Cultivation of Shitake (*Lentinula edodes*)

*Lentinula edodes*, commonly called as shiitake is most popular and important cultivated mushroom in Japan. It is the 2nd largest mushroom cultivated in the world after *Agaricus bisporus*. It is liked by the consumers because of its unique taste and flavour and presence of a chemical which reduces plasma cholesterol level. It is mainly cultivated in Japan, People's Republic of China, Taiwan, S. Korea and United State of America. It grows in nature on the wood of broad leaf trees mainly oak and chestnut.

**Contents**

- Spawn preparation
- Cultivation technique
  - Wood logs Method
  - Synthetic log cultivation
- Economics of shiitake cultivation
Spawn preparation

Mainly there are two types of spawn, sawdust and wood plug spawn are used. Saw dust spawn is prepared using any of the following formulae:

a. Saw dust 65%  
   Wheat bran 15%  
   Used tea leaves 20%  
   Water 65%

b. Saw dust 78%  
   Wheat bran 15%  
   Used tea leaves 20%  
   Water 20%

   Calcium carbonate 1%
   Water content 65%

c. Saw dust 800 g  
   Rice bran 200 g  
   Sucrose 30 g  
   Potassium nitrate 4 g  
   Calcium carbonate 6 g  
   Water 2 litres

Saw dust after properly sieving to remove bigger size wood particles and other impurities, it is thoroughly mixed with water. Normally one or two drops of water should ooze out when pressed between the fingers. It is then filled into either empty spawn bottles or in polypropylene bags. With the help of a road, one inoculation hole is made into the centre of the substrate. The spawn containers are plugged with non absorbent cotton and covered with aluminium foil. It is then autoclaved at 20 pounds pressure p.s.i. for 2 hours. The actively growing mycelium of 10 days old culture is inoculated aseptically and incubated for 30 days at 24 ± 2°C contaminated bottles are discarded for further use.

Wood plug spawn is prepared by inoculating mycelium on small wedge shaped or either small cylindrical wood pieces. When the fungal mycelium impregnates the wood pieces, they are ready for inoculation.

Cultivation methods

A. Wood logs Method

*Lentinula edodes* grows in nature on the dead wood of a number of hard wood trees mainly *Quercus* spp. (Oak), *Castenopsis* spp. (*C. chinensis*, *C. tissa*, *C. fordil*, *C. lamontii* etc.). *Elaeocarpus* spp. (*E. chinenses*, *E. japonicus*, *E. lancaefolius*), *Lithocarpus* spp. (*L. calophylla*, *L. glaber*, *L. spicatus*), *Betula* spp. and *Carpinus* spp. various plant species graded for cultivation adopting suitable strains either those which can fruit at or above 10°C. The detailed cultivation technique is as follows:

1. Log preparation
2. Spawn preparation
3. Spawning logs
4. Crop management
Log preparation

The *Lentinula edodes* mycelium is saprophytic and wood rotting. It mainly grows on dried wooden logs of different trees (Table 1) absorbing nutrients from the cambium. Although it grows on any size and age of logs, but the log with 9-18 cm diameter and from 15 to 20 years old tree are most suitable. The time of failing or cutting the trees is also equally important. The most suitable period is from autumn (December-January) to early spring when the logs contain highest amount of carbohydrates and other organic substrates. Moreover the outer protective layer, bark is also tightly attached with the woody portion. The logs should contain a moisture content of 44-55% at the time of felling. If the moisture content of the log is less than 20% then there will be no growth. If the moisture contents are more than 60% with a pH of 7-8, it will be contaminated with other moulds. The pH of the logs should be between 4.5 and 5.5. The felled logs are left as such for 25-45 days which results in the lowering the moisture contents to 40-45%. Sometimes the logs are immediately inoculated, if the moisture content is optimum and further drying will result in excessive moisture loss.

