Project Report

For

20 TPA Mushroom Spawn Unit Project

Rs. 22.12 lakhs

Prepared by:
Directorate of Mushroom Research
Chambaghat, Solan, (H.P.)-173213

FEASIBILITY REPORT

INTRODUCTION

-- is a registered firm. The address of the unit is --. The total capital out lay of the spawn Unit is Rs. 22.12 lakhs.

SECTOR BACKGROUND

Mushrooms have been identified as priority item in government's recent programme of promoting production of vegetables and fruits in the country. Though there are many types of mushrooms produced and marketed worldwide and the white button mushroom (*Agaricus bisporus*) contributes 15% of the total world production and more than 70% of Indian production is of this mushroom. Cultivation of mushrooms in India is of recent origin and it started in the year 1962 in the state of HP. Since then country has progressed tremendously at this front, and today we are producing around 1,81,000 tons of mushrooms per annum. However, this much production does not stand anywhere compared to China that is producing around 33 million tons of this mushroom. Mushrooms are very good source of proteins and are having many medicinal values. In India this sector is growing at the rate of 10% per annum.

PROJECT REPORT FOR SETTING UP OF SPAWN CULTIVATION UNIT

BROAD OUT LINE OF THE PROJECT

1.	**Name of the company**	:	--
2.	**Registered office and address**	:	--
3.	**Proposed location**	:	--
4.	**Promoter(s)**	:	--
5.	**Cost of the project**	:	Rs. 22.12 lakhs
6.	**Proposed capacity**	:	Around 20 tons of Mushroom Spawn production
7.	**Future planning**	:	
	The capacity will be doubled to 40 tons in the 5th year of operation by extending the Equipment's and incubation rooms.		
8.	**Raw materials**	:	
	Main raw materials for production of mushrooms spawn i.e. wheat/paddy/bajra/maize grain, gypsum, calcium carbonates, etc are available throughout the year in the region where project is proposed to be setup.		
9.	**Marketing**	:	
	Seed is one of the most important requirements for the cultivation of mushrooms like any other plant species. In mushrooms, seed is a spore which is a microscopic structure and can not be used directly for seedling as these spores germinate only under specific conditions and on some suitable medium. Hence, for mushrooms, vegetative mycelium offers a great scope for propagation. Mixing of mycelium as such is not practically feasible therefore, a suitable carrier is required which not only help in proper distribution in the substrate but also provides initial nutrition to the growing mycelium. Therefore, ***mushroom seed is a vegetative mycelium developed from a specific mushroom and grown on a convenient medium or substrate. This seed is popularly known as 'spawn'.*** In India, mushroom cultivation started in 1962 and has gradually developed with improvement in composting techniques, cultural practices and use of improved strains. The present		

	production of mushroom is 1.20 lakh metric tonnes per annum and white button mushroom alone contributes about 80 per cent of the total mushroom production in India. Rest of the 20 per cent is mainly contributed by Oyster, Paddy Straw and Milky mushrooms. In white button mushroom, if we take a conversion rate of 15 per cent the compost required for the production of above quantity of white button mushroom is 8.0 lakh metric tonnes. The recommended spawning rate for white button mushroom is 0.5-0.7 per cent of fresh weight of compost and taking into account the average at 0.6 per cent, the requirement of spawn for this mushroom comes to about 48,00,000 kg. The average requirement of spawn for this variety is therefore, approximately 1,92,00,000 bags of 250 g each or 96,00,000 bags of 500 of each or 48,00,000 bags of 1 kg each. Big units having a mushroom production capacity of 500 tonnes or more per annum, have their own spawn laboratories. However, smaller units depend entirely on spawn laboratories run by State Universities, Indian Council of Agricultural Research, New Delhi and private organisations for their spawn requirement. Smaller units need not to produce spawn of their own because of the involvement of highly sophisticated technology and/or due to lack of infrastructures. Therefore, there exists a large scope for private entrepreneurs to enter into this industry to cater to the needs of small mushroom growers.		
10.	**Employment**	:	2 persons
11.	**Electricity units needed per annum (kw)**	:	18900 Unit

WHY MUSHROOM AND SPAWN PRODUCTION

1. Mushroom is an excellent source of good quality proteins to fight protein malnutrition in the Indian masses. Highest producer of protein per unit area and time.
2. Profitable and environmentally sustainable way of recycling abundant agro wastes for food.
3. To reduce pressure on arable land (grown indoors utilizing space also)
4. Excellent medicinal value (diabetes, cardiac diseases anticancer etc.)
5. Labour intensive providing gainful employment.
6. Foreign exchange earner through exports.
7. Mushroom spawn is one of the key inputs for mushroom production.
8. In India, less availability of quality spawn is the major reason for slow growth of mushroom industry.
9. Spawn Industry is a high profit venture for educated youth.

TECHNOLOGY ENVISAGED

Various levels of technologies are available for production of button mushroom- right from cottage industry of China to automated and mechanized technology of the developed countries. The present project proposes to adopt the modern technology of mushroom growing under controlled growing rooms with necessary mechanization and automation owing mainly due to large size of the project and handling of the raw materials in bulk on regular basis to achieve uniform and constant production. This shall cut down the cost of production and improve the quality of mushrooms. Low cost of production will boost competitiveness in the national and international market.

MUSHROOMS - CULTURE AND SPAWN

Mushroom cultivation for food and medicine is a well established profitable industry in many countries of the world Nevertheless, strain improvement of cultivated mushrooms and the development of artificial cultivation of wild mushroom demand a well-planned system for the maintenance, preservation and availability of genetic diversity. Successful mushroom production depends upon the proper maintenance of pure culture spawn capable of providing higher yields, excellent flavour, palatable texture, colour and resistance to pests and diseases. Maintenance of vigour and genetic characteristics of a strain in form of a pure culture is very important. The isolation, purification and maintenance of mushroom cultures require technical expertise and aseptic high-tech laboratory facilities. Therefore, small mushroom growers can't

maintain their own pure culture and/or spawn. They have to rely entirely on commercial spawn producers, reliable governmental or non-governmental organizations that play a vital role in supplying reliable spawn of the desired mushroom strain or variety. Hence training any established organization is a prerequisite before starting a spawn lab.

Culture isolation

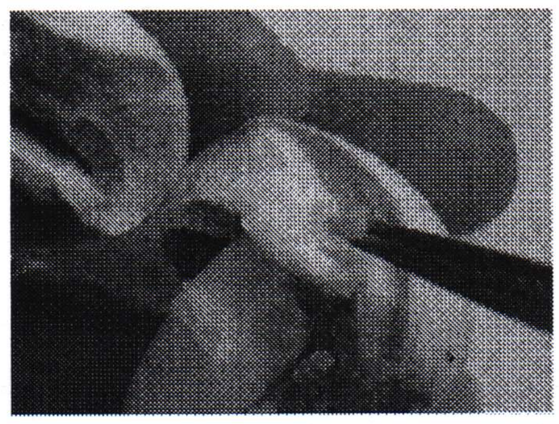
In nature all types of mushroom use dead plant materials as a source of nutrients, which are made available through different degree of decomposition. The culture media or substrate for isolation and culturing must meet the nutritional requirement of mycelium. Some of the commonly used media are: Potato Dextrose Agar, Malt extract, Oat meal, agar, Compost agar and Wheat extract agar. These can be used to isolate and multiply the mycelium of edible mushroom fungi in petridish/bottles/test tubes. A tissue from apex (rapidly growing cells) of the stipe or inner tissue of the cap is often used for obtaining the mycelial cultures. Tissue from other parts however, can also be used. Often 1 or 2 tissue cubes are placed per surface of replicated bottles or plates. These `inoculated' bottles or plates are then incubated at room temperature (supra-optimal mycelial growing condition) until mycelium reaches to the required stage. In laboratory, the edible mushroom strains are cultured on various culture media such as:

Potato Dextrose Agar (PDA) Peeled potato - 200 g Agar agar - 20 g Dextrose - 20g Distilled water - 1 lit	**Malt Extract Agar** Malt Extract – 20 g Agar agar – 20 g Dextrose – 20 g Distilled water – 1 lit
Oat meal agar Oat meal flakes - 30 g Agar agar - 20 g Distilled water - 1 lit	**Compost agar** Pasteurized compost - 150 g Agar agar powder - 20 g Distilled water - 1 lit
Wheat extract agar Wheat grain - 32 g Agar agar powder - 20 g Distilled water - 1 Lit.	**Rice bran decoction medium** Rice bran - 200 g Agar agar - 20 g Distilled water - 1 Lit

Wheat grain and compost extract are most suitable culture media for maintaining *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.

Fresh and healthy mushroom fruit body (basidiocarp) showing all the desirable attributes of that species/strain should be used to raise mycelial cultures by the following methods:

(i) Vegetative mycelium culture (tissue culture)



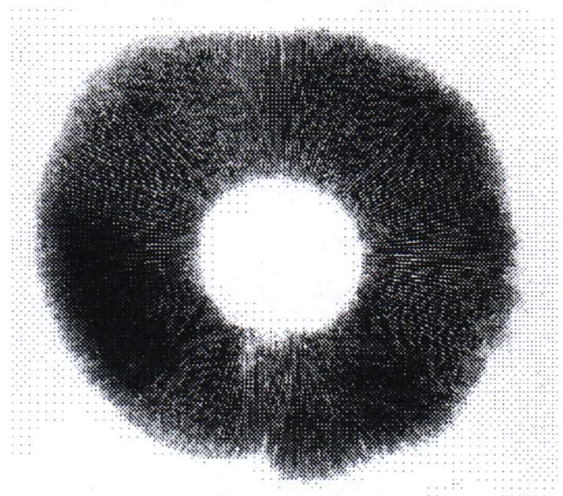
Under aseptic conditions using laminar flow, young basidiocarp is cleaned with sterilized distilled water and dipped into 0.1% murcuric chloride or 2.5% sodium hypochlorite solution for 1 min. In case of button mushrooms the basidiocarp is air dried and split open longitudinally from centre and vegetative 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. These bits are then placed in oven sterilize petriplates having culture media. Inoculated plates are incubated at 25 ± 2°C in a BOD incubator. Within 4-5 days the new mycelium growing from the tissue is observed.

The pure cultures are made by carefully transferring young mycelium from growing edge of the colony from petriplate to test tubes and again incubating at 25C±2°C for 10-14 days (35°C for *Volvariella* spp.).

It is important to check the cultures from time to time for growth and contamination. Mycelial disc of appropriate agar block size are then transferred to new bottles or test tubes and used as the first multiplication of that culture line. Various workers have observed variation in tissue culture propagated strains and reported that majority of the tissue cultures isolates (TCIs) produced low yield in comparison to parental strains. The variation for yield was also revealed among TCIs raised from different parts of fruit bodies. Pileus cultures exhibited minimum variability and yielded better than cultures from stipe and gill tissues. Somaclonal variation can also arise during propagation of various strains via tissue culture due to occurrence of somatic recombination, increased activities of transposons (jumping genes), mutation, gene frequency. The researchers use tissue cultures mainly for domestication of wild germplasm and maintenance of existing varieties.

