4 Production of Edible Mushrooms

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I. Introduction

Among all the edible commodities, mushrooms have attracted the attention of human beings, not only because of their fascinating shape, size, colours and structures, but also because of their edible nature, neutraceutical properties and their practical applications in various industrial products. Mushrooms are a source of food, nutrition and minerals, and nowadays they are also valued because of their bioactive molecules to fight against several diseases. Among the novel protein sources, mushrooms, yeasts and algal foods are frequently mentioned as alternative protein sources. Out of these, mushrooms, due to their unique flavour, palatability and direct utilization supersedes all non-conventional foods (Worgan 1968; Bano 1978). Mushrooms either belong to the Ascomycotina or the Basidiomycotina and may be hypogeous (below soil) or epigeous (above soil). They can be found growing in nature as saprophytes on any kind of organic matter or on fresh or dead wood or associated with plant roots as mycorrhizal fungi (ectomycorrhiza). They are found in all types of ecosystems in the tropical, sub-tropical and temperate regions of

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Table 4.1. World production of cultivated edible mushrooms in 1986 and 1997 (modified according to Chang 1999)

Species	1986		1997		Increase (%)
	Fresh weight (× 1000 t)	Worldwide (%)	Fresh weight (× 1000 t)	Worldwide (%)	
Agaricus bisporus	1227	56.7	1956	31.8	59.4
Lentinula edodes	314	14.4	1964	25.4	398.1
Pleurotus sp.	169	7.7	876	14.2	418.3
Auricularia spp.	119	5.5	485	7.9	307.6
Volvariella volvacea	178	8.2	181	3.0	1.7
Flammulina velutipes	100	4.6	285	4.6	130
Tremella spp.	40	1.8	130	2.1	225
Hypsizygus sp.	–	–	74	1.2	–
Pholiota nameka	25	1.1	56	0.9	124
Grifola frondosa	–	–	33	0.5	–
Others	10	0.5	518	8.4	5080
Total	2182	100	6158	100	182.2

the world. Many species produce a set of lignocellulolytic enzymes like peroxidases, laccases, aryl alcohol oxidase, endoglucanases, laminarinase and xylanases. These enzymes help the mycelium of white-rot and brown-rot mushrooms to recycle and degrade complex lignocellulosic materials including agricultural by-products. One has always to keep in mind that the "typical mushroom" just represents a temporarily formed sexual reproduction unit, the fruiting body, and that the actual fungus is growing, mostly invisible to us, as a hidden mycelium in the ground.

II. Present Scenario of Mushroom Production

Agaricus bisporus (white button mushroom) and *Pleurotus* spp. (oyster mushroom) are commercially cultivated world-wide due to their broad acceptability in the society, while other edible mushrooms like *Auricularia* spp. (black ear mushroom), *Volvariella* spp. (paddy straw mushroom) and *Tremella* spp. are in particular popular in China and *Grifola* and *Hypsizygus* spp. are cultured in Japan. The total world production of all mushrooms was around 6×10^6 t in 1997 and was estimated to have reached 22×10^6 t in 2009 (Chang 1999; http://opaals.iitk.ac.in:9000/wordpress/index.php/mushroom-cultivation). Twenty years ago, *A. bisporus* was contributing 70% of the total world mushroom supply but, by the mid-1990s, this has decreased to 37%. In 2001–2002, the United States alone produced about 393000t of mushrooms. *A. bisporus* alone contributed to 90% of total production, while *Pleurotus*, *Lentinula*, *Grifola*, *Hypsizygus*, *Flammulina* and *Hericium* were other mushrooms gaining popularity. The market share of mushroom trade in the world in 2001 was worth US $ 40×10^9, of which 70% was from the cultivation of edible mushrooms, while 20% was from medicinal mushrooms and 10% from wild edible mushrooms collected from nature (e.g. *Boletus* spp., *Cantharellus* spp.).

The seven most important cultivated mushrooms are: *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus* spp., *Auricularia* spp., *Volvariella volvacea*, *Flammulina velutipes* and *Tremella fuciformis*. The total world production of individual species for 1986 and 1997 is given in Table 4.1 (Chang 1999). In China, the main thrust is on *Lentinula edodes* and *Pleurotus* spp., while *A. bisporus* ranks fourth among all the cultivated mushrooms. There has been diversification of cultivated edible mushroom species, which is responsible for increased world production during the 1980s and 1990s. Several new wild-type mushroom species, subspecies and strains have been collected over the past decade and are now subject to domestication.

III. Main Genera of Cultivated Mushrooms

In the literature there are about 15 000 macrofungi, and 5000 of them are considered edible while 2000 mushroom species are prime edibles, belonging to 31 genera. Researchers have experimentally grown about 100 species, 50 species are economically used and 20–30 species are

cultivated in different parts of the world. The main genera of edible basidiomycetous mushrooms are *Agaricus*, *Agrocybe*, *Albatrellus*, *Auricularia*, *Boletus*, *Cantharellus*, *Calvatia*, *Clavaria*, *Clitocybe*, *Coprinus*, *Dictyophora*, *Flammulina*, *Gloesterium*, *Hericium*, *Hydnum*, *Kuehneromyces*, *Lactarius*, *Lentinula*, *Lepista*, *Lyophyllum*, *Marasmius*, *Podaxis*, *Phellorina*, *Pleurotus*, *Pholiota*, *Polyporus*, *Ramaria*, *Rhizopogon*, *Russula*, *Scleroderma*, *Sparassis*, *Stropharia*, *Termitomyces*, *Tremella*, *Trappeinda*, *Tricholoma* and *Vovariella*. The edible/medicinal ascomycetes are species of *Cordyceps*, *Tuber*, *Morchella*, *Terfezia*, *Tirmania*, *Elaphomyces* and *Helvella*.

Most of the species of *Amanita* are poisonous but some species like *A. ceasarea* and *A. hemibapha* make excellent eating. The majority of *Agaricus* species are edible but e.g. *A. xanthodermus* is poisonous. So, it is not necessarily the case that all species of a genus are edible and, vice versa, not all species of a poisonous group are poisonous. One has to be very careful while picking wild mushrooms for consumption because deaths due to mushroom poisoning are reported every year. For example, during 1996 in the Ukraine, 2860 people were poisoned due to consumption of wild mushrooms and 166 people died. Sometimes deaths are due to insect- and pest-infected mushrooms.

It is not possible to compile and discuss the cultivation methods of all edible mushroom species. However, a few of them, which are widely cultivated in Asian countries as well as a few promising species not paid much attention so far are described in detail here. This chapter does knowingly not deal with *Agaricus bisporus*, since a comprehensive literature exists about this mushroom (e.g. see Wood and Goodenough 1977; Whiteford and Thurston 2000), and likewise some other edible fungi, though commercially produced, are just touched in the margin (*Tremella ficiformis*, *Hypsizygus marmoreus*, *Pholiota nameko*, *Grifola fondosa*, etc.).

IV. Cultivation of *Calocybe indica* P. & C. (Milky Mushroom)

C. indica was first reported from India by Purkayastha and Chandra in 1974 (Fig. 4.1). This litter-decomposing agaric naturally grows on the humus-rich soil under roadside trees and agricultural fields in West Bengal, India. During rainy seasons, wild collections are gathered by the local people and sold on the Calcutta market as "Dudhi chata", which means milky mushroom. The natural occurrence of *C. indica* has been also reported from plains of Tamilnadu and Rajasthan in India (Doshi et al. 1989; Krishna Moorthy 1995). *C. indica* is a tropical mushroom that has not find a place in the global mushroom industry so far, but is presently commercially produced in India. It is cultivated in the tropical and subtropical parts of India during the summer months. This mushroom is particularly gaining popularity in South Indian states of Tamilnadu, Karnataka and Andhra Pradesh due to its similarity in colour and morphology to *Agaricus bisporus* and has readily been accepted by the people there as a "tropical variant" of the white button mushroom.

A. Substrate and Substrate Preparation

C. indica can be cultivated on non-composted lignocellulosic residues like *Pleurotus* spp. It can be grown on substrates containing lignin, cellulose and hemicelluloses. The substrate should be always fresh and dry; substrates exposed to rain or harvested premature (green plant material) are prone to various weed moulds, which may result in poor spawn growth and crop failure.

Suitable substrates for growing are straw of paddy, wheat, ragi, maize/bajra, sorghum, palm rosa grass, groundnut haulms, soybean hay, black gram hay, cotton stalks and leaves, sugarcane bagasse, cotton/jute waste, dehulled maize cobs, tea/coffee waste etc. However, cereal straw (paddy/wheat) easily available in large quantities is the most widely used substrate. Singh et al. (2009) reported higher mushroom yields from paddy straw followed by wheat straw, sugar cane bagasse and gram straw. Straw is chopped into small pieces (2–4 cm) and soaked in fresh water for 10–12 h. This period can be reduced when pasteurization is done by steam. Main purpose of soaking is to saturate substrate with sufficient moisture. It is easier to soak when the straw is filled into gunny bags and dipped into water.

The purpose of pasteurization is to kill harmful microbes. This can be achieved in three ways.

Hot Water Treatment: Water is boiled in a wide-mouth container and chopped wet straw filled into gunny bags is submerged into the hot water for 40 min at 80–90 °C to achieve pasteurization. This is a very popular method particularly used by small growers.

Steam pasteurization: Wet straw is filled inside a insulated room or pasteurization tunnel either on perforated shelves or in wooden trays. Steam is released under pressure from a boiler and temperature inside chamber and substrate is raised to 60–65 °C and maintained for 5–6 h. Air inside the room should be circulated to have uniform temperature in the substrate.

