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# Cultivation of Mushrooms

## Production of spawn

For the successful cultivation of any mushroom, one of the most important requirements is the seed of that species/variety. **Spawn**- a pure culture of the mycelium grown on a special medium- is the mushroom seed. The production of spawn is done by professionals in the laboratory under controlled conditions of temperature, light and humidity

Mushrooms for spawn production can be grown on sterilized cereal grain (wheat, rye, sorghum or bajra), but usually grain colonized with mycelium (*grain-spawn*) is used as an inoculum for composts. Grain is preferred as a substrate for mushroom spawn because grain gives a large number of inoculation sites, each with a high inoculum potential derived from the nutrient base the outgrowing fungi can utilize. This helps to ensure that the compost is rapidly permeated, which is important for the exclusion of competitors as well as for the rapid production of fruit-bodies.

The media used for maintenance, multiplication and preservation of mushroom culture are: Potato Dextrose agar (PDA), Yeast Potato Dextrose Agar (YPDA), Malt extract agar and Rice bran decoction medium.

Spawn production mainly consists of three steps: (i) substrate preparation; (ii) substrate inoculation; and (iii) incubation of the inoculated substrate for spawn production or growth of the mycelium on the substrate. Preferably fresh spawn should be used for mixing with compost for better results. Protocol for the spawn production is given below:

## Requirements

- Pure culture of mushroom (Source: NCMRT, Chambaghat, Solan)
- Cereal grain (wheat, rye or sorghum)
- Calcium sulfate (gypsum)
- Calcium carbonate (chalk)
- Glucose bottles/milk bottles/polypropylene bags
- Cotton
- Alkathene sheets
- Rings for bags
- Pressure cooker/autoclave
- Laminar flow cabinet
- Incubator/storage room
- Wire gauge balance
- Bunsen burner
- Water

## Procedure

### A. Substrate preparation

1. Take 600 gm of grain (wheat, rye or barley) in 400-600 ml of water in a container.
2. Boil the grain for 15-20 minutes to bring the moisture content of grain to 40-50%.
3. Remove the excess water of grain by spreading the grain in a sieve.

4. Allow the grain to surface dry by spreading over alkathene sheets, in shade, for a few hours.
5. Mix the grain thoroughly with chemicals (e.g. Calcium sulfate and calcium carbonate at 2% and 0.5% respectively on dry weight basis of the grain), to adjust pH of the grain at 7-7.8. The grain must not be coagulated at this stage.
6. Fill the grain-chemical mixture in 500 ml glucose/ milk bottles/polypropylene bags (300-350 g boiled grains/container). However, the first generation spawn (Master spawn) must be prepared in glass bottles due to their convenience in handling for further subculturing.
7. Plug the bottles/containers with non-absorbent cotton.
8. Sterilize the substrate by autoclaving at 121<sup>o</sup> C (15 psi) for 30 minutes.
9. Repeat the process of sterilization after 24 hours of first autoclaving.
10. Allow the substrate container to come to room temperature for making the substrate ready for inoculation.

#### **B. Inoculation of Substrate**

11. Inoculate the substrate (grain in containers) with the mycelium of the mushroom grown on a specific medium by transferring mycelium in agar on the grain under aseptic (sterile) conditions.
12. Shake the containers, after plugging, to distribute fragments of the mycelium.

#### **C. Incubation**

13. Store (incubate) the inoculated containers at 20-25<sup>o</sup>C in darkness for 3 weeks.
14. Shake the containers for an even distribution of mycelium, after a few days of incubation or as soon as mycelium is visible on grain.

### **Results**

Appearance of silky whitish growth completely covering the grain indicates the preparation of spawn.