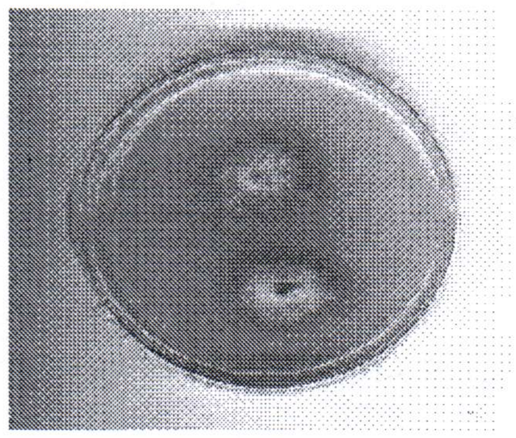
(ii) Multispore culture

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 shaked to obtain uniform spore suspension. A few drops of this suspension are 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 ± 2°C for 3-4 days (35°C for *Volvariella volvacea*). The germinating spores are transferred carefully to culture tubes along with a piece of agar containing a culture medium recommended for the mushroom species being isolated. The culture tubes are then incubated at 25°C for 10-14 days in case of *Agaricus bisporus* and *A. bitorquis*, and at 32°C for *Volvariella volvacea* for 7 to 10 days.



(iii) Single spore culture

A. bitorquis and *Pleurotus* spp. are heterothallic with tetraspored basidia, therefore single spore are self sterile but this technique can be successfully used for breeding new strains. *A. bisporus* being secondary homothallic with bispored basidia and majority of its spore being self fertile, can be used to raise fertile cultures. Single spore cultures are procured in same way as that in multispore cultures. Nevertheless, for single spore culture isolation, the spore suspension is serially diluted to obtain 10-15 spores per plate so that individual germinating spore is marked and could be lifted and transferred to culture medium and incubated at 25 ± 2°C for 10-14 days. Cultures of edible mushrooms can be preserved either as spores or vegetative mycelia. The techniques for maintenance of mycelia for short, medium and long term are described below.

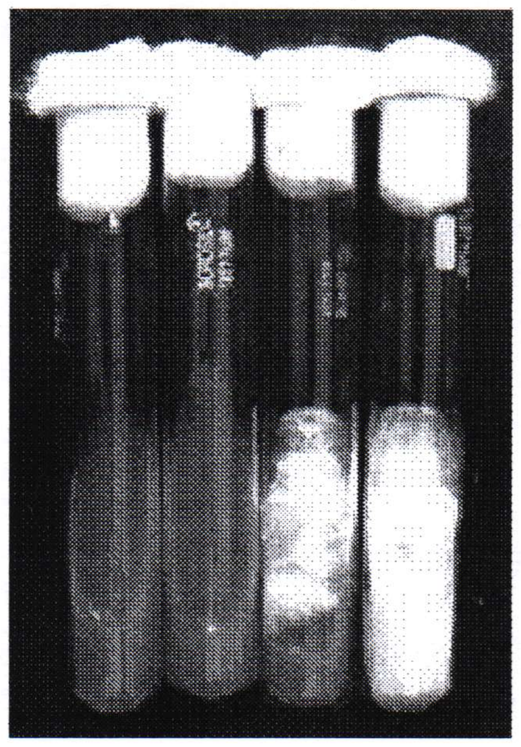


Culture maintenance

(i) Frequent sub-culturing

Under recommended temperature and pH conditions, most mushroom mycelium continues to grow till the nutrients of a suitable culture medium are exhausted. Therefore, these cultures remain viable only for few months depending upon the growth rate, substrate, method of storage, etc. Using a system of periodic transfer at reasonable intervals, stock cultures are often maintained in an actively growing state under

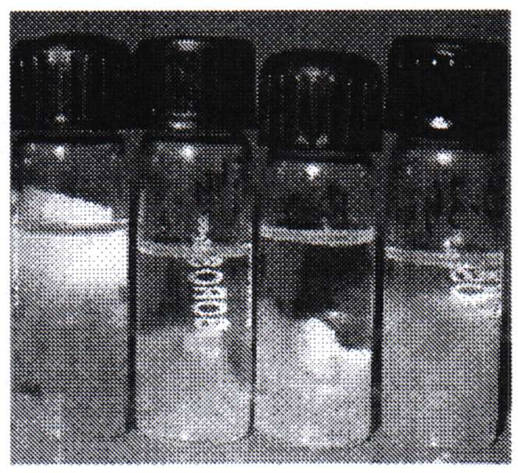
optimum laboratory conditions. After obtaining optimum mycelial growth, mushroom cultures are stored until sub-culturing is necessary. For storage purposes cultures are prepared on agar slants in culture bottles and or test tubes. These cultures can be stored in racks at room temperatures for one to few weeks. The periods between sub culturing can be extended up to 4-6 months by storage at cooler temperatures, i.e., at 4-7°C in a refrigerator or cold room.



In laboratory, the edible mushroom strains are subcultured on suitable culture media. *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. Once fully grown culture of *V. volvacea* has been obtained, they are to be kept at room temperature. *V. volvacea* should be subcultured every 2 months. Species of *Lentinula, Pleurotus* and *Agaricus* strains can be kept in a refrigerator at 4C, and they should be subcultured every 6 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. Cultures tubes of *Volvariella* spp. forms clamydospores, which are brownish in colour. Culture tubes showing more clamydospores indicates 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. Furthermore, such conventional procedures of conservation of living fungi are time consuming, costly and risky. Ultimately repeated subculturing may result in preserving a culture different from the original one. 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.

(ii) Storage under mineral oil

The mineral oil (Liquid Paraffin or Medical Paraffin specific gravity 0.830 – 0.890 g) is sterilized in an autoclave at 121C for 15 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 upto 1 cm above the slant level. 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. The mineral oil blocks the exchange of oxygen between the mycelial surface and the atmosphere in the container, thus retards metabolism and also prevents desiccation of the agar medium. In conjunction with maintenance of the culture in a refrigerator at 4°C, this is an effective method of preserving fungal cultures. Retrieval is done by removing a small piece of the fungal colony 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 culture is usually required before a good culture is obtained.

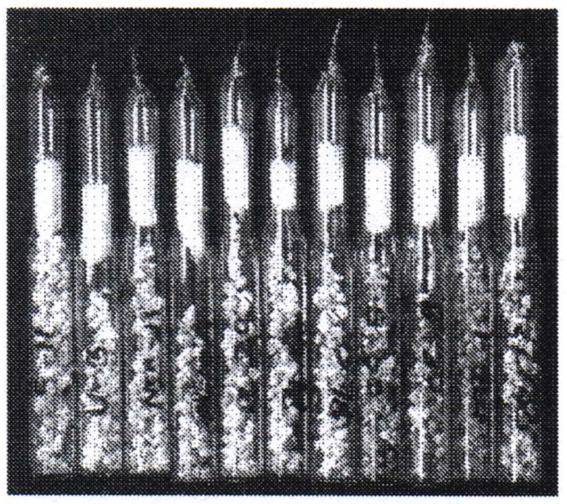
Two disadvantages of oil storage are contamination by airborne spore and retarded growth on retrieval. Nevertheless, in our laboratory, this method is working very well for conservation of most mushroom cultures satisfactorily for several years. A simple and economic method of long-term preservation of mushroom culture for tropical countries using *Pleurotus pulmonarius* involves taking 3 mm diameter discs from agar cultures of fungus in petridishes 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.

(iii) 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 pre-cooled and sterilized McCartney bottles containing simple 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. 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. In another study water storage of 650 plant pathogens belonging to the Phycomycetes, Ascomycetes, Fungi Imperfecti and Basidiomycetes remained viable for 7 years. Growth may sometimes occur during storage in water. This can be reduced if the spores or hyphae are removed from the surface of agar without medium and transferred in water blanks.

(iv) Lyophilization

Lyophilization, also known as freeze-drying, is a method of choice 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 are frozen and water is removed by sublimation. The drying of the spores 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.



Most commonly used suspending media for freeze-drying are skimmed milk (10%), or (trypticase soy broth (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 at 15 lb psi. 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 –40°C for 4 hours. Temperature is then raised in 10°C increments keeping at each temperature for at least half an hour and at 20°C for one hour. 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

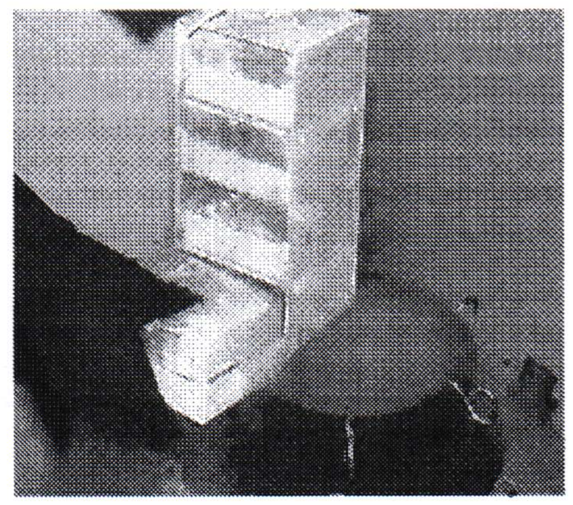
freeze-dryer for secondary drying under vacuum at 20°C for 2 hours and sealed while attached to the lyophilizer itself with the help of a gas-air torch. The ampoules are then stored at 4°C to 6°C for longer shelf life inside a refrigerator. A representative ampoule can be cut from the top to check post-freezing count before finally storing the ampoules for longer duration. Viability of most organisms does not change much upon freeze-drying of viable spores. Rehydration 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.

It has been demonstrated that hyphal cooling at the rate of –1xC/minute to temperatures of –45C and then –75°C, produced fully freeze-dried mycelia. Freeze drying was performed for 2 hours at –40C followed by 20 h at –2C and 8 hours at 20C, resulting in a residual moisture content of 2%. Hyphae of Ascomycetes as well as Basidiomycetes survived freeze-drying.

(v) 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. DMSO penetrates more rapidly and is often more satisfactory and may also be used as cryoprotectant in place of glycerol. Many culture banks are maintaining mushroom cultures by this method satisfactorily for several years (e.g. Microbial Type Culture Collection, Chandigarh).

(vi) Cryopreservation in liquid nitrogen



The storage of micro-organism at ultra low temperatures (-196°C in liquid nitrogen) is at present regarded as the best method of cryopreservation. 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. Although little metabolic activity takes places below –70°C, recrystallization of ice or ice crystal growth can occur at temperature above –139°C, 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. At –196°C dormancy is induced, during which the organism does not undergo and 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.

The temperature of liquid phase of nitrogen remains at –196°C and average temperature of the vapour phase is around –140°C. 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. At some culture banks 0.5 mm disc are suspended in 10% glycerol solution. Programmed cooling at 1C to 10C 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. Glycerol may be replaced by 5% DMSO. Cultures may be recovered by rapid thawing at 37°C. Presence of liquid nitrogen in storage vials may cause explosion while thawing.

