Mushroom Spawn Production and Infrastructure Requirements

R.C. Upadhyay, S.K. Singh and R.P. Tewari

भाकृ अनुप
ICAR

National Research Centre for Mushroom
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Technical Bulletin

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INTRODUCTION

In nature, mushroom spores help in survival of a species from one generation to the next. These spores are extremely small microscopic propagules and therefore difficult to handle as seed. Spores need time and specific conditions to germinate and during this time competitor fungi might germinate and grow faster to exhaust the available substrate. Therefore, a pure culture of the desired mushroom mycelium is first raised on a convenient artificial culture medium and then added to the substrate to give it an advantage. The term spawn has been defined as the vegetative mycelium from a selected mushroom grown on a convenient medium (Klingman, 1950). The spawn comprises mycelium of the mushroom and a supporting medium, which provides nutrition to the fungus during its growth. Spawn is used as inoculum or "seed" for the substrate in mushroom cultivation. Right kind and quality of spawn is very important for mushroom cultivation.

The rapid rate of development of mushroom production technology from a primitive cave culture in France to a high-tech industry during the last three centuries is a success story which has kept pace with the ever increasing demand for this commodity and there is every reason to be optimistic about its further growth in the years to come (Rai and Verma, 1997). The world mushroom production has registered a 3-fold increase from two million tonnes in 1986 to about six million tonnes in 1997. Five major mushroom species *Agaricus bisporus*, *Pleurotus* spp., *Volvariella volvacea, Lentinula edodes* and *Auricularia* spp. accounted for 82 per cent of the total world mushroom production (Chang, 1999).

Not all mushrooms are edible, but some are highly poisonous, while edible fleshy fungi are called mushrooms, poisonous ones are termed 'toadstools'. It has been estimated that out of 10,000 species of fleshy fungi (Kendrick, 1985) about half of them are edible (Chang, 1993) and as many as 100 species are highly poisonous. Therefore, utmost care should be taken in procuring pure cultures. Preferably, to start with, pure desired mushroom cultures should be procured from an authentic mushroom culture bank. *Agaricus bisporus*, popularly known as the white button mushroom, has the widest acceptability in India and its present production level is estimated between 50,000 to 60,000 tonnes per annum. The second most popular oyster mushroom species (dhingri) accounts for approximately 7000-8000 tonnes per year.

The isolation, purification and maintenance of mushroom cultures require technical expertise and aseptic high-tech laboratory facilities. Therefore, small mushroom growers can not maintain their pure culture and or spawn. They have to rely entirely on commercial spawn producers, reliable governmental and non-governmental organizations that play a vital role in supplying reliable spawn of a desired mushroom strain or variety.

This technical bulletin shall provide technical guidance and information to mushroom growers, entrepreneurs and young individuals those who are

interested in taking up spawn production as a source of income, business or part of sustainable agricultural activity.

HISTORY OF SPAWN PRODUCTION

Culpeper in 1652 described spawn as mycelium of mushroom. From 1652 to 1894 A.D. spawn was gathered from the wild rather than made (Flegg *et al.*, 1985). Before the advent of grain spawn, different kinds of spawn were natural or virgin spawn (from the pastures and meadows), flake spawn (breaking of beds through which mushroom mycelium has run), Mill track spawn (bricks dried and made from mixture of horse dung, cow dung and loam soil) and manure spawn (on sterilized horse manure or compost manure). Hamlin and Co. (UK) were the first to produce spawn spores or tissues free from contaminating microorganisms in 1886 and sold as brick spawn in UK and exported it to Australia, Germany and USA.

The first pure culture spawn was produced by Constantin in France (1894) on horse manure compost. In 1905 Duggar prepared pure culture from mushroom tissue. Later on Mycelial culture was used to inoculate sterilized horse manure in bottles (1918). The process of making spawn on grain was introduced by the Pennsylvania State University, which held two patents on it. These patents were assigned to the university by the inventor Prof. J.W. Sinden in 1932. License under the patent were available to any laboratory qualified to make the grain spawn. The grain spawn was further perfected by Stoller in 1962. Since the process for the production of grain spawn, the fundamentals have not changed. You still need a starter culture, cereal grain, the grain is sterilized, cooled and the product grown out. Simple 'Yes', easy 'No'. It is no secret that anyone can make spawn, just as anyone can grow mushrooms.

RAISING OF PURE CULTURES

a) Preparation of culture media

Besides infrastructure, raw materials and equipments, healthy vegetative mycelial culture is required to produce quality spawn. The vegetative mycelium is raised on to a convenient culture media. A variety of culture media can be used to grow vegetative mycelium before it is inoculated on a suitable substrate to grow mushrooms. These culture media are also used as substrate for isolation, multiplication, maintenance and preservation of mushroom cultures. For the convenience of the practical users a few compositions of different media are given below, which can be used for making slants or Petri dishes.

Malt Extract Agar (MEA)

Malt extract	:	25g
Agar-agar powder	:	20 g

Water	:	1 Litre
pH	:	7.0

Water is boiled, mixed with malt extract and the ingredients are dissolved using clean glass rod by stirring water to avoid lump formation. The pH of the medium is adjusted between 6.5 to 7.0 before autoclaving.

Wheat Extract Agar (WEA)

Wheat grains	:	32g
Agar-agar powder	:	20g
Water	:	1 Litre
pH	:	7.0

Wheat grains are boiled in water for 1-1½ hours and are filtered through the muslin/cheese cloth and discarded. The volume of grain extract is raised to 1 litre with water and then agar-agar powder is added by stirring continuously and pH is adjusted.

Potato Dextrose Agar (PDA)

Peeled and diced Potato	:	200g
Dextrose	:	20 g
Agar-agar Powder	:	20g
pH	:	7.0

Potatoes are peeled, washed, cut into small pieces (3-4cm) and boiled in water for 20-25 minutes. The potato extract is filtered through a cheese cloth and potato cubes discarded. The volume of this extract is raised to one litre. Dextrose and agar-agar powder is then mixed by stirring and pH is adjusted. Green potatoes should not be used as they contain anti-fungal alkaloids, which may be harmful to mushroom mycelium.

Compost Extract Agar

Pasteurized and conditioned compost	:	150 g (fresh weight)
Agar-agar powder	:	20g
Water	:	1 Litre
pH	:	7.0

Pasteurized compost is boiled in 1½-2 litre of water until the volume of water is reduced to half. It is filtered through cheese cloth and the volume of the compost extract is raised to one litre. Agar-agar powder is mixed by stirring and

pH adjusted. Streptomycin sulfate @ 50mg/litre may be added after autoclaving to eliminate bacterial contamination.

Oat Meal Agar

Oat meal flakes	:	30g
Agar-agar powder	:	20g
Water	:	1 litre
pH	:	7.0

Oat meal flakes are boiled in sufficient water for 2 hours. The volume of the supernatant is raised to one litre. Agar-agar powder is mixed by stirring and pH is adjusted.

Rice Br-an Decoction Medium

Rice bran	:	20g
Agar-agar powder	:	20 g
Water	:	1 litre
pH	:	7.0

Rice bran is boiled in water for 15-20min, filtered through cheese cloth and then agar-agar powder is mixed by stirring. pH is adjusted.

If the pH of the medium is below 6.5, $N/_{10}$ NaOH is added drop by drop to raise it to 7.0. Whereas, if the pH is above 7.0 it can be brought down by adding $N/_{10}$ HCl. The pH is to be adjusted to 7.0 before sterilization. It has been observed that the pH declines approximately 0.5 upon autoclaving. After autoclaving the pH comes down to 6.5 level, which is most suitable for mushroom mycelium to grow. If the cultures are to be raised in Petri plates then the medium is first sterilized in 500ml conical flasks, 15-20ml of lukewarm sterilized culture medium is poured in each pre-sterilized Petri plate. If the cultures are to be multiplied in test tubes then the medium is poured in large number of culture tubes @ 15ml/ tube. The culture tubes or conical flasks are then plugged with non-absorbent cotton and autoclaved under moist steam at 15 pounds per square inch (p.s.i.) pressure for 25-30 minutes. A pressure at 15 lb p.s.i. eliminates all microbes, which may later compete with vegetative mycelium of desired mushroom. After sterilization culture tubes are placed in slanting position to provide more surface area for the vegetative mycelial growth of a mushroom. The culture medium is allowed to solidify in culture tubes or in Petri plates for a few hours before they are used for inoculation or sub-culturing. The Petri plates are sterilized in an oven at 180°C for 2½ hours and allowed to cool down to room temperature before they are used for plating media.

b) Isolation from fruit body

We need sterilized culture medium both in Petriplates and culture tubes, scalpel, inoculation needle, wide mouth test tubes, spirit lamp, scissors, forceps, matchbox, 70% ethanol (rectified spirit), laminar flow, fresh basidiocarps (fruit-bodies) in an inoculation room. Inoculation room should be provided with a double door entry or air curtains at the entry point to cut direct and forceful entry of air into the room. Most of the contamination enters through direct air currents entering into inoculation room. The laminar flow or the clean bench has micro-filters that help in eliminating microbes and dust particles present in the air. When the laminar flow is put on, all dust particles and microbial contamination, more than 4 microns in size are retained with the filter and clean air is blown out in the working area. Ultra violet (UV) lights should be put on for about half an hour before working in the laminar flow. All material except the living cultures and fruit-body should be exposed to UV light to eliminate contamination present on the surface of culture tubes, Petri plates, scissors, forceps etc. The ultra violet lights should be switched off before using laminar flow because exposure to UV light is harmful to all living organisms including human beings and can cause mutation, skin diseases or even cancer. Separate foot ware and apron (coat) should be worn before entering into inoculation room and these wares should not be used outside spawn lab. Before starting the inoculation, the surface area of inoculation table should be swabbed with cotton dipped in rectified spirit. After putting off UV lights hands should be washed with rectified spirit (70% ethanol) and air-dried before lighting spirit lamp to avoid accidents.

Pure cultures of fleshy fungi/mushrooms can be prepared either by tissue culture or multi-spore cultures.

Tissue culture

In case of *Agaricus* species, a button stage young fruit body should be freshly harvested and brought to the laboratory for tissue culture. The fruit-body should be cleaned and adhered casing soil may be removed with the help of cotton swab. The fruit-body is held with the help of scalpel/inoculation needle and dipped in 0.1% mercuric chloride ($HgCl_2$) or 70% ethanol for 30 seconds for a while on spirit lamp flame to kill microbes present on its surface. The scalpel is surface sterilized by dipping it in 70% ethanol and then heating it on flame. After cooling scalpel, the fruit-body is split longitudinally and tissue bits of approximately 3-5mm are cut from pileus-stipe junction (collar region). The bits are aseptically transferred onto pre-sterilized Petri plates containing

Fig. 1. Culture slants showing culture medium and pure mycelial cultures

convenient culture medium with the help of a sterilized forceps. These Petri plates are wrapped with parafilm and incubated in a BOD incubator at 25°C+1°C for 6-7 days. The actively growing mycelium from edges along with culture medium of about 5mm size should be transferred to a number of test tubes (Fig. 1). These test tubes are then incubated in a BOD incubator at 25 ±1°C for 2-3 weeks. This pure culture can then be used as inoculum for spawn preparation. In case of *Pleurotus* spp., the tissue culture is raised from the junction of pileus and stipe portion. Rest of the procedure is same. In case of tropical mushrooms like *Volvariella* and *Calocybe* species the Petri plates and test tubes containing bits of fruit-body are incubated at 30-32°C instead of 25°C.

Spore culture

There exist difference in the sexuality pattern in cultivated mushrooms; *Agaricus bitorquis, Lentinula edodes, Pleurotus* spp. and *Auricularia* spp. are heterothallic, whereas, *Agaricus bisporus* and *Volvariella* are secondary and primary homothallic species, respectively. Single spore cultures may not eventually result in fruiting in heterothallic species. Therefore, in most of the spawn labs, the pure mycelial cultures are raised from tissue culture. Nevertheless, multi-spore cultures can be raised from any mushroom species for spawn preparation. It has its advantages, for raising a spore culture, mass of spores known as spore print is required. The spore print can be obtained from a fruit-body on to a sterilized Petri plate or plain paper. In case of *Agaricus*, a healthy fruit-body whose veil is still intact is harvested and cleaned with a cotton swab to remove the casing soil. Bell jar, Petri plates and beakers are sterilized in an oven at 180°C for 2½ hours before taking a spore print. The fruit-body is mounted on a coil and kept in an open Petri plate, covered with a beaker and enclosed in a Bell jar for 48 hours. Millions of spores are shed inside the Petri plate or on a plain paper. This spore print can be stored at a dry place until used. In case of *Pleurotus*, spore print can be obtained by simply placing the mature fruit body with gills facing on paper side, in a Petri plate or on sterilized plain paper within 48 hours. Pure cultures from spores can be raised in two ways.

Multi spore culture

Multi spore culture is also used for raising pure culture. It is raised from a mass of germinated basidiospores. From a spore print, a loop full of spore mass is picked using sterilized inoculation needle, suspended in 10ml sterile distilled water in 18x150 mm test tube, and mixed thoroughly. One ml of this suspension is remixed in nine ml sterile water. Again, one ml of diluted spore suspension is spread with the help of a glass rod on to a Petri plate containing 20ml of a convenient culture medium. These Petri plates are incubated in BOD incubator at 25°C+1°C for one week. The fast growing multi-spore colonies are picked up with piece of agar-agar and transferred to freshly prepared convenient culture medium in test tubes under aseptic conditions. These test tubes are incubated for 2-3 weeks in a BOD incubator. Multi spore culture is then ready for inoculation in the wheat grain substrate for spawn preparation.

Single spore culture

In secondary homothallic mushroom species like *Agaricus bisporus*, only 65% of its spores are fertile. In such cases, fertile spores can be identified and can be used for cultivation purpose, whereas, species of *Pleurotus, Lentinula, Auricularia* and *A.bitorquis* are heterothallic and single spores are not fertile. Single spores in such cases are not used for preparation of culture and spawn, as they may not eventually result into fruiting. Nevertheless, single spores are of immense importance in developing new varieties, hybrids and single spore selections using modern breeding techniques. The process of raising single spore culture is the same as that of multi spore culture. In single spore isolation, the spore suspension is further diluted to obtain a concentration of 20-30 spores/ml before spreading them in Petri plates. Instead of picking up a growing multi spore colony, only germinating single spore mycelium is carefully marked, picked and transferred to sterile test tubes. These test tubes are incubated for 2-3 weeks in a BOD incubator. The single spore culture colony will be very slow growing and can be identified visually.

c) Sub-culturing

Fig. 2. Pure culture of *Agaricus bisporus*

As a result of isolation and purification, a pure vegetative mycelial culture is established. These cultures are maintained and multiplied for their use in inoculation of grain substrate for large-scale production of spawn and use in future for a variety of purposes. Pure culture of *Agaricus bisporus* and *Pleurotus sajor-caju* are shown in figures 2 and 3, respectively. Sub-culturing is done by transferring a small piece of growing pure culture along with culture medium on a suitable medium. The compositions of most commonly used media have already been described. In order to maintain vigour of the mycelium the culture media may be changed in subsequent sub-culturing. It is desirable that cultures are kept in

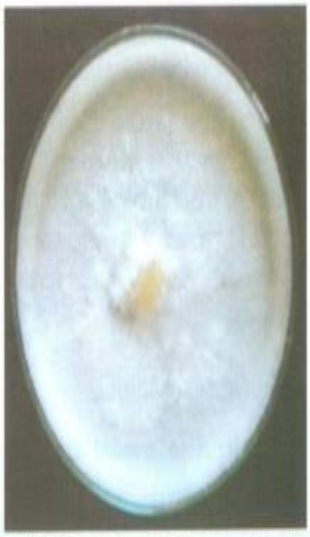

Fig. 3. Pure culture of *Pleurotus sajor-caju*

refrigerator at 4°C for 2-3 months and again sub-cultured in fresh culture tubes for use in making spawn.

