Production process

i. Spawn (mushroom seeds)

Spawns are readily available in the markets. If desired, the same can be produced and sold commercially. Separate training may be attended to get mastery in spawn production.

Compost preparation: There are several mixtures for compost formation and anyone that suits the entrepreneur can be chosen. Agricultural by products like cereal straw (wheat, barley, paddy, oat and rice), maize stalks, hay, sugarcane bagasse or any other cellulose wastes can be used for compost preparation. Wheat straw is used, should be freshly harvested, shining yellow in colour and should not have been exposed to rains. The straw should be in about 5-8cm long pieces, otherwise heap prepared by long straw would be less compact which may lead to improper fermentation. Conversely; too short straw makes heap too compact to allow enough oxygen to enter the centre of the heap leading to anaerobic fermentation. Wheat straw or any materials provide cellulose, hemicellulose and lignin, which are utilized by the mushroom mycelium as the carbon source. These materials also provide physical structure to the substrate needed to ensure proper aeration during composting for the buildup of microflora, which is essential for the fermentation. Rice and barley straw are very soft, degrade very quickly during composting and also absorb more water as compared to wheat straw. While using these substrates, care should, therefore, be taken on the quantity of water to be used, schedule of turnings and adjustment to the rate and type of supplements. Since the by products used in composting do not have adequate nitrogen and other components required for the fermentation process, compounding mixture is supplemented with the nitrogen and carbohydrates, to start this process. In synthetic compost straw is supplemented with nitrogen nutrients, organic and inorganic matter. In organic compost, horse dung is added. The compost can be

prepared by long or short composting method. Only those who have pasteurizing facility can employ short method. In long method 7-8 turns at regular intervals are required for a period of 28 days. Good compost is dark-brown, ammonia free, little greasiness and having 65-70% moisture.

Spawn in the bottles

For mixing spawn with compost any of the three procedures can be followed:

a) **Layer spawning**: Compost is divided into equal layers and spawns spread in each layer. Result is spawning in different layers.

b) **Surface spawning**: 3 to 5 cms of compost is remixed, spawns spread and covered with compost.

c) **Through Spawning**: Spawns are mixed with compost and pressed. Trays are then arranged in tiers in the cropping room and covered with newspapers. 2% formalin is sprinkled over them. Desired room temperature is around 23^0C with 85-90% humidity.

ii.Casing: Spawned compost is covered with sterilized hay, chalk powder etc.

iii. Cropping: Besides temperature and humidity mentioned above, proper

room ventilation should be ensured. Mushrooms prop up in 30-35 days. Usually 3 to 4 days after opening the bags, mushroom primordia begin to form. Mature mushrooms become ready for harvesting in another 2 to 3 days. These fungal fruit bodies appear in flushes and harvested when buttons are tightly closed. In a cropping cycle of 8-10 weeks an average yield of 10 kg mushroom/sq meter is feasible. An average biological efficiency (fresh weight of mushrooms harvested divided by air-dry substrate weight x 100) can range between 80 to mushrooms, they are grasped by the stalk and gently twisted and pulled. The mushrooms remain fresh up to 3 to 6 days in a refrigerator/cool place. Cropped mushrooms can be packed for marketing.

Spawn production

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

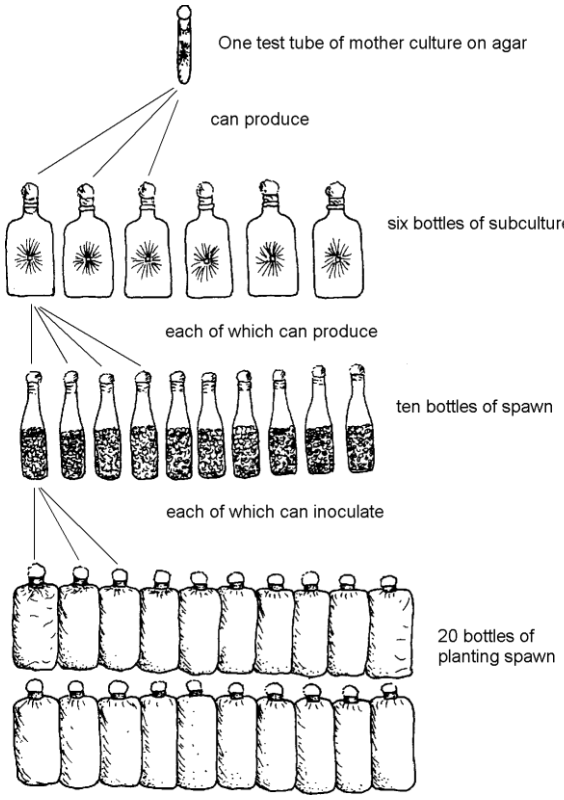
Availability of spawn culture

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.

