



2

MUSHROOM SPAWN (SEED) PRODUCTION/PROCUREMENT

In the first lesson we have learnt about different types of mushroom and basic steps involved in their cultivation. We came to know that seed of mushroom is called spawn and producing or procuring good quality seed of mushroom is the first step in cultivation of mushroom. Many big commercial units make their own spawn. However, small scale growers normally purchase it. While purchasing, transporting and storing seed of any crop, we take precautions like selecting the correct variety, checking the quality and transport and store it in a way that its quality is not affected. Same is true for mushroom seed. We will discuss about these aspects in the current lesson.



OBJECTIVES

After reading this lesson you will be able to:

- describe the steps involved in preparation of grain spawn;
- identify the equipments required in spawn lab;
- take precautions during purchase, transport and storage of spawn.

2.1 SPAWN PREPARATION

Before starting spawn production, we can prepare or procure the pure culture of mushroom. Culture of mushroom is the fungus obtained from the mushroom fruit body under sterile conditions. We have equipment Laminar Flow/Clean air station that gives sterile air. You will see further that we use autoclaves to sterilize grains. Hence, the spawn is produced in the lab and cannot be made in open like in other crops.



Notes



Fig. 2.1: Taking mushroom from pileus and stipe junction for tissue culture

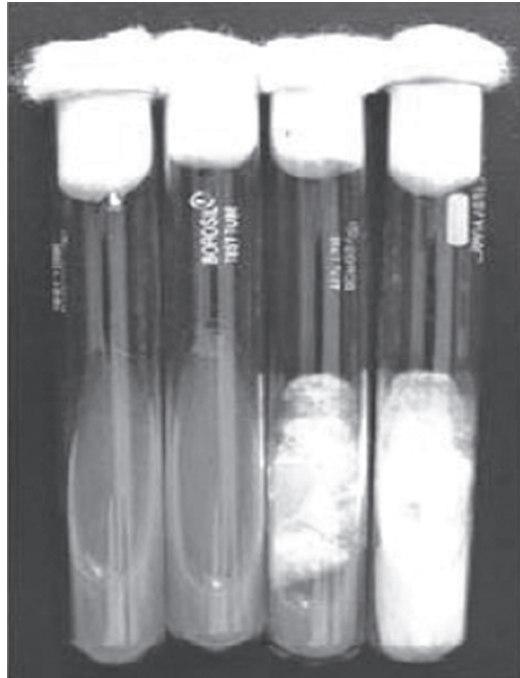


Fig. 2.2: Growth of the part of fruit-body into mycelium after transfer to test tubes on media

To prepare the pure culture we take a mushroom (Fig. 2.1) or its spores and allow growing it artificially in a tube containing the culture media (Fig. 2.2). The tubes have media like Potato dextrose agar, malt extract agar, etc. The media is made free from living organisms (i.e. sterilized) and all the work of preparing cultures is done under sterile conditions. For preparation of spawn, the fungus from the culture is then grown on grains or any other suitable medium.



- Cereal grains like wheat, sorghum, pearl millet, etc can be used for making spawn. Wheat grains are the most commonly used substrate. The wheat grains are boiled partly or half cooked after soaking overnight so that the grains become slightly soft but do not burst. After this grains are kept on a sieve to drain out the excess water and are allowed to dry for evaporation of surface water (Fig. 2.3).

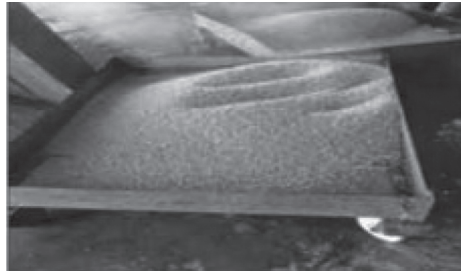


Fig. 2.3: Boiled grains on sieve

- Next step is to add chalk/calcium carbonate (0.5%) and gypsum (2%) mix thoroughly (Fig. 2.4) and fill in bottles/ polypropylene bags. However, now a day's only calcium carbonate is enough to mix in the grains and its mixing maintains the desired pH.



