricularia*, *Ganoderma* and *Collybia*, fishpond fertilizer for tilapia (*Oreochromis niloticus*) and feed for broiler chickens (Reyes and Abella, 1997; Abella *et al*., 1996; Divina *et al*., 1996a and b; Reyes and Abella, 1993).

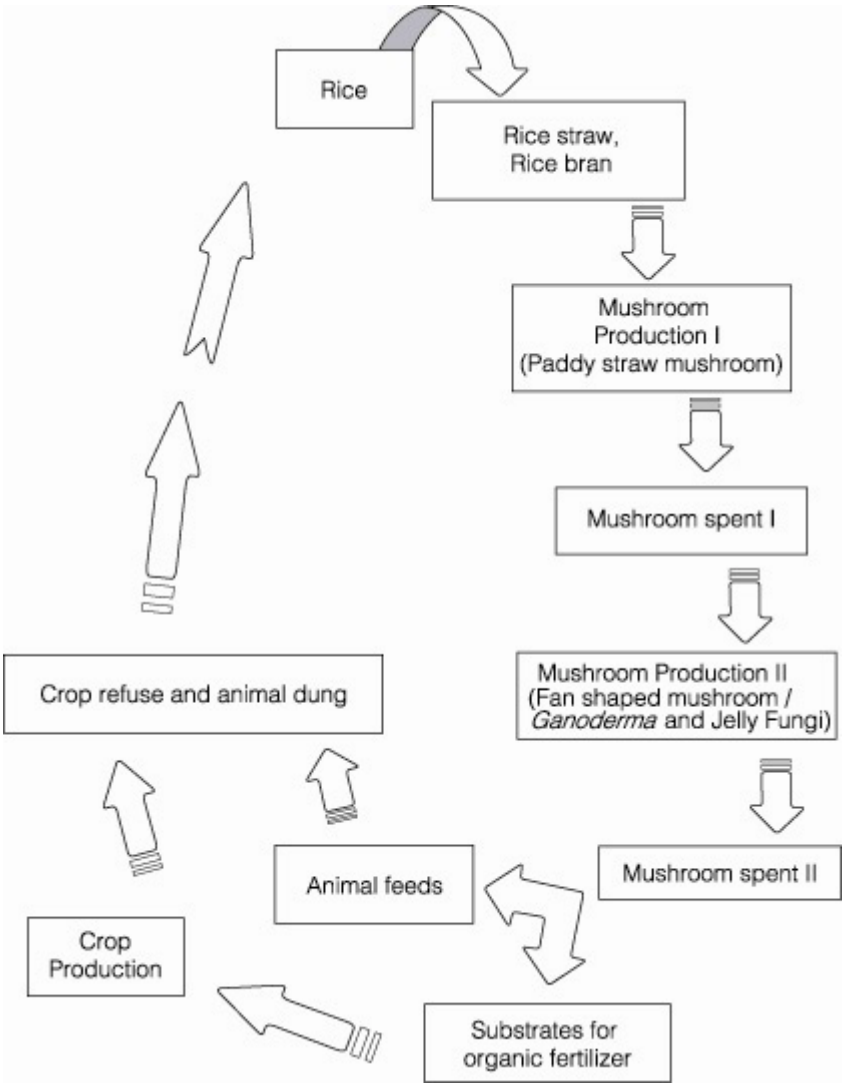


Figure 15. Efficient utilization of agricultural wastes for mushroom, crop and animal production

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Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 11

Mushrooms for the Tropics

GROWING *PLEUROTUS TUBERREGIUM*

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Introduction

History and distribution

The King Tuber Oyster Mushroom, *Pleurotus tuberregium* (Fr.) Singer is a tuberous basidiomycete. Consumption is widespread in Nigeria and across many tribes in sub-Saharan Africa. Unlike most other wild and cultivated mushrooms, it has a sclerotia stage in its lifecycle that is resistant to extreme environmental conditions found in some parts of its distribution areas, for example, the hot dry season in West Africa. Figure 1 shows 2kg sclerotium collected in the wild.



Figue 1. Anwar Ibrahim holding a 2kg sclerotium

Sclerotia can be stored for many years before used as food, medicine or inoculum and induced to produce sporophores (fruitbodies) . Fruitbodies (Fig. 2) harvested at a young stage are delicious edibles that may be eaten fresh or dried for future use. The local people who use this fungus for food and medicine usually collect the sclerotia from the wild, but it is getting difficult to find sclerotia due to the depletion of its forest habitat. However, easy growing method of this fungus is established to produce sclerotia using many lignocelullosic agricultural wastes as cultivation substrates.



Figure 2. Ebikare Isikhuemhen holding large mushrooms induced from sclerotium

P. tuberregium is indigenous to tropical Africa and the Australasian-Pacific regions of the world (Pegler, 1983; Isikhuemhen *et al*., 2000a) including sub-Sahara Africa, Madagascar, Malaysia, Papua New Guinea, Northern Australia, New Caledonia, Indonesia, Myanmar and the Yunnan province of China.

Demand for sclerotia all over the world remains high while harvest from the wild is reduced. The natural habitat of this mushroom is declining with deforestation and the conversion of forests into agricultural fields; meanwhile wild sclerotia have become scarce and very expensive, especially during the dry season (October to

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April) when it is not easy to collect them in the wild. Africans who have left their home and reside in foreign lands usually, take their eating habits with them, which is why today powdered sclerotia can be found in African food stores across the US, Canada and many European. Powdered sclerotia sold at such stores are always imported from Africa. As the wild source is lost, the demand for cultivation of this mushroom is likely to continue its increase. Wide adoption of cultivation by indigenous people will permit continued use of *P. tuberregium* in the diet and provide income. Various kinds of available agricultural wastes can serve as an ideal substrate for its growth; the cost of production can be quite low and adaptable to low technology methods.

Mushroom collection and usage by the local people

Indigenous peoples of Nigeria incorporate collected mushrooms, including *P. tuberregium*, into their diet and medicine (Oso, 1977, Ogundana, and Fagade 1982; Akpaja *et al*., 2003). Similarly, people in Ghana (Obodai *et al*., 2004), Cameroon (Kuyper *et al*., 2002) and Republic of Benin (Victor Ekun, personal communications) collect wild mushrooms and use as food and medicine. Sclerotia and fruitbodies of *P. tuberregium* are also collected from the wild and then either consumed or preserved for later use. Women and children collect and sell sclerotia at roadside stands for seasonal income and some financial autonomy. The Igbo people are the major consumers of *P. tuberregium* throughout Nigeria, using the fungus in place of meat as well as for its medicinal properties (Akpaja *et al*., 2003).