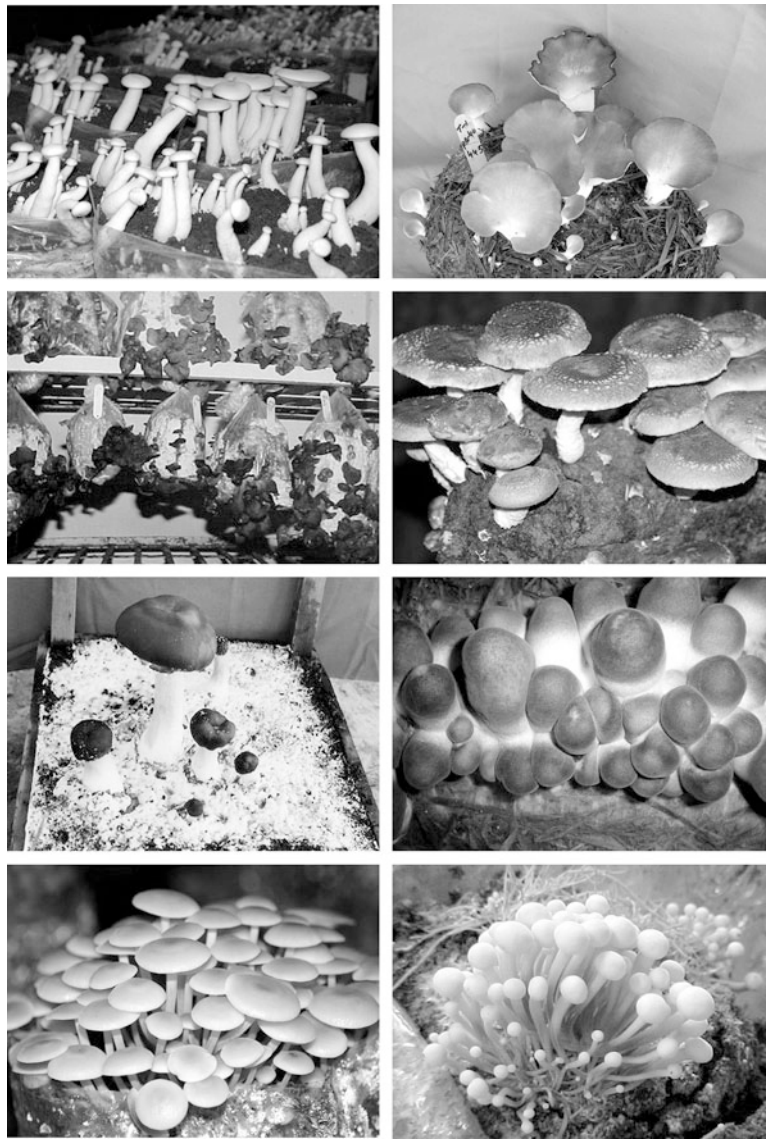

Fig. 4.1. Fruiting bodies of commercially used mushrooms. Left column, top down: *Calocybe indica*, *Auricularia polytricha*, *Stropharia rugoso-annulata*, *Flammulina velutipes* (wild type). Right column: *Pleurotus ostreatus*, *Lentinus edodes*, *Volvariella volvacea*, *F. velutipes* (cultivated type)

Sterilization: Substrate is filled in polypropylene bags (35 × 45 cm, holding 2–3 kg wet substrate) and sterilized. Once pasteurization/sterilization is over, straw is shifted to the spawning room for cooling, bag-filling and spawning.

B. Spawn Preparation

Cultures of *C. indica* are susceptible to low temperatures, and prolonged storage at low temperature (+4°C) inhibits the culture vigour. The optimum temperature for culture storage is between 16 and 18°C. Pandey et al. (2000) found that wheat, ragi, sorghum and bajra grains can be used for spawn preparation. The spawn preparation method is similar to other mushrooms, however, incubation temperature for *C. indica* is higher (30–35°C). The mycelial growth is slower than that of *Pleurotus* or *Volvariella* spp. and it takes 15–22 days for complete mycelial colonization of wheat grains.

C. Spawning

Spawning methods are similar to oyster mushrooms, however, the spawn rate is higher. An amount of 5% spawn (wet weight of substrate) in cultivation bags should be used and spawn should not be older than 45 days. After autoclaving and

spawning, bags are shifted to the spawn running room and kept in the dark at 25–35 °C and relative humidity above 80%. Substrate supplemented with maize meal or wheat bran (5%) gives higher production. It takes about 20 days until the substrate is fully colonized and bags are ready for casing. Bags are shifted to special cropping rooms for casing and cropping. Yield reduction has been reported at temperatures below 25 °C during incubation, while supplementation of soybean meal (4%) at the time of spawning gives earlier and a higher number of basidiocarps (= sporophores, fruiting bodies) and yield (Singh et al. 2007).

D. Casing

As in the case of *A. bisporus*, casing is also required for fructification in *C. indica*. Casing means covering the top surface of bags with a pasteurized casing material after the mycelium has fully colonized the substrate.

The casing soil is applied after opening the bags on the top surface to a thickness of about 10–15 mm. The casing provides physical support and moisture and allows gases to escape from the substrate. The composition and quality of casing mixtures (pH, water holding capacity, C:N ratio etc.) directly affects the initiation of pinhead formation and further fruiting body development. Casing material (soil 75% and sand 25%) having a pH adjusted to 7.8–7.9 with chalk powder is pasteurized in compost tunnels at 60–70 °C for 4–6 h or chemically treated with formaldehyde (4%) for about a week in advance of casing. Formaldehyde solution should be added in amounts enough to saturate the soil. Casing soil is covered with a polythene sheet to avoid volatilization of chemicals and, at 2-day intervals, the casing soil is turned so that at the time of casing the soil is free from formalin fumes. A mixture of old spent compost, farmyard manure, sand and garden soil in a 1:1:1:1 ratio has been found to be the best material, giving the highest mushroom yields (Singh et al. 2007). The bag's top surface is made uniform by ruffling and sprayed with the fungicide carbendazim (0.1%, active against moulds) and formaldehyde solutions (0.5%). Casing material is spread in uniform layers of 1–1.5 cm thickness and the cropping room temperature is maintained at 30–35 °C and relative humidity of 80–90% after casing.

E. Cropping

It takes about 10 days for the mycelium to reach the top of the casing layer. Complete darkness during incubation favours fruiting body formation. Light should be provided for 6–8 h. Krishnamoorty et al. (2000) reported daily light of 1600–3200 lux for 6 h during daytime for higher mushroom production. The changes thus made in the fungus' environment result in the initiation of fruiting body formation. Pinheads start to appear within 3–5 days and usually mature in about a week. Mushroom heads (7–8 cm in diameter) are harvested by twisting, cleaned and packed in perforated polypropylene bags for marketing. Mushrooms can also be wrapped in clingfilm for longer storage.

F. Precautions during Cropping

- The substrate is the major source of weed moulds and disease-causing organisms. Hence substrates should be chopped and soaked at a distance from bag filling/spawn running and cropping areas. The worker chopping the straw should not be involved in bag filling and spawning without taking a bath and change of clothes.
- Bag filling and spawning rooms should be sprayed with formaldehyde (1%) twice a week. There should not be much air movement in the room. For large-scale production it is advisable to have air circulation through filters.
- At the time of casing, the open top surface should be sprayed with carbendazim (0.1%) plus formaldehyde (0.5%) before casing and repeated on casing soil and inside the room and again after a week; solutions should not be sprayed on the mushrooms. The insecticide malathion (0.1%) can be sprayed in the evening or next day to protect the material from flies.
- If any patch of mould (it may be green/blue/brownish) is noticed, spot treatment with formaldehyde (4%) should be performed with soaked cotton.
- During rainy seasons, controlled watering once a day may be enough; during summer months twice watering is necessary as the loss of water is higher and it becomes difficult to maintain the required humidity and moisture of the substrate. During such periods, one should spread sand on the floor and use mist sprayer and frequently check the moisture of the casing material by touching. Watering should also be done to maintain a relative humidity of 80–85% inside the cropping rooms.

G. Harvesting

The picking of fruit bodies is usually done at the stage of beginning cap opening. The first flush appears 2–3 weeks after casing and 4 weeks after spawning. Mature and fully grown mushrooms with expanded caps are picked up gently without disturbing the young pinheads. After a first harvest the trays or bags give their next flush within 7–12 days. The second flush yield is less than the first one. With a high-yield strain, 0.7–1.0 kg fresh mushrooms can be obtained from 1.0 kg dry substrate. The mature fruiting bodies contain about 15–17% protein on a dry weight basis. Chemical analysis revealed 12 amino acids and one amide. Among the detected amino acids, glycine appears to be predominant (Purkayastha and Nayak 1981). The fruit bodies of *C. indica* can be easily air/sun dried. No much work on the medicinal properties of this mushroom has been carried out. However, antibacterial properties against human pathogens, namely *Bacillus* spp., *Escherichia coli*, *Vibrio cholerae* and *Salmonella typhi*, have been reported from dried fruiting bodies. Significant levels of pyridine 3-carboxylic acid (nicotinic acid) were also observed in *C. indica* fruiting bodies (Mallavadhani et al. 2005). Selvi et al. (2007) found non-enzymatic antioxidant properties in both fresh and dried fruit bodies of the mushroom.

There are several advantages of growing *C. indica* namely:

- It is suitable for warmer climates and can be easily cultivated in tropical and subtropical areas of Asia, Africa and South America.
- The crop duration is short (4–8 weeks).
- The fresh mushrooms have a shelf life of 2–4 days and the fruiting bodies never turns brownish or black.
- No serious incidence of any mould or insect has been reported so far.
- The production costs are relatively low and therefore the fungus is suitable for "poor" farmers in developing countries.

V. Cultivation of *Pleurotus* spp. (Oyster Mushrooms)

Oyster mushrooms (Fig. 4.1) are the most suitable fungal organisms for producing protein-rich food from various agro-wastes without composting. These mushrooms are cultivated in about 25 countries of Asia, Europe and America. It is the third most important cultivated mushroom in the world and annual world production was almost 900 000 t in 1997 (Chang 1999). China alone contributes to about 90% of the total world production. The other major producing countries are South Korea, Japan, Italy, Taiwan, Thailand and Philippines. *Pleurotus* mushrooms irrespective of the particular species are generally referred to as "oyster mushrooms". Oyster mushrooms are lignocellulolytic fungi causing a white rot of wood and grow naturally in temperate, subtropical and tropical forests on dead wooden logs of deciduous and sometimes coniferous trees. They can also grow on decaying organic matter. Their fruiting bodies are distinctly shell-, fan- or spatula-shaped with different shades of white, cream, grey, yellow, pink or light brownish depending upon the species. However, the colour of the basidiocarps is extremely variable and influenced by temperature, light intensity and the nutrients of the substrate.

A. Advantages of Growing Oyster Mushrooms

Variety of substrates: *Pleurotus* mushrooms are white-rot fungi, degrading and growing on any kind of agricultural or forest waste material which consists of cellulose, hemicelluloses and lignin, and due to the secretion of a set of extracellular enzymes they can be used without fermentation or composting stage beforehand.

