Cultivation Techniques of Shiitake

(A Medicinal Mushroom with Culinary Delight)

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Foreword

Over the past few years, a great interest has been developed in the world around the cultivation and consumption of shiitake mushroom. These meaty-tasting fungi are a product of the Orient world. Production of shiitake mushroom has increased faster than any other specialty mushroom with the proven therapeutic benefits. With its increasing popularity, shiitake became the leading contributor to the world mushroom production. Since this mushroom is grown in small quantities in India, the dried shiitake is fetching high price in the urban and semi urban markets. Shiitake mushroom cultivation in India is still at budding stage and the farmers who entered into its cultivation can tap the huge potential in the domestic market. Shiitake mushrooms could potentially be a very important in future food supplies and in new dimensions of sustainable agriculture and forestry.

This publication describes the techniques for shiitake mushroom production both on natural logs and artificial bag logs. It describes the common techniques based on the experimental findings, as well as insights collected from the growers in India and China. In addition to the production methods, this publication also gives an overview on the pharmacological properties of shiitake and discusses the methods for genetic improvement for those interested in mycological research.

V.P. SHARMA
Director, ICAR-DMR, Solan (HP)

Contents

1. Shiitake Mushroom – An Introduction

Lenitnula edodes (Berkeley) Pegler popularly known as shiitake is a cultivated edible mushroom native to East Asia region. The name shiitake was derived from the two Japanese words 'take' and 'shii' which means mushroom (take) associated with the shii (*Castanopsis cuspidate*) tree. Shiitake are the mushrooms that the Chinese fondly named "Xiang-gu" which means the fragrant mushrooms; dong-gu, the winter mushrooms and hua-gu, the flower mushrooms. In USA, it is popularly known as black forest mushroom and in France called as lectin. The mushroom may be found growing naturally in Japan, China, Taiwan, the Himalayan Mountains, Borneo and Papua New Guinea. Shiitake mushrooms are prominent in these Far East Asian countries as a food and as a part of traditional medicine for thousands of years.

The origin of shiitake cultivation has a long history traced back to Far East, especially in Japan. They have been grown and eaten in Japan and other Asian countries for centuries. Historical reports showed that, in the year 199 AD Kyusuyu, a native tribe of Japan, offered shiitake mushrooms to the Japanese Emperor Chuai (Singer, 1961). The practice of shiitake cultivation originated by Kwung in ancient China during the Sung Dynasty (960-1127 AD) had a detailed record in the Chinese history. Even today, every mushroom growing village in China has a temple in the honor of Kwung (Miles and Chang, 1997). The beginning of the 20th century marked the spread of shiitake mushroom to the Western world. The consumers in this region gradually endear shiitake due its enticing aroma, unique culinary characteristics and firm texture. The growers are increasingly curious about producing this gourmet mushroom to catch up the expanding market in the edible mushrooms.

1.1 Overview on shiitake mushroom production

Being native to Japan, shiitake mushroom cultivation is popular in this country and till mid 1980's Japan stood as the major producer and exporter. Farmers in Japan pioneered in the natural cultivation on fallen wood logs of shii tree (Yamanaka, 2011). However, the development of sawdust based artificial log cultivation technique gradually replaced the natural log cultivation with an advantage in production efficiency. From the late 90s to early 21st century Chinese

growers took the advantage of sawdust based cultivation technology and increased the shiitake mushroom production to manifolds. At present China accounts for more than 95% of the total shiitake mushroom produced in the world which is about 7.6 billion kg (Royse *et al*., 2017). Interestingly, the shiitake mushroom production in the United States has also increased by 24% over the last 10 years (USDA, 2015). Shiitake mushroom is the only edible mushroom in the world which registered more than 100 percent growth in a span of 35 years. The production has increased from a mere 0.2 billion kg in 1980 to 7.6 billion kg in 2013 (Fig 1.1) (Royse et al. 2017). Increased shiitake mushroom production and its consumption in the Western world is an indication of its popularity beyond the orient region. In India, where the mushroom industry is dominated by the production and consumption of white button mushroom, the shiitake mushroom is gradually making its inroads. The recent production trends indicated that, shiitake mushroom occupied more than 1% of the total mushroom produced in the country (Sharma *et al*., 2017). Cultivation of shiitake mushroom is confined to few pockets of North Eastern states and other Himalayan states only. Since the mushroom is grown in very small quantities in India, the demand for it is mostly met through imports from Thailand, Bhutan, Korea and China. As a result, the cost of one kg of dried shiitake mushroom is fetching almost Rs 1500 - 1,600 per kg.

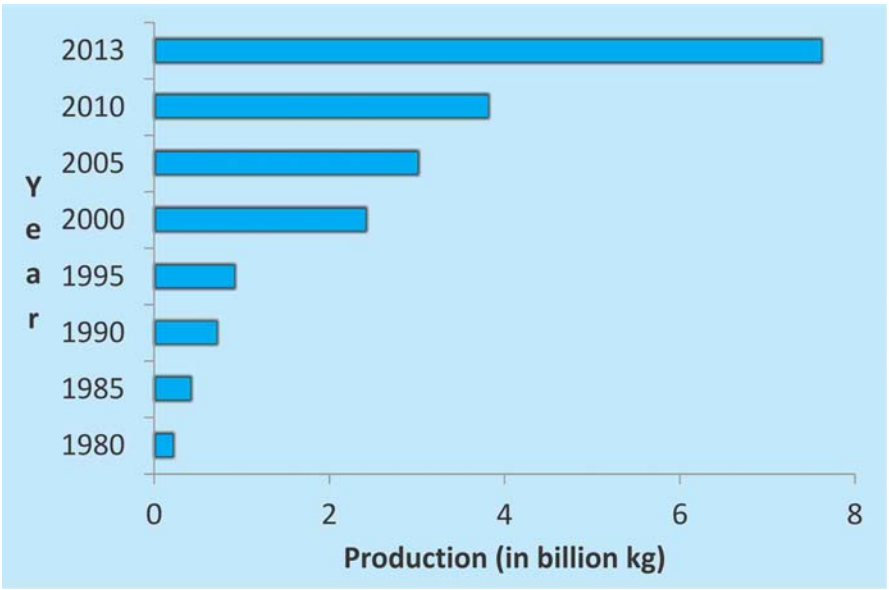


Fig 1.1 Growth in total shiitake mushroom production at global level

1.2 Habitat and life cycle

Shiitake mushroom grows naturally in the temperate climate on declining or dead hard wood (Stamets, 2000). The life cycle of shiitake begins as an invisible network of pale, mycelial threads that colonizes through the dead tissue of wide variety of deciduous hardwoods especially shii (from which the mushroom derives its name), oak, beech, maple, sweet gum, poplar, alder, horn beam, iron wood. The species of beech family (Fagacea) are especially preferred by shiitake mycelium (Leatham, 1982).The mycelia digest the wood and convert it into fungal tissue. After reaching the maturity by digesting the wood sufficiently, the fungus produces the fruit bodies. The fruit body usually has a central stalk attached to circular-shaped mushroom caps. The cap colour varies with the strain. Some strains produce light coloured mushrooms and other strains with honey to chocolate brown colour. They are also usually speckled with white spots around the rim of the cap. The gills and stipe are in creamy white colour.

1.3 Taxonomy

The shiitake has been referred to under a variety of common and scientific names such as *Cortinellus shiitake, C. edodes, C. berkeleyanus, Armillaria edodes* and *Lentinus edodes* (Singer 1941; 1961). Among them, *C. shiitake* was the most commonly used name. Synonymy of these scientific names created uncertainty among the scientific community. Then, Singer (1941) had raised a question as to whether shiitake belongs to the genus *Cortinellus.* He classified it as *Lentinus edodes* (Berk.) Sing based on the taxanomical features as the correct scientific name for the mushroom known under the common name of shiitake. Under the old system of classification both *Lentinus edodes* and *Volvariella volvacea* (paddy straw mushroom) were placed in the family Agaricaceae. But under the modern system of classification *Lentinus edodes* has been placed in the family Tricholomataceae. Till 1975, shiitake was known as *Lentinus edodes* (Berk.) Singer. Later, Pegler has proposed the genus name *Lentinula* considering the monomitic mycelium character. In the mushroom gill trama, the cells are arranged in a parallel and descending fashion instead of having highly irregular or interwoven cells as in the genus of *Lentinus*. Now, the shiitake mushroom belongs to the genus of *Lentinula*, species *edodes*, the family of Tricholomataceae, the order of Agaricales and the subphylum of Basidiomycotina.

1.4 Shiitake at a glance

Form: Fleshy convex cap, lightly tough stalk

Colour: Light to dark brown cap, creamy white gills, light brown stem (Fig 1.2 & 1.3)

Average size at harvest: 6-8 cm diameter cap, 3 to 4 cm stalk.

Flavour: robust/ earthy

Best grown on: *Toona*, poplar, oak, beech, maple, sweet gum, alder, horn beam, iron wood

Fig. 1.2 Shiitake fruit body (cap)

Fig. 1.3 Shiitake fruit body (gills)

2. Nutritional and Pharmacological Properties

Shiitake mushrooms are traditionally well known edible mushrooms favored by the Asian people. Shiitake's popularity is increasing day by day because of its high nutritious value, unique flavor, taste and enticing aroma. They are also well-known for their medicinal benefits in strengthening the human immune system. The level of nutritional and the activity of various compounds differ in strains and it is highly influenced by the method of cultivation and the growing conditions. In commercial markets across the world, the shiitake is available in dried form. In comparison to regular vegetables, shiitake mushroom have higher amounts of nutritional properties on a dry weight basis.

2.1 Nutritional properties

The fresh fruit bodies of shiitake contain relatively less amount of waster i.e. 88–90% and have more dry matter content in the fruit body. Proximate composition of fruit bodies showed that, the dried mushrooms contains 58–60% carbohydrates, 20–23% protein and 9–10% of fiber. The protein extracted from the shiitake has high digestibility. Shiitake mushrooms are a rich source of vitamin B complex viz.,

Table 2.1 Vitamins and mineral contents of shiitake mushroom

Vitamins (mg or µg/ 100 g)	FW	DW	Mineral contents (g, mg, or µg/ kg)	FW	DW
Vit C, mg	2.1	25	Ca, g	0.004	0.05
Vit B_1, mg	0.05	0.6	K, g	2.24	26.7
Vitamin B_2, mg	0.15	1.8	Mg, g	0.13	1.55
Folates, µg	25	300	P, g	0.73	8.7
Niacin, mg	2.6	31	Na, g	0.01	0.13
Vitamin B_{12}, µg	0.07	0.8	Cu, mg	0.44	5.2
Vitamin D, µg	0.1	1	Fe, mg	2.8	33
			Mn, mg	1.74	21
			Zn, mg	7.7	92
			Se, µg	3.3	39

*FW- On fresh weight basis; DW- on dry weight basis
Ref: Mattila et al. 2001 (J. Agric. Food Chem. 49: 2343-2348)

vitamins B_1 (thiamin), B_2 (riboflavin) and B_3 (niacin) (Mizuno, 1995; Hobbs, 2000). The high amounts of ergosterol in fresh shiitake make it as a vital source of vitamin D. Ergosterol (pro-vitamin D_2) converts in to vitamin D_2 in the presence of sunlight. The studies have showed that five times increase in vitamin D_2 content when fruit bodies exposed to direct sunlight for three hours/day. Exposure to sunlight also increases the free amino acid content in the dried shiitake (Kiribuchi, 1991). Hence, sun dried shiitake will be sweeter than oven dried shiitake. Shiitake contains almost all the major and micro nutrients needed for the human metabolism (Table 2.1).

