

**Agrodok 40**

**Small-scale mushroom  
cultivation**

oyster, shiitake and wood ear mushrooms

Peter Oei  
with contributions by Bram van Nieuwenhuijzen

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# Foreword

Mushroom cultivation fits in very well with sustainable farming and has several advantages:

- It uses agricultural waste products
- A high production per surface area can be obtained
- After picking, the spent substrate is still a good soil conditioner

This Agrodok contains detailed information on how to grow three kinds of mushrooms: oyster, shiitake and wood ear mushrooms. These mushrooms are rather easy to grow on a small scale. Cultivation of the common white button mushroom and of the rice straw mushroom is very different and will therefore be dealt with in another Agrodok.

Much of the information presented here is from my book “Mushroom cultivation and appropriate technologies for commercial mushroom growers”. By concentrating on three mushroom species only and on relatively simple technologies, I hope readers can obtain a sustainable profit from mushroom growing.

Bram van Nieuwenhuijzen was the director of the Mushroom Growers’ Training Centre (nowadays known as C Point) at Horst, The Netherlands, for a number of years. He is currently involved in mushroom cultivation projects in several countries as an adviser through PUM Netherlands Senior Experts in The Hague.

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# 1 Introduction

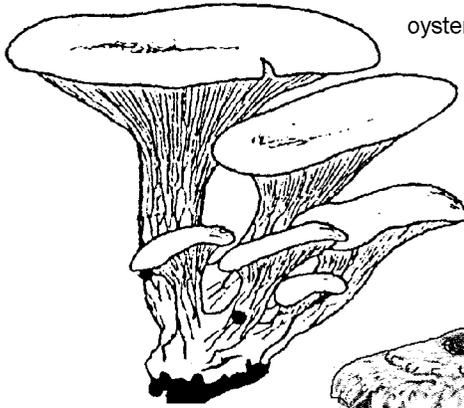
Do you want to grow mushrooms? There are plenty of reasons to do so. Mushrooms are a good cash crop; they are rather easy to grow and are brimming with protein, B vitamins and minerals. They even have medicinal properties. Time between spawning and harvesting can be as short as three weeks. Furthermore, after the cultivation, you can still use the substrate as a good soil conditioner.

This Agrodok gives you detailed information on the cultivation of oyster, shiitake and wood ear mushrooms. Although many other types of mushrooms can be grown, we have chosen the ones that can easily be cultivated in developing countries using appropriate technology.

When choosing your method to grow mushrooms, you have to find an answer to the following questions:

- 1 Which of the mushrooms do you want to grow? Check the market and the temperature ranges for fruiting (see paragraph 2.4).
- 2 Can you obtain mushroom spawn (the “seed”) of the species you want to grow? Chapter 4 shows you how to produce your own spawn. If you cannot obtain or produce spawn it will not be possible to grow mushrooms.
- 3 What kind of substrate would you need to be able to grow the desired mushrooms? See Chapter 5.
- 4 How should you treat the substrate? This affects the investments you have to make. Details can be found in the chapters on the specific mushroom species.

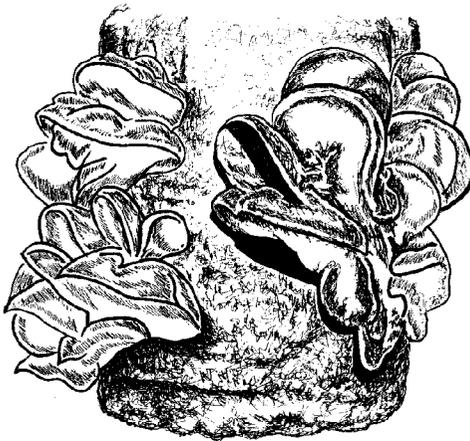
To understand mushroom growing and the properties of mushrooms, some biological knowledge of the crop is necessary. So, we will start with the biology of mushrooms.



oyster mushroom



shiitake



wood ear mushroom

*Figure 1: The three mushroom species dealt with in this Agrodok*

# 2 Biology of mushrooms

## 2.1 Fungi

Mushrooms belong to the kingdom of Fungi, a group very distinct from plants, animals and bacteria. Fungi lack the most important feature of plants: the ability to use energy from the sun directly through chlorophyll. Thus, fungi depend on other organisms for food, absorbing nutrients from the organic material in which they live. The living body of the fungus is mycelium made out of a tiny web of threads (or filaments) called hyphae. Under specific conditions, sexually compatible hyphae will fuse and start to form spores. The larger spore-producing structures (bigger than about 1 mm) are called mushrooms. In nature this is the most striking part of the organism, but in fact it is just the fruiting body and the major part of the living organism is found under the ground or inside the wood.

### **Scientific and colloquial names of mushrooms**

The scientific names of mushrooms are often used in this Agrodok, as they give rise to less confusion than colloquial names. For example, the name oyster mushroom applies to more than 20 different species of mushroom, each with its own cultivation characteristics such as optimal temperature range, colour and growth rate.

For mushroom growers, the most practical approach to the subject of taxonomy is to rely on taxonomists. It is best to order strains from renowned spawn producers or culture collections.

## 2.2 Fungus ecology

Fungi depend on other organisms for their food. Three modes of living can be recognised:

- Saprophytes: degrading already dead material
- Symbionts: living together with other organisms (especially trees) in a close, mutually beneficial relationship
- Parasites: living at the expense of other organisms

The mode of living has nothing to do with edibility: both edible and poisonous mushrooms can be found in all three groups. This Agrodok only deals with saprophytes.

### **Saprophytes**

Saprophytic fungi need organic matter to decompose. In nature they will grow on fallen leaves, animal droppings, or stumps of dead wood. Some are specialised in breaking down the hairs of mammals, while others may decompose birds' feathers. Saprophytes decompose the complex organic structures left behind by plants and animals. And in the natural run of things, plants or animals regain access to minerals and other nutrients present in the substrate. Oyster mushrooms degrade dead wood in nature. They can be cultivated on a wide range of ligno-cellulose waste materials.

## **2.3 Life cycle of fungi**

Fungi multiply by producing millions and millions of spores. When a spore settles in a suitable environment, it can germinate and branch to form a mycelium. When two sexually compatible mycelia meet, they may fuse to form a so-called secondary mycelium, which is capable of forming fruiting bodies.

### **Mycelial growth and spawn**

In the practice of edible mushroom cultivation no use is made of spores. Their small size makes them difficult to handle and their genetic characteristics may differ from those of their parent. Moreover, it takes some time for mushroom spores to germinate, whereas other fungi such as green moulds germinate and spread much faster.

The desired mushroom must be able to colonise the substrate before other fungi or bacteria do so. To achieve this, pre-grown mycelium (free of any contaminants) of the mushroom is inoculated on a sterile substrate. This material is referred to as spawn. Using spawn will give the cultivated mushroom an advantage in growth over other fungi.

## Spawn run

The mycelium will colonise the substrate and use the available nutrients. This is commonly referred to as the spawn run. When some nutrients run out, or when the weather changes, the mycelium will reach a different phase: the reproductive stage. A temperature of about 25 °C is optimal for the spawn run of most species. The environment can also enhance the growth of the desired mycelium: a high CO<sub>2</sub> concentration is favourable for mycelial growth (but not for cropping).

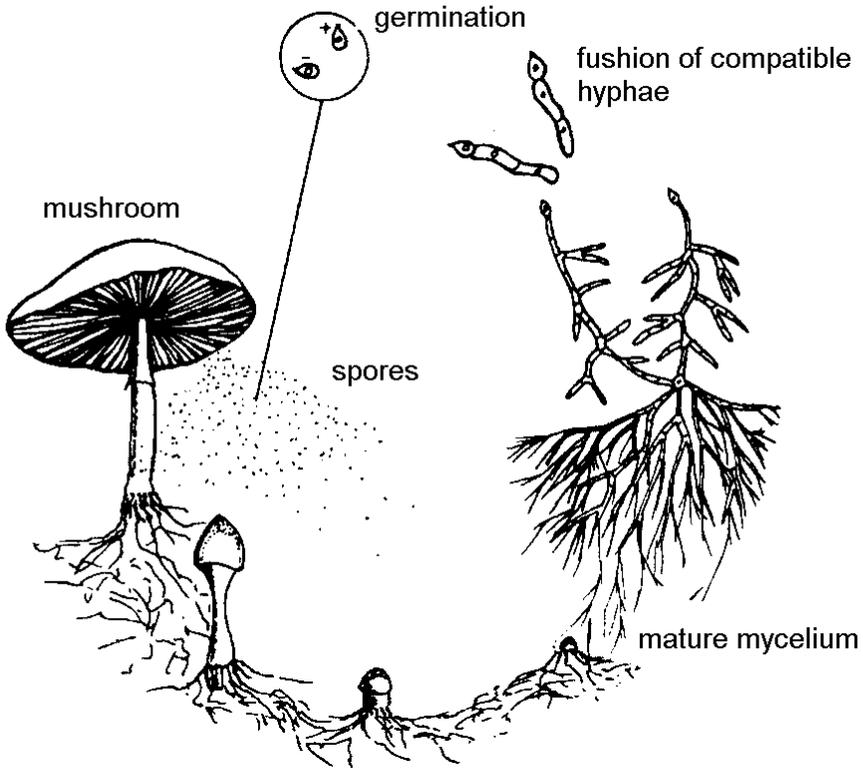
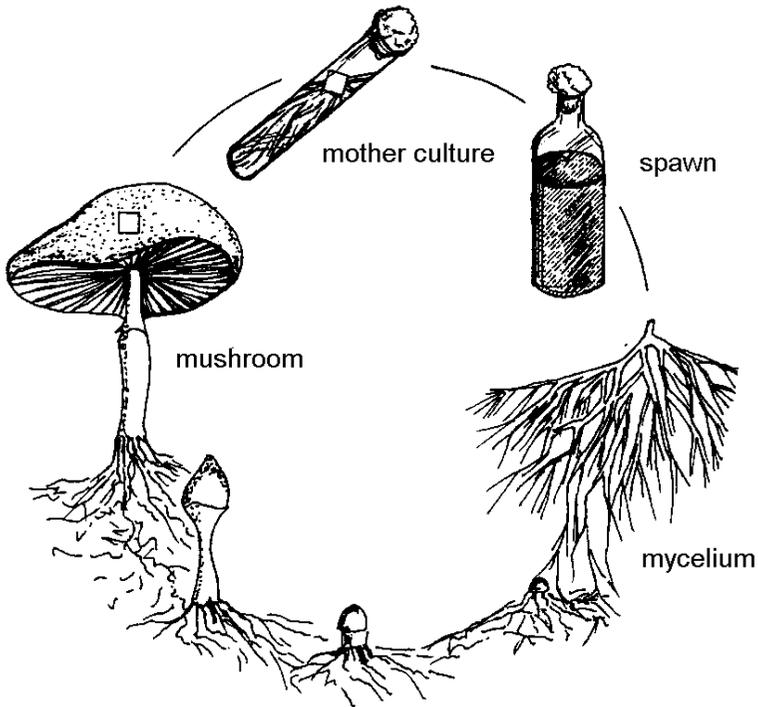


Figure 2: Life cycle of mushrooms in nature



*Figure 3: Life cycle from mushrooms to spawn. Tissue cultures are isolated from a mushroom and propagated on a suitable substrate. This full-grown substrate is then used in mushroom growing.*

After having colonised the substrate, the mycelium is capable of producing fruiting bodies. The number and quality of the fruiting bodies will depend on the environment.

Key factors to induce fruiting bodies are:

- changing temperature
- high humidity
- deficiency of a nutrient
- CO<sub>2</sub> concentration in the air
- light
- physical shock

These factors differ from mushroom to mushroom. Most of the changes that stimulate fruiting have a negative effect on the vegetative growth of the mycelium. Changes should therefore only be made when the mycelium has completely grown through the substrate. It is actually the less favourable condition for vegetative growth that will stimulate the mycelium to fruit.

Two examples to induce fruiting in different mushrooms:

- Some oyster mushrooms (for example *Pleurotus ostreatus* strains) will fruit reliably when, after mycelial growth, they experience a cold shock (a difference of 5 °C to 10 °C). The CO<sub>2</sub> concentration has to be lowered as well. Mycelial growth can take place in the dark, but light is essential for fruiting.
- Fully-grown shiitake (*Lentinula edodes*) mycelium in substrate bags are soaked in water for one or two days and receive a physical shock to stimulate fruiting. The shock will remove captured CO<sub>2</sub>.

Small primordia (initial fruiting bodies) will be formed at the beginning of the reproductive phase. Under the right conditions, these primordia will develop into fruiting bodies. Nutrients are transported from the mycelium to the fruiting bodies by a steady moisture flow. Water has to evaporate on the surface of the mushrooms in order to allow the flow to continue. This explains why spraying water on maturing mushrooms or a too high relative humidity of the air can spoil the crop.

## 2.4 Temperature ranges of cultivated mushrooms

Choose a species that fruits at temperatures near your outdoor temperatures. This limits investments in climate control and reduces energy costs. As the table shows there are actually few species suited to really tropical conditions. The only mushrooms currently being cultivated at temperatures around or just below 30 °C are: oyster mushrooms (*Pleurotus cystidiosus* / *abalones* / *ostreatus* var. *florida*) and

*Volvarella volvacea*, *Agaricus bitorquis*, *Stropharia rugoso-annulata* and wood ear mushrooms (*Auricularia polytricha*).

**Table 1: Temperature ranges and techniques for mycelial growth, optimal growth and fruiting for specific mushroom species.**

Mushroom species/ Common name	T <sub>mg</sub>	T <sub>optimal mg</sub>	T <sub>fruiting</sub>	Techniques
<i>Lentinula edodes</i> /Shiitake	5-35	20-30	8 -25*	1, 2, 3,
<i>Pleurotus abalonus</i> /Abalone oyster mushroom	15-35	20-30	25-30	2, 3
<i>Pleurotus cystidiosus</i> /Oyster mushroom	10-35	25-28	25-30	2, 3
<i>Pleurotus ostreatus</i> /Winter oyster mushroom	5-35	20-25	5-25	2, 3
<i>Pleurotus pulmonarius</i> /Oyster mushroom	5-35	20-25	13-20	2, 3
<i>Pleurotus cornucopiae</i> # / Branched or yellow Oyster mushroom	15-35	20-28	15-25	2, 3
<i>Pleurotus djamor</i> ^ /Pink oyster mushroom	15-35	24-30	20-30	2, 3
<i>Pleurotus eryngii</i> /King oyster mushroom	10-35	20-25	15-22	2, 3
<i>Auricularia polytricha</i> /Wood ear mushroom	20-35	35-30	23-28	2

#: Including *Pleurotus citrinopileatus*

^: Including probable synonyms: *P. ostreatus*, *P. salmoneo-stramineus*, *P. flabellatus*

T<sub>mg</sub>: The range at which the mycelium stays viable; the growth speed declines at both high and low ends of this range.

