

## SHIITAKE MUSHROOM CULTIVATION

*Lentinus edodes* is known as oak mushroom in English and Sokey Shamu in Dzongkha. In Bhutan, the tree species used for its cultivation are *Quercus griffithii*, (*Sisi* in Dzongkhag, *Benangshing* in Sharchopkha) which is followed by *Q.semicarpifolia* (*Bjishi*), *Q.lanata* (*Ghum* in Dzongkha and *Betsenang shing* in Sharchopkha). *Q.glauca*, (*Thom* in Dzongkha and *Thongpa shing* in sharchopkha) and lastly on *Castanopsis* species (*Sokeshing*).

Normally 7 to 25 years old trees are preferable for the cultivation but it depends on the thickness of the bark and the health condition of the tree. If the bark is too thick the spawn may not reach the woody part of the log and if it is too thin it can scale off. All ideal branches, regardless of age limitation are used if they satisfy the above conditions.

### SEASON

Mushroom can be grown through out the year but the best season for cutting logs for shiitake cultivation is during autumn and winter. The wood is dormant and the barks are firm compared to the season when the sap is active in the plant. During this period the logs have food stored in it which is used for production of new shoots once the plant becomes active. Emphasis is also made to inoculate the logs with mushroom spawn before it dries up too much.

#### CUTTING LOGS

In Bhutan, only the branches and tops and logs of the tree are used. The new shoots that sprout can be used for cultivation within five to six years. This is the sustainable way of harvesting oak logs for mushroom cultivation. The ideal length preferred is 90 cm to 120 cm with a diameter of 6 cm to 20 cm. If longer and larger logs are used, it becomes difficult to manage as they can become too heavy upon soaking in water. In case of smaller logs it can break off or be drilled through. There is no restriction otherwise.



Freshly cut logs

After cutting logs, measures are taken to protect moisture inside by keeping it under a roof or shade. Drying of logs at this time, reduce the growth rate of mycelium, and thereby delay the logs from maturing.

## EQUIPMENTS AND MATERIALS

A mushroom grower would require the following equipments:

- a. Electric drill b. Drill bits c. Spawn injector d. Paraffin wax and rosin  
e. Plastic sheet f. Tripod stand



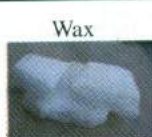
Electric drill



Hammer



Automatic injector



Wax



Drill bit



Drill bit



Hand injector

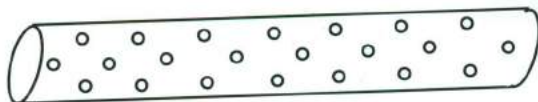


Rosin

Where electric facilities are not available, power generators would be required for drilling holes into the logs.

### DRILLING HOLES

To inoculate spawn it is required to drill holes into logs. On a normal length of log, 7 and 8 holes in each row would be required to be alternately drilled and number of holes will vary according to size and length of log. The holes are drilled with the help of electric drill provided with a drill bit, which has a diameter of 12 mm and a length of 20 mm.



Drilling the logs

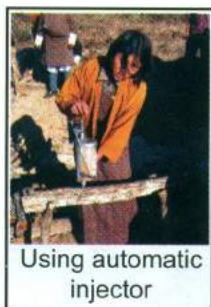
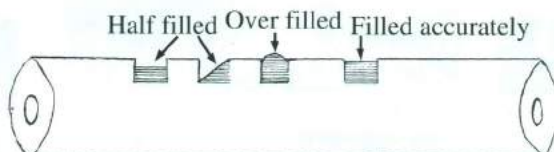


Drilling the logs with hammer



## **INOCULATION OF SPAWN**

After drilling holes, the logs should be spawned immediately. Otherwise it will result in exposure to disease and other competitive fungi besides drying up. Mushroom spawn is inoculated with the help of hand injector or automatic injector, which are designed to press adequate spawn to fill the holes. Care should be taken not to overfill the hole or leave it half filled as shown in the figures below:



Using automatic injector

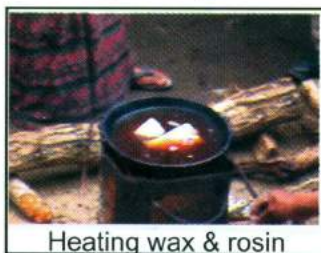


Using hand injector

Use of right type of injector for the specific holes has to be practiced for proper spawn inoculation. It will provide proper contact of spawn with the surface of wood in holes and enhance penetration of mycelium into the logs. On the contrary, loose contacts do not allow mycelium growth besides causing damage to the spawn while waxing.