Fig. 2.4: Grains mixed with chalk & gypsum

- The bottles are plugged using plugs made of non-absorbent cotton. These days most of the growers use polyfill (a synthetic material which you must have felt in soft pillows) instead of cotton as it is cheaper and gives better results (Fig. 2.5).



Fig. 2.5: Plugging of bottles/ bags



Notes

- These filled bottles/bags are sterilised in special equipment called autoclave. Autoclave can be imagined as a big pressure cooker. The bottles/bags are autoclaved for 2.5 hour at pressure of 22 pounds per square inch (psi). The temperature inside the autoclave reaches to about 127.6°C. There is pressure gauge attached to the autoclave and you can directly read the pressure.
- After autoclaving is complete and it has cooled down, the bottles/bags are taken out, cooled and inoculated with mushroom culture and this operation is done in laminar flow (an equipment that has filters so that only sterile air comes out) or under totally sterile conditions (Fig. 2.6).

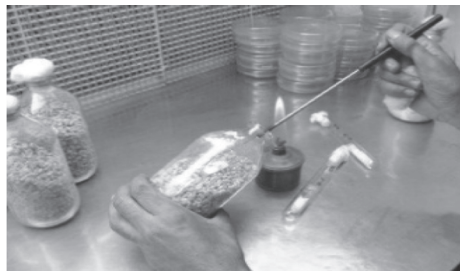


Fig. 2.6: Inoculation

- After inoculation, the bottles are incubated (kept) at 25°C for 2-3 weeks when the mushroom mycelium fully colonizes (covers) the wheat grains (Fig. 2.7). To begin with, spawn is prepared in bottles using the culture grown in the test tube and this is called Mother Spawn. The first generation grain spawn prepared in glass bottles is Mother Spawn.



Fig. 2.7: Mother spawn

- Mother spawn is used to make commercial spawn which is prepared in polypropylene (PP) bags (Fig. 2.8). That is, few grains of readymade spawn are added in each bag (Fig. 2.9). It is not recommended to multiply spawn to spawn beyond two generations. Polypropylene bags are used as these can be

Mushroom Spawn (Seed) Production/Procurement

autoclaved and are cheap and easy to transport (normal plastic bags will melt). These are then incubated for 3 weeks (Fig. 2.10). In between we inspect these bags, shake once or twice and remove bags that might have gone bad/contaminated.



Notes



Fig. 2.8: PP bags



Fig. 2.9: Spawn to spawn



Fig. 2.10: Incubation

Normally it takes 15-20 days for complete spread of mycelium on the grains. Major steps in preparation of spawn have been shown in Fig. 2.11.