T<sub>optimal mg</sub>: The optimal temperature range required for fruiting; the most important temperature.

Substrate preparation techniques:

- 1 Wood logs (not treated in this Agrodok)
- 2 Pasteurised or pre-heated substrate
- 3 Sterilised substrate

# 3 Mushroom farms

Certain factors should be kept in mind when selecting a site for a mushroom farm:

- distance to the market
- availability of good quality substrate material
- transportation of both product and substrate material
- ready availability of clean water

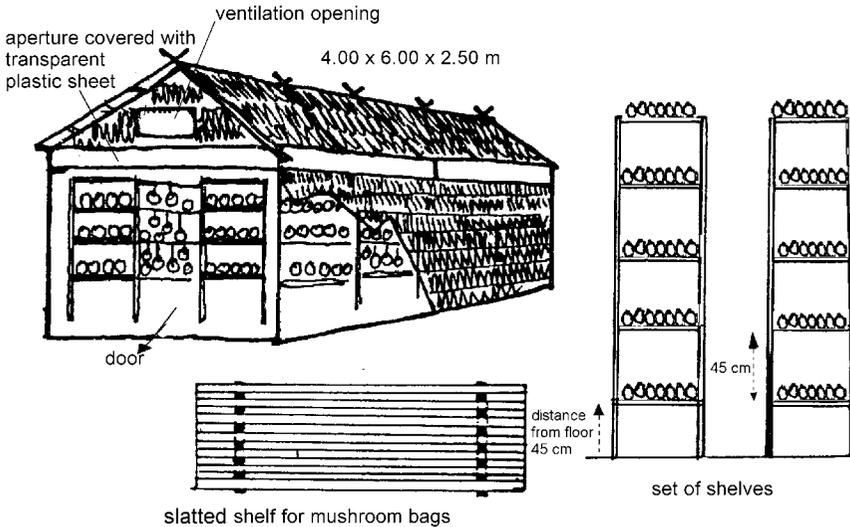


Figure 4: Cropping house

## 3.1 Farm layout

Before one can start to plan the layout, the processes to be performed at the mushroom farm will have to be listed. For example, whether or not an inoculation room is required depends on whether growers prepare their own substrate or buy inoculated substrate.

The farm layout should also include:

- An efficient flow of substrate materials
- Measures to prevent contamination on the farm
- Efficient use of space

The mushroom farm should provide suitable climatic conditions. It is possible to adapt existing structures such as defence tunnels, bunkers, caves, chicken houses, old milk factories and slaughterhouses. Some successful mushroom cultivation operations take place in old defence or railway tunnels.

### **Floors**

On a low investment level, mushroom houses are just built on arable land. On a higher investment level, cemented floors are used. Slightly inclined cemented floors provide a smooth surface that can easily be cleaned and allow excess water to drain.

A screened basket could be used to collect the coarse debris from the drained water. The drainage system of the different rooms should not be connected to prevent a disease in one growing room from easily spreading to other rooms. The floors should also be smooth to facilitate handling and transport of materials.

### **Doors, windows and other openings**

Doors and walls should close properly to prevent insects from entering the growing rooms. A double door, with a wire mesh for the second entrance, can help to keep insects out. The same rules apply for windows. The openings through which air is either blown in or out of the rooms should have at least a simple filter or cloth as barrier.

The smell of mushroom mycelium is very attractive to mushroom flies.
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*Figure 5: Double door at the entrance of the incubation unit*

## 3.2 Farm hygiene

Hygiene is vital on a mushroom farm. Since chemical control of pests and diseases is not possible in small-scale mushroom cultivation, the only preventive measure is hygiene, and to some extent disinfection. This goes for a spawn production unit, the site for substrate production, the incubation rooms and production units.

Therefore checking a suitable site for a mushroom farm is very important. The surroundings of a farm should be clean and free from possible contamination from insects, moulds etc. This means that building a new farm close to other mushroom farms should be avoided. Insects and diseases from these farms could easily spread to the new farm.

If possible separate the various units of the new farm.

The spawn laboratory should be separate from the growing site. The growing rooms ought to be separated by (plastic) walls to keep the different stages of cultivation apart. As a matter of fact no incubation or spawn running should take place in the same room where the mushrooms are harvested.

Debris, contaminated bags and spent substrate must be removed immediately from the rooms and from the farm itself, preferably to a place far away.

All these measures are necessary to avoid pests such as flies and other insects as well as diseases spreading from these waste dumps. If the spent substrate is to be used for gardening soil, it should be used as soon as possible.

## 4 Spawn production

The mushroom “seed” (*propagation material*) is generally referred to as spawn.

### **Availability of spawn culture**

The availability of good quality spawn is the limiting factor for mushroom cultivation in many developing countries. Customs’ bureaucracy, high shipping costs and the difficulty to keep the spawn cooled during transport, often hinders imports. It might therefore be necessary for the mushroom grower to produce his own spawn.

If good quality spawn of the desired mushroom species can be obtained at a reasonable price, it would be wiser to concentrate on the mushroom growing process. If not, spawn will have to be produced or multiplied by the mushroom grower.

The complete procedure of spawn production involves preparation of the medium, filling the test tubes or Petri dishes and sterilising them, and the process of inoculating larger containers with this culture.

Spawn production requires a clean laboratory and specialised knowledge.

*Basically, spawn production is nothing more than putting mycelium of the desired mushroom in suitable sterilised substrates under aseptic conditions.*

In practice, however, producing spawn is not that simple. Suitable strains from the required mushroom species have to be maintained under strict conditions to avoid degeneration. If this is not possible tissue culture from a fresh and healthy mushroom should be used for spawn production. In addition, the spawn production room has to be kept meticulously clean to avoid any contamination.

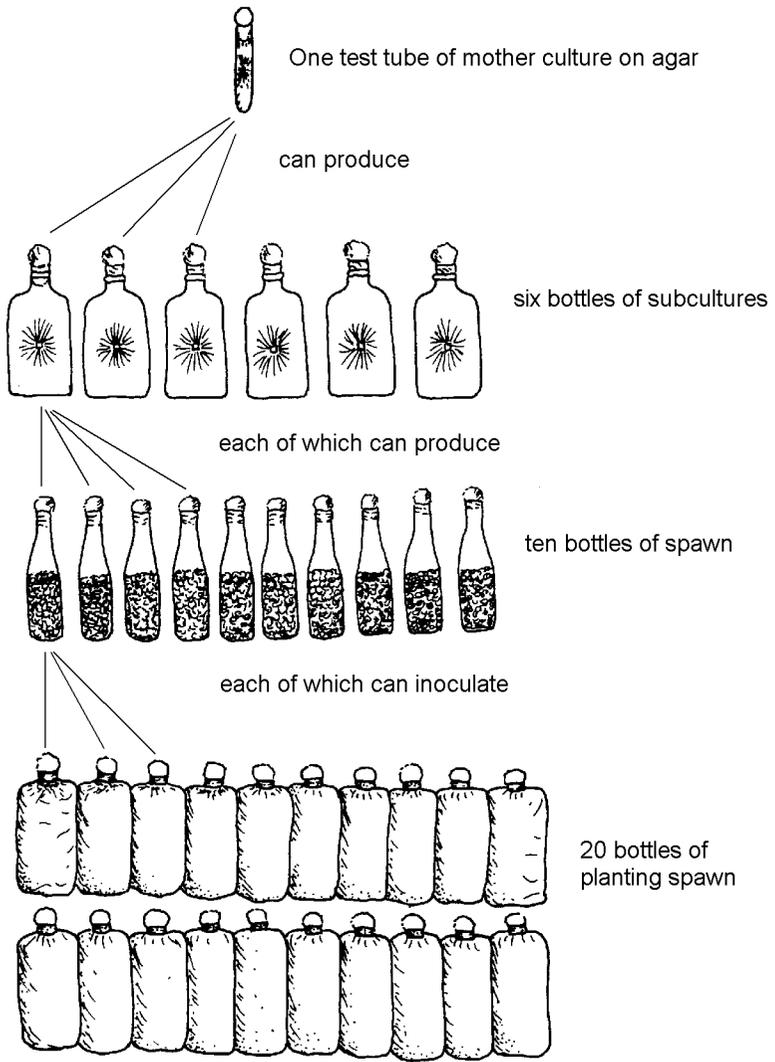


Figure 6: Multiplication of spawn

## 4.1 The starter culture

The starter culture (or mother culture) can be made from a fresh and healthy fruiting body or obtained from a spawn producer or laboratory. More agar cultures are then made from this starter culture. These serve to inoculate larger containers (like bottles) with mother spawn, which can be used to inoculate the final spawn substrate.

The minimal requirements for a spawn production unit are:

- a sterilisation unit (pressure cooker, autoclave)
- sterile environment: inoculation box or laminar airflow cabin
- laboratory equipment like Petri dishes, test tubes, scales, alcohol, flame
- incubation room

The above equipment is commonly available in hospitals, research stations and universities.

The raw materials include:

- ingredients for media preparation
- substrate material (grain, wooden sticks (skewers), sawdust, or even oil palm fruit fibre)
- pure culture or fresh mushroom of the desired mushroom species strain
- spawn containers (such as bottles or plastic bags)

In countries lacking mushroom production, spawn may be obtained from a spawn producer, a university or a research station at the start of a project.

For addresses of spawn producers see Useful Addresses.

## 4.2 The sterilisation process

Grain, sawdust and compost contain large numbers of contaminants. A single grain kernel may contain thousands of bacteria, fungi and actinomycetes.

Every one of these undesired agents, which are called contaminants, is capable of spoiling substrates that have not been properly sterilised or inoculated under unhygienic conditions.

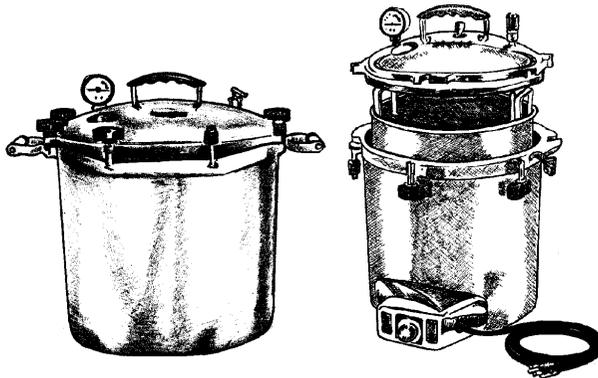
A heat treatment of 15 minutes at 121 °C is usually sufficient to kill all organisms. It takes quite some time for the steam to heat the inner core of substrates to this temperature, depending on the way the sterilisation/pasteurisation unit is filled and on the capacity of the burner.

Steaming in an oil drum for at least 6 hours is usually necessary to ensure proper heating of the inner core of the substrate bags. Sterilise 4-liter bags filled with 2kg spawnsubstrate for at least 2 hours at 121°C.

### **Pressure cookers**

The cheapest option is to obtain one or more large pressure cookers. Select pressure cookers that maintain the pressure when the final temperature has been reached.

The simplest pressure cookers blow out steam when the pressure is too high. The pressure inside will then often drop below 1 atmosphere overpressure, causing the media to boil.



*Figure 7: Pressure cooker for use on a burner and an electric pressure cooker*

This should be avoided. Petri dishes or bottles with agar media may become messy if this type of pressure cooker is used. The pressure cookers should have an inside rack, which will effectively ensure a more even temperature distribution inside the pressure cooker. The heat source is either external (gas burners, coal, wood) or built-in (electric). The advantage of pressure cookers with thermostatically controlled electric heating elements is that they allow for precise temperature regulation.

### **4.3 Clean environments**

A clean environment is absolutely essential to spawn production. In particular, whenever the containers with sterilised media need to be opened it must be done under aseptic conditions. The air carries numerous contaminants, which easily infect the sterilised media. It is therefore necessary to use special cabinets and inoculation rooms for performing the handling and the preparation of the (tissue) cultures.

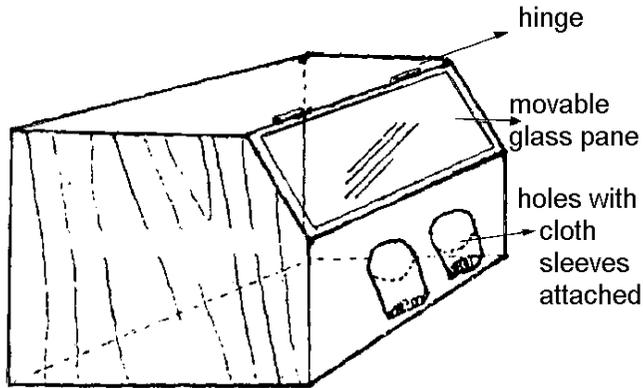
#### **Inoculation rooms**

The interior of the inoculation room should consist of non-biodegradable materials. All the surfaces should be smooth and easy to clean. Shelves should be designed in such a way that the floor beneath can be cleaned easily. Shelves are typically made of galvanised iron or Formica.

#### **Inoculation cabinets**

These simple inoculation cabinets are widely used all over the world. They can be constructed cheaply from locally available materials. The front glass pane can be opened to fill the cabinet with the sterilised media. The inside is disinfected by cleaning with a 10 % *Clorox solution*, a 2% *Formalin solution* or 70% *ethyl alcohol*.

Take care when using these chemicals. Some of them are poisonous and/or irritating to nose and eyes. Cautiously follow the instructions to ensure safe use.