Choice of species: Among all cultivated mushroom genera, *Pleurotus* comprises the largest number of species and varieties. Most of them grow best at less than 20 °C and some others prefer temperatures between 24 and 30 °C. So cultivation of oyster mushrooms can be done round the year, and variation in shape, colour, texture and aroma can be achieved in dependence of the particular species/variant.

Simple cultivation technologies: *Pleurotus* mycelium can also grow on fresh straw and it does not require a specific substrate for growth. Substrate preparation for oyster mushrooms is quite simple and cultivation does not require controlled environmental conditions as in case of *A. bisporus*, because most *Pleurotus* species have a wide temperature, relatively humidity and CO_2 tolerance.

Storage and shelf life: Unlike the white button mushroom, oyster mushrooms' fruit bodies can be easily dried and stored. Dried oyster mushrooms can be instantly used after soaking in hot water for 5–10 min or used in powdered form for several preparations. Fresh mushrooms have a shelf life of 24–48 h at room temperature.

Productivity: *Pleurotus* productivity is high as compared to all other cultivated mushrooms. One can harvest a minimum of about 500–700 kg of fresh oyster mushroom from one ton of dry wheat or paddy straw within 45–60 days (note: the same quantity of straw gives only 400–500 kg of the white button mushrooms within 100–120 days). Fruiting body yield can further be increased by supplementing the substrate with a suitable nitrogen source such as soybean and/or cotton seed meal or by using highly productive strains.

B. History of Oyster Mushroom Cultivation

The history of oyster mushroom cultivation is of recent origin in comparison to *Auricularia* spp. (600 A.D.), *Lentinula edodes* (1100 A.D.) and *Agaricus bisporus* (1650). The present day cultivation technology is a result of various successive steps evolved throughout the world during the twentieth century. A simple form of growing *Pleurotus* spp. was adopted by lumberman in Europe in the nineteenth century. They used to collect wooden logs and stumps showing fructification in nature and kept them in cool, moist places. It allowed them to harvest periodically oyster mushrooms from these logs under convenient conditions. The first successful modern cultivation of *Pleurotus ostreatus* was achieved in Germany by Falck in 1917. He inoculated tree stumps and wooden logs with mycelium of *P. ostreatus* (at that time *Agaricus ostreatus*) and could harvest fresh fruiting bodies, and later Etter (1929) produced fruiting bodies on different wood materials. On sawdust medium, formation of sexual spores of *P. corticatus* Fr. were reported by Kaufert (1935); and Block et al. (1958) cultivated *P. ostreatus* for the first time under laboratory conditions on saw dust. They used a mixture of oatmeal and saw dust for cultivation and found best results on *Eucalyputs* wood followed by pine saw dust. They observed some growth abnormalities in fruit bodies due to insufficient light conditions and no mushroom formation when the temperature was $<10^{\circ}$C or $>32^{\circ}$C. In India, cultivation of *P. flabellatus* on paddy straw was first reported by Bano and Srivastava in 1962. Corn cobs were used under sterile conditions for growing *P. ostreatus* (Toth 1970). This method was modified by Gyurko (1969) for non-sterile conditions. A Hungarian method for growing oyster mushrooms based on sterile production was patented in 1969 (HTTV patent). Stanek and Rysava (1971) developed a method of application of thermophilic microorganisms in the fermentation of substrates for the subsequent cultivation of *P. ostreatus*. Zadrazil (1974) developed a method for continuous preparation of substrate and industrial production of *Pleurotus* mushrooms. Jandaik and Kapoor (1976) grew *P. sajor-caju* on various substrates including wheat and banana pseudo stems. Finally, Leong (1982) successfully developed a method for cultivation of *P. sajor-caju* using cotton waste from the textile industry.

C. Biology of the Oyster Mushroom

Visually the basidiocarps (fruiting bodies) of an oyster mushroom has three distinct parts – a fleshy shell or spatula shaped cap (pileus), a short or long lateral or central stalk called stipe and long ridges and furrows underneath the pileus called gills or lamellae. The gills stretch from the edge of the cap down to the stalk and bear the spores. If a fruiting body is kept on a paper directly (gills facing the paper), a dirty deposition of powdery spores will be seen. This spore print colour may be whitish, pinkish, lilac or grey. The spores are smooth, cylindric or allantoid and germinate easily on any kind of mycological medium, and within 48–96 h, whitish thread-like colonies develop (primary mycelium). Fusion between two compatible primary mycelia (homothallic) develops into a secondary mycelium (heterothallic = dikaryotic), which has clamp connections and is again fertile; the primary mycelium is clampless and non-fertile. The mycelium of *Pleurotus* is pure white in colour except those of *P. cystidiosus, P. smithii* and *P. columbinus*, which form coremia-like stalked structures (asexual spores). *P. tuber-regium* forms a tuber-like structure in the substratum, which is also edible and has positive medicinal properties.

D. Varieties of Oyster Mushrooms

All the varieties and species of oyster mushrooms are edible except *P. olearius* and *P. nidiformis*, which were reported to be poisonous. There are 38 species of the genus recorded throughout the world (Singer 1986). In recent years, 25 species have been commercially cultivated in different parts of the world, among which the most important are as follows: *P. ostreatus* (Fig. 4.1), *P. flabellatus*, *P. florida*, *P. sajor-caju*, *P. sapidus*, *P. cystidiosus*, *P. eryngii*, *P. fossulatus*, *P. opuntiae*, *P. cornucopiae*, *P. yuccae*, *P. platypus*, *P. djamore*, *P. tuber-regium*, *P. australis*, *P. purpureo-olivaceus*, *P. populinus*, *P. levis*, *P. columbinus* and *P. membra-naceus*.

E. Cultivation

The procedure for oyster mushroom cultivation can be divided into the following four steps:

1. Preparation or procurement of spawn
2. Substrate preparation
3. Spawning of substrate
4. Crop management.

1. Preparation or Procurement of Spawn

The spawn preparation technique for oyster mushrooms is similar to that of the white button mushroom (*A. bisporus*). Pure cultures of *Pleurotus* spp. are transferred to sterilized wheat grains, which are then incubated for 10–15 ays. It has been reported that jowar and bajra grains are superior over wheat grains. The mycelium of oyster mushroom grows fast on wheat grains and 25- to 30-day-old spawn already starts forming fruit bodies. Sometimes the mushroom farmers use active mycelium already growing on the substrate for spawning fresh oyster mushroom bags. This method can be used only on a small scale.

2. Substrate Preparation and Nutrition Quality

A large number of agricultural, forest and agro-industrial by-products are useful for growing oyster mushrooms. These by-products or wastes are rich in cellulose, lignin and hemicelluloses. However, yield of oyster mushrooms largely depends on the nutrition and nature of the substrate. The substrate should be fresh, dry, free from mould infestation and properly stored. Substrates exposed to rain and harvested immature with green chlorophyll patches inhibit the growth of *Pleurotus* mycelium. Oyster mushrooms can utilize a number of agrowastes including straw of wheat, paddy and ragi, stalks and leaves of maize, jowar, bajra and cotton, sugarcane bagasse, jute and cotton waste, dehulled corn cobs, pea nut shells, dried grasses, sunflower stalks, used tea leaf waste, discarded waste paper and synthetic compost of button mushrooms. It can also be cultivated using industrial wastes like paper mill sludge, coffee by-products, tobacco waste, apple pomace and dried leaves of deciduous trees. The cellulose and lignin contents are important components influencing the yield of *Pleurotus* fruiting bodies; cellulose-rich substrates like cotton wastes are to prefer.

Methods of substrate preparation: The mycelium of *Pleurotus* is saprophytic in nature and it does not require specific substrates for its growth. Thus mycelial growth can take place on simply water-treated straw but there are always cellulolytic moulds present in straw, which may affect *Pleurotus* growth. These competitor moulds sometimes restrict the growth of *Pleurotus* mycelium due to secretion of toxic metabolites. There are various methods to get rid of undesirable microorganisms in the straw and to favour the growth of *Pleurotus* mycelium. The most popular methods of substrate preparation are as follows.

Steam pasteurization: Pre-wetted straw is packed in wooden trays or boxes and then kept in a pasteurization room at 60–65 °C for 4 h. Temperature of the pasteurization room is regulated with the help of steam through a boiler. The substrate, after cooling at room temperature, is seeded with spawn. The entire process takes 3–5 days. There are various minor variations of this methods adopted in Europe.

Hot water treatment: The substrate, after chopping (5–10 cm), is soaked in hot water (65–70°C) for 1 h (Bano *et al.* 1987), or 60–120 min at 80°C, or in the case of paddy straw at 85°C for 30–45 min. After draining excess water, the spawn is added. The leached water contains a lot of soluble sugars and phenolic compounds. Hot water treatment makes compact substrates like maize cobs or stems softer so that mycelial growth can proceed more easily. However, this method is not suitable for large-scale cultivations.

Sterile technique: The chopped substrate, after soaking in cold water, is put in heat-resistant polypropylene bags and sterilized in an autoclave for 1–2 h, followed by spawning under aseptic conditions. This method is rather suitable for research labs than for large-scale production due to energy costs.

Fermentation or composting: This method is a modification of composting techniques used for the white button mushroom. It is most suitable for substrates like cotton stalks, maize stalks and leguminous stubbles. Both aerobic and anaerobic fermentation of the substrate is suitable for *Pleurotus* cultivation. Composting should be done on a covered area or shed. The chopped substrate (5–6 cm) is supplemented with ammonium sulfate or urea (0.5–1.0%) and lime (1%) on a dry weight basis of the ingredients. Horse or chicken manure (10% on a dry weight basis) can also be used instead of nitrogenous fertilizers. The addition of lime improves the physical structure of the compost that is sprinkled with water and put into triangular heaps (75–90 cm in height). After 2 days of incubation, the pile is turned over, and 1% superphosphate and 0.5% lime is added. The compost is ready after 4 days and can be spawned as such or used after pasteurization.

Chemical sterilization techniques: It has been observed that mould infestation due to various species of *Trichoderma*, *Gliocladium*, *Penicillium*, *Sclerotium*, *Aspergillus* and *Stysanus* is a common problem during oyster mushroom cultivation. Sometimes these moulds prevent

the growth of mushroom mycelium resulting in complete crop failure. When wheat straw is treated by steeping in a chemical solution of carbendazim 50% (37.5 ppm) and formaldehyde (500 ppm) for a period of 16–18 h, most of the competitor moulds are either killed or their growth is suppressed for 25–40 days. The technique was standardized in India by Vijay and Sohi in 1987. The authors of this chapter also obtained good results with lower concentrations of carbendazim.