MAINTENANCE AND CONSERVATION OF STOCK CULTURES

There are various methods of maintenance and conservation of mushroom culture and a good culture collection centre adopts more than one method to preserve them. These mushrooms might be of academic, industrial, medicinal or of horticultural importance. When a new genus or a species is discovered and described, it is generally deposited in a established germplasm bank. This ensures availability of the organism for use in future. In addition, mushroom strains having industrial importance are patented and preserved although availability of such strains became restricted (Jong and Birmingham, 1991).

Since there is no satisfactory method to check and evaluate the quality of spawn by rapid on-the-spot examination, a method of preserving selected strains tested and proved desirable is of primary importance. If no degenerative changes were to take place during the preparation or maintenance of mushroom cultures and of spawn, then the preservation would have been a relatively simple, routine process. Unfortunately, it is not true. Degeneration of culture or spawn refers to the loss of desired traits leading to slow development, poor rate of survival and low level of productivity (Chang and Miles, 1989; Stadelmann, 1986).

Spores of heterothallic or secondary homothallic species are produced through a sexual process will have genetic differences (Petersen, 1995). Spores from a primary homothallic species would be expected to be genetically similar. Of the cultivated fungi, only *Volvariella* basidiospores are primarily homothallic but still exhibit variations (Chang *et. al.*, 1981). If single spore cultures are maintained, mating tests for heterothallic species would be required routinely, as well as tests for fruiting ability, while, if spores are maintained for homothallic species, it would be necessary to check fertility of the cultures by regular fruiting tests. Consequently, in practice, vegetative mycelia of only known origin are stored (Snell, 1984).

After pure mycelial cultures are obtained a wide variety of methods are available for the conservation of mushroom cultures suitable for a particular need e.g., preservation for a relatively shorter period, or for a long period. The choice of preservation method depends upon many factors but the availability of necessary equipments and funds is commonly a determining factor in such decision.

Frequent sub-culturing

Under recommended temperature and pH conditions, most mushroom mycelium continues to grow until the nutrients of a suitable culture medium are exhausted. Therefore, these cultures remain viable only for few months depending upon the growth rate, substrate and method of storage etc. Using a system of periodic transfer at reasonable intervals, stock cultures are often maintained in an actively growing state under optimum laboratory conditions. After obtaining optimum mycelial growth, mushroom cultures are stored until sub-culturing become necessary. For storage purposes, cultures are prepared on agar slants in culture bottles and or 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 4-6 months by storage at 4°C in a refrigerator or cold room.

In laboratory, the edible mushroom strains are sub-cultured on suitable culture media. *Volvariella volvacea* and *Calocybe indica* are incubated at 30-32°C for 10 and 15 days, respectively. The other mushroom strains are incubated at 25°C for 2-3 weeks until the slants are fully covered with mycelium. Once fully-grown culture of *V. volvacea* has been obtained, they are to be kept at room temperature. *V. volvacea* should be sub-cultured between 6-8 weeks. Strains of *Lentinula*, *Pleurotus* and *Agaricus* species can be kept in a refrigerator at 4°C, and they should be sub-cultured every 3-4 months.

Deviation from the original characteristics of the cultures can be detected with mycelial cultures. The most common degenerative symptoms are sectors of slow growth, mycelium that is thin and weak in appearance, or matted or fluffy but has normal growth rate. A slow growing mycelium needs more time for colonization and tends to carry virus particles. A fluffy mycelium causes the grain to stick together and is harder to spread in compost than normal grains. It tends to form stroma and it gives lower yields. Mycelia of these types should be discarded (Chang and Miles, 1989). Culture tubes of *Volvariella* spp. forms chlamydospores, which are brownish in colour. Culture tubes showing more chlamydospores indicate that the culture has a good vigour and will be high yielding type. Nevertheless, partial loss of mushroom forming capacity and the desired qualities because of degeneration and mutation during prolonged vegetative propagation of stock cultures, or from genetic recombination and selection in continuous field cultivation of re-established culture is relatively common in the spawn produced from cultures maintained by these methods (Burnett, 1975). Furthermore, such conventional procedures of conservation of living fungi are time consuming, costly and risky. Ultimately repeated sub-culturing may result in preserving a culture different from the original one (Smith and Onions, 1983). The disadvantages of frequent sub-culturing are loss of desirable traits, chances of contamination by air borne spores or mites carried infections, constant specialist supervision, labour intensive and time-consuming process etc.

Storage under mineral oil

Covering cultures on agar slants with mineral oil prevents dehydration and slows metabolic activity and growth through reduced oxygen tension. The mineral oil (Liquid Paraffin or Medical Paraffin specific gravity 0.830 – 0.890

g) is sterilized in an autoclave at 121°C for 20 minutes for two consecutive days. Short slants require less oil to cover them. Coverage must be complete as strands of mycelium left exposed may act as wicks to dry out the culture. Therefore, actively growing mycelial cultures are covered up to 1 cm above the slant level. Alternatively, 0.5 cm mycelial discs or mycelium multiplied on wheat grains (spawn) are suspended in 1-2 ml of sterilized liquid paraffin (Singh *et al.*, 2001). Mineral oil preservation of cultures as mycelial disc is shown in figure 4. The mineral oil blocks exchange of oxygen between the mycelial surface and the atmosphere in the container, thus retards metabolism and prevents desiccation

Fig. 4. Mineral oil preservation of cultures as mycelial disc.

of the agar medium. In conjunction with maintenance of the culture in a refrigerator at 4C, this is an effective method of preserving fungal cultures. Retrieval is done by removing a small piece of the fungal colony, grain spawn or disc with a sterile needle, hook or loop, draining off as much oil as possible and streaking the inoculum onto agar in plates or tubes. Tilting the plate or bottle may facilitate drainage. The first subculture often has a reduced growth rate and a second sub-culture is usually required before a good culture is obtained.

There are two disadvantages of oil storage, namely, contamination by air borne spore and retarded growth on retrieval. Nevertheless, in our laboratory, this method is working very well for conservation of most mushroom cultures satisfactorily for several years. The method involves taking 3 mm diameter discs from agar cultures of fungus in Petri dishes and storing the discs at room temperature in glass tubes (9 x 75 mm) containing 1 ml of liquid paraffin and plugged with non-absorbent cotton covered with tin foil. The culture stored in this way remained viable for 8 years (Sathe and Dighe 1987).

Water storage

Boeswinkel (1976) reported water storage of 650 plant pathogens belonging to the Phycomycetes, Ascomycetes, Fungi Imperfecti and Basidiomycetes could remain viable for 7 years. The cultures are grown on a suitable culture medium and after full growth, 4-5 bits of 5 mm diameter are transferred aseptically to pre-cooled and sterilized Mc Cartney bottles containing simple water and the lids tightly screwed down and are stored at room temperatures. All mushroom cultures except *V. volvacea* can be stored by this method. Demineralized water proved better. Revival of culture is by removal of a block and placing the mycelium on a suitable growth medium. Survival of fungal cultures stored this way is reported for 2 to 5 years period satisfactorily at IMI, Kew, Survey, U.K. (Smith and Kolkowski, 1996). Growth may sometimes occur during storage in water. This can be reduced if the spores or hyphae are only removed from the surface of agar and no medium is transferred.

Lyophilization

Lyophilization, also known as freeze-drying, is a method of choice for long-term preservation of spore-bearing fungi. Mycelial mushroom cultures can also be preserved in this way. In freeze-drying, mycelia are frozen and water is removed by sublimation. The drying of the spores and or mycelia is accomplished by freezing under reduced pressure in a vacuum. Stability and long storage periods have been shown to be the main advantages of freeze-drying (Jong *et al.*, 1984).