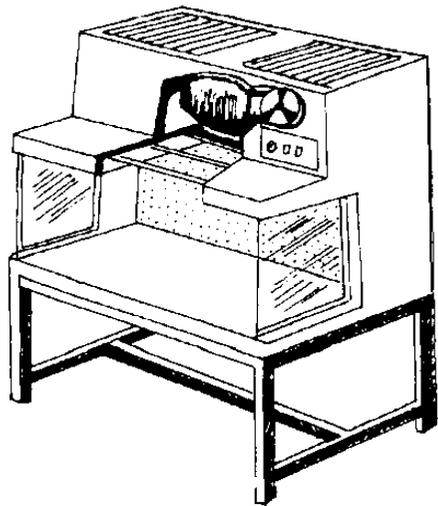
*Figure 8: Simple homemade inoculation cabinet showing front glass pane on hinges and holes (with cloth sleeves attached) for hands.*

### **Laminar airflow cabinets**

A laminar airflow system (LAF) consists of a fan, a duct, a HEPA (*High Efficiency Particle Air*) filter and a hood.

In laminar airflow contaminants can spread in only one direction. In turbulent airflow it is possible that spores move in different directions, causing more contamination.

The ventilators are rated by the producers according to the volume of air they can blow through materials of specified resistance. About 0.45 m/s air velocity is considered best for good laminar airflow. The fan



*Figure 9: A ready made laminar airflow cabinet*

should be regulated stepwise and have the capacity to push double the amount of required air through the filter to reach the required air velocity, to account for pressure losses when the filter gets loaded with particles.

In many countries both HEPA filters and these specific ventilators are not available and have to be imported.

The filters and ventilators are the heart of any laminar airflow system, but other factors have to be considered too: the operating persons, their skills and their hygiene; the construction of the ducts and filters to ensure that no contaminated air can be sucked in.

## 4.4 Cultures

The first steps in spawn production are performed on artificial media. These should contain sufficient nutrients for the mushrooms to grow, like saccharides and a solidifying agent (agar or gelatine). The mycelium grows on the surface of the medium and will later be used to inoculate larger amounts of substrates like sawdust or grain. Test tubes or Petri dishes (or flat whiskey bottles) can be used as culture containers.

Instead of working with cultures, one could also try to purchase small amounts of good quality mother spawn to prepare the final spawn.

### Tissue cultures

Young and vigorous mycelium can be obtained from a young fruiting body using a scalpel, alcohol, sterilised agar slants, Petri dishes or bottles with agar, flame (non-smoking), and a clean table to work on, or preferably a laminar airflow cabinet or inoculation box.

- Wash the mushroom thoroughly.
- Dip the scalpel in alcohol, and then flame it until red-hot.
- Let it cool down for 10 seconds.
- Now break or tear the mushroom lengthwise (do not cut it with a knife, since contaminants from the surface can stick to the blade). Do not touch the inner surface with your hands.
- Use the heated scalpel to remove a small piece ( $2 \times 2 \text{ mm}^2$  is sufficient) of the inner tissue. Take care that no outside surface tissue is included.
- Open the test tube/Petri dish.
- (When using test tubes: heat the mouth of the tube in the flame to kill unwanted spores). Then, gently replace the tissue on the scalpel in the middle of the agar.
- Immediately replace the plug.
- Inoculate at least three cultures, but preferably more.



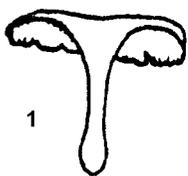
*Figure 10: Which part to use in shiitake (left) and oyster mushroom (right)*

Incubate the newly inoculated agar slants or Petri dishes at 25 °C for about ten days. Within three to four days mycelium will cover the tissue and branch out on the agar.

If no growth occurs on the agar, check the following:

- Possibly the mushroom was too old. Try a fresher specimen.
- Possibly the scalpel did not cool down before taking the tissue sample, thereby overheating the mycelium.

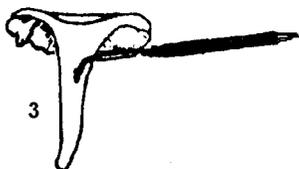
The mycelium should be white and grow out from the tissue. If yellow, blue, green or grey mycelia form on other places on the surface, then these are fungal contaminants. A creamy, shiny growth often indicates bacterial contamination.



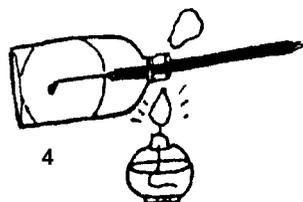
**1 Tear the mushroom in half**



**2 Flame the needle**



**3 Remove tissue from mushroom**



**4 Place it on the agar**



**5 Store in a dark place.  
Ready for use in 7 to 9 days**

*Figure 11: Preparation of the spawn*

## 4.5 Preparation of media

Most species grow on the following media:

### **Potato Dextrose Agar (PDA) extract medium**

Ingredients: *200g diced potato, 20 g agar powder, 20g dextrose or ordinary white sugar, 1 litre water.*

- 1 Wash and weigh the potatoes and cut them into small pieces.
- 2 Boil for about 15 to 20 minutes until they are soft.
- 3 Remove the potatoes.
- 4 Add water to the broth to make exactly 1 litre.
- 5 Add the dextrose and the agar. Be sure to add the right amount of sugar and agar, otherwise the medium will become either too soft or too hard.
- 6 Stir occasionally and heat gently until the agar has melted. The agar should be hot when poured into the test tubes or bottles otherwise it will become lumpy.
- 7 Fill about one fourth of the test tubes.
- 8 Then, seal the tubes or bottles with cotton plugs.

### **Rice bran broth medium**

The above recipe for PDA is commonly used for culture preservation, but for multiplying cultures, the following recipe is cheaper and easier to prepare. It is in use in the Philippines for oyster mushroom (*Pleurotus*) and wood ear mushroom (*Auricularia*).

Ingredients: *200 g rice bran, 1 litre water, 20 g gelatine.* Boil the rice bran for about 10 minutes in the water. Filter, save the broth and melt the gelatine and pour into bottles and sterilise.

## 4.6 Preparation of slants

After filling the test tubes or bottles with the medium, they must be sterilised before they can be used. The most commonly used sterilisation units in small-scale laboratories are pressure cookers, but autoclaves can be used as well.

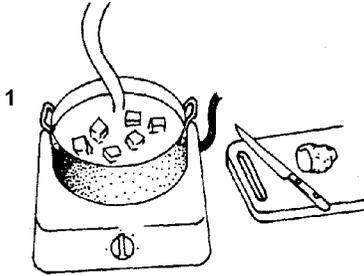
### *Procedure*

- Pour water into the pressure cooker to the level of the rack.
- Place the bottles/test tubes in the racks with a plastic covering to prevent water from wetting the cotton plugs.
- Then close the lid firmly.
- The air vent should be open at the beginning to allow the air to escape. This will take some minutes from the moment of boiling and steam escape.
- Close the air vent. A pressure gauge shows the pressure rise.
- Sterilise under pressure for 20-30 minutes.

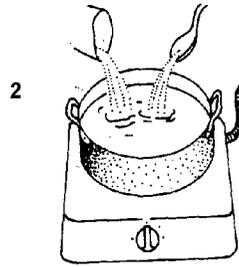
To increase the surface area, the test tubes or bottles are placed in an inclined position when the agar is still fluid.

Take care that the agar does not touch the cotton plug otherwise it might become contaminated.

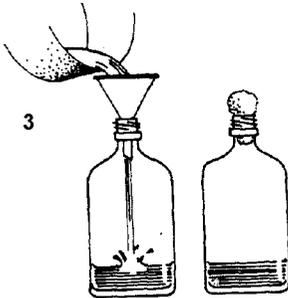
Do not move or handle the test tubes until the agar has solidified, otherwise a small portion of the agar may solidify at the other side of the slant or too close to the plug.



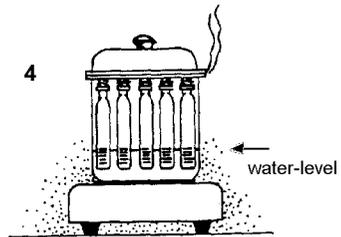
1 Boil diced potato until soft



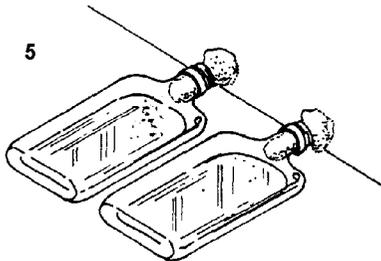
2 Remove potato and add dextrose and agar to its broth, make up to 1 liter.



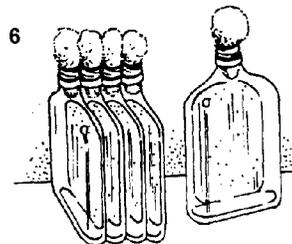
3 Pour into slants or flat bottles, plug with cotton.



4 Sterilize for 15 minutes in pressure cooker.



5 Place bottles in a slanting position, for the agar to stand and completely cover one side of bottle.

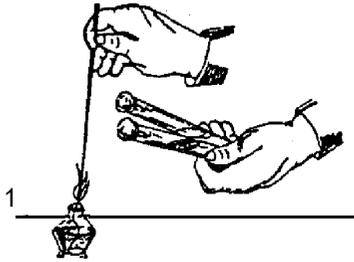


6 Store in cool place until ready to be inoculated.

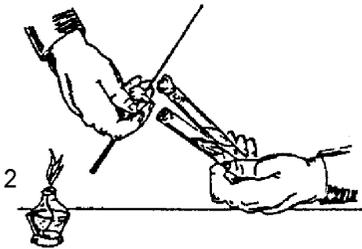
Figure 12: Preparation of the Potato Dextrose Agar (PDA) medium (1,2,3) and preparation of bottles (4,5,6)

## Sub-culturing

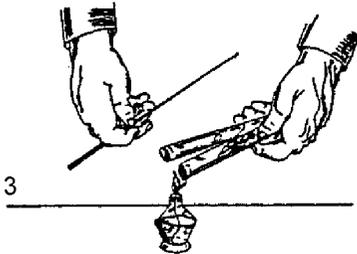
Inoculate more test tubes using the methods discussed above.



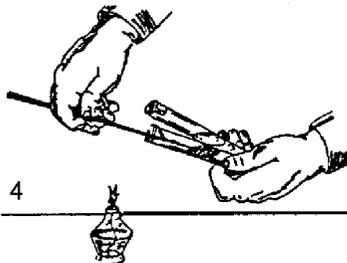
Sterilise the scalpel in the flame until red hot.



Take the plugs out of the test tubes (meanwhile the scalpel will cool down).

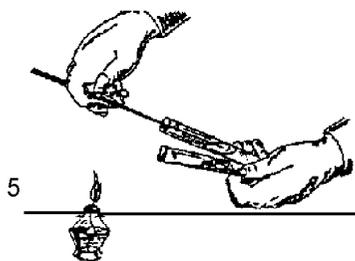


Keep the mouth of both test tubes above the flame.

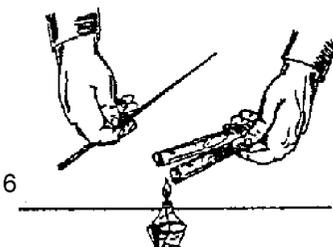


Cut a small square  $5 \times 5 \text{ mm}^2$  from the "mother" test tube culture.

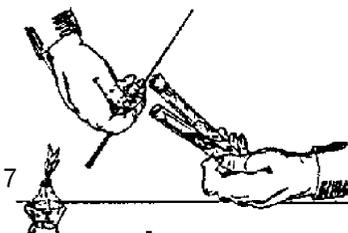
Figure 13: Sub-culturing (part 1)



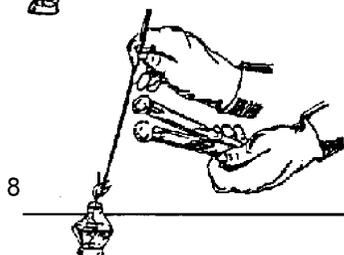
Put the square in the middle of the agar of the new test tube.



Hold the mouths of the test tubes above the flame for three seconds.



Put the plugs back in the test tubes.



Sterilise the scalpel again for the next transfer.

*Figure 14: Sub-culturing (part 2)*

For reasons of degeneration it is advisable not to transfer from one mother culture more than eight times or to use mother cultures on agar for more than two years.

The mycelium will degenerate after a certain number of transfers, so it is not possible to keep on transferring the cultures on agar forever.

### Spawn containers

Spawn containers should be made out of heat resistant material: mostly glass and polypropylene (PP). The spawn containers have to be tested to see if they can withstand the temperature inside the sterilisation unit. If the pressure is greater than 1 atmosphere overpressure, the temperature will be higher than 121° C. PP bags sometimes crack easily after having been subjected to the sterilisation process. Avoid bags with seams: these tend to split after the heat treatment.

Glass or heat-resistant plastic bottles are often used for the mother spawn. Wide-mouthed jars, milk bottles and dextrose bottles can also be used. Dextrose bottles are ideal, because they can be obtained for free from hospitals and they have air outlets that can easily be plugged with cotton wool. They can be used for final spawn too, but if the mycelium from the spawn material has grown into one big clump, the bottles have to be broken to get it out. Polypropylene bags with cotton plugs (or filters) to allow aeration are much in use for the final spawn (both sawdust and grain). Their size varies between 2.5 and 15 litres for grain spawn. The exchange of metabolic gases like CO<sub>2</sub> with ambient air has to be ensured; unwanted spores, however, must be prevented from entering the container..



*Figure 15: Bags sealed with a cotton plug.*

## 4.7 Mother spawn

Mother spawn can be used to inoculate either grain spawn or a second generation of mother spawn. In simple laboratories, grain mother spawn should not be used to inoculate another generation of grain mother spawn because the risk of contamination and degeneration will be too high.

## Preparation of grain spawn

The main advantage of grain is that it is very nutritious for fungi and forms kernels easily. The kernels can easily be dispersed in the substrate. The main disadvantage is that it provides an optimal substrate for other organisms too. The chances of contamination are therefore much higher compared to sawdust spawn.

### *Kinds of grains*

Different grains can be used such as *wheat, rye, millet, rice or sorghum*. Boil grain first, drain, then fill in containers and sterilise.

The moisture content of the grain, after boiling, should be around 50%. If it is higher, mycelial growth may be faster, but the danger of wet spot bacteria is also greater. If it is drier than 35% mycelial growth will be rather slow.

### *Grain spawn formula*

Grain in small containers can be moistened to a higher degree than grain in 15 litre bags. For 2 litre containers, use the following recipe: 480 g rye, sorghum or wheat, 400 ml water, 2 g gypsum (45% moisture). (See Appendix 1)

## Preparation of sawdust spawn

Sawdust spawn substrate: Sawdust 10 kg, CaCo<sub>3</sub> 147.5 g, Rice bran 1.25, Gypsum 0.1475g, Urea 0.5 g, Water 1.5 litres. (See Appendix 1)

### *Sterilisation*

Sterilise the spawn containers in an autoclave. The length of time depends on the autoclave, the way the spawn containers are packed (dense or loose) and the size of the containers. For instance, two hours for 500 g containers; three to four hours for 3 kg bags.

Shake the bottles when taking them out of the autoclave or the pressure cooker.

### *Inoculation*

Once the temperature in the centre of the container has dropped to below the maximum mycelial growth temperature, the spawn containers can be inoculated. Use at least one (for 250 ml bottles) or two (for bigger bottles) squares of 10 x 10 mm<sup>2</sup> from the full-grown agar of the mother culture for each bottle.

### *Incubation*

Incubate the bottles until the mycelium has grown all over the substrate. The temperature should be close to the optimal temperature for mycelial growth (consult Table 1 in Chapter 2).

Shake once (after eight days) or twice during the incubation period (or every three or four days) to distribute the mycelium evenly and to prevent kernels from sticking together.

### *Storage*

Keep the spawn in the refrigerator (except for certain strains of *Pleurotus djamor* spawn, which are cold sensitive and should be stored above 12 °C) and only take it out when needed.

Grain spawn can spoil in one night at temperatures above 25 °C.

## **4.8 Preparation of the final spawn**

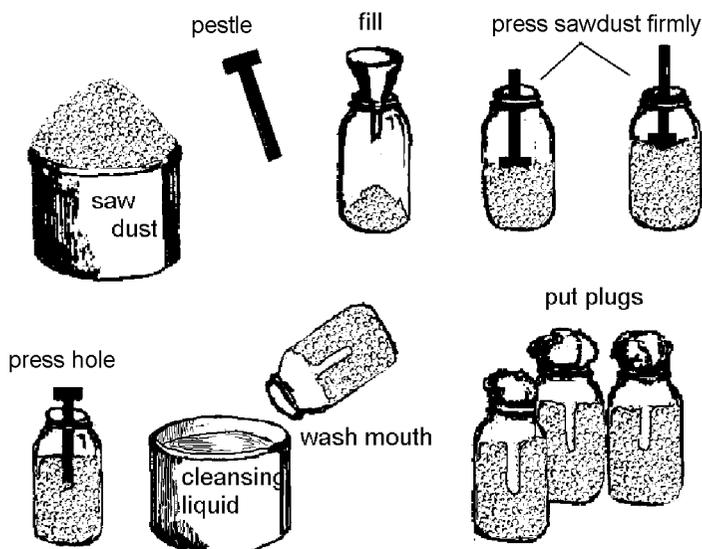
The choice of a specific spawn substrate depends on the chosen species and the cultivation method. The following table shows which spawn substrates are most frequently used.

*Table 2: Use of spawn substrates.*

<b>Species</b>	<b>Cultivation method</b>	<b>Final spawn substrate</b>
Shiitake / <i>Lentinula edodes</i>	Sterilised sawdust in bags	Grain, sawdust
Oyster mushroom / <i>Pleurotus spp.</i>	Pasteurised or sterilised substrates	Grain, sawdust or straw based spawn
Wood ear mushroom / <i>Auricularia spp.</i>	Sterilised substrate in bags	Sawdust

### Sawdust or grain spawn?

The advantage of sawdust spawn is that it can be kept at a higher temperature much longer before it spoils. The substrate material is also cheaper than grain. Sawdust based spawn is made in the same way as described in the chapter “sterilised” substrates, except that it needs to be sterilised at 121°C under pressure.



*Figure 16: Preparation of sawdust spawn in glass bottles. The mouth of the bottle is cleaned to prevent spores from germinating.*

An advantage of grain spawn is its vigour. A disadvantage is that it spoils rapidly and is very nutrient-rich and thus more susceptible to contamination. Grain spawn is unsuitable for outside use, as rodents will eat it. Grain spawn causes a faster temperature rise in the inoculated substrate than sawdust spawn, which may or may not be desirable. Grain is treated the same way as discussed above for the mother spawn. It may be inoculated by grain spawn or wooden sticks.

### *Storage and purity*

Good spawn shows vigorous mycelial growth and contains no other organisms. If it has been stored too long it will become less vigorous. Spawn from oyster mushrooms, for instance, will become very compact after prolonged storage. As a consequence it will be difficult to apply evenly during spawning.

# 5 Growing oyster mushrooms on pasteurised or ‘sterilised’ substrates

## Substrate

The material on which the mycelium of the mushrooms grows is called substrate. Agricultural waste like wood chips/sawdust, sugar cane bagasse, and different types of straw can be used as the main ingredients in the substrate for oyster mushrooms.

The properties of a substrate determine which mushrooms and microbes can grow in it. The more selective it is, the better the substrate meets the demands of a specific mushroom and the less suitable it is for others.

After mixing and adding certain supplements, the substrate undergoes a heat treatment to give the desired mushroom mycelium an environment with few competitors.

## 5.1 Preparation of the substrate

Oil drums and plastic bags are all you need to prepare the substrate. A cemented floor is the preferred underground for mixing and moistening the sawdust (or straw) and a fork for mixing the ingredients.

Substrate preparation equipment:

- substrate mixer (optional); the mixing of the substrate ingredients can be performed just as well manually
- a steam source or heating equipment like an oil drum

For the substrate:

- raw substrate materials, like sawdust, rice bran, wheat straw, dried banana leaves, dried elephant grass, dried grass pieces etc.
- substrate containers (plastic bags or bottles)

- depending on the type of bags/bottles: additional plugs and plastic rings and/or rubber bands

### **Mixing the substrate**

The aim of mixing is to distribute the different ingredients, including water, as evenly as possible. If adding a small quantity of one component like chalk, then it is better to mix it first with some of the substrate and only then add it to the large heap. Otherwise its distribution will probably remain non-uniform. Moreover, lumps might occur and the very high concentration of nutrients at these spots will result in contamination.

Mixing is also very important for the moisture distribution. The correct amount of water should be available everywhere in the substrate. After mixing, the moisture content should be 60 – 65%.

Sometimes a better distribution can be achieved if the substrate ingredients are mixed in a dry state (e.g. in “sterilised” substrates containing sawdust and supplements); the water is then added later.

A batch of up to 2000 kg can be mixed by hand on a cemented floor, similar to cement making. Two people are capable of manually mixing 2 tons of substrate per day. More people, however, should do filling.

Sterilise the substrate as quickly as possible after mixing in the supplements. Storage of the mixed ingredients for a period longer than 6 hours should be avoided to prevent fermentation of the substrate.

### *Sawdust substrate*

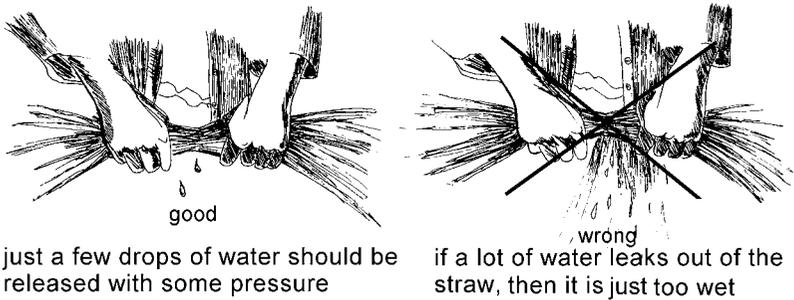
The sawdust (or other bulk substrate material) has to be stacked on a heap and moistened. By keeping the heap moist, the sawdust will soften. This will ease the absorption of water. Usually the sawdust is stacked for only one or two days.

If only fresh sawdust is available, like sawdust from recently felled trees, it should be stacked for a much longer period: up to several weeks.

The sawdust substrate should be free of splinters or bigger pieces of wood. These may damage the bags, offering contaminants easy access after sterilisation. On the other hand, several growers feel that a combination of fine and coarser sawdust or wood chippings provides the best starting material. Very fine sawdust should be avoided as it clogs the airflow when moistened.

*Straw substrate*

Moisten the finely chopped substrate ingredients and apply the squeeze test to determine whether the substrate is moist enough.



*Figure 17: Squeeze test*

*Filling the bags*

Fill small substrate containers (usually plastic bags) with the substrate before sterilising.

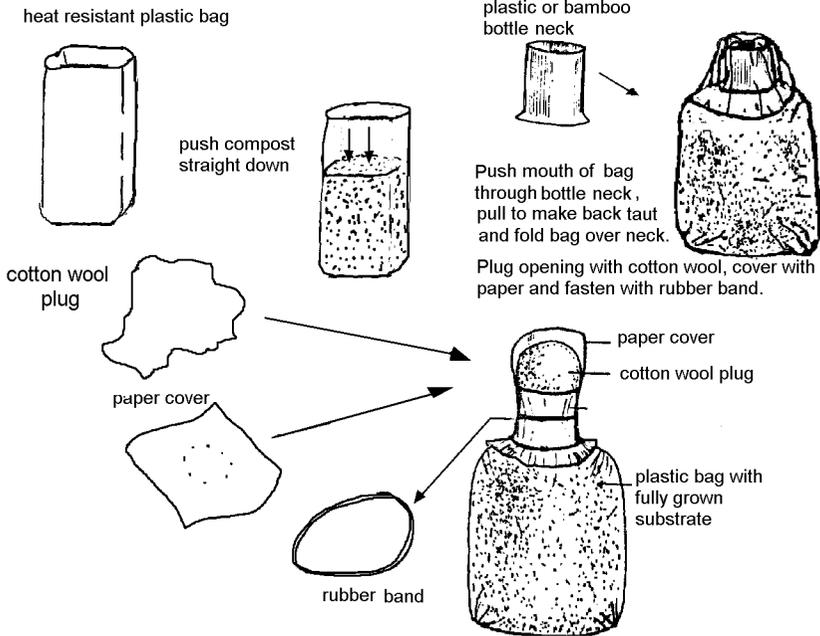


Figure 18: Filling the bags

## 5.2 Heat treatments

The aim of the heat treatment is to kill competing microorganisms and to get rid of soluble nutrients. Most substrates are given a heat treatment before spawning. It is an important measure to control pests and diseases.

In this Agrodok three methods are dealt with:

- pasteurisation by immersion in hot water
- pasteurisation by steam
- sterilisation

*Table 3: Advantages and disadvantages of different heat treatments.*

Heat treatment	Comments	Equipment
Fresh substrate pasteurised by immersion in hot water	Simple method  Feasible for several agricultural wastes, like coffee pulp waste, straw, sawdust  Little chance of contamination because easily soluble carbohydrates are removed by the immersion process	Wood fire or solar energy can easily be used
Fresh substrate pasteurised by steam	Good method to handle large amounts of substrate  Use of agricultural wastes like straw, corncobs, cotton seed hulls  More chance of contamination than with immersed or sterilised substrate	Steam boiler and pasteurisation room necessary  Oil drum on fuel burner
Fresh substrate "sterilised"	Good method for bags of sawdust	Simple method: oil drums on fuel burner Expensive method: autoclave

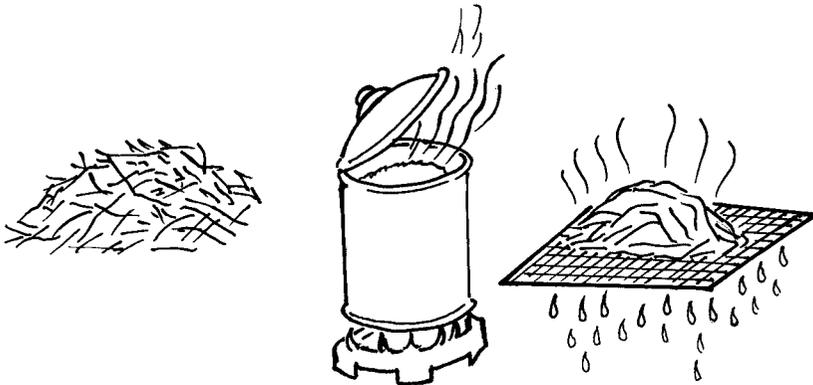
### **Immersion in hot water**

This method is a form of pasteurisation. The hot water will kill contaminants. Different types of straw can be treated in this way for the cultivation of oyster mushroom (*Pleurotus*)

The method is very easy: only hot water, containers and the means to keep the water hot are required.

Materials and equipment required:

- substrate material (see formulas in appendices 1 and 2)
- substrate containers (e.g. plastic bags or trays)
- containers for hot water and means to keep the water hot (fuel, solar energy, steam, etc.)
- wire mesh to let the substrate drain



*Figure 19: Immersion and draining of straw*

The substrate is put in wire mesh cylinders in hot water. The water has to be kept at 70 °C for at least 15 minutes, but 30-60 minutes is safer.

Immersion in water at lower temperatures and for periods shorter than 15 minutes is insufficient to kill all contaminants.

The size of the water containers depends on the scale of the operation. A 240-litre container can hold about 90 kg of wet straw substrate. The same container can be used 2-3 times a day, because the actual immersion time is only about 30 minutes to one hour.

The same batch of water should not be used for more than two or three batches of substrate.

### *Draining and cooling*

Drain the heat-treated substrate and let it cool on a clean plastic sheet on a table or on the floor inside the farm. Then spawn as described in paragraph 5.3 (Spawning pasteurised substrates).

## **Bulk pasteurisation by steam**

This method kills the unwanted organisms but keeps the favourable ones alive. To achieve this, a temperature of 60 °C to 70 °C has to be sustained for at least 8 hours; after which most pests and diseases (contaminants) will be eliminated.

Materials and equipment required:

- substrate material (see formulas 4-6 in appendix 2)
- substrate containers (e.g. plastic bags)
- oil drum and burner

Put a rack in the oil drum, with a fine mesh wire to prevent straw from falling through. Fill water below the rack to a height of 20 cm. Then add the moistened straw on top. Steam the straw for at least 8 hours. Take care that the straw has cooled down to 30 C before spawning.

Allow the steam to escape through small openings to prevent explosion of the drum.

## **Sterilisation**

This method too is used to kill unwanted organisms but here the temperature is much higher and there is overpressure in the container or oil drum.

When simple equipment is used the reached temperatures will not be higher than 90°C and the pressure in the container cannot build up very high. Good results, however, have been obtained by prolonged heating at this temperature when the substrate should be sterile.

To avoid an explosion, make sure that tightly closed drums or containers have a safety valve in the lid.

Materials and equipment required:

- substrate material (see formulas 1-3 in appendix 2)
- substrate containers (e.g. plastic bags)

- oil drum (re-enforced), or metal container. Make sure that materials used are able to withstand the temperatures

At higher altitudes water will cook below 100°C. In that case also, the period of heating should be prolonged.

### 5.3 Spawning pasteurized substrate

The substrate should have cooled down (whether pasteurised by steam or by immersion in hot water) to 30 C. The spawn (3% to 8% of the weight of the substrate) can be mixed in with when filling the bags. Or a layer of substrate can be topped with some spawn, layer by layer. See figure 27.

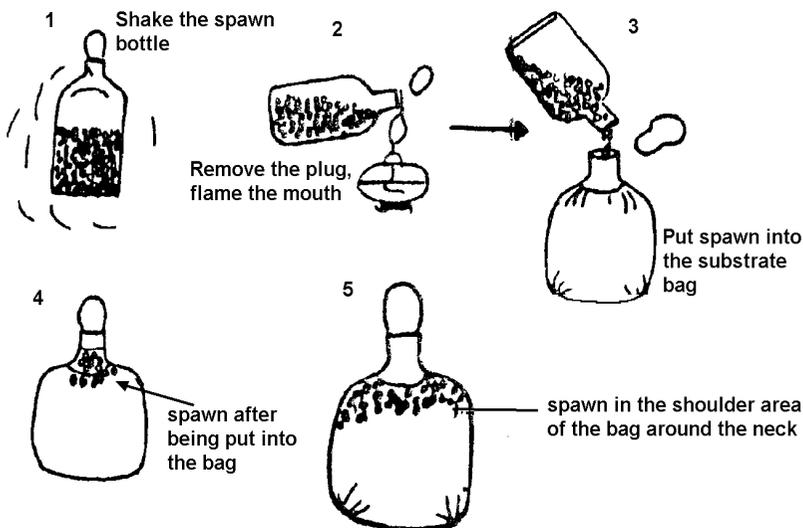
Different types of bags can be used to hold the substrate. Never fill to more than 20 kg per bag: spontaneous fermentation would raise the temperature inside the bags to more than 30 °C, the upper limit for mycelial growth of most oyster mushroom species. Make holes in the bags to ensure that enough oxygen can reach the substrate.

One type of bag used in China is made of cylindrical plastic, 20 cm in diameter, filled up to a height of 50cm, with a perforated pipe in the middle, right down to the bottom of the bag. The mouth of the bag is tied around the pipe, and aeration proceeds through the pipe. The aeration pipe will also allow heat to dissipate, even if it is formed in the core of the substrate. Spawn run: It will take the mycelium 20 days at 25 °C to colonise the substrate. The plastic and aeration channel can be removed entirely if a very humid environment can be created, for example in a shed. Alternatively, the plastic can remain around the substrate, in which case cuts have to be made in the plastic to enable the mushrooms to grow out.

### 5.4 Spawning sterilised bags

The substrate should be spawned as soon as it has cooled below 30 °C. Relatively large amounts of spawn are used: 7 to 10%. If smaller percentages give similar results, then there is no need to stick to these figures.

Spawning is performed by lifting the plugs from the bags containing the substrate (thus opening the bags) and putting in a small amount of spawn. This is the moment at which contamination is most likely to occur. So ensure that the time the bags are open is as short as possible!



*Figure 20: Steps in spawning procedure*

During the process of spawning the following measures need to be taken to control contamination of the substrate:

- Put on clean clothes.
- Put the hot bags in a special room with UV lights. Let the bags cool down without ventilation, or ventilate with filtered air.
- Do the spawning on the following day (do not forget to turn off the UV light).
- Hold both substrate and spawn containers in a horizontal position to prevent spores falling in.
- Use a flame near the mouths of the bottles of spawn and plastic bags to keep the environment more or less sterile.

- Spawning should take place at night when there is less contamination in the air.
- Clean with chemicals: formalin or alcohol.

Be careful not to come into contact with these chemicals. The use of chemicals can affect both health and environment; health measures should be considered first.

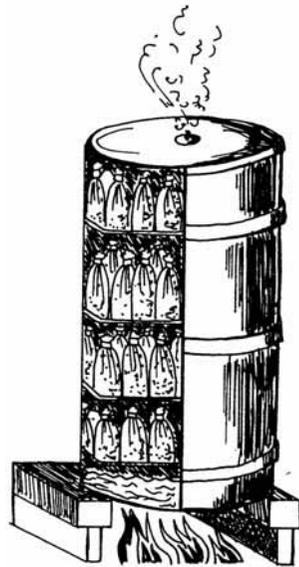
Misting with H<sub>2</sub>O<sub>2</sub> is an environment-friendly way to obtain a clean room for spawning, as its end products are oxygen and water.

### Using Oil drums

A simple oil drum can be used in the following way:

- Place a wooden rack on the bottom of the oil drum at a height of around 20 cm.
- Fill the drum with water up to the height of the rack (20 cm).
- Place the bags with the substrate on the rack inside the oil drum.
- Put the lid on the drum and steam for four to six hours by heating the drum with either wood or gas.

Allow the steam to escape by a few small holes. Each time about 75 bags can be steamed in this way. Take care to add enough water and to supervise the heating process in order not to boil away all the water.



*Figure 21: Simple sterilisation unit prepared from an old oil drum.*

### Steaming unit

Relatively simple tent-like constructions can also be used to semi-sterilise the bags. Prolonged heating at around 96-98 °C will sterilise the substrate sufficiently. Obviously the materials used should be able

to withstand the temperatures. Insulation panels can keep energy costs down. After the heat treatment the substrate should be sterile.

### *Autoclaves*

Autoclaves are double-walled steel containers, which are able to withstand an overpressure of 1 atmosphere. Large autoclaves require a high investment and are therefore not discussed further in this Agrodok.

## **5.5 Spawn run**

During spawn run stage the mycelium will grow through the substrate. The spawn run time is different for each species and depends on the size of the bag, amount of spawn, the strain used and the temperature.

Once the bags are spawned they should be placed on shelves in the incubation rooms. Depending on the strain and temperature the mycelium will colonise the substrate in two or three weeks and start to form small fruiting bodies.

As a consequence either the conditions in the growing room will have to change, or the bags need to be moved from the incubation room to the growing room.

Next, remove the cotton plugs and (a part of) the plastic and ensure that high humidity is maintained: 90 to 95%.

If, however, the relative humidity is rather low do not cut away too much plastic to prevent the substrate from drying out.

When the pinheads have grown to a size of 1 cm, the humidity should be lowered somewhat to 85% by passing fresh air through the room.

## 5.6 Fruiting/cropping

Several techniques are used for filling the mushroom house and making the bags ready for fruiting. A common practice is to make bamboo or wooden frames and stack the bags on them to form a wall of plastic bags.



*Figure 22: Oyster mushroom fruiting.*

If you want to get small mushrooms, a larger surface should be exposed to the open air. Note; this will result in the substrate drying more rapidly.

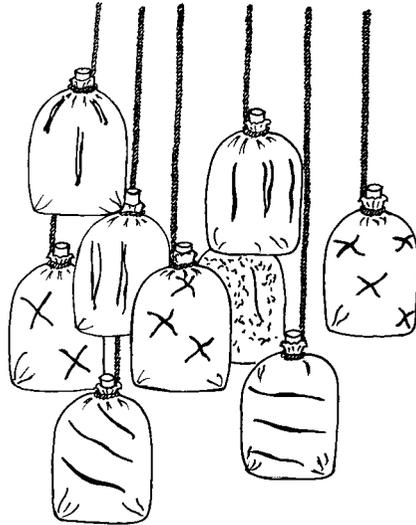
It takes three to four days after opening the bags before the primordial buttons/mushrooms will form.

### *Hanging the bags*

Another method is to slash each bag and hang them from the ceiling.

### *Opening the bags*

Open the bags as soon as the mycelium has covered the substrate completely. Remove the cotton plugs and cut away the plastic top of the bag (partially). Take care not to cut too deep or else you would damage the mycelium.



*Figure 23: . Different ways to cut the plastic of the spawned substrate bags after the mycelium has fully grown through the substrate.*

## **Temperature**

The ambient temperature has to fit the chosen mushroom strain. If the temperature in the mushroom house is too high for the chosen strain, it will be necessary to frequently mist the house. Opening the doors and windows at night will also help keep the temperature down.

## **Aeration/ventilation**

The mushroom house needs ventilation openings that may also provide light.

## **Light**

Oyster mushrooms are very sensitive to insufficient aeration and light. Required light (colour and intensity) depends on the strains. Some growers adhere to the rule of thumb that light should be sufficient to read a newspaper everywhere in the growing room.

When the small mushrooms emerge, their form will reveal whether they get sufficient light and aeration.

If the stems are long and the caps small, the aeration and light requirements were not met. In the complete absence of light, oyster mushrooms will form no cap but stipes (mushroom stalks) forming a coral-like structure.

## **Humidity**

Good control of the humidity during cropping is very important for all types of mushroom. Keep the humidity high (80 - 90%) by spraying water several times per day.

However, no water should be sprayed directly onto mushrooms that are ready for picking. Their shelf life will decrease drastically if they become too wet.
--



*Figure 24: Maintaining a high humidity during cropping is important for all mushrooms.*

## **5.7 Harvesting**

The mushrooms are ready for harvesting in five days (if the temperature is between 15 and 20 °C) or two to three days (at higher temperatures). It will take another five to nine days for the second flush.

There is so much variability among strains and substrates used that it is difficult to give periods for fruiting. Typically, it will take about one week before new primordia are formed, but much depends on the local climate conditions and the climate control in the growing rooms.

Harvesting is performed by gently pulling or twisting the mushrooms from the substrate. Only very little substrate should be pulled out.

### **Rubbing instead of scraping**

Some growers in the Philippines scrape off some of the substrate to free it from small, undeveloped primordia. These would easily become infected and have to be removed, but scraping the substrate will also retard the formation of new primordia. Rubbing the surface of the sawdust bags is a better method to remove the small and already dead fruiting bodies without causing harm to the mycelium.

Harvesting can continue as long as the mycelium remains white and firm. In total, three or four flushes can be harvested. When the substrate becomes soft and colourless, it is time to remove it from the house.

Do not throw the spent substrate near the mushroom houses!

All waste should be removed from the working areas immediately. Pests and diseases present in the used substrate can too easily spread to the fresh substrate.

Mushroom yields vary according to biological factors, environmental conditions, as well as pests and diseases present during cultivation. The yield from commercial production is about 20% of the weight of the wet substrate of fresh oyster mushrooms.

### **Product handling**

In order to avoid rapid deterioration, the fresh mushrooms should be marketed directly after harvesting. If this is not possible the mushrooms could be dried in a simple drying unit and marketed later. See Chapter 8, Post harvest handling.

## **5.8 Case description: Ahmedabad, India**

Aryan AgroTech organisation runs a spawn laboratory and a mushroom production farm. In addition to these activities Aryan AgroTech organises oyster mushroom growing projects for minority groups, which the Gujarat government financially supports to a certain extent.

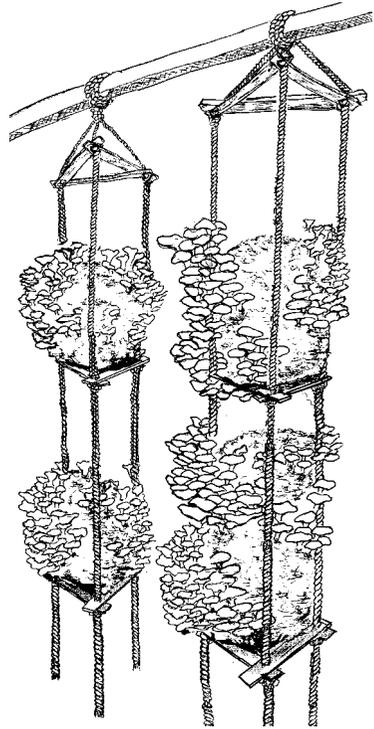
The projects are selected mostly in tribal regions and the groups receive information and training sessions. After training, the selected persons are provided with materials for construction of a growing unit as well as basic materials for the cultivation.

### *Growing House*

The growing house consists of a bamboo skeleton of about 2.5 metres high with a 50-m<sup>2</sup> surface. Plastic netting is placed over this skeleton, which is then covered with jute. Inside the growing rooms triangular platforms (4-high) of bamboo sticks hang from the bamboo roofing poles.

### *Temperature control*

Temperature control is done to some extent by wetting the jute covering. The evaporation that will take place will lower the temperature in the growing rooms. Temperature can be lowered by several degrees, depending on the outside temperature and air current through the netting.



*Figure 25: Triangular hanging constructions*

In the rainy period, however, the outside temperature is around 40 °C. During this period the cultivation is stopped there because the inside temperature cannot be lowered sufficiently to continue growing.

From the point of farm hygiene this seasonal break in the growing of mushrooms is a good way to prevent outbreaks of pests and diseases.



*Figure 26: Spraying the jute-covered roof*

### *Preparing the substrate*

The substrate is made of wheat straw that has already been chopped into short pieces during threshing. This wheat straw is immersed in a drum with hot water (70 °C) and kept there for 2 hours, maintaining the water temperature at 70 °C using a wood fire or a burner.

Then the straw is taken out and placed on a grid or a piece of plastic to drain excess water (see Figure 21).

### *Spawning the substrate*

Following the heat treatment and when drained, the moisture content of the straw substrate will be about 60%. The substrate is then put in layers in plastic bags. Spawn is placed on each layer. The spawning rate is about 10 % of the weight of the substrate (see Figure 29).

The grain spawn is produced in the spawn laboratory in Ahmedabad.

After filling and spawning the 3.5 kg bags are then moved into separate rooms for incubation. The incubation process will take 3 weeks, preferably at a temperature of 25 °C.

Once the bags are full-grown with mycelium, holes or cuts are made in the bags in order to provide aeration for the fruiting bodies to develop (see Figure 25).

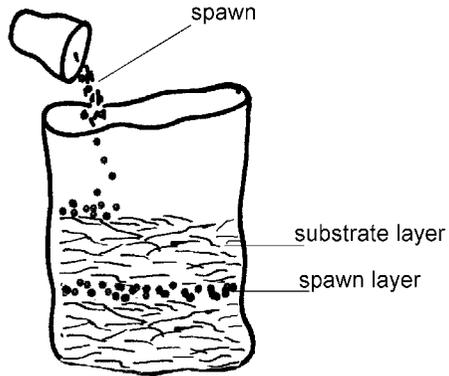


Figure 27: Spawning in layers.

### Harvesting

When the fruiting bodies have developed in mushroom clusters they are ready for harvesting. Harvesting of the mushroom clusters can be done for a period of at least 3 weeks. The stems are cut off. Stems and mushrooms are marketed separately. A part of the mushrooms is sold fresh in local markets. The rest is dried and sold at a fixed price to Aryan AgroTech.

## 5.9 Case description: Bogor, Indonesia

The Women’s Farmer group ‘Hanjuang’ at Bogor, Indonesia, was initiated some years ago in order to stimulate housewives to start agricultural activities in their free time. The revenues provide additional family income, which is mainly used for school fees and medical expenses. Various activities such as seedling nurseries for ornamental plants and fruit trees as well as home industry have been started.

One of the activities of the Women Farmers Group “Hanjuang” at Bogor is the cultivation of oyster mushrooms mainly *Pleurotus ostreatus*

*var. florida*. Spawn is produced on sawdust substrate in their own lab from tissue cultures.

### *Construction of the growing house*

The production houses have a surface area of about 35 m<sup>2</sup> and are about 3 metres in height. They are constructed out of wooden or bamboo poles and bamboo leaf mats. The roofing is often reinforced with plastic. The shelves in the houses (5 high) are also constructed out of bamboo.

### *Substrate preparation*

The substrate used is sawdust.

Formula: 10 kg sawdust, 1.5 kg rice bran, 200 gram chalk, 30 gram gypsum and 15 litres of water.

This well mixed substrate is put in 2-litre PP bags and pressed to make so-called bag logs weighing about 1.2 kg. The opening is closed with a PVC ring and a cotton wool plug after which these bag logs are sterilised for 8 hours in closed drums.

### *Spawning the bag logs*

After cooling down, the bag logs are spawned. The spawn is put through the top opening, which is then sealed with the cotton wool plug. The cotton wool is then covered with paper.

Spawn is produced on sawdust substrate in their own lab from tissue cultures.

### *Incubation*

After being spawned the bag logs are placed in an incubation room. The incubation room is clothed well in plastic sheeting along the ceiling and the walls in order to maintain a constant temperature of 30 C. The bag logs are kept in the incubation room for about 3 weeks.

### *Fruiting*

Once the bag logs are full grown with mycelium they are placed on bamboo shelves in the production house. The paper covers and cotton wool plugs are removed from the bags in order to provide aeration and stimulate cropping and fruiting.

### *Temperature*

During the daytime the temperature in the incubation room reaches about 26 °C with a relative humidity of 90%.

### *Harvesting and marketing*

When the mushroom clusters are mature they are picked, slightly trimmed and sold in the local markets and/or occasionally to supermarkets.

## **5.10 Juncao Technology turns grass into mushrooms**

In 1983, Professor LIN Zhanxi of Fujian Agricultural University recognised the rapid decline of forests in China as wood logs were much in demand for shiitake and other exotic mushrooms. He started to work with wild grasses, bagasse, rice and corn straw as basic materials for the mushroom substrate. In 1987 he decided to name the technique JUNCAO: Jun from fungi, and Cao being the Chinese word for grass(es). Now, 21 years later, the technique has led to a comprehensive growing system for more than 40 types of mushrooms, using some 33 kinds of leguminous plants as basic substrate material. The grasses are dried after the harvest, ground and stored until used. Specific substrate recipes have been developed for each mushroom. For example a patented process has been developed to use protein from fermentative bacteria instead of the commonly used wheat bran. Heat treatments and substrate containers also vary between species. This systematic set of technique has spread to at least 50 countries and helped to alleviate poverty while making sustainable use of resources which are readily available.

*Table 4: Common and scientific names of grasses and legumes*

<b>Common name</b>	<b>Scientific name</b>
alfalfa, lucerne	<i>Medicago sativa</i>
banana	<i>Musa nana</i>
brazilian lucerne	<i>Stylosanthus</i>
common reed	<i>Phragmites communis</i>
elephant grass	<i>Pennisetum purpureum</i>
foxtail millet	<i>Setaria italica</i>
giant reed	<i>Arundo donax</i>
peanut	<i>Arachis stylosanthus</i>
reed grass	<i>Arundinella nepalensis</i>
setaria grass	<i>Setaria sphacelata</i>
sudan grass	<i>Sorghum arundinaceum var. sudanensis</i>
swamp foxtail, fountain grass	<i>Pennisetum alopecuroides</i>
water lettuce	<i>Pistia stratiotes</i>
wild ranking fern	<i>Dicranopteris ampla</i>
wild sorghum	<i>Sorghum proquinum</i>

# 6 Shiitake cultivation on plastic bags

The cultivation of shiitake in sterilised plastic bags is rapidly gaining popularity. Mushrooms can then be harvested faster and the yield is higher compared to growing on wood logs. Filling the bags and sterilising them, however, is labour intensive and energy consuming. The main advantages of growing shiitake on bags are:

- Many types of organic waste can be used.
- Total cropping period is 6 months compared to 4 to 6 years with cultivation on woodlogs.

If the substrate has been compressed and only little spawn has been used, the incubation period is three to four months.

## 6.1 Substrate preparation

The most commonly used substrate formulations are:

- Sawdust, 3 to 4% rice bran, 1% corn meal or wheat bran, 1% CaCO<sub>3</sub>
- Sawdust, 10 to 25% corn waste, 1 to 2% CaCO<sub>3</sub>

Fresh sawdust from the trees of the genera *Quercus*, *Betula*, *Castanopsis*, *Castanea*, and *Carpinus* can be used without prior fermentation. Sawdust from other trees can also be used, but if the sawdust contains resins it has to ferment for a number of months (stack on a moist heap for 1 week, turn after one week, and then once every month for up to 6 months). When the sawdust is moist enough it has to be mixed with the supplements and the chalk.

Mix the chalk first with the rice bran, as it will be easier to get an even distribution.

The moisture content (apply the squeeze test, see figure 17) at the time of preparation is usually between 55-65% of the substrate and in-

creases during incubation; take care to compare the right data (e.g. always measure before sterilisation).

Some reports indicate that a high water-holding capability of the substrate combined with good aeration will give better results. Substantially higher yields have been reported when (tea)leaves were mixed with the above-mentioned substrate as described for oyster mushrooms.

If the substrate is too moist, the airflow will be clogged and even a long spawn run period will not deliver a high-quality substrate. If water collects at the bottom of the bags, the substrate is certainly too wet.

## 6.2 Filling and heat treatment

Check the general procedures for filling. In Taiwan steaming at a temperature of 96 - 98 °C showed better results than sterilisation under pressure at 121 °C, but both methods can be used. Steaming under low pressure is appropriate if more flushes are expected. Ample space between the crates and bags should provide sufficient steam circulation.

## 6.3 Spawning

Let the bags cool down and spawn them the next day. 10 g of sawdust spawn is sufficient to spawn one bag of 1.2 kg so one bottle of 550 ml is sufficient for about 50 bags. The strain for sawdust cultivation should be checked carefully.

Some serious losses in yield have occurred because spawn makers sold new strains that produced well on wood logs, but gave very low yields on sawdust.

Some strains will perform better on a substrate of corncobs; others better on a sawdust substrate.

Take the usual precautions when spawning; use the measures for spawn making if extreme levels of contamination occur. Not more than 5% of the bags should become contaminated.

## 6.4 Spawn run and mycelial development

It will take one to four months for the mycelium to colonise the substrate and mature, depending on the type and the amount of spawn (refer to the case studies).

For fruiting some light should be provided for at least the end of the spawn run. Growers who have completely dark spawn run rooms should illuminate the room with a day/light cycle at the end of spawn run. Problems can be avoided if a little light is present during all stages of the growth.

All strains show optimal mycelial growth at 25°C. The temperature inside the bags is usually a few or even ten degrees higher than the ambient room temperature. If many bags are packed in a room, extensive cooling may be necessary.

### Growth stages

Five different stages of mycelial growth of all strains of shiitake can be distinguished for all strains. The first phase is the normal spawn run as it occurs in all fungi. When the substrate has turned white, it is not ready to fruit. It has to mature first.

The following are the five stages:

- 1 **Mycelial running:** The spawn will give rise to white hyphae, which produce enzymes to degrade complex substances like cellulose, lignin and hemi cellulose into smaller fragments. The fragments will be consumed at later stages of mycelial growth. As soon as the complete substrate is colonised, the next phase is entered.
- 2 **Mycelial coat formation:** A thick, white mycelial sheet will develop on the surface of the substrate. This will occur in two to four

weeks after inoculation. If the CO<sub>2</sub> level is high, the sheet will be thicker.

- 3 **Mycelial bump formation:** Bumps are clumps of mycelium, commonly formed on the surface by most strains. These bumps can turn into primordia at a later stage, but most of them fall off. Fluctuating temperatures and a high CO<sub>2</sub> level promote bump formation. If many bumps are formed decrease the CO<sub>2</sub> level by slitting the plastic open. The bumps may become a problem at a later stage of the cultivation, because green moulds can easily contaminate them.
- 4 **Pigmentation phase:** Some aeration should be provided when the bumps have formed. The mycelium will turn reddish-brown. If, however, the plugs are removed entirely, the substrate may dry out too much.
- 5 **Coat hardening phase:** Remove the plastic when bags have partially (half or one-third) turned brown. The outside of the substrate (coat) will have gradually become hard, while the inside should be softer and moister. The moisture content of the substrate core (inside) can be as high as 80%. If the outside is relatively wet, contaminants will have easy access to the substrate. The brown hard skin acts like the bark in wood log production: it protects against contaminants and keeps the humidity in the substrate. It is important to regulate climate conditions to obtain a mycelial coat of the right thickness.

## 6.5 Fruiting

The same factors that promote fruiting in shiitake cultivation on wood logs are used to manipulate the flushes in plastic bag cultivation.

These are:

- temperature fluctuation
- high humidity
- soaking
- removal of CO<sub>2</sub>
- physical shocks

If the plastic is removed too early or too late, yields will be affected. De-formed fruiting bodies during the first flush are a sign of a spawn run being too short or CO<sub>2</sub> too high during incubation. Strains differ in mycelial growth rate. While 60 days is sufficient to mature one strain, another strain would yield many deformed mushrooms after the same period of maturing.

If the temperatures are rather low and a suitable strain has been used, high-quality donko mushrooms can be harvested. If the humidity is also relatively low (60 to 70%), then cracks may appear in the caps of the most expensive quality in the Far East, which is called “flower winter mushroom” (hua dong gu) in Chinese.

*Table 5: A typical time schedule for shiitake cultivation on sterilised substrates (from: B. Chalmers)*

Stage/activity	Days	Temperature (°C)	Light intensity (Lux)	Relative humidity
Incubation	30-120	20-30	None	65-70%
Induction of fruiting bodies	2-4	10-20 <sup>1</sup>	500-1000	85-95%
Harvesting	7-14	12-18 <sup>1</sup>	500-1000	60-80%
Recovery	7-21	20-30	None	65-70% <sup>2</sup>
Induction of fruiting bodies for second flush <sup>3</sup>	2-4	10-20	500-1000	85-95%
<p>1 The temperature range for fruiting is dependent on the strain.                  2 A dry period after harvesting will prevent contaminants from spoiling the substrate at the scars where the mushrooms have been picked.                  3 The artificial logs may be given a cold water bath to restore a high moisture content of the substrate. Substrate blocks do not need to be watered during incubation.</p>				

If substrate is spawned on top only, most mushrooms will emerge from the top (see Figure 28). If the spawn has been mixed thoroughly, shiitake mushrooms will emerge from all sides.

## 6.6 Harvesting

Hold the mushrooms by their stalks and break them off carefully from the substrate. Do not tear them from the surface otherwise too much substrate will be torn loose. Harvest the mushrooms at an early stage according to the quality requested by the buyers. Do not water the scars left behind for three or four days. White mycelium growing on the scar is a sign of recovery. Completely opened mushrooms have a much lower value in Asia, whereas buyers in Europe are less critical. Normal yields are 15 to 35% of the wet substrate weight.



*Figure 28: Shiitake fruiting on top of vertical bags*

## 6.7 Pests and diseases

### **Green moulds**

Green moulds are the most common contaminants at the moment of spawning. They will also grow if there are any cracks in the bags. The substrate should be kept dry in between the flushes. Moist conditions promote contamination, and contamination attracts flies, which spread contamination even further.

The shiitake mycelium will normally form a crust below the *Trichoderma* colony. It is best to spray the green moulds after the harvest with a strong flush of water. However, if the substrate is too soft (because the moisture content is too high), the block would be damaged and consequently it will be more difficult to obtain a good second flush.

### **Mushroom flies**

Mushroom flies are attracted by the odour of the mycelium. They may occur in batches of old bags. The flies as such do not harm the mushrooms but they lay eggs between the lamellae and on the mycelium. Larvae will hatch from the eggs and will spoil the crop.

The only solution to tackle this problem is to consistently remove the old bags as well as the contaminated bags, and to clean the rooms.

### **Mites**

Mites may crawl into the incubation bags (if bags with plugs are used) and contaminate the substrate. However, the plastic bags will generally form a good barrier against insects, which makes this method of substrate packaging most suitable for countries with a high infection pressure.

## 7 Wood ear mushrooms on 'sterilised' substrate

Wood ear mushrooms (*Auricularia spp.*) are commonly cultivated in Asia. Plastic bag cultivation is gaining popularity due to the scarcity of suitable logs and the ease with which different species of *Auricularia* can be cultivated on sawdust. The technology can be expected to spread in the near future.

There are many *Auricularia* species of which *Auricularia polytricha*, *Auricularia fuscusuccinea* and *Auricularia auriculu-judea* are the most commonly grown.

*Auricularia polytricha* is the most suitable species to cultivate in tropical regions where temperatures are high.

### 7.1 Substrate preparation

The formula for the sawdust substrate is about the same as for oyster mushroom and shiitake, but the moistening period (fermentation) of the substrate should be longer. The preparation of the bags is again the same.

### 7.2 Heat treatment

The filled bags are steamed in the same way as for oyster mushroom and shiitake.