Substrate supplementation: The nitrogen contents in most lignocellulosic substrates range between 0.5 and 0.8% and hence the addition of organic nitrogen to straw helps getting higher yields. Some of the common supplements (3–10%) are wheat bran, rice bran, cotton seedmeal, soybean cake, groundnut cake and ammonium nitrate. Supplements are thoroughly mixed with straw during spawning. It should be taken into account that supplements increase the substrate temperature, which can be risky during the summer season.

3. Spawning of Substrates

Freshly prepared, 20- to 30-day-old grain spawn is best for spawning. Old spawn (3–6 months) stored at room temperature (20–30 °C) forms very thick mats due to mycelium aggregation and, sometimes, young pinheads and fruit bodies start developing in the spawn bottle itself. The spawning should be done in a pre-fumigated room (48 h with 2% formaldehyde). The spawn should be mixed at 2–3% to the wet substrate, i.e. 300 g spawn is sufficient for 10–12 kg substrate. Spawn can be mixed thoroughly or in layers. Spawned substrates are filled into polyethylene bags (60 × 45 cm) of 125–150 gauge thicknesses. Ten to 15 small holes (0.5–1.0 cm in diameter) are made on all sides, especially two to four holes in the bottom to leach out excess water. Perforated bags give a higher and earlier crop (4–6 days) than non-perforated bags because of accumulation of CO_2, which inhibits fruiting. The bags can also be tightly pressed and tied with a nylon rope. Such blocks are incubated intact and, after mycelial growth, the polythene sheet is removed.

4. Crop Management and Incubation

The spawned bags or blocks are kept in incubation rooms for mycelial growth. They can be kept on a raised platform or shelf or hanged in cropping rooms for mycelial colonization. Although mycelium can grow at 10–30°C, the optimum temperature lies between 22 and 26°C. Higher temperatures (>30°C) in the cropping room inhibit growth and kill the mycelium. The daily maximum and minimum temperature of cropping rooms and beds should be recorded. The bed temperature is generally 2–4°C higher than the room temperature. During mycelial growth, the bags are not to be opened and no ventilation is needed. Moreover, there is no need for high humidity or water spraying.

Fruiting body induction: Once the mycelium has fully colonized the substrate and formed a thick mycelial mat, it is ready for fruiting. Contaminated bags with moulds may be discarded while bags with patchy mycelial growth may be left for a few more days to complete mycelial growth. Bags should not be opened before days 16–18, except in the case of *P. membranaceus* and *P. djamor* var. *roseus* which form fruiting bodies within 10 days, even in closed bags with small holes. There is no need for casing the substrate. All the bundles, cubes or blocks are arranged on wooden platforms or shelves with a minimum distance of 15–20 cm between each bag. Some cultural conditions required for fruiting are as follows.

Temperature: Mycelial growth of all *Pleurotus* spp. can take place between 20 and 30°C. However, for fruiting, different species have different temperature requirements. Depending upon the temperature requirement of a species, they can be categorized into two groups: winter or low-temperature species (10–20°C) and summer or moderate-temperature species (16–30°C). Summer varieties can fructify at low temperatures as well, but the winter varieties cannot do so at higher temperature; they need a low temperature shock to induce fructification. Commercial varieties which can be cultivated during summer are *P. flabellatus*, *P. sapidus*, *P. citrinopileatus* and *P. sajor-caju*. Low-temperature species are *P. ostreatus*, *P. florida*, *P. eryngii*, *P. fossulatus* and *P. cornucopiae*. The growing temperature affects not only the yield but also the quality of the product. The pileus (cap/cup) colour of *P. florida* is light brown when cultivated at low temperature (10–15°C) but changes to white pale and yellowish at 20–25°C. Similarly, the fruit body colour of *P. sajor-caju* when cultivated at 10–19°C is white to dull white with a high dry matter content, while at 25–30°C it is brownish to dark brown with a low dry matter content. The temperature requirements of different *Pleurotus* spp. are given in Table 4.2.

Relative humidity: All *Pleurotus* species require high relative humidity (70–80%) during fruiting. To maintain relative humidity, water spraying is to be done in the cropping rooms by hand or using humidifiers.

Oxygen and carbon dioxide requirements: The oyster mushroom can tolerate high carbon dioxide concentrations during a spawn run (up to 20 000 ppm, or 20–22%) while it should be less than 600 ppm or 0.6% during cropping. Therefore, sufficient ventilation should be provided during fructification. If the CO_2 concentration is high, the mushrooms will have long stipes and small convoluted pilei (like a trumpet).

Light: Light is required to initiate fruiting body formation. For primordia formation, the light requirement is 200 lux intensity for 8–12 h. Inadequate light conditions can be judged by long stalks, small caps and poor yield.

Table 4.2 Temperature requirement of different *Pleurotus* spp. and their yield performance (Upadhyay, unpublished data)

Species	Optimum temperature of mycelial growth on substrate (°C)	Optimum temperature for fruiting (°C)	Temperature range for growth	Yield performance (% B.E.)
P. flabellatus	25–28	22 ± 2	16–28	60–90
P. sajor-caju	25–28	24 ± 2	17–30	50–70
P. sapidus	25–28	24 ± 2	17–30	40–70
P. membranceus	30	27 ± 2	20–30	60–80
P. citrinopileatus	30	26 ± 2	20–30	50–80
P. djamor	30	27 ± 2	16–32	70–90
Winter-cultivated species				
P. ostreatus	25–28	20 ± 2	18–22	30–50
P. florida	25–28	20 ± 2	12–22	60–90
P. eryngii	18–25	20 ± 2	12–22	50–70
P. cornucopiae	18–25	20 ± 2	12–25	40–70

The colour of the pileus is also influenced by light intensity and duration of exposure. Fruit bodies raised in bright light are dark brown, grey or blackish. If the light intensity is less than 100 lux, the mushrooms will be pale yellowish.

Hydrogen ion concentration (pH): The optimum pH during mycelial colonization is between 6 and 7, and the water for spraying should not contain too much salt. Rusted iron drums used for substrate treatment or storing water for spraying delay fructification due to the presence of excess iron in the water.

F. Harvesting and Post-Harvest Practice

Oyster mushrooms are harvested before spraying water. The right stage for picking can be judged by the shape and size of fruiting bodies. In young mushrooms, the edge of the cap is thick and the cap margin is enrolled, while the cap of mature mushrooms becomes flat and inward curling starts. It is advisable to harvest all the mushrooms at one time from a bag so that the next crop of mushrooms starts early. After harvesting, lower parts of the stalks/stipes with adhering debris should be cut using a knife. Stipes should be kept as short as possible because they are tough and not liked by the customers. Fresh mushrooms are packed in perforated polythene bags for marketing. They can also be dried in sunlight or drying rooms. Dried products with 2–4% moisture can be stored for several months.

G. Medicinal and Nutritional Value of Oyster Mushrooms

Oyster mushrooms are 100% vegetarian and their nutritive value is as good as that of other edible fungi like the white button mushroom (*A. bisporus*), shiitake (*Lentinula edodes*) or the paddy straw mushroom (*Volvariella* spp.). They are rich in vitamin C and B complex. Protein content varies between 1.6 and 2.5% on a fresh weight basis (15–18% in dried mushrooms). It has most of the minerals required by the human body, such as potassium, sodium, phosphorus, iron and calcium. The niacin content is about ten times higher than that of common vegetables. A polycyclic aromatic compound with antibiotic properties, pleurotin, has been isolated from *P. griseus*.

H. Precautions While Growing Oyster Mushrooms

Oyster mushrooms produce millions of spores, which can be easily seen as spore clouds in the cropping rooms in early morning. Several growers working in cropping rooms have complained of headache, high fever, joint pains, nausea and coughing due to *Pleurotus* spores. Mushroom pickers are therefore advised to open the doors and ventilators or switch on exhaust fans 2–3 h before entering the cropping rooms. Furthermore, they should use respiratory masks in growing rooms and change clothes after coming out from growing rooms.

VI. Cultivation of *Auricularia* spp. (Black Ear Mushroom)

The genus *Auricularia* belongs to the "jelly fungi" and they are commonly known as wood ear or

black ear mushrooms (Fig. 4.1). The fungi cause a moderate white rot of different kinds of wood. The basidiocarps are gelatinous, mostly growing on logs, branches and twigs of deciduous or less frequently coniferous trees. The black ear mushroom is one of the oldest cultivated fungi and was already cultured 600 A.D. in China. It is widely distributed in tropical, sub-tropical and temperate forests where it grows both on fresh and dead wood. It is a traditional food in China and one of the main constituents of Chinese dishes. It is consumed fresh as well as after drying. *Auricularia* spp. are cultivated mainly in China, Taiwan, Thailand, Indonesia and Japan. Its annual world production is around 500 000 t/year and constitutes about 8% of the world-wide mushroom production (Chang 1999). During the monsoon season people in the North-eastern states of India collect wild *Auricularia* spp. and sell them on local markets.

A. Biology of Black Ear Mushrooms

The basidiocarps (fruiting bodies) of *Auricularia* spp. are 2–12 cm (in diameter), 1–3 mm thick and they are attached laterally to the substrate wood without a stipe. They are gelatinous, slimy, small cup-like and creamish brown to purple or reddish-brown in colour. The colour becomes darker after drying. There are 15 *Auricularia* spp. worldwide (Kirk et al. 2001), which differ in the shape, size and colour of the cup, length and thickness of basidiocarp hairs and its internal anatomy. The outer basidiocarp surface is mostly hairy while the inner part is bright and shinning bearing the basidia and basidiospores. The basidiospores are hyaline, oblong, curved, cylindrical, smooth, heterothallic and readily germinate within 2–3 days. The basidiocarps rapidly appear during rainy season growing singly or in large bunch-like clusters on deciduous or coniferous wooden logs, branches and twigs or on the outer bark of living trees. Quimio (1984) observed a considerable decrease in viability of older spores from spore prints compared with fresh spores. Spores germinate best at 28–30°C, while at 10°C and 40°C, spore germination is totally inhibited.

Cultures of *Auricularia* spp. can be obtained by transferring basidiocarp pieces or basidiospores on natural, synthetic or semi-synthetic media. Best mycelial growth is obtained on glucose yeast extract, malt extract or yeast/potato/dextrose media. The colonies on agar plates are dirty white and flattened, changing to brownish during ageing. Glucose, fructose and galactose at 1% are well assimilable carbon sources and calcium nitrate, urea, asparagine or alanine are the best nitrogen sources. The spawn of *Auricularia* can be prepared on wheat grains or saw dust.

