CERTIFICATE PROGRAMME IN MUSHROOM CULTIVATION

LABORATORY MANUAL & RECORD

COURSE - 3

BIOLOGY AND CULTIVATION METHODS OF MUSHROOMS

Block - I	:	Laboratory Methods
Block - II	:	Edible and Poisonous Mushrooms
Block - III	:	Spawn Preparation and Cultivation Methods
Block - IV	:	Pests and Diseases and Preservation

DR. B.R. AMBEDKAR OPEN UNIVERSITY
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PREFACE

This Laboratory Manual & Record deals with the Biology and Cultivation Methods of Mushrooms included in the syllabus of six months duration Certificate Programme in Mushroom Cultivation offered by Dr. B.R. Ambedkar Open University. Keeping in view the requirements and pattern of practical examination, necessary information of the subject is provided in this manual. To acquaint the students with laboratory and microbiological techniques, common laboratory methods are included. To enable the students to identify various wild mushrooms, morphology and identification of commonly occurring edible and poisonous mushrooms are included. Raising of pure culture, mother spawn preparation and cultivation methods of button mushroom, oyster mushroom, paddy straw mushroom, shiitake mushroom, black ear mushroom and milk white mushroom are included to train the students in these aspects. Some of the commonly occurring weed moulds, pests and diseases of edible mushrooms, their control measures are included. Certain preservation methods of mushrooms are also dealt with.

The students are expected to submit this Manual & Record at the time of Examination after completing the exercises given to them. Critical suggestions for improving the text are most welcome and they will be incorporated in the future edition.

CONTENTS

BLOCK - I : LABORATORY METHODS

In order to acquaint the students with various preliminary microbiological techniques used in the isolation of mushroom pure cultures, spawn preparation and mushroom cultivation this block is introduced. In this block, the student shall know about the type of glassware used in the laboratory, glassware cleaning, various sterilization methods, preparation of culture media, isolation and maintenance of pure cultures and identification of the common laboratory contaminants. These techniques shall help the students in the preparation of media, raising of pure cultures of mushrooms and spawn preparation.

UNIT - 1 : COMMON LABORATORY METHODS

1.1. AIM

In this unit you will study the type of glassware used in the laboratory, cleaning of glassware, methods of sterilization, preparation of various types of culture media, preparation of pure cultures, maintenance of cultures and identification of common contaminants like *Aspergillus* spp., *Neurospora* spp., *Penicillium* spp., *Rhizopus* spp., *Trichoderma* spp., etc.

1.2. OBJECTIVES

After completing this unit you will be able to :

- mention and describe various types of glassware used and method of cleaning the glassware,
- list out the various sterilization methods,
- describe the preparation of culture media,
- describe the method of preparation of pure culture and the methods of maintaining them,
- identify the common contaminants like *Aspergillus, Neurospora, Penicillium, Rhizopus, Trichoderma* etc.

1.3. INTRODUCTION

In nature, different kinds of microorganisms occur and to study various physico - chemical and metabolic activities in the laboratory it is essential to grow or culture them in a suitable culture medium. Any material in which microorganisms find nourishment and able to grow and reproduce themselves is known as culture medium. Culture medium may be of liquid or solid type. The growth of these microorganisms obtained in a medium is called culture. A culture that contains only one kind of organism is called pure culture.

Like any other microorganism, the development of mushroom pure culture on culture media is dependent upon a number of important factors like the supply of readily utilizable food materials, availability of oxygen, proper moisture content, temperature and pH. In a microbiological sense, the medium must be sterile without any contamination. The usual range of temperature suitable for the growth of various mushrooms lies between 15°C and 43°C. The pH of the culture medium which is nothing but its Hydrogen ion (H^+) concentration, is very important for the growth of mushroom pure cultures. Most of the mushrooms are able to grow with the pH range of 5.0 to 8.0. Initial pH should be determined after the addition of all ingredients. The pH may be regulated by the addition of solid calcium carbonate or acids like hydrochloric acid or sulphuric acid. The preparation of different media and culturing of different kinds of microorganisms are to be done in glassware.

1.4. GLASSWARE AND THEIR CLEANING

The important glassware used for mushroom cultures comprise of test tubes, petriplates and conical flasks. Apart from these, pipettes, measuring cylinders, beakers, funnels, glass rods etc., are also used. The description of different glassware items are mentioned below:

1. **Test tube** : A test tube is a tube like glass container with a opening on one inside (Fig. 1.1A). This is mainly used for the culturing of microorganisms.
2. **Petriplate** : Petriplate is a glass container with a spherical flat dish having vertical sides and a similar and slightly larger cover that fits over it. This is mainly used for growing microorganisms (Fig. 1.1B). The diameter usually ranges from 2" to 5".
3. **Conical flask** : This is a glass flask which is conical in shape (Fig. 1.1C) with different sizes (100 ml, 250 ml, 500 ml, 1000 ml etc.) and these are used for the preparation of solutions and media.
4. **Beaker** : Beaker is a cylindrical glass container of different sizes (100 ml, 250 ml, 500 ml, 1000 ml etc.) used for preparing various stains or solutions (Fig. 1.1D).
5. **Pipette** : Pipette is a graduated, long, narrow tube like apparatus used for measuring small quantities of liquids (Fig. 1.1E). The larger pipettes which are used for measuring 25 to 50 ml, have bulging in the middle portion.
6. **Measuring cylinders** : It is a graduated, cylindrical container used for measuring liquids. Measuring cylinders are of various capacities viz., 10 ml, 50 ml, 100 ml, 250 ml, 500 ml, 1000 ml etc.
7. **Glass rod** : Glass rod is an elongated rod used for stirring or mixing the solutions.
8. **Funnel** : This is a funnel shaped glass apparatus used for pouring solutions into test tubes, conical flasks, beakers and also for filtering solutions with the help of filter papers.

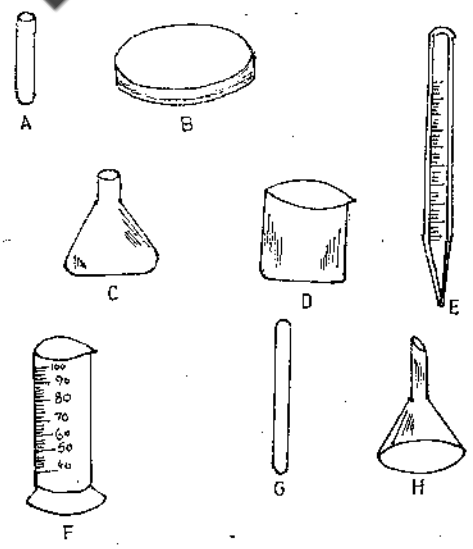

Fig.1.1. Glassware. A. Test tube. B. Petriplate. C. Conical flask. D. Beaker. E. Pipette. F. Measuring cylinder. G. Glass rod. H. Funnel.

Glassware used for sterilizing culture media should be heat resistant. As it has to withstand about 160°C in hot air oven or 121°C in Autoclave either Corning or Borosil make is to be used. The glassware should be thoroughly cleaned before they are used for culture media preparation. The following methods are followed for cleaning of glassware.

1. The glassware has to be kept overnight in cleaning solution. Cleaning solution (Chromic-Sulphuric acid) is prepared by the addition of commercial grade potassium dichromate (powder) to the concentrated sulphuric acid. Twenty grams of Potassium dichromate is dissolved in 1000 ml. of tap water to obtain a clear solution of potassium dichromate to which 100 ml. of concentrated sulphuric acid is gradually added.
2. The glassware should be washed with soap water.
3. They are then washed in tap water for 3-4 times respectively.
4. Finally the glassware should be rinsed with distilled water and used after drying.

1.5. METHODS OF STERILISATION

Method of keeping the desired apparatus and the environment absolutely free from living organisms is usually called sterilisation. Various methods of sterilisation are : (1) Flame, (2) Dry heat, (3) Steam, (4) Steam under pressure, (5) Chemicals and (6) Light.

1. **Flame**

   Needles, scalpels, forceps, slides, glassrods and cover slips are sterilised by this way.

2. **Dry heat**

   Hot air oven is used for sterilising glassware. They are heated at 160°C for one hour.

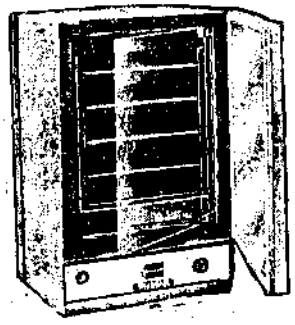

Fig. 1.2. Hot air oven

3. **Steam**

   This is also called intermittent sterilisation. In this method steaming is done for 30 minutes for three successive days. It is usually used for sterilising vegetable and sugar media.

4. **Steam under pressure (Autoclave)**

All types of media, petriplates, test tubes, conical flasks etc are sterilised in this way. The instrument used for this purpose is called an autoclave.

1. Autoclave (Fig. 1.3) sterilisation for 20 minutes at 15 lb pressure (121°C temperature) or 20 lb (127°C) is recommended for quantities upto one litre of the media.
2. The medium is prepared according to the formula and poured in test tubes or conical flasks upto 2/3 of its capacity.
3. They are then plugged with non-absorbent cotton, wrapped with brown paper or aluminium foil and placed properly in an autoclave.
4. Before operating the autoclave one should check the water level in the autoclave in order to avoid burning of the heating coils.
5. After closing the lid of the autoclave opposite screws should always be turned simultaneously (in case of screw type autoclave).
6. Care should be taken that the autoclave is closed properly before operating the instrument.
7. The temperature in the autoclave slowly starts raising.
8. One should note the sterilisation time only when all the residual air in the chamber has been displaced by steam and when the autoclave has reached the required temperature i.e. 121°C (15 lb) or 127°C (20 lb). In case of culture media the sterilisation is to be done for 20 minutes at 15 lb pressure by adjusting the necessary screws or the pressure meter can be fixed at that pressure.
9. Pressure - temperature relations of a properly working autoclave are shown below :

Table 1.1. Pressure - temperature equivalents in autoclave.

Pressure in lb (pounds)	Temperature (°C)	Temperature (°F)
5	108	226
10	116	240
15	121	250
20	127	260
25	131	267
30	134	274

10. After the completion of sterilisation period, the source of power is cut off and the pressure of the autoclave is allowed to return to atmospheric level without removing the steam. If you remove the steam the media will boil blowing out the cotton plugs from the tubes or flasks.

11. The media should be removed from the autoclave shortly after pressure reaches zero pounds. It should not be permitted to remain in the autoclave for longer periods as the agar medium forms a precipitate.

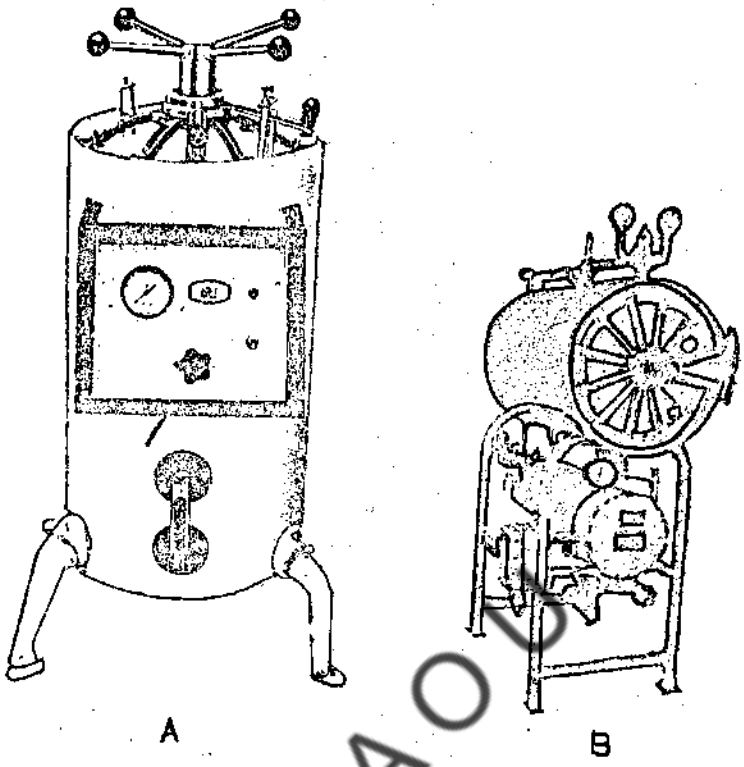

Fig. 1.3. Autoclaves. (A) Vertical autoclave. (B) Horizontal autoclave.

5. **Chemical Sterilisation**

1. Chemicals like mercuric chloride (0.1%), ethanol (80% v/v), sodium hypochlorite (NaOCl) or calcium hypochlorite ($Ca(OCl)_2$) etc. are usually used for surface sterilisation.

2. However, one should be cautious while using these chemicals.

3. Ethanol is highly inflammable. Care should be taken about the spilling of ethanol or any other alcohol in the vicinity of an open flame. The vessel containing alcohol should be placed in a metal can to avoid burning of alcohol in case the glass container breaks.

4. Hypochlorite solution on inhalation may produce bronchial irritation and skin contact may also be harmful.

5. Mercuric chloride solution is slightly volatile at room temperature and causes mercury poisoning to laboratory workers.

6. **Light**

1. Direct sunlight is used for drying glassware at places where hot air ovens are not available.

2. Ultraviolet lamps are employed in inoculation chambers or Laminar flow chambers to provide aseptic conditions or for killing the microorganisms present inside the chambers. Microbial inoculation can be done only after sterilising the chambers with ultraviolet rays for about 20-30 minutes, before inoculation.

3. The life of ultraviolet tube is about 500 hours. Even after 500 hours violet light will be emanated from the bulb but it will not be effective. So, it is to be replaced by a new one.

4. Laminar flow chambers (Fig. 1.4) provide a gentle flow of ultrafiltered sterile air across the working area to reduce air-borne contamination during inoculation.

5. Few precautions are to be taken while using UV-lamps since UV irradiation causes health hazards.

6. One should not look at a live UV-lamp with the naked eye.

7. A glass barrier between the eyes and the UV light source gives complete protection.

8. UV irradiation can produce irritation to unprotected skin, hence avoid placing hands in the chamber when the lamp is on.

9. In presence of UV-light atmospheric oxygen shall be converted to ozone due to photochemical reaction.

10. UV lamp should never be switched on for longer periods of time with the chamber closed.

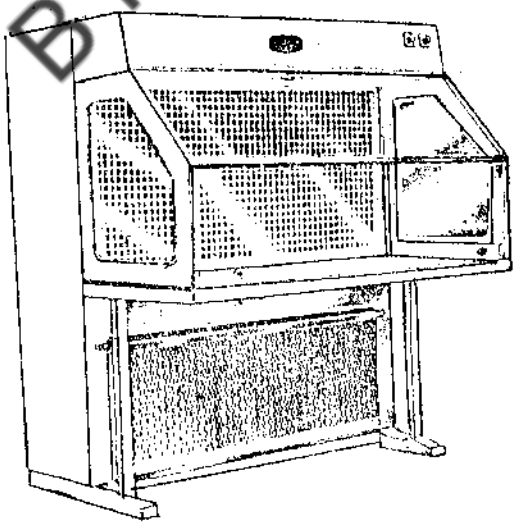

Fig. 1.4. Laminar flow chamber

1.6. PREPARATION OF CULTURE MEDIA

The culture medium is a common substrate consisting of necessary food substances on which the microorganisms are grown. The resultant growth is called culture. The composition of the medium for growing pure mushroom mycelium vary widely. However some common media that are often used for growing mushroom mycelium are given below :

1.6.1. Potato Sucrose Agar or Potato Dextrose Agar Medium

Necessary Ingredients

Peeled and sliced potatoes	250 g
Sucrose / Dextrose	20 g
Agar - Agar powder or flakes	20 g
Distilled water	1000 ml.

1. Wash, peel and cut the potatoes into small cubes of one cm. or one inch.
2. Boil cut potatoes in 1000 ml. of distilled water for 15-20 minutes till they are soft but should not be over cooked.
3. Filter it through a cheese or muslin cloth and collect the liquid in a measuring cylinder of 1000 ml. capacity.
4. Restore the volume to 1000 ml. by adding fresh distilled water if necessary.
5. Add 20 g. of sucrose / dextrose and 20 g. of agar powder.
6. Boil with occasional stirring until sucrose or dextrose and agar dissolve.
7. Check the pH of the medium with the help of pH paper and adjust the pH to 6.5-7 by adding dilute hydrochloric acid if the pH is more than 7 and sodium hydroxide (base) if the pH is less than 6.5.
8. Transfer the medium into test tubes depending upon the size. It may usually be about 10 ml. per tube. Medium is to be poured with the help of pipette. The medium should not touch the top edge of the tube as there will be a chance for contamination.
9. Plug the test tubes with non-absorbent cotton.