The nutritional value of the shiitake mushrooms is highly influenced by the growing method. It was found that, the carbohydrate and mineral contents (Ca, Cu and Mn) were significantly higher in the fruit bodies harvested from the natural wood logs than those from the sawdust substrate logs (Aoyagi *et al.*, 1993). The protein content of the fruit bodies is having positive correlation with the nitrogen content of the growing substrate. As in the sawdust log technology, substrate is enriched with the nitrogenous compounds; the fruit bodies harvested from these mini logs contains relatively higher protein content. Even there is a variation in the mineral contents within the fruit bodies. Concentrations of minerals were generally higher in caps than in stipes of the fruit body (Vetter, 1995).

Shiitake contains almost all the essential amino acids. Among them, lysine and arginine are found abundant (Liu and Bau, 1980) and methionine and phenylalanine are less abundant (Lasota and Sylwestrazak, 1989). On dry weight basis, the total amino acid concentration was 15.24% in caps whereas in stipes it is 11.35%. The analytical results by Fasidi and Kadiri, 1990 showed the amino acids, protein, glycogen, lipids, ascorbic acid and mineral contents increased with the maturity of the fruit bodies. Hence, it is always desirable to consume fully matured fruiting bodies for maximum nutritional gain.

The delicious flavor and aroma in the shiitake mushroom is contributing by the alcohols, ketones, sulfides, alkanes, fatty acids, etc. Matsutakeol (octen-1-ol-3) and ethyl-n-amyl ketone were found to be the major flavor causing compounds and 1,2,3,5,6- pentathiepane was identified as the agent responsible for the typical aroma of the shiitake. Linoleic acid, palmitic acid, oleic acid, tetradecenoic acid, stearic acid and myristic acid are the major fatty acids reported in the shiitake (Mizuno, 1996). Among the free sugars present in the shiitake, trehalose, glycerol, mannitol, arabitol, mannose, and arabinose were reported (Mizuno, 1995; Hobbs, 2000).

2.2 Bioactive compounds of shiitake

Lentinan (β-1,3 glucan with β-1,6 and β-1,3 glucopyranoside branching), is a water-soluble polysaccharide extract from the shiitake, is an important bioactive compound majorly responsible for antitumor activity of the shiitake mushroom. It is widely used as an anti tumor drug which can be used as an intravenous injection in Japan. These polysaccharides amount to 1–5% of the dry weight of the mushroom. In addition to lentinan, other potential active compounds *viz.*, heteroglucans, heterogalactans, heteromannans, xyloglucans, etc. have also been identified.

2.3 Pharmacological properties

People from orient region have enjoyed shiitake mushroom for ages as folk medicine. Though, many years raw forms of shiitake was in use, more concentrated and purified derivates of shiitake come up and playing important role in modern medicine. Among them, lentinan, a polysaccharide extracted from the fruit bodies or from the mycelium was the most frequently studied compound. Chihara *et al*., 1970 isolated a more refined form of water soluble antitumor polysaccharide from the fruiting bodies of shiitake and named the compounds as lentinan after the genus *Lentinula*. In the following section of this chapter, the available literature with respect to the pre clinical and clinical studies on various therapeutic effects is presented.

Table 2.2 Bio active compounds of shiitake isolated in pure form

Compound	Properties
Lentinan	➢ It is a cell-wall constituent extracted from the fruiting bodies or from the mycelium. ➢ Lentinan is a highly purified, high molecular weight polysaccharide containing only glucose molecules with β-(1-3)-D-glucan linkages
Lentinula edodes mycelium extract (LEM)	➢ It is a preparation from the powdered mycelial extract ➢ It contains a heteroglycon protein conjugate
KS-2	➢ A polysaccharide containing α-linked mannose and a small amount of peptide
Eritadenine	➢ A nucleic acid derivative

2.3.1 Anti carcinogenic effects

Majority of health conscious people who enjoy the mushrooms as dietary supplements are curious about the medicinal effects of these mushrooms specially shiitake has in its whole, dried or in a purified extract form. The development of modern medicine comes with the sound proof for the antitumor effects possessed by the various shiitake derivatives based on the animal based studies and preclinical studies conducted on the human beings. The anticancer activity of mushroom polysaccharides was observed for first time in farmers who were engaged in cultivation of medicinal mushrooms. The death rate caused by cancer of those farmers was exceptionally low than the general population (Ikekawa 2001). Several studies were conducted to understand the reasons for this phenomenon.

Lentinan and LEM were found to enhance the immune system in the patients suffering from various types of cancers rather than attacking the tumor cells. According to Chihara *et al.*, 1970, lentinan was found to regress the solid type of tumors of sarcoma180 and methylchloranthrene-induced fibrosarcoma in synergic host–tumor system. The xenobiotic compounds such as polycyclic aromatic hydrocarbons (PAHs) will induce the activity of cytochrome P450 (CYP) and metabolically activate the procarcinogens. The cytochrome P450 (CYP) is a xenobiotic metabolizing enzyme which expresses mainly in the liver. Hence, it was hypothesized that suppression of the activity of CYP by the mushroom compounds will reduce the risk of procarcinogenesis. Accordingly, a laboratory study was conducted by injecting lentinan intra peritoneally to female mice. The liver cells were examined to investigate the effect of lentinan on expression of CYPs (Hashimoto *et al*., 2002). The shiitake polysaccharide reduced the level of CYP1A activity induced by 3-methylcholanthrene, a PAH accompanied by the TNF-α (tumour necrosis factor) production through the suppression of DNA-binding activity of aryl hydrocarbon receptor and an increase in the DNA-binding activity of nuclear factor-κB (Hashimoto *et al*., 2002; Okamoto *et al*., 2004). These results clearly indicated the anticarcinogenic activity of *L. edodes* polysaccharides as it has down regulated the activity of CYP and further to preclude the metabolic activation of procarcinogenesis. In addition to this, inhibition of telomerase activity by lentinan also attributed the anticarcinogenic activity (Sreenivasulu *et al*., 2011).

2.3.2 Immune-Modulating Effects

As discussed above, the bioactive compounds of shiitake mushrooms do not attack the tumor cells directly, but these compounds activate the different immune responses within the host itself. Lentinan acts as a host defense potentiator which is able to restore the host cells response to lymphocytokines and other substances and thereby improves the host defense mechanisms (Yap, 2001; 2003). Host defense potentiators are functionally different from biological response modifiers. Thus, lentinan is able to increase host resistance against various kinds of cancer and infectious diseases, including acquired immuno deficiency syndrome (AIDS) (Nagi, 2003).

2.3.4 Cardiovascular Effects

The major cause of death in the modern sedentary world is coronary artery disease (CAD), a primary risk factor for which hypercholesterolemia is a factor contributing to hardening of the arteries. Disturbance in blood platelet augmentation, higher blood pressure levels, diabetes and hypercholesterolaemia are the main risk factors associated with CAD. Along with these factors, the low-density lipoprotein (LDO) and very-low-density lipoprotein (VLDL) cholesterol levels in the blood will increase in patients suffering from CAD. It is estimated that the source of 60–65% of the blood cholesterol is originated from endogenous dietary sources. Hence, many clinical studies indicate the need of therapeutic measures than drug therapy to correct the hypercholesterolaemia by modification of diet pattern. The diet with a nutritional regime including foods with low saturated fatty acids and high crude fibres is gaining popularity for treating the patients with hypercholesterolaemia. Because of the low calorific value, high protein and crude fibre contents, mushrooms are considered as ideal diet for treating cardiovascular diseases. It was reported that, eritadenine content of shiitake mushroom is able to lower the blood serum cholesterol (BSC). In an rat based study, it was observed that the eritadenine has better cholesterol-lowering activity in rats fed with a high fat diet than in those on a low-fat diet. Although feeding studies with humans have indicated a similar effect, further research is needed.

2.3.4 Hepatoprotective Effects

A polysaccharide fraction from shiitake mushrooms demonstrated the ability to protect the liver cells in animals and also showed the ability to improve liver function to enhance the production of antibodies when infected with hepatitis B (Wasser, 1999). Lentinan improved serum glutamic pyruvic transaminase (SGPT) and completely restored GPT levels in the livers of mice with toxic hepatitis. Crude extracts of shiitake mushroom have also established the liver-protecting actions (Hobbs, 2000). The injection of LEM slowed the proliferation of tumor cells in the cancerous liver of rats (Mizuno, 1996).

2.3.5 Antiviral, Antibacterial, and Antiparasitic Effects

Lentinan and its derivatives are found to be effective against various kinds of bacterial, viral (including AIDS), and parasitic infections. An important area of this polysaccharide research deals with its ability to mobilize the body's humoral immunity to ward off bacterial infections resistant to antibiotics (Wasser, 1997). Many cancer and AIDS patients die of opportunistic infections due to immunodysfunction. It is extremely important to protect AIDS patients from these various infections. According to Tuchikura *et al*. 1988 when lentinan was used in combination with azidothymidine (AZT), it suppressed the surface expression of human immunodeficiency virus (HIV) on T cells more so than did AZT alone. Lentinan has exhibited potent anti-HIV activity under in vitro conditions resulting in an inhibition of viral replication and cell fusion. AIDS therapy must include a strategy to enhance the immune system. Among the various therapeutic approaches used, prevention of the development of AIDS symptoms in carriers should be stressed. Based on these *in vitro* studies, it is possible that such prevention may be realized by the use of HDPs such as lentinan or its related substances. In addition, lentinan has showed several antimicrobial activities as listed below (Wasser, 1997).

a. Antiviral activity in mice against vesicular stomatitis virus (VSV) encephalitis virus, abelson virus, and adenovirus type 12
b. Stimulated nonspecific resistance against respiratory viral infections in mice;
c. Conferred complete protection against an LD75 challenge dose of virulent mouse influenza A=SW15

d. Increased resistance against the parasites *Schistosoma japonicum* and *S. mansoni*

e. Exhibited activity against *Mycobacterium tuberculosis*, bacilli resistant to antituberculosis drugs, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Candida albicans* and *Saccharomyces cerevisiae*

f. Increased host resistance to infections with the potentially lethal *Listeria monocytogenes*.

2.4 Shiitake as a drug and its dosage

Apart from the fresh and dried forms, shiitake mushroom is available in different commercial forms in the health food stores and urban markets. Teas, syrup, wine, powdered extract, capsules and concentrates are some of the forms available in the market. The clinical studies indicate that the pharmacological activity of the shiitake mushroom is dependent on its form and the dosage given. A pure form of lentinan is injected through i.v @ 1mg/vial for the patients suffering from various cancers. In the form of tablets, the dosage is usually 2-4 tablets per day containing 2gm of the dried water extract. As the drying process concentrates the lentinan and other active compounds, dried water extract of the fruiting bodies is used for the preparation of tablets. The standardized commercial formulations with definite levels of lentinan content are preferable over the crude formulations.

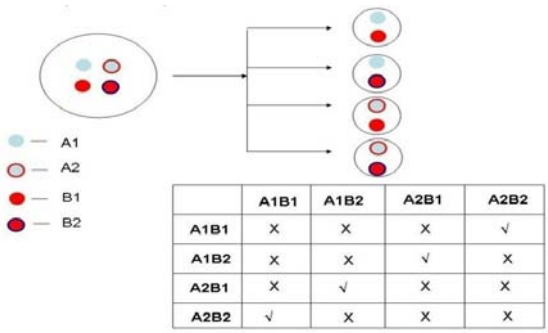
The consumers may have an opinion that, the fresh mushroom as a dietary supplement can be used to achieve the potential medicinal benefits. But to achieve the therapeutic doses, it needs relatively higher amounts of fresh mushroom consumption on a daily basis (Hobbs, 2000). To obtain a concentrate, whole fruit bodies or powdered mushrooms are to be boiled in water. The extract is then concentrated and is used as a drink (Rahman and Chowdary, 2012). An alcohol extraction product is obtained by preserving fresh or dried shiitake mushroom in alcohol, which has been mixed with sugar or molasses. Some products, including healthy shiitake wine are sold as a nightcap or as a tonic drink. Many of these new bioactive compounds extracted and purified from shiitake mushrooms have undergone the basic clinical trials and showed considerable effectiveness in curing many ailments, especially different kinds of cancer tumours. However, it further warrants a much systematic research in combination with advanced clinical trials to accept and promote the medicinal mushroom based extracts as an approved drug compositions for curing the targeted diseases.