**Table 1: Trees suitable for wood log cultivation of shiitake**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Scientific Name</th>
<th>Common Name</th>
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<tbody>
<tr>
<td>1.</td>
<td><em>Carpinus laxiflora</em></td>
<td>Hornbean</td>
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<td>2.</td>
<td><em>Castanea crenate</em></td>
<td>Japanese</td>
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<td>3.</td>
<td><em>Castanopsis cuspidata</em></td>
<td>Shii</td>
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<td>4.</td>
<td><em>C. sieboldii</em></td>
<td>Shii</td>
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<td>5.</td>
<td><em>Ostrya virginiana</em></td>
<td>Ironwood</td>
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<td>6.</td>
<td><em>Quercus abla</em></td>
<td>White oak</td>
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<td>7.</td>
<td><em>Q. acutissima</em></td>
<td>Oak</td>
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<tr>
<td>8.</td>
<td><em>Quercus spp.</em></td>
<td>Oak</td>
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<td>9.</td>
<td><em>Salix nigra</em></td>
<td>Black willow</td>
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<td>10.</td>
<td><em>Betula lutea</em></td>
<td>Sweet birch</td>
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<tr>
<td>11.</td>
<td><em>B. nigra</em></td>
<td>Red birch</td>
</tr>
<tr>
<td>12.</td>
<td><em>Alnus serrulata</em></td>
<td>Hazel alder</td>
</tr>
</tbody>
</table>

Spawning the logs

For spawn inoculation, small holes of 1 x 1 cm and 1.5 to 2 cm deep are made on the logs with the help of drilling machine. The holes are made at a distance of 20-30 cm (long axis) and 6 cm between each row. The holes between two rows are alternate in position. Saw dust spawn is filled in the holes or wood plug spawn is inserted by cutting out similar size pieces. The sawdust spawn should be kept soft and not tightly pressed. The holes are sealed with paraffin wax. The spawning should be mostly done in aseptic condition. Shiitake mycelium grows between 5 to 30°C but the most optimum temperature is 20-26°C. Low temperatures (14-20°C) are favoured during spawning logs, so that there is minimum growth of mould competitors.
**Crop management**

Inoculated logs are kept in open at a place where the physical conditions are most favourable for the mycelial growth. The inoculated logs are kept in a flat pile so that there is minimum light exposure. The pile should be covered with either straw, or gunny bags to prevent excessive water loss of the logs. The vegetative growth in the logs will be completed within 8-12 months depending upon the culture strain and the type of wood used.

For fruit body induction it requires, temperature shock of temperature drop, high humidity and enough light. The logs for fruiting are either sprayed with cold water or immersed in a tank of cold water. If the logs are immersed in cold water, then during summer they should be kept for 24 hours in cold water (15-18°C) while during winter they should be kept for 2-3 days at 10-15°C.

The logs are then leaned against the supports. The cropping area is kept moist to maintain high relative humidity. The temperature should be 15-20°C and humidity around 80-90%. Fruit bodies are harvested by first pressing and then twisting. Mushrooms are harvested up to 3 times and after a rest for 30-40 days they again watered to get more mushrooms. It can be repeated upto 3-4 times per year and these logs will produce crop upto 4-6 years.

**Synthetic log cultivation**

This method is practised in Taiwan, Mainland China, Singapore, New Zealand, USA, Finland, Netherlands, Germany, Philippines, Sri Lanka and Thailand.

**Substrate preparation**

The commercial cultivation can be carried out on sawdust of broad leave trees mainly tuni, mango, safeda, oak, maple and poplar. Some of the common formulations are as under

**i.**

- Saw dust 80%
- (Maple and birch 60: 40)
- Rice bran 20%
- Water content 65%

**ii.**

- Saw dust 80%
- Millet 10%
- Wheat bran 10%

**iii.**

- Hard wood 89.8%
- Water content 65%

**iv.**

- Hardwood saw dust 32.8%
- Rice bran 2.7-5.2%
- Corn powder millet rice 1.4-2.6%
- CaCO₃ 0.2-0.3%
- Water content 59-62%

**v.**

- Corn cobs 40 kg
- Saw dust 10 kg
- Wheat bran 12.5 kg
- Cane sugar 1 kg
- Pectin 15 g
- Urea 20 g

