SEC IV

Mushroom Culture technology –Part II

(Mushroom Spawn Preparation)

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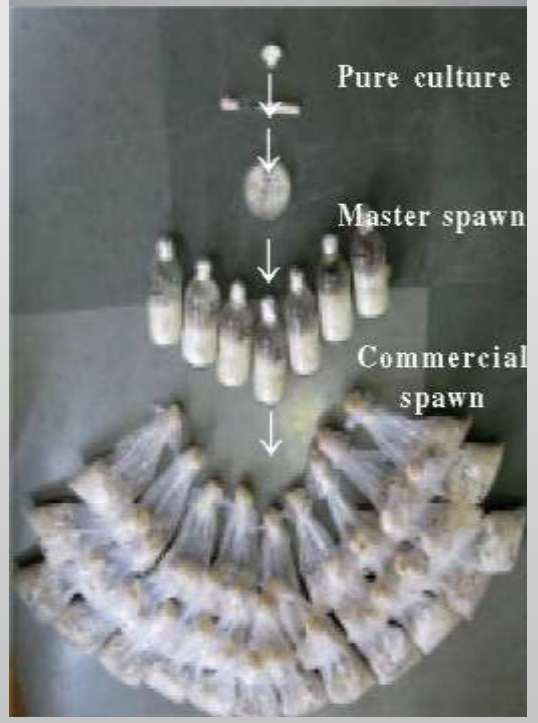


Mushroom cultivation consists of 5 phases

- **Establishment of fruiting culture**
- **Spawn/Seed /inoculum preparation**
- **Compost preparation**
- **Spawn running**
- **Mushroom development**

- **Culture of mushroom (having capacity to form fruit bodies) Stock culture**
- **A medium with mycelia/fungal body**
- **Substrate suitable for growth of mushroom**
- **The phase during which fungal body ramifies within the substrate**
- **Development of primordia / mushroom fruit body under suitable condition**

METHOD OF SPAWN PREPARATION

- There are three steps involved in spawn production:
  - ✓Raising pure culture raising pure culture,
  - ✓Preparation of mother spawn and Multiplication of spawn.
- Preparation of agar media is basic for mushroom cultures.
- There are several types of agar media such as potatoes, malt extract and corn meal.
- Potato- dextrose-agar and malt extract-agar are available in market and could be prepared in laboratory.

MEDIA PREPARATION

PDA medium in petriplate and slants

- Potato Dextrose Agar (PDA)
- We can prepare our own medium from fresh potato:
- Wash and slice about 200 g of potato and place in 1 liter of boiling water in a flask and boil for 15 minutes.
- Filter the potato broth using a piece of cloth.
- Add 20 g of glucose or sucrose and 20 g of agar and adjust the volume to one liter by adding water
- Sterilize the agar mixture and petridishes or test tubes in the autoclave
- Pour the agar carefully in to petridish (20ml) and test tubes (10ml) in the hood
- Keep the test tubes in a slant position so that the agar slants are solidified
- Next day use the agar slants for pure

PURE CULTURE PREPARATION

- There are two ways of raising pure culture
- a. Tissue culture
- b. Spore culture

Cullturing tissue of mushroom

Culturing spores of mushroom

TISSUE CULTURE

Mushroom tissue

- Well grown young mushroom is selected
- Clean the mushroom from any debris with alcohol ☐
- Split the mushroom lengthwise with knife and avoid any contact of the knife with the area we want to take tissue. ☐
- Sterilize the scalpel on aflame and take a small piece of mushroom tissue using forceps. ☐
- Inoculate the mushroom tissue to PDA or MEA media slants or plates aseptically ☐
- In a few day hyphea will grow out from the tissue and covers the entire surface and ☐
- The culture becomes ready for further multiplication.

SPORE CULTURE

- Well developed fruiting body are selected and cut the stalk of the mushroom
- Laid the gills down on a clean typing paper, glass or similar surface
- ☐ After 12 hours most mushrooms have released thousands of spores. ☐
- The spores are collected by spore map techniques. Pick up the spores by the inoculating loop
- ☐ Inoculate the spore to the PDA or MEA slants as in tissue culture under aseptic condition and incubate at room temperature. ☐
- The spores germinate and will form mycelium in a few days.