1.6.2. Oat Meal Agar Medium

Necessary Ingredients

Oat Meal flakes	30 g
Agar - Agar powder or flakes	20 g
Distilled water	1000 ml

1. Wrap the oat meal flakes in cheese cloth and boil in distilled water for 2 hours.
2. Remove the cheese cloth and make the volume of water up to 1000 ml.
3. Add 20 g. of agar powder and boil with occasional stirring till the agar dissolves.

4. Adjust the pH to 6.5-7.0 as described earlier.

5. Fill the medium in test tubes @ 10 ml. per tube and plug the tubes with non-absorbent cotton taking necessary care.

1.6.3. Malt Extract Agar Medium

Necessary Ingredients

Malt extract	25 g
Agar - Agar powder or flakes	20 g
Distilled water	1000 ml
OR	
Malt extract agar	50 g
Distilled water	1000 ml

1. Add 25 g. of malt extract and 20 g. of agar powder or flakes in 1000 ml. of distilled water and boil the mixture till malt extract and agar dissolves.

OR

Dissolve 50 g. of malt extract agar which is available commercially in 1000 ml. of distilled water and boil the mixture till malt extract agar dissolves.

2. Adjust the pH to 6.5 - 7.0.

3. Pour the medium into test tubes @ 10 ml. per tube and plug with non-absorbent cotton.

1.6.4. Complete Medium

Necessary Ingredients

Magnesium Sulphate ($Mg\ SO_4.\ 7H_2O$)	0.050 g
Potassium dihydrogen Orthophosphate (KH_2PO_4)	0.46 g
Dipotassium hydrogen Orthophosphate (K_2HPO_4)	1 g
Bacteriological Peptone or Bactopeptone	2 g
Dextrose	20 g
Agar (Sigma type IV)	20 g
Thiamin - Hcl	0.50 g
Distilled water	1000 ml

1. Add all the ingredients to 1000 ml. of distilled water.

2. Boil the solution till agar dissolves by stirring occasionally.

3. Pour the medium into test tubes @ 10 ml. per tube and plug with non-absorbent cotton.

The complete medium is usually used for raising *Volvar* cultures. The pH range for *Volvariella* should be between 6.8 to 7.8.

1.6.5. Sterilisation Process

1. Cover the mouths of plugged tubes with brown paper or aluminium foil and tie with rubber band.
2. Sterilise the tubes in an autoclave at 15 lb/sq. inch pressure for 20 minutes.
3. When the pressure meter of the autoclave comes back to zero, remove the tubes and keep in a slanted position as shown in the figure (Fig. 1.5) with the help of a long stick or rod. Slanted position will increase the surface area of the medium.
4. While keeping the test tubes in slanted position, care should be taken that the medium should not touch the cotton plug as it may lead to contamination.
5. The test tubes are to be kept in slanted position for about one day by which time the medium will be solidified.
6. If there is any need to culture the microorganism in petriplates the medium should be sterilised in conical flasks of required size and poured in sterilised petriplates under aseptic conditions, when the medium is in molten (liquid) state.
7. Media should always be stored in cool atmosphere (room temperature is alright) to prevent evoperation of water and drying up of medium.
8. The medium which is stored for prolonged period is not advisable for culturing any organism.

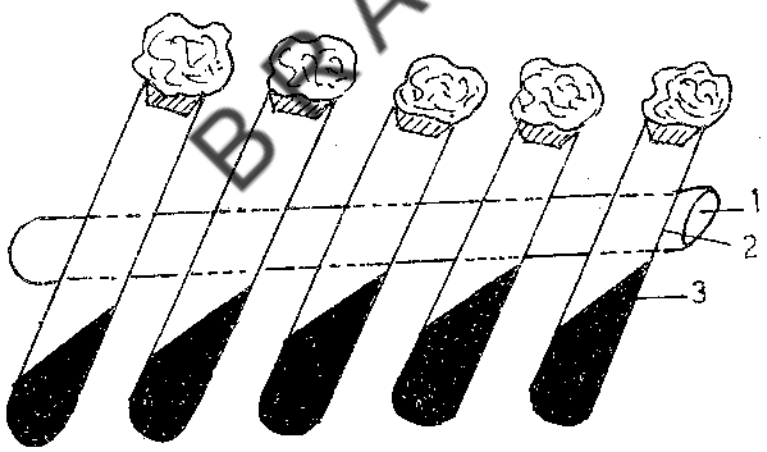

Fig. 1.5. Preparation of test tube slants. 1. Wooden block. 2. Test tube. 3. Medium.

1.7. PREPARATION OF PURE CULTURES

1. When only one species or strain of any one organism is present in the culture, it is called a pure culture.
2. Pure cultures of mushroom mycelium are prepared by two methods viz., tissue culture and spore culture. Tissue culture method is the widely followed one for commercial production.

3. For raising a pure culture, select an young fruit body and clean with cotton swab to remove straw or dust particles adhering to the mushroom.

4. Hands, instruments, working area should be clean and disinfected with rectified spirit to avoid surface contamination.

5. Dip the fruit body in 0.1% mercuric chloride solution (100 mg. mercuric chloride in 100 ml. distilled water) for 30 seconds with the help of a sterilised forceps.

6. Wash the fruit body for 3-4 times serially in sterile distilled water to remove excess chemical. For each washing, fresh distilled water is to be used.

7. Dry the fruit body with the help of sterile filter papers to remove superficial moisture.

8. Cut the fruit body longitudinally into two equal halves.

9. Lift a small piece (2 x 2 mm) of tissue from inner cut area at the junction of stipe and pileus with the help of a sterile scalpel or blade.

10. Open the tube or petriplate with medium over the flame and transfer the bit of mushroom tissue onto agar surface and plug the tube immediately.

11. Incubate the tubes/petriplates at 25 ± 1°C and observe for the growth of mycelium from the tissue.

Observations

1. White mycelial growth can be seen in the tubes and petriplates.
2. Any coloured growth in the tubes, petriplates indicate contamination.
3. Slimy growth accompanied by foul odour indicates bacterial contamination
4. Discard the contaminated tubes and petriplates.

1.8. MAINTENANCE OF CULTURES

The fully grown cultures are preserved in different ways. Maintenance of mushroom cultures require technical expertise in different fields like Genetics, Mycology, Microbiology and Taxonomy. Hence small growers cannot maintain cultures effectively. The following are some of the important methods to maintain mushroom cultures.

1. **Under Refrigeration** : The mushroom cultures can be stored in refrigerator at around 5°C (at 15°C in case of *Volvariella*) upto six months.

2. **Method of Storage in Mineral oil**

   1) Mineral oil having specific gravity of 0.865 to 0.89 is sterilised for 30 minutes.
   2) After cooling, the oil is poured over the cultures under aseptic conditions.
   3) Then the tubes should be screw capped.
   4) The agar bits should be used for further inoculation after draining out the oil.
   5) By this method the cultures can be stored upto 10-12 months.

3. **Method of Storage in Compost**

1) 300 g. of well pasteurized, dried compost is ground to small pieces (5 mm).

2) One litre of tap water should be added to compost and the moistened compost should be washed with hot water serially for three times to remove unwanted metabolites and gases.

3) The compost is filled up to 1/3 of the test tubes and sterilised at 15 lb/sq. inch pressure (121°C) for two hours for two consecutive days to kill thermophilic bacteria.

4) After cooling, the compost is inoculated with the culture aseptically and after full growth the tubes are stored below 5°C.

5) By this method the cultures can be stored for 2 years. This method is mostly used for preserving *Agaricus* cultures.

4. **Method of storage in demineralized water**

1) The bottles having demineralized water are sterilised for 2 hours at 15 lb/sq. inch pressure (121°C).

2) After cooling, small bits of (0.5 cm^2) culture from fully grown culture tube are transferred aseptically at the rate of 3-4 bits per bottle and stored in refrigerator below 5°C. This method is not suitable for preserving *Volvariella* cultures.

5. **Cryogenic freezing**

1) Storage of cultures in liquified nitrogen is called cryogenic freezing. Small vaccum ampules containing mycelial cultures should be kept in liquid nitrogen at a temperature of -196°C or in nitrogen vapours at -150 to -180°C.

2) By this method mushroom cultures can be stored for about 10 years.

6. **Freeze drying or Lyophilization**

Drying cultures or spore suspension at the frozen state under reduced pressure is called lyophilization. The cultures are freeze dried at -20°C. Only spores of young mushrooms can be collected and stored by this method for a period of 20 years or more.

1.9. IDENTIFICATION OF COMMON CONTAMINANTS

Common contaminants that occur in mushroom cultures are the species of *Aspergillus, Neurospora, Rhizopus, Trichoderma* etc. The identification characters of different contaminants are given below :

1.9.1. *Aspergillus spp.*

The common species of *Aspergillus* that occur as laboratory contaminants are *A. flavus, A fumigatus, A. nidulans* and *A. niger.*

Aspergillus flavus : Colonies of *A. flavus* (Fig. 1.6) grow rapidly with big conidial heads. Centre of the colony is yellow and greenish towards periphery. Yellow-green colours are mixed in older cultures. Reverse of the colonies in test tubes or petriplates are colourless to pale yellow brown. If you observe the cultures under microscope, conidiophores can be seen singly but not in groups. They measure 0.5-1.5 mm. long with pitted walls, almost spiny in appearance, gradually broadening into vesicles of 28-40 µm. in diameter; sterigmata in single series, 5.0-7.8 x 2.3-3.5 µm. in size; Conidia globose to sub-globose, yellowish green, 3.2-4.6 x 3.5-4.2 µm. in size.

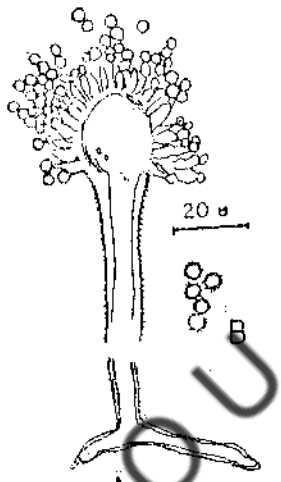

Fig. 1.6. *Aspergillus flavus.* (A) Conidiophore. (B) Conidia.

Aspergillus fumigatus : *Aspergillus fumigatus* (Fig. 1.7) spreads rapidly, colonies white at first, becoming dark green, floccose, reverse of the colony colourless to light pinkish red; conidiophores smooth, short, upto 300µm. in length and 3.5-6.0µm. in breadth. Septate gradually enlarging into flask shaped vesicle. Vesicles fertile only on the upper half, 17-24µm. in diameter, bearing a single series of sterigmata. Sterigmata closely packed, 4.5-6.0 x 1.5-2.5µm. Conidia globose, green in mass, smooth, 2-3µm. in diameter. Sclerotia and cleistothecia absent.

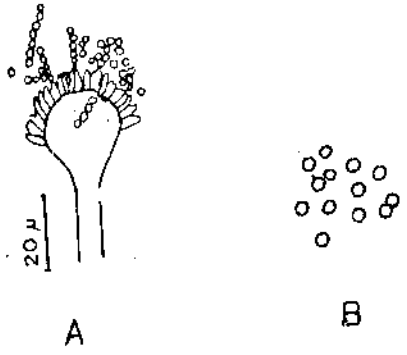

Fig. 1.7. *Aspergillus fumigatus.* (A) Conidiophore. (B) Conidia.

Aspergillus nidulans : *Aspergillus nidulans* (Fig. 1.8) grows rapidly with cinnamon brown conidiophores, 3-4µm. in diameter, the tips broadening gradually into hemispherical vesicles. Vesicles are 7.5-9µm wide. The sterigmata are in two series, primary sterigmata (9-9.5 x 3-4µm) and secondary sterigmata (7.5-12 x 1.5-3µm). The conidia are yellowish green (2.3-3.5µm in diameter). Reverse of the colony appears purplish red. Cleistothecia (250-320µm) appear in abundance from the centre towards the periphery. Walls of Cleistothecia are purplish red. Hulle cells are present and measure upto 22µm in diameter. Asci are 8-spored (19 x 11µm). Ascospores (6-7µm) are lenticular, purplish red, smooth walled with two equatorial ridges (0.5-1µm in width).

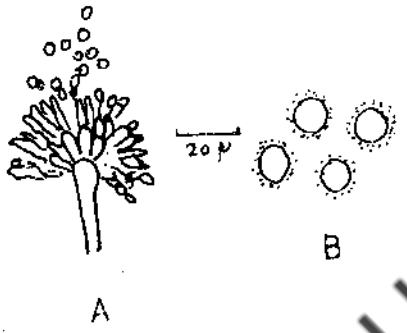

Fig. 1.8. *Aspergillus nidulans.* (A) Conidiophore. (B) Conidia.

Aspergillus niger : *Aspergillus niger* (Fig. 1.9) grows rapidly with abundant submerged mycelium. Reverse of the colony colourless to pale yellow. Smooth, non-septate conidiophores (1-2µm long, 17-23µm diameter) arise directly from the substratum. Vesicles globose, thickwalled, 77.5-93µm in diameter, vesicles bear two series of fully packed sterigmata, primary (1.5-21.7 x 8-9.3µm) and secondary (8-11.5 x 5-7µm). Conidia black in colour, globose, spinulose 6.2-7.5µm in diameter. Sclerotia not observed.

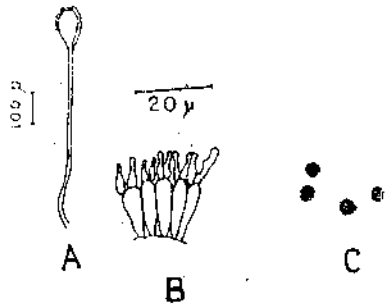

Fig. 1.9. *Aspergillus niger.* (A) Conidiophore. (B) Conidia.

1.9.2. Neurospora spp.

The species of the genus *Neurospora* that invades the bacteriological or mycological laboratories and is difficult to eliminate due to its easily dispersed conidia and rapidly growing hyphae is *Neurospora sitophila* (Fig. 1.10). It is commonly known as bakery mould or red bread mould, because it often infests the bakeries and causes damage. The mycelium grows rapidly with numerous branched hyphae. The aerial hyphae produce pink masses of oval conidia. Conidia are borne in chains on branched conidiophores. The perithecia are dark coloured, pyriform and beaked. Asci are octosporous. At maturity paraphyses are not seen. The spores are dark brown or black with ridges on the outer wall. Presence of ridges on the spore wall characterize the genus and give its name.

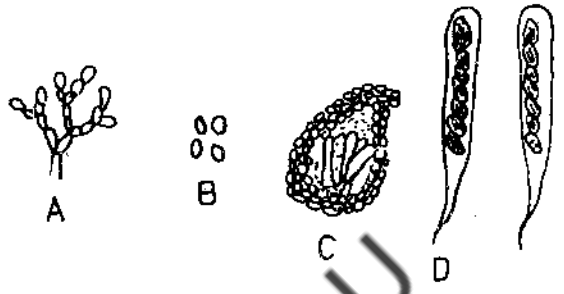

Fig. 1.10. *Neurospora sitophila.* (A) Conidiophores. (B) Conidia.

1.9.3. Penicillium spp.

Common species of *Penicillium* that occur as laboratory contaminants are *Penicillium chrysogenum*, *P. citrinum* and *P. notatum*.

Penicillium chrysogenum : Colonies of *Penicillium chrysogenum* are greyish green, cottony to subfloccose, loose in texture, wheel-like in appearance having radial furrows. Reverse of the colonies yellow in colour, conidial areas yellowish green to bluish grey green shades. Conidiophores (Fig. 1.11) smooth walled, colourless 150-175µm in length and 3-4µm in diameter. Penicillia biverticillate and asymmetrical with one or more branches ending in verticils of 2-5 metulae.

Metulae bear phialides. Branches 15-25µm long and 3-4µm in diameter. Metulae 10-15µm long, 2.5-3.5µm wide; phialides 4-6 in number, 8.5-11 x 2-3µm; conidia in chains upto 200µm long; unicellular, elliptical, smooth walled, yellowish green conidia (3-4.5 x 2.5-4µm).