**vi.**

- Sugar cane bagasse 50 kg
- Rice bran 12.5 kg
- Gypsum 1.5 kg
- Potassium Sulphate 15 g
- Urea 15 g
- Magnesium Sulphate 10 g
vii. Rice straw 50%  
Wheat Straw 20%  
Saw dust 20%  
Cane sugar 1.3%  
CaCO$_3$ 1.5%  
Citric acid 0.2%  
CaSO$_4$ 0.5%  

Water should be adjusted to 60-65% and pH should be stabilized by gypsum and lime at 5.5 to 6.0. Soluble ingredients (citric acid, sugar, sulphates) are usually dissolved first in water before mixing; saw dust has to be soaked at least for two days and rice straw for three hours. All the ingredients are thoroughly mixed.

**Filling the bags**

Fill the bags (1.5 to 2 kg) immediately after mixing and wetting the substrate. Otherwise fermentation and contamination will start. Polypropylene (heat resistant) bags are used. The ends of the bags are sealed with heat. The bags are first loosely filled and later by putting the pressure, cylindrical shape is given to the bags. Some growers make holes for later inoculation before semi-sterilization; other will make holes after the heat treatment. Two 15mm diameter 20 mm deep holes are punched on opposite sides with an auger. The holes are covered with 33 mm square adhesive medical tape. The time between mixing the supplements and sterilization should be less than six hours to avoid fermentation. Bags can also be fitted with iron ring and plugged with non-absorbent cotton.

**Sterilization**

Sterilization is carried out in an autoclave at 22 psi for 2 hour.

**Spawn running**

The bags are placed in cropping rooms where the bags are generally incubated in a 4 hr/20 hr light/dark cycles at 23-25°C. Spawn run (Fig.1) may take 60-80 days. After longer spawn runs (more that 40 days) the surface of the colonized substrate may begin to turn brown, some exudates may be recognised in the spawn run of shiitake.
**Mycelial coat formation**

A thick mycelial sheet will develop (Fig. 2) on the surface of the substrate. This will occur 2-4 weeks after inoculation.

**Mycelial bump formation**

Bumps are clumps of mycelium, commonly formed on the surface of most strains (Fig.3). These bumps can turn into primordial at a later stage but most of them abort. Fluctuating temperatures and high CO$_2$ promotes bump formation.

**Pigmentation**

Some aeration should be provided when the bumps have formed.

**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. The core of the substrate has a moisture of about 80%.

**Fruiting**

Various factors which affect induction of fruiting are:

a. Temperature fluctuation
b. High humidity
c. Soaking
d. Removal of CO$_2$
e. Physical shocks

The logs do not require watering during incubation. Keep humidity low (60-70%) to prevent contamination if the plastic is removed too early or too late, yields will be affected. Deformed fruit bodies during the first flush are a sign of a too short spawn run or high CO$_2$ during incubation.

**Table 2: Various fruiting parameter (Chalmers)**

<table>
<thead>
<tr>
<th>Stages/Activity</th>
<th>Days</th>
<th>Temperature °C</th>
<th>Light intensity (Lux)</th>
<th>Humidity (%)</th>
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<tbody>
<tr>
<td>Incubation</td>
<td>30-120</td>
<td>20-30</td>
<td>500-1000</td>
<td>65-70</td>
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<tr>
<td>Induction</td>
<td>2-4</td>
<td>10-20</td>
<td>500-1000</td>
<td>85-95</td>
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<tr>
<td>Fruiting</td>
<td>7-14</td>
<td>12-18</td>
<td>None</td>
<td>60-80</td>
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<tr>
<td>Rest</td>
<td>7-21</td>
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<td>Induction</td>
<td>2-4</td>
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i. The temperature range for fruiting is strain dependent.

ii. A dry period after harvesting will prevent contamination.

iii. The artificial logs may be given a water bath to restore high moisture content of the substrate.

**Harvesting**

Take the stalks of the mushrooms and break them from the substrate. Don't tear them from the surface. Harvest the mushrooms at early stage. Don't water the scars left for 3-4 days. Normal yields are 15-30% of the wet weight of the substrate.
Special features of plastic bag method

a. The materials used to prepare synthetic logs are mainly saw dust and other agricultural by-products or residues such as bagasse, sugarbeet residue, cotton seed hulls, peanut hull and corncobs.

b. This method shortens the production period and gives a high yield. Using natural logs, the time from spawning to first harvest is about 8 to 12 months and harvesting is completed in about 3 years. About 100 kg of natural logs can produce about 10-15 kg fresh mushrooms. In synthetic logs, mushrooms can be harvested in about 80 days after spawning. Completion of harvesting is generally within 8 months. Biological efficiency of 80-145% is obtainable in this period.

c. Bag cultivation is relatively easy to manage.

d. Quality of mushrooms produced on synthetic log is poorer than on natural logs.

Systematic flow chart of cultivation of shiitake mushroom is given in Fig. 5.

Flow chart of cultivation of shiitake mushroom

- **Substrate**
  - Sawdust + Wheat bran
  - Wetting 65%
  - Pasteurization (22 psi for 2 hr)

- **Spawn**
  - Sawdust or wheat grain
  - Spawning @ 3% dry wt. basis

- **Incubation** (25-30°C, high CO₂, dark)

- **Pinning** (12-25°C, RH 85%, 1500 ppm CO₂, light > 800 lux)

- **Maturation** (12-25°C, RH 80%)

- **Harvesting**

- **Sun drying or in Oven 50-60°C**

Flow chart of cultivation of shiitake mushroom
**Spent Mushroom Substrate**

1. **Substrate for other mushroom** The shiitake SMS supplemented with 10% wheat bran and 10% millet can be utilized for *Pleurotus sajor-caju* cultivation but it required air-drying, grinding, supplementation, pasteurization and spawning. The higher yield (79% Biological efficiency) of *P. sajor-caju* could be obtained by supplementing the spent shiitake base medium with 12% soybean and 1.0% calcium carbonate. Increase in both biological efficiency and mushroom size showed positive correlation with increasing levels of calcium carbonate addition to the basal medium. It has also been used for *Agaricus* substrate preparation.

2. **Bioremediation**: The degradation of various chemicals in environment depends upon the prevailing physical and chemical conditions and the nature of microorganisms thriving within the system. SMS has the ability to chemically adsorb the organic and inorganic pollutants, and in addition it also contains diverse category of microbes having capability of biologically breaking down of the organic xenobiotic compounds present in soil and water. The SMS from shiitake mushroom has been found effective against degradation of pentachlorophenol (PCP) contaminated soil. It has been recorded in one study that when aliquots of spent sawdust cultures of shiitake mushroom, supplemented with nutrient solution of glucose, thiamine and mineral salt were mixed with PCP contaminated soil, it results in disappearance of about 44.4-60.5% of PCP within 21 days of incubation. The SMS from shiitake mushroom has been found to ability to treat acid mine drainage and effluents from olive mill.

3. **Manure for field crops**: The SMS from shiitake mushroom has been found effective as manure for production of tomatoes. It has also been pelletized for organic fertilizer or mixed with *Agaricus* spent substrate for organic fertilizer.

4. **Animal feed**: The ground waste logs from natural log shiitake cultivation, rice straw fermented with waste shiitake sawdust media, corn and molasses have been studied for its use as animal and fish feed.
v) **Biocontrol**: The SMS from shiitake mushroom has been found effective in suppression of *Rhizoctonia* damping-off disease of cabbage and disease incidence in tomato.

vi) **Alternative fuel**: The SMS from shiitake mushroom, *Lentinula edodes* has been studied for production of alternative fuel and vermiculture.
Economics of shiitake cultivation

Production facility (6 Cropping rooms)  = 6 (10’x40’x10’)
No of bags to be accommodated  = 10000 (2kg each)
Cycle to be completed in a year  = Two
Expected yield from two cycles (20000 bags)  = 130q/ annum

Farm lay out

![Diagram of farm layout]

Production Cycle

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<th>Jan</th>
<th>Feb</th>
<th>March</th>
<th>April</th>
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Cost and Benefit

- Cost of production  = 501800/-
• Returns @ Rs 80/ Kg ( 80 X 13000 ) = 10,40,000/-
• Net return per year = 5,38,200/-
• Net return per month = 44,850/-