It has been reported that culture viability and mushroom production were not affected by cryogenic storage for 9 years. In a study in the Glasshouse Crops Research Institutes, U.K. where 1,012 cultures of *Agaricus bisporus* and related species for 3 to 4 years were stored and reported 95% recovery rate. Researchers found that 10% aquous 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, it was found that a 10% aqueous DMSO (dimethyl-sulfoxide) solution gave constant and reliable retrieval of *V. volvacea*.

(vii) Cryopreservation in mechanical freezers

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, which 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 when 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.

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 stored in liquid nitrogen, the storage period appears to be indefinite, because no chemical and or physical changes can occur at such low temperatures.

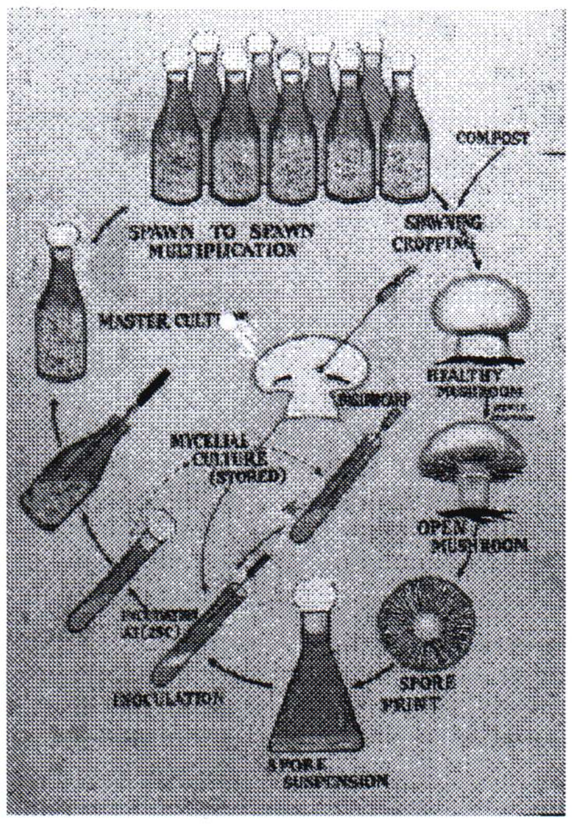
Culture Multiplication as Spawn

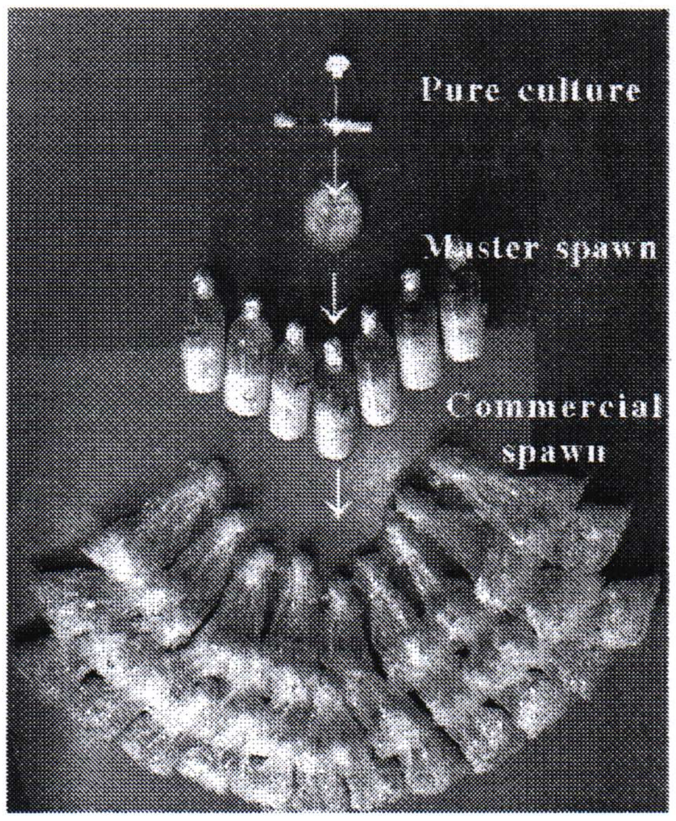
(i) History and types of spawn

The term spawn has been defined as the vegetative mycelium from a selected mushroom grown on a convenient medium. The spawn comprises mycelium of the mushroom and a supporting medium, which provides nutrition to the fungus during its growth. Culpeper described spawn as mycelium of mushroom. From 1652 to 1894 A.D. spawn was gathered from the wild rather than made. Before the advent of grain spawn in 1932, different kinds of spawn used were natural or Virgin spawn (from the pastures & 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 loan soil), etc. In the beginning of 20th century pure mycelial culture were made and used for making manure spawn on sterilized horse manure or compost manure.

The first pure culture spawn was produced by Contratin in France (1894) on horse manure compost. In 1905 Duggar prepared pure culture from mushroom tissue. Later on spawn were 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, Professor J.W. Sinden in 1932. Licenses under the patent were available to any laboratory qualified to make the grain spawn. Grain spawn had an advantage over manure spawn as it could be mixed easily and provided many inoculum points. 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. It is no secret that anyone can make spawn, just as anyone can grow mushrooms. Today most of the traditional spawn laboratories world over are using wheat, rye and millet grains as substrate for spawn production and are following the standard technique of mother spawn from pure culture mycelium grown on synthetic medium.

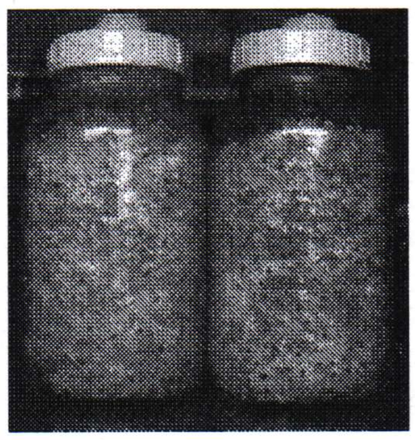
At present, the pure culture spawn has been the basis of modern spawn production units all over the world. The manufacture of the pure culture spawn is done under scientifically controlled conditions which demand a standard of hygiene as in a hospital operation theatre. Equipment and substrate used for spawn are autoclaved and filtered air is passed during the inoculation ensures complete freedom from contamination.





(a) Manure spawn

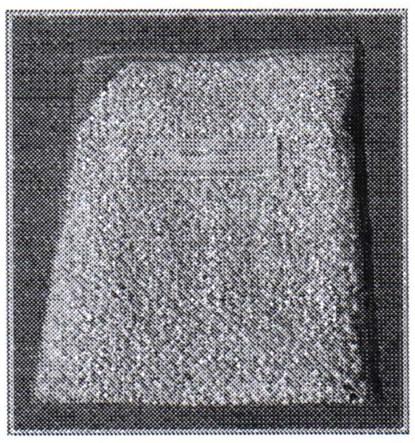
Either composted horse-dung or synthetic compost may be used. The composted manure is thoroughly washed to remove such substance in compost which retards growth. The excess water is squeezed out and moisture content adjusted to 60%. The manure is packed in half-litre milk bottles or heat-resistant polypropylene bags of suitable size. The bottles or bags plugged with non-absorbant cotton-wool/poly-fill and sterlized in an autoculave at 121°C for 2 hr or on 2 consecutive days for an hour each. They are then inoculated with a large bit of agar-containing mycelium and incubated at 22-24°C in a dark place..the spawn can be used to inoculate fresh bottles or bags to obtain the second generation spawn.



(b) Grain spawn

Ten kilograms of wheat grains are boiled for 15 min in 15 litres of water and then allowed to soak for another 15 min without heating. The excess water is drained off and the grains are cooled in sieves. Turn the grains several times with a spoon for quick cooling. The cooled grains are mixed with calcium carbonate. The gypsum ($CaSO_4.2H_2O$) and 30 g of calcium carbonate. The gypsum prevents the grains from sticking together and calcium carbonate is necessary to correct the pH. The prepared grains are filled into half-litre milk bottles or polypropylene bags (at the rate of 150-200 g per bottle or bag) and autocalved for 2 hr at 121°C. After sterlization, the material should have a pH value of 6.5 to 6.7. The bottles are inoculated with grains spawn or with bits of agar medium colonized with mycelium and incubated at 22-24°C in a dark place. the mycelium completely permeates the grains in about 2 weeks. Other grains like sorghum and pearl millet can also be used for spawn making.

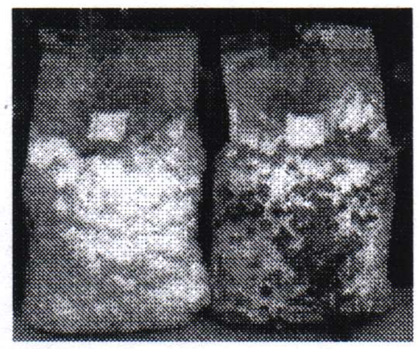
(c) Perlite spawn



This was developed by Lemke (1971). Perlite is a mineral which expands at temperature more than 1000°C. The ingredients of the spawn are: Perlite (1,450

g), wheat-bran (1,650 g), gypsum (200 g), calcium carbonate (50 g), and water (665 cc). The ingredients are mixed, filled in bottles and sterilized. Thereafter, the process is the same as for grain spawn. Perlite spawn is easy to disperse and can be produced at a cheaper cost. This spawn can be stored for a long time.

(d) Saw dust spawn



Sawdust spawn is a sterilized mixture of sawdust and bran fully colonized with mushroom mycelium. This kind of spawn is normally used for the cultivation of wood rotting mushrooms like Shitake, Reishi, maitake, etc.