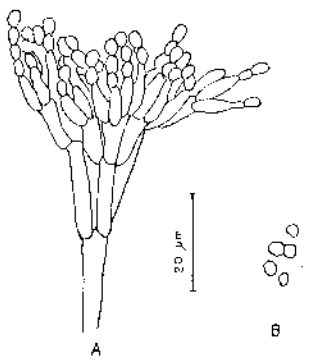

Fig. 1.11. *Penicillium chrysogenum.* (A) Conidiophore. (B) Conidia.

Penicillium citrinum : Colonies on the medium are furrowed, floccose or leathery. Bluish green to clear green conidial areas, reverse yellow. Conidiophores (Fig. 1.12) unbranched, upto 175µm long and 2-3µm in diameter. Penicilli have terminal group of 3,4 or rarely more metulae. Metulae 15-25 x 2-3µm, each producing a cluster of parallel phialides. Phialides 7-10 x 2-3µm, conidial chains in columns, upto 150µm long, conidia unicellular, globose, smooth walled or slightly granular, 2.4-3.5µm in size.

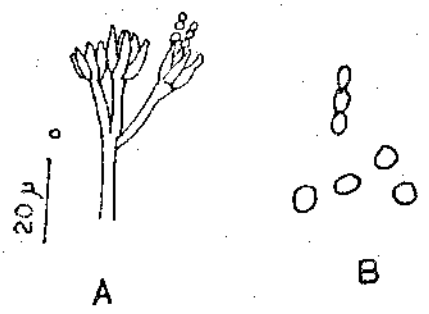

Fig. 1.12. *Penicillium citrinum.* (A) Conidiophore. (B) Conidia.

Penicillium notatum : Colonies are close textured, grow rather slow and attain a diameter of 2-2.5cm in two weeks at 26-28°C. Conidial areas are meadow green with fairy green margin. Light yellow colour exudate is produced. Reverse of the colony golden yellow, attaining yellowish brown with age. Conidiophores arise from basal felt, length is variable 3-8.8µm in width (Fig. 1.13) colourless, smooth, sparsely septate. Penicilli biverticillate, metulae usually in groups of 3-5, variable in length 12.5-19 x 3-3.8µm. Sterigmata in verticils of 3-6, 7-9.3 x 2.3-3µm, terminating abruptly. Conidia globose or sub-globose, smooth, green coloured, 2.3-3.5µm in size.

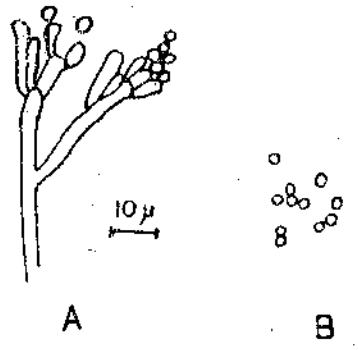

Fig. 1.13. *Penicillium notatum.* (A) Conidiophore. (B) Conidia.

1.9.4. *Rhizopus spp.*

The species of *Rhizopus* that usually appear as laboratory contaminant is *Rhizopus nigricans* (Fig. 1.14). It is commonly known as black bread mould. Colonies are white at first, turning brownish black later on. Stolons are far spreading, internodes are brown in colour, brown rhizoids at each node. 3-5 unbranched sporangiophores arise in clusters. Sporangiophores 0.4-2µm long and 10.5-17µm in breadth, white at first, black at maturity, columella globose, 45-100µm in diameter. Spores round to oval, angular or irregular, 5-6.2 x 3-4.6µm.

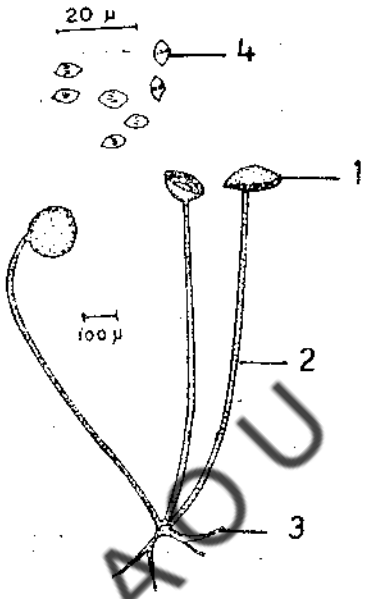

Fig.1.14. *Rhizopus nigricans*. 1. Sporangia. 2. Sporangiophores. 3. Rhizoids. 4. Spores.

1.9.5. *Trichoderma spp.*

Species of *Trichoderma* that usually appear as contaminant is *Trichoderma viride*. Colonies grow rapidly with submerged vegetative mycelium, white at first, later turning from light green to deep green at the conidial areas. Conidiophores (Fig.1.15) are not distinct from vegetative hyphae, indefinite in length, di-or-trichotomously branched. The branches that bear conidia are called phialides. Phialides are pin shaped and arise singly or in whorls. Conidia are produced in groups, green in colour, smooth, globose or oval, 3-4µm or 3-5 x 2.5-3.5µm. Old cultures produce chlamydospores and emit typical coconut smell when the cultures attain more than one week old.

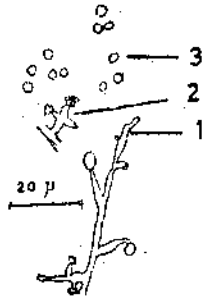

Fig. 1.15. *Trichoderma viride*. 1. Conidiophores. 2. Phialides. 3. Conidia.

1.10. EXERCISE

Describe the procedure for the preparation of PDA medium and slants and precautions to be taken while preparing slants.

1.11. SELF ASSESSMENT

To check your progress on your own answer the following questions in the space provided below without referring the text above.

1. Write the names of different sterilization methods that are carried out in a microbiological laboratory.

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2. Write the various contents of the PDA medium.

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3. What is meant by tissue culture?

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4. What are the different ways of storing the cultures?

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5. Write the names of various contaminants in mushroom cultures.

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BLOCK - 2 : EDIBLE AND POISONOUS MUSHROOMS

The mushrooms are distributed over a wide geographical area. There are nearly 2000 edible fungi reported throughout the world. There is no thumb rule to distinguish an edible mushroom from poisonous one. The rules of edibility of wild mushrooms have emerged over centuries of experience.

In this laboratory manual, an attempt has been made to acquaint the students with morphological and identification characters of various edible mushrooms like *Agaricus bisporus*, *Agaricus bitorquis*, *Auricularia spp.*, *Calocybe indica*, *Lentinus edodes*, *Pleurotus florida*, *P. sajor-caju*, *Volvariella spp.* and *Morchella spp.* and poisonous mushrooms like *Amanita spp.* and *Coprinus atramentarius*.

The students can get additional information from the first course *viz.* Biology of mushrooms.

UNIT - 2 : MORPHOLOGY AND IDENTIFICATION OF EDIBLE AND POISONOUS MUSHROOMS

2.1. AIM

In this unit you will study the morphology and identification of edible and poisonous mushrooms.

2.2. OBJECTIVES

After completing this unit, you will be able to :

- describe the morphological and identification characters of edible mushrooms,
- describe the morphological and identification characters of poisonous mushrooms and
- list out the guidelines to recognize the poisonous mushrooms.

2.3. INTRODUCTION

Mushrooms are the fleshy fruit bodies of some members of the sub-division Ascomycotina and Basidiomycotina. Usually, in nature, the fungal mycelium is buried under soil and during favourable conditions fleshy fruit bodies i.e., the so called mushrooms appear above ground.

Among the mushrooms included in this unit only *Morchella* belongs to Ascomycotina and all others belong to Basidiomycotina. The fruit bodies of Ascomycotina are called ascocarps and the fruit bodies of Basidiomycotina are termed as basidiocarps or basidiomata. The ascocarps consists of sac-like structures called asci in which ascospores are present. The basidiospores arise externally over the club shaped basidia present in basidiocarps. However, for a lay man, it is easy to distinguish in a typical mushroom the basal stalk (stipe) and the upper cap (pileus).

The mushrooms are usually identified on the basis of macro-characteristics like colour, shape, surface, texture, presence or absence of volva, annulus, margin of pileus, colour, arrangement of gills, position, size, colour of the stipe etc. Micro-characters being size, shape and number of basidia, basidiospores, spore print, presence or absence of sterile structures and their shape.

2.4. MORPHOLOGY AND IDENTIFICATION OF EDIBLE MUSHROOMS

The morphological and identification characters of white button mushrooms (*Agaricus bisporus* and *A.bitorquis*), black ear or jews ear mushroom (*Auricularia* spp.), milky white

mushroom (*Calocybe indica*), Shiitake mushroom (*Lentinus edodes*), Oyster mushroom (*Pleurotus florida* and *P. sajor-caju*), paddy straw mushroom (*Volvariella* spp.) and morel (*Morchella* spp.) are given below.

2.4.1. White Button Mushroom

1. *Agaricus bisporus* and *A. bitorquis* are popularly known as white button mushrooms.
2. They grow in nature on manure piles, damp marshy soils and garden lands.
3. a) *Agaricus bisporus* is umbrella like in shape. When young, pileus is convex, measures about 2.5 to 4cm, get flattened at maturity and expands to about 10cm across.
   b) The colour of the mushroom is white to light brown.
   c) Gills are free and brown in colour. Gills are the spore-bearing, plate-like structures present on the under surface of the pileus.
   d) Annulus is present. Annulus is a ring-like structure on the stipe formed by the broken veil.
   e) Volva is present.
   f) The cap (pileus) and the stalk (stipe) are easily separable.
   g) Spores are elliptical, smooth and thick walled.
4. a) *Agaricus bitorquis* is a temperature tolerant white button mushroom than *A.bisporus*.
   b) It grows at a higher temperature (28-30°C) and is resistant to viruses.
   c) The mushroom produces firm and larger (pileus diameter between 3.94cm to 4.28cm) fruit bodies.
   d) The other characters are exactly similar to that of *A.bisporus*.

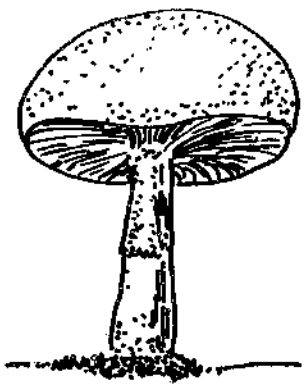

Fig.2.1. *Agaricus bisporus*.

2.4.2. Black Ear or Jew's Ear Mushrooms

1. Black ear or Jew's ear mushroom *(Auricularia* spp.) appears on tree trunks under natural climates.

2. Commercially cultivated species of *Auricularia* are *A.polytricha*, *A.auricula* and *A fucosuccinea*.

3. Due to their gelatinous nature, they are also known as jelly fungi.

   a) *Auricularia polytricha* produces ear shaped fruit bodies which are usually sessile or with a short stalk.

   b) The fruit bodies are rubbery, gelatinous, pale reddish brown when fresh and becomes grey to black, cartilaginous after drying.

   c) The size of the fruit body ranges from 6cm to 12cm length and 1 to 2mm thickness.

   d) The lower surface of the fruit body is smooth and consists of vein-like folds.

   e) The upper surface is hairy.

   f) On the lower surface, hymenium (fertile region) with basidia and basidiospores is present (Fig. 2.2).

   g) Basidia are divided into cells by transverse septa.

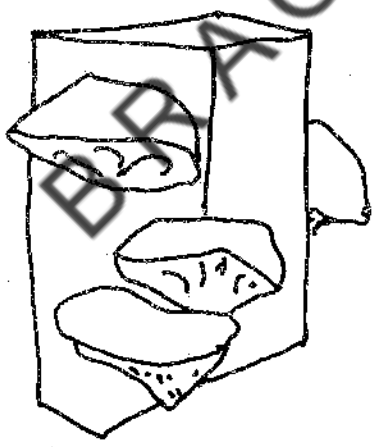

Fig. 2.2. *Auricularia polytricha*.

2.4.3. Milky White Mushroom

1. Milky white mushroom (*Calocybe indica*) was reported from the forests of West Bengal in 1974.

2. The mushroom grows on humus rich soil in nature.

3. The fruit bodies are mainly white in colour, solitary and have long shelf life.

4. The stalk (stipe) is central, 8cm long, thick, sub-bulbous base, surface dry, without annulus and volva.

5. The diameter of cap (pileus) is about 8cm.

6. Gills are separable, distinct, unequal and white in colour.

7. Spores are thin walled, broadly ellipsoidal, hyaline, non-amyloid, 5.9-6.8 x 4.2-5.1μm and spore print is white in colour.

Fig. 2.3. Milky White Mushroom.

2.4.4. Shiitake Mushroom

1. Shiitake mushroom (*Lentinus edodes*) grows on the wood logs of dead, deciduous trees like oak, chestnut, beech etc.

2. The fruit bodies are with eccentric or lateral stipe, sometimes central stipe.

3. Pileus (cap) is upto 11cm in diameter, convex when young, depressed at maturity with dark scales in the centre.

4. Lower surface of pileus is white, fleshy when young, becomes tough with age.

5. Gills crowded, white, turning to brown later on, easily separable from stipe.

6. Stipe is solid, reddish brown, 3-4 x 0.8-1.5cm in size.

7. Spores non-amyloid, smooth and cylindrical.

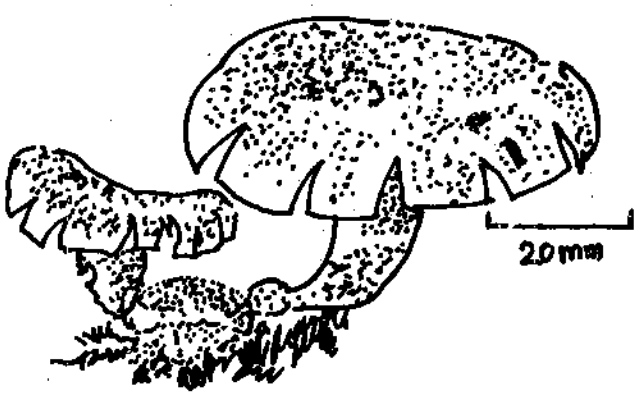

Fig. 2.4. Shiitake mushroom.

2.4.5. Oyster Mushrooms

1. Fruit bodies appear on tree trunks in large numbers and look-like oysters, hence called oyster mushrooms (*Pleurotus* spp.).

2. The most important species of *Pleurotus* that are commercially cultivated are *P florida, P.flabellatus, P.citrinopileatus* (white oyster mushrooms) and *P.sajor-caju* and *P.sapidus* (grey oyster mushrooms).

3. a) *Pleurotus florida*, one of the white oyster mushrooms grows on dead trunks or branches.

   b) When cultivated at low temperature (5-15°C) the fruit bodies are light brown and at 20-25°C it is white to pale yellow.

   c) The cap is convex and slightly depressed in the centre.

   d) The stalk is white, short (2-4 cm) and attached to the pileus at the centre or slightly away from the centre.

   e) Spores are pale cream coloured, oblong and 7-10µm long.

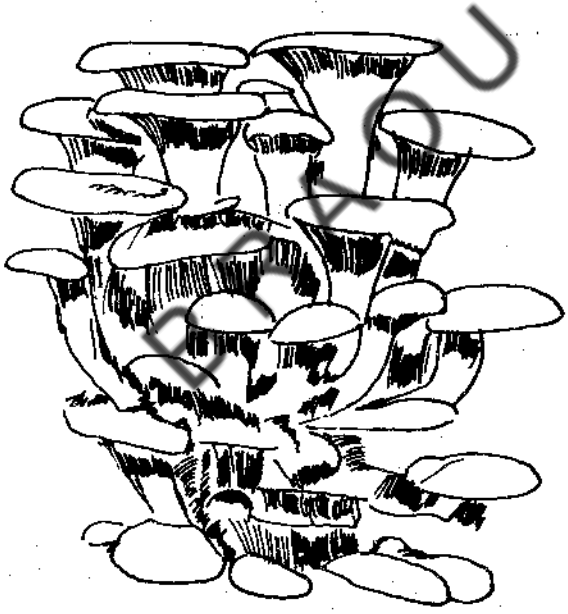

Fig. 2.5. White Oyster Mushroom (*P.florida*).

4. a) *Pleurotus sajor-caju* appear on dead and decaying plants.

   b) The fruit bodies are shell-like or spathulate.

   c) They grow either singly or in clusters, greyish brown, stalk is 1.5-3.5cm long and white, pileus diameter is 4-12cm.

   d) Gills run down on the stalk.

   e) Spores 6.5-7.5µm in diameter and colourless.

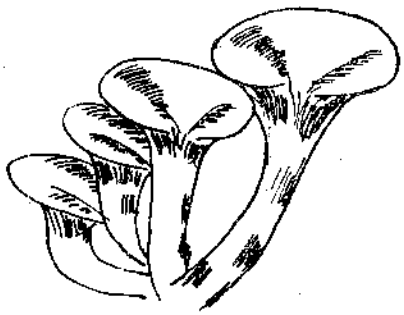

Fig. 2.6. Grey oyster mushroom (*P. sajor-caju*).

2.4.6. Paddy straw mushrooms

1. Paddy straw mushroom (*Volvariella* spp.) grows abundantly on paddy straw heaps.
2. Commonly cultivated species of *Volvariella* are *V.volvacea, V. diplasia* and *V. esculenta.*
3. a) *Volvariella volvacea* produces centrally stipitate (stalk is central) usually solitary fruit bodies with a characteristic volva.
   b) Pileus greyish, 5-12cm in diameter.
   c) Gills are free, distinctly formed.
   d) Stipe is solid, cylindrical, smooth, shining white, bulbous base with well developed, persistent volva (Fig. 2.7).

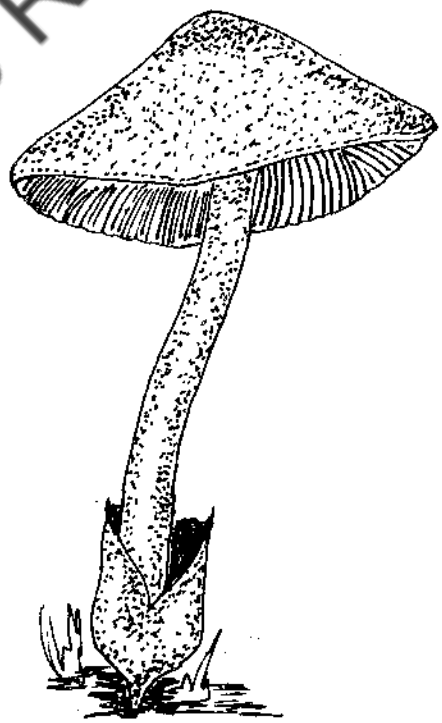

Fig. 2.7. *Volvariella volvacea.*

2.4.7. Morels

1. Morels are the species of *Morchella*.

2. The most prized wild edible species of *Morchella* are *M. esculenta* and *M. conica*.

3. a) *Morchella esculenta* usually produces solitary fruit bodies under the woods and in grass lands.

   b) Pileus is oval in shape, grey in the beginning, later on changing to yellowish brown colour.

   c) The surface of the pileus consists of narrow ridges arranged in honey comb like fashon.

   d) Pileus ranges from 3-5cm in diameter and 3.5-6cm in length.

   e) Stipe is cylindrical or slightly enlarged at base, white in colour changing to brown with age, hollow and brittle and measures about 4-8cm length and 1.5-2.5cm width.

   f) Ascospores are elliptic, long and smooth, 17-25 x 11-13.5µm in size.

   g) Spore print is in cream colour.

Fig. 2.8. *Morchella esculenta.*

4. a) *Morchella conica* produces fruit bodies on forest soils and are usually found scattered on the ground.

   b) Pileus is conical in shape with a pointed tip, buff to yellow when young and dark coloured at maturity.

c) The diameter of the pileus is 3-5cm and consists of 5-8cm long pits arrang in regular rows.

d) Stipe is white in colour, cylindrical and fragile, 4-8cm long and 1-3cm thi ..

e) Ascospores are elliptical, yellow in colour, 20-24 x 12-14µm in size.

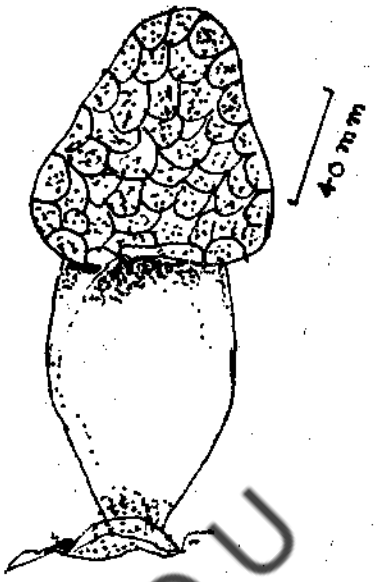

Fig. 2.9 *Morchella conica.*

2.5. MORPHOLOGY AND IDENTIFICATION OF POISONOUS MUSHROOMS

The morphological and identification characters of poisonous mushrooms like Amanitas (*Amanita* spp.) and common ink cap mushroom (*Coprinus atramentarius*) are given below:

2.5.1. Amanitas

1. The mushrooms that belong to the genus *Amanita* are commonly known as Amanitas.
2. *Amanita phalloides, A.muscaria, A.verna* and *A.virosa* are highly poisonous species of this genus.
3. Many species of *Amanita* consist of both volva and annulus.
4. a) *Amanita phalloides* is popularly called death cap.
   b) The pileus is flat to subspherical, deep olive green to olive brown, 5-20cm in diameter.
   c) Gills are white or slightly yellowish.
   d) Stipe is tapering towards top.
   e) Volva is white and membranous.

f) Spores ovoid or nearly round, smooth, 8-11 x 7-9µm in size.

5. a) *Amanita muscaria* (fly-agaric) consists of red cap with white pyramidal warts, slightly concave.

b) Gills are crowded, white in colour. Stipe is bulbous at the base with volva.

c) Spores ovoid, smooth, white in colour and measures 9-11 x 6-8µm in size.

6. a) *Amanita verna* (fool's mushroom) is deadly poisonous.

b) The symptoms appear very late. However, it is pure white in colour.

c) Volva encloses the base of the stipe.

d) The annulus (ring) hangs like a skirt from the apex of the stalk.

7. a) *Amanita virosa* (destroying angel) is deadly poisonous.

b) The pileus is sticky, 10cm in diameter, egg shaped, white at first, later turns yellowish.

c) Stipe is slender with swollen base and surrounded by volva.

d) Gills are pure white and free from the stipe.

e) The flesh has an offensive smell.

f) Spores are round, white, smooth, amyloid, 9-12µm in size.

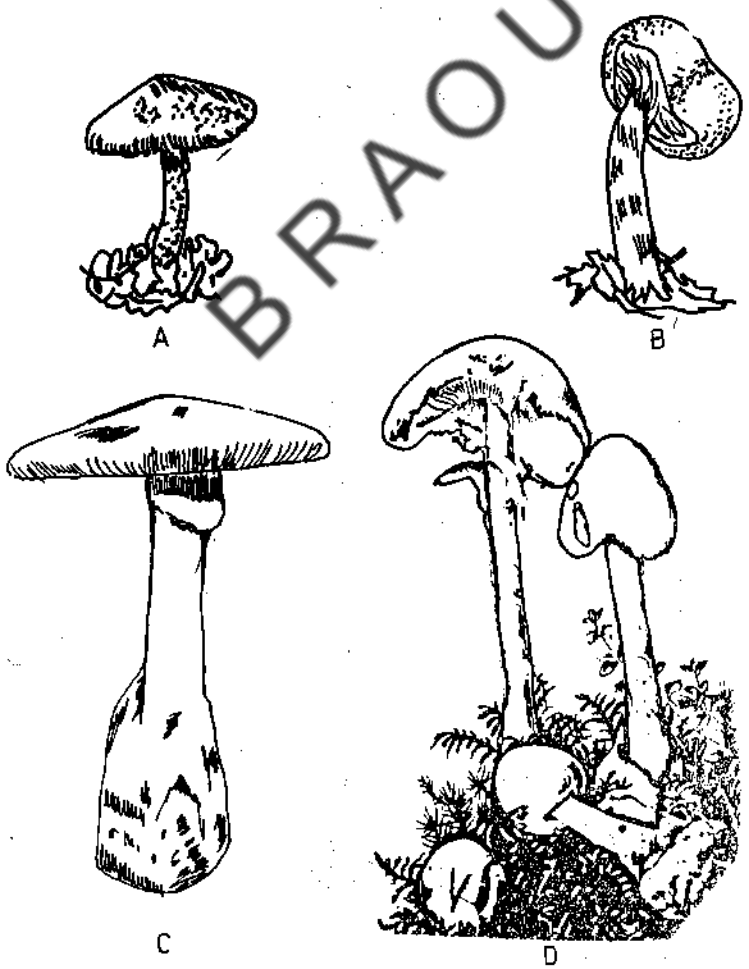

Fig. 2.10. *Amanita* spp. A) *A.phalloides.* B) *A. muscaria.* C) *A. verna.* D) *A. virosa.*

2.5.2. Ink Cap Mushrooms

1. The genus *Coprinus* (ink cap mushroom) consists of many species and common of them are *C. atramentarius, C. micaceous* and *C. plicatilis*.
2. They are commonly called ink cap mushrooms due to the conversion of cap and gills into black liquid when they mature (autodigestion).
3. *C. atramentarius* (common ink cap) is poisonous when consumed with alcoholic beverages.
4. It is conical to ovate when young, centrally stipitate, brown to different shades of grey, 3-8cm. in diameter.
5. Annulus is not distinct on the stipe, spores elliptical, black in colour.
6. Spore print is black.

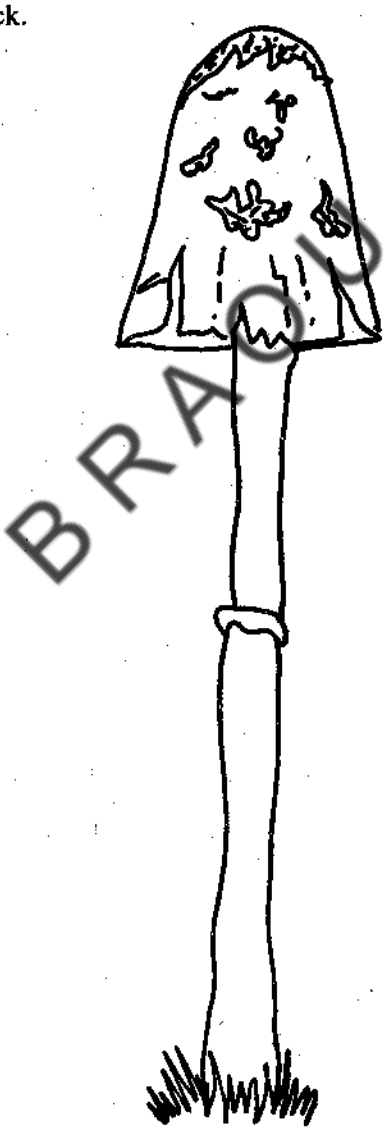

Fig. 2.11. *Coprinus atramentarius.*

2.6. FEW GUIDELINES FOR IDENTIFYING POISONOUS MUSHROOMS

Poisonous Mushrooms cannot be identified so easily by a layman. Though not reliable quite often, few guidelines or tests are given below :

1. Mushrooms having both volva and annulus are said to be poisonous.
2. Damaged fruit bodies exude milky substances in case of poisonous mushrooms.
3. A silver spoon or silver coin would turn black in colour when dipped in a dish of poisonous mushrooms.
4. When poisonous mushrooms are cooked with onions, one can observe an unusual change in colour of onion.

2.7. EXERCISE

Procure a wild mushroom from your area and identify it. Write its morphological characters. Draw a labelled diagram.

2.8. SELF ASSESSMENT QUESTIONS

To check your progress on your own, answer the following questions in the space given below without referring to the text above.

1. What are the morphological differences between *Agaricus bisporus* and *A.bitorquis*.

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2. List out the commonly cultivated species of *Pleurotus*.

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3. What is the mushroom that belongs to Ascomycetes? Name the important edible species of it.

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4. List out the highly poisonous species of *Amanita*.

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5. When is *Corprinus atramentarius* poisonous?

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Ms. K. Prasunamma

BLOCK - III : SPAWN PREPARATION AND CULTIVATION METHODS

Spawn preparation and cultivation methods are the key aspects of mushroom cultivation. Isolation of mushroom pure cultures, mother spawn preparation and spawn preparation are the highly technical aspects and must be carried out with utmost care. Hence, methods of raising pure cultures, mother spawn preparation and preparation of spawn of various mushroom species are included in the block. Apart from these, in order to acquaint the student with cultivation methods of mushrooms, cultivation procedures of white button mushroom, oyster mushroom, paddy straw mushroom, shiitake mushroom, black ear mushroom and milk white mushroom are given in this laboratory manual.

UNIT - 3 : RAISING OF PURE CULTURE OF MUSHROOMS

3.1. AIM

In this unit you will study the method of raising pure culture of Mushrooms.

3.2. OBJECTIVES

After completing this unit you will be able to :

- describe the procedure of raising pure cultures for button mushroom, oyster mushroom and paddy straw mushroom by tissue culture and spore culture methods and.
- list out the materials required for raising pure cultures.

3.3. INTRODUCTION

In microbiology, culture refers to the growing of microorganism in glassware (test tube/petridish) on an artificial medium. Pure culture means growing of only a single microorganism without any contamination. In nature, since many organisms grow together, it is very difficult to get a pure culture of a particular organism.

The pure culture of a mushroom mycelium can be obtained by means of either tissue culture or spore culture methods. The materials required and the method of raising pure culture is similar for button, oyster, paddy straw mushroom, shiitake mushroom, black ear mushroom and milky white mushroom.

3.4. MATERIALS REQUIRED

Healthy mushroom; test tubes containing medium; 0.1% mercuric chloride solution (100mg mercuric chloride dissolved in 100ml water); alcohol; sterile water; sterilised petriplates; filter paper (sterilised in oven); scalpel; blade; scissors; inoculation loop; sterile cotton; sterile beakers and gas burner or spirit lamp.

3.5. METHOD OF RAISING PURE CULTURE

Pure culture of a mushroom can be obtained by two ways. They are tissue culture and spore culture methods.

3.5.1. Tissue Culture Method

1. Prepare suitable medium (PDA or Malt Extract Agar medium) and make test tube slants (refer Unit-I).
2. Select an young and healthy mushroom.

3. Clean the mushroom externally with a clean cotton swab to remove straw and dust particles.

4. With the help of a sterilised forceps, dip the mushroom in a sterilised beaker containing 0.1% mercuric chloride solution for 30 seconds. Mercuric chloride will kill all surface contaminants.

5. After 30 seconds, remove the mushroom from mercuric chloride solution and wash 3-4 times in fresh sterilised water serially to remove excess chemical.

6 Use fresh sterilised water for every change.

7. To remove excess water, dry the mushroom on a sterilised filter paper.

8. Cut the fruit body length wise with the help of a sterile blade (Fig. 3.1)

9. From the inner cut surface, at the junction of stipe and pileus, remove small bits of tissue (2 x 2mm) with the help of a scalpel.

10. Open the tube having medium over the flame and place a bit of tissue on the medium with the help of an inoculation loop. Plug the tube immediately.

11. Incubate the test tubes at 22-25°C in case of button, 25 ±1°C in case of oyster mushroom, 30-32°C in case of paddy straw mushroom, 25°C in case of shiitake mushroom, 25-28°C in case of black ear mushroom and 28-30°C in case of milky white mushroom respectively.

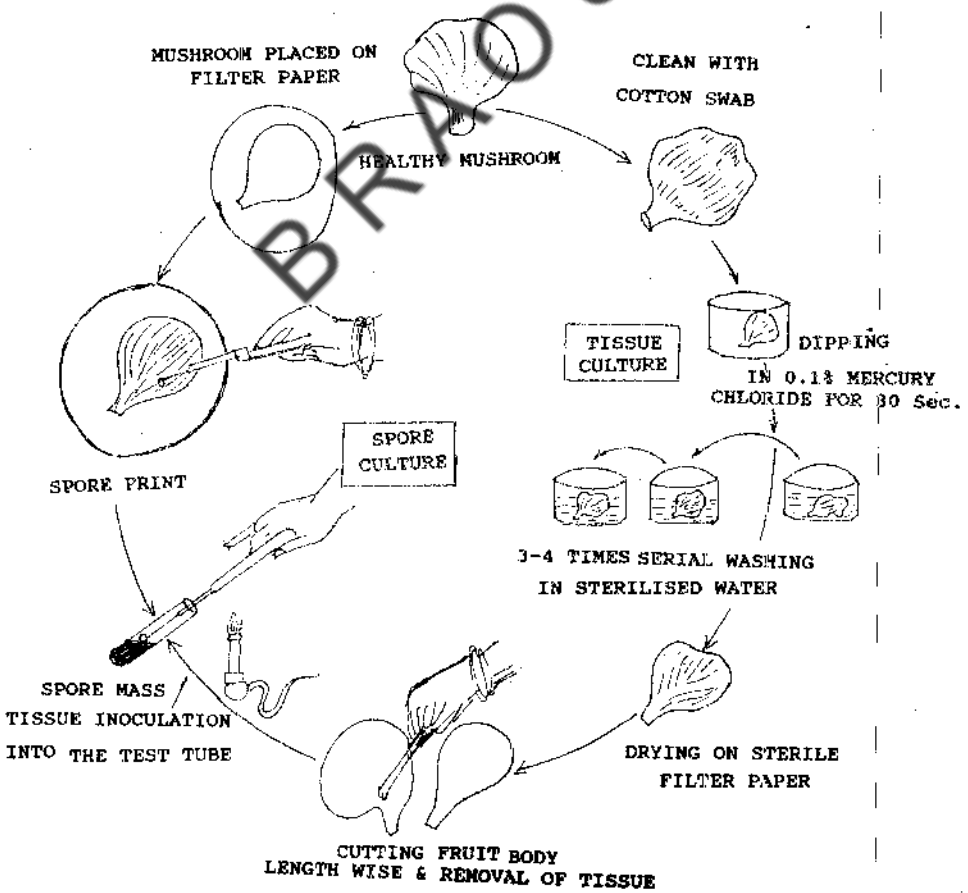

Fig. 3.1. Raising pure culture of oyster mushroom.

3.5.2. Spore Culture Method

1. Collect the spores of mushroom by taking spore print. A spore print is nothing but a mass of spores, collected from the fruit body on a clean surface. The technique of taking spore print is as follows :

   a) Take a mature opened mushroom and cut its stipe to half of its length.

   b) Place the mushroom on a clean, sterile paper. After ten minutes spore print is observed. Discard it since there are chances of occurrence of external microorganisms in it.

   c) Place the mushroom in a sterile Petriplate and cover with a sterile beaker.

   d) Observe the Petriplate after 20-30 minutes. You will find the spores shed on the Petriplate which are ready for inoculation.

2. Lift the spore masses with the help of a sterilised inoculation loop and transfer them onto an agar slant (Fig. 3.1) under sterile conditions. This process is called inoculation.

3. Incubate the test tubes at the respective temperatures for different mushrooms as mentioned in tissue culture technique.

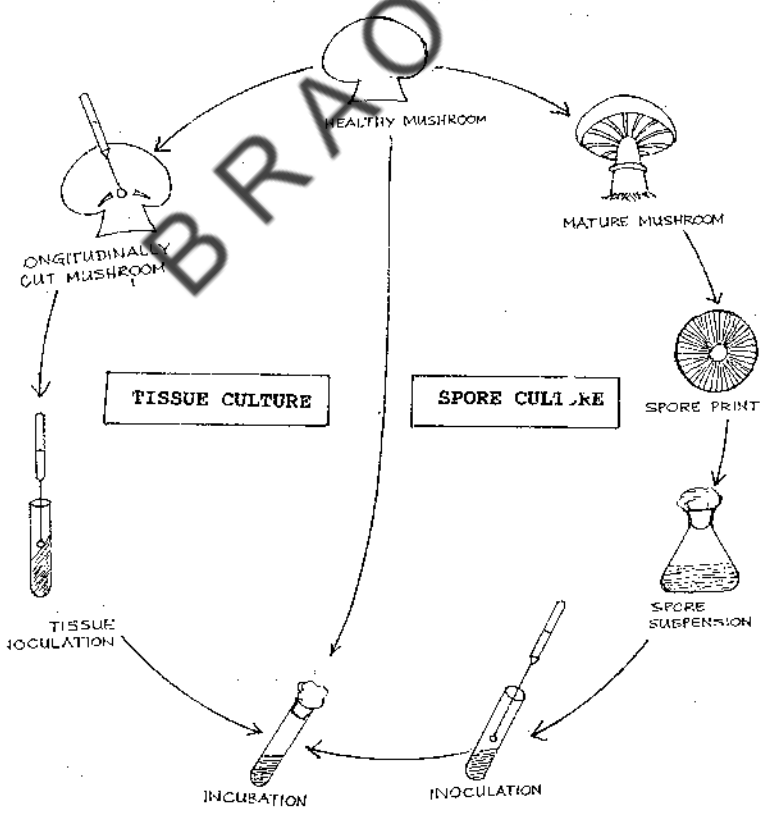

Fig. 3.2. Raising of pure culture of button mushroom by tissue culture and spore culture methods.

3.6. PRECAUTIONS TO BE TAKEN

1. All the above operations should be carried out under sterile conditions in an inoculation chamber.
2. The persons involved in the isolation of pure cultures should maintain cleanliness.
3. Strict hygiene of the area should be maintained.

3.7. OBSERVATION

1. Within 4-5 days of inoculation you would observe white mycelium spreading out of the tissue or from the spores onto the medium.
2. Coloured growth in the tubes indicate fungal contamination. Creamy slime-like growth indicates bacterial contamination. Such tubes are to be discarded.

3.8. EXERCISE

Procure a mushroom (button, oyster or paddy straw) and raise the pure culture by tissue culture method. Write the flow chart of different steps of the method. Draw the diagrams related to tissue culture method & label them.

3.9. SELF ASSESSMENT QUESTIONS

To check your progress on your own, answer the following questions in the space provided below without referring the text above.

1. What is meant by pure culture?

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2. What is the use of Mercuric Chloride during isolation of pure culture?

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3. What is meant by contamination? How do you differentiate the fungal contaminant from bacterial contaminant?

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Ms. K. Prasunamma

UNIT - 4 : SPAWN PREPARATION

4.1. AIM

In this unit, you will study the preparation of mother spawn and spawn from pure culture of mushrooms.

4.2. OBJECTIVES

After completing this unit you will be able to :

- define spawn, stock culture and mother spawn,
- list out various substrates used for making spawn,
- list out the precautions to be taken while making spawn.

4.3. INTRODUCTION

The word spawn in mushroom industry means a medium through which the mycelium of a fruiting culture (mushroom) is grown and it serves as the inoculum or 'seed' for mushroom cultivation. The term 'stock culture' means a strain or stock of known origin, free from contaminants. The growth of fungal mycelium in the medium (sorghum grain, straw etc.) after being inoculated from the stock culture is known as mother spawn. From the mother spawn further spawn upto 2nd or 3rd generation can be produced. The efficiency of the spawn will be reduced if it is continuously sub-cultured for a number of generations. It is very important that the quality of the spawn should be good. Failure to achieve a satisfactory harvest may frequently be traced to an unsatisfactory spawn. If the spawn has not been made from a genetically suitable fruiting culture, or if a stock culture has degenerated, or if it is too old, the yield of mushrooms will be less than the optimum.

Though the potential of a spawn is set by the genetic constitution of the fruiting culture used in its manufacture, the substrate material is also very important. Some of the substrates used in spawn making include various grains (rye, wheat, sorghum), rice straw cuttings, cotton waste, rice hulls, cotton seed hull etc. Spawn is frequently referred to as grain spawn or straw spawn, which tends to over emphasize the importance of the substrate; whereas, in reality, it is the strain or stock of the mushroom that is of prime importance in determining the merits of a spawn. The spawn substrate serves mainly as the vehicle that carries the vegetative mycelium of the mushroom that is used to inoculate the growing beds. This does not mean that the spawn substrate has no effect upon the success or failure of a spawn. The growth pattern of the mycelium may be influenced by the spawn substrate, as is seen by the more rapid growth and filling up of the beds (spawn running) with some spawn substrate than with others

4.4. MULTIPLICATION OF SPAWN FROM PURE CULTURE

1. A number of materials, alone and in different combinations are used as spawn substrates e.g., cotton waste, rice hulls, cotton seed hull, paddy straw cuttings, used tea leaves, coffee pulp, rye, wheat and sorghum grains

2. The material may vary from place to place and on economics too. However, grain as the substrate for spawn production is the most popular and most widely used method.

3 The materials required and the method of preparation of mother spawn and spawn are given below :

4.4.1. Materials Required

1. Autoclave
2. Inoculation chamber
3. Room or chamber for spawn growth
4. Autoclave
5. Gas stove or hot plate
6. Vessel for boiling the grains
7. Jowar (*Sorghum*) or wheat grains
8. Chalk powder
9. Gypsum
10. Empty glucose bottles
11. Non-absorbent cotton
12. Spirit lamp
13. Rectified spirit
14. Inoculation needle
15. Stock culture

4.4.2. Mother Spawn and Spawn Preparation

1. Wash and boil jowar or wheat grains in water.
2. When they are half cooked (grains should slightly break when gently pressed between two fingers), remove from fire.
3. Drain off excess water and spread evenly over a fine wire mesh or a thick cloth for about one hour. This helps to reduce excess moisture and lumps will break.
4. Add and mix 6% chalk powder (Calcium carbonate) and 2% Gypsum (Calcium sulphate) to the cool, boiled grains.
5. Fill the mixture in glucose bottles upto 2/3rd of the bottle (250-300g).
6. Clean the mouth of the bottles and tightly plug (close) the mouth of the bottles with non-absorbent cotton.

7. Cover the cotton plug with a piece of paper and fasten it around the neck of the bottle with a rubber band.

8. Sterilise the bottles at 22lb. pressure in an autoclave for 2 hours which gives an uniform temperature of 127°C.

9. Transfer the sterilised bottles with grains to inoculation chamber and spray 2% formaldehyde inside the chamber.

10. Next day before inoculation switch on the ultraviolet tube for 20-30 minutes.

11. Switch off the ultraviolet tube before entering the chamber for inoculation.

12. In the inoculation chamber, with the help of sterilised inoculation needle take out small piece of fungus culture from the stock culture in the slants and transfer it into each bottle. About 6-8 bottles can be inoculated with one slant containing good growth of the mycelium. Inoculation should be done over the burning spirit lamp to avoid contamination.

13. Transfer the bottles after inoculation to the spawn growing chamber/room. Its temperature should be 25±2°C.

14. Examine the inoculated bottles at 5-7 days interval and check for contamination. Discard the contaminated bottles.

15. The mother spawn prepared from the stock culture can be further used to multiply spawn. Open the mother spawn bottle over the spirit lamp, loosen the spawn with the help of a sterile spathula or iron rod above the flame and transfer about 10g of mother spawn into the sterilised bottle containing grains. From one bottle of mother spawn 25-30 bottles can be inoculated, depending upon the mushroom species. Buttor mushroom being slow growing requires more inoculum, whereas oyster mushroom which grows faster requires less inoculum.

16. The advantages of grain spawn are that it contains more nutrients and more initial growing points, the spawning can be handled more quickly and the spawn can be distributed more easily over the compost/substrate. Therefore, the mycelium from grain spawn can colonize the compost substrate rapidly and form fruiting bodies early.

4.4.3. Precautions

1. A major source of contamination of spawn is the grain used for making the spawn. The grain should be clean, healthy and unbroken.

2. Contaminated bottles showing unacceptable differences in appearance, growth, colour or odour should be discarded.

3. Care should be taken to see that the culture/spawn is free from virus.

4. The grains should be half cooked and unbroken.

5. Do not keep the boiled grains mixed with chalk powder and lime unsterilised for more than 10 hours.

6. Plug and sterilise the bottles properly.
7. Inoculate the bottles after 2-3 days of sterilisation.
8. Before inoculation, keep the bottles under UV lamp for 30 minutes.
9. Carry out the whole process in a double chambered, closed air tight inoculation room.
10. Remove and replace the plugs quickly.
11. Always inoculate over the flame.
12. After inoculation shake the bottles thoroughly to get early and uniform growth.
13. After inoculation incubate the bottles at 25±2°C.
14. Store the spawn bottles at 4°C, after they are ready.

4.4.4. *Agaricus* spp.

The mycelium of *Agaricus* spp. grows very slowly, therefore more inoculum is needed. From one bottle of mother spawn 10-15 bottles can be inoculated. Spawn will be ready in about 30 days. The pH of the medium should be 7.2-7.4. The temperature should never go beyond 32°C and it is lethal to the mycelium of *Agaricus* spp.

4.4.5. *Pleurotus* spp.

The mycelium of oyster mushroom grows reasonably fast, so less inoculum is needed. From one bottle of mother spawn 25-30 bottles can be inoculated. Spawn will be ready in about 18-20 days. The pH of the medium should be 5.6-6.5. White plaster of paris can also be used instead of chalk powder. It will lower the pH, therefore bacterial contamination will be less.

4.4.6. *Volvariella* spp.

For paddy straw mushroom, in addition to grain spawn, straw spawn and rice bran spawn can also be used.

Straw spawn : Well soaked paddy straw is chopped into 1 inch bits, 4% Gypsum is added by weight. Compactly fill them in bottles and sterilise in an autoclave. The grain and straw spawn is ready in 20-25 days after inoculation with culture.

Rice bran spawn : Add red gram powder (1% w/w) to rice bran, moisten with water, fill in bottles and sterilise in an autoclave. The spawn is ready in 7-10 days after inoculation. This spawn gives increased yield.

4.4.7. *Lentinus edodes*

The spawn substrates used for shiitake mushroom are saw dust and wood plugs (refer Unit-13, Course-2. CPMC for the composition). The pH should be 5.5-6. The substrate after inoculation should be incubated at 25°C for 25-30 days.

4.4.8. *Auricularia* spp.

The spawn substrate used for black ear mushroom consists of saw dust (100g), rice bran (25g), Potassium nitrate (5g), Calcium carbonate (7g) and sufficient water to make the medium wet. The substrate after sterilisation and cooling is to be inoculated with the pure culture. Incubation should be done at 25-28°C for 3 weeks.

4.4.9. *Calocybe indica*

Wheat or sorghum grains are used as spawn substrate with the addition of 2% (wet weight of grains) Gypsum and 6% (wet weight of grains) Calcium carbonate. The inoculated bottles or bags should be incubated under light (6-7 hr/day) for 3-4 weeks at 28-30°C.

4.5. EXERCISE

Procure a pure culture of any one type of mushroom mentioned above and prepare mother spawn from it. Write the materials required and method of preparation of mother spawn. Observe for any contamination and if there is any contaminant, identify with the help of morphological characters.

4.6. SELF ASSESSMENT QUESTIONS

To assess your progress on your own, answer the following questions in the spaces provided below without referring the text above :

1. What is mother spawn? What is the difference between spawn and mother spawn?

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2. Which is the most popular substrate for spawn preparation?

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3. At what temperature and pressure and for how long the bottles are sterilised in the autoclave?

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Dr. I. Kunwar

UNIT - 5 : CULTIVATION OF MUSHROOMS

5.1. AIM

In this unit you will study the methods of cultivation of button mushroom (*Agaricus bisporus* & *A. bitorquis*), oyster mushroom (*Pleurotus* spp.), paddy straw mushroom (*Volvariella volvacea*), shiitake mushroom (*Lentinus edodes*), black ear mushroom (*Auricularia* spp.) and milky white mushroom (*Calocybe indica*).

5.2. OBJECTIVES

By the end of this unit you will be able to :

- list out the mushrooms that are more suitable for Indian conditions,
- describe the method of cultivation of oyster mushroom and various steps involved in it,
- describe the procedure for composting, spawning and casing necessary for button mushroom cultivation,
- explain the method of preparation of substrate for the cultivation of paddy straw mushroom and
- describe the cultivation procedure of shiitake, black ear and milky white mushrooms.

5.3. INTRODUCTION

Cultivation of mushrooms is becoming increasingly popular in India. Most commonly cultivated mushroom is the white button mushroom (*Agaricus bisporus*). It is followed by oyster mushroom (*Pleurotus* spp.) and paddy straw mushroom (*Volvariella* spp.). Cultivation of these mushrooms is done indoors and requires little land area. *Volvariella* spp. can be cultivated outdoor also in shade and therefore requires very little investment. Optimum temperature for the growth is 30-36°C. If optimum temperature, humidity, aeration, pasteurization of substrate, quality of spawn etc., are maintained properly, the yield of mushrooms would be higher. The cultivation of oyster and paddy straw mushrooms can be started with very little investment, and it is highly profitable. Other three important edible mushrooms, which are becoming popular in India are shiitake, black ear or Jew's ear and milky white mushrooms.

5.4. OYSTER MUSHROOM

1. *Pleurotus sajor-caju* and *P.florida* are two important oyster mushrooms, exploited for commercial production. These can be grown on any type of agricultural waste or bye products of agricultural industry like paper waste, sugarcane bagasse, cotton waste, hulled maize cob etc.

2. The cultivation is easy, investment is less, yield is good and the profit margin is quite high.

3. The cultivation is done indoors and requires very little land area. As such, oyster mushroom cultivation is recommended for our state.

4. An ordinary thatched shed can be used for cultivation of oyster mushroom.

5. Temperature in the room should be between 24-28°C and should not be more than 30°C.

6. High humidity is needed. About 75-80% humidity is congenial for oyster mushroom production.

7. A thatched shed with gunny curtains provides a congenial environment. The walls on the inside are also lined with hanging gunny bags. The floor may be covered with sand. The floor and gunny should be wetted with water twice a day. This keeps the room cool, besides increasing the humidity.

8. The room should have diffused light and good ventilation.

9. Spawn running room is the room where the beds after spawning are kept for the spawn run (spread/grow).

10. The temperature in the spawn running room should be around 25-28°C. During spawn running relative humidity of around 70% is to be maintained.

11. The cropping room is the one where open beds are kept after completion of spawn running for production of mushroom.

12. The temperature of cropping room should be 23-25°C. Some growers use the same room for spawn running and cropping.

13. Spray the room with formalin solution (20ml commercial grade formalin in 100ml water) one day before making the beds for spawn running. Afterwards spray two times in a week as long as mushrooms are cultivated.

5.4.1. Bed Preparation

1. Select fresh paddy straw as substrate. Care should be taken to ensure that the paddy straw is not too old.

2. Cut the paddy straw into 3-5 cm bits. One kg. dry straw is required for one bed (bag).

3. Wash the straw with fresh and clean water.

4. Soak the straw for 8-24 hours. Remove it from water and allow the excess water to drain off (do not dry in the sunlight).

5. Boil the water in a big vessel. Keep the pre-soaked straw immersed in boiling water for 30 minutes; or alternatively add 125ml formalin (37-40%) and 7.5g bavistin in 100 litres of water while soaking the straw.

6. Drain the water and allow the straw to loose excess moisture. The straw gets pasteurized by this method and all the harmful germs and insects get killed.

7. When the straw has lost all the excess moisture and reached room temperature, it is ready for spawning. Test the straw by squeezing it by hand and if the water does not drip, it is ready for use.

5.4.2. Spawning

Spawning is the process of broadcasting the spawn on substrate. The following are the various steps of spawning :

1. Take a polypropylene bag of 60 x 30cm size of 80 gauze, tie the bottom of the bag with a thread or rubber band. This gives a circular bottom for the bed when prepared.
2. Make 10-20 small holes (0.5-1.0 cm. diam.) in the sides and bottom of the bag.
3. Disinfect the spawn bottle, spathula, polypropylene bag, hands, plastic tray and iron rod with an antiseptic solution.
4. Open the spawn bottle and loosen the grains with a spathula.
5. Collect the spawn in disinfected tray and break the solid spawn with fingers to individual grains.
6. Fill the pasteurized paddy straw in the polypropylene bag to a height of 5cm.
7. Spread or broadcast a handful of the spawn uniformly over the entire surface of the straw (first layer).
8. Spread the second layer of straw to a height of 10cm and broadcast a handful of spawn over the surface uniformly (second layer).
9. Spread the 3rd and 4th layer of straw and spawn.
10. Gently press the straw down and tie the top of the bag with rubber band or thread. One spawn bottle (250-300g. spawn) is sufficient for spawning of two beds (½kg. dry paddy straw in each bed).

5.4.3. Spawn running

1. Shift all the bags to spawn running room and arrange them on racks or on elevated surface (on two bricks or wooden blocks).
2. Do not keep on the ground.
3. Observe daily the growth of the fungus. It grows as white mycelium and spreads in the entire bed.
4. Spawn running is completed in about 18-20 days. The bags become filled with mycelium and gives white appearance.
5. Before opening the bed, check for contamination if any.
6. Normally beds get contaminated either before or after opening.
7. Remove contaminated beds, treat them with 2% formalin and discard to avoid the spread of contaminants in the atmosphere.

5.4.4. Opening of Beds

1. Cut open the polypropylene bag with a disinfected blade after 18-20 days of spawning. The bed will be intact after removing the polypropylene bag. Sometimes mushrooms may come out piercing through the polypropylene bag even before 18-20 days. In that case the bag should be opened immediately.

5.4.5. Cropping

1. Arrange the opened beds on racks or on two bricks or two wooden blocks.
2. The gunny bag lined walls and the floor of the cropping room should be sprayed with water twice a day to maintain relative humidity of 80-85%.
3. For about two days there is no need to spray water on the beds.
4. Afterwards, spray water (light mist spray) on the beds every day in the morning and evening using a sprayer.
5. Mushroom beds can be observed with pin heads on the 3rd or 4th day of opening the beds.
6. Once pin heads are of 2-3cm size, a little heavier watering is done on the beds and further watering is stopped to allow them to grow. This helps to avoid bacterial rotting. However, the optimum relative humidity should be maintained inside the cropping room by spraying water on the gunny bags. Fully grown mushrooms develop within 3-4 days after the appearance of pin heads.

5.4.6. Harvesting

1. Pluck fully grown mushrooms early in the morning before spraying water on beds.
2. Continue spraying water twice a day.
3. Second crop of mushrooms will be ready after about 8-10 days of the first crop.
4. 3rd and 4th crop of mushrooms can also be harvested like the 2nd crop after 8-10 days intervals.
5. Entire harvesting of mushrooms is over in 35-40 days. Almost 80% of the mushrooms are harvested in 1st and 2nd crop.
6. Care must be taken so that the beds do not become too wet, otherwise rotting may occur.
7. Harvested mushrooms should not be washed in water.
8. Cropping room must be provided with cross-ventilation and diffused light in the day time.

5.4.7. Packing and storage

1. After harvesting, remove straw bits and root portions from the mushrooms.
2. Pack the mushrooms in perforated (5-6 small holes) polythene or polypropylene bags. Now they are ready for marketing. Small packings of 100-250g of fresh mushrooms are ideal for marketing.

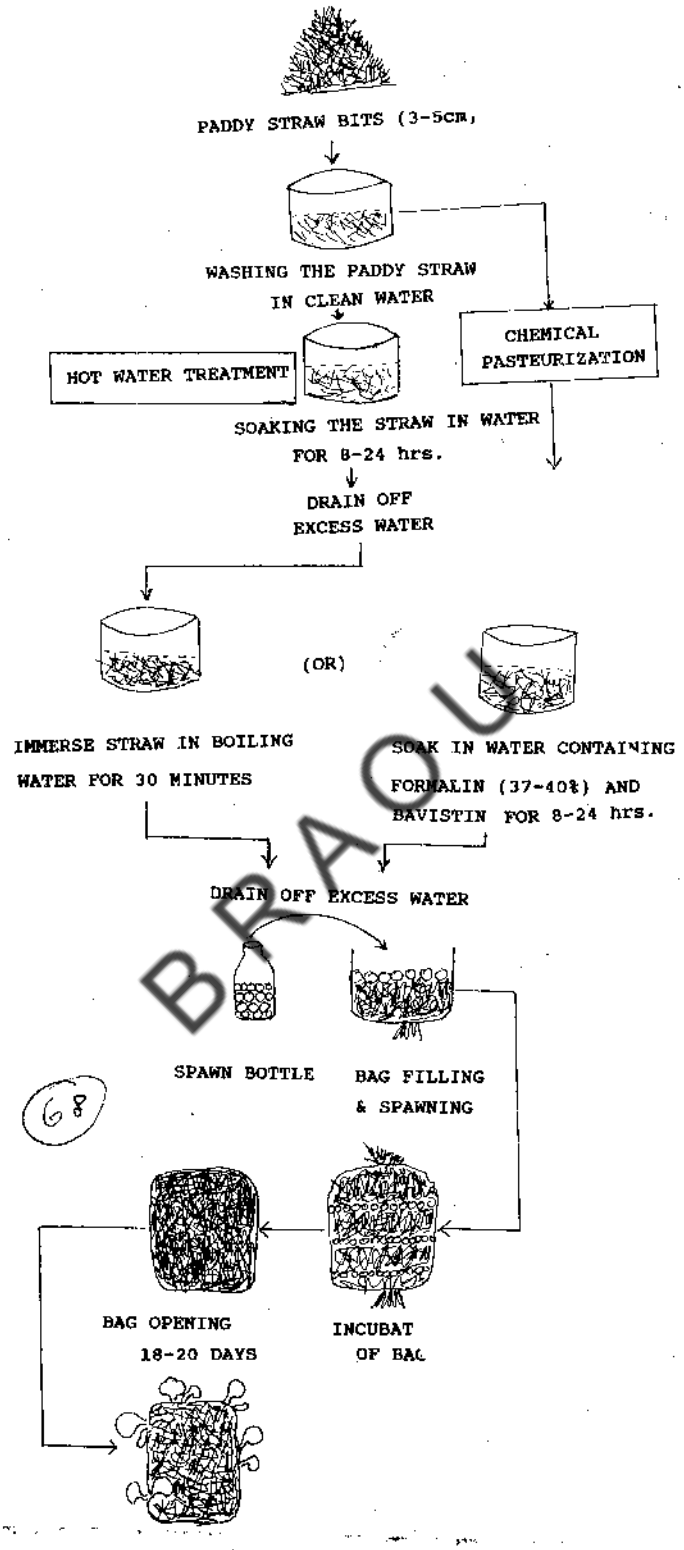

Fig. 5.1. Method of oyster mushroom cultivation.

3. Normally mushroom should be used/marketed on the same day of harvest. Normal shelf life is about 12-16 hours at room temperature, but mushrooms can be stored for about 3 days in the refrigerator.

5.5. BUTTON MUSHROOM

1. *Agaricus bisporus* and *A.bitorquis* are the two important button mushrooms grown commercially. White button mushroom is also called as temperate mushroom because it is grown at low temperature i.e. temperature within the range of 16 to 18°C is most favourable for its cropping.

2. In India 90% of the production is white button mushroom followed by oyster and paddy straw mushroom.

3. Various agricultural, poultry and animal wastes can be used with some fertilizer and other ingredients for growing button mushroom.

4. Its cultivation is done indoors.

5. It requires some investment.

6. Good compost, pure and productive spawn, optimum temperature and relative humidity, right amount of CO_2 in the air are essential for growing button mushroom. Various steps involved in growing the button mushrooms are given below.

5.5.1. Composting

1. Button mushroom cannot grow on paddy straw but requires a manure made specially for it. It is known as compost. The process of making compost is termed as composting. There are several formulae for making compost and four important formulae are given below :

Formula-1

Wheat or paddy straw	150 kg.
Ammonium sulphate or Calcium ammonium nitrate	9 kg.
Superphosphate	3.5 kg.
Urea	4.5 kg.
Wheat bran	30 kg.
Rice bran	50 kg.
Gypsum	12 kg.
Calcium carbonate (Chalk powder)	10 kg.

If paddy straw is used in this formula, 6kg of cotton seed meal should also be added.

Formula-2

Paddy straw	600 kg.
Rice bran	100 kg.
Urea	10 kg.
Gypsum	24 kg.
Cotton seed meal	12 kg.

Formula-3

Paddy straw	3,000 kg.
Chicken manure	1,500 kg.
Wheat bran	125 kg.
Gypsum	90 kg.

Formula-4

Horse manure	1,000 kg.
Urea	6.8 kg.
Rice bran	20 kg.
Gypsum	25 kg.

There are two popular methods of making compost.

1. Long method.
2. Short method.

Long method

1. Twenty six days are needed to make compost by the long method. The pile of compost is turned on 0, 6th, 10th, 13th, 16th, 19th, 22nd and 25th days. Pasteurization is not needed in this method. Two days before the start of the composting (day -2) paddy straw is kept wet for 48 hours on a concrete floor and stacked in a heap of 6' x 5' x any length. The mixture of fertilizers and rice/wheat bran are mixed and watered 24 hours separately in advance of zero day.
2. On zero day wet straw and wet mixture of fertilizers and bran are mixed thoroughly and a stack (6' x 5' x length) is made.
3. At this stage the moisture content of the straw should be 75-77%.
4. To make a compact pile, press down the pile with a wooden frame.
5. The compost is given first turning on the 6th day for free exchange of air. The water is sprinkled if needed.
6. The growth of white mycelium can be seen in the compost. It is a good indication. Usually species of *Coprinus* appear initially. Growth of *Coprinus* indicates the presence of Ammonia.
7. Pile up the compost again. The compost is turned for the second time on the 10th day.
8. Gypsum is added while turning the compost for the 3rd time on 13th day.

9. The compost is given turnings like this for the 4th, 5th, 6th and 7th times on 16th, 19th, 22nd and 25th days respectively.

10. The smell of ammonia should not be there on 22nd day during 6th turning or it should be very mild. Spray water if needed.

11. On 26th day if ammonia smell persists, before filling the compost in trays, turn the pile again. By this time *Coprinus* is not seen in the compost, its absence indicates the disappearance of ammonia.

12. In a good compost the moisture content is about 65-75%; its colour is dark brown and it does not have ammonia smell. In this method of composting unwanted fungi, insects, mites and nematodes come and the nutrients become less; but this method is popular with small growers, who are unable to install boilers for pasteurization.

2. **Short method**

Short method is more popular for commercial production of button mushroom. It is completed in two phases. In the first phase compost is made in the open. It is like long method. It takes 7-16 days. In the second step the compost is pasteurised to kill unwanted organisms.

1. Day -4 : Four days before composting, straw and horse/chicken manure are piled on concrete floor. The stack should be 1-2' height. Sufficient water is added to wet the stack.

2. Day -2 : Water is poured to soak the substrate and the stack size is to be reduced to half.

3. Zero day : Compost is turned after two days and urea and rice bran are mixed thoroughly and stacked again (6' x 5' x any length).

4. Day + 2 : First turning is given and water is added if required.

5. Day + 4 : Gypsum is added and the compost is turned.

6. On day + 6, day + 8 and day + 10 also turnings are given. Water is sprayed if required. In this method a strong smell of ammonia comes.

7. Day + 12 : On 12th day the compost is ready for the second phase.

8. Compost is filled quickly in trays before it cools down.

9. Compost filled trays are kept in rows in pasteurization room.

10. Sufficient space is left in between top tray and the ceiling of the room, in between trays and the walls of the room, so that air can move freely.

11. Water is sprayed on the floor, walls of the room and the sides of the trays, so that relative humidity of the room becomes 100%.

12. Turn the blower on and open the ventilators upto 1/3 or 1/4th. Steam goes into the room through pipe having holes which increases the air temperature upto 40-45°C.

13. By closed ventilation and higher air temperature, microbial activity increases, as a result, the compost temperature raises to 50-55°C which is maintained for 24 hours.

14. Now close the ventilators and slowly raise the temperature to 60°C and maintain it for 2-4 hours. This is the upper limit of temperature in the pasteurization of compost.

Conditioning of the compost

1. The ventilators are open (1/3rd) after the pasteurization is completed so that the temperature of the air inside the room could come down and 50-55°C is to be maintained for 2-3 days.
2. It is very important to supply fresh air during conditioning of the compost.
3. Absence of ammonia smell and whitish grey mycelium in the compost indicate that the process of composting is over.
4. Let the compost cool down for two days before filling in trays/bags so that the temperature comes down to 25±2°C.

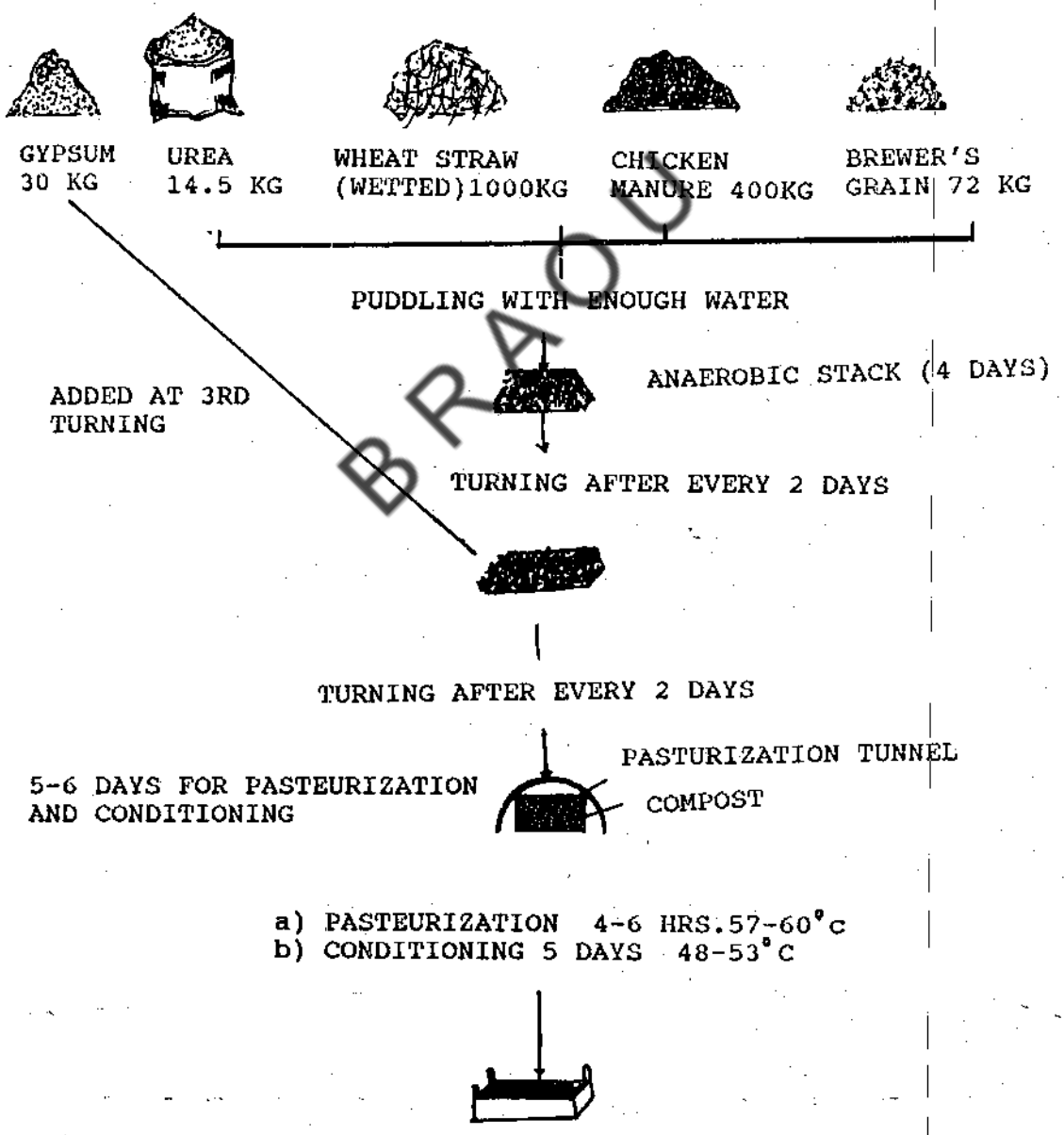

Fig. 5.2. Compost preparation.

5.5.2. Spawning

It is done by 3 methods. They are (1) Top spawning, (2) Layer spawning and (3) Through spawning.

Top Spawning

1. Remove 1-2" layer of compost from the top.
2. Sprinkle spawn.
3. Cover the spawn with 1-2" layer of compost.
4. Press slightly.

Layer Spawning

1. In this method spawning is done in layers.
2. Remove 3-4" compost from the top. Sprinkle spawn in a layer.
3. Cover once again with compost (1½-2" thick).

Through Spawning

1. The spawn is mixed uniformly in the compost.
2. Cover trays with news paper after spawning and keep in spawn running room.
3. Examine the compost everyday to check its moisture.
4. If the surface looks dry spray water lightly.
5. Maintain 90-95% Humidity by spraying water on the walls, floor, sides of trays and on paper.
6. Ideal temperature for spawn running is 25±2°C. Temperature above 34°C is lethal for the mycelium of the mushroom.
7. After spawning, the mycelium starts growing in the compost.
8. After 14-18 days, white and fluffy mycelium covers the surface of the compost.
9. After the spreading of the mycelium remove newspapers and cover the compost with casing soil.

5.5.3. Casing & Casing soil

1. Casing means covering the compost with protective covering of soil or any other material.
2. Casing has many advantages. It maintains humidity, gives support, prevents drying of compost, enhances exchange of air, controls the temperature of compost and encourages the growth of mushrooms.
3. Casing material should be free from microorganisms and insects, and its pH should be neutral or alkaline (pH 7.0-7.5).

Preparation of Casing Soil

1. In casing soil many materials are to be mixed in definite ratio. The following are some of the casing materials.

a) Soil and sand	1:1
b) Cowdung and soil	3:7
c) Farmyard manure and gravel	4:1
d) Farmyard manure and clay	1:1
e) Used compost, sand and lime	4:1:1
f) Peat and soil	1:2

2. Casing material can be pasteurised with the help of formaldehyde.
3. Casing material is spread on the cemented floor and soaked with 5% formaldehyde solution.
4. Casing soil can form a lump but it should be easily broken.
5. Casing soil can also be pasteurized in a small chamber by passing steam through pipes having holes.
6. Before pasteurization pH of the casing soil should be adjusted to 7-8 by adding chalk powder.
7. Fully grown mushroom mycelium is covered with casing material to a depth of 2.5 to 4cm.
8. After casing, the trays are kept in cropping room. But the present day commercial growers are using shelves and polythene bags for cropping.
9. In the cropping room, Humidifier to increase the humidity and Air Conditioner to lower the temperature are being used.
10. For quick spread of mycelium in the casing soil, the temperature is to be maintained at 23-24°C and humidity at 85-90%.
11. On 6th or 8th day the casing soil is ruffled a bit.
12. On 10th day, the temperature is brought down to 16±2°C.
13. Fresh air is circulated with the help of ducts. Water is sprayed once or twice a day on casing soil.

5.5.4. Cropping Cycle

1. Cropping cycle starts after white mycelium is on the casing. Pinning and cropping are two stages of this cycle.
2. Pinning starts 10-15 days after casing. It is a very important stage.
3. It requires low temperature and fresh air (3.6mt^3/meter^2 of beds area/hour).
4. Circulation of air should be good at this stage.

5. The quantity of CO_2 in the air should be less than 0.1%. The CO_2 level in air is important for the formation of fruit bodies. Air should be changed 3-4 times/hour.

6. With the help of fans and ducts fresh air is introduced for 10-15 minutes/hour.

7. Avoid spraying water in pinning stage. Spray gently only in those trays which have less moisture.

8. When mushrooms are of pea size little more water can be sprayed.

9. In 3-5 days button mushrooms can be harvested. At this stage the size of the button mushroom is 2.5-4cm.

10. After a gap of 7-10 days button mushrooms crop up in plenty. Mushrooms are plucked in button stage.

11. Cropping of mushrooms continues for 8-10 weeks. On an average 3.5 Kg mushrooms per 15Kg compost can be harvested in 10 weeks.

12. If pasteurization, air circulation, humidity and temperature are properly maintained, 5-6Kg of mushrooms can be harvested in 10 weeks.

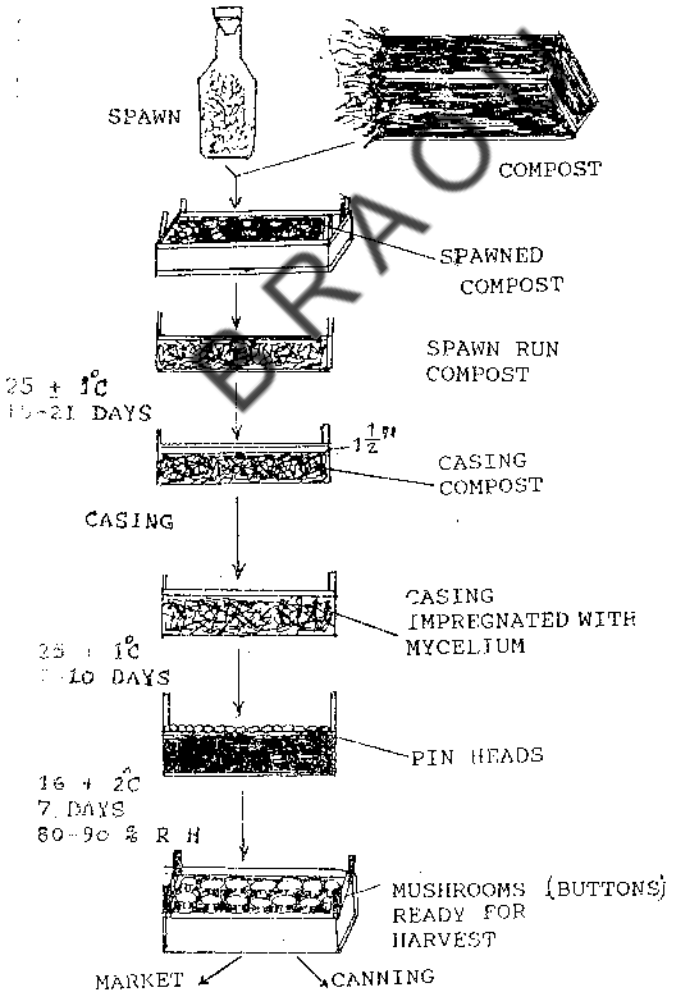

Fig. 5.3. Method of Button mushroom cultivation.

5.5.5. Precautions to be taken during cultivation

1. Ammonia smell should not be there in compost.
2. Spawn should be pure and productive.
3. Only if required, spray water with the help of sprayer during spawn running.
4. Casing soil should be free from microorganisms and pests.
5. Used air should be replaced with fresh air.
6. Remove contaminated trays.
7. Holes should be closed after plucking mushrooms.
8. Mushroom cultivation rooms should not be broomed when in use, otherwise the contamination will increase.

Another species of button mushroom *Agaricus bitorquis* can be grown at higher temperature. It is more suitable for Indian climate. Same procedure given for *A. bisporus* will be followed for *A. bitorquis* cultivation except temperature regulation. *A.bitorquis* requires 28-30°C during spawn running and casing and 24±2°C during pin head formation. Fresh button mushroom can be packed and marketed like oyster mushroom.

5.6. PADDY STRAW MUSHROOM

Paddy straw mushroom (*Volvariella volvacea*) is commonly cultivated in tropics and subtropics.

1. The cultivation of paddy straw mushroom is simple and costs less.
2. It is harvested in 8-10 days.
3. The yields are usually less when compared with oyster mushroom and it is about 3-4Kg/bed of 30-32 Kg paddy straw.
4. The cultivation can be undertaken from March to October under normal conditions.
5. Suitable temperature is in the range of 30 to 35°C, but it can withstand upto 45°C. There is decline in the yield after 36°C.
6. Optimum relative humidity is 80-90% and pH required is 6-7.
7. Straws of paddy, wheat, sorghum, finger millet etc., dried banana leaves, cotton waste, sugarcane bagasse, water hyacinth and semi dried leaves can also be used as substrate. Dry banana leaves are as good as paddy straw.
8. It can be cultivated both outdoors and indoors.

5.6.1. Paddy Straw Method

1. Fresh, dried, golden yellow, whole paddy straw is used.
2. It is tied into 32 uniform bundles (about 800g-1kg. each). Bundles are soaked in clean water for 24-48 hours (preferably in running water) and drained.
3. The paddy straw can be pasteurized by dipping in boiling water for about 30 minutes and then drained.

4. Alternatively the bundles may be directly soaked in water containing 5% formaldehyde (chemical pasteurization).

Bed preparation

5. Place four straw bundles side by side over the bamboo or cement platform in one direction, i.e., with butts on one side.

6. Pile up four more bundles over these in the opposite direction, so that the level becomes uniform.

7. Press with a clean plank to compress.

8. Trim the sides of the 8 bundles and they form the first layer.

9. Spawning is done all around the bed at about 3-4 inches away from the edges.

10. Sprinkle clean "Turdal" powder (red gram) between and over the spawn. About 150g powder is needed for each layer. Cover the spawn with a thin layer (3-4") of cut, soaked and pasteurized paddy straw pieces.

11. Interior parts of the bed should not be spawned.

12. In a similar manner place the second and subsequent layers, placing the bundles at right angles to the previous layer so that the bed remains more firm, now the substrate is ready for spawning.

13. Two spawn bottles are necessary for a bed.

14. Gently sprinkle water over the bed and cover with a transparent polythene sheet (180 x 90 cm.) of 150-200 gauge. Horse gram or tapioca starch powder can also be supplemented as organic amendment. The beds can be kept outdoors under the shade of the trees or indoors.

15. Bed size should not be either too small (less than 20kg.) or too big. In bigger beds the temperature becomes more and the yield is affected.

16. Every morning or evening sprinkle water to maintain optimum moisture (70-75%) by removing polythene cover.

17. Remove the polythene cover on 10th day.

18. In one or two days "pin heads" start appearing. After 3-4 days they become egg sized.

19. Under proper temperature and moisture conditions, the cropping period is between 25-30 days.

20. Room should never be dark if cropping is done indoors, as it affects fruiting.

21. Every 7-10 days fresh flushes appear. There may be 3-5 flushes.

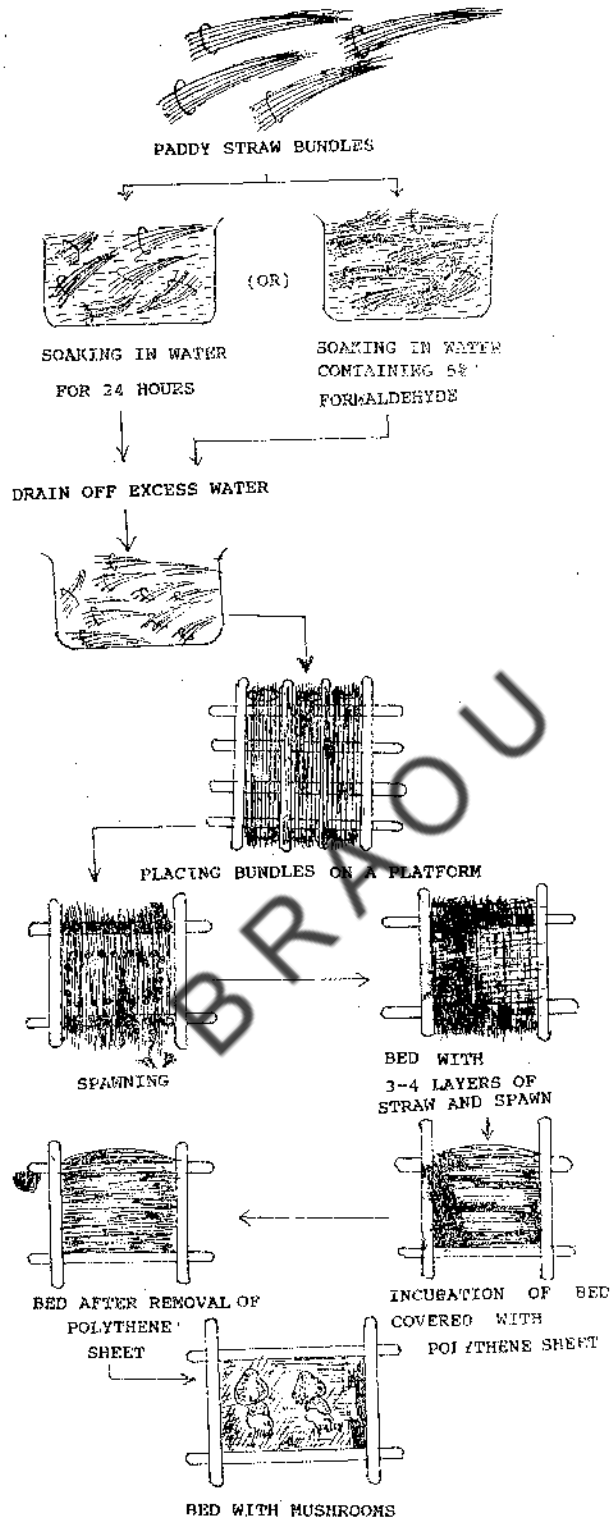

Fig. 5.4. Paddy straw mushroom cultivation.