Notes

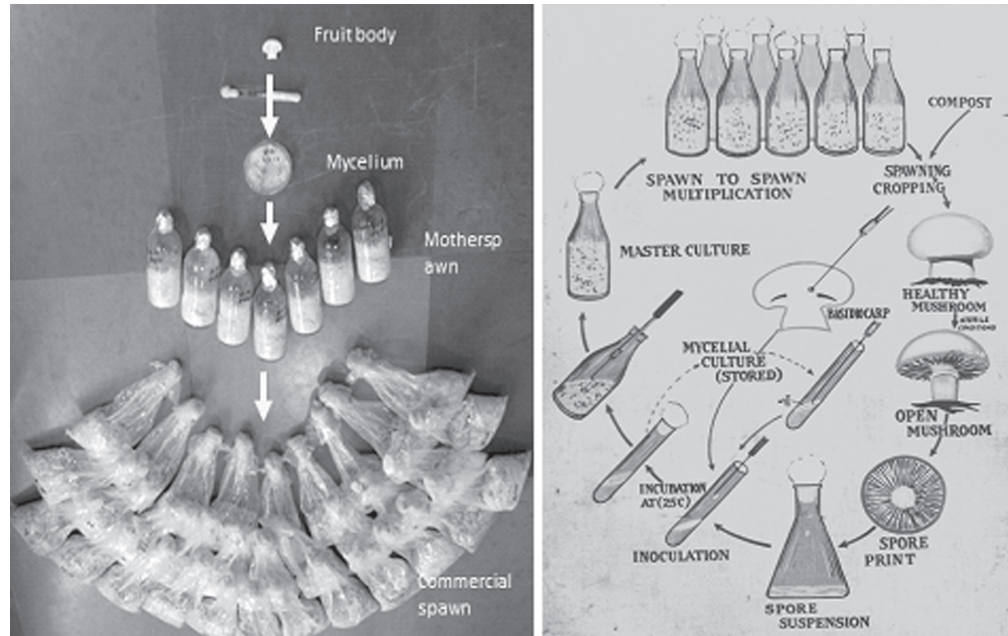


Fig. 2.11: Major steps in spawn multiplication

Some of the important precautions while preparing spawn are:

1. Always keep the inoculation chamber (area where we prepare culture or transfer culture from tube to bottles or from bottle to bags) and its surroundings very clean.
2. Switch on UV tube in the inoculation chamber for 30 minutes before inoculation by keeping sterilized substrate, forceps, etc. inside the chamber. Switch off UV tube 15 minutes before you enter the inoculation room.
3. Inoculation should always be done near the spirit lamp flame to avoid contamination.
4. You should swab your hands and clean inoculation area using alcohol.
5. Mother spawn should not be used beyond two generations as it starts to degenerate. Fresh spawn gives higher yield; therefore, spawn may not be stored for more than a month.
6. Shake bottles and bags after 10 days or so to ensure uniform white silky growth and also to remove contaminated bottle (that is bottles in which we see green, black or other unwanted growth which is due to entry of spores of other fungi despite our precautions).
7. All the bottles must be labelled indicating firms name, species, variety, date of inoculation to know the age and type of spawn.



INTEXT QUESTIONS 2.1



Notes

State True or False

- (i) Mother spawn is the spawn produced from pure culture in test tubes.
- (ii) Poly-fill can be used instead of non-absorbent cotton for plugging the bottles.
- (iii) Pure culture can be prepared either from fruit-body of mushroom or its spores.
- (iv) Mother spawn should not be used for spawn to spawn multiplication beyond 2-3 generations.
- (v) When the pressure in autoclave is 22 lb psi, the temperature inside is 127.6° C.

2.2 EQUIPMENTS REQUIRED IN SPAWN LAB

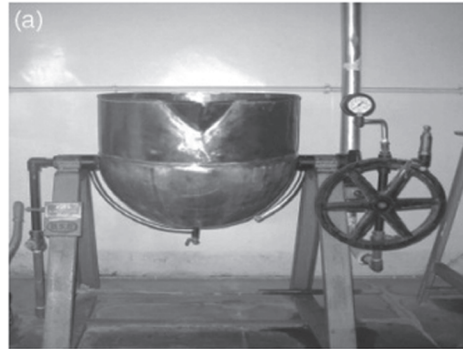
Different equipments are required in a spawn laboratory (Fig. 2.12). These include:

1. Boiling pans/boiling kettle (vessel) for boiling the grains. We may boil grain in big utensils or boil in steam operated kettle if baby boiler is available. Otherwise kettles working on electricity, kerosene or gas can be installed.
2. Gas stove or steam line for boiling of wheat grains and preparation of medium.
3. A digital pH meter to check pH of the medium.
4. Autoclave for sterilization of spawn/medium and oven for sterilization of glassware. Two electrically operated autoclaves with 100-145 bottle capacity having a diameter of 2½' and height of 3¼' are sufficient. If boiler is available, steam operated autoclaves can be used for better efficiency. A small clinical autoclave can also be kept for sterilization of culture medium.
5. BOD (Biological Oxygen Demand) incubator is needed to incubate cultures.
6. Laminar flow cabinet (normally 4 ft. horizontal) is needed for isolation and multiplication of cultures and spawn.
7. Refrigerator is needed for short-term preservation of mycelial cultures.
8. Other items like glassware, chemicals for medium preparation, non-absorbent cotton, polypropylene bags (or bottles), disinfectant (formaldehyde), calcium carbonate, calcium sulphate are also required.