Most commonly used suspending media for freeze-drying are skimmed milk (10%), or trypticase soybroth (0.75 g) with sucrose (10 g) and Bovine-serum albumin (5.0 g) in 100 ml distilled water which are used with equal volume of culture suspension. Freeze-drying of basidiospores of mushroom can be done by adopting following procedure of freeze-drying. The glass ampoules are first sterilized in a hot air oven at 130°C for 2-3 hours and are plugged with cotton. These ampoules are autoclaved for 15-20 min. at 121°C (15 lb. p.s.i.) Culture suspension in case of mushroom or spore suspension in other fungi is prepared in skim milk or suitable medium. Each sterilized ampoule is then filled with 0.2 ml of culture suspension. A few aliquots are serially diluted to determine pre-freezing viable count. Rest all the ampoules with spore suspension are placed in a freezer (-70°C) for 1 to 2 hours. When shelf temperature of the freeze chamber reaches –40°C, ampoules with frozen samples are placed inside the chamber of freeze-dryer (Lyophilizer) and vacuum is created. Primary drying is achieved at –60°C for 4 hours. Vacuum is released and ampoules are stored at –20°C (or –70°C). Next morning samples are dried at least for 2 hours and vacuum released. Cotton plugs are then pushed inside down and constrictions are made in the ampoules above the cotton plug. The ampoules are then attached to the freeze-dryer for secondary drying under vacuum at 20°C for 2 hours and sealed while attached to the lyophilizer itself with the help of a gas-air torch. The ampoules are then stored at 4°C to 6°C for longer shelf life inside a refrigerator. A

representative ampoule can be cut from the top to check post-freezing count before finally storing the ampoules for longer duration. Viability of most organisms do not change much upon freeze-drying of viable spores. Re-hydration of the fungi with sterile distilled water should be carried out slowly for 30 min. for absorption of moisture before plating on a suitable culture medium.

Tan *et al.* (1991) demonstrated that hyphal cooling at the rate of –1°C/minute to temperatures of – 45°C and then –75°C produced fully freeze-dried mycelia. Freeze drying was performed for 2 hours at –40°C followed by 20 h at –2°C and 8 hours at 20°C, resulting in a residual moisture content of 2%. Hyphae of Ascomycetes as well as Basidiomycetes survived freeze-drying. Recently, a new lyophilization protocols has been developed at this center by lyophilization of mushroom mycelium multiplied on pearl millet grains instead of culture suspension in glass ampoules (Fig. 5) to improve survival rates reaching almost 100% (Singh *et al.*, 2004).

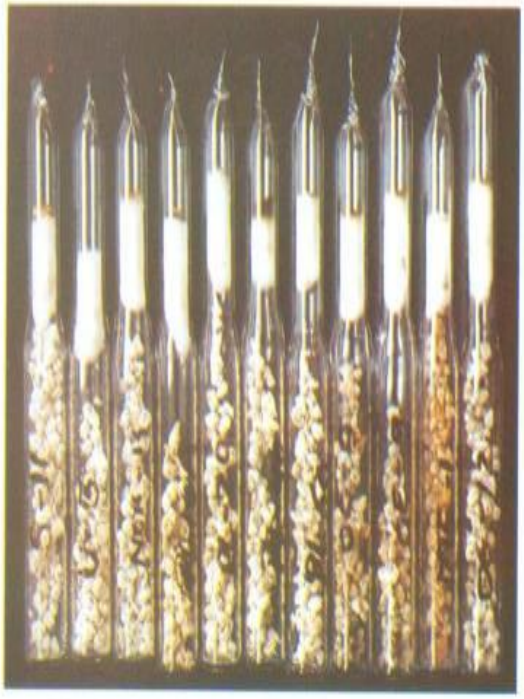

Fig. 5. Lyophilized cultures multiplied on pearl millet grains

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 suspension of cultures is 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. Many culture banks are maintaining mushroom cultures by this method satisfactorily for several years (Microbial Type Culture Collection, Chandigarh).

Cryopreservation in liquid nitrogen

The storage of microorganisms at ultra low temperatures (-196°C in liquid nitrogen) is at present regarded as the best method of culture preservation (Kirsop and Doyle, 1991, Singh *et al.*, 2004). Lowering the temperature of living cells reduces the rate of metabolism until, when all internal water is frozen, no further biochemical reaction occurs and metabolism is suspended (Franks, 1981). Although little metabolic activity takes places below –70°C, re-crystallization of ice or ice crystal growth can occur at temperature above –139°C (Morris, 1981), and this can cause damage during storage. The volume occupied by water increases by 10% when water crystallizes and form ice. This puts the cell under mechanical stress (Grout and Morris, 1987). At –196°C dormancy is induced, during which the organism does not undergo any change either phenotypically or genotypically, provided adequate care is taken during freezing and thawing. This method can be applied to both sporulating and non-sporulating cultures. Optimization of the technique for individual strain has enabled the preservation of organisms that have previously failed (Morris *et al.* 1988).

The temperature of liquid phase of nitrogen remains at –196°C and average temperature of the vapour phase is around –140°C. Liquid nitrogen preservation of cultures in cryovials is shown in Fig. 6. Glycerol (15%) suspension of young mushroom mycelium is prepared and distributed in aliquots of 0.5 ml to 1 ml in plastic screw cap cryovials (2 ml) that can withstand ultra cold temperature. At some culture banks 5 mm disc are suspended in 15% glycerol solution. 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. Cultures may be recovered by rapid thawing at 37°C (Singh *et al* 2001). Presence of liquid nitrogen in storage vials may cause explosion while thawing.

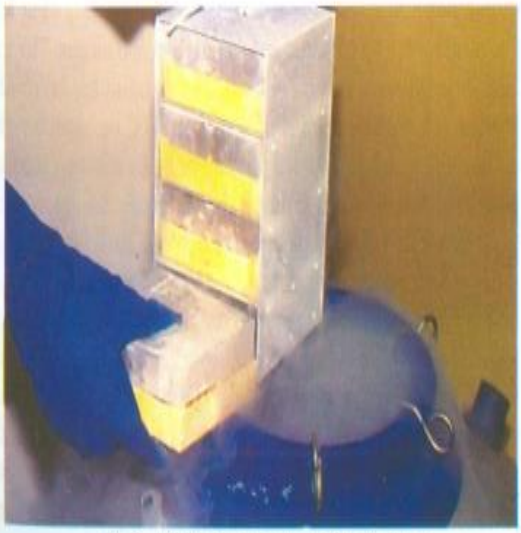

Fig. 6. Liquid nitrogen preservation of cultures

San Antonio (1979) reported that culture viability and mushroom production were not affected by cryogenic storage for 9 years. Elliott and Challen (1979) stored 1,012 cultures of *Agaricus bisporus* and related species for 3 to 4 years in the Glasshouse Crops Research Institute, Littlehampton U.K. and reported 95% recovery rate. Jodon *et al.* (1982) also reported that eight cultures of the commercial mushroom *A.brunnescens* (*A.bisporus*), were preserved in liquid nitrogen for 10 years with no apparent change in morphological or physiological characters. Challen and Elliott (1986) found that 10% aqueous glycerol solution used as cryoprotectant was good in preserving the cultures of *Agaricus* spp., *Coprinus* spp., *Lentinula* spp., *Pleurotus* spp., *Schizophyllum commune*, *Tremella* spp., *Polyporus* spp., and *Volvariella bombycina* but not suitable for *Volvariella volvacea*. However, they found that a 10% aqueous DMSO (dimethyl-sulfoxide) solution gave constant and reliable retrieval of *V.volvacea*. Chen (1987) reported that cultures of all 122 strains from 42 species of Basidiomycetes including important edible mushrooms survived better with slow cooling (@ 1°C/min.) than rapid freezing. Slow freezing and rapid thawing generally gave the highest viability count. Singh *et al.*(2004) preserved mushroom mycelium multiplied on wheat grains instead of mycelial disc in liquid nitrogen and tested survival, yield and genetic stability of 11 edible mushroom stock cultures after several years. The modified cryopreservation protocols, gave experimental demonstration of genetic stability of stock cultures, and validated the use of liquid nitrogen cryopreservation for long-term preservation of mushroom cultures.