B. Cultivation Techniques

Several *Auricularia* species have been reported as cultivars: *A. auricula-judae*, *A. polytricha*, *A. delicata*, *A. mesentrica*, *A. cornea*, *A. rosea*, *A. peltata* and *A. fuscosuccinea*. Sohi and Upadhyay (1990) identified several light *Auricularia* spp. from the North-western Himalayas (India) suitable for commercial cultivation. There are mainly two cultivation methods: (1) conventional wood log cultivation and (2) compost bag or composted sawdust cultivation.

1. Wood Log Cultivation

Wood of most broad leaf trees can be used for black ear cultivation but *Quercus* spp. (particularly *Q. variabilis*, *Q. acutissima*) are preferred trees in Asia. Generally, the tree should be 6–10 years old and 10–15 cm in diameter and are cut during autumn into logs 1–2 m in length. The cut surface is smeared with Bordeaux mixture (copper sulfate, hydrated lime) to prevent attack by other wood-decay fungi. Then holes are made in the logs in two or three rows at a distance of 10–12 cm (holes are 1.0–1.6 cm in diameter, 1.0–1.5 cm in depth). The holes are inoculated with sawdust spawn and sealed with wax. The inoculated logs are arranged in shades and covered with grass to maintain humidity (35–40%), and during dry periods, watering once per week is necessary. The temperature required for fruiting is 15–27°C. The logs produce primordia after getting sufficient water by rain or through spraying water. After the first flush of fruiting bodies, the logs must be rested for a short time to get the next flush. The logs produce fruiting bodies again in the second year, and mushroom production just slowly reduces in the third and fourth year.

2. Composted Sawdust Method ("Synthetic Log" Cultivation)

Wood log cultivation is no longer feasible at larger scale due to non-availability of suitable logs and inconsistent yields. Moreover, it takes longer time to get fructification on logs so that this method has been replaced by the composted sawdust method, also named "synthetic log" cultivation. There are various formulations using treated sawdust, cereal straw or other lignocelluloses as basic material for synthetic log preparation, as well as sugars, salts and minerals as supplements.

3. Composting of Saw Dust, Spawning and Culture Conditions

Saw dust has to composted prior to use. All ingredients are thoroughly mixed with sawdust and water is added to

a final moisture content of 65–70%. The wet material is piled in pyramidal shape and allowed to ferment for 6–10 days, with turning on alternate days. Composted sawdust is filled in polypropylene bags and sterilized for 2 h and, after cooling, is inoculated with the spawn. Cereal straw and other ingredients do not require composting but it may help by softening the straw for easy penetration of the mycelium.

Spawn of *Auricularia* spp. can be prepared on sawdust or cereal grains (e.g. wheat). Saw dust spawn is more suitable for wood logs and grain spawn for composted saw dust. The spawn should not be older than 30 days. Spawn bottles are thoroughly shaken so that the grains become loose. For 2 kg of composted saw dust or straw, about 25–30 g of spawn will be sufficient. The bags after spawning are plugged again. Spawned bags are incubated in dark growing rooms where the temperature is 20–25 °C. The mycelial growth is inhibited and stopped if the temperature falls below 12 °C or rises above 35 °C. During the incubation stage, no fresh air is required and mycelial growth is better at higher CO_2 concentration. The spawn bags are full of whitish mycelium within 20–25 days and are then ready for fruiting. The bags are given four or five vertical slits with a knife and are hung in the growing room. Light is required for initiation of fruiting, and daily 2–4 h diffuse light is sufficient. Relative humidity at 75–80% in the cropping room is provided by the spraying of water. Fresh air circulation is maintained by a air-handling unit or simply by opening the windows. The pin heads develop scantly and they do not expand fully under deficient light conditions. It has been observed that, if there is excess carbon dioxide in the cropping room, the basidiocarps do not expand but form long and thin coral-like structures.

4. Harvesting

The mushrooms usually appear 15 days after slitting the bags. It takes 3–7 days from a pinhead to the mature cup suitable for harvesting. Mushrooms are harvested manually and the adhering straw or saw dust is removed from the base. Biological efficiencies of 137% and 174% have been reported to be reached within 4 and 8 weeks, respectively (Upadhyay 1999a, b). Interestingly, freshly collected wild-type strains gave higher yields than strains obtained from GenBank (Quimio and Guzman 1984). The yield of *A. auricula-judae* is generally somewhat lower than that of *A. polytricha*; and in the Philippines *A. fuscosuccinea* is the preferred and most productive species.

C. Nutritional Value of *Auricularia* spp

Black ear mushrooms are equally nutritional as other mushrooms despite their cartilage consistency. 100 g of dried *Auricularia* contains 14% protein, 1.4% fat, 72% carbohydrates, 4% fibres, and 5.4% ash; furthermore 0.2 mg thiamine, 0.6 mg riboflavin, 4.7 mg niacin, 240 mg calcium, 256 mg phosphorus, 65 mg sodium and 72 mg iron and 984 mg potassium. There are some advantages of growing *Auricularia* spp. Harvesting can be prolonged up to 4 days, because these fruiting bodies do not rapidly lose quality as in the case of *Pleurotus*, *Volvariella*, and *Agaricus* spp. Bhandal and Mehta (1989) reported that *Auricularia* fruiting bodies remained in good condition even 1 week after harvest. The dried fruit bodies can be easily re-hydrated, which decreases transport cost and eliminate spoilage problems. *Auricularia* spp., besides being edible, have interesting medicinal effects. In traditional Chinese medicine, the black ear mushroom is considered mild and sweet in nature and is thought to activate blood and stop pain. It is often used to treat haemorrhoids, to stimulate bowel movement and to act as an anticoagulant (Ying et al. 1987). Polysaccharides of *A. auricula* have been claimed to have various positive effects on human health, e.g. they act against ulcer, diabetis and tumours, they are immunomodulatory and antibiotic, and they lower cholesterol and triglycerides (Hobbs, 1995).

VII. Cultivation of *Lentinula edodes* (Shiitake)

The wood mushroom *Lentinula edodes* (Fig. 4.1), commonly called shiitake, is the most popular mushroom in Japan and other East Asian countries. It is the second most important mushroom cultivated in the world after *A. bisporus*. In 1997, its annual production was about 1.6×10^6 t, of which almost 90% were produced in China (Chang 1999). The shiitake mushroom is liked by the consumers because of its unique taste and flavour and the presence of metabolites, which are thought to reduce the plasma cholesterol level.

A. Cultivation Technique on Wood Logs

Lentinula edodes is a white-rot fungus that grows in nature on dead logs of a number of hardwood trees, mainly *Quercus* spp. (Oak), *Castenopsis* spp. (*C. chinensis*, *C. tissa*, *C. fordil*, *C. lamontii* etc.), *Elaeocarpus* spp. (*E. chinenses*, *E. japonicus*, *E. lancaefolius*), *Lithocarpus* spp. (*L. calophylla*, *L. glaber*, *L. spicatus*), *Betula* spp. and *Carpinus* spp. It is commercially grown on oak wood logs or "synthetic logs" (see also *Auricularia*).

1. Log Preparation

The *Lentinula edodes* mycelium is saprophytic and cause a strong white rot. It mainly grows on dried wooden logs

absorbing nutrients from the cambium. The outer bark layer protects the growing mycelium from the mould competitors. Although it grows on any size and age of timber, logs 9–18 cm in diameter and 15–20 years in age are most suitable. The time of cutting the trees is also important. The most suitable period in East Asia is from autumn to early spring when the logs contain a maximum amount of carbohydrates and other organic substrates. The logs should have a moisture content of 44–55% at the time of felling. If the moisture content of the logs is <20%, there is no growth, likewise at moisture contents >60% (impending mould infections). The felled logs are left as such for 25–45 days, which results in the lowering of the moisture content to 40–45%.

2. Spawn Preparation and Spawning the Logs

There are two types of spawn: saw dust and wood plug spawn. The former is prepared by inoculating mixtures of water, saw dust, cereal bran, sugar and minerals with pure cultures of the fungus. The material is filled into either empty spawn bottles or in polypropylene bags and incubated for 30 days at 24 ± 2°C. Wood plug spawn is prepared by inoculating mycelium on small wedge shaped or either small cylindrical wood pieces. When the fungal mycelium impregnates the wood pieces, they are ready for inoculation. The shiitake mycelium grows between 5 and 30°C with optimum temperature of 20–26°C. Low temperatures (14–20°C) are favourable during spawning the logs. For spawn inoculation, small holes of 1 × 1 cm and 1.5–2.0 cm in depth are made on the logs with the help of a drilling machine. The holes are made at a distance of 20–30 cm (long axis) and 6 cm between each row. The holes between two rows alternate in their position. Saw dust spawn is filled into the holes or wood plug spawn. The holes are sealed with paraffin wax.

3. Crop Management

Inoculated logs are kept outdoors in flat piles at a place where the conditions are most favourable for mycelial growth. The pile is covered with either straw, or gunny bags to prevent excessive water loss of the logs. The vegetative growth in the logs will be completed within 8–12 months depending on the fungal strain and the type of wood. For fruiting body induction, temperature shock, high humidity and/or light exposure are required. The logs are either sprayed with cold water or immersed in a tank of cold water (1–3 days at 10–18°C). The cropping area is kept moist to maintain a high relative humidity (80–90%). Optimal temperature for fruiting is between 15 and 20°C at a humidity around 80–90%. Mushrooms can be harvested several times from one log; after a rest for 30–40 days, the logs are again watered to get a second flush. This can be repeated up to four times per year and logs may produce crops up to six years.

B. "Synthetic Log" Cultivation

This method is practiced in East Asia, Thailand, Sri Lanka, New Zealand, the United States and Europe, and it is becoming more and more important for commercial shiitake production. Cultivation is carried out in plastic bags or substrate blocks, also called "synthetic logs".

1. Substrate Preparation

Commercial cultivation is mostly carried out on saw dust of oak *(Queruus* sp.), maple (*Acer* sp.), birch (*Betula* sp.) or other hardwood trees. Various formulations have been recommended for growing shiitake. These complex substrate mixtures consist of saw dust, cereal bran, grains, sugars, minerals (sulfates) and sometimes urea in different concentrations. The most suitable formulation can be selected after conducting productivity tests. The material is filled in plastic bags (1.5–4.0 kg) immediately after mixing and wetting the substrate. The bags are first loosely filled and later by putting some pressure giving them a cylindrical shape. Some growers make holes (15 mm in diameter, 20 mm in depth) before, others after heat treatment. The holes are covered with adhesive medical tape. The time between substrate preparation and sterilization should not exceed 6 h to avoid unwanted fermentation. Heat treatment can be carried out in an autoclave at 121°C for 1 h or on a brick- and cement-lined tower at 90–95°C for 5–7 h.