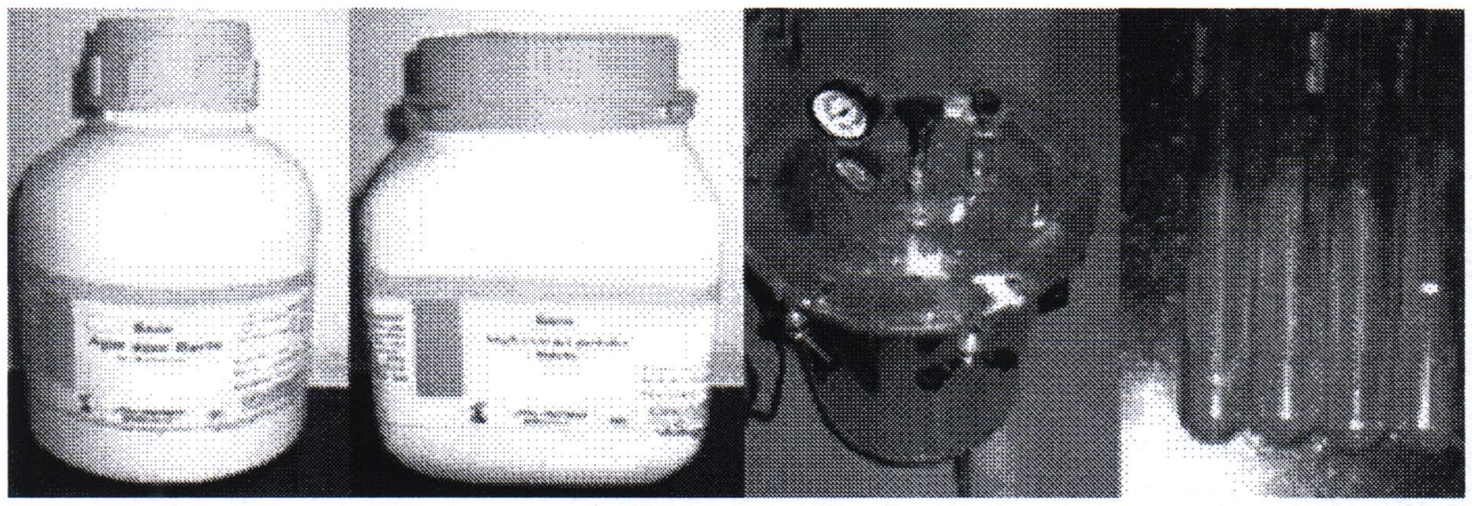
(e) Liquid spawn

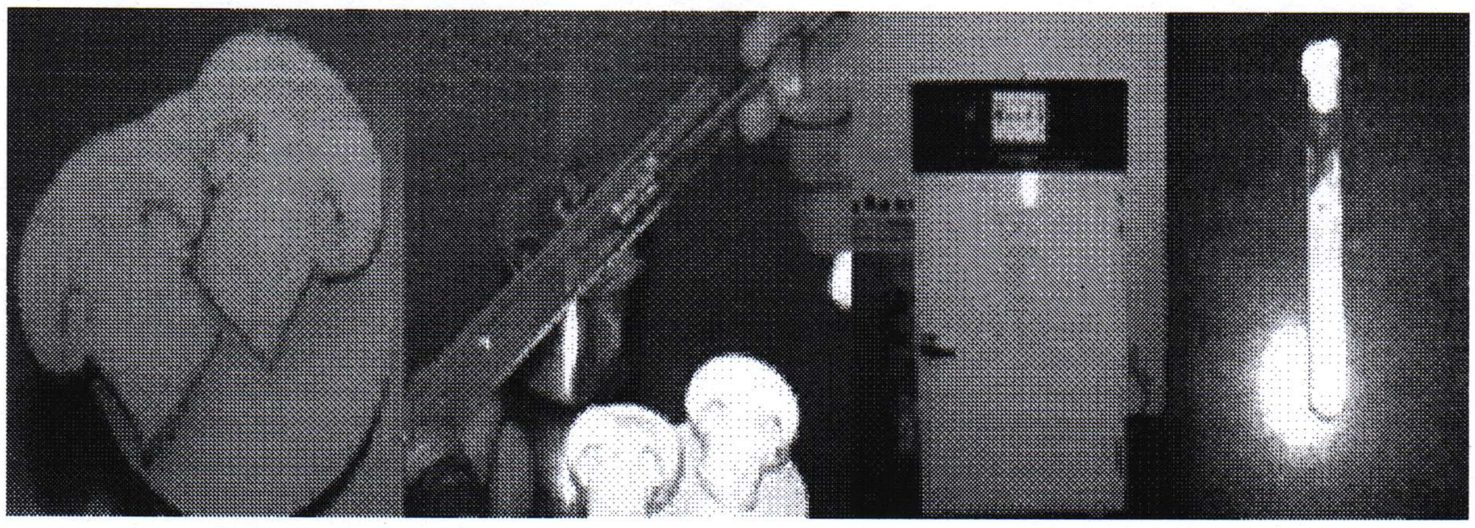
Mycelium cultured in liquid medium followed by maceration/ homogenization can also be used for spawning. This is commonly referred as liquid spawn. It can be used for mechanizing inoculation process of spawn multiplication or can be used for inoculating substrates.

Today most of the traditional spawn laboratories world over are using wheat, rye and millet grains as substrate for spawn making and are following the standard techniques of mother spawn from pure culture mycelium grown on synthetic medium. Strandy cultures showing good growth and not showing fluffy growth, sectoring or slow growth are desirable. During cropping bare patches on bed, deformed fruit bodies with no or few gills, weeping mushrooms indicate strain degeneration. Multispore cultures degenerate faster than single spore cultures. Hence, it is important to properly maintain the culture at desired temperature and rejuvenate them by change of media and replace them in case of any sign of degeneration.

Pure Culture Preparation

Pure culture of fleshy fungi/mushrooms can be prepared either by multi-spore culture or tissue culture. Multi-spore culture is obtained by placing a fresh fruit body after alcohol sterilization on a petriplate/sterilized paper. Millions of spores are collected within 48hr. Serially diluted loop full of spores are then transferred to sterile Potato-dextrose-agar (PDA) or Malt-extract-agar culture slants. These slants are then inoculated at 25°C ± 2°C for 2 weeks to obtain pure culture. For tissue culture, the basidiocarp after alcohol sterilization is cut longitudinally into 2 halves and bits from collar region are transferred to pre sterilized PDA or MEA culture medium. The petriplates are incubated at 25°C ± 2°C in BOD incubator for one week. Mycelium from growing edges is carefully transferred to MEA/PDA slants and again incubated for 2-3 weeks to obtain pure cultures. Basic materials and equipment required for obtaining pure culture is given in Fig. that follows.



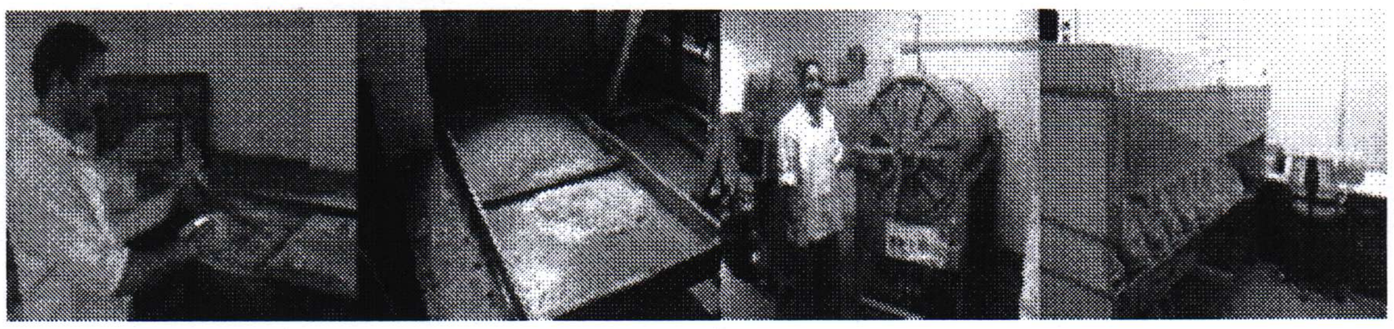


(ii) Substrate Preparation

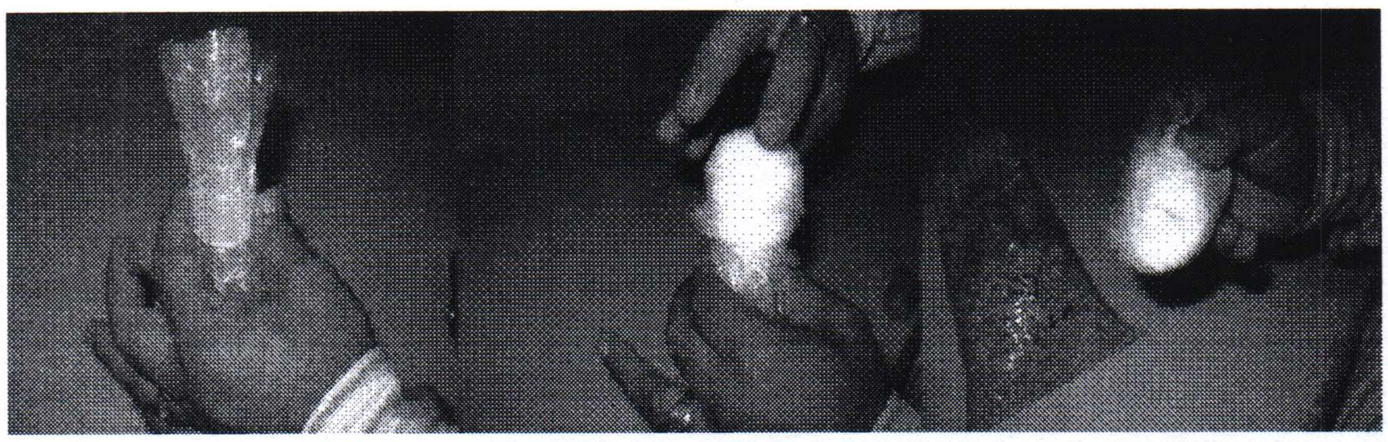
Mushroom spawn can be prepared on any kind of cereal grains like wheat, jowar, bajra or rye and agricultural waste like corn cobs, wooden sticks, rice straw, saw dust and used tea leaf etc. Spawn substrate should have following desirable characteristics.

i) It should not contain any inhibitory compounds to desirable mushroom species.
ii) Large surface area of substrate should be available for fungal colonization.
iii) It should provide essential nutrients required by mushroom mycelium to grow.
iv) Cereal grains should be free from diseases.
v) Cereal grains should not be broken, old, and damaged by insect pests.