New Granular structure medium

A new granular structure culture medium developed by Xiang (1991) can be used as an economic substitute for the traditional cereal grain medium used in spawn manufacture and as medium for the preservation of mushroom strains. The ingredients suggested are sawdust or mixed straw powder (72%), wheat powder (20%), soybean powder (5.5%), complex additives (2%) and adhesive (0.5%). The medium can be prepared and sterilized in 500 ml jars. 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. When the spawn is prepared for cultivation by this new method, less inoculum needs to be removed each time from the specific 500 ml jar. Removed inoculum is then transferred and reproduced into spawn. Each time the inoculum is taken it has to be done at a temperature of 2-4°C in a sterile operation. Using this method, a 500 ml jar of the preserved strain can provide the original and pure, inoculum for long time. Alternatively, fresh granular medium is replaced into the jar and mycelium grows upwards from the jar bottom to top when kept at 20°C to culture the inoculum. When the mycelial growth reaches the surface, the jar is immediately returned to 2-4°C. This cycle can be continued for many years. This method was claimed to be practically superior and helps in rejuvenation of mycelium of the preserved mushroom strain.

Cryopreservation in mechanical freezer

Because viability of stored cells increases dramatically with lower temperature, the ultra low temperature mechanical freezers are recently designed by leading multinational companies to operate efficiently at –140°C or –150°C. Cells may be stored indefinitely at sufficiently low temperatures, safely below –130°C that is glass transition temperature of water. Below this temperature enzyme activity is completely suspended and thermally driven reaction cannot occur. 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. The culture preservation by this method is as good as in liquid nitrogen. It is cost effective as compared to the cost of per litre refill charges of liquid nitrogen, thus reducing operating expenses. Nevertheless, ultra low temperature freezers are run on electricity and therefore are not very successful in developing countries where electricity supply is erratic and on the spot repairs are inaccessible.

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 techniques are best suited for preservation

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
Ultra-low mechanical freezers (-150°C)	High	Low	(-)	(-)

1 = Not a regular practice with mushroom culture bank.

(-) = Ultra low temperature freezers are new. Longevity & genetic stability yet to be proved superior to other methods of preservation.

of edible mushrooms. The handling techniques, freezing protocols, cryo-preservation and thawing rates can be optimized for a particular strain to obtain maximum survival. Once the mushroom has been successfully frozen and stored in liquid nitrogen, the storage period appears to be indefinite, because no chemical and or physical changes can occur at such low temperatures (Grout and Morris, 1987).

Mushroom repositories

The maintenance and production of the reliable pure culture spawn with desirable qualities is a key operation and the first critical stage in the success of mushroom cultivation. Mushroom culture repositories/banks play a vital role in supply of pure and authentic culture to most of the mushroom spawn producing units. One can obtain pure cultures for making spawn from any of the National or International repositories. Some of them are listed below:

National

i) National Research Centre for Mushroom (ICAR), Chambaghat, Solan (HP).

ii) Division of Mycology and Plant Pathology, IARI, Pusa, New Delhi, 110012.

iii) Indian Institute of Horticulture Research (ICAR), Bangalore, Karnataka.

iv) Institute of Microbial Technology(IMTECH), Sector 39D, Chandigarh.

v) Department of Microbiology,Punjab Agricultural University, Ludhiana, Punjab.

vi) Department of Plant Pathology, Maha Rana Pratap Rajasthan Agricultural University, Udaipur, Rajasthan.

International

i) American Type College Collection (ATCC), Rockville, Maryland, USA.

ii) International Mycological Institute, Kew, Surrey, UK.

iii) National Regional Research Laboratory (NRRL), USDA, Peroria, Illinois, USA.

iv) Fermentation Research Institute (FRI), Japan.

v) Canadian Collection of Fungus Culture (CCFC), Canada.

vi) College of Agricultural Sciences, Pennsylvania State University, USA.

vii) Dutch Mushroom Experimental Station, the Netherlands.

viii) Deutsche Sammlung von Mikroorganismen-Braunschweig, Germany.

SPAWN PRODUCTION

Substrate

Cereal grains

Mushroom spawn can be prepared on any kind of cereal grains like wheat, maize, bajra (pearl millet), jowar (sorghum), or rye etc. The large grains carry a greater reserve of food material per grain to sustain the inoculum of mushroom mycelium until it is established and feeding on the compost, so they may be more effective in poor compost or adverse conditions. Whereas, the small grains provide more points of inoculum per gram of spawn, so if all the grains of both types grow equally well, the small ones will penetrate the compost sooner.

Mushroom species	Cultivation method	Spawn substrate	Grain colonization period
Agaricus spp.	Trays, plastic bags/shelf system	Cereal grains	20-25 days
Auricularia spp.	Wood logs/ synthetic bags	Sawdust/grain	18-20 days
Lentinula spp.	Wood logs/ synthetic bags	Saw dust/cereal grains/wood sticks	20-25 days.
Pleurotus spp.	Synthetic bags	Cereal grains	10-15 days
Volvariella spp.	Outdoor/ indoor in cages	Used tea leaves/ Ipil ipil leaves/ paddy straw + saw dust/cereal grains/cotton waste	10-12 days

Other substrates

Although cereal grains are suitable for making spawn of any mushroom variety but due to prohibitive cost a variety of agricultural wastes like corn cobs, wooden sticks, rice straw, sawdust and used tea leaves etc. have also been used. A variety of substrates for different mushrooms have been suggested.

Sawdust of red wood tree species is preferred for making spawn. Wheat bran ranging from 10 to 20% is mixed with sawdust to increase moisture percentage. Used tea leaves are washed and dried before using them for spawn preparation. Ipil ipil leaves are mixed with saw dust in the ratio 3:1 and used as substrate. Paddy straw can also be cut into small pieces of 2-3 cm and soaked overnight before using it as spawn substrate.

Substrate preparation

Spawn substrate should have following desirable characteristics:

- It should not contain any inhibitory compounds to desirable mushroom species.
- Large surface area of substrate should be available for fungal colonization.
- It should provide essential nutrients required by mushroom mycelium to grow.
- Cereal grains should be free from diseases.
- Cereal grains should not be broken, old or damaged by insects.

Spawn-preparation using cereal grains as substrate is described in details. The cereal grains are thoroughly washed in sufficient water 3-4 times to remove soil debris, straw particles and undesirable seeds and grasses etc. washed grains are then soaked in sufficient water for 20-30 minutes to save energy. These grains are taken in a wide mouth container and boiled in sufficient water for 20-25 minutes (Fig. 7). Normally, for soaking and boiling 20 kg of wheat grains, 35 litres of water is required. After boiling, grains should absorb 55-60% moisture. Excess water from the boiled grains is removed by spreading on sieve made of fine wire mesh or muslin cloth. The grains are allowed to leave as such for a few hours so that the water on the surface is evaporated (Fig. 8). Now the grains are mixed with Gypsum (calcium sulfate, $CaSO_4$) and chalk powder (Calcium carbonate $CaCO_3$) so that the pH of the grains is around 7 to 7.5 and they do not form lumps (Fig.9). Different people have given different ratios for mixing gypsum and calcium carbonate. The best results have been obtained by using 200 g gypsum and 50g calcium carbonate for 10 kg grains. These calculations are on dry weight basis of grains used for spawn prepa-ration. First gypsum and calcium carbonate are mixed separately and then the mixture of both is thoroughly mixed with the grains. This mixing should be done on a smooth surface after wearing gloves to avoid contamination.

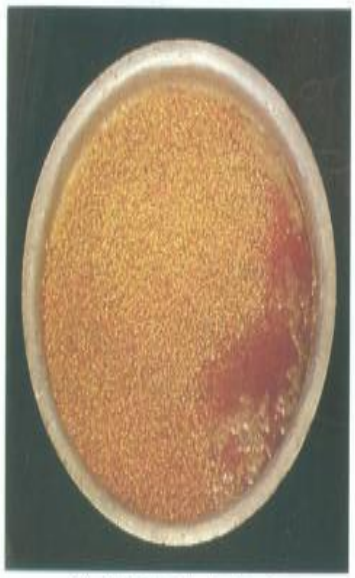

Fig. 7. Boiling of wheat grains

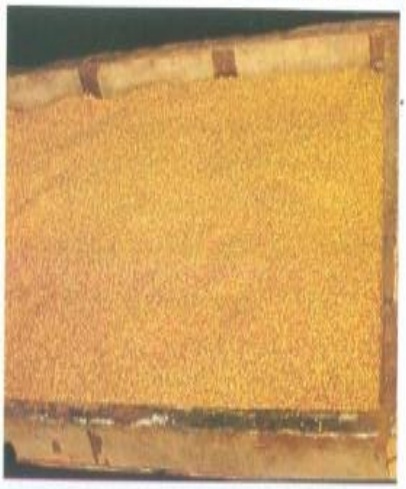

Fig. 8. Draining of water from boiled grains

Fig. 9. Mixing of $CaSO_4$ and $CaCO_3$ with boiled wheat grains.