2. Spawning and Spawn Run

If no holes had been made before sterilization, the bags are cleaned with 70% ethanol and forceps are used to make small holes. The amount of saw dust spawn per inoculation hole is about 1 cm^3, i.e. a typical spawn bottle (750 g) can inoculate 25–30 bags. Grain spawn is introduced at concentrations of 2–5%. Spawn run may take 18–100 days. During this period, the bags are incubated in a 4 h/20 h light/dark cycles at 23–25°C. There are several stages of fruiting body formation:

1. **Mycelium formation.** A thick mycelial sheet/mat develops on the surface of the substrate. This usually occurs 2–4 weeks after inoculation.
2. **Formation of mycelial bumps.** Bumps are clumps of mycelium, commonly formed on the surface of mycelial mats. These bumps can turn into primordia at a later stage but most of them abort. Bump formation is promoted by fluctuation of temperature and high concentrations of CO_2.

3. **Pigmentation.** The colour of the "synthetic logs" turns brownish 5–6 weeks after inoculation. At this stage, aeration should be provided to promote fruiting.
4. **Coat Hardening.** In the last stage, the outer part of the substrate including the mycelial mats becomes gradually hard whereas the inner part of the "synthetic log" stays soft and moist (80%); browning continues and encompasses the whole bag. At this stage, the plastic sheets are removed and fruiting body formation starts.

3. Fruiting and Harvesting

Factors affecting the induction of fruiting are: temperature fluctuation, humidity, CO_2 concentration, light and physical shocks. These parameters may vary in each stage (1–4) and from strain to strain. Some general data are summarized in Table 4.3. The "synthetic logs" do not require watering during incubation and fruiting. Humidity should be kept low (60–70%) to prevent microbial contamination when the protecting plastic sheets are removed. Deformed fruiting bodies obtained in the first flush are a sign of too high a CO_2 concentration during incubation and/or too short a spawn run. For harvesting, the stalks are held tightly and the whole mushroom is broken from the substrate. Generally, the mushrooms should be harvested at an early stage (young fruiting bodies with closed caps). Good yields range between 15% and 30% of the wet weight of the substrate.

4. Special Features of the Plastic Bag ("Synthetic Log") Method

- The materials used to prepare synthetic logs are mainly saw dust and other agricultural wastes and by-products such as bagasse, sugarbeet residues, cotton-seed hulls, peanut hulls and corn cobs.
- This method shortens the production period and gives high yields. Using natural logs, the time from spawning till first harvest is about 8–12 months and harvesting will be completed within 3–4 years. About 100 kg of natural logs can produce about 10–15 kg of fresh shiitake mushrooms. Using synthetic logs, mushrooms can already be harvested 80 days after spawning. Harvesting is possible over a period of 8 months and biological efficiencies of 80–145% can be reached within this period.
- Bag cultivation is easy to manage and does not require special logistics as in case of natural log cultivation.
- Negative effect: the quality of mushrooms produced on synthetic logs is poorer than those obtained from natural logs (in particular concerning the size and performance of fruiting bodies).

Table 4.3. A schedule of various cultural requirements of *Lentinus edodes* for different stages of fruiting body formation (according to Upadhyay, unpublished data)

Stage/ activity	Days	Temperature (°C)	Light intensity (Lux)	Humidity (%)
Incubation	30–120	20–30	None	65–70
Induction	2–4	10–20	500–1000	85–95
Fruiting	7–14	12–18	500–1000	60–80
Rest	7–21	20–30	None	65–70
Induction	2–4	10–20	500–1000	85–95

VIII. Cultivation of *Stropharia rugoso-annulata* (Wine-Cap Stropharia)

Stropharia rugoso-annulata (Farlow apud Murrill) was first described in the United States in 1922, and in the 1930s it was collected in Germany, Czechoslovakia and Japan (Szudyga, 1978). From the eco-physiological point of view, this agaric fungus has an intermediate position between the white-rot (i.e. wood-decaying) and litter-decomposing basidiomycetes; thus it grows well on bark and straw-like materials but not on logs (Steffen et al. 2000). Cultivation of *S. rugoso-annulata* was first attempted in East Germany (Puschal, 1969), then in Poland, Czechoslovakia and Hungary, and it has been cultured in India since the 1989 (Upadhyay and Sohi 1989). The mushroom is particularly popular among hobby farmers.

The basidiocarp of *Stropharia rugoso-annulata* is relatively large (up to 30 cm), agaricoid, stipitate and annulate (Fig. 4.1). The pileus (cap) is fleshy, 4–19 cm in diameter, differently coloured (withish, yellow, brown to wine-red), initially conic, then applanate or with slight depression. The stipe lies central, is cylindrical, 10–25 cm long and 1.0–2.5 cm in diameter, white to creamish in colour with a slightly swollen base. The form of basidiospores is broadly ovoid to sub-ellipsoid; they are smooth with a broad germ pore and violaceous to dark purple. Spore prints are purplish black.

Clamp connections are always present in the heterothalic mycelium. Spores easily germinate at 30–35°C within 2–4 days. *S. rugoso-annulata* has a bifactorial hetrothallic mating system (Upadhyay, unpublished data). The mycelium can be grown on malt extract or potato dextrose agar and forms whitish cottony colonies. Maximum growth occurs between 20 and 30°C, with some variation between particular strains.

Spawn can be prepared on any kind of cereal grains, e.g. wheat, rye, sorghum or bajra, as described above for *C. indica*; sterilized corn cobs, wheat straw and pine bark are also suitable for spawn preparation (see also Chapter 22 of this book). Various agriculture residues, namely wheat straw, paddy straw, sugarcane bagasse or dehulled maize cobs, are suitable for fruiting body production.

The lignocellulosic materials are either sterilized (autoclaved) or composted prior to use and the fungus grows without supplements of organic nitrogen (Upadhyay and Sohi 1989). However, synthetic compost as used for *A. bisporus* does not support mycelial growth of *S. rugoso-annulata*. Composted wheat and paddy straw was found to give a biological efficiency of almost 130% BE (Upadhyay and Sohi 1989). There are reports recommending to cover the top surface of the straw substrates with soil to promote fructification; however, efficient fruiting body formation occurs also without casing (Upadhyay and Sohi 1989). However, casing may help to induce synchronized fruiting and the development of uniform mushrooms required for market. Mushrooms should be harvested in the button stage (i.e. before the caps starts to open), because mature ("over-ripe") basidiocarps have a strong taste and may develop a pungent stench. Only a few pests and deformations have been recorded for *Stropharia rugoso-annulata*. Incidence with the fungal parasite *Mycogone rosea* causes damage to the primordia, gills and/or stipe (Sohi and Upadhyay 1989).

The wine-cap *Stropharia* has a promising potential as market mushroom, since it is easy to cultivate on cheap straw-like materials without nitrogen supplementation. To this end, it will be necessary to establish a collection of fast-growing wild-type strains isolated from suitable natural habitats in order to select new production strains. Furthermore strain selection/improvement should be done towards a more pleasant aroma.

IX. Cultivation of *Volvariella* spp. (Paddy Straw Mushrooms)

Paddy straw mushrooms (Fig. 4.1) are suitable market fungi for tropical and subtropical regions (e.g. south India, south-east Asia) and about 180000t were produced in 1997, mostly in China (Chang 1999). In India, *Volvariella* spp. were first cultivated by Thomas et al. in 1943 near Chennai. As *S. rugoso-annulata*, it is an intermediate species between white-rotters and litter-decomposers. The fungus requires higher temperatures than most other mushrooms (30–35°C), both for growth and fructification. It is a fast-growing mushroom, and it takes only 10 days to complete the cycle from fungal inoculum via mycelial growth on/in a substrate to full fruiting body formation. Although *V. volvaceae* is called the paddy straw mushroom, it was shown to grow also on other straw-like materials such as sorghum and wheat straw, maize debries, sugarcane bagasse as well as leaves of banana and water hyacianth (Chang and Mok 1971). The mycelium of *V. volvacea* does seemingly not produce ligninolytic enzymes (neither lignin nor manganese peroxidases; see also Chapter 15 of this book) and growth is therefore inhibited by lignin-related phenolic compounds (Cae et al. 1993). For this reason, the fungus prefers lignocelluloses with low lignin and high cellulose content (e.g. straw, cotton wastes) and can also be grown on waste paper. Spawn is prepared analogously to *S. rugoso-annulata* or *C. indica*.

There are two methods for commercial growing of *Volvariella*: (1) out-door and (2) in-door cultivation. Outdoor cultivation has several disadvantages like insect and pest damages, unstable yields and non-regulated environmental conditions. Nevertheless, this method is widely used in subtropical and tropical regions of Asia. The in-door method produces mushrooms in straw-beds in growing rooms. For one bed, 22 bundles of paddy straw are soaked overnight in water. These bundles are arranged on a raised platform in four layers of five bundles at a right angle, with two bundles on the top. On each layer, gram dal powder (1.5% of dry weight of straw) along with the spawn is added. The inoculated straw-bed is covered with a polyethylene sheet. Already, 10 days after spawning, fruiting bodies start to appear from all sides of the bed. Usually between 500 and 1000 g mushrooms can be harvested at one time from a bed. A new technique using cotton wastes was developed in the 1980s (Chang 1982). There are various substrate formulations combining cotton wastes as basic material with rice bran and limestone. The substrate is soaked in water and then stacked to form piles 1–2 m in height. After 2–4 days of composting, the material is transferred to a "mushroom house" made of plastic sheets and filled to a 10–20 cm layer. Then live steam is introduced to raise the temperature in the tent to 60–62°C and to sterilize the surface of the substrate. After cooling down to 35°C, the substrate is inoculated with fresh spawn. The biological efficiency of this method is 40% (Chang 1982).

X. Cultivation of *Flammulina velutipes* (Enokitake)

Flammulina velutipes (Curt. ex Fr.) Singer is also known as winter mushroom or golden mushroom and as enokitake in Japan (Fig. 4.1). It grows all over the world as a saprophyte (white-rot fungus) on trunks and stumps of deciduous trees, preferably on poplar trees (*Populus* spp.), elms (*Ulmus* spp.), willows (*Salix* spp.), plum trees (*Prunus* spp.), maple (*Acer* spp.) and birch (*Betula* spp.). Fruiting bodies typically develop from the end of autumn till early spring when the temperature ranges between –2°C and +14°C (Zadrazil 1999). *F. velutipes* was already cultivated as early as 800 AD in China and the mushroom has spread all over the world over the past 30 years. It is well suitable for growing in temperate regions as well as in hilly and mountain areas, where the temperatures are most of the time between 6–14°C. *F. velutipes* is known for its pleasant taste and aroma as well as its medicinal properties. The total world production of *F. velutipes* has increased from 143 000 t in 1990 to 285 000 t in 1997 and Japan is the biggest producer of this mushroom.

F. velutipes belongs to the Tricholomataceae of the order Agaricales. The fruit bodies are caespitose, arising in a large group. The cap or pileus is 1–3 cm in diameter and convex to hemispheric in the initial stages which later on flattens. The pileus is viscid with yellowish brown to dark brown in the centre, while the margin is lighter. The stipe is very long (4–10 cm), thin (3–7 mm) and velvety; its colour is light brownish at the top and dark brown at the base. Lamellae are adnexed, initially white to light cream and later light brown. The spores are smooth, hyaline, thin-walled and ellipsoid (5–7 × 3–4 m). The spore print is white and the spores germinate easily on common mycological media. The fungus has a bifactorial heterothallic mating system and clamp connections are always present in the dikaryotic hyphae.

The vegetative mycelium of *F. velutipes* is white and strandy. The optimum temperature for growth is around 25°C (though the fungus tolerates even temperatures below zero!). Spawn can be prepared on saw dust or cereal grains. The spawn can be prepared within 12–15 days, depending upon the vigour of the strains used. In earlier times, the cultivation of *F. velutipes* was carried out using wooden logs, but the production was just moderate and the quality of mushrooms not assured. Therefore, artificially composted substrates based on saw dust and rice bran were developed in the 1920s (Morimoto 1928). Interestingly the saw dust of coniferous trees, *Chamaecyparis obtuse* (hinoki cypress) and *Pinus* spp. (pine trees), gave best yields. However, saw dust from other trees can be also used; cotton-seed hulls are nowadays the preferred substrate in China.

Synthetic substrate consisting of sawdust and rice bran (4:1 w/w) is mechanically mixed and, after soaking with water to a moisture content of 57–62%, it is filled into polypropylene bottles with a capacity of 800 to 1200 ml. The substrate can be also filled into polypropylene bags, vinyl bags, jars or filter bags. The sterilized containers are spawned with a mechanical mixers via a central hole made at the time of filling. The spawn rate should be 2–3% of dry substrate. The spawned bags are incubated in dark rooms with a temperature range of 20–25°C. The mycelium can grow at up to 34°C; however, a low temperature during incubation helps to overcome the incidence of contaminants. The initiation of pinheads takes place in the dark; however, light is required for maturation of pinheads and pileus expansion. It takes 25–30 days for mycelium to spread on the substrate. Then the temperature of incubation rooms is lowered to 10–14°C, which is the ideal temperature for fructification. The stipe starts elongation, so a plastic collar is placed around the neck of bottle or bags to hold the mushrooms in place and straight. Aeration is required during cultivation because, when the CO_2 concentration is more than 5%, pileus expansion occurs. For better-quality mushrooms, the temperature is reduced to 3–8°C until harvest. The relative humidity in the cropping room is maintained at 80–85% by spraying water. When the stipe length is 13–14 cm, the collar or rolled paper around neck is removed and fruiting bodies are harvested. Three or four flushes of mushrooms are obtained and the yield is about 80% in relation to the dry substrate. The yield of mushrooms in the first flush is 100–140 g from 250 g of dry substrate, while the second flush still gives 60–80 g, depending upon the strain quality. The entire crop cycle is around 90–100 days. The mushrooms have a long shelf life of 8–10 days and they are usually vacuum-packed for sale.

Gramss (1981) successfully used mixtures of beech saw dust, wheat straw, flour and bran as well as pea haulm to cultivate *F. velutipes*. Green mould caused by *Trichoderma harzianum* and related species is the most important pest associated with *F. velutipes*, causing poor mycelial growth. The addition of wood vinegar (a by-product from charcoal production using *Quercus acutissima*) to the saw dust helps to control the incidence of *Trichoderma* spp. and at the same time stimulates mycelial growth of *F. velutipes* (Chang et al. 1995). *F. velutipes* is nutritionally rich and contains over 30% protein, 6% fat and 3% fibres. Fresh fruiting bodies of *F. velutipes* are also rich in enzymes (peroxidase, superoxidase

dismutase) and bioactive compounds and have been claimed to prevent cancer and coronary heart diseases.

A. Future Prospects

- Fruiting bodies of *F. velutipes* are long-stiped with small pilei and very light; strains forming heavier mushrooms with larger pilei and smaller stipes would be helpful to mushroom growers.
- The fructification temperature is 12–16°C, which is a limiting factor for its cultivation in sub-tropical regions. Therefore new strains of *F. velutipes* (and closely related species) will have to be isolated aiming at fungi with fructification temperatures of 20–25°C.
- The available strains grow well on saw dust but their growth is limited regarding agricultural waste materials (Gramss 1981); improved/new strains colonizing different kinds of straw would be useful for *F. velutipes* production.

XI. Conclusions

Mushroom cultivation is one of the most suitable and safest commodities suitable for tropical, subtropical and temperate climatic conditions. It helps in recycling agricultural wastes and their conversion into protein-rich food. Mushroom farming is a labour-intensive activity, so it can help in employment generation, in particular in developing countries. The production of mushrooms during the past three decades has achieved a phenomenal growth not only regarding production as such but also in productivity. Thus, not only the total production but also the number of commercially cultivated fungal varieties have increased and several new edible species are under the process of "domestication" and commercialization throughout the world.

Against the background of a raised interest of consumers in fungal products, mushroom production will further increase in the course of this decade. May be molecular tools will help in the future to improve fruiting body formation (though the genetics of basidiomycetes is more complicated than that of bacteria, yeasts and ascomycetous moulds; Kothe 2001). It is equally important, however, to further study and protect fungal biodiversity. This should ideally be done in worldwide programmes, because the mushroom flora is rapidly depleting everywhere due to global climate change, deforestation, soil erosion, forest fires, grazing by domestic animals and human interference. Still, it is possible to find new species and strains of edible mushrooms. Some of these fungi (and already available mushrooms as well) will be exploited, beyond fruiting body production, in the recycling of agricultural wastes, in bioremediation or for the development of new enzymes and bioactive molecules. Not least, cultures of edible mushrooms should be freely exchanged for their beneficial utilization in all countries.

References

Badalyan SM, Innocenti G, Garibyan MG (2002) Antagonistic activity of xylotrophic mushroom against pathogenic fungi of cereals in dual culture. Phytopathol Med 41:220–225

Ball AS, Jackson AM (1995) The recovery of lignocellulose-degrading enzymes from spent mushroom compost. Bioresour Technol 54:311–315

Bano Z (1978) The nutritive values of mushrooms. Ind Mush Sci 1:471–487

Bano Z, Srivastava HC (1962) Studies on cultivation of *Pleurotus* sp. on paddy straw. Food Sci 12:363–365

Bano Z, Rajarathnam S, Nagaraja N (1987) Some important studies on *Pleurotus* mushroom Technology. Ind Mush Sci 12:67–71

Barron GL, Thorn RG (1987) Destruction of nematodes by species of *Pleurotus*. Can J Bot 65:774–776

Bhandal MS, Mehta KB (1989) Cultivation of *Auricularia polytricha* (Mont.) Sacc. (Jew's ear mushroom) on wheat straw. Mush Sci 12:383–394

Block SS, Taso G, Han L (1958) Production of mushroom from sawdust. J Agric Food Chem 6:923–927

Cae YJ, Buswell JA, Chang ST (1993) Effect of lignin related phenolic monomers and tannin derivatives on the growth of edible mushroom *Lentinus edodes, Pleurotus sajor caju* and *Volvariella volvacea*. World J Microbiol Biotechnol 9:503–507

Chang HY, Kang AS, Cha DY, Sung JM, Maringa T (1995) Effects of wood vinegar on the mycelial growth promotion of some edible mushroom and *Trichoderma* pathogen inhibition. RDAJ Agric Sci Farm Manage Agric Eng Sericult Farm Prod Util 37:766–771

Chang ST (1982) Cultivation of *Volvariella* mushroom in Southeast Asia. In: Chang ST, Quimio TH (eds) Tropical mushroom - biological nature and cultivation methods. Chinese University Press, Hong Kong, pp 221–249

Chang ST (1999) World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinula edodes* (Berk) Sing. in China. Int J Med Mush 1:291–300

Chang ST, Mok SH (1971) Preliminary experiment of water hycianth used as a medium for the cultivation of paddy straw mushroom. J Hort Soc Chin 17:194–197

Chihara G (1992) Immunopharmacology of lentinan, a polysaccharide isolated from *Lentinus edodus*: its application as a host defence potentiator. Int J Orient Med 17:57–77

Chiu SW, Ching ML, Long KL, Moore D (1988) Spent Oyster mushroom substrate performs better than many mushroom mycelial in removing the biocide pentachlorophenol. Mycol Res 102:1553–1562

Doshi A, Sidana N, Chakravarti BP (1989) Cultivation of summer mushroom *Calocybe indica* (P&C) in Rajasthan. Mush Sci 12:395–400

Doshi A, Sharma SS, Trivedi A (1993) A promising edible mushroom for the tropics *Calocybe indica* P. & C. Mush Info 86:14–22

Eicker A (1993) Mushrooms: a source of protein for Atrica? Afr J Mycol Biotechnol 1:12–23

Etter BE (1929) New media for developing sporophores of wood rot fungi. Mycologia 21:197–203

Falck R (1917) Uber die wald kulture des Austern pilze (*Agaricus ostrestus*) auf Laubneolz stubben. Z Forst Jagdwes 49:159–165

Fanz G (1989) Polysaccharides in pharmacy: current applications and future concepts. Plant Med 55: 493–497

Gramss G (1981) Experiences with new cultivated fungi II. Velvet stemmed agaric. Champignon 237:14–22

Gyurko P (1972) Die Rolle der Belichtung bei dem Anbau des Austernseitlings (*Pleurotus ostreatus*). Mush Sci 8:461–469

Hikino H, Mizuno T (1989) Hypoglycemic actions of some heteroglycans of *Ganoderma lucidum* fruit bodies. Plant Med 4:339–340

Hobbs C (1995) Medicinal mushroom: an exploration of tradition, healing and culture. Botanic, Santa Cruz

Ito T (1978) Cultivation of *Lentinus edodes*. In: Chang ST, Hayes WA (eds) The biology and cultivation of edible mushroom. Academic, London, pp 461–473

Jandaik CL, Kapoor JN (1976) Studies on cultivation of *Pleurotus sajor caju* (Fr.) Singer. Mush Sci 9:667–672

Jong SC, Birmingham JM, Pai SH (1991) Immunomodulatory substances of fungal origin. J Immunol Immunopharm 11:115–122

Jong SC, Birmingham JM, Ma G (1994) ATCC catalogue of filamentous fungi, 18th edn. American Type Culture Collection, Rocksville

Kaufert F (1935) The production of asexual spores by *Pleurotus corticatus*. Mycologia 27:333–340

Kirk PM, Cannon PF, David JC, Staplers JA (2001) The fungi. CAB, Wallington

Kobayashi HK, Mastsunaga K, Oguchi Y (1993) PSK as a chemopreventive agent (review). Cancer Epidemiol Biomark Prevent 2:271–276

Kothe E (2001) Mating-type genes for basidiomycete strain improvement in mushroom farming. Appl Microbiol Biotechnol 56:602–612

Krishnamurthy AS (1995) Studies on cultivation of milky mushroom (*Calocybe indica* P&C). Dissertation, Tamil Nadu Agriculture University, Coimbatore

Krishnamurthy AS, Muthuswamy M (1997) Yield performance of *Calocybe indica* (P&C) on different substrates. Mush Res 6:29–32

Lelley J (1987) Edible mushrooms as weapons against starvation. Mush J Trop 7:135–140

Leong PC (1982) Cultivation of *Pleurotus* mushrooms on cotton waste substrate in Singapore. In: Chang ST, Quimio TH (eds) Tropical mushrooms - biological nature and cultivation. Chinese University Press, Hong Kong, pp 349–361

Lou LH (1982) Cultivation of *Auricularia* on logs in China. In: Chang ST, Quimio TH (eds) Tropical mushrooms - biological nature and cultivation methods. Chinese University Press, Hong Kong, pp 437–441

Mallavadhani UV, Shudhakar AVS, Satyanarayana KVS, Mahapatra A, Li W, Bruemen, RBV (2005) Chemical and analytical screening of some mushroom. Food Chem 95:58–64

Mishra C, Leatham GF (1990) Recovery and fractionation of the extracellular degradative enzymes from *Lentinula edodes* cultures on a solid lingo cellulosic substrates. J Ferm Eng 69:8–15

Mizuno T, Kim BK (1995) A medicinal mushroom, *Ganoderma lucidum*, Ganoderma, Polyporaceae and others: oriental tradition, cultivation, breeding, chemistry, biochemistry and their utilization of *Ganoderma lucidum*. IL Yang Pharmaceutical Co., Seoul

Mori K (1974) Mushroom as health foods. Japan Publications, Tokyo

Morimoto H (1928) Nametake to Shiitake noogakuzubaiyoko (culture method of nametake and Shiitake with sawdust). Sanrin (Tokyo) 551:15–18

Oleke BC, Smith JE, Peterson A, Waston (1993) Aerobic metabolism of pentachlorophenol by spent sawdust culture of "Shiitake" mushroom (*Lentinula edodes*) in soil. Biotechnol Lett 15:1077–1080

Ooi VCE (2000) Medicinally important fungi. In: Griensven Van (ed.) Science and cultivation of edible fungi. Balkema, Rotterdam, pp 41–51

Pandey M, Lakhanpal TN, Tewari RP (2000). Studies on spawn production of *Calocybe indica*. Ind J Mush 18:15–18

Parent G, Thoen D (1977) Food value of edible mushrooms from Upper-Shaba region. Econ Bot 31:436–445

Purkayastha RP, Chandra A (1976) A new technique for in vitro production of *Calocybe indica* as edible mushroom from India. Mush J 40:1–2

Quimio TH (1984) Physiological consideration of *Auricularia* spp. In: Chang ST, Quimio TH (eds) Tropical mushrooms - biological nature and cultivation. Chinese University Press, Hong Kong, pp 397–407

Quimio TH, Guzman RDE (1984) Taxonomy and basidiocarp development of *Auricularia* mushrooms. In: Chang ST, Quimio TH (eds) Tropical mushrooms – biological nature and cultivation methods. Chinese University Press, Hong Kong, pp 383–395

Royse DJ, Schislerb LC, Diehle DA (1985) Shiitake mushrooms. Consumption, production and cultivation. Interdisc Sci Rev 10:329

Selvi SS, Devi U, Suja PS, Murugan S, Chinnaswamy (2007) Comparison of non enzymatic antioxidant status of fresh and dried from of *Pleurotus florida* and *Calocybe indica*. Pak J Nutr 6:468–471

Sharma SR, Uapdhyay RC (2002) Scope and cultivation of specialty mushrooms in India. In: Verma RN, Vijay V

(eds) Recent advance in the cultivation technology of edible mushrooms. Nirmal Vijay, New Delhi, pp 229–244

Sharma SS, Doshi A, Trivedi A, Kothari KL (1997) Evaluation of the suitability of different casing materials for *Calocybe indica* P&C. Mush Res 6:81–82

Shukla PN (2007) Effect of casing soil thickness on crop duration and yield of milky mushroom (*Calocybe indica* P&C). Ind Phytopathol 60:537–539

Singer R (1986) The Agaricales in modern taxonomy, 4th edn. Bishan Singh Mahendra Pal Singh, Dehradhun

Singh M, Singh AK, Gautam RK (2007) Effect of casing and supplementation on yield of milky mushroom (*Calocybe indica*). Ind Phytopathol 60:191–193

Singh M, Singh AK, Gautam RK (2009) Screening of substrates for growth and yield of *Calocybe indica*. Ind Phytopathol 62:109–111

Sohi HS, Upadhyay RC (1988) Laboratory cultivation of *Lentinus edodes* (Berk) Sing. in India. Ind Phytopathol Soc 10:3

Sohi HS, Upadhyay RC (1989) New and noteworthy disease problems of edible mushrooms in India. Mush Sci 12:611–614

Sohi HS, Upadhyay RC (1990) Natural occurrence of different species of *Auricularia* in Himacha Pradesh. Mush J Trop 10:47–51

Stanek M, Rysava J (1971) Application of thermophillic microorganism in the fermentation of the nutrient substrate for the cultivation of *Pleurotus ostreatus* Jacq. ex Fr. Kummer Mykol 8:59–60

Steffen, KT, Hofrichter, M, Hatakka, A (2000) Mineralisation of ^{14}C-labelled synthetic lignin and ligninolytic enzyme activities of litter-decomposing basidiomycetous fungi. Appl Microbiol Biotechnol 54:819–825

Szudyga K (1978) *Stropharia rugoso-annulata*. In: Chang ST, Hayes WA (eds) The biology and cultivation of edible mushrooms. Academic, New York, pp 559–571

Takashi M (1999) Bioactive substances in *Hericium erinaceus* (Bull: Fr.) Pers. (Yamabushitake), and its medicinal utilization. Int J Med Mush 1:105–119

Thorn RG, Barron GL (1984) carnivorous mushrooms. Science 224:76–78

Tokimoto K, Komatsu M (1978) Biological nature of *Lentinus edode*. In: Chang ST, Hayes WA (eds) The biology and cultivation of edible mushroom. Academic, London, pp 445–459

Upadhyay RC (1993) Cultivation of *Calocybe indica*. In: Sharma SR, Dhar BL, Thakur K (eds) Recent developments in the cultivation technology of edible mushrooms Nirmal Vijay, New Delhi, pp 179–180

Upadhyay RC (1999a) Non-conventional substrates for growing oyster mushrooms. Mush Res 9:35–38

Upadhyay RC (1999b) Studies on cutivation of *Auricularia mesenterica* Pers. Mush Res 8:43–45

Upadhyay RC, Sohi HS (1989) Natural occurrance of *Stropharia rugoso annulata* Farlow Apud Murrill in Himachal Pradesh (India) and its artificial cultivation. Mush Sci 12:509–516

Viella LC, Silverio CM (1982) Cultivation of *Auricularia* on composted sawdust in the Philippines. In: Chang ST, Quimio TH (eds) Tropical mushrooms - biological nature and cultivation methods. Chinese University Press, Hong Kong, pp 427–435

Vijay B, Sohi HS (1987) Cultivation of oyster mushroom *Pleurotus sajor-caju* (Fr.) Singer on chemically sterilized wheat straw. Mush J Trop 7:67–75

Whiteford JR, Thurston CF (2000) The molecular genetics of cultivated mushrooms. Adv Microb Physiol 42:1–23

Wood DA, Goodenough PW (1977) Fruiting of *Agaricus bisporus*. Arch Microbiol 114:161–165

Worgan JT (1968) Culture of the higher fungi. In: Dlokenthull DJ (ed) Progress in industrial microbiology, vol 8. Churchill, London, pp 74–139

Ying JZ, Mao XL, Ma QM, Zong YC, Wen HA (1987) Icons of medicinal fungi from China. Science, Beijing

Yohallem DS, Harris RF, Andrews JH (1994) Aqueous extracts of spent mushroom substrate for foliar disease control. In: AMI (ed.) Environmental agricultural and industrial uses for spent mushroom substrates from mushroom farms. American Mushroom Institute, Washington, D.C., pp 98–105

Zadrazil F (1974) The ecology and industrial production of *Pleurotus ostreatus, Pleurotus florida, Pleurotus cornucopiae* and *Pleurotus eryngii*. Mush Sci 9:621–652

Zadrazil F (1999) *Flammulina velutipes*, a dangerous parasite and a good, cultivable edible fungus. Champignon 407:34–35

Zhang C, Cao H, Chen L, Wang J (2005) Preliminary study on the inhibition of polysaccharide of edible fungi to plant virus. J Anhui Agril Univ 32:15–18

Zhang L, Sun F, Zhang Y (1994) The influence of chemical modification on antiviral (CB5) activity of polysaccharide from *Pleurotus citronipileatus*. Shengwu Huaxue Zazhi 10:150–154