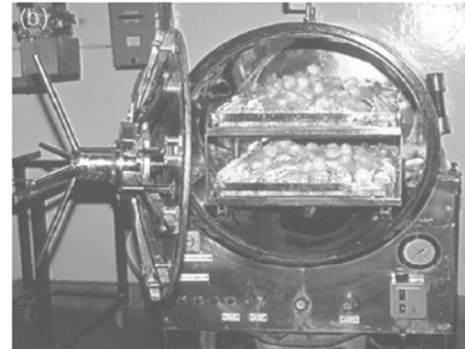


Notes

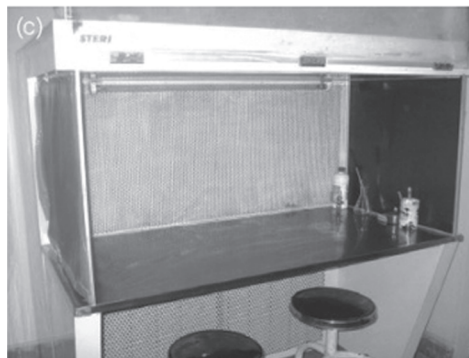
9. Steel racks in incubation room and cold storage for keeping bags/ bottles, exhaust fans, filters, office table, working tables, troughs, sieves, inoculating needles, scalpels, test tubes, Petri plates etc. are also required.
10. Air Handling Units (AHUs) fitted with HEPA filters can be installed for creating positive pressure in spawn lab, particularly in inoculation area. Similarly, air curtains are desirable to keep aseptic conditions in the lab.



Boiling kettle



Autoclave



Laminar flow



BOD Incubator

Fig. 2.12: Equipments required in spawn lab



INTEXT QUESTIONS 2.2

State True or False

- (i) A pH meter is used to sterilise the grains.
- (ii) BOD incubator is used for getting sterile air.
- (iii) Laminar Flow Cabinet is used to store cultures.
- (iv) Baby boiler is must for boiling the grains.



2.3. SPAWN STORAGE

Fully colonized bags, that is, the bags in which mycelium has covered all the grains and spawn is ready to use, can be stored in cold room (+4-6°C) for 1-2 months for future use. The spawn of button mushroom, *Pleurotus* can be stored at this temperature. However, neither the culture nor spawn of *Volvariella*, *Ganoderma* and *Calocybe* is stored below 15°C (Table 2.1). The contaminated bottles/ bags/ tubes etc. are autoclaved before emptying and discarding. Wherever possible, freshly prepared spawn should be used because the mycelium is in the state of active growth.

Table 2.1. Temperature requirement and storage and incubation of different mushrooms

	<i>Agaricus</i>	<i>Pleurotus</i>	<i>Lentinula</i>	<i>Volvariella</i>	<i>Calocybe</i>
Days for complete colonization of mother spawn	20-21	8-12	20-22	6-8	15-17
Days for complete colonization in commercial spawn	12-14	8-10	15-16	5-7	12-14
Incubation temperature (°C) during colonization	25	25	25	32	25
Storage temperature (°C)	4	4	4	>15	>16
Shelf life of spawn	Two months	One months	Three months	< 15 days	15 days



INTEXT QUESTIONS 2.3

State True or False

- (i) Culture of paddy straw mushroom and milky mushroom is stored at 20°C.
- (ii) Spawn of paddy straw mushroom and milky mushroom can be stored for two months.
- (iii) To prevent build up of contaminants, the contaminated bottles/ bags/tubes etc. are autoclaved before emptying and discarding.
- (iv) Spawn of paddy straw mushroom needs 5-7 days for complete colonisation of grains.