3. Genetic Improvement

Mushrooms belongs to the kingdom Fungi, a group very distinct from the plants, animals and bacteria. Hence, the activity of growing mushrooms is entirely different from the field grown crops. To become a successful mushroom grower, it is wise to understand the basic life cycle of the desired mushroom species. As the breeding techniques for genetic improvement is also linked to the reproductive behavior of the targeted organism, knowing the basic life cycle of the shiitake mushroom is of prime importance to develop the high yielding strains. The living body of the fungus is a mycelium made out of a tiny web of threads (or filaments) called hyphae. Under suitable conditions, compatible hyphae will fuse and start to produce basidiospores. The spores released from the gills of the fruiting body again will germinate and develop to form the hyphae, which is the main mode of fungal vegetative growth. The mushroom fruit bodies produces millions of spores in its life span. The life cycle will continued each time the spores germinate to form the mycelium.

3.1 Sexuality in shiitake mushroom

Takemaru (1961) demonstrated that, *L.edodes* exhibits a heterothallic bi-factorial mating system. Heterothallic species have different sex forms in two different individuals. It requires two compatible monsporous mycelia to produce the sexual spores. This condition is comparable with the dioecious nature of the plants in which male and female organs are present in different individuals. This kind of heterothallism is also referred as bipolar incompatibility or tetrapolar incompatibility. Two unlinked mating type factors were found to be operative in this system of mating. When homokaryotic mycelia germinated from the single basidospores confront together plasmogamy takes place. If the plasmogamy takes place between the compatible mycelia, nuclear migration happens from one mycelium to another. A dikaryotic condition establishes when the nucleus reaches to the tip cell of the resident mycelium. The two nuclei co-exist



	A1B1	A1B2	A2B1	A2B2
A1B1	x	x	x	√
A1B2	x	x	√	x
A2B1	x	√	x	x
A2B2	√	x	x	x

Fig. 3.1 Bifactorial tetrapolar heterothallism

in the same cell without fusion. The dikaryotic condition can be maintained by development of clamp connections and growing into the secondary mycelium.

3.2 Genetic improvement studies in shiitake

Till 1930s the natural log cultivation of shiitake is popular using the wild strains. The development of pure culture technique using mycelia as inoculum marked the beginning for the modern cultivation methods and also created the scope for the genetic improvement. Efforts were made to develop the high yielding crossbreed strains in shiitake after identifying the mating behavior of the shiitake by Takemaru, 1961. Many useful genes are being introgressed into the cultivated strains through systematic breeding efforts (Hasebe 1991). These strains were well characterized for their economic traits and remains as a useful genetic resource in shiitake growing regions.

Genetic diversity studies

Techniques used	Major Contribution	Region of the study	Reference
Yield traits	Identified a quick growing strain DMRO-388s which came for first harvesting within 50 days of inoculation.	India	Sharma et al. 2018
RAPD, ISSR and SRAP markers	Indicated that cultivated strains of shiitake in China are genetically very homogeneous	China	Fu et al. 2010
RAPD & RFLP			Wai et al (1996)
ISSR			Liu et al. 2014
AFLP	Identified two distinct groups suitable for cultivation on wood logs and sawdust substrate.	Japan	Terashima et al (2002)
SCAR	Identified the molecular markers linked to the mating factors	Japan	Tanaka et al (2004)

3.3 Selection of strains based on enzyme activity

L. edodes being a white rot fungus produces wide range of oxidases and hydrolases for degradation and utilization of various lignocellulosic wastes. Babasaki et al (1991) developed a screening technique to select the potential strains that can degrade the lignin preferentially. The strain that was able to degrade lignin

up to 38.4% in one month was selected based on the wood-decay tests. The activity of peroxidases and laccase enzymes in degradation of lignin which is a major structural component of the wood material was demonstrated in this study. The mycelial growth abilities and extra cellular enzyme activities of nine different strains of shiitake cultivated on wheat straw were measured to establish predictable information on strain-substrate compatibility by Annepu *et al.* 2018. The experimental results showed that the strains varied significantly in their mycelial growth rate, enzyme activities and in turn the biological efficiency. Fast mycelial colonization and higher amount of cellulase activity was observed in strain DMRO-327 with the highest mean fresh mushroom yield of 202.32 g per kg of wetted substrate. Correlation study was found significant between the initial speed of linear growth rate and biological efficiency of various strains. The rise in activity of carboxy methyl cellulase, FPase and xylanase during the primordial formation illustrated the role of these enzymes to promote fruiting of shiitake in straw based substrate. While decrease in the lignolytic enzymes with the time showed that these enzymes are responsible for substrate degradation.

3.4 Conventional breeding techniques

Detecting the mating type behavior in shiitake is an important step for the genetic improvement through hybridization. Owing to the heterothallic nature, mating types determine the compatibility of the parental strains in a cross. Bak et al (1996) carried out a hybridization process to develop the high yielding strains of shiitake suitable for cultivation on oak wood sawdust. Germplasm from Korea Republic and other countries were used in the breeding programme. A total number of 12 hybrid strains were developed by Di-mon mating method. The hybrid strains were evaluated for their temperature suitability and three high temperature and two mid temperature strains and seven low temperature strains were identified with high yield potential. Mating between monokaryotic isolated obtained from the 21 strains was attempted by Fox *et al.* (1994). These strains were well characterized for the different mating factors and further utilized in the hybridization process.

3.5 Biotechnology tools

Gong *et al.* 2018 mapped a total number of 25 QTLs (Quantitative Trait Loci) responsible for precocity and fruiting in segregating population of shiitake using

the composite interval mapping (CIM). QTLs for precocity, fruiting and yield traits were mapped on five different linkage groups and these findings hastened the process of marker assisted breeding (MAS) for high yielding cultivars in shiitake. The genome of *L.edodes* was sequenced by Kwan *et al.* 2012 using Roche 454 and ABI SOLiD genome sequencing. The study compiled the genome sequence into a searchable database for annotating the genes and for analyzing the metabolic pathways. The diversity, population structure and genetic loci associated with the major agronomic traits of *L.edodes* were examined by Li *et al.* 2017 by genotyping 297 molecular markers of 89 cultivars of shiitake. The study provided a range of markers useful for MAS and also enables us to understand the genetic architecture of agronomic traits in the shiitake mushroom. Whole genome *de novo* sequencing and genome annotation of the *L.edodes* was done by Shim *et al.* 2016. In this study first draft genome consists of 46.1 Mb genome comprising several predicted gene models were presented.

High yielding strains of shiitake mushroom released by ICAR-DMR, Solan

DMR- Shiitake 38

DMR-38

Fruit body characteristics

- Fruit body shape: spherical, centre dark brown, outer light brown white scars uniformly distributed throughout the cap
- Fruit body size: Cap dia. 6.5-8.0cm; stipe length 5-6cm
- Fruit body weight: 40-45g
- Fruit body colour: Brown

Spawn run conditions

- Temperature – 23-25°C (bag temperature)
- Relative humidity – 75-80% (cropping room)
- CO2 – 5000-8000 ppm (cropping room)
- Light – 8-10 h daily

Fruiting/cropping

- Temperature - 22-24°C (bag temperature)
- Relative humidity – 80-85%
- CO2 – 600-800ppm
- Light – Fluorescent light 8-10 hours/day
- Air circulation/exchange – Two times/day (5-10 min/exchange)
- Light watering daily is required
- Harvesting once in week

- Packaging in perforated polythene/polypropylene or paper bags

Yield: 31-40 kg/100 kg saw dust

DMR- Shiitake 388

DMR-388

Fruit body characteristics

- Fruit body shape: spherical, initially fruit bodies are pale yellow in colour, turns light brown with maturity, ring of white scars on the cap
- Fruit body size: Cap dia. 6-7cm stipe length 5-6cm
- Fruit body weight: 35-39g
- Fruit body colour: Light brown
- Veil opening: opened veil from beginning

Spawn run conditions

- Temperature – 23-25°C (bag temperature)
- Relative humidity – 75-80% (cropping room)
- CO2 – 5000-8000 ppm (cropping room)
- Light – 8-10h daily
- Air circulation/exchange- Nil
- No watering during spawn run

Fruiting/cropping

- Temperature - 22-24°C (bag temperature)
- Relative humidity – 80-85%

- CO2 – 600-800ppm
- Light – Fluorescent light 8-10 hours/day
- Air circulation/exchange – Two times/day (5-10 min/exchange)
- Light watering daily is required
- Harvesting once in week
- Packaging in perforated polythene/polypropylene or paper bags.

Yield: 22.3-43.9kg/100kg substrate

DMRO - 356

DMRO-356

Fruit body characteristics

- Fruit body shape: Fleshy mushrooms with round caps and firm stipe
- Fruit body size: Cap dia. 8.2-8.6 cm stipe length 5.9-6.1 mm
- Fruit body weight: 23-27g
- Fruit body colour: Light to dark brown cap, creamy white gills, light brown stem
- Veil opening: opened veil from beginning

Spawn run conditions

- Temperature – 25±2°C (bag temperature)
- Relative humidity – No role
- CO2 – 5000-6000 ppm (cropping room)
- Light – 4-6h daily

- Air circulation/exchange- Nil
- No watering during spawn run

Fruiting/cropping

- Temperature - 20±2°C (bag temperature)
- Relative humidity – 75-80%
- CO2 – 1000-1500ppm
- Light – Fluorescent light 8-10 hours/day
- Air circulation/exchange – Two times/day (5-10 min/exchange)
- Light watering daily is required
- Harvesting once in week
- Packaging in perforated polythene/polypropylene or paper bags.

Yield: 40-46kg/100kg of dry substrate in three flushes within a cropping period of 110 days

Other cultivated strains of *L. edodes* presently under evaluation and their characteristics

Strain no.	Gene bank accession	Source of collection	Main agronomic traits
1	DMRO-7	Philippines	S, Lm, My, G_2
2	DMRO-12	USA	S, Mm, Ly, G_3
3	DMRO-20	Nepal	S, Lm, Ly, G_3
4	DMRO-22	Nepal	S, Mm, Ly G_3
5	DMRO-23	Nepal	S, Lm, Ly, G_1
6	DMRO-35	Switzerland	S, W, Mm, My, G_2
7	DMRO-51	Korea	S, W, Mm, Ly, G_3
8	DMRO-297	Japan	S, W, Lm, Ly, G_3
9	DMRO-327	Manipur	S, W, Mm, My, G_1
10	DMRO-328	Manipur	S, W, Mm, Ly, G_3
11	DMRO-329	Raipur	S, Mm, Lm, Ly, G_3
12	DMRO-330	Raipur	S, W, Lm, Ly, G_3
13	DMRO-331	Raipur	S, Mm, Ly, G_2
14	DMRO-410	Delhi	S, W, Mm, My, G_2
15	DMRO-412	Udaipur	S, W, Mm, My, G_3
16	DMRO-623	Kerala	S, Mm, Ly, G_3

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S-sawdust based substrate, W-wheat straw based substrate; Em- early maturity (< 60 required for first harvest from the date of spawning), Mm-medium maturity (60-90 days required for first harvest from the date of spawning), Lm- late maturity (> 90 days required for first harvest from the date of spawning); Ly- low yielding strains (Biological efficiency <20%), My- medium yielding strains (BE 21-50%), Hy- high yielding strains (BE> 51%); G_1- Grading based on pileus thickness (>15 mm thickness), G_2 (pileus thickness 12-15mm), G_3 (pileus thickness <12mm).