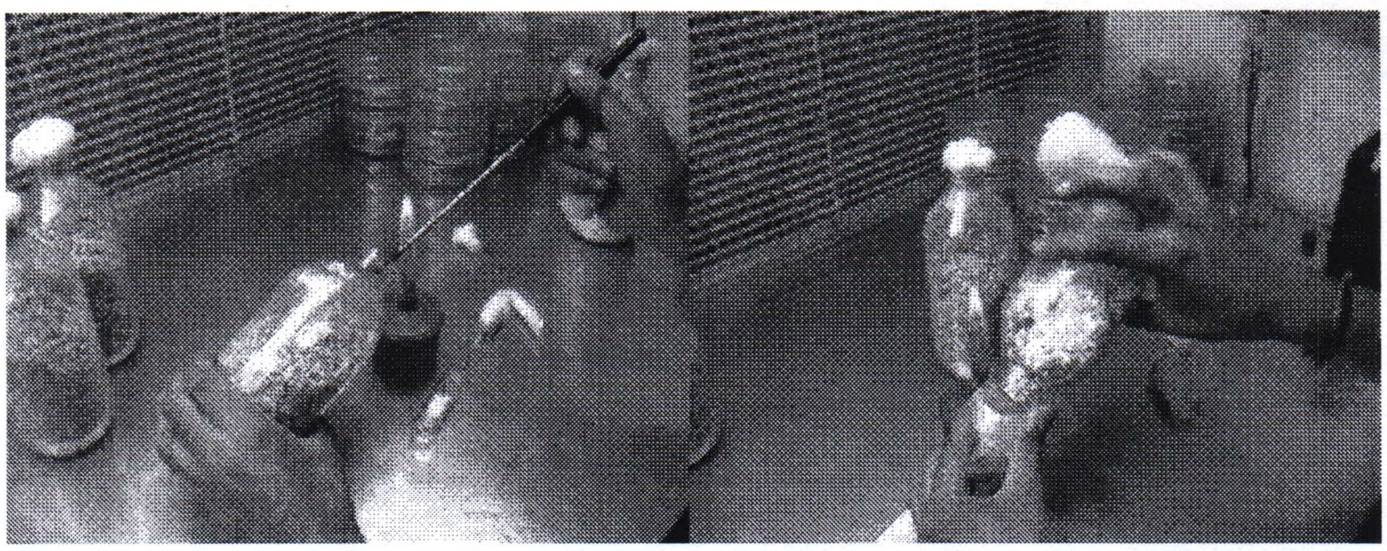
Most of the cereal grains are good substrate for spawn production of white button mushroom (*Agaricus bisporus* and *A. bitorquis*), oyster mushroom (*Pleurotus* spp.) and paddy straw mushroom (*Volvariella volvacea*, but wood rotting fungi like shiitake (*Lentinula edodes*) and black ear mushroom (*Auricularia* spp.) grow better on saw dust based substrates over cereal grains. The grains are thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses etc. washed grains are then soaked in sufficient water for 20-30 minutes and boiled in some container for 20-25 minutes. Normally for soaking and boiling 20kg of wheat grain, 35 litres of water is required. 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 few hours so that the water on surface is evaporated. Now the grains are mixed with Gypsum (Calcium sulphate) and chalk powder (Calcium carbonate) so that the pH of the grains is around 7 to 7.8 and they do not form clumps. Different people have given different ratios for mixing Gypsum and Calcium carbonate. The best results have been obtained by using 200g Gypsum and 50g chalk powder for 10kg grains (dry weight basis). The quantity of Gypsum and chalk powder influences their ratio for mixing. First Gypsum and chalk powder are separately mixed and then they are thoroughly mixed with the grains. This mixing should be done on a smooth surface after wearing gloves to avoid contamination. For commercial spawn production rotating drums can be used for uniform mixing.



(a) Boiling, sieving, autoclaving and inoculation



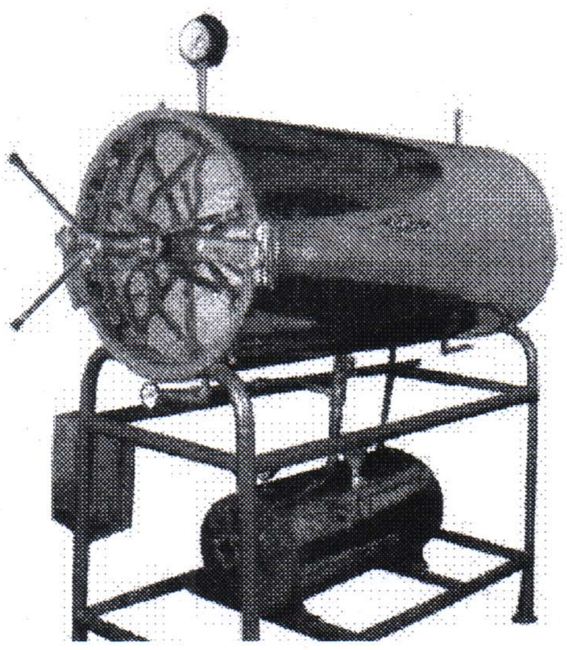
(b) Method of inserting ring, folding bag and plugging



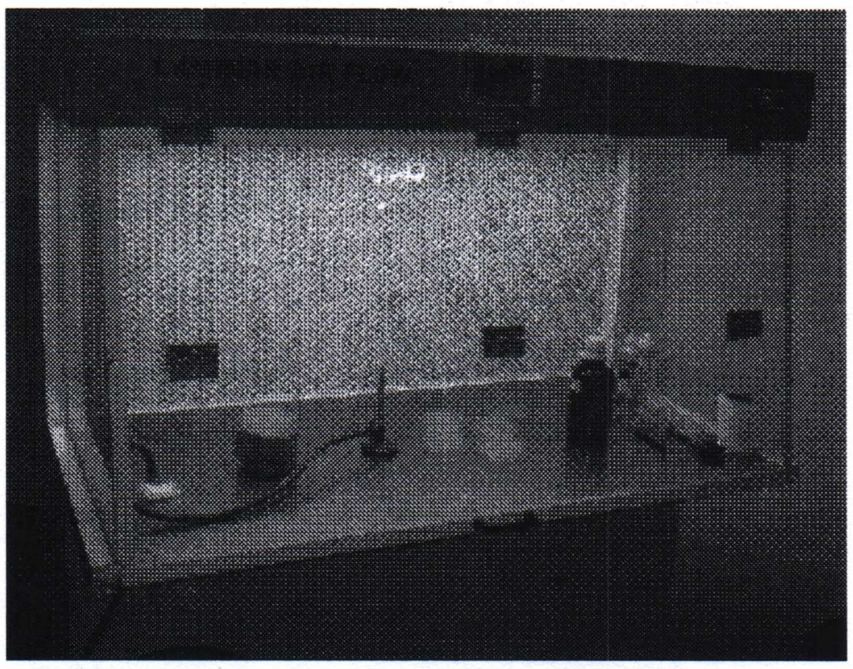
(c) Preparation of master spawn and commercial spawn

(iii) Mother Spawn Preparation

About 300g prepared substrate 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 1.5 to 2 hr. These autoclaved bottles are left in the room for 24 hours so that they are cooled and kept on laminar flow under U.V. tube for 20-30 minutes. A piece of growing mycelium is aseptically transferred to these bottles and inoculated bottles are incubated at 25°C. Inoculated bottles are gently shacked on 5th and 10th day. Fully grain colonized mother spawn bottles can be used for inoculating commercial spawn bags after two to three weeks. For colonization these bottles are incubated at 22-25^{0}C for *Agaricus bisporus, Pleurotus* spp. and *Lentinula edodes* but at 30^{0}C for *Volvariella* spp.

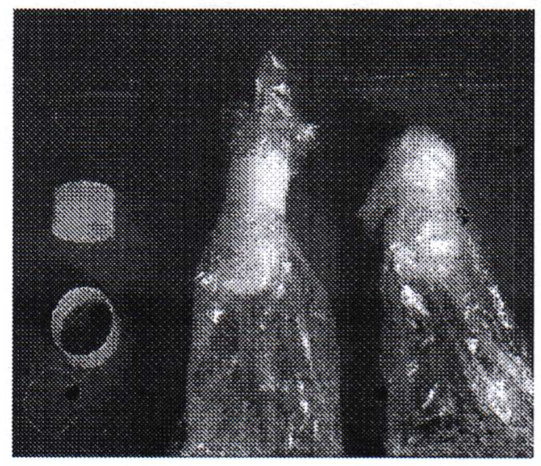


Autoclave for sterilization of grains



Laminar Flow - Inoculation Chamber

(iv) Commercial Spawn Preparation



Commercial spawn can be prepared in polypropylene bags (heat resistant). 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 PP neck and non-absorbent cotton. The bags are then sterilized at 22lb p.s.i. pressure for 1.5 to 2 hours. Autoclaved bags are shaked well before inoculation so that the water droplets accumulated inside the bags is being absorbed by the grains. The sterilized bags are kept on the laminar flow under U.V. tube for 20-30 minutes. Ten to fifteen gm of grains from master spawn bottle is inoculated per bottle under aseptic condition or one bottle of master spawn is sufficient for inoculating 25 to 30 commercial spawn bags.

Spawn Incubation

Inoculated bags are again shaked so that the inoculum is well mixed with other grains. Then the bags are kept in incubation room for mycelium spread. During incubation the bags are regularly examined for mould infestation. Contaminated bags should be immediately removed before discarding the bags to avoid build-up of contamination in the vicinity. Normally it takes 15-20 days for complete spread of mycelium on the grains. Fully colonized bags should be kept in cold room (+4^{0}C) for future use. The spawn of button mushroom, *Pleurotus* can be stored at this temperature. However, neither the culture nor spawn of Volvariella, Ganoderma and Calocybe is stored below 15^{0}C (Table). The contaminated bottles/ bags/tubes etc. are autoclaved before emptying and discarding.

Table. Temperature requirement and storage and incubation of different mushrooms

	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

(v) Spawn Storage and its Transport

Wherever possible, freshly prepared spawn should be used because the mycelium is in the state of active growth. The spawn bags after completion of log growth phase are maintained of 2-3 months. The planting spawn should be systematically packed. It is transported for long distances during night so that the temperature of planter spawn does not rise beyond 30-32°C.

Scores of improvements have been reported in various step involved in spawn production. Earlier spawn was prepared in milk or glucose bottles, which was difficult to transport from one place to another.

Heat resistant polypropylene bags have revolutionized the spawn industry. High-tech spawn labs now use polypropylene bags with microfilm windows for aeration. Though 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. The mature spawn bags, that is polypropylene bags with grains fully colonized by mycelium should be packed in well ventilated cardboard cartons and stored at 2-4°C. The spawn is transported from one place to another in refrigerated vans or during night when temperature does not rise above 32°C. It is important that spawn bags are not exposed to heat and dust during transport.

Precautions

1. Always keep the inoculation chamber and its surroundings very clean.
2. Switch on UV tube in the inoculation chamber for 30 minutes before inoculation by keeping sterilized substrate, forceps, cultures inside the chamber
3. Inoculation is always done near the spirit the spirit lamp flame to avoid contamination.
4. The working person should swab his hands and inoculation chamber using alcohol.
5. Spawn should grow fast in the bottles, should be silky white in colour and should never show fluffy growth.
6. All grains should be covered by the mycelial growth and fresh spawn should have mushroom odour.
7. Mother spawn should not be used beyond 3-4 generations as it starts degeneration. Fresh spawn gives higher yield, therefore spawn should never be stored for more than a month.
8. All the bottles must be labeled indicating firms name, species, date of inoculation to know the age and type of spawn.

Characteristics of good spawn

Quality of spawn is mainly determined by the biological value of the strain used and technology involved at different stages of spawn production. The spawn should be fast growing in the compost, early cropping after casing, high yielding and should produce better quality mushrooms. Mushroom growers can not guess or predict the quality of spawn is being supplied to them unless the crop is grown from spawn. So it is the duty and responsibility of the spawn producing laboratories to stick to the schedule and standard of the quality spawn. There are few under mentioned characteristics of good quality spawn which can be judged by visual observation of spawn: Spawn prepared on Jowar or wheat grains gives higher yield over spawn prepared on bajra, barley or kodo grains.