Mother/master spawn preparation

About 300 g prepared substrate (boiled cereal grains coated with gypsum and calcium carbonate) is filled in glucose/milk bottles up to 2/3 volume and plugged with non-absorbent cotton. These bottles are then autoclaved at 22 lb p.s.i. pressure at 126°C for 2 hours (Fig. 10). These autoclaved bottles are left in the room for 24 hours so that they are cooled to ambient temperature and the excess moisture accumulated inside the bottle walls is evaporated. These autoclaved bottles containing sterilized grains are then exposed to UV light inside the inoculation chamber for about 30 minutes. A piece of growing mycelium is

Fig. 10. Horizontal autoclave for sterilization of spawn substrate.

aseptically transferred to these bottles and inoculated bottles are incubated at 25°C. Inoculated bottles are gently shaked on 5th and 10th day after inoculation. Fully colonized mother spawn bottles can be used for inoculating commercial spawn bags after two to three weeks. Inoculated bottles are incubated at 22-25°C for *Agaricus, Pleurotus, Lentinula* and *Auricularia* species but at 30-32°C for species of *Volvariella* and *Calocybe*. Uninoculated and ready master spawn in bottles is shown vide Figure 11.

Fig. 11. Uninoculated and ready master spawn in bottles

Commercial spawn preparation

Commercial spawn can be prepared in heat resistant polypropylene bags. Normally for half and one kg spawn, the bags should be of 35x17.5cm and 40x20cm size, respectively. Polypropylene bags should have double sealing at the bottom and after filling the grains they are plugged with the help of a polypropylene neck and non-absorbent cotton. The polypropylene bags of 150 gauge should be used. The bags are then sterilized at 22 lb p.s.i. pressure for 1.5 to 2 hours in an autoclave. Autoclaved bags are shaked well before inoculation so that the water droplets accumulated inside the bags are reabsorbed by the grains. The sterilized bags are kept in the laminar flow under UV light for about 30 minutes. Ten to fifteen grams of grains from master spawn bottles is inoculated per bag under aseptic conditions (Fig. 12). One bottle of master spawn is sufficient for inoculating 25 to 30 commercial spawn bags of half kg capacity. Inoculated bags are again shaked so that the inoculum is well mixed with other grains. However, to reduce the time period for spawn preparation, the quantity of inoculum may be increased. Then the bags are kept in incubation room or in BOD incubator for mycelium spread at 25°C (Fig. 13 and 14). During incubation the bags are regularly examined for mould infestation, contaminated bags should be immediately removed and autoclaved before discarding the bags to avoid build-up of contamination in the vicinity. Normally it takes 20-25 days for complete spread of mycelium on the grains for *Agaricus* species. Ready to use commercial spawn in polypropylene bags is shown in Figure 15.

Fig. 12. Inoculation of commercial spawn with master spawn

Fig. 13. Pure cultures, master spawn and commercial spawn in incubation room at 25°C

Fig 14. Incubation of pure cultures and spawn in BOD incubator

Fig. 15. Ready to use commercial spawn in polypropylene bags.

SPAWN STORAGE AND TRANSPORT

If possible, freshly prepared spawn should be used while the mycelium is in the state of active growth. The spawn bags after completion of log growth phase can be maintained up to 3-4 months at 4°C. The commercial spawn should be systematically packed in ventilated bags or cardboard boxes. The spawn should be carried in refrigerated vans to large distances. Alternatively, it can also be transported to long distances during night by public transport or private vehicles so that the temperature of planter spawn do not rise beyond 30-32°C. The spawn of *Volvariella* and *Calocybe* should not be stored at refrigerated temperatures as these mushrooms are sensitive to low temperatures. Old spawn bags of *Auricularia* may also ooze brownish liquid at cooler temperatures that may result in crop losses.

SPAWN PRODUCTION AT A GLANCE

Preparation of Mother / Master Spawn

Step-1: Healthy and cleaned cereal grains

↓

Step-2: Boil grains in water (20-25min.)

↓

Step-3: Remove excess water through sieve

↓

Step-4: Dry grains in shade (4-6 hrs.)

↓

Step-5: Mix $CaCO_3$ (0.5%) and $CaSO_4$ (2%)

↓

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

↓

Step-7: Plug cotton and autoclave at 22 p.s.i for 2 hr.

↓

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

↓

Step-9: Inoculate in BOD at 25°C for 20-25 days (Tropical mushrooms at 30-32° C)

↓

Step-10: Mother / Master spawn is ready.

Preparation of Commercial spawn

Step-1: Use polypropylene bags instead of bottles for grain filling

↓

Step-2: Up to autoclaving Step 1 to 7 are same as of master spawn

↓

Step-8: Inoculate about 10 gm from mother spawn to polypropylene bag containing grains

↓

Step-9: Shake bags after 7-8 days

↓

Step-10 Incubate at 25°C in incubation room or BOD (Tropical mushrooms at 30-32° C)

↓

Step-11: Commercial spawn is ready in 20-25 days.

RECENT ADVANCES IN SPAWN PRODUCTION TECHNOLOGY

Scores of improvements have been reported in various steps involved in spawn production technology. Earlier commercial spawn was prepared in milk or glucose bottles, which used to be difficult to carry from one place to another. Heat resistant polypropylene bags have revolutionized the spawn industry. High-tech spawn labs now use polypropylene microfilm windows for aeration. However, polypropylene translucent bottles of 5-10 litre capacity are also used in Europe and USA for spawn production, but it has not been introduced in India due to high cost of the material. Shiitake (*Lentinula edodes*) cultured continuously in liquid medium and has been used as liquid spawn. Shiitake mushroom was cultivated on synthetic sawdust substrate and this technique is still practiced in developing countries. Normal fruit-bodies were harvested from the colonized substrate block after 120 days incubation with solid spawn. The incubation time was reduced to 90 days with the use of liquid spawn. Liquid culture can be a useful for breeding of this mushroom.

PRECAUTIONS IN SPAWN PREPARATION

Although a number of precautions have been suggested in relevant chapters, still the major precautions are listed below:

1. Strict hygiene should be maintained throughout right from raising of pure culture to storage and transport of spawn.
2. Excess of visitors to inoculation room should be denied.
3. Inoculation room should be disinfected regularly by exposing it to formalin or by installing ozone generator to eliminate chances of contamination.
4. During incubation of spawn, bottles and bags should be inspected frequently to remove contaminated bags.
5. To avoid spread of contamination in the vicinity, the contaminated bags must be autoclaved and buried in the soil.
6. Before spawning into the substrate the stored spawn under refrigerated conditions should be brought to ambient temperatures.
7. It is necessary for each growing cycle to start with a fresh spawn.
8. Regular floor cleaning with surface disinfectants like dettol, lysol etc. should be done twice a week.
9. Cultures should be wrapped with aluminum foil to prevent contamination of cultures during storage under refrigerated conditions.
10. Cultures and spawn should never be stored at sub-zero temperatures in deep freezers. However, cultures can be preserved at ultra low temperatures using cryo-protectants following proper protocols already described separately.

11. Over autoclaving of culture medium beyond the prescribed pressure and time should be avoided as it may cause charamalization of sugars in the medium.

MANAGEMENT OF CONTAMINATION

The most common contaminants encountered during spawn preparation are species of *Aspergillus, Penicillium, Trichoderma, Cladosporium, Chaetomium, Alternaria, Mucor, Rhizopus, Fusarium* and *Drechslera* (Mazumdar and Rathaiah, 2001; Oi, 1991; Thapa *et al.* 1996). Grains are the main source of contamination. The fungal contamination can be usually recognized with the typical colours of their mycelium, spores or conidia. At times a distinctive zone can be recognized as lesion between inoculated mushroom mycelium and contamination. If the contaminants are allowed to grow, they may spoil a large number of spawn bags. If such contaminated bags are not timely removed and disinfected, it may become a perennial source of contamination. Selection of good quality grains, proper autoclaving and strict hygiene in spawn lab can reduce the contamination to a great extent. If the problem continues, carbendazim or thiophanate-methyl @ 0.05g/kg of boiled grains can help in reducing the losses caused by fungal contaminants.