Life Cycle

The life-cycle described here is based on previously reported observations (Isikhuemhen *et al*., 2000a, Oso 1977, Pegler 1983) in nature and experiments conducted on the cultivation of *P. tuberregium* (Isikhuemhen *et al*., 2000b). In nature, dead wood is colonized either by mycelia in the soil or airborne spores discharged from individual fruitbodies. *P. tuberregium* is a white rot fungus, which derives nutrients from the degradation of lignocelullosic material (usually hardwood). During the rainy season it is possible to see a decaying log filled with fruiting mushrooms during early years of its colonization. Once the substrate is fully colonized and has reached an advanced stage of decay, sclerotia will form at the end of the rainy season. The sclerotia survives the dry and hot season until the rain returns, at which point the sclerotia will either continue to enlarge or form sporophores (mushrooms).

Sclerotia are a compact mass of mycelia tissue that serves to store food during unfavorable conditions and able to fruit when favorable conditions return. Sclerotia are dark brown on the surface, white inside, spherical to ovoid in shape and as large as 30cm in diameter. Sclerotia weighing up to 6kg are commonly harvested and sold between June and September in Nigeria (Oso, 1977). These are harvested from the wild from decaying logs and adjacent soil. These are either used as sclerotia or buried and watered regularly to induce fruit bodies (Okhuoya and Etugo 1993; Oso, 1997).

The sclerotia stage is not common among white-rot fungi. Among cultivated mushrooms, only *Morchella* spp. is known to form sclerotia as part of their lifecycle. However, only the sclerotia of *P. tuberregium* are valued as food and medicine independent of its ability to produce mushrooms. Not all strains of *P. tuberregium* form sclerotia; some strains from Australia and Indonesia have been observed to fruit directly without ever forming sclerotia (Isikhuemhen, 2000a). The sclerotia stage provides a unique advantage to the cultivation of this species because it eliminates the need for costly fruiting conditions and because sclerotia may be stored for many years without loss of viability.

Under cultivation, it is common to find 1 and up to 3 sclerotia per 1kg (dry weight) bag of sawdust substrate. Aborted sclerotia primordial less than 10cm diameter may be more numerous on wheat straw substrate, which is incapable of generating new mycelium therefore unsuitable for use in propagation techniques described below (Isikhuemhen *et al*., 2000b). Wild strains from Nigeria have exhibited slower colonization and greater yield on

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wheat straw when compared to strains from Australasian-Pacific regions; some hybrid strains were highest yielding (Isikhuemhen *et al*., 2000b). Recently, we have obtained biological efficiency of 73.5% using improved strain grown on supplemented sawdust in laboratory experiments (Isikhuemhen and Vaughans, unpublished).

Generally, *P. tuberregium* as a tropical mushroom grows best around 30°C. Fruiting requires high humidity to provide the large volume of water required for mushroom fruit body development.

Products and Uses

Food

Sclerotium can be soaked in water for 12-24 hours and after the skin is peeled off, the whitish inner tissue is milled into paste. This paste is used to substitute in part or whole for the melon seeds (*Citrulus lanatus*) in the preparation of 'egusi' soup, or mixed with corn flour and fried (Isikhuemhen and Okhuoya 1995; Nwokolo 1987). When sclerotia are induced to form sporophores, the ideal stage for harvest is 5-7 days after emergence, before the cap becomes upturned. These mushrooms are cut into pieces and used as meat in soups (Nwokolo 1987). Analysis of both sclerotia and sporophores shows that they are rich in carbohydrate, protein, vitamins, and minerals, while low in fats (Nwokolo 1987; Okhuoya and Ajerio 1988).

Medicine

The sclerotium of *P. tuberregium* has long been used for food and medicine by various tribes of Nigeria (Akpaja *et al*., 2003; Isikhuemhen and Okhuoya, 1995). The Igbo use it to treat heart problems, while it is used to treat asthma, cough, and obesity among people of the Edo State (Isikhuemhen 1995, Isikhuemhen *et al*., 2000a). Other known uses include treatment of headache, stomach ailments, colds and fever, smallpox and high blood pressure (Oso, 1977). Okhuoya *et al*., (1998) reported that in Ghana they are used in medicine for illness that relates to malnutrition and anemia in children and in the rural areas it is used as one of the ingredients in the embalming of dead bodies.

Materials

In this chapter, we discuss specific materials used as substrates. However, it should be noted that *P. tuberregium* can be cultivated on a variety of lignocellulosic substrates, including many agricultural byproducts, sawmill waste, and even cardboard. The farmer should first assess what substrates are readily obtainable at low cost, and experiment with combinations of substrates to determine which produce greatest yields. Corn, rice, and wheat straw (*Zea mays*, *Oryza sativa*, and *Tritiucm aestivum*), oil palm fruit fiber, cassava (*Manihot escunlenta*), banana leaves, corncobs, cotton waste, hardwood sawdust, paper, and cardboard are all suitable substrates. Where available, oil palm fruit fiber is not matched for yield and low incidence of contamination (Okhuoya and Okogbo 1990).

Bags: Bags made specifically for mushroom cultivation with gas-exchange filters are available from Unicorn (www.unicornbags.com), though grocery bags are a suitable alternative.

Spawn

Starter culture

The method you want to employ in the cultivation of this fungus can determine your starter culture or material. If you want to cultivate this fungus on sterile substrate using a semi-intensive or intensive cultivation process, you can isolate pure culture from mushroom fruit bodies germinated from sclerotia. Obtaining a pure culture from wild fruit bodies, sclerotia, or spores is possible using standard tissue culture techniques outlined by Stamets (1984). A pure culture of the mycelium of this fungus can be obtained by incubating tissue from inside the stipe near the base of

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the cap or from inside the sclerotia on standard malt extract agar in a Petri dish incubated at 30℃. We have observed that isolating pure cultures directly from sclerotium can be difficult at times due to contamination problems associated with inherent microbial load in wild sclerotia. Pure cultures of this fungus are available from the author and major culture banks all over the world.