1. There should be proper coating of mycelium around grains used as substrate for spawn production. No loose grain should be visible in a bottles/bags. The grains left over without mycelial coating will invite contamination in the compost during spawn running period.
2. The growth of the mycelium in the spawn bags/bottles should be silky/strandy type. It should not be cottony type because there is likelihood of stroma formation on the casing layer, which interferes with air exchange and absorption of water in the casing material resulting in low yields.
3. The growth of fresh spawn is more or less white. Brown colouration develops as spawn gets older. Fresh spawn gives higher yield than the old one. Spawn should not be more than one month old in any case.
4. There should not be any slimy growth in spawn bags/bottles which is an indication of bacterial contamination.
5. There should not be any greenish or blackish spot in the spawn bottles/bags. Such spots indicate that the spawn is contaminated with moulds.

6. When the spawn bags/bottles are opened for spawning it should emit typical mushroom smell.

Spawn Production (Flow chart)

Preparation of Mother Spawn

Step-1 Select healthy and cleaned cereal grains
↓
Step-2 Boil Grains in water (15-20 min.)
↓
Step-3 Remove excess water on sieve
↓
Step-4 Dry grains in shade (4 hrs.)
↓
Step-5 Mix $CaCO_3$ (0.5%) and $CaSO_4$ (2%) on dry wt basis
↓
Step-6 Fill 300g grains in glucose/milk bottle
↓
Step-7 Plug cotton and autoclave at 22 p.s.i. for 1.5 to 2 hrs.
↓
Step-8 Inoculate growing mycelium of desired strain using laminar flow
↓
Step-9 Incubate in BOD at 23 ± 2^0C for 20-25 days
↓
Step-10 Master spawn is ready

Preparation of Commercial spawn

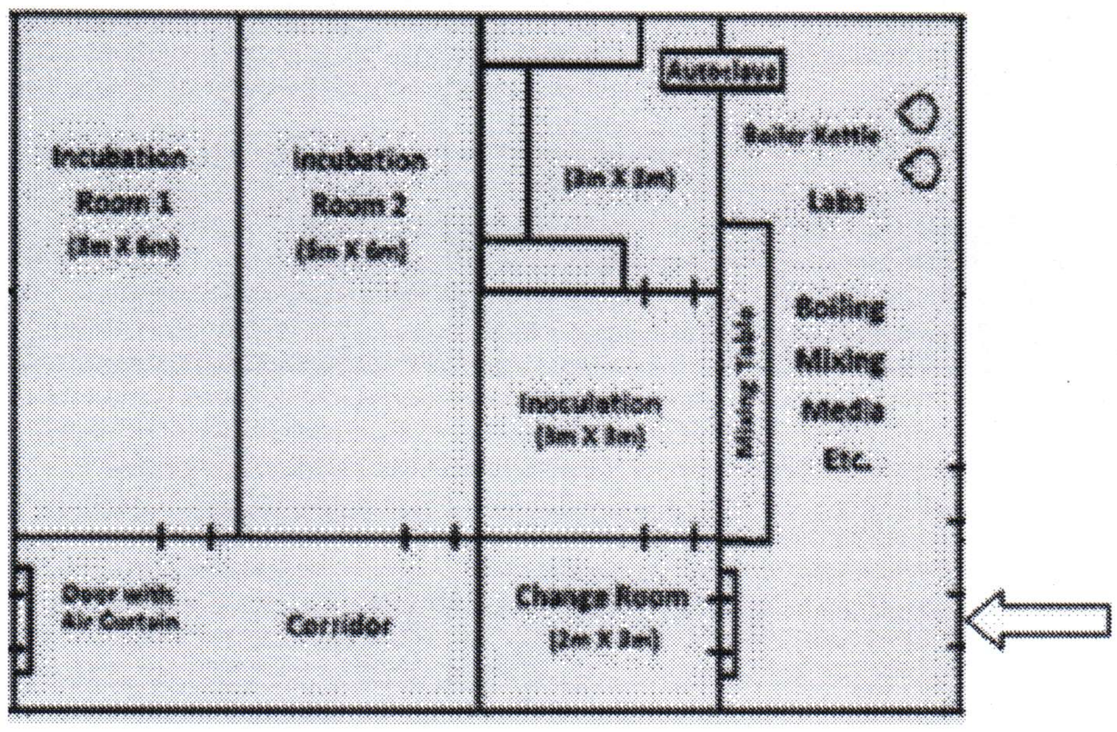
Step-1 Use polypropylene bags instead of bottle
↓
Step-2 Upto autoclaving (Step 1 to 7) is same as of mother spawn
↓
Step-8 Inoculate 15-20 grains from mother spawn per PP bag
↓
Step-9 Incubate at 23 ± 2^0C in incubation room
↓
Step-10 Shake bags after 7-8 days
↓
Step-11 Commercial spawn is ready in 20-22 days.

Table. Problems faced, causes and solution during preparation of pure culture/spawn

Problem	Cause	Solution
Agar medium very soft or hardly solidifies	Quantity of agar insufficient i.e. too low or agar is of inferior quality	Use branded and proper quantity of agar in medium
Agar surface in the plates not smooth or lumpy	Agar medium partially solid when poured	Pour agar medium when it is still hot
Contaminants appear after 2-3 days on the surface of the medium after sterili- and before inoculation	Medium not sufficiently sterilized Medium not aseptically poured	Sterilization should be carried for the recommended period and at recommended temperature pressure Medium should be poured aseptically
Transferred mycelilial bit/ tissue resume no growth	Non- viable inoculumn/ culture Wrong type of medium Incorrect formulation or pH Needle or scalpel used to transfer the culture bit too hot or culture exposed to flame for prolonged period during transfer	Use viable culture/ actively growing culture Use correct medium Properly check the formulation and pH of the medium Cool the flamed needle before picking the inoculum and carefully transfer it
Contamination develops on the plugs after 2-3 days	Cotton too moist or not of non-absorbent quality Filters of the laminar flow damaged Incubation room too much loaded with air born inoculum	Properly cover plugs before autoclaving and use quality non absorbent cotton Filters should be checked or replaced as per recommendation Sterilize incubation rooms from time to time
Resulting mycelial growth slow and fluffy	Strain degenerated	Obtain another culture or retrieve stock culture
Grains contaminated after sterilization and before inoculation	Highly infected seeds Grains not fully sterilized Sealing of PP bags improper or plugs too loose	Use fresh and clean seed Prolong sterilization period Use quality PP bags (may use double bags) and properly plug the bottles/bags
Mycelial growth very thin and hardly penetrates the grains	Grains too dry	Boil the grains sufficiently and adjust proper moisture levels
Mycelial growth does not continue upto the bottom of the bag	Excessive grain moisture	Adjust proper moisture level
Mycelia do not grow thoroughly on the substrate or patchy growth	Grains contaminated with bacteria / yeast due to improper sterilization Less vigorous strain	Use recommended sterilization time Use vigorous strain
Contamination appears on the surface of the inoculum (grain or mycelia bit)	Contamination occurred during inoculation	Inoculation should be performed in a more aseptic way and observe complete cleanliness
Mycelia growing very slowly	Unsuitable substrate or processing improper, pH not correct, poor quality of gypsum or chalk Incubation temperature not suitable	Use recommended substrate Check the temperature requirement Use vigorous culture

G. Design of a Spawn Laboratory (Production Capacity 100 TPA)

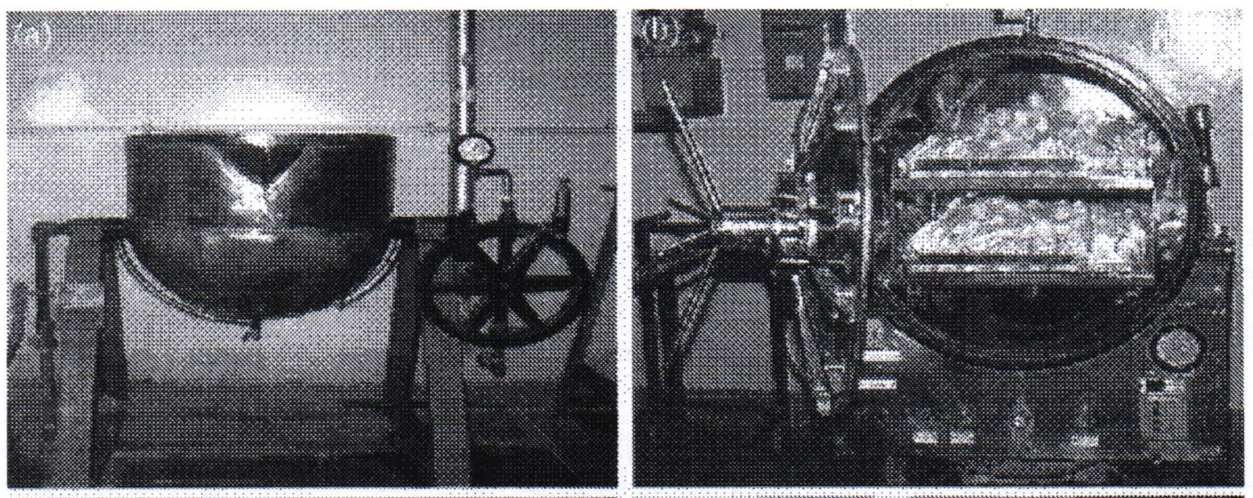
The medium size spawn laboratory (production capacity at least 100 TPA) should have a total built up area of 96 x 8 x 3.2 m (L x B x H). This area will be divided into different work areas like cooking/autoclaving room, inoculation room, and incubation room, washing area. Cold storage room of 3 x 3 x 3.2 m (L x B x H) can also be added and it is enough to store the spawn at 4-5°C. The walls, roof floor as well as door is provided with heavy insulation (7.5-10 cm thickness) and two air conditioner (each of 1.5 tonnes capacity) are required to maintain temperature inside the room. Two incubation rooms of 3 x 6.0 x 3.2 m (L x B x H) with entire surface area (wall, floor, ceiling, doors) insulated with 5-7.5 cm thick insulation. Two air conditioners (each 1.5 tonnes capacity) are required to maintain temperature (25°C) in the incubation room.