Notes

2.4 SPAWN TRANSPORT

Most of the growers procure spawn from government or private labs specializing in spawn production. It is not only important to purchase good quality spawn, but it is also important to transport and store properly. Many a times we may commit mistake in transport and storage and then blame the spawn producer.

The mature spawn bags, that are polypropylene bags with grains fully colonized by mycelium, should be packed in well ventilated cardboard cartons and stored at 4°C wherever applicable. The spawn is transported from one place to another in refrigerated vans or during night when temperature does not rise above 32°C. It is important that spawn bags are not exposed to heat and dust during transport.

Starting spawn production is one of the vocations and is first step in mushroom cultivation. However, it is not necessary that every mushroom grower should make his or her own spawn. However, while procuring, transporting and storing the spawn there are some important aspects that should be kept in mind.

- We should prefer to use freshly prepared spawn because the mycelium is in the state of active growth.
- We should inspect all the bags to ensure that no bag is contaminated, that is, having green, black growth of other fungi.
- We should record name of the strain, when it was inoculated and on which date it was ready, etc.
- We may pack the mature spawn bags in well ventilated cardboard cartons.
- We may transport spawn from one place to another in refrigerated vans or during night when temperature does not rise above 32 °C.
- It is important that spawn bags are not exposed to heat and dust during transport.
- The spawn can be stored in any cool place away from dust but should not be frozen.
- We should also ensure that the cotton plugs are opened only when the spawn is to be used.
- During spawning if we notice some bag with contamination, it should be removed and hands sterilised before continuing with the spawning.



INTEXT QUESTIONS 2.4

State True or False

- (i) While purchasing we should inspect the spawn by opening the plugs.
- (ii) During spawning if we notice some bag with contamination, the contaminated part can removed and rest of the spawn may be used.
- (iii) As far as possible, freshly prepared spawn should be used.
- (iv) While transporting spawn, it may not be exposed to temperature above 32°C.



WHAT YOU HAVE LEARNT

Let us recapitulate the important points we have learnt in this lesson:

- Pure culture of any mushroom can be made using part of tissue from stipe – pileus junction of its fruit-body or from its spores.
- Mother spawn can be prepared by inoculating pure culture to sterilized wheat or other type of grains in bottle.
- Mother spawn can be used for spawn to spawn multiplication.
- Commercial spawn is usually made in polypropylene bags.
- Incubation time & temperature, time for colonisation and temperature at which spawn can be stored is different for different mushrooms.
- While purchasing spawn it is important to see that bags are fully colonized and free from contaminants.
- Bags should be transported at low temperature and may not be exposed to dust.
- Bags may be stored in cool place and opened only when these are to be used for spawning. Any contaminated bag, that is bag having black or green patches or foul smell, may be discarded.



TERMINAL EXERCISE

1. Write down the steps involved in preparation of grain spawn.
2. Enlist the equipments required in spawn laboratory alongwith their use.



Notes

3. What are the grains that can be used for making spawn?
4. What are the materials that can be used for making plugs in place of absorbent cotton?
5. What precautions may be taken while purchasing spawn?
6. What are important precautions required during transport and storage of spawn?



ANSWERS TO INTEXT QUESTIONS

2.1

- (i) True (ii) True (iii) True (iv) True (v) True

2.2

- (i) False (ii) False (iii) False (iv) False

2.3

- (i) False (ii) False (iii) True (iv) True

2.4

- (i) False (ii) False (iii) True (iv) True

SUGGESTED ACTIVITY

Collect information about government and private organisations supplying spawn in your area. If possible, visit any nearby spawn lab and enquire about species of which spawn is prepared and their rates.

Key Learning Outcomes

- Produce quality spawn for different mushroom.
- Procure mushroom spawn from authentic source.