Preparation of substrate for spawn production

Many substrates can be used for spawn preparation. Those reported so far are oil palm fruit fiber (*Elaeis guineensis*) hereafter referred to as OPF, wheat bran (*T. aestivum*) supplemented sawdust and whole grains: untreashed rice (*O. sativa*), millet (*Setaria macrochaeta*), sorghum or Sudan grass (*S. bicolor*) and wheat (*T. aestivum*). Use of the above grains follows standard methods for using grains to prepare spawn (Stamets 1984). Here we provide a variety of spawn production methods that have been used in the past to prepare spawn, starting with our standard wheat grain.

Wheat grain

Unthreashed wheat grain is first soaked in water overnight and then parboiled for 10 min. Excess water is drained. Calcium carbonate ($CaCO_3$) is added at 1% w/w and grains are spread out to air dry for 20 minutes. Spawn bags are made with 500g of these grains and sterilized at 121℃ for 30 minutes. After cooling, bags are inoculated with 8-10 agar blocks (approximately 1cm2) colonized by actively growing mycelium of *P. tuberregium*. This inoculation process is best done under sterile airflow such as under a laminar flow hood to decrease contamination. Inoculated spawn materials are incubated either in incubators or clean rooms at 30℃. Colonization of 500g spawn substrate bags usually takes less than ten days (Isikhuemhen *et al*., 2000b). At lower temperatures, total colonization will require more time. Care should be taken to avoid contamination, and any contaminated bag should be removed and autoclaved before disposal. Colonized spawn can be stored at 5-10℃ without loss of vigor or vitality for up to six months. However, longer storage periods can increase the chances of spawn becoming contaminated. Using pieces of agar from sclerotia collected in the wild is not recommended for inoculation spawn of this nature, because in most cases you will have contamination problems. It is possible to use pieces of sclerotium cultivated on sterile substrate for spawn inoculation, but the rate of contamination is still much higher than when pure cultures on agar blocks are used.

Sawdust

The supplemented sawdust recipes used by commercial mushroom growers (Stamets, 1984) work well for this species. Hardwood sawdust supplemented with 20% w/w wheat or rice bran and wet to 65% moisture content supports healthy spawn growth. Under less sterile conditions, spawn can be made by adding sclerotia pieces to sawdust at 65% moisture, which has been boiled for 3 hours. When sterile conditions are not available, sawdust should not be supplemented.

Oil palm fruit fiber

Oil palm fruit fiber (OPF) can be used for spawn preparation. It is known to have low incidence of contamination, which is attributed to its nutrient content that allows *P. tuberregium* to grow quickly to colonize the substrate (Okhuoya and Okogbo, 1990). OPF is an abundant waste generated from oil palm fruit processing, a major industry in many tropical countries where *P. tuberregium* is found. Fresh OPF is soaked in water for six hours, drained and loaded into spawn bags, sterilized, inoculated and incubated as per grain spawn above. Usually, 2kg bags prepared as such achieve total colonization within seven days at 30℃, forming a compact mass that can be easily broken down into smaller pieces and used for inoculation.

OPF is also a very good material to use for spawn preparation under sub optimal sterile conditions. Fresh OPF

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can be soaked in water for six hours, boiled at 100°C for 3 hours and the water drained before inoculation with the inner parts of freshly collected or presoaked sclerotia pieces. In a similar manner, sawdust spawn can be prepared. However such sawdust should not be supplemented to reduce incidence of contamination. In some cases, we have been able to make spawn from fresh sawdust that was wetted to 65% water content and inoculated with pieces of sclerotia. These methods of spawn preparation under sub-optimal or non-sterile conditions can be very useful in developing countries where sterile facilities may be too expensive to obtain.

The direct use of sclerotia as spawn, i.e. inoculation of cultivation substrate with pieces of sclerotia is also common. However, the contaminant load from wild collected sclerotia is very high. To do this we recommend soaking the sclerotia in water over night if they are dried or have been stored for over three months (this is not necessary if collected fresh). The soaked sclerotia are clean washed in boiled water followed by removing the outer skin with a clean knife. The whitish inside core of the sclerotia are cut into pieces (usually not less than $2cm^3$ a piece) and used for inoculation.

It is reported that soaking in bleach solution have been used to achieve surface sterilization, but we do not recommend it due to the fact that compounds or by products resulting from its degradation or breakdown may be toxic to human health This is especially so if the produce is to qualify as organic food or aimed at export to countries where there are stringent control on the chemicals used in food production and processing.

Substrate

Bulk substrate preparation

Many lignocellulosic wastes can support growth of *P. tuberregium* (Fasidi and Olorumaive 1994; Isikhuemhen and Okuoya 1995; Okhuoya and Etugo 1993; Okhuoya and Okogbo 1990). Sawdust and straw are readily available at little or no cost, easy to pre-treat, and are less prone to contamination compared to other agricultural wastes.

Straw

Dried straw is shredded with a wood chipper or lawn mower and placed in a pile. As the pile grows, periodic watering combined with frequent turning over of the substrate can help to bring the water content to optimum (ratio of 1:3, straw: water) within a 24hour period. Alternatively, proper moisture content can be achieved by soaking (complete immersion of substrate in water) over night and allowing the soaked substrate to drain for at least 3 hours before heat treatment. After achieving proper water content, substrate can be loaded into cultivation bags before pasteurization or bulk heat-treated. Cultivation bags that can contain up to 5kg wet substrate can be heat sterilized at 100°C for 1 hour on 2-3 consecutive days. Steam sterilization can be done in 55gallon drums filled to 20cm with water and heated over a fire or propane stove, making sure to maintain water in the drum during steaming. Bricks or wire should be used to keep bags above water. Bulk pasteurized substrate can be achieved by steaming the bulk substrate at 100°C in a suitable chamber, but bulk substrate of more than 5kg should be given longer hours of exposure to steam to ensure all substrate reaches 100°C during heat treatment on 2-3 consecutive days.

Heat-treated substrate should be allowed to cool down to at least 45°C before inoculation with spawn. In the case of bags, spawn should be added (5-10% w/w) and carefully shaken down to allow even spawn distribution throughout the bag. Afterward, the bag is either heat-sealed or the mouth of the bag is folded to a tight knob and closed with a rubber ban. For the bulk substrate, spawn materials should be added at similar rate and carefully mixed into the substrate before or during loading into cultivation bags. This process is more efficient, but is not as effective at controlling contamination, which may be introduced during spawning. Usually substrate inoculation with spawn is done under sterile airflow in a laminar flow hood. However, a clean room can be used for this process with low incidence of contamination, provided strict personal hygiene rules are observed.

