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MUSHROOM SPAWN PRODUCTION TECHNOLOGY

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General requirements

In general, the layout plan of a spawn laboratory should have a total built up area of 19x8x3.6 m (LxBxH). This area will be divided into different work areas like cooking/autoclaving room, inoculation room, and incubation room, washing area, store, office and one cold storage. Cold storage room of 3x3 x3.6 m (LxBxH) is enough to store the spawn at 4-5°C. The walls, roof floor as well as door is provided with heavy insulation (7.5-10 cm thickness) and two air conditioner (each of 1.5 tonnes capacity) are required to maintain the temperature inside the room. Incubation room 2 (3x6.0x3.6 m, LxBxH) with entire surface area (wall, floor, ceiling, doors) insulated with 5-7.5 cm thick insulation is required. Two air conditioners (each 1.5 tonnes capacity) are required for maintenance of temperature (25°C) in the incubation room. Besides these, some ancillary structures like office, small lab space, delivery area etc. may also be required.

The equipment and other miscellaneous items required in a spawn laboratory are:

- ¶ **Big Autoclave (Horizontal type):** It is required for the sterilization of grain bottles and substrates filled in polypropylene bags for producing spawn and also the non-composted substrates for production of specialty mushrooms.
- ¶ **Small Autoclave (Standing type):** It is for the sterilization of culture media in tubes / flasks and the substrates, including grains for production of Master culture and spawn in glass bottles / PP bags on a small scale.

- ¶ **Pressure Cooker:** A big size pressure cooker (5-10 litre capacity) will be required for sterilization of media for routine laboratory work.
- ¶ **Boiler:** Baby boiler run by wood fuel, electricity or diesel will be required for production of pressure steam for boiling, sterilization of grains and pasteurization of casing mixture.
- ¶ **Laminar Flow:** It is required for isolation of fungi and inoculation of grain bags / bottles with master cultures under aseptic conditions.
- ¶ **Weighing Machine:** Weighing machines is necessarily required for the exact measurement of raw materials for producing spawn and compost.
- ¶ **Steel or cemented racks:** Racks are required in the incubation and storage rooms on which the inoculated bags are to be kept at a particular temperature for mycelial run and their storage at different temperatures.
- ¶ **Steel Trolleys:** About 5-6 pushing type steel trolleys will be required for easy movement and carriage of grain bags, spawn bottles, compost bags and other materials from one room to another room.
- ¶ **BOD Incubators:** These are required to incubate cultures inoculated or transferred in tubes, Petri dishes, flasks and Master culture bottles for their speedy growth at a fixed temperature.

- Ñ **Oven:** The oven is required for the sterilization of glasswares, including Petri plates, pipettes, beakers, glass tubes etc.
- Ñ **Refrigerators:** In order to maintain purity of the fungal cultures for a considerable period, these are to be kept in the refrigerators in a cool environment.
- Ñ **Wire mesh Tray:** One or two wire mesh trays will be required for removing excess water from boiled cereal grains or the boiled substrates like straw or sawdust used for mushroom production.
- Ñ **Boiling pans/boiling kettle/Gas/ kerosene stove or electric stove:** These are required for boiling the grains/ preparation of media.
- Ñ **pH meter** to check the pH of the medium.
- Ñ **Microscope** for diagnosis of microbial contaminations and infections.
- Ñ **Hot plate/ Heater:** It is used to heat the media and boil the contents of culture media.
- Ñ **Glassware** viz., Petridishes, test tube, culture tube, beakers, funnels, measuring cylinders' glucose bottles, Glass slides, cover slip and conical flasks.
- Ñ **Chemicals** for medium preparation, calcium carbonate, calcium sulphate and disinfectant (formaldehyde) etc.
- Ñ **Furniture** like steel racks in incubation room and cold storage for keeping bags/bottles, exhaust fans, filters, office table, working tables etc.

- Ñ **Other miscellaneous items** like Bunsen burner, inoculating needle/ loop, non-absorbent and absorbent cotton, polypropylene bags (or bottles), rubber band, sieves, inoculating needles, scalpels, culture tube rack, tripod with asbestos mat, butter paper sheets, muslin cloth, Petridish can, wire basket, plastic mist sprayer, razor blades, cork-borer, forceps, scissors, troughs etc. are also required in a spawn laboratory.

Spawn (mushroom seed) is the vegetative mycelium from a selected mushroom cultured on a convenient medium/ substrate like wheat, pearl millet, sorghum grains, rye etc. In simple words spawn is grains covered with mushroom mycelium. Mixing of spawn in compost or substrate is known as spawning. The spawn production technology can be divided into following steps.

Substrate preparation

Spawn can be prepared on any kind of cereal grains like wheat, sorghum, pearl millet and on agricultural wastes like corn cobs, wooden sticks, rice straw, saw dust and used tea leaves etc. Spawn substrates should be free from diseases and should not be broken, old and damaged by insect pests. Most of the cereal grains are good substrate for spawn production of white button mushroom (*Agaricus bisporus* and *A. bitorquis*), oyster mushroom (*Pleurotus* spp.) and paddy straw mushroom (*Volvariella volvacea*), but wood rotting fungi like shiitake (*Lentinula edodes*) and black ear mushroom (*Auricularia* spp.) grow better on saw dust based substrates over cereal grains.

Table 1. Temperature requirement for storage and incubation of different mushrooms

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

Spawn Preparation

Preparation of master stock or mother spawn

Step-1 Select healthy and clean wheat grains

Step-2 Boil grains in water (15-20 min.)

Step-3 Remove excess water on sieve

Step-4 Dry grains in shade (4 hrs)

Step-5 Mix CaCO_3 (0.5%) and CaSO_4 (2%) on dry wt. basis

Step-6 Fill 300 g grains in glucose/milk bottle

Step-7 Plug and autoclave at 22 p.s.i. for 1.5 to 2 h

Step-8 Inoculate growing mycelium of desired strain using laminar flow

Step-9 Incubate in BOD at $23 \pm 2^\circ\text{C}$ for 20-25 days (shake bottles after 10 days)

Step-10 Master stock is ready

Fig. 1. Flow chart for preparation of master stock or mother spawn

Preparation of commercial spawn

Use polypropylene bags instead of bottle

Up to autoclaving (**Step 1 to 7**) is same as of mother spawn

Step-8 Inoculate with 10-15 grams of mother spawns per PP bag (500g grain)

Step-9 Incubate at $23 \pm 2^\circ\text{C}$ in incubation room (Shake bags after 7-8 days)

Step-10 Commercial spawn is ready in 2-3 weeks

Fig. 2. Flow chart for preparation of commercial spawn

Liquid Spawn

In this, mycelium cultured in liquid medium followed by maceration/ homogenization can also be used for spawning. This is commonly referred as liquid spawn. It can be used for mechanizing inoculation process of spawn multiplication or can be used for inoculating substrates. Strandy cultures showing good

growth and not showing fluffy growth, sectoring or slow growth are desirable.

Mushroom Spawn Standards

Spawn standards as such have not been set out in India. Pure culture can be equated to nucleus seed, master spawn to breeder seed and commercial spawn to foundation seed.

Following standards appear reasonable based on researches.

1. Pure culture (Nucleus seed)

- Culture should be obtained from authentic source.
- The culture should be genetically pure and true.
- Free from any kind of fungal and viral contamination.
- Culture should indicate specific growth rate on defined medium and at defined temperature.
- Visually the culture should be strandy and off white in colour in *Agaricus*, pure white and thick fluffy growth in *Pleurotus*, cottony fluffy with brown sclerotia (after 12-15 days) in *Volvariella*, pure white, dense, thick and fluffy growth in *Calocybe indica* and pure white later on turning to light brown pigmentation in *Lentinula edodes*.
- Culture should be stored at 4-6°C for *Agaricus*, *Pleurotus* and *Lentinula* and between 18-22°C in *Volvariella* and *Calocybe indica*.
- The incubation temperature should be between 32±2°C for *Volvariella* and *Calocybeindica* and 25°C for *Agaricus*, *Pleurotus* and *Lentinula*.

2. Master spawn (Breeder seed)

- It should be produced in autoclavable transparent glass bottles
- Breeder seed should always be prepared from pure culture.
- Free from any kind of contamination.
- It should be multiplied on wheat, sorghum, pear millet or barley grains.
- Breeder seed should be incubated at 25±2°C for *Agaricus*, *Pleurotus*, *Lentinula* and 32±2°C for *Calocybe indica* and *Volvariella*.
- The master spawn should be stored at 4-6°C for 40-45 days in *Agaricus*, *Pleurotus*,

Lentinula and at 18-20°C for 30-40 days in *Calocybe indica* and *Volvariella*.

3. Commercial spawn (Foundation seed/ Certified spawn)

- It should always be prepared from master spawn (Breeder seed).
- It should be multiplied on wheat, sorghum, pear millet or barley grains.
- It should be free from any kind of contamination.
- The incubation temperature should be 25±2°C for *Agaricus*, *Pleurotus*, *Lentinula* and 32±2°C for *Volvariella* and *Calocybe indica*.
- Spawn should not be older than 60 days in *Agaricus*, 30-45 days in *Pleurotus*, *Lentinula* and 30-40 days in *Calocybe indica* and *Volvariella*.
- Certified spawn should be stored at 4-6°C in *Agaricus*, *Pleurotus* and *Lentinula* and 18-20°C in *Calocybe indica* and *Volvariella*.
- Commercial spawn may not be used for further multiplication of seeds as it may lead to higher contamination and decline in yield. Fresh master spawn (breeder seed) should be used for every new lot of commercial seed.
- The bag should indicate lot no., date of inoculation, variety/strain and quantity.

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