4. History of Shiitake Cultivation

Cultivation of shiitake has a fascinating history. During the Sung dynasty (1100 A.D) in China, a famous personality Wu San Kang, noticed the shiitake growing on the fallen dead wood logs (Luo, 2004). As a first milestone in shiitake cultivation, domestication of these naturally grown wild mushrooms was started by Wu San Kang on the fallen logs of deciduous wood which is popularly known as the "Hatchet-notching" technique. When he cuts the logs, he observed that, the mushrooms grown on the cut logs were of better-quality. However, there were no mushrooms during certain times despite of his cuttings. With frustration one day he beat the logs feverishly. Surprisingly, mushroom started producing profusely after some days. This was considered to be origin of the shocking method, cutting and beating of natural logs in shiitake cultivation. Later Chinese growers introduced this cultivation technique of shiitake to the Japanese farmers. From there it spread to the other Asian countries.

Eight centuries later the development of hatchet notching cultivation technique, during 1936; Kitajima in Japan (Chang and Miles, 2004) started working with the pure culture technique in log production. He demonstrated the use of pure culture free from other microbial contamination and its inoculation in the logs. This approach brought the control over the crop production in the hands of the growers and led the way for extensive natural log cultivation. The third and significant historical milestone was the invention of synthetic bag log cultivation methodology. This technique was evolved and improved by Peng in China during 1983 (Ting, 1994). Compared to the natural log technique, the grower can harvest 3-4 times more mushrooms in sawdust log method in just one-tenth of time (Royse, 2002). The adoption of sawdust cultivation method became more rapid and faster due to the greater productivity with shorter growing cycles.

4.1 Hatchet-notching cultivation technique

"Hatchet-notching" is a relatively primitive and semi-artificial method of cultivation. In this method, a hatchet (a small axe with a short handle) was used to make cuts in the felled broadleaf tree trunks (Fig 4.1). The cut ends are exposed to wild *L. edodes* spores floating down with the wind for inoculation. The yields levels in this technique depends greatly on the density and quality of the wild *L. edodes*

spores in the natural environment and also on the prevailing local climatic conditions (Tan *et al*., 2014).

Fig. 4.1 Hatchet-Notching cultivation of shiitake (Source: WSMBMP Bulletin No. 13, 2015)

4.2 Natural wood log cultivation

With the isolation of pure culture of *L.edodes*, production of this mushroom moved from the hatchet-notching method to the natural wood log cultivation technique with controlled inoculation. This production practice on natural logs started with the placing of spawn into the holes drilled on hardwood logs. After spawning, these logs are then shifted to laying yard under shade for about one year before fruiting begins. The logs will continue to yield the fruit bodies in three to four flushes for the next three to four years. This technique managed to shorten the length of cultivation cycle with substantial increase in the mushroom yields. However, this technique consumed huge amounts of forest resources and threatened the ecological stability.

4.3 Synthetic bag log cultivation

In 1978, Shanghai Academy of Agricultural Sciences (SAAS) developed sawdust based brick system for cultivating shiitake mushroom under controlled conditions.

This system was focused on the use of an artificial substrate formulation based on the saw dust waste, inoculated aseptically with a pure mushroom strain and careful management of the growing conditions. It reduces the demand for scarce wood resources typified by cut log procedure and facilitated the expansion of shiitake cultivation from forest regions to accessible areas. Inspired by the sawdust brick technique, ICAR-DMR, Solan developed a short duration production technology in a bag log method using a specific substrate and strain combinations. It is possible to get the fruit bodies within 45-60 days using this technology compared to the earlier synthetic log technologies where it took 80-110 days.

As a consequence of rapid scientific developments, diverse cultivation models suitable to different regions and countries have emerged. Genetic improvement coupled with the standardized pure spawn production, improved inoculation procedures and the crop management practices have helped to come into sight an industrialized cultivation mode. At present, shiitake cultivation became more intensive and mechanized in countries like China, Japan and Korea.

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5. Natural Wood Log Cultivation Technique

Cultivation of shiitake on wood logs has been the most common method since, 1100 A.D. The recent advances in the production technology of shiitake by using the synthetic blocks made up of sawdust, many growers have turned to bag log cultivation. However, log cultivation that uses the natural environment conditions still has several advantages over the bag cultivation. It requires less initial expenditure as it grows under natural conditions. The quality of the fruit bodies are relatively superior with fragrant odour compared to the fruit bodies harvested from the bag log cultivation. The polysaccharide content (i.e lentinan) is higher in the mushrooms harvested from the natural logs (Braner *et al.,* 2002).

Shiitake production on natural logs involves several steps starting from selection of the suitable wood species and placing of the spawn followed by incubation of the spawned logs in the laying yard under shade. The vegetative growth of mycelium continues up to one year and the mushrooms starts appearing on the logs from second year onwards. The logs will continue to yield the mushrooms in three to four flushes for the subsequent three to four years. Following are the steps in natural log cultivation technique of shiitake mushroom.

1. Selection of the laying yard
2. Selection of host tree species
3. Choice of logs and felling time
4. Preparation of the logs
5. Spawn inoculation
6. Stacking method
7. Conditions for primordial formation
8. Crop management
9. Harvesting

5.1 Selection of laying yard

Availability of year round shade and high humidity are the critical factors to start this activity. Choose a location with minimum 60% shade for staking and

storing of the inoculated logs. The location should have enough space to permit the movement of logs and other small machinery. The laying yard located in North to East facing slopes will help protecting against sun and heat. Care should be taken that the selected location should have accessibility to the road. Proximity to water source is another important factor which is necessary for fruiting induction. Presence of the wind breaks will have an additional advantage by keeping the logs from drying out in the long run.

5.2 Selection of host tree species

The host tree species selected for logs preparation has strong influence on the mushrooms produced in each flush. Even the taste and size of the mushrooms are also influenced by the properties of the host tree species. A matured log contains

Table 5.1 Tree species tested for suitability for shiitake cultivation

Common name	Family	Genus	Species
High suitability			
Oak	Fagaceae	*Quercus*	*acutissima, alba, brandisiana, crispula, dentate, garryanna, kelloggii, kerii, kingiana, mongolica, muehlenbergii, pinus, rubra, semiserrata, serrata, variabilis*
Chinkapin	Fagaceae	*Castanopsis*	*accuminatissima, argentea, chrysophylla, cuspidate, indica*
Tan oak	Fagaceae	*Lithocarpus*	*auriculatus, densiflorus, lanceafolia, lindleyanus, polystachyus*
Horn beams	Fagaceae	*Carpinus*	*betula, caroliniana, japonica, laxiflora, tschonoski*
Medium suitability			
Alder	Betulaceae	*Alnus*	*glutinosa, japonica, rubra, serrulata, tinctoria*
Aspen , Poplar, Cotton wood	Betulaceae	*Populus*	*balsamifera, deltoids, grandidentata, nigra, trichocarpa*
Beech	Fagaceae	*Fagus*	
Birch	Betulaceae	*Betula*	*nigra, pendula*
Chestnut	Fagaceae	*Castanea*	*Crenata*
Hickory	Juglandaceae	*Carya*	
Maple	Aceraceae	*Acer*	*rubrum, macrophyllum*
Sweet gum	Hamamelidaceae		*Liquidambar Styraciflua*
Tupelo	Nyssaceae	*Nyssa*	*Silvatica*
Willow	Salicaceae	*Salix*	*Nigra*

Source : Przybylowicz and Donoghue, 1990

three layers viz., external bark, sapwood and hard wood. This ratio of sapwood to heartwood impacts the mycelia colonization and subsequent mushroom production. Quick mycelium colonization can be seen in the sap wood and cambium because they contain the most readily available nutrients and have higher moisture content than the heart wood. Trees having higher sapwood than the heartwood are most preferred for shiitake production. The site on which trees grow can influence the nutrient content of logs. The best shiitake logs come from the trees grown on fertile sites.

5.3 Choice of logs and felling time

Trees selected for log preparation should be felled between late autumn and early spring. This is the period in which the tree will be in dormancy stage. During this stage the sapwood contains high sugar levels. At this point of time, the bark is tightest around the trunk and will prevent the early bark loss at later stages of its planned use. A log with an intact bark is very important component, in order to retain the optimum moisture level of 30-35% inside the log. Trees should be cut about 2-4 weeks before the intended time for inoculation. The logs should be kept in rest after felling to inactivate the natural defense system of the tree against the invasion by the shiitake fungus. Oga *et al.* (1977) observed that fatty acids and phenolics in *Pinus densiflora* and myricitrin in *Myrica rubra* inhibited the growth of the fungus. Hence, the wood logs should be kept intact for a minimum period of one month, during which any inhibitory characteristics of new wood will be lost.

5.4 Preparation of the logs

After felling the logs, it is important to avoid the mechanical damages to the bark. Any such damages to the bark may invite other invading fungi inside the wood. The logs can be cut into suitable lengths for operational convenience and easy handling. Log length can vary from 36 to 48 inches, and the diameters can range from 2.5 to 10 inches. Logs that are 40 inches in length and 5 inches diameter are easy to handle and are

Fig. 5.1 Preparation of the wood logs (Photograph courtesy: Dr Susheel Kumar, Scientist AICRP Mushrooms, Manipur Centre)

reported to be most productive. If the area selected for natural log cultivation of shiitake is comes under dry climate, it is preferable to prepare the logs with a length of 4 to 6 feet. A smaller log tends to lose the moisture quickly resulting in poor spawn run. The prepared logs must be inoculated as early as possible to reduce the contamination by other fungal organisms and also prevents the moisture loss before the point where mycelium establishes in the wood. Montini *et al.* (1998) inoculated shiitake mycelium in *Eucalyptus saligna* logs with 2.07-2.30 m length and 6-11 cm diameter and observed that productivity was inversely proportional to the diameter of logs.

5.5 Spawn inoculation

For inoculation of spawn, small holes of 1x1cm size with 1.5 to 2 cm deep should be made on the logs with the help of drilling machine. The holes on the logs should be made at a distance of 20-30cm (long axis) and 5-6cm in between the rows. Making holes close around the circumference, supports the best colonization as the fungus grows along the length of the log (Przybylowicz and Donoghue 1988). The spawn prepared on the sawdust or wood plugs made out of this sawdust spawn is preferable for inoculation. The sawdust spawn is filled in the holes or the wood plugs can be inserted directly into the holes. The spawning operation is to be carried out under clean environment. After placing the spawn in the holes, it should not be pressed tightly. Then seal the inoculated holes with a thin coat of hot wax. This will prevent the lateral entry of the contaminants into the logs. Paraffin

Fig. 5.2 Addition of wood plug spawn to the logs (Photograph courtesy: Dr Susheel Kumar, Scientist AICRP Mushrooms, Manipur Centre)

wax is the most commonly used wax for this purpose. Once inoculated, the mycelium will establish in the logs and gradually starts colonizing the wood. A temperature range of 20-25°C favours the vegetative growth of the mycelium. However, a temperature range of 14-20°C is favoured during this phase, to keep the growth of mould competitors to the minimum level. Boztok and Erkip (2002) reported that an incubation perod of 6-18 months after inoculation on logs is needed for fruiting, depending on species of tree, spawn and climate. The productive life of logs was 3-5 years.