#### **Storage of Spawn**

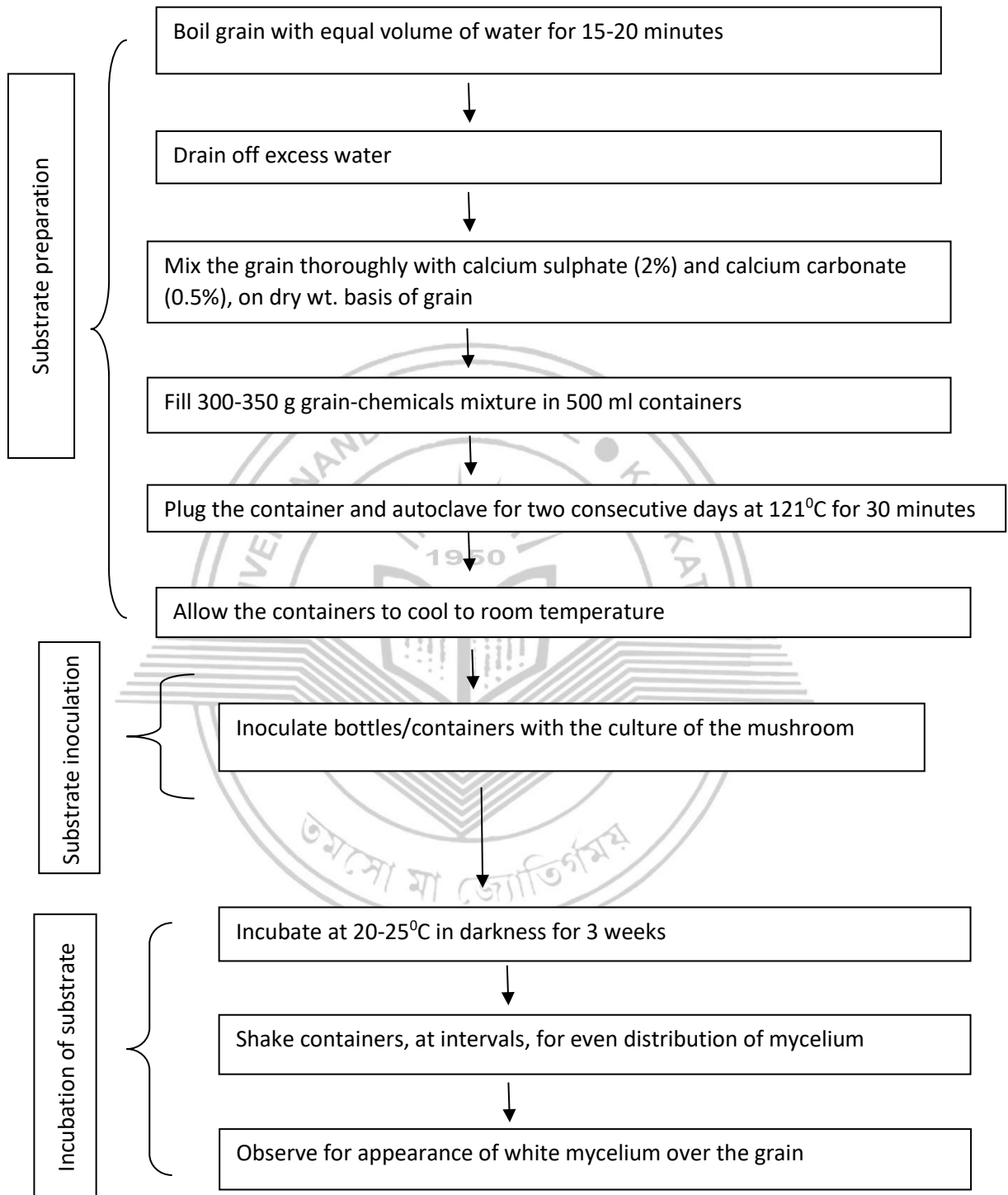
Store the spawn, if not needed immediately, at 0-4<sup>o</sup>C in a refrigerator for a maximum period of 6 months. Spawn, if stored at low temperature, should be allowed to attain room temperature (25<sup>o</sup>C) before being used for spawning the compost.

#### **Transportation of Spawn**

Transport the spawn in refrigerated vans after its purchase or at night when the temperatures are low, as higher temperature (above 32<sup>o</sup>C) is detrimental to mushroom mycelium.

#### **Precautions**

1. Always use healthy and whole grain for spawn making.
2. Remove the damaged grain, weed seeds and inert material before boiling of grain.
3. Never boil the grain for a longer period.
4. Check the master spawn for contamination; contaminated as well as suspected to be contaminated bottles should be discarded immediately.
5. Always keep a check for microbial contamination of the mushroom culture during incubation.
6. Always use cotton plugs to maintain aerobic conditions in the spawn containers.



**Diagram: Protocol for the spawn production**