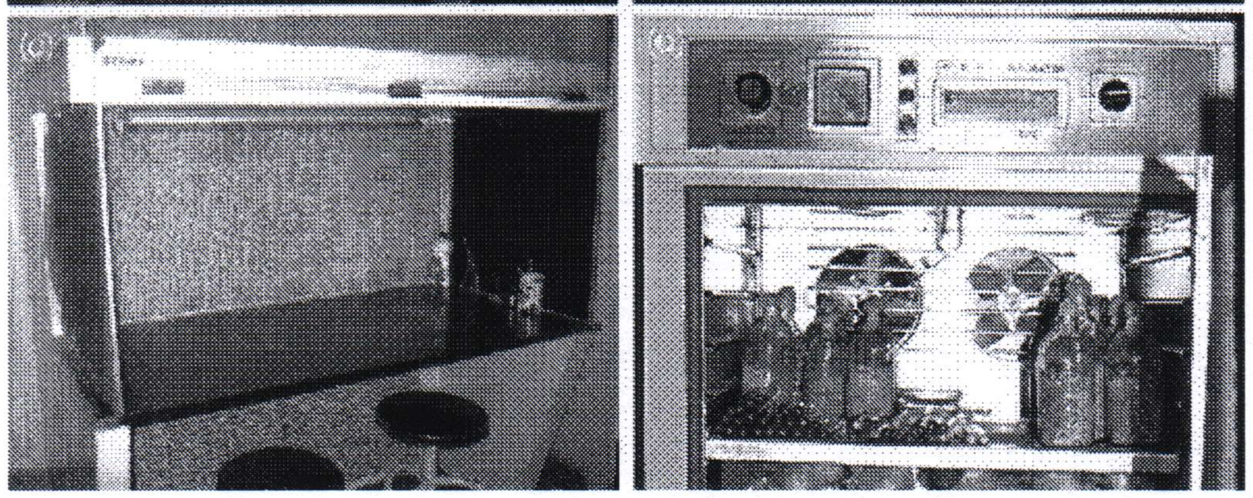


H. Equipments Required

The equipment required in a spawn laboratory are:

1. Boiling pans/boiling kettle (vessel) for boiling the grains. Kettle can also be used if baby boiler is available otherwise kettles working on electricity, kerosene or gas can be installed. Pans for preparation of medium are also required.
2. Stove or steam line for boiling of wheat grains and preparation of medium.
3. pH meter to check pH of the medium.
4. Autoclave for sterilization of spawn medium and oven for sterilization glassware. Two electrically operated autoclaves with 100-145 bottle capacity having a dia. of 2 ½' and 3 ¼' height are sufficient. If boiler is available steam operated autoclaves can be used for better efficiency. A small clinical autoclave can also be kept for sterilization of culture medium.
5. BOD incubator is needed to incubate cultures and master cultures.
6. Laminar flow cabinet (normally 4 ft. horizontal) is needed for isolation and multiplication of cultures and spawns inoculation.
7. Refrigerator is needed for short-term preservation of mycelial cultures.
8. Other items like glassware, chemicals for medium preparation, non-absorbent cotton, polypropylene bags (or bottles), disinfectant (formaldehyde), calcium carbonate, calcium sulphate are also required.
9. Steel racks in incubation room and cold storage for keeping bags/bottles, exhaust fans, filters, office table, working tables, troughs, sieves, inoculating needles, scalpels, test tubes, petri plates etc. are also required.
10. AHUs can be installed for creating positive pressure by filtered air. Similarly air curtains are desirable to keep aseptic conditions in the lab.





COST OF PROJECT

	Items	Rs in lakhs
1.	Land	Owned/Leased
2.	Land and Site Development (986 sq ft)	1.98
3.	Buildings	6.30
4.	Plant and Machinery	13.23
5.	Misc fixed asset	0.60
	Grand Total	22.12

1. **Land and site development**: A piece of land measuring 977 sq ft will be required. Land will be leveled and developed including boundary wall/barbed wire making the total cost of Rs. 1.98 lakhs.

2. **Buildings:** Design and layout of the buildings to be constructed are given in the figures enclosed and annexure –A (Rs. 6.30 lakhs)

3. **Plant and machinery:** Cost Rs 13.23 lakhs (Annexure – B)

4. **Miscellaneous fixed assets:** Rs 0.60 lakhs (Annexure –C)

List of Annexure	
Annexure No.	
	Summary
I	Infrastructure
II	Plants and Machinery
III	Recurring expenses
IV	Cost of Project
V	Repayment of term loan and interest calculation
VI	Depreciation
VII	Revenue Generation
VIII	Profitability Estimates
IX	Calculation of Break Even Point
X	Debt Service Coverage Ratio
XI	Calculation of Internal Rate of Return and Net Present Value
XII	Projected Balance Sheet
XIII	Projected Cash Flow Statement
XIII	Debt Equity Analysis

Summary

Production and Cost

PRODUCTION AND COST		
Spawn production /day	**66.5**	kg
No of operational days/year	**300**	days
Selling price per kg in year 1 (Rs)	**100**	per kg
Rate of income tax on income	**0%**	
Spawn production per year	**20**	tons
Total cost of the project	**22.12**	lakhs
DEPRECIATION		
Depreciation rate Buildings	5%	
Depreciation rate Equipment's	10%	
Maximum amount eligible for subsidy	As applicable	
LOAN		
Months after which loan re-payment will start	**12**	months
Rate of interest on loan	**11.00%**	
Percent of total admissible as loan	**75.00%**	
Max loan that can be taken from bank	**16.60**	lakh
Total admissible loan from bank out of Rs 16.6 lakh	**16.50**	lakh

SUMMARY					
	1st Year	**2nd year**	**3rd year**	**4th Year**	**5th Year**
Annual installment (in Lakhs)	4.75	4.39	4.03	3.66	3.30
Pay back period	2 years 1.9 months				
Discounted PBP	2 years 7 months				
Break Even Point (1st year)	13.00%	%			
DSCR (1st year)	2.33				
NPV	35.92	lakh			
IRR	41.0%				
IRR/NPV	113.11				
Discounted IRR	25.8%				
BC Ratio	2.62				
Current Ratio (1st Year)	1.33				
Debt to Equity Ratio (1st Year)	0.98				
Discount rate	10%				

Annexure-I

Infrastructure

Infrastructure	No.	Area (Sq Ft)	Unit Cost (Rs)	Total cost (Rs in Lakhs)
Boiling, mixing area	1	100.00	700.00	0.70
Inoculation room	1	120.00	900.00	1.08
Incubation rooms	1	114.91	1200.00	1.38
Corridor	1	30.00	600.00	0.18
Store/Facilities	1	120.00	700.00	0.84
Grain Storage area	1	71.82	700.00	0.50
Sale Room	1	90.00	700.00	0.63
Total	-	**647**		**5.31**
Boundary wall and flooring of open area (50% of above)	1	330	300	0.99
Total area (sq ft) (Lab+Open area)	-	**977**		**6.30**

Annexure-II

Plant and Machinery

Equipment required and cost	No/kg	Rate	Cost (Rs. in Lakhs)
Autoclaves horizontal	1	600000	6.000
Autoclave vertical small for lab	1	50000	0.500
Laminar flow	1	150000	1.500
Stands for incubation rooms (sq ft area)	193.68	400	0.775
Stands for cooling room (sq ft area)	96.84	400	0.387
AC Unit for Incubation and cold room (TR)	1.9576	50000	0.979
AC system for cooling room	1	60000	0.600
Incubator	1	100000	1.000
Fridge	1	30000	0.300
pH meter	1	25000	0.250
Lab coats	4	500	0.020
Gloves, caps, footwears, masks, disposable coats, etc	2	1000	0.020
First aid box	1	5000	0.050
Fire safety equipment	1	10000	0.100
Gas/LPG cylinder, burner/spirit lamps, misc equip (per ton spawn)	20	200	0.040
Temperature and humidity meters	2	1000	0.020
UV tubes	2	2000	0.040
Hot air Oven	1	50000	0.500
Weighing balance	1	5000	0.050
Microwave oven/induction cooktop	1	10000	0.100
Total	-	-	**13.23**

Annexure-III

Recurring expenses (Salary, Raw material and Energy)

	Amount/month	No	Months	Cost
Labour	8000	**1**	12	96000
Skilled labour	10000	**1**	12	120000
Total Wages	**18000**		**12**	**216000**
Total Salary & wages				**216000**
Raw Material	**Quantity**	**Rate (Rs.)**	**Cost (Rs.)**	
Wheat/Sorghum/Bajra/ Paddy grains, etc (quintals)	111	2600	288889	
Glass bottles (No.)	200	5	1000	
flasks (No.)	4	500	2000	
Plastic petri-plates (pre-sterilized) (No.)	200	15	3000	
Culture tubes (No.)	20	30	600	
Non absorbent cotton/Polyfill (Kgs)	250	300	75000	
PP bags/Microbial filter bags (kgs)	200	400	80000	
PP rings (No.)	20000	1	20000	
Parafilm for petri-plates (No.)	1	2500	2500	
Butter paper, tissue paper, rubber bands, etc (No.)	20000	1	20000	
Data registers, standard labels for record (No.)	20000	0	6000	
Forceps, inoculation needles, dispensers (No.)	4	500	2000	
Misc items	2000	1	2000	
Total			**502989**	
Cost (Rs. in Lakhs)			**5.03**	
Chemicals required (per annum)	**Quantity**	**Rate (Rs.)**	**Cost (Rs.)**	
Calcium carbonate (kg)	556	1	556	
Calcium sulphate (kg)	2223	3	6669	
Spirit/sanitizer/alcohol (Bottle of half litres) (No.)	4.00	100	400	
Agar-agar (kg)	0.16	10000	1600	
Glucose (kg)	0.12	1000	120	
Malt extract (kg)	0.16	6000	960	
Potato (kgs)	1.60	30	48	
Sodium hydroxide (500 g)	1.00	1000	1000	
Hydrochloric acid (500 ml)	1.00	1600	1600	
Floor cleaner (litres)	2.00	60	120	
Yeast extract (kg)	0.50	4500	2250	
Misc	0.40	5000	2000	
Total			**17323**	
Cost (Rs. in Lakhs)			**0.17**	
ELECTRICITY REQUIRED AND COST				
Electricity units needed per annum (kw)	18900	7	132300	
Total			132300	
Total (Rs. in lakhs)			1.32	
Items			Rs. In Lakhs	
Raw material and Electricity		6.53	**652612**	
Wages		2.16	lakh	
Salary		0.00	lakh	
Raw materials, electricity, etc		6.53	lakh	
	Total	**8.69**		

Annexure-IV

Cost of Project

COST OF LAND, BUILDINGS & MACHINES		
Land	OWNED	
Building	6.30	Lakhs
Machinery	13.23	Lakhs
Total Cost of Project	22.12	Lakhs
Working Capital	2.21	Lakhs
MEANS OF FINANCE		
Proprietor's Capital	5.62	Lakhs
Term Loan	16.50	Lakhs
Total	22.12	Lakhs
SUBSIDY		
Rate of Subsidy	As applicable	
CROP- PRODUCTION AND SALE PRICE		
Spawn production /day	66.50	kg
No of operational days/year	300.00	
Spawn Production / year	20.00	
Selling price per kg in year 1 (Rs)	100.00	Rs

Annexure-V

Repayment of term loan and interest calculation

Particulars	**Opening Balance**	**Interest Capitalized**	**Repayment Principal**	**Closing Balance**	**Interest (11%)**	**Total Repayment**
Year	16.50			16.50		
1st Year	16.50		3.30	13.20	1.45	4.75
2nd year	13.20		3.30	9.90	1.09	4.39
3rd year	9.90		3.30	6.60	0.73	4.03
4th year	6.60		3.30	3.30	0.36	3.66
5th year	3.30		3.30	-	-	3.30

Annexure-VI

Depreciation (in Lakhs)