METHOD OF SPAWN PREPARATION (CONT……)

- The mushroom seed is generally referred to as spawn. ☐
- **Spawn is the mycelium of the mushroom with its substrate**, which is the propagating material, used for initiating mushroom production.
- Its production is a precise laboratory procedure where maintaining sanitation and purity of the spawn are critical. ☐
- Cereal grains and sawdust are common materials used
- for spawn making. Commonly utilized grain types wheat,
- sorghum and millet.
- Grain spawn is made of cooked or soaked grains with small amount

GRAIN SPAWN PREPARATION

- Select clean wheat, wash the grains and soak overnight
- Next day remove the soaked grains and wash gently and drain the water on a sieve
- Check the moisture content by hand; it should not be too dry or too wet
- Mix the grain with 2% calcium sulfate and calcium carbonate mixtures
- Fill the supplemented grain in sterilizable bottles or flasks or poly propylene packets and loosely cover the mouth.
- Then sterilize the bottles in an autoclave at 121 °C for about 15 -30 minutes or for one hour in pressure cooker.
- Take out the bottles from the autoclave and let it to cool for a day.

PREPARATION OF MOTHER SPAWN

- After sterilization and cooling, inoculate the bottles with pure culture by taking a piece of agar with the mycelium.
- Mix the culture and the grains by shaking to uniformly distribute the mycelium.
- Write the name of the species and the day of inoculation.
- Incubate the inoculated bottles in incubator or at any clean table that maintains 25°C.
- After 15-20 days the grain is fully covered with the mycelium

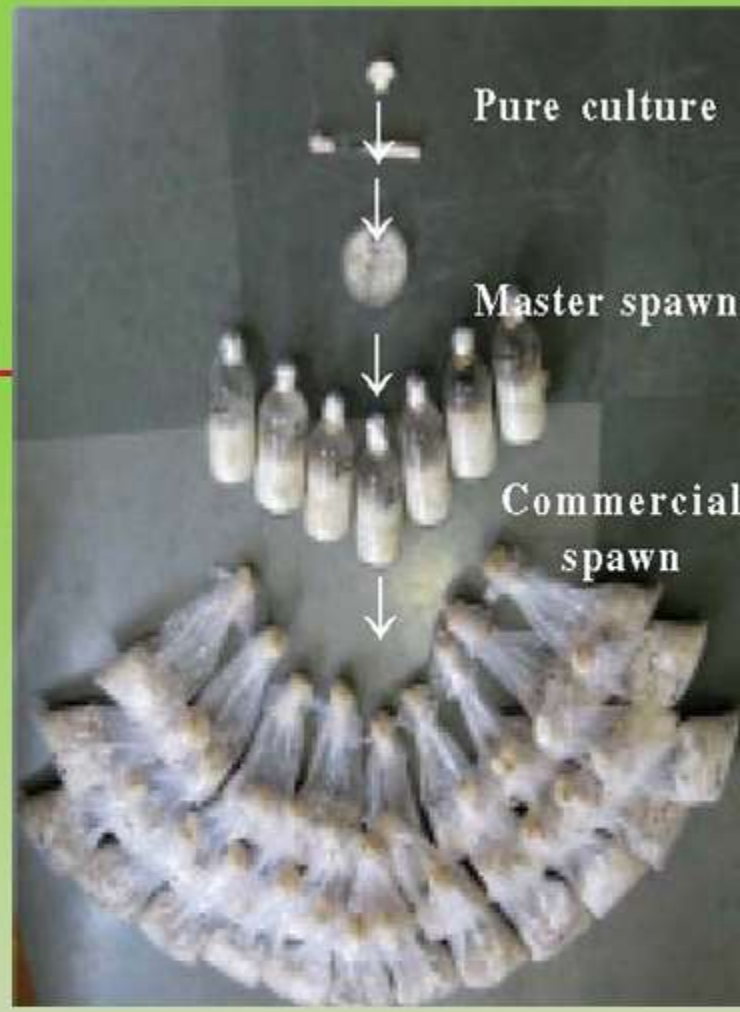
During spawn preparation wheat grains become covered with off white mycelia within 12-14 days and consider ready to use as spawn

Stock culture inoculation under aseptic Hood

Spawn in polypropylene packet

MULTIPLICATION OF MOTHER SPAWN

- Select well grown mother spawn
- Open the mother spawn bottles on a flame and stir the spawn using sterilized forceps to get the individual grains.
- Transfer few grains with the mycelium in to sterilized substrate bottles under aseptic conditions and cover the mouth.
- Mix the grains by shaking to uniformly distribute the mycelium.
- Incubate the inoculated bottles at 25°C till all the grains is covered with the mycelium. Inspect the bottles regularly and discard contaminated ones.
- Within 10-15 days of inoculated mycelial growth covers the entire substrate and the **spawn is ready for use on mushroom beds/compost.**



Multiplication of mother/master spawn