Composted sawdust and OPF

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Figure 3. cultivation of *P. tuberregium* in column bags

Fresh hardwood sawdust and OPF is collected and sun-dried. On a cement platform, sawdust and OPF are mixed in a ratio of 1 : 1 (w/w). Water is added at a substrate: water ratio of 1 : 2. Substrate is piled into 2m diameters and 1.5m tall heaps and covered with a black plastic sheet. Solar and metabolic heating will occur. The pile is turned and mixed once per week for four weeks. Substrate becomes partially fermented and low in contaminants. Substrate can then be loaded into bags and steamed sterilized as above before spawning (Fig. 3).

Substrate not used immediately after fermentation can be dried in the sun and stored for future use, but will require bring water content to optimum capacity (1 : 2 to 1 : 3, substrate: water ration) and sterilized immediately prior to use. In more rural areas where sterilization equipment is not available, pasteurized substrate can be inoculated with sclerotia pieces. In this case, sclerotia pieces should be larger (5-10cm^3) and rate of spawning at least 10% per weight of substrate. When sclerotial is used to inoculate semi or unsterilized substrate, it has high power to overcome contaminants like blue mold (*Trichoderm* sp.). The mycelia growing out of the sclerotial inoculums aggregates into rhizomorphic strands that is used to penetrate and colonize contaminated areas of the substrate (Fig. 4).

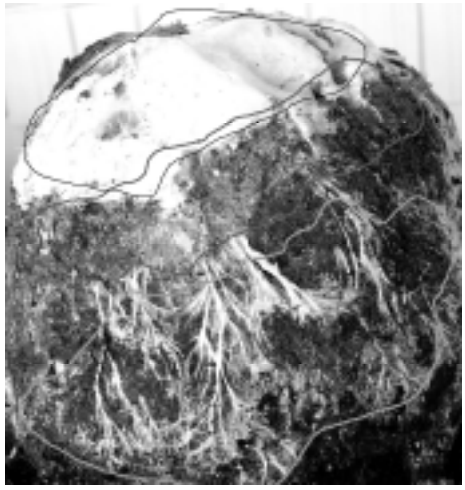


Figure 4. Rhizomorphic mycelia from sclerotia inoculated sawdust colonizing unsterile substrate

Supplemented sawdust

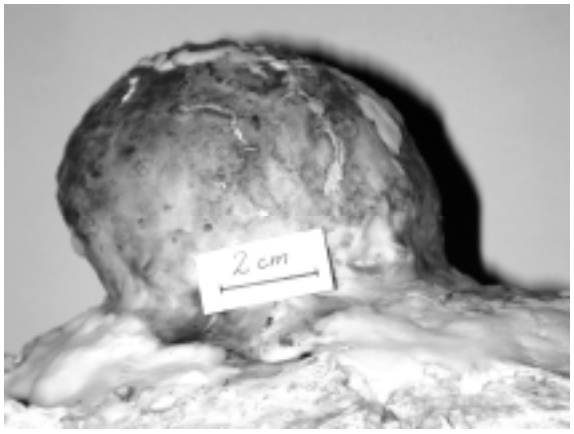


Figure 5. Sclerotia growing on supplemented sawdust substrate.



Figure 6. cultivation of *P. tuberregium* in sawdust bed cased with garden soil. Marked spots are points where sclerotia are forming and breaking through the casing material.

Different hardwood sawdust has been tested in the cultivation of *P. tuberregium*. Okhuoya *et al*., (1998) reported various levels of sclerotial yield on different tropical hard wood sawdust. Even for the best sawdust substrates, supplementation with different source of organic nitrogen can improve sclerotia yield significantly, for example using one of our selected strains, we have obtained 735g fresh sclerotia from 1kg sawdust substrate with 5% wheat bran supplementation (Fig. 5). Contaminant loads may be high without suitably sterile facilities. A 20% w/w supplementation is recommended, and cultivation on this substrate should be done in bags to minimize contamination. However, fresh un-supplemented sawdust can be laid in beds and used for cultivation either on

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floors of unused rooms, forest floors or under plantation crops like rubber (*Hevea brasilenses*) Cocoa (*Theobroma cacao*) or OPF. In a study of this type of cultivation method, sawdust beds of dimension 1m2 laid in rooms gave yield of 4.0 ± 0.57kg (fesh weight) per bed (Isikhuemhen, 1993). Similarly, Akpaja and Begho (1999) have produced sclerotia from cultivation beds laid under a rubber plantation. Sawdust of suitable hardwood is collected, adjusted for water content (1 : 3 subsrate: water ratio) and lay out in beds on concrete floors or on plastic sheets lay on forest or plantation floors. In this case no heat treatment may be done and inoculation is best by direct sclerotia pieces as described above.

Bed cultivation of *P. tuberregium* requires casing, usually done soon after complete colonization on the bed is observed. This is because sawdust beds can dry up very easily under tropical weather conditions. Generally, when sawdust substrate colonized by mycelia it is difficult to water as applied water will just flow away or stay on top the bed till it evaporates. However, by casing, water content of the substrate is conserved and the casing layer helps to hold the water adder to maintain water levels in the bed until it is slowly absorbed into the bed. Normal casing material used in *Agaricus* production can be used: peat moss supplemented with lime. However there are reports of the use of top garden soil (Fig. 6). Sclerotia formation will be visible within two weeks of casing. It takes longer for colonized and uncased bed to begin sclerotia formation. However, the mechanism by which casing hastens sclerotia formation on beds is not understood.

Logs

Hardwood logs approximately 10-20cm diameter and 1m long can be used to produce both sclerotia and fruit bodies. Okhuoya and Okogbo (unpublished data) tested different logs from different hardwood species and found that almost all tested logs were able to produce sclerotia. However relatively less dense hard wood was earliest to produce sclerotia and in some case, like oil palm log, both sclerotia and non-sclerotia associated fruit bodies were produced. Logs of less economic values, like leftover branches from logging operations and stumps or storm knocked down logs can be used for the cultivation of *P. tuberregium* to obtain sclerotia. The time it takes to produce sclerotia on logs depends on the tree species, log diameter, and strain of *P. tuberregium*.

Standard methods for log inoculation used in shiitake cultivation are applicable to this process. On suitable logs, holes (0.5 × 2cm) are drilled into a diamond pattern with 15cm spacing and spawn injected into such holes with a plunger, followed by waxing with cheese or bee wax. Logs are stacked for one year in shade and prevented from drying out. Any local material of organic origin can be used to cover the inoculated holes, since its function is mainly to prevent the injected spawn from drying up before the mycelium grow into the logs. In the Esan tribe of Nigeria, pomade from palm kernel, Ori-eyo (pronounced ori-ehyor) could be for this purpose. Inoculated logs can be laid on forest floors for colonization and production of sclerotia. Large fallen trees can be inoculated *in situ* and allowed to colonize and produce sclerotia. In such cases, the log can be source of sclerotia year after year till the logs are completely rotted.

We have observed that sclerotia produced on logs under natural conditions are more condensed, heavier and generally store longer than those from on straw or hardwood sawdust substrates.

Cultivation Methods

Inoculation of bulk substrate

When spawn material is fully colonized, it will be a compact mass of mycelia and substrate. In front of a laminar flow hood, spawn is cut into bits 2-4cm^3, which are then ready to inoculate the final substrate. In unsterile or semi-sterile environments, where for example, OPF is used to generate spawn from sclerotia, the spawn can as well be

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cut into pieces and used for bulk substrate inoculation.



Figure 7. Sclerotium under formation in a cultivation bag filled with sawdust substrate.

Spawn run and sclerotia formation

Spawn run is fastest at 30℃ and > 80% relative humidity. Spawn run will take 20-30 days depending on substrate. Spawn run and sclerotia production can be done in beds if an appropriate growing facility is available or in bags similar to those used for the production of spawn. For large-scale production, growing houses will maximize productivity and efficiency. Alternatively, using bags allows production to be adapted to a variety of scales and eliminates the need to maintain humidity. Cultivation in bags can also decrease contamination. Light is not required for sclerotia formation (Fig. 7).

Cased beds of bulk substrate

Substrate is laid into 1m^2 beds to a depth of 20cm. Bamboo (*Bambusa* sp.) houses with cement floors and palm leaf roofs are suitable. Racks can be used to stack beds and maximize the use of space. Substrate beds are inoculated at a rate of 5-15% (w/w) depending on contaminant load and sterilization method used.

In tropical climates, water loss from beds can be high. Water should be added at a rate of 5L/m^2 every other day for the first three weeks before colonization is complete. After three weeks, bowls containing water placed on available spaces will maintain humidity. Until colonization is complete, windows are kept closed except for one hour during the night.

Casing is done when substrate is completely colonized, usually six-eight weeks after spawning. Topsoil from the garden, sand, or peat is used to cover the colonized beds to a depth of 4-5cm. Casing moisture is maintained by occasional watering. 2-4 weeks after casing, sclerotia will emerge through the casing material. Casing has been shown to increase yields in a number of mushroom species (Stamets 1984) including *P. tuberregium* fruit bodies (Okhuoya and Okoobo, 1990) though the effect of casing on sclerotia yield has not yet been reported. At this stage, watering is increased to prevent sclerotia from drying out. Windows can be opened for six hours during the day to allow ventilation and lower the ambient temperature. Young sclerotia are white, turning dark brown with time. Using this method with proper management can yield 2-4kg sclerotia/m^2.

Bag method

Substrate may also be inoculated in bags up to 8kg each. A rate of 5% (w/w) spawn is added. Substrate may be inoculated from spawn or directly from sclerotia pieces. However where sterile facilities are not available and the possibility of even pasteurization of substrate is remote, pieces of sclerotia can be used to inoculate sawdust substrates loaded into bags after mixing with water. Under such condition, inoculation at the rate of 10% spawn can be successful. When unsterilized substrate is used, supplementation with organic nitrogen sources such as grain should never be used, because contaminants will out-compete the fungus and reduce yields. Sclerotia can be cultivated in the bags with filters similar to those used for spawn production, with the same variety of substrates discussed and not requiring casing. Cultivation in bags requires only that temperature be maintained around 30℃, as moisture will be retained. Sclerotia will be ready for harvest within 12 weeks, when fast growing and high yielding strains are used.

Harvest and storage

At maturity, the sclerotia are harvested, washed clean with water, and air dried (or sun dried) for one week. Fresh sclerotia are approximately 50% water. Dried sclerotia will maintain viability for at least two years if stored at 5-

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10℃, or for one year at 28℃. Dr Roy Watling was able to fruit a sclerotium collected in Cameroon after seven years in the green house in Edinburgh (personal communication). Yields of 10% fresh sclerotia from wet substrate (approximately 5% dry mass from dry substrate) can be expected.

Induction of sporophores from sclerotia

To induce mushroom fruiting from sclerotia, first soak the sclerotia in water for 6-12 hours (depending on size). It is necessary to bury the sclerotia in peat, sand, or soil. Sclerotia may be placed in trays or into shallow holes dug in the ground and covered with a casing layer of peat and/or soil. Ambient humidity should be greater than 85%, and it may be necessary to water the casing layer daily with approximately 40mL/ sclerotia. Fruit body formation should occur within 5-15 days.

Mushrooms intended for eating should be harvested before the cap expands, approximately 3-5 days after emergence under temperature conditions of 25-30℃. Two and possibly more flushes can be expected depending on the size of the sclerotium. Sclerotia will form if substrate is colonized at a high temperature, usually in less than 90 days. However, fruiting may be inhibited at this temperature, and will not occur while inside the bag. We have found that 25℃ is ideal for fruiting and Okhuoya and Okogbo (1990) reported that the use of casing material increases yield on a variety of substrates. Mushroom yield will be approximately 25% of sclerotia weight, and 90% moisture. Mushroom yield from sclerotia and mushroom quality is not comparable to other Pleurotus spp. on similar substrates.

Marketing and Export

P. tuberregium is not as widely cultivated or well understood as other cultivated mushrooms such as *Agaricus bisporus*(button mushroom) and *Lentinula edodes*(shiitake), which are multi-billion dollar industries in the western world. However, the sclerotia of *P. tuberregium* are very important because of its multiple uses as food and medicine (Akpaja *et al*., 2003, Nwokolo 1987, Oso 1977). The sclerotia are an essential commodity for local consumption, especially in the West African sub-region, has also become a commodity for export to developed countries, since they are sold in specialty food shops across the many continents. The possibility for increased export is predicted on two major facts:

1. People from the regions of the world where the sclerotia is used for food and medicine, who now live in Europe and America actively search for this material in African food shops in those continents. Isikhuemhen has found powdered sclerotia in shops in the UK, US and Canada.

2. The second drive for exports are due to recent scientific studies that is showing sclerotia of this fungus to contain polysaccharides and other compounds with positive medicinal benefits. Publications from Asia have shown that fresh sclerotia of this fungus have high content of useful compounds like β-glucan and lectins that are promising medicinal properties (Zhang *et al*., 2003; Wang and Ng, 2003). In our laboratories we are finding this fungus to have antibiotic properties as well as inhibition of different cancer cell lines *in vitro* (Isikhuemhen and Goktepe, unpublished). At the IMC 7 in Oslo, 2002, Dr. Jae Sung Lee of Department of Biochemistry, Yeungnam University, Korea told us that they were testing incorporating sclerotium powder into bread, as a cheap source of supplement to increase the protein content of bread.

The world is becoming aware of the importance and health benefits of natural foods and supplements. The sclerotia and sporophores of *P. tuberregium* are some of such natural foods. It is very expensive to produce sclerotia in places where temperatures are suboptimal for this fungus because of the overhead cost in environmental control. The production we do to obtain sclerotia for our research is done in incubators set at 30℃ all year round. That will

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be expensive for a commercial operation to adopt in a place like central and northern Europe and most part of North America and Asia. However, the same fungus can be produced, even under open or outdoor cultivation, without need for environmental control in places across sub-Saharan Africa (from West to East), Madagascar, southern India, China and Southeast Asia. Therefore, wherever there will be need for the sclerotia of this fungus, it will be more economical to import form the regions where they are easily cultivated. Fortunately, the regions mentioned above are mostly in developing countries.

The method described above for the cultivation of *P. tuberregium* is simple and cheap to operate and it is suitable for use in developing tropical countries. However, this model can be adjusted in both directions with little or no modifications, either for use by rural dwellers (especially women), as small or hobbyist producer, small-scale enterprise or a large commercial producer.

Feasibility of these Methods for Developing Tropical Countries

The methods outlined here were developed specifically to provide methods of cultivating fungi for food and medicine in developing countries with limited technological resources. To this end, we have described the use of agricultural wastes, which are readily available at little or no cost. We have described a variety of methods applicable to various situations. OPF and OPF/sawdust are recommended for greatest yield, low contamination, ease, and applicability to farmers with limited resources. If good sawdust spawn can be produced, log cultivation maximizes substrate inoculation and production from small amounts of spawn, and has a minimal risk of contamination. Another advantage to logs is that they produce for many years. When a sterile lab is available, use of sawdust spawn to inoculate straw, OPF, or other bulk substrates is recommended.

A beginner or untrained mushroom cultivator may have difficulty in the development of pure cultures and pure spawn because this requires some technical skill and is most effective when a laminar flow hood and autoclave are available. This can be overcome by obtaining spawn from commercial spawn manufacturers or creating a cooperative to develop a single spawn production facility to serve many farms. Otherwise, low nutrient substrates such as un-supplemented OPF or sawdust will not host most contaminants.

Contamination by other weed fungi and pathogenic organisms can be minimized by following the instructions given here for spawn and substrate preparation and by maintaining strict hygienic conditions within and around the cultivation premises. A clean room for spawn production and substrate inoculation is recommended. Growing rooms should be thoroughly cleaned between crops. Screened windows and doors can keep the majority of insects away. We have not observed insects attracted to fruit bodies of this fungus as much as we see for other *Pleurotus* species.

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Oyster Mushroom Cultivation

Part IV. Information Sources

Chapter 12

Mushroom Cultivation Information Sources

RECOMMENDED BOOKS

- Cultivation of Mushrooms by B.M. Duggar
  published by Shorey Pubns

- Edible Mushrooms and Their Cultivation by Shu-Ting Chang *et al.*
  published by CRC Press

- Edible and Poisonous Mushrooms of the World by Ian R. Hall (Editor) *et al.*
  published by Timber Press

- Genetics and Breeding of Edible Mushrooms by S. T. Chang (Editor) *et al.*
  published by Taylor & Francis

- Growing Gourmet and Medicinal Mushrooms by Paul Stamets
  published by Ten Speed Press

- Growing Mushrooms for Profit by Warren Kilby, Suzanne Kilby
  published by Fox Valley Publishing

- Growing Your Own Mushrooms : Cultivation, Cooking and Preserving by Jo. Mueller
  published by Storey Books

- International Journal of Medicinal Mushrooms
  published by Begell House

- Manual on Mushroom Cultivation (Fao Plant Production and Protection Paper, No 43)
  by Angelo Rambelli

- Mushroom Biology and Mushroom Products by World Society for Mushroom Biology and Mushroom Products

- Mushrooms : Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact,
  Second Edition by Shu-Ting Chang *et al*.
  published by CRC Press

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- Mushroom Cultivation : With Special Emphasis on Appropriate Techniques for **Developing Countries** by Peter Oei
  published by Backhuys Publishers

- Mushroom Terms : Polyglot on Research and Cultivation of Edible Fungi by H.C. Bels-Koning *et al*.
  published by Backhuys Publishers

- Mushroom Cultivator : A Practical Guide to Growing Mushrooms at Home
  by Paul Stamets, J. S. Chilton

- Mushroom Research and Development - Equality and Mutual Benefit
  by S.T. Chang, 1966

- Penn State Handbook for Commercial Mushroom Growers by Paul J. Wuest *et al*.
  published by Pennsylvania State Univ. Press

- Pennsylvania Mushroom Integrated Pest Management
  published by Pennsylvania State Univ. Press

- Shiitake Growers Handbook : The Art and Science of Mushroom Cultivation
  by Paul Przybylowicz, John Donoghue
  published by Kendall/Hunt Publishing Company

- Simon & Schuster's Guide to Mushrooms by Gary H. Lincoff
  published by Fireside

- Science and Cultivition of Edible Fungi (Mushroom Science) by International Soceity Mushroom Science

- Technical Guidelines for Mushroom Growing in the Tropics by T.H. Quimio *et al*.

- The Biology and Cultivation of Edible Mushrooms by S. T. Chang , W. A. Hayes
  published by Academic Press

- The pests of protected cultivation ; the biology and control of glasshouse and mushroom pests by N. W. Hussey
  published by American Elsevier Pub. Co.

- Tropical Mushrooms : Biological Nature & Cultivation Methods : Volvariella, Pleurotus, & Auricularia by S. T. Chang, T. H. Quimio
  published by The Chinese Univ. Press

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Oyster Mushroom Cultivation

Part IV. Information Sources

Chapter 12

Mushroom Cultivation Information Sources

RECOMMENDED WEBSITES

Cultivation

Fungi Perfecti	http://www.fungi.com
Growing Mushrooms the Easy Way	http://www.mycomasters.com
Teagsac-Irish Agriculture & Food Development Authority	http://www.teagasc.ie/agrifood/mushrooms.htm
HRI mushroom research	http://www.hri.ac.uk/sile2/research/path/pathmic.htm
Mushroom Council	http://www.mushroomcouncil.org/
Mushroom Cultivation and Marketing	http://www.attra.org/attra-pub/mushroom.html
Mushroom Cultivation Information	http://www.mycosource.com/info.htm
Mushroom Growers' Newsletter	http://www.mushroomcompany.com/
Mushrooms.com	http://www.mushrooms.com/
Mushrooms on the Move	http://aginfo.psu.edu/PSA/s99/contents.html
Penn State Mushroom Spawn Laboratory	http://mushroomspawn.cas.psu.edu/
Specialty Mushrooms	http://www.hort.purdue.edu/newcrop/proceedings1996/v3-464.html
The International Society for Mushroom Science	http://www.hri.ac.uk/isms

Pest & disease

Crop Profile for Mushrooms in California	http://cipm.ncsu.edu/ cropprofiles/docs/camushrooms.html
Growers' Information Site	http://mushgrowinfo.cas.psu.edu/siteindex.htm
HRI mushroom research	http://www.hri.ac.uk/sile2/research/path/pathmic.htm
Mushrooms on the Move	http://aginfo.psu.edu/PSA/s99/contents.html

Medicinal mushroom

Beta Glucan Health Center	http://www.glucan.com/catalog/catalog.html
Christopher Hobbs' The Virtual Herbal	http://www.christopherhobbs.com/

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DXN International	http://www.dxn2u.com/
Elitelands.com	http://www.elitelands.com/
Fungi Perfecti	http://www.fungi.com
Garuda International Inc.	http://www.garudaint.com/amush.htm
Gloria's Health Castle	http://www.geocities.com/HotSpring/spa/6638/herb_previous.html
Hawaiian Health Products, Pacific Myco Products	http://alohamedicinals.com/
Home of PSP	http://www.psp.bc.ca/index.html
International Journal of	http://www.begellhouse.com/journals/708ae68d64b17c52.html

Medicinal Mushrooms

Mushroomcity	http://www.mushroomcity.com/
Mushroom Science	http://www.mushroomscience.com/msstore/about_mushrooms.htm
MycoBiotech Ltd.	http://www.everbloom-mushroom.com.sg/
North American Medicinal Mushroom Extracts	http://www.nammex.com/MedicinalMushroomBooks.html
Pub Med	http://www4.ncbi.nlm.nih.gov/PubMed/

Societies & organizations

Alberta	http://www1.agric.gov.ab.ca/app21/rtw/index.jsp
Australian Mushroom Grower's Associastion	http://www.oz-mushrooms.com.au/
British Columbia Mushroom Marketing Commission	http://www.bcmushrooms.org/
Boston Mycological Club	http://www.bostonmycologicalclub.org/
Copenhagen School of Shiatsu	http://www.shiatsu.dk/
Department for Environment, Food and Rural Affairs (United Kingdom)	http://www.defra.gov.uk/
Food and Agriculture Organization	http://www.fao.org/
Forestry Agency (Japan)	http://www.rinya.maff.go.jp/
Fujian Edible Fungi Strains Station (Japan)	http://www.edible-mushrooms.com/
German Mycological Society	http://www.dgfm-ev.de/
Horticulture Research International	http://www2.hri.ac.uk/
Institute of Microbiology	http://www.biomed.cas.cz/mbu/mbu.html
Katholieke Hogeschool Sint-Lieven	http://www.kahosl.be/
Kinokonet	http://www.kinokonet.com/
Korean Society of Mycology	http://www.mycology.or.kr/
Mantar_kulu (Turkish)	http://www.mycology.or.kr/
Mushrooms of Middle and South Ural	http://www.geocities.com/VoyagerServiceSoftware/artemiev/mushroom/index.htm
Mycological Society of San Fransisco	http://www.mssf.org/index.html

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Pecurke.cjb.net (Cudesni Svet Gljiva) (Yugoslavia)	http://www.pecurke.cjb.net/
Persian Gulf Biotechnology Research Center	http://www.pgbrc.com/
P R Bureau Champignons	http://www.champignons.org/
Skal, certification organic production	http://www.skal.com/
Swedish Mycological Society	http://www.svampar.se/index.asp
USDA - Annual Mushroom Production Report	http://usda.mannlib.cornell.edu/reports/nassr/other/zmu-bb/
The American Mushroom Institute	http://www.americanmushroom.org/
The Australasian Mycological Society	http://www.munchkinsoftware.com/mycology/
The International Society for Mushroom Science	http://www.hri.ac.uk/isms/
The Mushroom People, Ireland's Mushroom Community Online	http://www.themushroompeople.com/default.asp
The WWW Virtual Library	http://mycology.cornell.edu/
United States Department of Agriculture's Home Page	http://www.usda.gov/
Vancouver Mycological Society	http://www.geocities.com/ RainForest/Andes/8896/

Wild mushroom

Eleen's Mushroom Mania	http://www.geocities.com/ kitonka/MushroomMania/
Fungus Image on the Net	http://www.in2.dk/fungi/
Kinoko-ya	http://www.cx.sakura.ne.jp/~kinoko/01eng/0e_home.htm
Mykoweb	http://www.mykoweb.com/
Nathan's Fungi Page	http://collectivesource.com/fungi/
R.A. Chilton's Homepage	http://www.chilton.u-net.com/fungi.htm
Red Angels	http://fly.to/redangels
The Hidden Forest	http://www.hiddenforest.co.nz/
Tom Volk's Fungi	http://botit.botany.wisc.edu/toms_fungi/
Treasures from the Kingdom of Fungi	http://www.fungiphoto.com/

Spawn

Amycel	http://www.amycel.com/
Field and Forest Products	http://www.fieldforest.net/
Golden Oak Spawn	http://www.oakshire.com/
Hesco-Inc.	http://www.hesco-inc.com/
ItalSpawn	http://www.italspawn.com/english/home_eng.html
Laboratorium Grzybni	http://www.grzybnia.pl/
Mushroom Adventures TM	http://www.mushroomadventures.com/
Mycelia	http://www.mushroomadventures.com/
Northwest Mycological Consultants	http://www.nwmycol.com/
Sylvan Inc.	http://www.sylvaninc.com/
Wylie mycologicals	http://www.interlog.com/%7Ewylie/low/main.html

Compost

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McArdle group	http://www.mcardle-mushrooms.com/
McGeary's Group	http://www.compost-ireland.com/
Sylvan Inc.	http://www.sylvaninc.com/
Thilot Holland	http://www.thilot.nl/
Walsh mushrooms	http://www.walshmushrooms.com/

Supplement & casing

Bas Van Buuren	http://www.euroveen.nl/
Champfood	http://www.champfood.com/
Fafard	http://www.fafard.com/
Harte peat	http://www.hartepeat.com/
McArdle Group	http://www.mcardle-mushrooms.com/
micelios FUNGISEM s.a	http://www.fungisem.es/
Premiere Pro - Moss	http://www.premierhort.com/
Stockosorb	http://www.stockosorb.com/
Sun Gro	http://www.sungro.com/
Sylvan Inc	http://www.sylvaninc.com/
Theriault & Hachey	http://www.theriault-hachey.com/
Topterra Holland bv	http://www.topterra.com/
Trouw nutrition	http://www.trouwnutrition.com/

Culture tools & growing kits

Field & Forest Products, Inc.	http://www.fieldforest.net/
Gourmet Mushrooms and Mushroom Products	http://www.gmushrooms.com/
Mushroom Adventures TM	http://www.mushroomadventures.com/
Myco Supply	http://www.mycosupply.com/
SporeWorks.com	http://www.sporeworks.com/index.html
Wylie Mycologicals	http://www.interlog.com/%7Ewylie/low/spawn.html

Bag & container

Fardis	http://www.fardis.org/
MycoBag	http://www.mycobag.com/
Norseman Plastics	http://www.norsemanplastics.com/
SACO2 Microsac	http://www.mycelia.be/
Unicorn Bag	http://www.unicornbag.com/

Machinery & equipment

Alcoa	http://www.alcoa.com/
Bran + Luebbe	http://www.bran-luebbe.de/
Chrisko	http://www.christiaensgroup.com/

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Christiaens	http://www.christiaensgroup.com/
Dalsem	http://www.dalsem.nl/
DLV	http://www.dlv.nl/
Dofra	http://www.dofra.nl/
double T equipment	http://www.doubletequipment.com/
Embridge	http://www.cgc.enbridge.com/
Fancom	http://www.fancom.com/
Geraedts	http://www.geraedts.nl/
Gicom	http://www.gicom.nl/
Grundfos	http://www.grundfos.com/
GTI	http://www.gti.nl/
Hoving Holland	http://www.hoving-holland.nl/
Installatievedrijf Verhoeven-Drunen	http://www.verhoeven-drunen.nl/
JanssenKessel	http://www.christiaensgroup.com/
Kubota	http://www.kubota.com/
Limbraco B.V.	http://www.limbraco.nl/
Modern Mushroom Farms	http://www.modernmush.com/
Namsan E.N.G.	http://www.namsan.co.kr/
Noord-Oost Nederland bv	http://www.non.nl/
Panbo system bv	http://www.panbo.nl/
RTM products Pty., Ltd.	http://www.rtmproducts.com.au/
Steam Engineering	http://www.steamengineering.ca/
Techmark Inc.	http://www.techmark-inc.com/
Ten cate nicolon	http://www.tencate-nicolon.com/
Traymaster	http://www.traymaster.co.uk/
Ummels	http://www.etrade.nl/etrade/klant/1541/
Van rens bv	http://www.christiaensgroup.com/
Viscon	http://visserite.com/viscon/

Mushroom producers

All Seasons Mushrooms	http://www.allseasonsmushrooms.com/
Continental Mushroom Corporation Ltd.	http://www.continentalmushroom.ca/
Elitelands	http://www.elitelands.com/
Garden City Fungi	http://www.gardencityfungi.com/
Golden Gourmet Mushrooms	http://www.goldengourmetmushrooms.com/
Highline Mushrooms	http://www.highlinemushrooms.com/
Karol Kania I Synowie Sp. z. o. o	http://www.kania.net.pl/
Lost Creek Mushroom Farm	http://www.cowboy.net/cmf/
Money's Mushrooms Ltd.	http://www.moneys.com/indexa.htm
Monterey Mushrooms, Inc.	http://www.montereymushrooms.com/
MushMush	http://www.mushmush.nl/
Myco Biotech Ltd	http://www.everbloom-mushroom.com.sg/
Myco Supply	http://www.mycosupply.com/

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Oakshire Mushroom Farm Inc.	http://www.oakshire.com/
Organic mushroom Co.	http://www.organicmushroom.com/
Ostrom's	http://www.ostromfarms.com/
Phillips Mushroom Farms	http://www.phillipsmushroomfarms.com/
Prairie Mushrooms	http://www.prairiemushrooms.com/
Shanghai Edible Mushroom	http://www.sh-mushroom.com/
Shiitake Mushroom Center	http://www.shiitakecenter.com/
To-Jo Fresh Mushrooms, Inc.	http://www.to-jo.com/
Verbruggen Paddestoelen	http://www.verbruggenpaddestoelen.nl/

Consultants

Blaak	http://www.eblaak.com/
C point http://www.cpoint.nl/	
Northwest Mycological Consultants	http://nwmycol.com/

Chemicals

Amvac chemical corporation	http://www.amvac-chemical.com/
Aventis	http://www.aventis.com/main/home_static.asp
CERTIS	http://www.thermotrilogy.com/
Curtis Dyna-fog	http://www.dynafog.com/
Drummond American	http://webapp1.drummondamerican.com/drummond/homePage
Ehrlich Distribution	http://www.ehrlichdistribution.com/
International Dioxcide Inc.	http://www.idiclo2.com/
Micro Bio	http://www.microbiogroup.com/
Notional Chemical Laboratories	http://www.nclonline.com/
Sylvan Inc.	http://www.sylvaninc.com/
Well mark	http://wellmarkinternational.com/

Distributors

Florida Mushrooms, Inc.	http://www.flmushroom.com/
Lil' Shop of spores	http://lilshopofspores.com/
Melissa's/World Variety Produce, Inc.	http://www.melissas.com/
Micron Magick Mycology Market	http://www.micronmagick.com/
MycoLogical Natural Products	http://www.mycological.com/

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