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.

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.



Multiplication of spawn

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 sterilization 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)

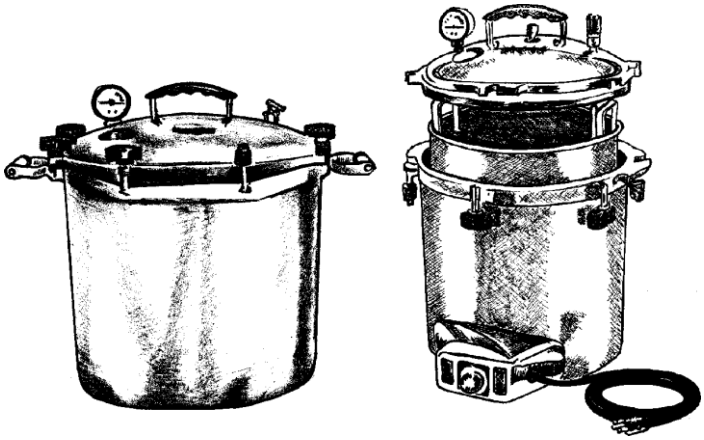
The sterilization processes

- ❖ Grain, sawdust and compost contain large numbers of contaminants.
- ❖ A single grain kernel may contain thousands of bacteria, fungi and actinomycetes.
- ❖ 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 sterilization/pasteurization unit is filled and on the capacity of the burner.

Pressure cookers

The cheapest option is to obtain one or more large pressure cookers. Select pressure cookers that maintain the pressure when the final tem- perature 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.



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.

Clean environments

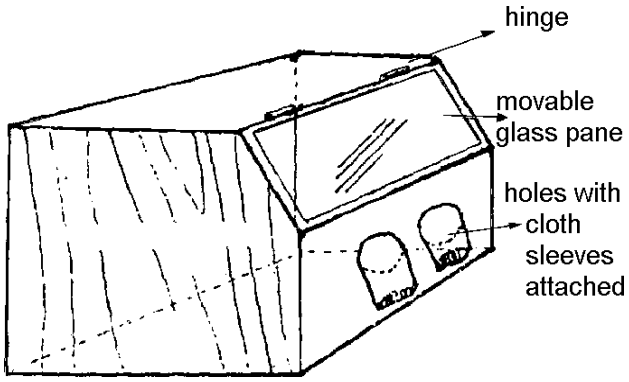
A clean environment is absolutely essential to spawn production. In particular, whenever the containers with sterilised media need to be opened it must be done under aseptic conditions. The air carries nu- merous contaminants, which easily infect the sterilised media. It is therefore necessary to use 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 be- neath can be cleaned easily. Shelves are typically made of galvanised iron or Formica.

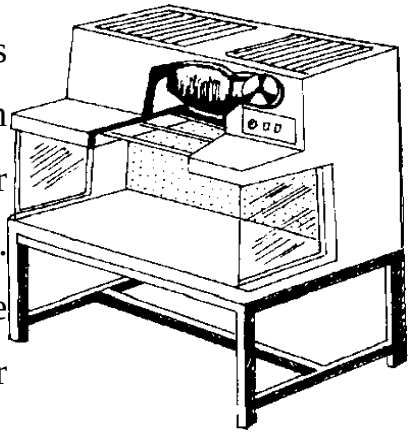
Inoculation cabinets

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 solu- tion,* a *2% Formalin solution* or *70% ethyl alcohol.*



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

Laminar airflow cabinets



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 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.

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.

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.

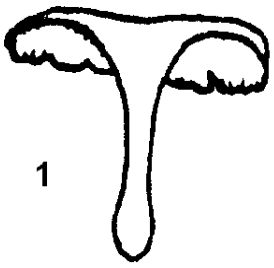
Tissue cultures

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

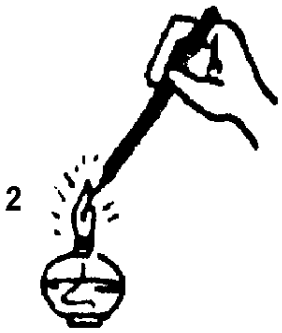
- Take care that no outside surface tissue is included.
- Open the test tube/Petri dish.
- (When using test tubes: heat the mouth of the tube in the flame to kill unwanted spores). Then, gently replace the tissue on the scalpel in the middle of the agar.
- Immediately replace the plug.
- . Inoculate at least three cultures, but preferably more

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 tis- sue and branch out on the agar.