Year	**Depreciation**	**Building**	**Plant & Machinery**	**Total WDV**	**Dep. For Year**	**Total Dep**
		5%	**10%**			
	Value	**8.28**	**13.83**	**22.12**		
1st Year	Depreciation	0.41	1.38		1.79	**1.79**
	WDV	**7.87**	**12.45**	**20.32**		
2nd year	Depreciation	0.39	1.24		1.64	**3.43**
	WDV	**7.48**	**11.20**	**18.68**		
3rd year	Depreciation	0.37	1.12		1.49	**4.92**
	WDV	**7.11**	**10.08**	**17.19**		
4th year	Depreciation	0.36	1.01		1.36	**6.29**
	WDV	**6.75**	**9.07**	**15.83**		
5th year	Depreciation	0.34	0.91		1.25	**7.53**
	WDV	**6.41**	**8.17**	**14.58**		

Annexure-VII

Revenue from Sale of Mushroom

	Year 1	Year 2	Year 3	Year 4	Year 5
Total Spawn sold/annum (ton)	20	20	20	20	20
Selling price Rs./kg (5% increase)	100	105	110	116	122
Revenue from sale of Spawn (Rs in lakhs)	**20.00**	**21.00**	**22.05**	**23.15**	**24.31**

Annexure-VIII

Profitability Estimates

lakhs

Sr. No.	Particulars	1st Year	2nd Year	3rd Year	4th Year	5th Year
A	**Sales (Revenue)**	20.00	21.00	22.05	23.15	24.31
	Total (A)	**20.00**	**21.00**	**22.05**	**23.15**	**24.31**
B	**Cost of Production/ Expenses**					
	Variable					
	Raw Material	5.20	5.46	5.74	6.02	6.32
	Fuel & energy	1.32	1.39	1.46	1.53	1.61
	packing cost	0.00	0.00	0.00	0.00	0.00
	Wages	2.16	2.32	2.50	2.68	2.88
	Direct Operating Exp.	**8.69**	**9.17**	**9.69**	**10.24**	**10.82**
	Fixed					
	Total (B)	8.69	9.17	9.69	10.24	10.82
	Gross Profit (A-B)	**11.31**	**11.83**	**12.36**	**12.91**	**13.49**
	Salary	0.00	0.00	0.00	0.00	0.00
	Admn. Expenses	0.00	0.00	0.00	0.00	0.00
	Depreciation	1.79	1.64	1.49	1.36	1.25
	Total	**1.79**	**1.64**	**1.49**	**1.36**	**1.25**
	Profit before Interest and tax	**9.52**	**10.19**	**10.86**	**11.55**	**12.25**
	Interest on term loan	1.45	1.09	0.73	0.36	0.00
	Interest on working capital	0.24	0.00	0.00	0.00	0.00
	Profit before Tax	**7.83**	**9.10**	**10.14**	**11.19**	**12.25**
	Income Tax@0%	0.00	0.00	0.00	0.00	0.00
	Net Profit after Tax	**7.83**	**9.10**	**10.14**	**11.19**	**12.25**
	Depreciation	1.79	1.64	1.49	1.36	1.25
	Cash Accruals	**9.62**	**10.74**	**11.63**	**12.55**	**13.49**
	Cumulative Profit	**7.83**	**16.93**	**27.07**	**38.25**	**50.50**
	Operating profit	**11.31**	**11.83**	**12.36**	**12.91**	**13.49**
	Net Profit ratio	39.14	43.32	45.98	48.32	50.38
	Interest Coverage Ratio	5.39	8.35	13.96	30.82	0.00
	PAY BACK PERIOD					
	Payback period=	**2 years 1.9 months**				
	Discount rate=	10%				
	Discounted PBP=	**2 years 7 months**				

Annexure-IX

Calculation of Break Even Point

(Based on 1st Year Working Results)

		Amount (In Lacs)				
		1st Year	**2nd Year**	**3rd Year**	**4th Year**	**5th Year**
Sales		20.00	21.00	22.05	23.15	24.31
Less: Variable Exp.		8.69	9.17	9.69	10.24	10.82
Contribution		11.31	11.83	12.36	12.91	13.49
fixed Expenses						
Salary Expenses		0.00	0.00	0.00	0.00	0.00
Administration Expenses		0.00	0.00	0.00	0.00	0.00
Interest		1.45	1.09	0.73	0.36	0.00
Total Expenses		1.45	1.09	0.73	0.36	0.00
Total Expenses (Fixed in Nature)		1.45	1.09	0.73	0.36	0.00
Break Even Point(Cash, Operating B.E.P)						
	(At Sales Value)	**2.60**	**1.89**	**1.32**	**0.69**	**0.00**
	(At Capacity Level)	**13.00%**	**9.00%**	**6.00%**	**3.00%**	**0.00%**

Annexure-X

Projected Debt Service Coverage Ratio (DSCR)

	Particulars	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**
	Profit after tax	7.83	9.10	10.14	11.19	12.25
Add:	Depreciation	1.79	1.64	1.49	1.36	1.25
	Cash Profit	**9.62**	**10.74**	**11.63**	**12.55**	**13.49**
Add:	**Interest**					
	Interest on Term Loan	1.45	1.09	0.73	0.36	0.00
	Cash Available to (Repayment Interest & Installment)	**11.08**	**11.83**	**12.36**	**12.91**	**13.49**
	Repayment					
A.	**Principal**					
	Repayment of Term Loan	3.30	3.30	3.30	3.30	3.30
B.	**Interest**	1.45	1.09	0.73	0.36	0.00
	Total Repayment(A+B)	**4.75**	**4.39**	**4.03**	**3.66**	**3.30**
	DSCR	**2.33**	**2.69**	**3.07**	**3.53**	**4.09**
	Avg. DSCR Ratio	**3.14**				

Annexure-XI

Calculation of Internal Rate of Return and Net Present Value

Particulars	1st Year	2nd Year	3rd Year	4th Year	5th Year
CASH INFLOWS					
Net Profit after Tax	7.83	9.10	10.14	11.19	12.25
Depreciation	1.79	1.64	1.49	1.36	1.25
	9.62	**10.74**	**11.63**	**12.55**	**13.49**
CALCULATION OF IRR	**Interest rate (10%)**				
			Yr	**cash flow**	
Year o (cash outflow)			**0**	**-22.12**	**-22.12**
1st Year (cash inflow)			**1**	**9.62**	**9.62**
2nd Year (cash inflow)			**2**	**10.74**	**10.74**
3rd Year (cash inflow)			3	11.63	**11.63**
4th Year (cash inflow)			4	12.55	**12.55**
5th Year (cash inflow)			5	13.49	**13.49**
NPV (check)					35.92
NPV					**35.92**
INTERNAL RATE OF RETURN (IRR)					**41%**
BC Ratio or PI					**2.62**
IRR/NPV					113.11%
Modified IRR					
Discount rate of reinvestment		10%			
Discounted IRR					**25.83%**

Annexure-XII

Projected Balance Sheet

Particulars	Year 1	Year 2	Year 3	Year 4	Year 5
Sources of Fund					
Owner's Funds	5.62	13.44	22.54	32.68	43.87
Profit & Loss a/c	7.83	9.10	10.14	11.19	12.25
Closing Capital	13.44	22.54	32.68	43.87	56.12
Loan Funds					
Term Loan	13.20	9.90	6.60	3.30	0.00
Total	**26.64**	**32.44**	**39.28**	**47.17**	**56.12**
Applications Of Funds					
Fixed Assets					
Gross Block	22.12	22.12	22.12	22.12	22.12
Less:-Depreciation	1.79	3.43	4.92	6.29	7.53
Net Block	20.33	18.69	17.19	15.83	14.58
Net Working Capital					
Net Current Assets	6.32	13.76	22.09	31.34	41.53
Total	**26.64**	**32.44**	**39.28**	**47.17**	**56.12**

Annexure-XIII

Projected Cash Flow Statement

Particulars	Year 1	Year 2	Year 3	Year 4	Year 5
Cash From Operating Activities					
Profit Before Tax	7.83	9.10	10.14	11.19	12.25
Add:					
Depreciation	1.79	1.64	1.49	1.36	1.25
Interest on Term Loan	1.45	1.09	0.73	0.36	0.00
Cash before working capital Change	11.07	11.83	12.36	12.91	13.49
Change in Working Capital	0.00	0.00	0.00	0.00	0.00
Tax	0.00	0.00	0.00	0.00	0.00
Cash from Operating Activities	**11.07**	**11.83**	**12.36**	**12.91**	**13.49**
Cash From Investing Activities					
Purchase of Fixed Assets	22.12	0.00	0.00	0.00	0.00
Cash generated from investing activities	**-22.12**	**0.00**	**0.00**	**0.00**	**0.00**
Cash From Financing Activities					
Promoter's Contribution	5.62	0.00	0.00	0.00	0.00
Capital Expenditure Loan	16.50	0.00	0.00	0.00	0.00
Repayment of Term Loan	3.30	3.30	3.30	3.30	3.30
Interest on Loan	1.45	1.09	0.73	0.36	0.00
Cash generated from Finance Activities	**17.36**	**-4.39**	**-4.03**	**-3.66**	**-3.30**
Opening Balance	0.00	6.32	13.76	22.09	31.34
Cash generated during the year	6.32	7.44	8.33	9.25	10.19
Closing Balance (Cash & Bank Balance)	**6.32**	**13.76**	**22.09**	**31.34**	**41.53**

Annexure-XIV

Debt Equity Analysis

Particulars	Year 1	Year 2	Year 3	Year 4	Year 5
Debt Funds					
Term Loan	13.20	9.90	6.60	3.30	0.00
Debt Fund	**13.20**	**9.90**	**6.60**	**3.30**	**0.00**
Equity Fund					
Owner Funds	13.44	22.54	32.68	43.87	56.12
Debt Equity Ratio	**0.98**	**0.44**	**0.20**	**0.08**	**0.00**
Avg. D/E Ratio	**0.34**				
Net Current Asset	6.32	13.76	22.09	31.34	41.53
Net Current Liability	4.75	4.39	4.03	3.66	3.30
Current Ratio	**1.33**	**3.13**	**5.49**	**8.56**	**12.59**

CONCLUSION

Mushroom cultivation has tremendous potential in the country due to vast availability of raw materials and cheap labour. Mushrooms have been kept under a major thrust area by the govt. There is a vast potential of export of this commodity to Europe, America and middle East countries. Production figures envisaged in the project (Promoter: --, --) are based on 100% capacity utilization, which can easily be achieved in a year's time if the farm is managed properly and there is a constant supply of raw materials. Yield realization are based on the cultivation of advanced hybrids by providing exact environment conditions to derive optimum potential of the strain. These projections can very well be achieved, however DMR, Solan (HP) in no way guarantees for the above production and profit since it will not be directly involved in the execution of the project and in the production phases. Much would depend upon the skill and management of the entrepreneur for the success of the project. At the moment most of the projects of similar capacity or more are running successfully and earning handsome profits.

(Shwet Kamal)
Principal Scientist
ICAR-Directorate of Mushroom Research
Chambaghat, Solan (H.P.) – 173213.

(V.P. Sharma)
Director
ICAR-Directorate of Mushroom Research,
Chambaghat, Solan (H.P.) – 173213.

Dated: