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Mushroom: Culture preparation and spawn production technology

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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. It is a medium that is impregnated with mycelium made from a pure culture of the chosen mushroom strain. Spawn production is a fermentation process in which the mushroom mycelium will be increased by growing through a solid organic matrix under controlled environmental conditions. The purpose of the grain spawn is to boost the mycelium to a state of vigour such that it will rapidly colonise the selected bulk growing substrate. Each individual grain becomes coated with the mycelium and in fact becomes a mycelial capsule. An extensive technology has been developed throughout the world to ensure the production of high quality mushroom spawn. The availability of good quality spawn is the limiting factor for mushroom cultivation in many developing countries. The complete procedure of spawn production involves spawn laboratory and basic requirements; preparation of the media, culturing and maintenance of mushroom fungi, substrate preparation and it's spawning etc.

Spawn laboratory and general requirements: In general, the layout plan of a spawn laboratory should have a total built up area of 19x8x3.6m (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 conditioners (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: 1) autoclave (horizontal type) 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, 2) small autoclave (standing type) 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, 3)pressure cooker required for sterilization of media for routine laboratory work, 4) baby boiler run by wood fuel, electricity or diesel and required for production of pressure steam for boiling, sterilization of grains and pasteurization of casing mixture, 5) laminar Flow required for isolation of fungi and inoculation of grain bags / bottles with master cultures under aseptic conditions, 6) weighing machine required for the exact measurement of raw materials for producing spawn and compost, 7) steel or cemented racks 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, 8) steel trolleys required for easy movement and carriage of grain bags, spawn bottles, compost bags and other materials from one room to another room, 9) BOD incubators required to incubate cultures inoculated or transferred in tubes, Petri dishes, flasks and Master culture bottles for their speedy growth at a fixed temperature, 10) oven is required for the sterilization of glasswares, including Petri plates, pipettes, beakers, glass tubes etc., 11) refrigerator for maintain purity of the fungal cultures for a considerable period, these are to be kept in the refrigerators in a cool environment, 12) wire mesh Tray required for removing excess water from boiled cereal grains or the boiled substrates like straw or sawdust used for mushroom production, 13) boiling pans/boiling kettle/gas/kerosene stove or electric stove required for boiling the grains/ preparation of media, 14) pH meter to

check the pH of the medium, 15) microscope for diagnosis of microbial contaminations and infections, 16) hot plate/ heater to heat the media and boil the contents of culture media, 17) glassware viz., Petridishes, test tube, culture tube, beakers, funnels, measuring cylinders' glucose bottles, Glass slides, cover slip and conical flasks, 18) chemicals for medium preparation, calcium carbonate, calcium sulphate and disinfectant (formaldehyde) etc., 19) furniture like steel racks in incubation room and cold storage for keeping bags/bottles, exhaust fans, filters, office table, working tables etc. and 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.

Media for mushroom fungi : The pure cultures are raised on a convenient culture medium which is generally in solidified state due to the addition of Agar-agar. In laboratory, the edible mushroom strains may be cultured on different media. The composition of media and the methods of preparation are as given under:

Potato - dextrose Agar medium (PDA):

– Peeled and sliced potato: 250g
– Dextrose: 20g
– Agar –agar powder: 20g
– Water: 1000 ml.

About 250g potatoes are peeled, cut into small pieces, boiled in water for 25-30 minutes and filtered through a muslin cloth. The volume of the extract is raised to 1000 ml with water and boiled along with dextrose and agar-agar powder so as to get a thoroughly mixed solution. Before pouring in the test tubes or flasks, the pH is adjusted to 7.0 and then after plugging with non-absorbent cotton and sterilization at 15 p.s.i. for 15 – 20 minutes in an autoclave.

Potato -dextrose Yeast Agar Medium (PDYA): Just like preparation of PDA, PDYA can be prepared by adding 2g Yeast extract in the solution for selected fungi.

Malt Extract Agar medium (MEA) :

– Malt extract ------25g
– Agar- agar powder ----- 20g
– Distilled water ------ 1000ml

Malt extract and agar are mixed in 1 litre water and boiled by continuously stirring with a glass rod so as to avoid formation of clumps followed by sterilization at 15 p.s.i. for 15 – 20 minutes in an autoclave

Compost Extract Agar medium (CEA) :

– Pasteurized compost ------ 150g
– Agar –agar powder ------ 20g
– Water ------ 1000ml

Compost is boiled in 1.5 to 2.0 litre water for few minutes till volume of the water is reduced to half and after filtering through muslin cloth, the volume is again made to 1 litre and autoclaved after mixing agar powder in it and filling in the test tubes.

Malt Peptone Grain Agar Medium (MPGA) :

– Malt extract ----- 20g
– Rye or Wheat grains ----- 5g
– Yeast (Optional) ------ 2g
– Agar-agar powder ----- 20g
– Peptone ------ 5g (pH -7.0)

Wheat or rye grains are boiled in water for 1-1.5 hours; the filtrate is mixed with other ingredients and continuously stirred while heating before filling and autoclaving.

Oat meal agar :

– Oat meal flakes 30g
– Agar-agar: 20g
– Distilled water: 1000ml

Cook oatmeal in water for 15 – 30 minutes. Filter through three or four layers of cheesecloth and bring filtrate back to volume with water. Add agar and autoclave it at 15 p.s.i. or 121°C for 15 minutes.

Wheat extract agar :

– Wheat grain: 32g
– Agar-agar powder: 20g
– Distilled water: 1000ml

Boil 32g wheat grains with 1 litre of distilled water for about 2 hrs and filter after 24 hrs. Bring filtrate back to volume with water. Add agar and autoclave it at 15 p.s.i. or 121°C for 15 minutes.

Rice bran decoction medium :

– Rice bran: 200g
– Agar-agar: 20 g
– Distilled water: 1000ml

Boil 200g rice bran with 1 litre of distilled water for about 2 hrs and filter it. Bring filtrate back to volume with water. Add agar and autoclave it at 15 p.s.i. or 121°C for 20 minutes.

The pH of the medium adjusted by adding N/10 NaOH or N/10 HCl drop by drop to raise it to 7 or brought down to be adjusted to 7.0, respectively before sterilization. Wheat grain and compost extract are most suitable culture media for *A. bisporus* and *A. bitorquis* cultures. Cultures of *Volvariella spp.* and *Pleurotus spp.* can be maintained on PDA or Malt extract agar medium. It is desirable that

cultures are not maintained on the same type of culture medium in each sub-culturing.

Culturing of mushroom fungi :

Culture isolation : Fresh and healthy mushroom fruit body (basidiocarp) showing all the desirable attributes of that species/strain or their spores are used to raise mycelial cultures by following methods:

Vegetative mycelium culture (tissue culture) :

Step 1 : Cleaning of young basidiocarp with sterilized distilled water and dipping in 2.5% sodium hypochlorite solution for 1 min under aseptic conditions using laminar flow.

Step 2 : In case of button mushrooms, the basidiocarp is air dried and split open longitudinally from centre and vegetative mycelial bits are cut from the collar region (junction of pileus and stipe). Whereas, in black ear mushrooms, the ear is cut along the edge with a sterilized scissor and inner tissues are scraped and small bits of tissues are removed.

Step 3 : These bits are then washed in sterilized water to remove sodium hypochlorite and placed in oven sterilize Petriplates having culture media.

Step 4 : Incubation of inoculated plates at 25°C ± 2°C in a BOD incubator.

Step 5 : New mycelium growth over the tissue is observed within 4-5 days.

Step 6 : Purification of cultures by carefully transferring young mycelium from growing edge of the colony from Petriplate to test tubes.

Step 7: Incubation of inoculated tubes at 25°C± 2°C for 10-14 days (35°C for *Volvariella volvacea*).

Multispore culture :

Step 1 : Under aseptic conditions, spore mass is scraped from a fresh spore print or basidiocarp and suspended in 100 ml of sterilized distilled water in flasks and shake to obtain uniform spore suspension.

Step 2 : A few drops of this suspension is added to lukewarm culture medium and poured into oven sterilize Petriplates. Petriplates are rotated to homogenize the spore suspension into culture medium. The culture medium is allowed to solidify and then Petriplates are inoculated at 25°C± 2°C for 3-4 days (35°C for *Volvariella volvacea*).

Step 3 : The spore germination is observed under microscope and germinating spores along with a piece of agar are transferred carefully to culture tubes.

Step 4 : Incubation of culture tubes of*Agaricus bisporus and A. bitorquis*at 25°C for 10-14 days and *Volvariella volvacea*at 32°C for 7 to 10 days.

Single spore culture: *Agaricus bitorquis* and *Pleurotus spp.* are heterothallic with tetraspored basidia, therefore single spore is self-sterile but this technique can be successfully used for breeding new strains. *Agaricusbis porus* being secondary homothallic with bispored basidia and majority of its spores being self-fertile, can be used to raise fertile cultures. Single spore cultures are procured in the same way as that in multispore cultures except serial dilution of spore suspension for single spore culture isolation. Its methodology is given below.

Step 1 : Single spore culture isolation: serial dilution of spore suspension to obtain 10-12 spores/petriplate so that individual germinating spore is marked and could be lifted under aseptic conditions.

Step 2 : Transfer of above individual germinating spore into culture tubes and its incubation at 25°C for 10-14 days in BOD incubator.

Step 3 : Procurement ofpure mycelial cultures followed by their preservation for a particular need.

Maintenance and storage of mushroom culture :

There are following methods used for preservation of mushroom cultures.

Regular sub-culturing : For storage purposes cultures are prepared on agar slants in culture bottlesor test tubes. These cultures can be stored in racks at room temperatures for one to few weeks. The periods between sub-culturing can be extended up to 46 months by storage at cooler temperatures, *i.e.,* at 4-7°C in a refrigerator or cold room. However, all mushroom species cannot be stored at low temperature. For example, *Ganoderma* and *Volvariella* sp. should be kept at temperature less than 15°C or so. *Volvariella volvacea* is incubated at 32°C for 7 to 10 days. The other mushroom strains are incubated at 25°C for 2-3 weeks until the slants are fully covered with mycelium. *V. volvacea* should be sub-cultured every 2 months. Species of *Lentinula, Pleurotus* and *Agaricus* strains can be kept in a refrigerator at 4°C, and they should be sub-cultured every 6 months.

Storage under mineral oil : In this, actively growing mycelial cultures are covered upto 1 cm above the slant level, with the help of mineral oil (liquid Paraffin) sterilized in an autoclave at 121°C for 15 minutes for two consecutive days. Alternatively, 0.5 cm mycelial discs are suspended in 1-2 ml of sterilized liquid paraffin. Covering cultures on agar slants with mineral oil prevents dehydration, slows down metabolic activity and growth through reduced oxygen tension. In conjunction with maintenance of the culture in a refrigerator at 4°C, this is an effective method of preserving fungal cultures. The

culture stored in this way remained viable for 8 years.

Water storage : The cultures are grown on a suitable culture medium and after full growth, 4-5 bits of 0.5 mm diameter are transferred aseptically to precooled and sterilized McCartney bottles containing demineralized water and the lids tightly screwed down and are stored at room temperature. All mushroom cultures except V. volvacea can be stored by this method. Revival of culture is by removal of a block and placing the mycelium on a suitable growth medium. Survivals of fungal cultures stored this way are reported for 2 to 5 years' period satisfactorily.

Lyophilization (freeze-drying) : It is a method for long-term preservation of spore-bearing fungi. Mycelial mushroom cultures are not well preserved in this way. However, spore collected from a young and healthy mushroom aseptically can be stored for several years by this method. In freeze-drying, spore is frozen and water is removed by sublimation. The drying of the spores is accomplished by freezing under reduced pressure in vacuum. Primary drying is achieved at -40°C for 4 hours. Vacuum is released and glass ampoules are stored at -20°C (or-70°C). Secondary drying is done in freeze-dryer under vacuum at 20°C for 2 hours. The ampoules are then stored at 4°C to 6°C for longer shelf-life inside a refrigerator.

Preservation at -70°C : Glycerol (10%) in aqueous solution is sterilized by autoclaving at 121°C for 15 minutes. Alternatively, Dimethyl sulfoxide (DMSO) is sterilized by filtration using 0.22 micron Teflon filter. Usually, 10% glycerol suspensions of cultures are made (0.5 ml to 1 ml) and the aliquots are distributed in small vials or tubes. The vials/tubes are placed at -70°C.

Cryopreservation in liquid nitrogen : Glycerol (10%) suspension of young mushroom mycelium is prepared and distributed in aliquots of 0.5 ml to 1 ml in plastic screw cap cryo-vials, which can withstand ultra-cold temperature. Programmed cooling at 1°C to 10°C per minute is ideal. In case where programmable freezer is not available, vials are first placed in a mechanical freezer (-70°C) for an hour and then to check viability of a culture before and after freezing. In cryopreservation, the prepared suspension of mushroom mycelium is stored at ultra-low temperatures (-196°C in liquid nitrogen).

Granular structure medium : The mycelial viability and the economic properties of mushroom strains can be retained for at least five years, if the mycelium is preserved at 2-4°C on granular structure medium. The ingredients of granular structure culture medium are saw dust or mixed straw powder (72%), wheat powder (20%), soybean powder (5.5%), complex additives (2%) and adhesive (0.5%).

Cryopreservation in mechanical freezers : In this method, the cultures are prepared in the same way as for liquid nitrogen preservation and placed first at -20°C and then at -70°C and finally in freezers maintained below -130°C (-140°C or -150°C).

The choice of methods will depend on the requirements of the collection, the equipment and facilities available. Table 1 compares different methods of preservation with regard to costs of materials, labour, longevity and genetic stability. It is recommended that each mushroom strain/ isolate should be maintained by at least two different methods. In general, storage in liquid nitrogen and mineral oil preservation technique are best suited for preservation of edible mushrooms. The handling techniques, freezing protocols, cryopreservation and thawing rates can be optimized for a particular strain to obtain maximum survival. Once the mushroom has been successfully frozen and

Table 1: Comparison of mushroom culture preservation methods

Method of preservation	Cost		Longevity	Genetic stability
	Material	Labour		
Storage at room temperature	Low	High	4-6 weeks	Variable
Storage in refrigerator	Medium	High	4-6 months	Variable
Storage under oil	Low	Low/medium	4-5 years	Moderate
Storage in water	Low	Low/medium	2-3 years	Moderate
Storage in deep freezer (-70°C)	Medium	Low/medium	4-5 years	Moderate
Freeze-drying of[1]Basidiospore	High	Initially high	20 years	Good/medium
Liquid nitrogen	High	Low	Indefinite	Good[1]
Ultra-low mechanical freezers (-150°C)	High	Low	(-)	(-)

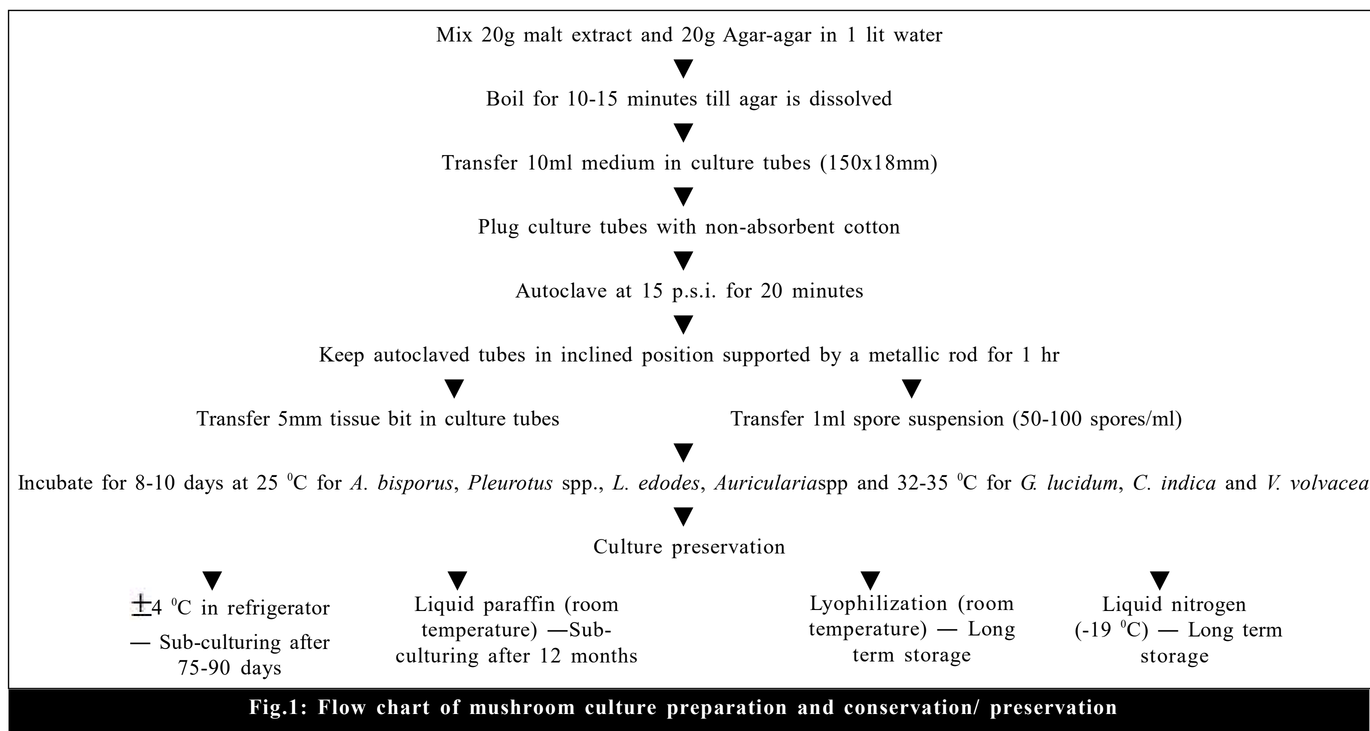

Fig.1: Flow chart of mushroom culture preparation and conservation/ preservation

Table 2 : 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

stored in liquid nitrogen, the storage period appears to be indefinite, because no chemical and or physical changes can occur at such low temperatures. The flow chart of mushroom culture preparation and conservation is given below:

Spawn production methodology :

In simple words spawn is grains covered with mushroom mycelium. Mixing of spawn in compost or substrate is known as spawning. 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.

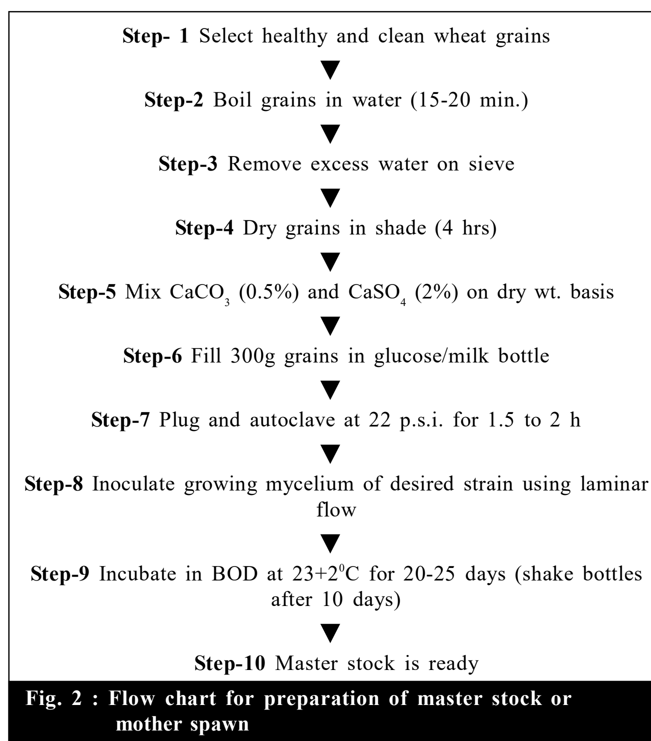

Fig. 2 : Flow chart for preparation of master stock or mother spawn

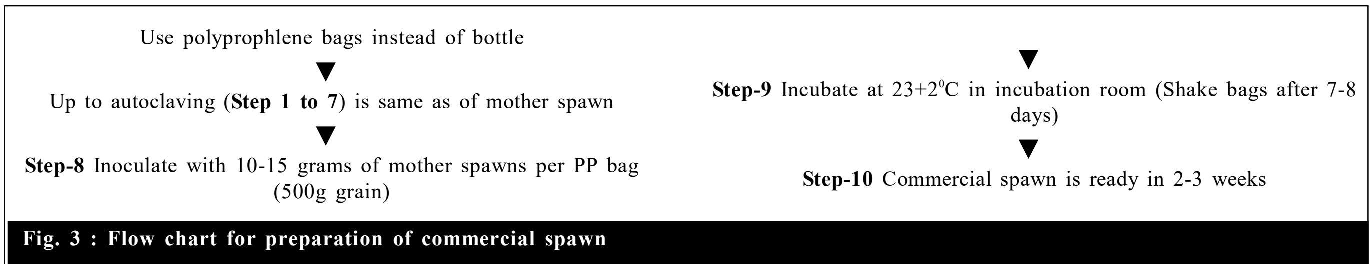

Fig. 3 : Flow chart for preparation of commercial spawn

Cleaning of grains

Boiling of grains

Draining of water

Drying of grains

Mixing of CaCOand CaSO$_4$

Steam sterilization of grains

Drying and sterilization under Laminar

Inoculation of sterilized grains

Spawn is ready for use

Fig. 4 : Spawn production technology

Preparation of master stock or mother spawn:

Preparation of commercial spawn :

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.

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^{0}C for *Agaricus, Pleurotus* and *Lentinula* and between 18-22^{0}C in *Volvariella* and *Calocybe indica*.

– The incubation temperature should be between 32±2°C for *Volvariella* and *Calocybeindica* and 25^{0}C for *Agaricus, Pleurotus* and *Lentinula.*

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^{0}C for *Agaricus, Pleurotus, Lentinula* and 32±2^{0}C for *Calocybe indica* and *Volvariella.*

– The master spawn should be stored at 4-6^{0}C for 40-45 days in *Agaricus, Pleurotus,Lentinula* and at 18-20^{0}C for 30-40 days in *Calocybe indica* and *Volvariella*.

– 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^{0}C for *Agaricus, Pleurotus, Lentinula* and 32±2^{0}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^{0}C in *Agaricus, Pleurotus* and *Lentinula* and 18-20^{0}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.

References :

Ahlawat, O.P. and Rai, R.D. (2000). Growth regulators for mushroom yield enhancement. *Mushroom Science,* **15**:695-699.

Aneja, K.R. (2004). Mushroom production technology. In: *Experiments in microbiology plant pathology and biotechnology* (4th Ed.). *New Age International Limited*, Publishers. New Delhi. pp. 496-519.

Chadha, K.L. and Sharma, S.R. (1995). Mushroom research in India: History, Infrastructure and Achievements. *Advances in Horticulture,* **13** : 1-12.

Chang, S.T. and Miles, P.G. (2004). Mushrooms. Cultivation, nutritional value, medicinal effect, and environmental impact (2nd Ed.). *CRC Press*. 451 pp.

Dhar, B.L. (2002). Infrastructure for seasonal and round the year cultivation of white button mushroom. In:*Recent advances in the cultivation technology of edible mushrooms* (ed. RN Verma and B. Vijay): 71-94pp.

Ekman, J. (2017). Pre and postharvest management of mushrooms: A Review. *Applied horticultural research*. Horticulture Innovation Australia. 60pp.

FAO. (2009). Make money by growing mushrooms, Rome.

https://iasri.icar.gov.in/

https://www.agrimoon.com/

Singh, M., Vijay, B., Kamal, S., Wakchaure, G.C. (2011). Mushrooms: cultivation, marketing and consumption. Directorate of Mushroom Research. ICAR-Chambaghat, Solan –173213 (HP), 266pp.

Upadhyay, R.C., Singh, S.K. and Tewari, R.P. (2004). Mushroom spawn production and infrastructure requirements. Technical Bulletin, DMR, Solan. pp. 38.

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