Bacterial contamination is more difficult to detect. Some bacteria give a greasy appearance and emit a pungent or foul order smell. If the bacterial contamination is not detected in master spawn bottles, all the commercial spawn or compost prepared from them will become useless and may result into total loss of spawn or crop. Wet spot disease caused by *Bacillus* species is a common bacterial contamination problem in mushroom spawn (Ahlawat *et al.*, 1999). The disease can be managed by maintaining pH of spawn substrate near 6.0 and by incubating it between 20-25°C. If the problem continues, antibacterial compounds like neomycin, streptomycin or streptocyclin @ 10-50 µg/gram of spawn can be added after boiling of wheat grains to manage the disease.

INFRASTRUCTURE AND RUNNING COST

Proposed Installation Capacity : 20000 kg/annum (20 tonnes/annum)

Now a days there is a lot of awareness about mushroom cultivation in India and people are coming forward to take up commercial cultivation both as self employment venture and subsidiary source of income. The quality of mushroom seed or spawn directly affects the mushroom production. Each mushroom species has its specific seed. Only four species namely *Agaricus bisporus, Pleurotus* spp., *Volvariella volvacea* and *Calocybe indica* are commercially cultivated. The yearly mushroom production and the spawn requirement in India for different species are shown in Table 2.

Table 2 : Spawn requirement for growing different mushroom species in India (2004-2005)

Mushroom Species	Annual Production (Tonnes)	Spawn rate per tonne of prepared substrate/ compost (kg)	Annual spawn requirement (Tonnes)
Agaricus bisporus	50,000	5	1700
Pleurotus spp	8,000	25	1000
Calocybe indica	3,000	50	750
Volvariella volvacea	1,000	25	125
Total	62,000		3575

The total annual mushroom production in India by the end of 2006 has been estimated around 90,000 tonnes and for meeting out this demand about 5200 tonnes of mushroom spawn will be required. In India, spawn is produced and supplied by Research Institutes and Agriculture Universities and private spawn laboratories. Most of th e export oriented units have their own spawn production facilities. The cost of the spawn is the major economic factor for cultivation of specialty mushrooms like *Pleurotus* and *Calocybe indica*. If the spawn is prepared by the producer himself then the profit will be more and one is assured of the quality mushrooms.

Selection of site and layout of a spawn production unit

It is always desirable that the spawn unit should be isolated from composting yard and growing rooms. A separate block for spawn unit can be constructed above the office rooms. The land required for 3666 bags of 500 gm spawn per month are as follows:

Store room

Storage room is required for storage of wheat grains, chemicals, PP bags, PP necks, cotton bundles etc. There should be minimum 4-5 shelves for maximum space utilization. The room should be 4x4x4 m size.

Boiling and filling rooms

The room should be 4x4x4 m size that can be used for washing, boiling and filling grains with sufficient water supply and drainage. Boiling cattle or vessel should be placed near the water drainage. One or two fans and exhaust should also be provided so that the grains could be dried quickly. A platform made of marble or cement should be on the opposite side of the boiling cattle. The platform is used for mixing chemicals with grains, filling and plugging bags. The platform should be 1.10 m (h) x 0.9 m (b) x 4 m (l).

Autoclaving room

The bags after filling are brought for sterilization. Autoclave of 750 mm X 550 mm size should be used for sterilization. The bags after autoclaving should be kept for 6 hours so that water droplets on the PP bags are absorbed before inoculation. There should be small window between autoclaving room and inoculation chamber. Sterilized bags are to be transported only through this window directly to the inoculation room. The autoclaving room should be 3 x 3 x 4 m in size.

Inoculation room

The sterilized bags before inoculation are to be kept in the laminar flow in a double door room located between autoclaving and incubation room. The bags before inoculation should be kept for minimum 15 to 30 minutes under UV light before inoculation. A small BOD (6 cft) and a refrigerator is also required for storing master spawn and culture tubes. The inoculation room should be 4 x 3 x 3 x 4 m in size.

Incubation room

Incubation room should be environmentally controlled for incubation of inoculated bags at desired temperature. Iron racks with 5 tiers at a distance of 30 cm between two tiers, 37.5 cm wide and 1.5 m long could accommodate 72 bags of half kg spawn in each tier. On a single rack about 350 to 360 bags could be kept. An incubation room of 4 x 3 x 4 m size could accommodate 15 racks of the above size. The size and number of incubation room can be increased as per the requirement of the spawn laboratory.

Cold room

The cold room of 3 x 3 x 4 m is used for storing fully colonized spawn bags. Cold room should be fully insulated and the temperature should be maintained between 4 to 6°C. The cold room should have one door for taking prepared spawn out of the cold room. One air curtain should be fixed above the door.

Corridors

A glazed Corridor of 6 x 2 x 4 m should be at the main entry of the spawn unit. An air curtain at the main entry should also be provided.

Equipment

The following major equipments are required for commercial spawn production:

1. Grain storage bins (5 quintal capacity 6 Nos.)
2. Boiling and soaking pans – four Nos. (50 litres capacity)

3. Autoclaves (750 mm depth & 550 mm dia.) - one.
4. Laminar flow – 6'size – One
5. BOD incubator (90 x 90 x 90 cm)- one
6. Refrigerator (210 litre capacity) – One
7. Racks for keeping bags (6'h x5'l x 15" b) with 5 tiers = 15 No.
8. Trolleys for transporting bags - one No.
9. Exhaust fans- Two
10. Air curtain - three Nos.

Minor instruments

Spirit lamps, (2 Nos.) inoculation needles (4 Nos.), big size sieves mounted on iron frame (4' x 4'), (6 Nos.) and lab sitting stools (2 Nos.).

Consumables

Consumables should always be purchased in bulk and the total quantity required for one month should be kept in the stores. Consumables required are wheat grain, rectified spirit, polypropylene bags (14"x 7" or 16"x10"), PP necks. Apron, sleepers, glucose bottles, petriplates, culture tubes, aluminum foils, gloves and non absorbent cotton etc.

Manpower requirement

Two persons are required for preparing 160 bags of 500 g each using 50 kg wheat grain daily.

Economics of spawn production Unit (20 tonnes per annum)

Land and building (Total Area 90sq. m.)

Facility	Unit	Size (Sq. m)	Total area (Sq. m)
Corridor cum office	One	$6^{1}X2^{0}X4^{H}$	12
Storage room	One	$4^{1}X4^{H}X4^{H}$	16
Boiling and filling room	One	$4^{1}X4^{H}X4^{H}$	16
Autoclaving room	One	$3^{1}X3^{H}X4^{H}$	9
Inoculation room	One	$4^{1}X3^{H}X4^{H}$	12
Incubation room	One	$4^{1}X4^{H}X4^{H}$	16
Cold room	One	$3^{1}X3^{H}X4^{H}$	9
Total			**90**

Cost of building, equipment, consumables and manpower

A. Non-Recurring expenditures

a) **Land and building cost**

Item	Area (m)	Cost/sq. mt. (Rs.)	Total Cost (Rs)
Cost of land and development	10X9	Rs. 2000	180,000
Cost of construction of office cum corridor	6X2	Rs. 4,500	54,000
Cost of storage room	4X4	Rs. 4,500	72,000
Cost of boiling room	4X4	Rs. 4,500	72,000
Cost of autoclave room	3X3	Rs. 4,500	40,500
Cost of insulated incubation room	4X4	Rs. 5,000	80,000
Cost of cold room including insulation	3X3	Rs. 5,000	45,000
Total			**5,43,500**

b) **Equipments**

Machinery/Equipment	No. of units	Unit Price (Rs.)	Total Price (Rs.)
Boiling and soaking pans (50 ltr. capacity)	04	500	2,000
Disel/Kerosene operated burner or LPG connection	01	5,000	5,000
Sieves (Mounted on wooden frame 1 x 1.5m)	04	1,000	4,000
Autoclaves (horizontal 550 mm dia x 750 mm depth, 152 liters	01	1,50,000	1,50,000
Small autoclaves (20-25 liters capacity)	01	5,000	5,000
Laminar Air flow (900 x 600 x 600mm size)	01	60,000	60,000
B.O.D. incubator (6cu. Ft.)-	01	50,000	50,000
Hot Air oven (455 x 455 x 455mm) up to 200C.	01	12,000	12,000

Contd...