5.6 Stacking method

Next step in the process of natural log cultivation is stacking of the spawned logs in the incubation yard. Staking is a process that helps in best utilization of the space during the incubation. There are many stacking systems followed in the shiitake cultivation and the choice of stacking depends on the spawn run site. Two commonly adopted systems are crib-stacks and lean to stacks. In crib stacking, the logs are arranged in a horizontal layers perpendicular to each other. Lean to stacks is composed of vertical rows of logs supported against a horizontal rail or wire. Each stacking method has its own advantages and disadvantages. The space utilization is very efficient in crib-stacks compared to the lean to stacks, but there is a pronounced difference in temperature and humidity.

5.7 Conditions for primordial formation

Inoculated logs should be stacked and kept for incubation in the places where suitable humidity and shade are available. Artificial shade nets can be used to create such environment. Direct sunlight to the logs must be avoided, because this can raise the log temperatures to over 35°C which causes heat damage to the shiitake mycelium. If the temperature falls below 15°C during this incubation period, the log piles may be covered with plastic films to raise the temperatures to the optimum levels. The moisture content of the logs should be maintained at 35 -45% by weight. Under natural conditions, fruiting may be induced by heavy rainfall or temperature changes in the environment. But under natural intensive cultivation models growers controls the fruiting by a pre planned log rotation schedule.

A sudden change in the key environmental factors, such as lower temperature and high humidity are required to trigger the mycelium from vegetative stage to

the reproductive stage. After completion of the incubation period, the logs are transferred to a growing house where the temperature, humidity and light are provided at optimum levels for primordial initiation. Although, temperature requirement for fruiting is strain specific, a range of 15-25°C was found appropriate for fruiting induction (Komatsu and Tokimoto, 1982). The higher humidity levels may be achieved by increasing the free water levels beyond 10%. Similarly, the inner bark, need to receive the 0.01-0.001 Lux intensity of luminous for primordial formation (Ishikawa, 1967).

Fig. 5.3 Stacking of the spawned wood logs under favourable conditions

5.8 Crop management

Once primordia formed, they should develop into fruiting bodies. Low temperatures ranging from 5-20°C and the relative humidity more than 65% will stimulate the further development of fruit bodies. The lower temperature levels at the time of primordial initiation may accompany the enhancement of acid protease enzyme activity. This will results in the accumulation of nutrients around the developing fruit body (Tokimoto *et al*., 1989). Some growers are adopting the physical shock method by beating the logs and/or cold water soaking at 15-20°C to promote the fruit body development. Log beating in synchronization with water soaking promotes the greater fruit body production. Generally, strains which induces the fruiting at higher temperatures found to be more sensitive to physical shock and shows higher response to the forced fruiting under intensive cultivation.

5.9 Harvesting

Shiitake mushrooms are to be harvested when the cap is still curled and somewhat closed. The optimum stage for harvesting will be decided based on the growth progress rather than the size of the mushroom. Gills of the fruit body should be visible and the outer edge of the mushroom should be slightly curled under. Flattened edge of the fruit body indicates the over-ripe stage, but still edible. However, if the cap is completely unveiled and curved upwards, the fruit bodies lose its appearance and fetches very less price in the market.

From the point of nutrition, many compounds including sugars and polysaccharides found maximum during the middle stage of development (Minato *et al*., 2001). The fruit bodies harvested at this stage of development can be preserved for longer periods than those harvested during the later stages. The mushrooms are harvested by twisting the stem gently by hand, so it breaks freely. Using a sharp knife ensures a clean cut on the stem and the dirt or debris should be removed without washing the mushrooms under water. While harvesting the mushrooms, proper care should be taken to avoid the mechanical injuries to the bark. To obtain the next flush, a rest period of more than one month is required for the recovery of mycelium to accumulate the nutrients.

6. Bag Log Cultivation Technique

Till 1980's Japan was the leading producer of shiitake worldwide. After 1980's rapid developments took place in the cultivation techniques of shiitake. The invention of bag log cultivation technique led the China to overtake the Japan in fresh shiitake mushroom production. The bag log technology became wide spread among the mushroom growers in the Orient region and the trend of worldwide shiitake production is leaning towards sawdust bag log cultivation. Development of bag log cultivation technology makes it possible to produce shiitake round the year to meet the market demands. Consistent market supply coupled with increased productivity per unit of time is the major advantages of this method. Though, cultivation of shiitake in bag log technique needs relatively higher initial investment, these advantages far outweighs this limitation.

In Indian perspective, shiitake mushroom cultivation is still at nascent stage. ICAR-Directorate of Mushroom Research, Solan made significant progress in the cultivation technique of shiitake suitable to the Indian farming conditions. New strains with rapid growth and higher productivity were developed by this institute which paved the way for development of short duration synthetic log production technology of shiitake mushroom with the precised control over the crop. By adopting this technology, it is possible to get the first harvest within 55-60 days compared to the earlier technologies, where it took 80-110 days. The steps involved in synthetic log cultivation are explained in detail under the following steps.

1. Substrate preparation
2. Bag filling and sterilization
3. Spawning
4. Incubation
5. Crop management
6. Harvesting

6.1 Substrate preparation

Sawdust is the main ingredient for the preparation of substrate blocks. Similar to the natural log cultivation, selection of the suitable host tree species for obtaining

the sawdust is an important factor. Based on the experimental findings and repeated cultivation trails conducted at ICAR-DMR, Solan it has been observed that sawdust of tuni (*Toona sinensis*) is highly preferable by the shiitake strains available at ICAR-DMR, Solan. In regions where, this sawdust is not available, other non-aromatic, broadleaved hardwood species such as poplar (*Populus* spp.), mango (*Mangifera indica*), safeda, oak (*Quercus* spp.), maple (*Acer* spp.) and willow (*Salyx* spp.) can be used for commercial cultivation of shiitake using bag lag technology.

Nutritional requirements for shiitake cultivation

a. **Carbon source**: *L.edodes* is a wood rotting fungi. It utilizes the wood substrates composed of lignin, cellulose and hemicelluose as the source of carbon required for metabolism.

b. **Nitrogen source**: *L.edodes* can utilize only organic nitrogenous sources such as peptone, amino acid, urea and it cannot utilize nitric nitrogen and nitrite forms of nitrogen. Cereal bran can be used as an organic nitrogen source in shiitake cultivation.

c. **Vitamin B_1**: The fungi require vitamin B_1 during mycelium growth. Fresh and coarse rice or wheat bran supplies this nutrient to the mycelium.

The organic nitrogen supplements such as wheat bran or rice bran should be added to the sawdust to support the mycelial growth of shiitake. The formulae standardized by ICAR-DMR, Solan for preparation of substrate is 80% sawdust; 19% cereal bran and 1% of calcium carbonate on dry weight basis. For the preparation of substrate, the sawdust should be soaked in the water overnight (14-16 hours) followed by the draining out of excess water in the next day morning. The wetted sawdust should be mixed with the cereal bran and calcium carbonate. Ingredients are mixed thoroughly in a mixer or by hand to hold a moisture level of 60-65%. The pH level of the substrate is to be maintained at 6.5-7.0. An optimum water holding capacity of the substrate combined with good aeration helps in achieving higher yields. Moisture in the substrate beyond the recommended levels prevents the airflow within the substrate. For a beginner, it is recommended to test the moisture levels of the substrate using the hot air oven drying method. However, it can be manually observed under field conditions by pressing the wetted substrate in a fist. The substrate is considered as too wet, if water oozing out of the fist.

Kalberer and Griensven (2000) reported that the supplementation of a sawdust-corn flour substrate with urea or ammonium chloride increased the crop yield of *L. edodes*. From supplemented substrates heavier fruiting bodies were harvested. The duration of the incubation influenced the crop yield and the size of the fruiting bodies. The supplements slowed down the spawn run and prolonged the incubation. Urea added to the substrate caused the failure of some primordia to develop and the deformation of some fruiting bodies. Royse *et al.* (1990) amended substrate formulations of mixed hardwood sawdust, wheat bran and millet with sucrose, fructose or glucose. Addition of sucrose (0.6 to 1.2% DW) to the substrate stimulated mushroom yield by 11 to 20% or more. Addition of fructose at 1.2% and glucose at 0.6% resulted in similar yield increases. The substrate amended with 1.2% sucrose tended to have a more synchronous maturation for the second break resulting in fewer days when mushrooms were harvested.

According to Fomina *et al.* (1999) addition of 20% rye bran to sawdust substrates was found to increase *L. edodes* yield. The sterilization (1 h, 125 °C) is the best method of the sawdust substrate preparation for *L. edodes* cultivation. Royse (1985) inoculated sawdust substrate (a 60:40 mixture of maple and birch) supplemented with millet or wheat bran or both, with spawn made from an isolate (PSU 305) of *L. edodes*. Biological efficiencies were two to three times greater for the longer incubation period. Larger mushrooms generally were produced with longer spawn runs.

6.2 Bag filling and sterilization

The substrate must be filled in the bags immediately after mixing. Otherwise the wetted substrate starts fermentation and renders the substrate unfit for mushroom cultivation. Heat resistant polypropylene bags of 150-180 gauge are to be used for filling the substrate. The bags are first loosely filled with 1.5 kg of the substrate material and later by putting the pressure, cylindrical shape is given to the bags. Some growers practicing to make the holes in the substrate block for inoculation of the substrate in post sterilization stage. After filling the substrate, the bags are then sealed with the non absorbent cotton with the help of polypropylene rings. These substrate bags are shifted to the autoclave for high pressure sterilization at 121°C for two hours. The sterilization time and pressure should be strictly maintained to prevent the chances for further contamination

during the early stages spawn run. Then allow the bags for cooling at room temperature. Filling the substrate more than 1.5 kg in the pp bags needs more sterilization time.

6.3 Spawning

In general top or surface spawning offers better results in bag log technology. In this method, the spawn should be placed on the surface of the substrate by removing the cotton plugs under the aseptic conditions. The grain spawn prepared on wheat grains or any other suitable cereal grain should be added @ 3% on wet weight basis (i.e., 45 grams per each bag log weighing 1.5 kg). Care should be taken to avoid the mold contamination during the spawning. It is always advisable to use the fresh spawn for better output. If the stored spawn is to be used for inoculation, the spawn must be brought down to the room temperature for few and then proceed for spawning. Terashita *et al.* (1997) investigated the effects of storage of spawn (15-200 days) at 4°C, 15°C and at room temperature (24°C) on mycelial growth, fruit-body yield, chemical constituents and enzyme activities of the mycelia of 3 strains of *L. edodes*. No significant decrease in mycelial growth and fruit-body yield was observed following with long term storage. However, storage of spawn for longer periods at low temperatures (4 or 15°C) decreased the formation of morphologically normal fruit-bodies. These results indicate that, abnormalities in the fruiting bodies have often traces its origin in the spawn procured from the long term storage.

6.4 Incubation

The next step followed by the spawning is incubation. Incubation facilitates the vegetative mycelium growth of the mushroom. Optimal mycelia growth of shitake during spawn run takes place at a temperature range of 25±2°C. The duration of spawn run varies with the strains selected for cultivation. Depending on the strain it ranges from 45-90 days. After adding the spawn under the aseptic conditions, the bags should be shifted to the incubation room. Even though the mycelium grows in the darkness, exposure to the light for 4-6 hours during the first 2-3 weeks helps in browning of the mycelia blocks. Unlike other edible mushrooms, completion of spawn run does not mean that, it is ready for fruiting. Following by spawn run, aging and maturation of mycelium is also required before primordial

Fig. 6.1 Steps in substrate preparation and filling of the substrate material

Fig. 6.2 Sterilization of the substrate bags

Fig. 6.3 Steps in addition of grain spawn to the sterilized substrate

initiation. Shiitake has a complex vegetative growth pattern composed of different stages. There is no clear demarcation between each growth phase. However, the growers need to pay personal attention to understand these physiological process which leads to the fruiting initiation.

i) **Colonization of mycelia in the substrate**: The complex substrate material comprising of cellulose, hemicelluose and lignin are degraded by the extracellular enzyme system of mycelium.

ii) **Physiological maturation of mycelium**: The mycelia growth will be stopped, while physiological metabolic changes occur during this phase.

iii) **Mycelial coat formation**: After completion of mycelial run in the substrate, white colour thick coat is formed on the substrate surface. Higher CO_2 levels in the incubation room promote the thicker mycelial coat formation.

iv) **The popcorn stage/ mycelia bump stage**: Clumps of mycelia are developed in some strains giving a pop-corn like surface. Primorida are produced at the tips of these bumps. Fluctuation in temperature and high CO_2 levels promotes more bumping.

v) **Browning/ pigmentation**: The surface of the substrate blocks turns into reddish brown colour and eventually forms a dark brown and dry outer protective surface.

vi) **Coat hardening phase**: Remove the plastic when bags have partially (half or one third) turned brown. The coat will gradually become hard. The outside of the substrate should be hard, the inside should be softer and more moist.

6.5 Crop Management

As the browning process nears completion, pinheads start to form about 1-2 mm beneath the surface. Regardless of the strain selected for cultivation, shiitake mushroom needs sudden change in the growing conditions for transition from vegetative phase to the reproductive phase. The changes in the growing environment such as temperature fluctuation, high humidity, soaking in cold water, removal of CO_2 and induction of fresh air by ventilation, physical shocks such as agitation, disturbance stabbing, and beating are some of the practices adopted by the growers in different regions.

Triggering the fruiting, by changing environmental growth parameters as mentioned below

1. Lower the temperature (from 25°C to 18-20°C; strain dependent)
2. Lower the CO_2 levels, to < 1000 ppm
3. Increase O_2 supply by raising the frequency to 4-8 air exchange per hour.
4. Increase the ambient humidity, to 60-80% R.H. (depending on the growing facility)
5. Increase the light intensity (1500-2000 lux)

The most commonly following practice at ICAR-DMR, Solan for stimulating the fruiting is by soaking the bag logs in ice cold water (4-6°C) for 15-20 minutes. After coat hardening phase, the polypropylene layers will be peeled off and the substrate blocks are to be immersed in the ice cold water taken in the suitable containers. After 15-20 minutes of time the logs are shifted to the cropping room. The logs do not require watering during incubation. Keep humidity low (50-60%) to prevent contamination. However, during the fruiting period the logs should be watered frequently to maintain the relative humidity at above 80%. A schedule of various parameters as given below:

Table 6.1 Various fruiting parameters

Stage / Activity	Time (Days)	Temperature (°C)	Light intensity (Lux)	Relative Humidity (%)
Incubation	45-90	23-27	500-1000	50-60
Induction	2-3	10-20	500-1000	85-95
Fruiting	7-14	16-18	1500-2000	75-80
Rest	7-10	20-30	None	60-70
Induction for next flush	2-4	10-20	500-1000	85-95

Note: The temperature range for fruiting is strain dependent.

Fig. 6.4 a) Mycelial run during the incubation phase; b) Mycelial bump formation as an indication of fruiting body initiation

Fig. 6.5 Cold water shock treatment to promote the fruiting

6.6 Harvesting

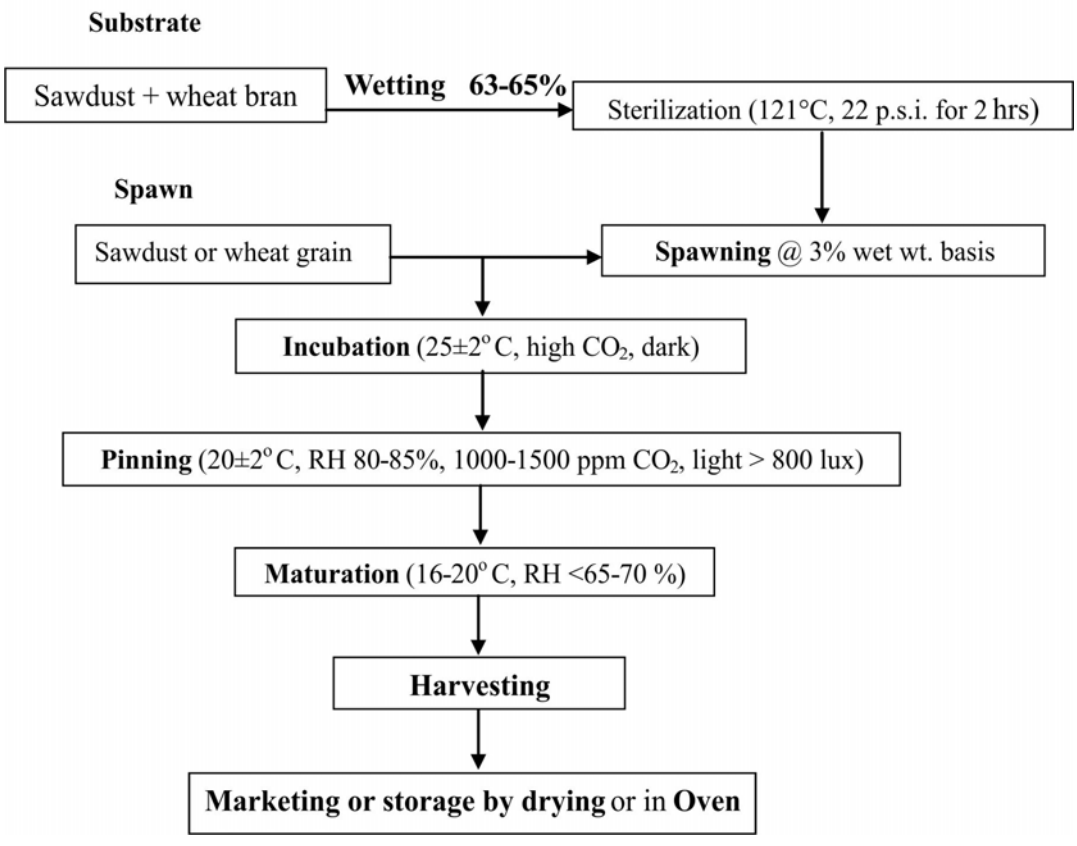
The desirable stage for harvesting of the fruit bodies of shiitake is when the cap margin of the fruit body is still rolled downward. Fruit bodies can be handpicked by holding the stalk in the hand and gently twisted in clock wise direction to separate it from the substrate. Residual stubs at the bottom of the stalk can be trimmed off using the sharp knife. To increase the post harvest quality and shelf life of the fruit bodies, the R.H should be lowered to <60%, 12 hours before harvesting. After harvesting the mushrooms, the substrate logs are allowed to rest for 7-10 days and no watering should be applied. Simultaneously, the R.H in the cropping room must be lowered to 30-40% to avoid the contamination during this dormant phase.

Fig. 6.6 Resting the substrate logs after harvesting the first flush

The yield levels are varying with the strain selected for cultivation. The strain DMRO-388s released for commercial cultivation by ICAR-DMR, Solan is giving a biological efficiency of 60-80% of dry substrate i.e., 360 to 480 grams of fresh mushroom per each substrate log weighing 1.5 kg. The total yield is distributed among three different flushes. More than 60% of the yield potential can be realized from the first flush itself. Remaining two flushes yields nearly 25% and 15% of mushrooms respectively. The cold water soaking is required for the subsequent second and third flushes also. But for induction of fruiting in substrate logs lying in rest phase, needs cold water soaking for longer duration. The dormant bag logs after harvesting the first flush can be soaked for 12 hours in the cold water to induce the second flush and further 18 hours for third flush. The mushrooms keep coming

Fig. 6.7 Harvesting and grading of the matured fruit bodies

Systematic flow chart for cultivation of shitake mushroom



from the substrate blocks till the exhaustion of the nutrition. However, it is advisable to retain the crop for three flushes to realize the economic yield and then discard the used material. A grower indented to harvest 50kg fresh mushrooms per day, he has to start the production activity with a minimum of 250 bags, 75 days prior to the targeted harvesting date. The cycle should be continued based on the desired production levels.

6.7 Alternate agro-wastes as growing substrate

Mushroom cultivation utilizes various agricultural by products as growing substrate for production of protein rich food. In common edible mushrooms species such as white button mushroom, oyster and milky mushrooms majority of the agricultural residues have been tested in large scale production. However, in shiitake mushroom cultivation relatively less is known about the use of different agro residues other than sawdust in bag log technology. Morais *et al.* (2000) and Curvetto *et al.* (2002) reported the use of sunflower seed hulls as growing substrate for production of shiitake with different levels of biological efficiency. Lee *et al.* (2008) successfully conducted the cultivation trails of shiitake using the corn waste as growing substrate. Growing shiitake mushroom in a substrate combination of sawdust and corn cob waste is increasing popularity in major shiitake growing countries. Barley straw, corn cobs, corn stover, rice bran, pineapple crown bracts, coffee husks, sugarcane bagasse and sugarcane leaves were tried as growing substrates by Salmones *et al.* (1999). The results varied greatly in all these experiments depending on the substrate formulation, accurate control of growth conditions, maintenance of non-infected substrate throughout incubation and it largely depends on the genotype of the strain employed (Chen *et al.*, 2000).

In the early 1990s, several researchers (Oliver and Delpech, 1990: Delpech and Olivier, 1991: Levanon *et al.*, 1993) reported the successful production of shiitake on bulk, pasteurized wheat straw. Olivier and Delpech (1990) were the first to develop cultivation techniques based on substrates containing wheat straw enriched with chicken feather meal. Mushroom yield improvements also were made through the addition of 15% sawdust to the wheat straw substrate. Levanon *et al.*, (1993) demonstrated that a mixture of bulk-pasteurized wheat straw and cotton straw could be used to produce shiitake. Royse and Sanchez (2003) reported the possibility to replace up to 16 % of the oak sawdust with chopped wheat straw and obtained

better yields. ICAR-DMR, Solan has developed a cultivation technology of shiitake using wheat straw as the substrate material supplemented with starch based cereal bran. The substrate composition was standardized by mixing wheat straw (chopped into 4-6 cm size), wheat bran and gypsum in the ratio of 80:19:1 on dry weight basis. Moisture content of the substrate was adjusted at 60-65%. The substrate of one kg is filled in the polypropylene bags and sterilized at 121°C temperature and 15 psi pressure for two hours. The spawn rate was optimized at 5% on wet weight basis. The temperature of 25±2°C and 17±2°C was maintained at incubation and fruiting, respectively. Fruiting was induced by dipping the colonized substrate logs in ice-cold water for 15 minutes. The mean average biological efficiency (fresh yield per dry weight of the substrate) of 55.50% was recorded with the strain DMRO-327 (Annepu *et al.*, 2018a).

In a comparative study conducted to evaluate the effect of substrate on the yield performance of different strains of shiitake, strain DMRO-388s recorded highest biological efficiency of 85.63% on saw dust and strain DMRO-327 with 53.02% on wheat straw (Annepu et al 2018b). Among the genotypes tested, sawdust gave relatively higher yield over the wheat straw. But the earliness of fruiting on wheat straw can economize the shiitake cultivation as it is cheap and abundantly available. This new initiative in shiitake mushroom production popularizes the specialty mushrooms in India.


Fig. 6.8 Fruiting of shiitake strain No – DMRO 327 on wheat straw-based substrate

7. Post Harvest Management

7.1 Shiitake for the fresh market

Having the delicacy and unique taste, fresh mushrooms are more preferable in the markets. Although shiitake can be dried for extending the shelf life, growers prefer to sell fresh mushrooms as they ensure better returns. Under ambient conditions, freshly harvested shiitake mushrooms have short shelf life, so they must be sold in the market as and when harvested or can be preserved in cold storage for 2 to 3 weeks. The shiitake can be stored for 3 days at 20°C and the shelf life can be extended up to 14 days by keeping them at 6°C (Minamide *et al*., 1980) packed in containers wrapped with microporus film. At refrigeration below 10°C being packaged in polystyrene trays covered with PVC plastic film the storage life can be as long as seven days (Santana, 2003). Under the same refrigeration conditions, the shelf can be extended up to 15 days by wrapping the trays with the films having lower permeability.

Fig. 7.1 Shiitake mushrooms packed for fresh market

7.2 Long term storage

There are many methods of long term preservation, including drying, pickling, canning, frozen slices, shiitake teas, shiitake powders, etc. The quality of the preserved product is marinated in comparable with the fresh ones to gain the market acceptance. Following are the successful methods used for long term storage of shiitake under Indian conditions.

a. Drying

One of the most common and best methods of extending shelf life of shiitake mushrooms for long term storage is by drying. Sun drying and thermal drying are the commonly employed methods for shiitake drying.

i. Sun drying

The freshly harvested mushrooms are spread on shelves or sheets and are directly exposed to the sunlight. While spreading, arrange the mushrooms with the gills facing upwards. The time required for drying depends on the initial moisture content of the fruit bodies and the prevailing weather conditions. The quality of the sun dried mushrooms is of lesser quality than that dried by the thermal method.

ii. Thermal drying

Another method of drying is thermal drying which utilizes the hot air blown into the cabinet air dryer. The temperature required for drying largely depends on the water content of the fruit bodies. Compared to the oyster mushrooms, shiitake mushrooms contain lesser moisture in their fruiting bodies. For effective drying the moisture should be lowered to the level of 13-15%. Air temperature of 30-50°C for a short period results in uniform drying with less shrunken shiitake mushrooms (Kawai, 1962). While drying, arrange the mushrooms with the gills facing upwards. Grading should be done as per thickness and size before spreading the mushrooms in the dryer. By the end of drying process, the dried shiitake should be cooled down and then put into the polyethylene bags. The packed bags should be sealed properly and can be stored in a cool and dry place. The dried shiitake produced by this method retains better quality in terms of color, appearance and flavour

compared to the sundried shiitake. The dried shiitake readily absorbs the moisture from the air, so they should be stored properly and examined frequently to discard the mold infected ones.

Li *et al* (2001) introduced a new drying method using far infrared in a vaccum. The combined drying method of far infrared in an air flow in the earlier stage and under vaccum and in exchange air in later stage proved best among all the tested methods. Minamide *et al* (1980) reported that browning of pilei, gills, stipe and polyphenol oxidase activity and total free amino acid content were markedly increased during storage at 20°C. According to Qing *et al* (2001) the activity of catalase was significantly increased while the activities of peroxidase poly phenol oxidase and ascorbic acid oxidase were significantly decreased by different concentrations of $CaCl_2$. Treating shiitake with 0.005mol/l of EGTA significantly decreased catalase activity whereas activity of peroxidase, polyphenol oxidase and ascorbic acid oxidase were markedly enhanced. According to Minamide *et al* (1980) when O_2 level was kept below 2% there was a marked reduction in gill browning polyphenol oxidase (PPO) activity. Keeping quality was best at 40% CO_2 and 1-2% O_2 with a shelf life up to 4 times longer than for control.

Fig. 7.2 Dried shiitake mushrooms for long term storage

When shiitake was stored in polythene bags of various thicknesses with or without hermetic sealing, shelf life of mushroom packed in non hermetically sealed bags was about 18 days at 1°C, 14 days at 6°C, 7 days at 15°C and 4 days at 20°C. Shelf life at 20°C was longest following pre cooling at 1°C for 24h in hermetically

sealed bags 80u thick containing CO_2. According to Sheng *et al* (1998) when fresh shiitake was irradiated with different doses, irradiation above 1.0kGy inhibited the growth and mould decay of fresh shiitake after harvesting. Treatment at 1kGy irradiation produced some new volatile compounds in dry product such as methylethyl disulfide, sulfinylbis methane, methyl ethyl disulfide and N-3 (methylbutyl) acetamide. The 8 carbon compounds mostly disappeared after drying. Lui *et al* (1989) reported that modified atmosphere method with a composite paper/ plastic bag with the inclusion of a natural substance to reduce the peculiar smell, enabled the fresh shiitake mushrooms to be stored at 5°C for 15 days.

a. Frozen slices

With the increasing urbanization frozen food products are gaining wide acceptance due to their convenient preparation. The freshly harvested mushrooms are cleaned properly and then sliced or cut into pieces uniformly and then cooked. Drain out the excess water off, the mushrooms are frozen immediately. They can be stored in frozen conditions for a long period.

b. Shiitake powder

Shiitake powder is generally used as a food additive. The broken shiitake pieces, cut stems and deformed fruit bodies are processed and they are ground into powder.

7.3 Recycling of spent shiitake substrate

Mushroom production is the largest solid state fermentation industry in the world with huge spent substrate being produced. It is extremely important to find a potential ways to utilize this barren material. Every tonne of mushrooms produced results in double the amount of dry spent residual material. In bag log cultivation technology, the sawdust based substrate is supplemented with the starch based additives from cereals to optimize the nutritional needs of the growing mycelium. After completing the cropping period, growers are discarding this material near the mushroom farm. Subsequently, this material is acting as a repository and breeding area for contaminants. Very few scientific reports are available for alternate re-uses of spent shiitake substrate.

Fig. 7.3 Disposal of spent shiitake substrate

Chang *et al*. 2000 reported the use of spent shiitake substrate in the treatment of acid mine drainage and its use in the treatment of effluents from olive mills by D'Annibale et al. (1998). Okeke et al. 1993 and Chiu et al. 1998 reported that, spent substrate of shiitake can be used to degrade the pentachlorophenol from the contaminated soil. Use of spent shiitake substrate as organic manure for growing tomato (Lin and Chuen, 1993), sugarcane (Pan et al. 1989), corn (Chang, 1997) radish (Cho *et al*., 1997), etc were reported. Huang, 1997 and Huang and Huang, 2000 reported the biological control of *Rhizoctonia* damping off of cabbage using the spent shiitake substrate. The experimental findings of Monika *et al*. (2012) proved the strong and unique possibility of two-, three- and four ring poly aromatic hydrocarbons degradation by spent compost from *A.bisporus* and *L. edodes*. In general, production of various field and horticultural crops benefitted from the re-use of spent mushroom substrate.

8. Crop Protection

In bag log cultivation technology, mushrooms are grown on sterilized substrates. Under such conditions, there is minimum possibility for the contamination by the competitor moulds. However, if any contaminant enters into the sterilized substrate, it flourishes well due to the absence of other competitors. If unnoticed, these contaminants often lower the productivity and under sever conditions causes complete crop failure. Green mold (*Trichoderma* spp.), red bread mold (*Neurospora* spp.) blue mold (*Pencillium* spp.) are the major fungal pathogens causing severe damage to the shiitake bag logs under controlled conditions.

8.1 Green mold (*Trichoderma* spp.)

Most problematic competitor mould in shiitake cultivation is green mould infection. Presence of wide diversity in the genus *Trichoderma* such as *T. viride*, *T. koningii*, *T. polysporum*, *T. longibrachiatum* and *T. glauscum* makes it complicated to develop the shiitake strains resistant to this disease. Green mould damage symptoms caused by *T.viride* are characterized by white and dense mycelia growth in the substrate during the initial stages. Later it is followed by extensive sporulation by the fungus which results in turning of whole substrate into green colour. Poorly sterilized substrate and poorly disinfected transfer during the spawning and transfer between the growing rooms are the primary cause for this infection. Once the contaminant entered into the substrate, it spreads rapidly into

Fig. 8.1 Green mould contamination in shiitake substrate logs

large patches and later results in green sporulation and darkened patches which in turn cannot be colonized by the shiitake mycelium. The remaining area in the bag log colonized by the shiitake mycelia often produces inferior quality fruit bodies.

Badham (1991) reported that *T. harzianum* have higher growth rate compared to the *L.edodes* and it tolerates pH fluctuations and lower water potential better than the shiitake. According to Balazs *et al* (1996) temperature, pH and N requirements of *Trichoderma, Aspergillus, Penicillum, Chaetomium* and *Nigrospora* are similar to those of shiitake indicating that modification of these factors would not help in control. They recommended that temperature should not exceed 25°C during the initial phases of incubation and pH should be kept below 6-6.5. Among the six fungicides tested (Fukui *et al*., 1974) benomyl inhibited the growth of *T. viride, Cephalosporium diospyri, Verticillium malthousei* and *Dactylum dendroides* without any harmful effect on the mushrooms.

8.2 Red bread mold (*Neurospora* spp.)

The symptoms of damage caused by the red bread mold are similar to the green mold. Initially the fungal colonization is characterized by white mycelail growth, later followed by extensive red or orange sporulation. Semi sterilized substrate coupled with high humidity in the growing environment favours the spread of this disease. The fungus is air borne and spreads rapidly under high temperatures coupled with high relative humidity.

Fig. 8.2 Symptoms of red bread mold

8.3 Blue mold (*Pencillium* spp.)

There are many species reported to cause damage in shiitake cultivation viz., *P.citrinum*, P. *funiculosum*, *P.chrysogenum*, *P.cyclopium*, *P.pallidium*, *P.digitatum* and *P.italicum*. Compared to green mold, damage caused by blue mold is less severe in bag log cultivation technology. The blue mold growth starts with white mycelia growth followed by blue colour sporulation of the fungus. Acidic conditions of the substrate favours the spread of this fungus.

8.4 Control measures

If the contamination occurs near the top of the bag, it is an indication that contamination gained entry during inoculation. If the contamination occurs at the bottom or side, there may be a leakage in the bag. If the contaminant scattered throughout the substrate it is an indication for insufficient sterilization of the substrate. The chemical control measures are not advisable for the management of these contaminants as such measures can affect the mycelail growth. The mushroom itself being a fungus, when fungal diseases appear, it is often very difficult to control as the chemicals used against the disease may affect the mushroom itself. Thus, stringent care has to be exercised from the very beginning of the cultivation to avoid the entry of any contaminants. Following are the basic preventive measures suggested for adoption.

1. The substrates and supplementation materials selected for cultivation should be free from pathogens.
2. Sterilize the substrate for 2 hrs at 121°C for elimination of pathogens
3. Maintain strict hygiene and sanitation in the growing rooms. The rooms where mushrooms are to be grown should be thoroughly washed and then whitewashed with lime. The floor should also be limed. High pH is not suitable for most contaminants.
4. At the entry of every room, there should be a trough filled with 2% formalin solution, wherein the shoes or feet must be dipped before entering the room.
5. In case of contamination, the contaminated block should be remove to a spot well away from the house and buried in a pit or burnt.

6. At the end of every cropping cycle, the room should be washed again and white washed and fumigated with formalin.
7. Any fallen bits of mushroom should not be left on the floor of the room. cleaning and cutting off the base of the mushroom stalk should be done outside the growing room and properly disposed off.
8. Broken pieces of the mushroom stalk, while harvesting should not be left on the blocks. If the stalk breaks, it should be removed entirely from the bed.
9. While preparing the block, care should be taken that is properly compressed. The more the compression, the better will be the spawn running.
10. Avoid high temperature and humidity which are favourable for contaminants. Usually development of fruit body requires low temperature and high humidity.
11. Inspect the bag logs in the cropping room regularly and eliminate the contaminated bags immediately. Before disposal, sterilize the contaminated bags by autoclave.

8.5 Incidence of insect pests in shiitake cultivation

In commercial cultivation of shiitake no major pest attack was reported so far. However, few reports mentioned in the scientific literature is presented here. Yoshimatsu and Nakata (2003) reported *Diomea cremata* as new pest of shiitake. The larvae consumed the thick brown mycelial coat formed on the surface of synthetic logs. Kumar and Sharma (1999) reported *Hypena* spp. damaging the mycelium and fruit bodies of shiitake. They also observed *Lycoreilla* spp., *Megaselia* spp., *Heteropeza* spp. and mites *Tyroglyphus* sp and *Pigmephorus* sp damaging the shiitake mushroom. Sato *et al* (1999) reported that *Dacne picta* is one of the most important pests of cultivated shiitake in Japan. Savary (1995) reported major infestation of *Dacne picta* in dried shiitake. Kumar *et al* (2004) reported *Lessioderma serricone* as a serious pest of dried shiitake stored at room temperature. Chin *et al* (1996) reported five insect and six other animal species associated with shiitake. Of the insect species *Achorutes armatus*, *Scaphidium amurense* and mollusk *Philomycus confosa* caused severe damage to fruit bodies. A great deal of damage was done to lamella by mites, *Rhizoglyphus* sp and *Histiostoma* spp. Yi *et al* (1998) reported

Aphelenchoides composticola and *Rhabditis* spp causing severe damage to *L. edodes*. Kumar and Sharma (2006) reported slugs, *Laevicantis alti* causing 40-60% damage to shiitake crop.

8.6 Abnormalities in fruit bodies of shiitake

Unfavourable growing conditions causes several abnormalities in the fruit bodies such as malformation of the fruit bodies, formation of double cap, cracked caps, elongated stipe with small cap, pale and small fruiting bodies, fruiting body without gills, etc. Malformation of the fruiting bodies is due to the low or high temperature than required for the growth of the shiitake strain. In double cap condition, another pin may form on the cap of the fruiting body. It is due to the higher moisture content in the growing substrate and relatively lower temperatures during the fruiting body development. Cracking of the cap is caused due to the lower humidity levels. This characteristic condition happens when the inner part of the cap outgrows the outer part. These cracked mushrooms are fetching higher prices in the market in the name of flower shiitake. When the growing room lacks the sufficient light, it results in production of elongated stipe with small capped fruiting bodies. Lack of nutrients in the growing substrate delays the primordial initiation and subsequent production of elongated fruiting bodies. Similarly, pale fruit bodies may be caused by dark conditions at fruiting body development. Fruiting bodies without gills are developed when fruiting is induced before the complete colonization of the substrate by the mushroom mycelium.

Fig. 8.3 Abnormal fruit body formation due to unfavourable growing conditions

In majority of the cases, manipulation of growing environment can reverse these abnormalities. During fruiting, fluctuating light and humidity improves the fruit body quality. Drastic fluctuation in the humidity during the fruiting will improve the fruiting body quality and also discourages the mold contamination. Shiitake fruit bodies developed under such fluctuating humidity conditions have a tougher leathery outer skin which also increases its post harvest shelf life.

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9. Economics of Shiitake Mushroom Cultivation

This chapter deals with the common terminology and cost factors involved in calculation of economics of mushroom production activity using the hypothetical models. The real costs and benefits involved in the mushroom production activity vary from region and region and also subjected to the price fluctuations.

9.1 Terminology used in economics of mushroom cultivation

a) **Breakeven point:** BEP is the production level of mushrooms at which the total revenues equals the total expenses incurred.

b) **Capital expenditure:** Capital expenditure is the funds used by entrepreneur to acquire the physical assets required to carry out the mushroom production activity. Physical assets are of permanent in nature and lasting longer with standard depreciation rates. These include physical structures such as buildings and machinery such as chilling units, boilers, blowers, steel racks, generators, etc.

c) **Cropping cycle:** It is the cycle of activities related to the growth and harvest of crop. The cropping cycle varies with the mushroom type selected for cultivation and the growing conditions.

d) **Cost of production**: It refers to the total sum of money required for the production of a specific quantity of output.

e) **Depreciation**: Depreciation is the value of physical assets such as civil structures, machinery and equipments over a period of time due to the ware and tare. The depreciation of machinery is calculated at 10% per year and on buildings and structures it will be 5%. In real terms calculation of depreciation is a method used to reallocate the funds over the useful life span of a tangible asset.

f) **Estimated project cost**: EPC is the practice of estimating the total cost of completing a project with a defined scope. EPC helps in planning, monitoring and controlling a project's monetary costs.

g) **Gross profit:** It is the profit made by a production unit after deducting the cost of production. It is calculated by subtracting the cost of production from the income gained through sale of mushrooms.

h) **Net profit:** Net profit is the final profit of a grower after deducting the depreciation, interest and other overhead expenses from the gross profit.

i) **Operative costs**: Operative costs are the expenses involved with the administration and maintenance of a production activity on a day to day basis.

j) **Professional charges**: Professional charges are the prices charged by experts for designing of the environmentally controlled units as per the desired technical specifications.

k) **Recurring expenditure / variable cost:** Recurring expenditure is the cost involved in meeting the production volume of the unit. These costs include direct raw material cost, labour wages, electricity cost and other miscellaneous expenditure necessary to complete the production cycle.

9.2 Technical parameters/ assumptions for proposed model

1. The budgetary parameters taken for financial calculations are flexible and vary according to the region and quality of the materials.
2. Shiitake mushroom cultivation under controlled conditions with a production capacity of 20 tons per annuum (TPA) is taken into consideration for working out economics.
3. The standard substrate formulae and crop management practices developed by ICAR-Directorate of Mushroom Research, Solan is taken for calculation of raw materials requirement.
4. The biological efficiency of 80% *i.e* 80 kg of fresh mushroom per 100 kg of dry substrate is taken into consideration for yield calculations.
5. The deprecation rates are calculated @ 5% on buildings and infrastructure and @ 10% on machinery.
6. The interest rate is calculated @ 12% per annum on fixed capital.

9.3 Estimated Project cost

Basis for calculation

Mushroom species	Shiitake
Substrate formulae	Sawdust + wheat bran (4:1)
No of cropping rooms	4
Bags in each room	2800
No of crops in each room	3
Biological efficiency	80%
Total fresh production	20 ton
Production facility	4 rooms (502 x162 x102 each) LBH
No of bags to be accommodated/ room	2800 (1.5 kg wet substrate/bag)
Cycle to be completed in a year	Three
Total bags to be used in three crops	33600
Expected yield of *Shiitake* from three cycles	20 ton

Capital Expenditure

Land: Available

a. Machinery/equipments

Machinery/equipments	Quantity	Rate	Amount (lakhs)
Autoclave	Two	7,00,000.00	14.0
Laminar flow	Two	1,00,000.00	2.0
Air conditioners and temperature control systems	Four	1,50,000.00	6.0
		Total	22.0

b. Cropping Rooms

Material/item	Qty	Size	Rate (Rs.)	Amount (Rs.)
Cropping rooms(including insulation)	4	502 x162 x102	1000/sq ft	32.00
Autoclave room	1	102 x202 x102	600/sq ft	1.20
Inoculation room	1	102 x102 x102	1000/sq ft	1.00
Mixing room	1	202 x102 x102	600/sq ft	1.20
Total				35.40

Total Capital expenditure = 22.0 + 35.40 = 57.40 lakhs

Recurring Cost

a. Expenses on raw materials and energy

Material/item (Unit)	Qty.	Rate/unit (Rs.)	Amount (Rs.)
Saw dust (T)	25 T	3000	75000
Wheat bran (T)	5 T	5000	25000
$CaCO_3$ (q)	2.5 q	700	1750
Rings 21000	(No)	35000	0.60
Spawn (q)	20 q	7000	140000
Non Absorbent cotton (q)	2 q	2000	4000
PP bags	3 q	17000	51000
Electricity charges/month(Rs.)	12	100000	1200000
Miscellaneous(Rs.)	50000		50000
Total			1567750
Cost of raw materials for three crops= Rs. 1567750.00			

b. Wages

Labour	Period	Salary/month/person	Total Amount (Rs.)
2 Person	12 months	9000	216000

Interest and depreciation

Item	Cost (Rs.)
Depreciation on buildings (@5%)	177000.00
Depreciation on machinery (@10%)	220000.00
Interest @ 12 %	688000.00
Total	1085000.00

Cost of production and return (Rs.)

Item	Total cost/annum (Rs. in lakhs)
1. Raw materials	15.68
2. Wages and salary	2.16
3. Interest and depreciation	10.85
Total	28.69

Cost and Benefit

	Total production/ annum	Selling price*	Total
Shiitake on saw dust	20 tons	2.0 lakh / ton	40.00 lakh
Total Sale			40.00 lakh

Cost of Production = Rs. 28.69 lakh
Net return (1st year) = 40.00 –28.69 = Rs.11.31 lakh
*Selling price is only an estimate based on the existing market price.

9.4 Marketing considerations

Shiitake mushrooms can be marketed through a number of different channels. Smaller-scale producers will typically sell either directly to consumers through a farmers' market or to restaurants, small independent groceries, and specialty/ natural/health food stores. Many marketing innovations are well suited for this product. Working with Community Supported Agriculture organizations that have a produce subscription distribution system may be a possibility. Promoting availability to restaurant buyers on a regularly maintained Web site also works in certain market areas. Larger-scale marketing is carried out through a number of cooperatives and larger grower-packers, selling primarily into wholesale markets.

Since shiitake mushrooms are unknown to many consumers, and considering that it will require some effort and resources to establish a reliable supplier presence with institutional customers, the grower should plan on committing some funds early in the enterprise development towards promotion. Promotional strategies targeted directly to consumers can include product information, recipes and product care inserts, samples, and cooking demonstrations. Promotional strategies targeted to larger buyers can involve help with cross merchandising, in-store point-of-purchase printed material, and logo development. There may be additional opportunities to promote a product as locally produced on a menu or with a display.

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Glossary

- **Basidiocarp -** The fruiting body of fungi that reproduce through basidia.
- **Clamp connection-** An elbow-like protuberance which arches over the walls separating cells in mated (dikaryotic) mycelia of some mushroom species.
- **Flush:** The sudden development of many fruiting bodies at the same time. Usually there is a resting period between flushes or breaks.
- **Heterothallic -** Having two or more morphologically similar pairs of strains within the same species. The combination of compatible spore types is essential for producing fertile offspring. Typically a spore on a four spored basidium is compatible with only one of its counterparts.
- **Incubation** - The period during which the logs are maintained under conditions favorable for the mycelium to grow throughout the sapwood of the log
- **Inoculation –** the process of introducing the mushroom mycelium into the substrate
- **Mycelium** - The vegetative part of a fungus, consisting of a network of fine white filaments (hyphae).
- **Pinning –** The process of forming primordia
- **Primordia –** Little mushroom buds visible on the surface of the logs
- **Spawn** - Vegetative mycelium cultured on sawdust and a little grain under sterile conditions, and used to inoculate logs.
- **Spawn run** - An incubation period bolts undergo after inoculation during which the shiitake mycelium colonizes the wood.
- **Strain –** A selected mushroom variety