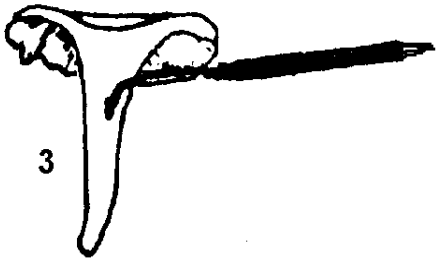
The mycelium should be white and grow out from the tissue. If yel- low, blue, green or grey mycelia form on other places on the surface, then these are fungal contaminants. A creamy, shiny growth often indicates bacterial contamination.



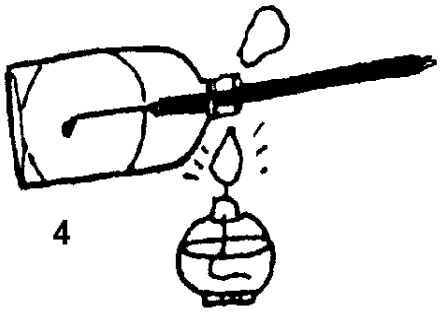
1 Tear the mushroom in half



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

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 (*Pleuro- tus***)** and wood ear mushroom (*Auricularia)*.

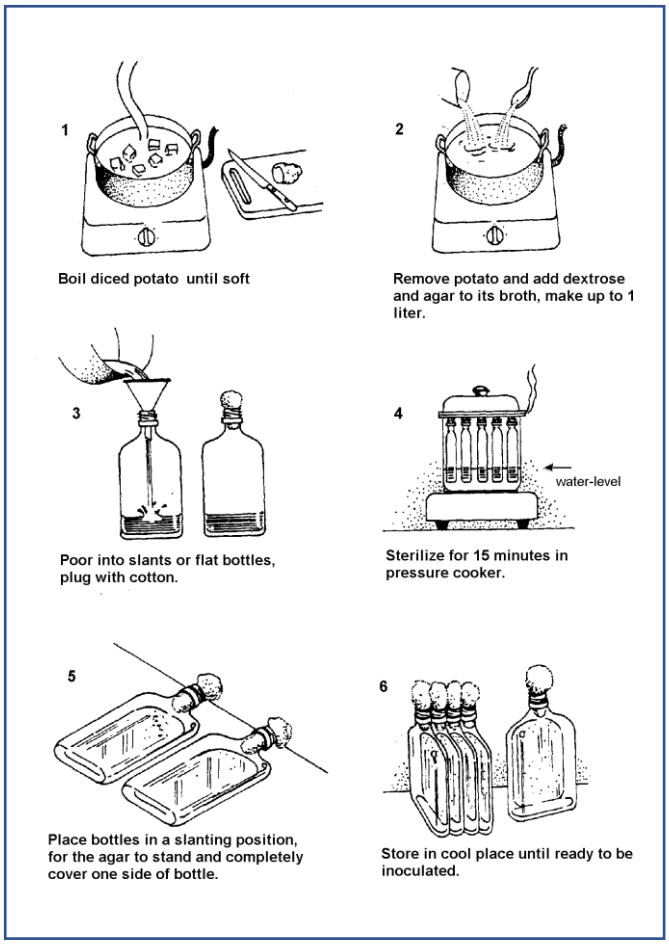
Ingredients: *200 g rice bran, 1 litre water, 20 g gelatine*. Boil the rice bran for about 10 minutes in the water. Filter, save the broth and melt the gelatine and pour into bottles and sterilise.

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 sterilisa- tion units in small-scale laboratories are pressure cookers, but auto- claves can be used as well.

Procedure

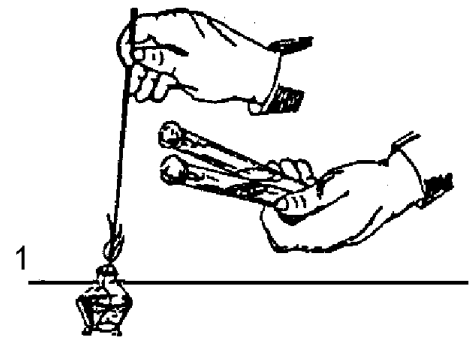
- Pour water into the pressure cooker to the level of the rack.
- Place the bottles/test tubes in the racks with a plastic covering to prevent water from wetting the cotton plugs.
- Then close the lid firmly.
- The air vent should be open at the beginning to allow the air to es- cape. This will take 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.



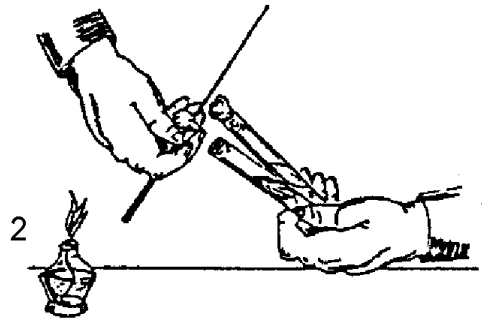
The mycelium will degenerate after a certain number of transfers, so it is not possible to keep on transferring the cultures on agar forever.

Preparation of PDA

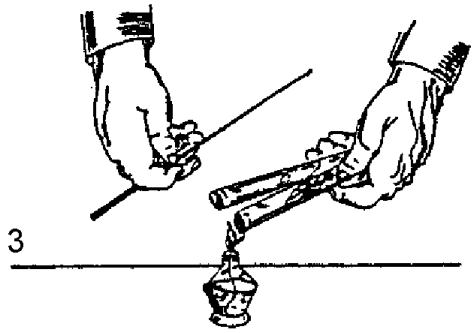
Subculturing



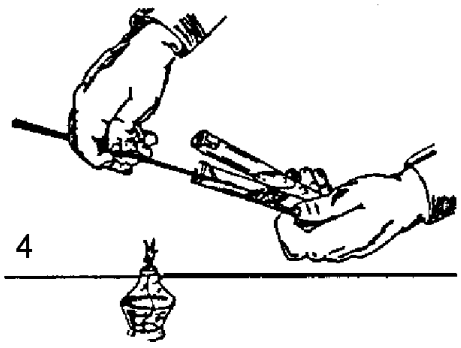
Sterilise the scalpel in the flame until red hot.



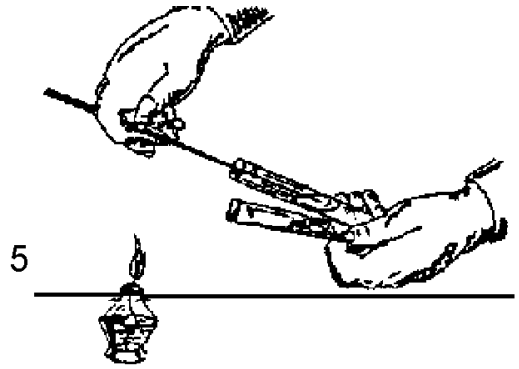
Take the plugs out of the test tubes (meanwhile the scalpel will cool down).



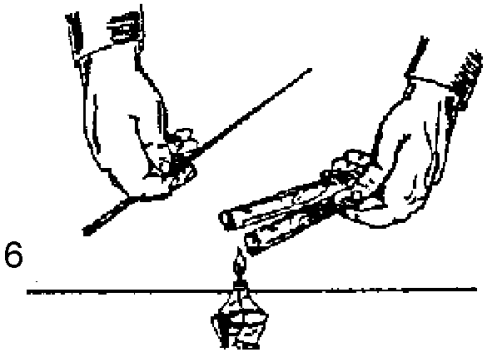
Keep the mouth of both test tubes above the flame.



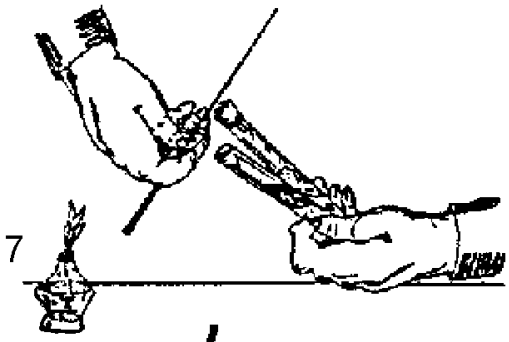
Cut a small square 5 x 5 mm^2 from the "mother" test tube culture.



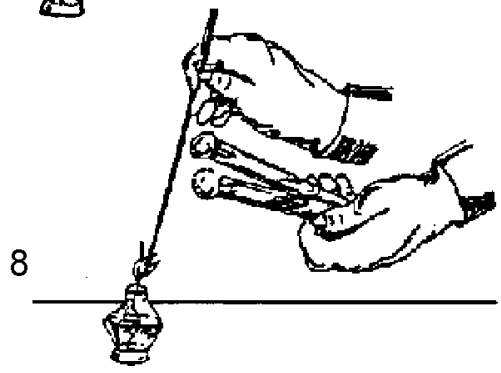
5 Put the square in the middle of the agar of the new test tube.



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



7 Put the plugs back in the test tubes.



8 Sterilise the scalpel again for the next transfer.