Refrigerator one (210 liters capacity)	01	15,000	15,000
pH meter	01	10,000	10,000
Exhaust fans	02	2,500	5,000
Air curtain	03	5,000	15,000
Stools	02	1,000	2,000
Almirah set	01	5,000	5,000
Minor equipments	05	1,000	5,000
Refrigeration in cold room.	01	2,50,000	2,50,000
Total			**5,95,000**

B. Recurring Expenditures

a. Consumables: Actual consumables for producing 20 tonnes per year spawn (40,000 bags X 500g) we have to produce minimum 44,000 bags of 500g are to be inoculated to avoid 10% contamination also.

Item	No of units	Unit Price (Rs)	Total cost (Rs)
Wheat grain	150 qt.	Rs 875/qt.	1,31,250
Non absorbent cotton	40 qt.	Rs. 1100/qt.	44,000
P P bags (150 guage thickness for 500 g spawn)	3.67qt.	Rs. 7000/qt.	25,690
P P necks	44,000 Nos	Rs 65/100 Nos	28,600
Gypsum (@2 Kg/qt dry wheat)	3.0 qt.	Rs. 300/qt.	900
Calcium carbonate (@0.5Kg/qt dry wheat)	0.75 qt.	Rs. 700/qt.	525
Methylated sprint	20 Litre	@Rs. 20/liter	400
Disel, kerosene or LPG	570 liters or 40 gas refil	@Rs 250	10,000
Electricity charges	7,000 units/ year	Rs 4.50	31,500
Water	1,00,000 liters/ year	Rs 500/ 10,000 Litres	5,000
Total			**2,77,865**

b. Manpower

Incharge spawn production Rs 5000/- per month 60,000/-	=60,000
Lab workers @ Rs. 2,500/ per month, 02 Nos 60,000/-	=60,000
Total	**1,20,000**

Interest and depreciation

On Land	Cost	Int. & Depr.
7.5% interest	180,000	13,500
On building		
5% depreciation	3,63,500	18,175
7.5% interest		27,262
On Machinery		
10% depreciation	5,95,000	59,500
7.5% interest		44,625
Total		**1,63,062**

Total cost of Production

Raw materials	2,77,865
Salary and wages	1,20,000
Interest & depreciation	1,71,750
Total	**5,69,615**

Production/Receipts

1. 20 tonnes spawn/ year
2. Selling price = Rs 50/kg
3. Total revenue from the sale = **10,00,000/-**
4. Cost of production for 1kg spawn = Rs. 28.48/kg
5. Net profit from the spawn sell = **Rs. 4,30,400** per annum

Note : **The cost of infrastructure, recurring and non-recurring expenditure including interest rates may vary from place to place and from time to time. The spawn production economics is only approximate. The centre shall not be responsible for any variation across time and at different places.**

LIST OF SOME SPAWN LABORATORIES IN INDIA

A) Andhra Pradesh

i) Department of Horticulture,
Public Gardens,
Hyderabad - 500 001.

ii) Department of Horticulture,
Vishakhapatnam, A.P.

B) Arunachal Pradesh

i) Directorate of Horticulture,
Govt. of Arunachal Pradesh,
Itanagar, Arunachal Pradesh

C) Assam

i) Assistant Horticulturist,
Department of Horticulture, Govt. of Assam,
Goalpara, Assam.

D) Bihar

i) Department of Microbiology,
Mushroom Centre, RAU,
Pusa - 848 125, Bihar.

ii) Mushroom Spawn Centre,
DRDA Dumka, Bihar.

E) Chhattisgarh

i) Mushroom Project,
Department of Plant Pathology,
Indira Gandhi Krishi Vishwa Vidyalaya,
Raipur - 492 012, Chhattisgarh.

F) Delhi

i) Division of Plant Pathology,
IARI, Pusa - 110 012, New Delhi.

ii) National Horticulture Research and Development Foundation,
Bagwani Bhavan, 47 Panka Road, Institutional Area,
Janakpuri, New Delhi- 110 058.

G) Haryana

i) Mushroom Spawn Laboratory,
Department of Plant Pathology,

CCS Haryana Agricultural University,
Hisar, Haryana.

ii) Haryana Agro Industrial Corporation,
Murthal Road, Sonepat, Haryana.

H) Himachal Pradesh

i) Director,
National Research Centre for Mushroom,
Chambaghat, Solan - 173 213, H.P.

ii) Department of Plant Pathology,
Dr. Y.S. Parmar University of Horticulture and Forestry,
Nauni, Solan (HP) 173 230.

I) Jammu and Kashmir

i) Spawn Production Laboratory,
Talab Tillo, Jammu, J&K.

ii) Spawn Production Laboratory,
Govt. of Jammu and Kashmir,
Lalmandi, Srinagar, Kashmir, J&K

J) Jharkhand

i) Horticulture and Agroforestry Research Programme,
Plandu, Ranchi - 834 010.

K) Karnataka

i) Mushroom Spawn Laboratory,
Indian Institute of Horticultural Research,
Hessarghatta Lake Post, Bangalore.

ii) Deptt. of Horticulture,
Lalbagh, Bangalore.

L) Kerala

i) Department of Plant Pathology (AICMIP),
College of Agriculture, Kerala Agricultural University,
Vellayani, Thiruvananthapuram, Kerala - 695 522.

ii) Director,
Tropical Botanical Garden Research Institute,
Palode, Thiruvananthapuram, Kerala - 695 522.

M) Maharashtra

i) Department of Plant Pathology,
College of Agriculture, Pune,
Maharashtra.

N) Meghalaya

i) Division of Plant Pathology,
ICAR Research Complex for NEH Region,
Umroi Road, Barapani, Meghalaya - 793 109.

O) Mizoram

i) Director of Horticulture,
Aizwal, Mizoram.

P) Nagaland

i) Spawn Laboratory,
Directorate of Horticulture,
Kohima, Nagaland.

Q) Orissa

i) Spawn Production Laboratory,
Orissa Agro Industries Corporation,
Rourkela, Orissa.

ii) Spawn Production Unit,
State Horticulture Department,
Bhubaneshwar.

R) Punjab

i) Department of Microbiology (AICMIP),
Punjab Agricultural University,
Ludhiana (Punjab).

S) Rajasthan

i) Department of Plant Pathology (AICMIP),
Maharana Pratap Rajasthan Agricultural University,
Udaipur - 313 001 Rajasthan.

T) Sikkim

i) Joint Secretary,
Science and Technology,
Govt. of Sikkim, Gangtok, Sikkim.

U) Shillong

i) Assistant Horticulturist,
Mushroom Development Centre,
Shillong - 793 009.

V) Tamil Nadu

i) Department of Plant Pathology,
Tamil Nadu Agricultural University,
Coimbatore - 641 002, Tamil Nadu.

W) Tripura

Mushroom Laboratory,
Gamnagar, Agartala, Tripura.

X) Uttranchal

i) Mushroom Research Centre,
Department of Plant Pathology,
G.P. Pant University of Agril. And Technology,
Pantnagar - 263 145, Uttranchal.

Y) Uttar Pradesh

i) Department of Plant Pathology (AICMIP),
N.D. University of Agriculture and Technology,
Kumarganj, Gaziabad - 224 229 (UP).

ii) Joint Director,
Industrial Training Centre,
Saharanpur, UP.

Z) West Bengal

i) Department of Plant Pathology,
North Bengal Campus,
B.C.K.V., PO Pundibari, Coochbehar, W.B.

ii) Department of Plant Pathology,
Bidhan Chandra Krishi Vishwa Vidyalaya, Mohanpur,
Nadia, W.B.

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