## **WAXING**

After inoculating spawn in the holes, it is required to be sealed to prevent the spawn from drying up and to protect from disease and other fungi entering with spawn. Wax and rosin are placed in a container in a ratio of 4:1 and then heated to 100 degree to 120 degree centigrade. It is then applied over the exposed spawn thereby sealing the holes.



Heating wax & rosin



Waxing

After completion of waxing, the logs are termed as billet (s). It is then stacked and irrigated at an interval varying upon different climatic conditions.

### **FORCING/ SPAWN RUN**

The billets have to be kept in incubation for the spawn to spread in the logs. During this stage the mushroom mycelium consumes and colonizes the logs. This is called forcing or spawn run. In order to provide ideal temperature and moisture during the incubation period the billets are stacked in upright position with pineleaves on the top and below the billets and kept covered with plastic sheet as per the figures below:



Adding pine needles



Billets in incubation

It should provide ideal temperature and moisture essential for mycelium inside the billets. The optimum temperature required is 18 degree centigrade to 25 degree centigrade with 65-70% moisture. The main purpose of this process is to provide a favourable condition for mycelium to spread while disease and competitive fungi are inactive. In order to provide such conditions, the following points need to be considered.

- ☞ Clean and disinfect the site chosen for forcing.
- ☞ Make stone soling to prevent the billets from becoming muddy.
- ☞ Stack the billets in upright position.
- ☞ Add pine needles on the top and bottom of the logs.
- ☞ Water just enough to keep the pine needles moist.
- ☞ Wrap the billets with plastic sheet and cover it with straw mat for insulation during the cold season.
- ☞ While covering the billets with plastic sheet, provide air passages to provide some aeration.
- ☞ Water billets periodically as per requirement.



## DIPPING BILLETS

Under good conditions (temperature and humidity) mentioned so far, the billets should take about 6 months to mature. The maturity could be observed by the softness of billet condition and by looking at the mycelium colour. When the logs are ready it becomes soft and light and the mycelium at the end of the billets turns brown.

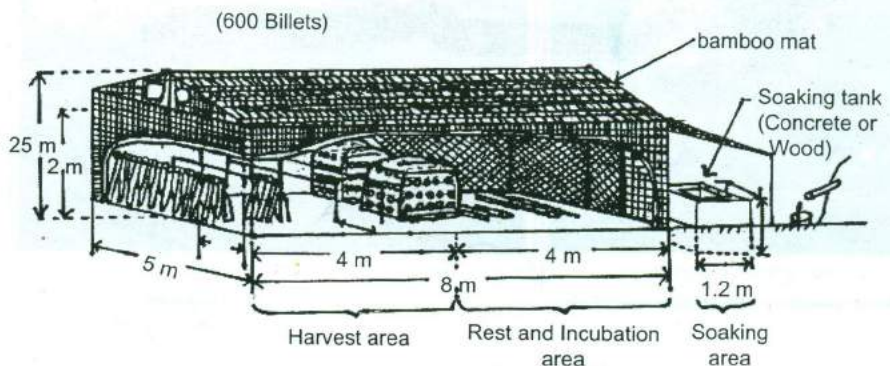
The billet is then ready for dipping. They are normally dipped in water tank for a period of 12 to 24 hours depending upon dryness of billets.

After completion of dipping hours, water is drained out from the tank and the billets removed from the tank and wrapped in plastic sheet. Within 3 to 6 days depending on temperature, mushroom (pin heads) will start to sprout from billets, which are then stacked for cropping.

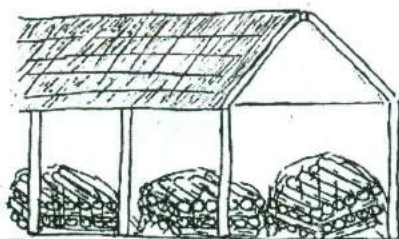
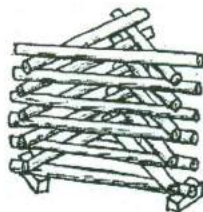
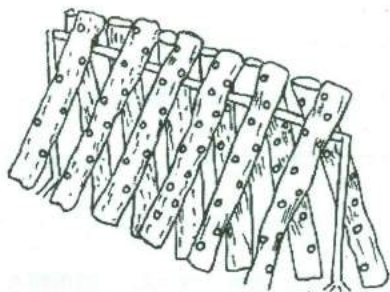
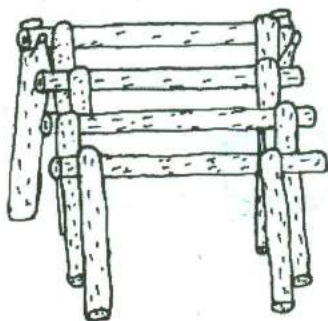
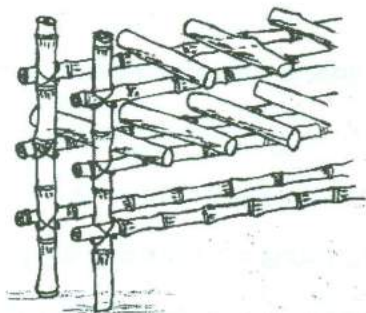
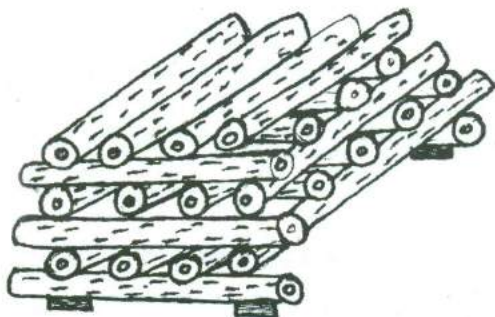
## HARVESTING SHIITAKE

After stacking the billet for cropping, mushroom will mature within 4 to 8 days. At the time of harvesting water should not be sprayed on the mushrooms as it will drastically reduce its shelf life. After the harvest the billets are restacked in a criss-cross position and given a rest period of one to two months. It is again soaked in water tank and sprouting induced. Likewise, the cropping pattern is repeated till the billets get exhausted.

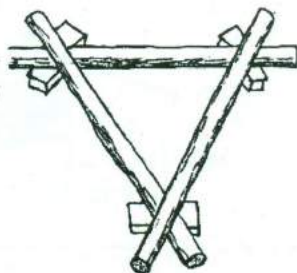
### a) Mushroom Cultivation Shed



## DIFFERENT STACKING OF BILLETS



BILLETS during the resting periods



The cultivation method described, is by far, a method of growing shiitake with the use of logs. This technique could be also applied to growing of *Auricularia* and *Pleurotus* species but with the use of different tree species eg;- soft-woods.

### **Mushroom cultivation in Straw**

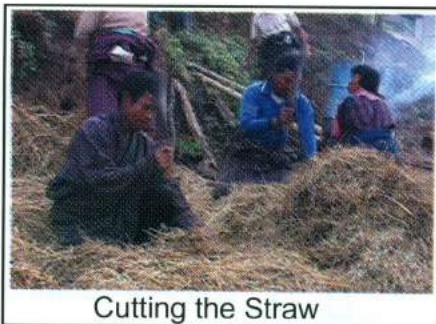
Paddy and wheat straw, grass and other agriculture residues can be used for mushroom cultivation. Mushrooms like oyster (*Pleurotus spp*), paddy straw mushroom (*Volvariella volvacea*) and wood ear mushroom can be cultivated in straw. In order to provide proper nutrients, additives has to be added. In Bhutan we normally add rice bran to provide additional nutrients.

### **PROCESS.**

1. The paddy straw or grass is cut into 5-6cm long.
2. It is then soaked in water for a certain duration depending upon the type of straw. In case of straw like paddy straw, few hours of soaking is enough, but in case of wheat straw and grasses need to be soaked for longer duration.
3. The straw is then heaped to drain out excess water in the straw. Water content in the straw should be about 65%.
4. Rice bran is added in the ratio 1: 10(1kg rice in 10kg straw).
5. After mixing thoroughly, it is filled in gunny bags and then sterilized in a drum. A small amount of water is added in the drum, inside which a wooden frame is placed at the bottom of the drum. Straw filled gunny bags are kept on top of this to avoid it from getting soaked with water. Sterilization is done to remove competitive and disease causing microorganisms. Sterilization can also be done in boiling water, but in this case nutrients and additives could get dissolved or washed away by the water.
6. Sterilization of straw is considered to begin once water starts boiling. At this time the open end of the drum is sealed with a plastic sheet. Note that the time for sterilization should be kept between 30 mints to 1 hour after reaching boiling point.