### 7.3 Spawning and spawn run

Sawdust spawn is generally used, 10 ml of spawn per bag is sufficient. During the spawn run the temperature should be 25° to 28° C. The mycelium will cover the substrate in about four weeks.

## 7.4 Fruiting

Cuts in the bags are made so the mushrooms can emerge. Take care when handling the bags, because the texture of the substrate will stay soft even after the mycelium has colonised it.

The mycelium is very sensitive to breakage.

Only little light should be present in the mushroom house. Three to four flushes can be expected. Per bag of 1.2 kg, 300 – 500 g can be harvested.

## 7.5 Case description: the Philippines

Although the market in the Philippines is more favourable for the smaller *Auricularia auricula-judae* (the black wood ear mushroom), the temperature range there is more suitable for growing *A. polytricha*. *A. auricula-judea* can only be grown in cooler areas.

### *Substrate preparation (weight percentages)*

- Dry sawdust (moisture content 15-18%)      78 kg
- Fine rice bran (first class)                      21 kg
- CaCO<sub>3</sub>    1 kg

The rice bran has to be sifted to break bigger particles into smaller pieces. The bigger particles would be the first to become contaminated. Weigh the substrate ingredients and mix CaCO<sub>3</sub> and rice bran well before mixing them with the sawdust. Add water slowly until the moisture content is 65-70%. (Check with the squeeze test, figure 17)

### *Fermentation*

Pile the substrate in pyramids and cover with plastic to retain its moisture. Let the heap ferment for five days and turn the heap on the third day. Sieve through 1.5 mm mesh to remove bigger particles and to break the clumps that may have formed during fermentation. The bigger particles might damage the plastic.

### *Filling*

Pack about 1 kg per 12 x 30 cm bag and add the ring and the plug.



*Figure 29: Putting rings on substrate bags.*

### *Heat treatment*

Sterilise the filled bags for 1.5 hours at 121° C or semi-sterilise for 10 hours at a temperature just below 100° C.

### *Spawning and spawn run*

Use one 500 ml of spawn for 50 bags.

The spawn run takes about one month at 25° – 30° C. Place the bags flat in rows on shelves.

A mushroom house (5 m wide, 12 m long and 4 m high) can hold 2640 bags. Each row has 55 bags per layer; four layers per row. Four rows with 220 bags each can hold 880 bags, so that three shelves may hold 2640 bags.

### *Fruiting and harvesting*

The optimum fruiting temperature of wood ear mushrooms (*Auricularia polytricha*) is 23° – 28° C. To promote primordia formation, the cotton plugs should be removed from the bags and holes cut in the bottom. Try to keep the temperature below 30° C by spraying water and opening the mushroom house at night. The primordia will develop into fruiting bodies in seven to ten days. Twist the fruiting bodies from the substrate by hand, leaving no bits of stem behind.

## 8 Post harvest handling

The edible mushroom is a highly prized product. It has a short shelf life. Special conservation methods have been developed, most of which are described in this chapter. This chapter pays attention to:

- Quality grades and harvest
- How mushrooms can be packed for the fresh market
- How they can be conserved for future consumption

### Quality grades and harvest

Mushrooms should be picked at the stage at which they have the highest profitability. Mushrooms should be dry on the surface when they are picked.

Spraying (or rain) a few hours before picking reduces the shelf life of most cultivated mushrooms.

### *Picking*

The pickers should gently break the mushrooms from the substrate or casing soil. Tearing away chunks of mycelium from the substrate or casing soil should be avoided. After picking, the mushrooms are cut to the desired stem length.

Since mushrooms can easily be damaged it is best if handling is kept to the minimum.

Immediate grading at picking and packing them in the same packages in which they will be sold will ensure that they are touched only once; at the moment of picking.

Instruct pickers to stringently stick to the following rules:

- Always pick mushrooms from newest beds/rooms first
- Do not touch sick fruiting bodies (collect them at the end of picking in a separate bag, disinfect hands and clothes of the involved worker afterwards)

Oyster mushrooms (*Pleurotus*) can either be harvested in bundles or as single fruiting bodies. Some concepts of oyster mushroom cultivation rely on harvesting whole bundles (e.g. oyster mushroom cultivation in Japan in bottles). This particularly applies to *Pleurotus ostreatus* and *Pleurotus cornucopiae*.

Harvesting and marketing young bundles of oyster mushrooms has the following advantages:

- Many mushrooms can be picked in a brief period
- The mushrooms look nice and stay fresh longer
- And the buyers also pay for the stems

Oyster mushrooms, however, are often sold as individually cut mushrooms. They should be picked when the outer margin of the fruiting bodies has only just rolled inwards, on the verge of becoming horizontal. Storage time will increase if they are picked at a stage just before maturation. Stipe length should be discussed with the buyer.

Shiitake (*Lentinula*)

Cut the stems immediately after picking. Cut the stems with a sharp knife (where the mushroom was attached to the substrate). Debris from stems will make the mushrooms dirty.

Wood ear mushrooms (*Auricularia*)

Twist the fruiting bodies from the substrate by hand leaving no pieces of stem behind.

## 8.1 Fresh Market

Under ideal conditions, packed mushrooms for the fresh market are covered with a plastic film and cooled rapidly after harvesting. The plastic film provides good protection from water loss, as long as the storage temperature is more or less constant. Repeated exposure to fluctuating temperatures should be avoided.

If the temperature rises, the mushrooms will lose water. If the temperature drops, water will condense inside the package and on the surface of the mushrooms. This will lead to fast wilting.

*Pleurotus spp.*: Experiments in the tropics have shown that keeping the mushrooms at 8-10 °C in pre-packs wrapped in perforated polyethylene films is a good way to keep mushrooms fresh. They can be kept for four days.

## Conservation methods

The taste and nutritional value of fresh mushrooms is usually better than that of conserved mushrooms. Nevertheless, conservation methods are necessary when only part of the harvest can be sold fresh.

Canning, brining and drying are the most common techniques, but not all conservation methods are equally suitable for all the different types mushrooms. Canned oyster mushrooms, for instance, taste horrible (except for *Pleurotus cystidiosus* and *P. abalonus*).

In some cases, the taste may become stronger after the conservation treatments.

Oyster mushrooms and shiitake, for example, give off a specific fragrance after drying.

## 8.2 Drying

This process is rather easy to perform. Drying has several advantages: it is easy, quick and safe and well-dried mushrooms can be stored for a long time. Among cultivated mushrooms, this conservation technique is mostly used for shiitake (*Lentinula*). The shiitake mushrooms get tastier after the drying process. Oyster mushrooms (*Pleurotus*) also become tastier. Nevertheless, the market for dried oyster mushroom is smaller compared to the market for dried Shiitake.

Wood ear mushrooms (*Auricularia*) can be dried as well and are often marketed in this way.

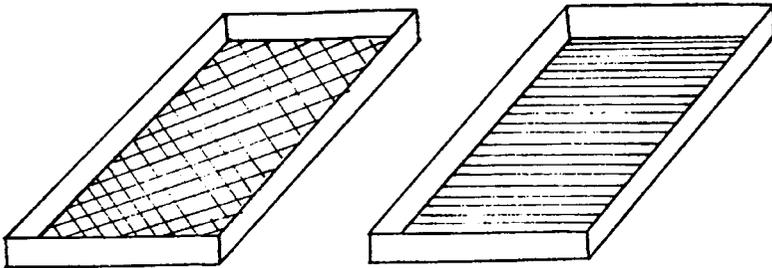
Watch the following points during drying:

- The mushrooms should not touch each other.
- Air circulation is very important; put the mushrooms on a grill rack or a metal grid.
- The area around the drying oven should be well ventilated to provide fresh dry air, while the moist air can flow out.

Mushrooms do not have to be crisp to the touch after drying; they should still be slightly flexible. As the mushrooms could become toasted at high temperatures, longer drying at low temperatures is safer than faster drying at high temperatures. If the fresh mushrooms are very wet, the starting temperature should not be low as they might start to rot. This is especially important for large whole mushrooms.

### *Drying by sun*

The quality of sun-dried mushrooms is generally less than that of artificially dried ones. The moisture content of sun-dried mushrooms is higher and therefore they can be kept for a shorter period than the artificially dried ones.



*Figure 30: Drying trays*

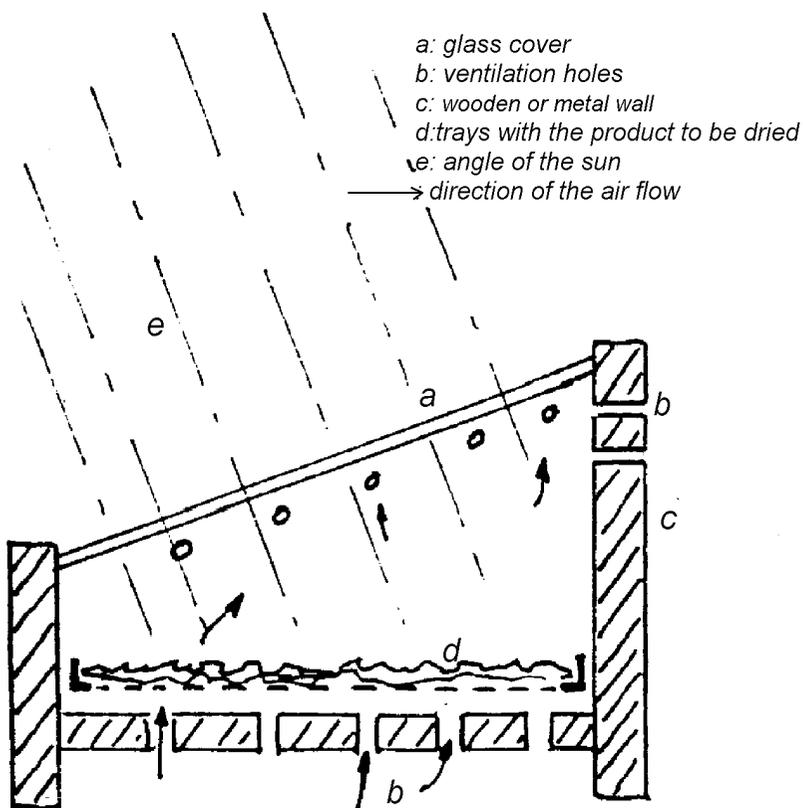


Figure 31: Indirect sun dryer

*Artificial drying:*

Revolving driers are suitable for mass production. The starting temperature for shiitake should be 30°C and increased hourly by 1 or 2°C until it reaches 50°C in 12 to 13 hours. The final touch is heating the mushrooms up to 60°C for one hour to increase the luster of the cap. Fluctuations in drying temperature will cause the cap to wrinkle, according to Chinese growers.

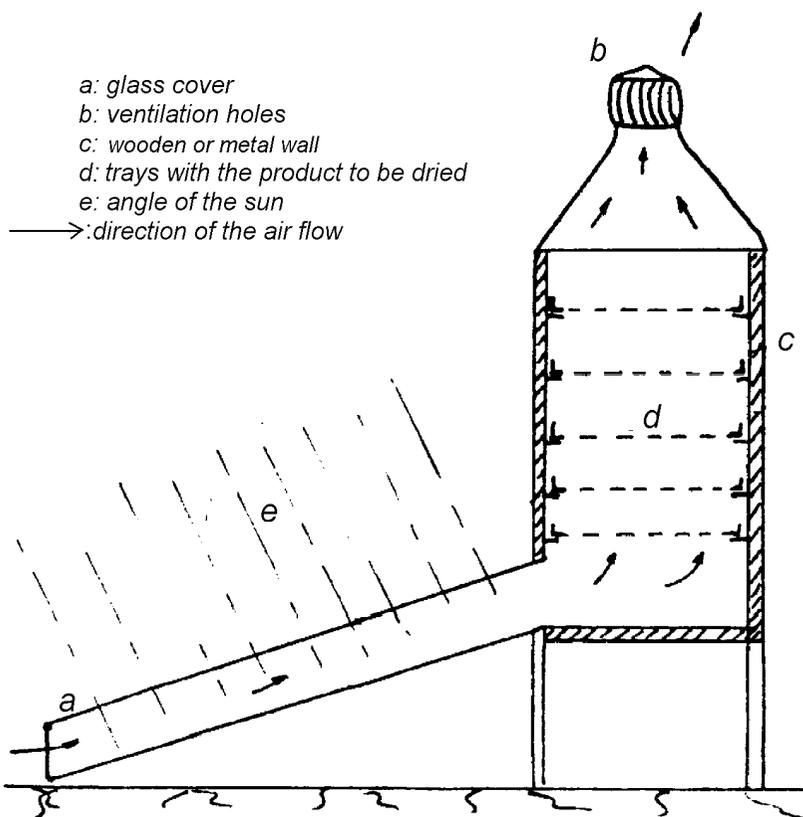


Figure 32: Improved indirect sun dryer

### Drying by ventilation:

A low energy input method of drying is by constructing a simple plastic tunnel and blowing in cold air from one side. The freshest mushrooms should be added downstream, as they lose much water through evaporation.

### Packing and storage

All foreign material should be removed at the end of the drying process. Dried products easily absorb water from the surrounding air because of their low water content, so packing has to take place in a dry

room. It is a good idea to finish drying during the warmest part of the day when the relative humidity is at its lowest. The product can be cooled in the shade and if the work is done hygienically, the cooled products can be packed immediately.

The packing material must be waterproof, airtight and insect-proof. The dried products will only remain good if stored in such a way that they are dry and protected from insects.

Normal plastic bags (properly sealed) will do for some time, but are not entirely gas and waterproof.

It is also possible to use polymer-coated cellophane bags, which are waterproof and airtight. These can be sealed with a hot iron or a sealing machine (where electricity is available). Unfortunately this kind of plastic cannot be easily obtained and is not too strong either.

<p>A plastic bag of thicker quality (polyethylene, 0.05 mm thick) is best. These can be closed tightly with a metal clip or with cellophane tape.</p>
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# Appendix 1: Formulas

## Formulas for media

### *PDA: Potato Dextrose Agar extract medium*

200 g diced potato, 20 g agar powder, 20 g dextrose or ordinary white cane sugar, 1 litre water.

### *Rice bran broth medium*

200 g rice bran, 1 litre water, 20 g gelatine. Boil the rice bran for about 10 minutes in the water. Filter, save the broth and melt the gelatine and pour into bottles and sterilise.

## Formulas for spawn substrate

### *Grain spawn substrate*

Grain in small containers can be moistened to a higher content than grain in 15 litre bags. For 2 litre containers, use the following recipe: 480 g rye, sorghum or wheat, 400 ml water, 2 g gypsum (45% moisture).

### *Sawdust spawn substrate*

Sawdust 10 kg, CaCo<sub>3</sub> 147.5 g, Rice bran 1.25g, Gypsum 0.1475g, Urea 0.5 g, Water 1.5 liter

## Appendix 2: Substrate preparation

### Substrate preparation (weight percentages)

1.	Dry sawdust (moisture content 15-18%)	78 %
	Fine rice bran (first class)	21 %
	CaCO <sub>3</sub>	1 %
2.	Sawdust	94 %
	Rice bran	4 %
	Cornmeal / Wheat bran	1 %
	CaCO <sub>3</sub>	1 %
3.	Sawdust	89 – 73 %
	Corn waste	10 – 25 %
	CaCO <sub>3</sub>	1 – 2 %

Recipes 1- 3 can only be used when the substrate is sterilized. The mentioned supplements like corn meal or bran would contaminate quickly in substrates which have been pasteurized only.

4.	Rice straw	98 %
	CaCO <sub>3</sub>	2 %
5.	Wheat straw	99 %
	CaCO <sub>3</sub>	1 %
6.	Wheat straw	100 %

# Further reading

## *Literature on mushroom cultivation*

**Edible and poisonous mushrooms of the world**, 2003, New Zealand Institute for Crop and Food Research, by I. Hall, et al. ISBN 0-478-10835-4. 370 Pages of general information on mushrooms: which wild mushrooms to pick, how to grow them, poisonous mushrooms of the world, and 250 high quality colour pictures.

**JUNCAO Technology**, 2001, by Z.X. Lin and Z.H. Lin. China Agricultural Sciencetech Press, Beijing. ISBN 7-80167 210-0.

250 pages on the use of different types of grasses for 13 different types of mushrooms, including shiitake, white button and oyster mushrooms. Very promising technology for developing countries.

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**Mushroom biology and mushroom products**, 1993, edited by S.T. Chang, J.A. Buswell and S.W. Chiu. Chinese University Press, Hong Kong. ISBN 962-201-610-3.

Contains the Proceedings of the First International Conference on Mushroom Biology and Mushroom Products in 1993 in Hong Kong, 37 scientific articles on fundamental aspects of mushroom biology, nomenclature of edible mushroom species, cultivation and bioconversion technology, post-harvest treatment, and nutritional and medicinal aspects. An interesting volume for research stations. Two articles deal with the implementation of mushroom projects.

**Mushrooms: Cultivation, Nutritional Values, Medical Effects and Environmental Impact**, second edition, 2004 by S.T.Chang and P.G.Miles. CRC Press ([www.crcpress.com](http://www.crcpress.com)) ISBN 0849310431. \$ 160.

**Mushroom Cultivation, Appropriate technology for mushroom growers**, third edition, 2003 by Peter Oei, Backhuys Publishers, Leiden, The Netherlands. Also available from CTA = no. 1146, 40 credit points. ISBN 90-5782-137-0

**Mushroom Growers' Handbook 1 : Oyster Mushroom Cultivation, 2004.** MushWorld ([www.mushworld.com](http://www.mushworld.com)) Obtainable from Mushworld.

**Mushroom Growers' Handbook 2 : Shiitake Cultivation, 2005.** MushWorld ([www.mushworld.com](http://www.mushworld.com)) Obtainable from Mushworld.

**Shiitake Growers Handbook: The Art and Science of Mushroom Cultivation**, Paul Przybylowicz and John Donoghue. ISBN 0-8403-4962-9 Price: approximately US\$ 25.

Gives detailed descriptions on how to grow Shiitake on both logs and sawdust substrates. It does not address sterile culturing and spawn production, assuming spawn is bought by the growers.

**Growing Gourmet and Medicinal Mushrooms, 2000 by Paul Stamets** third edition 2000 by Paul Stamets, Ten Speed Press, Berkely, United States. ([www.tenspeed.com](http://www.tenspeed.com)) ISBN 00-0242584

#### *Literature on taxonomy and identification of wild mushrooms*

**The atlas of cultivated Pleurotus mushrooms**, by J.T. Peng, et al. 1990. ISBN 957-9055-03-3. Description of the cultivation parameters of 50 different strains of oyster mushrooms from the culture collection of CCRC in Taiwan.

**Edible mushrooms of Tanzania. 1995**, by M. Härkönen, T. Saarimäki, and L. Mwasumbi. ISBN 951-45-6962-8

This work is a combination of ethno-mycological knowledge from Tanzania and modern natural sciences. The book (93 pages and colour pictures) covers the most important edible and poisonous mushroom species. It is the result of four field trips and hundreds of interviews

with Tanzanians. Vernacular names, modes of preparation and an introduction on how to identify mushrooms are all discussed. *KARSTENIA* Vol. 35 suppl. 1995, Helsinki. ISBN 951-45-6962-8.

**An introduction to the larger fungi of South Central Africa**, 1994 by L. Ryvarden, G.D. Pearce and A.J. Masuka. Published by Baobab, Zimbabwe. ISBN 0-908311-52-4

A guide to the most common edible and poisonous mushroom species in Malawi, Zambia and Zimbabwe. 200 pages and colour pictures. Contains more information than *Edible mushrooms of Tanzania*.

**The edible fungi south of the Sahara**, 1993, by J. Rammeloo and R. Walley. A literature survey. *Scripta Botanica Belgica* 5: 1-62.

**The poisonous and useful fungi of Africa south of the Sahara**, 1994, by R. Walley and J. Rammeloo. A literature survey. *Scripta Botanica Belgica* 10: 1-56.

# Useful addresses

## **International society for mushroom science**

ISMS Secretary

PO Box 11171, Centurion, Pretoria 0046, South Africa

Phone: +27 12 665 2210; Fax: +27 12 665 2212

Email: [secretary@isms.biz](mailto:secretary@isms.biz), Website: [www.isms.biz](http://www.isms.biz)

## **Kali Mata Women's Group, Gezaulole, Tanzania**

Women's development centre in Tanzania. This centre has developed a project on mushroom cultivation.

Kaifa Ally, secretary, POBox 36484, Dar es Salaam, Tanzania

Phone: 0744853351

## **Kali Mata Ki Jai! Foundation Netherlands**

Information also in English and Swahili.

Trui Goslinga-Lindeboom Houtlaan 25, 2334 CJ Leiden, Netherlands

Phone: 0031(071)5157279,

E-mail: [kalimata@vrouwen.net](mailto:kalimata@vrouwen.net), Website: [www.vrouwen.net/kalimata](http://www.vrouwen.net/kalimata)

## **Mushroom Business**

Mushroom Business is a two-monthly, international trade journal for the worldwide mushroom industry (growers and suppliers). It features articles on growing techniques, markets and marketing, cultivation tips, research, industry news, opinion, and more. The site of Mushroom Business has links to the major suppliers of mushroom equipment, training etc.

Reed Business Information bv

P.O. Box 16500, 2500 BM The Hague, The Netherlands

Phone: +31 (0)70 441 5060, Fax: +31 (0)70 441 5902

[www.mushroombusiness.com](http://www.mushroombusiness.com)

## **Mushworld: [www.mushworld.com](http://www.mushworld.com)**

Non-profit organisation devoted to poverty alleviation in the world through mushroom growing, especially in developing countries.

**Mycelia:** spawn manufacturer

Jean Bethunestraat 9, 9040 Gent, Belgium

Phone: +32 (0)9 / 228 70 90, Fax: +32 (0)9 / 228 80 28

E-mail: [info@mycelia.be](mailto:info@mycelia.be), Website: [www.mycelia.be](http://www.mycelia.be)

**Spore Mushroom Products / Stichting ECO Consult**

Gargouille 1, 4007 RE Tiel, Netherlands

Phone: + 31 (0)6 515 42 882, Fax 0344 630 225

Website: [www.spore.nl](http://www.spore.nl) Website of the author. Information on special plastic bags for spawn production and on international training activities. E-mail for training courses to [info@spore.nl](mailto:info@spore.nl).

**World Mushroom Society:** [www.worldmushroomsociety.com](http://www.worldmushroomsociety.com)

The objective of the WSMBMP is to promote knowledge related to mushroom biology and mushroom products.

[www.fungitec.com](http://www.fungitec.com), Website in English and Spanish

Advisory, workshops, short courses and mushroom projects.

**ZERI (Zero Emission Research Initiative)**

This initiative promotes sustainable human development in Africa, and also provides information on mushrooms.

ZERI Africa: UNDP/UNOPS Regional Project

University of Namibia, Private Bag 13301, Windhoek, Namibia

Phone: 206 3340, Fax: 206 3505, Website: [www.zeri.unam.na](http://www.zeri.unam.na)

**PUM, Netherlands Senior Experts**

PUM sends senior experts to more than 70 countries in Africa, Asia, the Middle East, Latin America and Central and Eastern Europe. Upon request, PUM's experts offer their skills and experience to businesses and organisations in places where these are most needed. In the course of their careers, PUM's advisers have gained extensive experience in nearly every conceivable field. These experts are independent and work on a voluntary basis (they receive no wages).

P.O. Box 93078, 2509 AB The Hague, The Netherlands

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# Glossary

- Agar:** An extract from a seaweed used to solidify media; alternatively, (cheaper) gelatin may be used. Agar is available in bar or powder form.
- Anaerobic:** Without oxygen (O<sub>2</sub>).
- Aseptic:** Sterile condition: no unwanted organisms present.
- Autoclave:** A container, the contents of which can be heated up to 121°C. It must be able to withstand an over-pressure of 1 bar; otherwise the temperature cannot rise sufficiently.
- Bacteria:** Micro-organisms that may cause contamination in culture work. Grain spawn is very easily contaminated with bacteria.
- Break:** See flush
- Button stage:** The young mushrooms are still fully closed.
- Cellulose:** An organic compound in wood, straw, etc. It is easier to degrade than lignin. Cellulose is probably best known as raw material to make paper. Cotton waste contains high amounts of cellulose; sawdust contains cellulose, hemi-cellulose and lignin.
- Culture:** See mother culture.
- Culture medium:** Micro organisms differ in their nutritional needs. A large number of different media have been developed; PDA-agar and Malt-agar can be used for most cultivated mushrooms.
- Fermentation:** The process of composting. Easily accessible nutrients will be degraded by micro organisms which makes the substrate more selective. Unwanted fermentation may occur if the compost is still very 'active' or if thick layers or large bags are used. In that case the temperature rise inside the substrate will become too high for the desired mycelium.

Flush:	The sudden development of many fruiting bodies at the same time. Usually there is a resting period between flushes or breaks.
Formol:	A 30% solution of formaldehyde used to sterilise areas. <i>The gasses kill living micro-organisms and spores.</i>
Free water:	The actual water available to the micro-organisms in the substrate. Water content is the absolute measure. Free water is related to the water film around each particle in the substrate and the concentration of salts in the water.
Fruiting:	The mycelium will form mushrooms in its reproductive stage. This is called fruiting as the mushrooms are actually the fruiting bodies of the mycelium.
Germination:	The spreading of hyphae from spores.
Gills:	the radially arranged, vertical plates below the cap of a mushroom on which spores are formed.
Hypha, hyphae:	Individual cells of mycelium.
Incubation:	The period after inoculation (preferably at a temperature optimal for mycelial growth) during which the mycelium slowly grows through the substrate
Inoculation:	Transferring an organism into a specific substrate.
Lamellae:	See gills
Lignin:	A difficult-to-degrade organic substance which, together with cellulose, forms the basis of wood, straw, etc.
Micro organisms:	Microscopic organisms which are abundantly present in the air. And stick to every surface.
(Mother) culture:	A pure strain of an edible fungus growing on a medium.
Mother spawn:	Spawn not meant for inoculating substrate, but for inoculating another batch of spawn.

Mycelium:	The network of hyphae that form the vegetative body of the fungus. Mushrooms are the fruiting bodies of the mycelium.
Mycorrhiza:	A symbiotic relationship between fungi and plants.
Parasite:	Organism that lives at the expense of others, usually causing diseases in its hosts. Ultimately it may cause the death of its host.
Pasteurisation:	Heat treatment applied to a substrate to destroy unwanted organisms but keeping favourable ones alive. The temperature range is 60-80°C. The treatment is very different from sterilisation, which aims at destroying all organisms in the substrate.
Petri dish:	A round glass or plastic dish with a cover to observe the growth of microscopic organisms. The dishes are partly filled with sterile growth medium (or sterilised after they have been filled). Petri dishes are commonly used to grow mycelium which will inoculate the mother spawn.
Ph:	A measure to describe acidity of a medium. Ph 7 is neutral; a higher figure means alkaline, lower acidic. Most wood-inhabiting mushrooms prefer a slightly acidic substrate.
Pinhead:	A term to describe a very young mushroom when the cap is pin-sized.
Primordium:	The initial fruiting body.
Pure culture:	An isolated culture of micro-organism without any other micro-organisms. Pure cultures are essential to the spawn production process.
Relative humidity:	The percentage of moisture in the air compared to the maximal amount that the air can hold at that temperature and pressure.
Slant:	A test tube with growth medium, which has been sterilised and slanted to increase the surface area.

Spawn:	Mycelium growing on a substrate used as planting material in mushroom cultivation.
Spawn run:	The period of vegetative growth of the mycelium throughout the substrate after spawning.
Species:	Fundamental unit of biological taxonomy. Generally spoken, two individuals belong to the same species if they can produce fertile offspring.
Spent substrate:	The substrate remaining after the mushrooms have been harvested.
Spores:	The means of reproduction in fungi. In cultivated mushrooms they are formed on the gills and dispersed in the air. One mushroom can produce millions of spores.
Stipe:	Stalk of the mushroom.
Sterile:	Conditions: see aseptic.
Sterilisation:	Destroying (completely) all micro-organisms present, by heat or chemicals. Spawn substrate always has to be sterilized prior to inoculation.
Strain:	A group of individuals within a species. Equivalent to “race” or “variety” in plants.
Subculture:	A culture derived from another culture.
Substrate:	the material in which the mycelium grows.
Test tube:	A tube of thin, transparent glass closed at one end used in chemical and biological experiments.
Tissue culture:	A culture made from the tissue of a young and healthy mushroom.