7. On the completion of the sterilization, straw is taken out and allowed to cool to temperature to about 25 degree C before the cultivation commence. Mushroom seed is added to the cooled material. This process is called SPAWNING.



Cutting the Straw



Sterilization of straw

**There are two methods of spawning.**

1. Thorough spawning,
2. Layer spawning.

In thorough spawning, spawn and straw are mixed thoroughly.

In layer spawning, a layer of straw is added to the container, pressed down and over which a small quantity of spawn is added. Number of layers depends upon the size of the plastic bags. The quantity of spawn added is about 2% of the material used.

The National Mushroom Center has promoted two types of cultivation;

1. Plastic bag method
2. Block size method.

### **PLASTIC BAG METHOD.**

In this method, the sterilized straw is layered with the spawn broadcasted evenly on the top of the each layer until the bag is filled. The mouth of the plastic bag is tied after placing a ball of cotton in it to allow aeration. The plastic bag is kept in a room away from



Spawn inoculated straw bags



direct sunlight for incubation. The ideal temperature required is between 20 and 25 degree Centigrade. In a week or two, white cotton like fluffy mass will grow through the substrate. This indicates good mycelium run. After three weeks or so the whole substrate should turn white indicating the completion of the spawn run or incubation. The temperature has to be brought down by watering to initiate pinhead formation. The plastic is removed to enable the mushroom (fruit body) to grow. Watering is done depending upon the humid content of the straw and the environment. Care should be taken to prevent the water from collecting at the base, as this will eventually initiate infection. Once the mushroom become fully developed it should be harvested without damaging the substratum.



After the first harvest, the substratum should be kept in a dry and cool place for resting for about a week after which it can be made to sprout again by spraying water. This growing pattern is called flush. There can be three or four flushes after which substratum can be thrown away or used for production of manure. The size of the substratum will go on reducing after every flush as it gets consumed by the mushroom mycelium.

### **BLOCK SIZE METHOD.**

In this method, a plastic sheet is placed on the floor to make straw bed. Boxes with open ends are placed over the plastic sheets. The straws are layered 3-4 cm high with spawn broadcasted evenly on the top of each layer and this process is followed till the top of the box is full. The straw has to be pressed down properly either with hand or by stamping in every layer. Once the last layer has been



Spawning in block



Spawning completed



Sprouted mushroom

spawned, the bed is ready. The box is then removed without disturbing the layer and can be used to prepare other blocks. The bed or block thus prepared should be covered with plastic sheet and kept in incubation or spawn run. In apple box size blocks four layering with spawn is recommended. The rest of the procedure is same as in the case of plastic bags.

### **DISEASE, WEEDS AND PESTS IN MUSHROOM CULTIVATION**

About 150 spp. Of problem, fungi can affect the log and mycelium. These fungi can be divided into three categories;

1. Disease fungi
2. Competitor fungi.
3. Weed fungi.

Other problems are insect pests, animal pests, bacteria and viruses.

### **APPROACH TO DISEASE FUNGI MANAGEMENT.**

A holistic approach looks at the overall system and tries to solve the problem at the source and not just focus on the symptoms. Air normally contains spores of wood-inhabiting fungi, among which *Trichoderma* is most prevalent.



**Trichoderma**

Cause:- Hot & Wet Condition



**Hypocrea nigricans**

Cause:- Hot & Wet Condition



## PREVENTION OF DISEASE.

- a) Provide conditions that encourage strong growth of shiitake at each stage of its life.
- b) Recognize and eliminate conditions, which lead to the establishment of disease and pests.
- c) Trichoderma outbreak is often attributed to overly wet conditions but can also be caused by overly dry conditions especially during the wet conditions.
- a) Chemical agents are of limited value; they are expensive, difficult to apply, over use can lead to resistance, often used as band- aid to cure symptoms, and not solve problem.

### 1. DISEASE FUNGI.

They are capable of attacking and killing the mycelium. They secrete anti-fungal compounds, which inhibit the growth of shiitake mycelium and can parasitize and kill shiitake hyphae. All the serious diseases are Ascomycetes. If the fungi infect the log before the spawn has established they can kill the spawn before it can grow into the log. This results in the total loss of the log.

Example; TRICHODERMA.

- They are member of Ascomycetes, genus Hypocrea and their asexual stages. Trichoderma and Gliocladium are most serious and widely distributed disease fungi.
- They occur naturally under forest soil; some species prefer logs in warm, dry locations, other do well under moist in both warm and cool conditions.

### 1. SYMPTOMS.

- Trichoderma and Gliocladium are referred to as "Green Molds" and start as white patches of or pads of fluffy mycelium.
- They initially appear in cracks in the bark, on the spawn, on wounds, on the ends of the logs and cut surfaces.
- As they age and colored conidia are produced, the colonies appear green to rich forest green.



*Steccherinum ochraceum*  
Cause: Hot and wet conditions

\*Hypocrea may appear as fleshy cushions of fungal tissues called stroma, which range from cream yellow to reddish brown.

\* Wood colonized by Trichoderma appears darkened and discolored and in advanced cases, the entire barks fall off.

\* Trichoderma can cause the spawn to turn black, and the shiitake mycelium may disappear giving the spawn hole a dead look.

### CONDITIONS FAVOURING TRICHODERMA COLONISATION.

1. Invasion by the disease prior to colonization by shiitake.
2. RH above 90% when shiitake is weakened by environmental stress.
3. Prolonged high or low LMC( Log Moisture Content )
4. Direct sunlight on the bark.
5. Low sugar content.

### PREVENTION;-

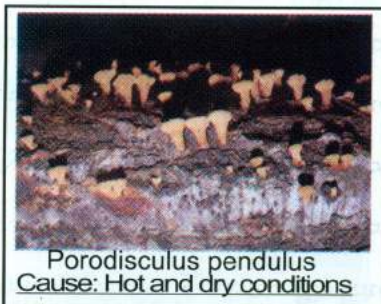
1. Maintain bark dry while maintaining high LMC.
2. Provide good air circulation among logs.
3. High nitrogen content favors Trichoderma.

### B. HYPOXYLON.

Widespread group of Ascomycetes, which is serious problem in shiitake. They invade logs during early spring month.

#### SYMPTOMS;

- Fruiting bodies start as tiny dark spots usually in cracks.
- These spots gradually develop into hard brick red to black mold.
- In the later stage, barks fall off.





## PREVENTION;-

\* Hypoxylon levels can be decreased by shading logs from direct sunlight. Severely infected logs should be removed to lower spore concentration.

## 2. COMPETITOR FUNGI.

They do not actually attack mycelium, but they do diminish the crop by occupying space and withdrawing nutrients from the logs. Most of them are member of Aphyllophorales. Example; Coriolus (polyporus) versicolor.

## SYMPTOMS.

These fungi produce leathery fruiting bodies, often colonize the end of shiitake log. Fruiting bodies do not appear until the end of spawn run.

## 3. WEED FUNGI.

Weed fungi include both Basidiomycetes and Ascomycetes. Although these fungi may slightly decay the wood, the damage is minimal. These fungi are often specific to trees and the area. Example; Bulgaria inquinans; common canker forming fungus on living oak.

## POST HARVEST FUNGI.

- Attack mushroom either on logs or during storage.
- Usually over mature and excessively wet mushroom are affected.
- Example; Gliocladium deliquescens; forms green colonies with slimy conidia. Affected mushroom fail to open fully, turn brown and becomes soft and shriveled.



**Schizophyllum  
commune**

Cause: Hot and dry conditions



**Bulgaria inquinans**

Cause: Wet conditions



**Schizopora flavipora**

Cause: Hot & Wet conditions



**Dentipellis macrodon**

Cause: Hot, & wet conditions

Other problems include bacteria, viruses and insect pests. Bacteria are seldom problem in shiitake cultivation..

However, browning disease of mushroom caused by *Pseudomonas fluorescens* may be severe under warm conditions. High RH, warm temperature and poor ventilation favor bacteria. Viruses are known to cause disease such as "Die- Back", and are found in common button mushroom.

### **INSECTS AND OTHER PESTS;**

**Termites:** They eat and consume shiitake logs. They can be controlled by chemical treatment or by keeping logs off the ground.

**Bark Beetle:** Their larvae feed on the cambium layer and leaves small holes in the bark and tunnels. Larvae are apparent under the bark. Heavy infestation can make the bark fall off.

**Ambrosia beetles:** They leave small holes in the bark often with small pile of sawdust beneath it. Attracted to freshly cut logs and may appear in the early part of spawn run.

**Fungus beetles:** Both larvae and adults feed on the mushroom directly.

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