Harvesting

1. Pick mushrooms in the morning gently by twisting at the base.
2. For marketing fresh mushrooms 'button' and 'egg' stages are preferred and for drying fully mature ones are preferred.
3. Keeping quality of these mushrooms is very less and should be consumed afresh within 6-8 hours. They should not be stored under refrigeration conditions.
4. They can be sun dried or dried mechanically at an optimum temperature of 50-55°C for 5-6 hours.

5.7. SHIITAKE MUSHROOM

The cultivation procedure of shiitake mushroom (*Lentinus edodes*) by polybag method is given below.

1. The following substrate formulations are commonly used.

a)	Saw dust (maple or oak)	80 kg
	Rice bran	20 kg
b)	Saw dust (maple and birch 60:40)	80 kg
	Millet	10 kg
	Wheat bran	10 kg
c)	Sugarcane bagasse	50 kg
	Rice bran	12.5 kg
	Gypsum	1.5 kg
	Potassium sulphate	15 g
	Urea	15 g
	Magnesium sulphate	10 g

2. Soak the saw dust for two days and paddy straw for three hours before use.
3. Dissolve the substances like sulphates, sugar and citric acid in water before mixing with the substrate.
4. Adjust the water to the above formulations till 60-65%.
5. Adjust pH to 5.5-7.0 by adding gypsum and lime.
6. Fill the saw dust mixture (1.5 to 4 kg.) in polypropylene bags (500 x 160mm) and press to form a cylindrical cake.
7. Tie both ends of the bag with twine.
8. Make two holes of 15mm diameter and 20mm deep on opposite sides with the help of an auger.
9. Cover the holes with square (33mm) adhesive medical tape.
10. Sterilise the bags in an autoclave at 121°C (15lb.) for 1 hour or on a brick and

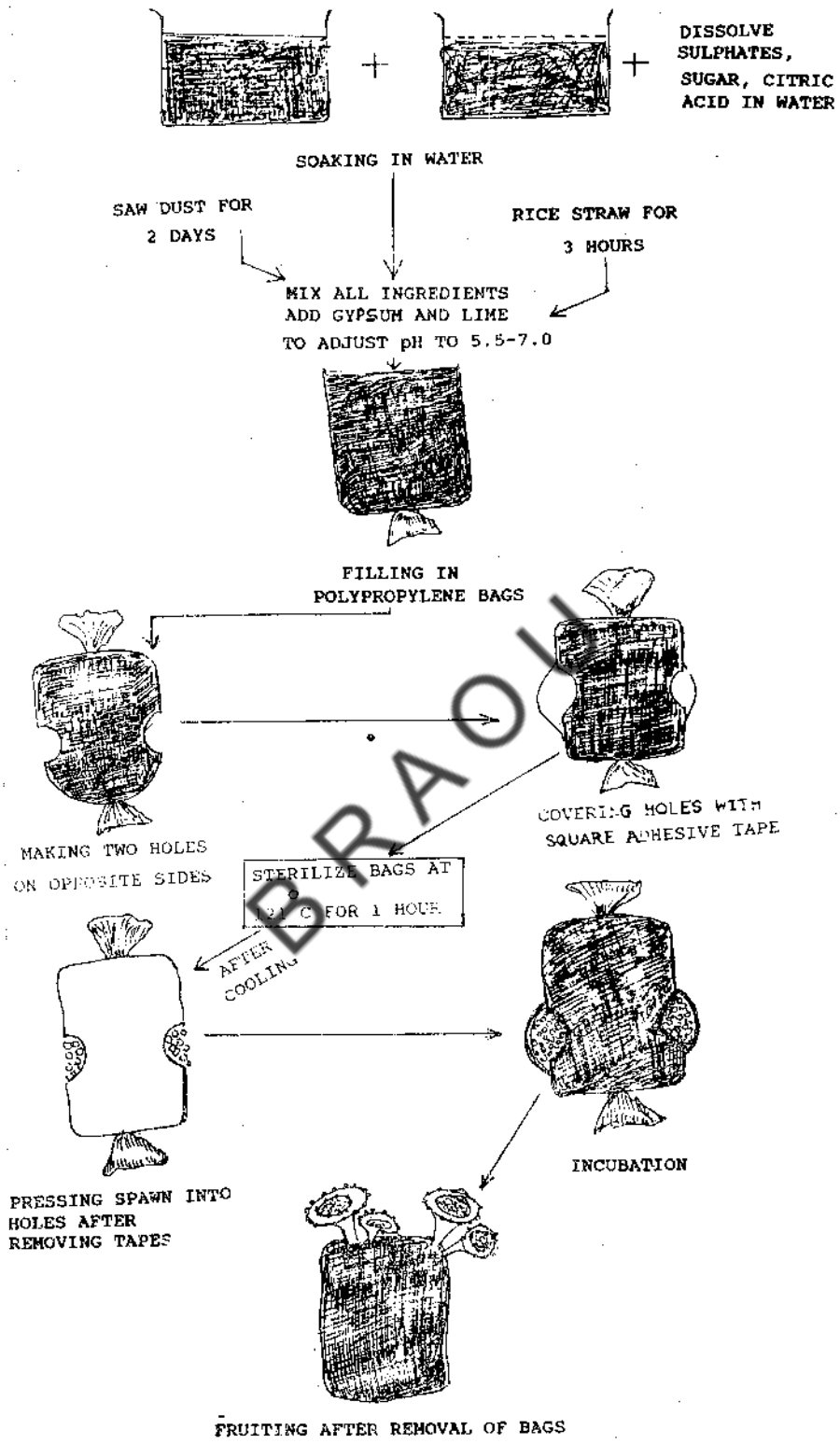

Fig. 5.5. Shiitake mushroom cultivation.

10. Sterilise the bags in an autoclave at 121°C (15Ilb.) for 1 hour or on a brick and cement lined-tower at 90-95°C for 5-7 hours. The time period between the preparation of substrate and sterilisation should not exceed six hours.
11. After cooling, remove the tapes in a sterile area and press the spawn into the holes. About 750g of spawn can be used to inoculate 25-30 bags.
12. Put back the tapes and incubate the bags in the growing rooms at 24-28°C.
13. Mycelial growth can be observed in about 100 days.
14. Remove the bags partially when the mycelial coat turns hard and brown.
15. Maintain the temperature at 12-20°C and relative humidity at 85-90%.
16. Fruiting occurs. Harvest them at an early stage.
17. The mushrooms are consumed afresh or in dried form.
18. Drying can be carried out under sun for 2-4 days or in a mechanical drier at 40-60°C for 12-18 hours.

5.8. BLACK EAR MUSHROOM

The method of cultivation of black ear or Jew's ear or wood ear mushroom or Jelly fungus by polybag method is described below.

1. Mix the saw dust with 2 to 20% rice bran and little calcium carbonate to adjust pH. Wheat straw supplemented with 4% rice bran can also be used.
2. Fill the substrate in polypropylene bags (15-20 cm length and 10-20 cm diam).
3. Sterilise at 100°C for one and half an hours.
4. Inoculate the bags with spawn after cooling. About 250g. of spawn is sufficient to spawn 3 beds.
5. Incubate the bags on racks in growing rooms. Maintain the temperature between 20-34°C.
6. Cut open both the ends of the bag when mycelial growth is complete. Spawn run is completed within 2-3 weeks.
7. Spray water once or twice a day. Maintain the temperature at 12-30°C and relative humidity at 85%.
8. Fruiting occurs within a week.
9. Harvest the mushrooms either by slight twisting or cutting with a knife.
10. The mushroom stays for 7-10 days on the substrate after maturity. It is less perishable and can be sun dried or dried in a mechanical drier.

5.9. MILK WHITE MUSHROOM

The method of cultivation for the milk white mushroom (*Calocybe indica*) is given below.

1. Soak the paddy straw bits (1cm. long) in water for 18-24 hours and drain off excess water.

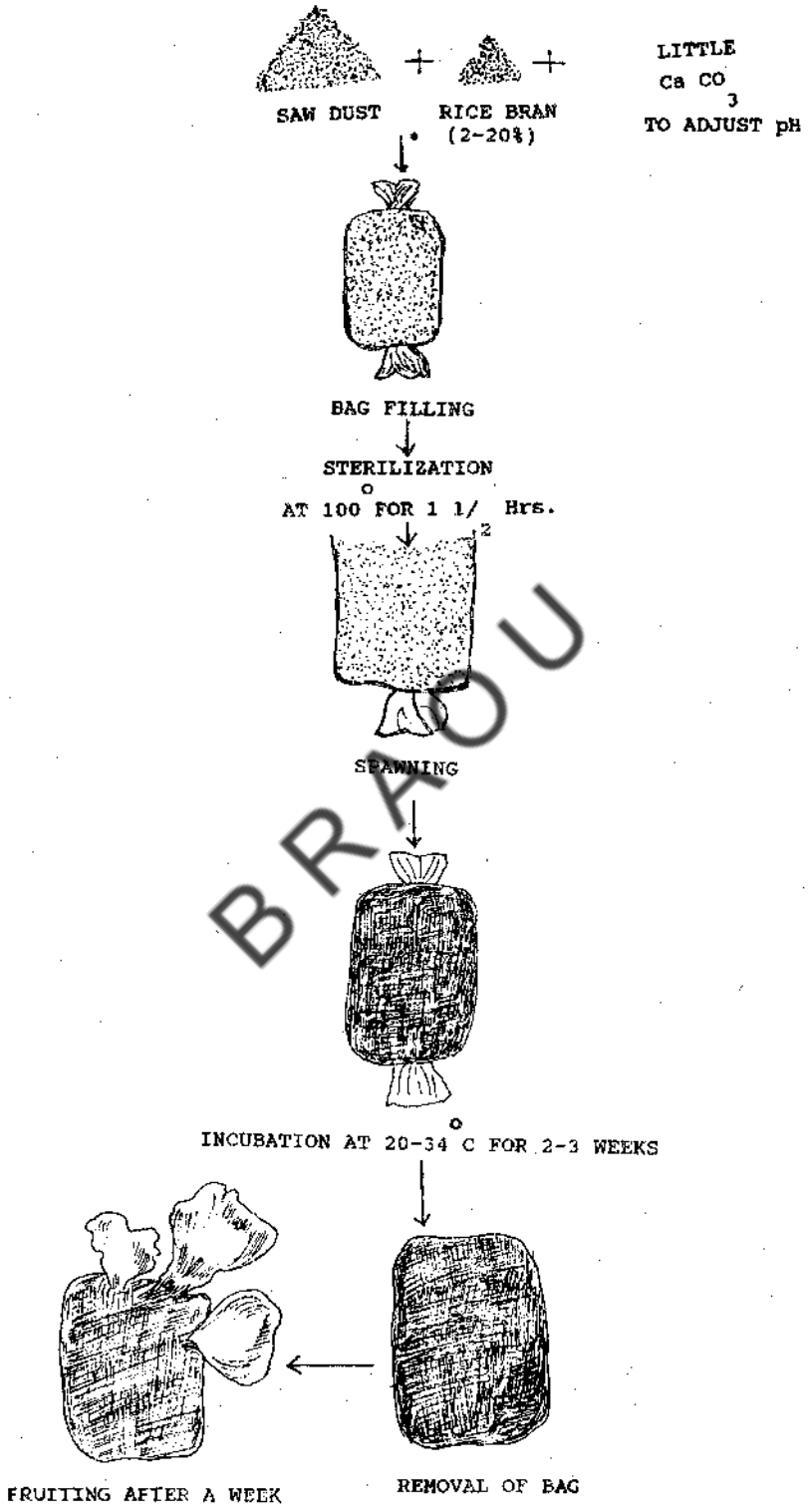

Fig. 5.6. Black ear mushroom cultivation.

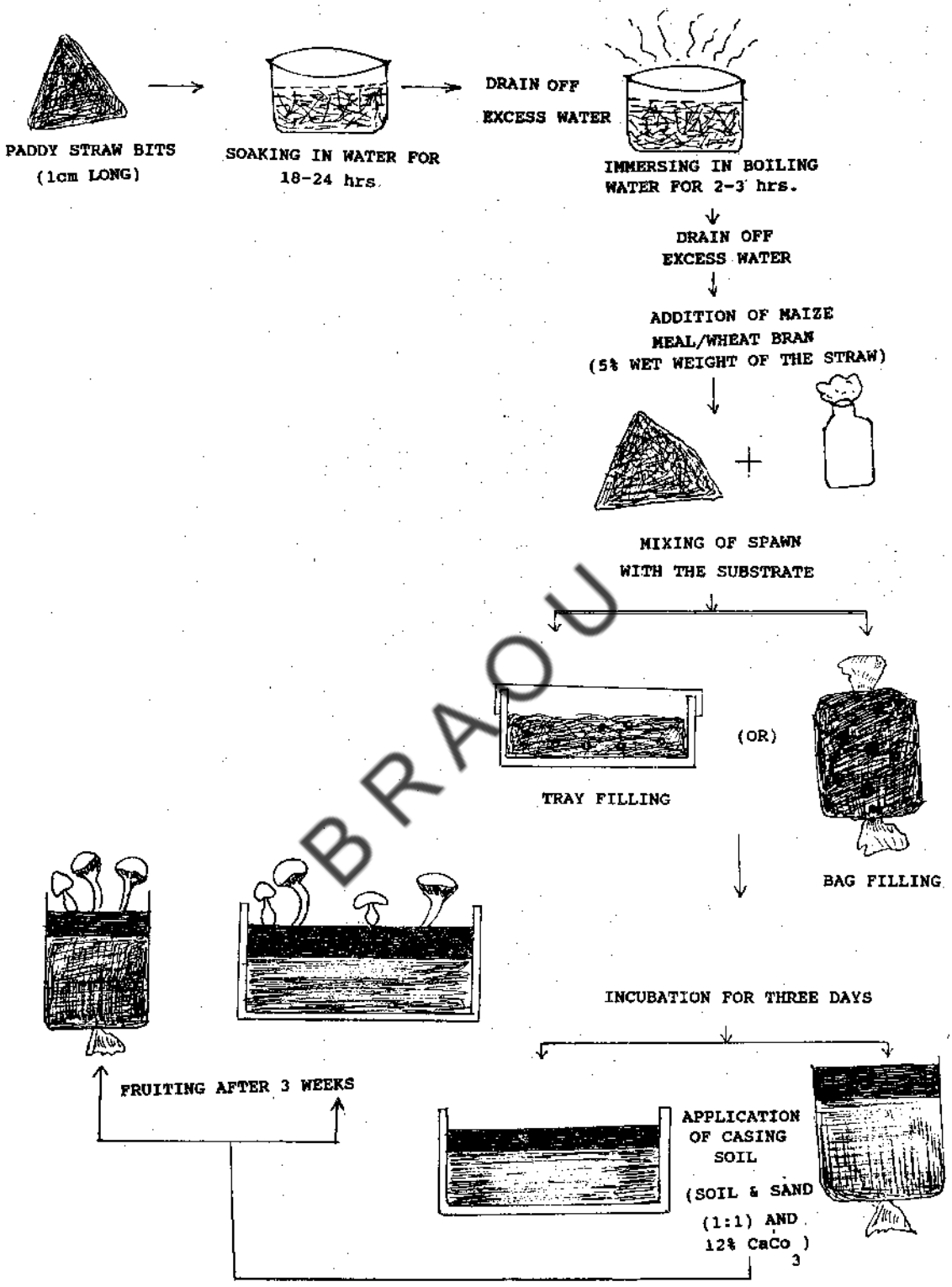

Fig. 5.7. Milk White Mushroom Cultivation.

2. Immerse them in boiling water for 2-3 hours. Drain off excess water.
3. Mix Maize meal or wheat bran with the treated straw (5% of the wet weight of the straw).
4. Mix the spawn thoroughly with substrate and fill in polypropylene bags or in small trays. For each tray of 2sq.ft. and for two polyprolene bags (60 x 30 cm) 250g of spawn is required.
5. Cover the trays with sterile newspapers for 2-3 days.
6. On fourth day, remove newspapers and apply casing soil over the growing mycelium. Casing soil is prepared by mixing dry loam soil or garden soil and sand (1:1) and 12% calcium carbonate of soil and sand mixture.
7. Water the beds periodically to keep the substrate wet.
8. Within 3 weeks after casing pinheads appear and attain maturity in another 5-6 days. Pluck the mushrooms by slight twisting at the base.
9. The harvested mushrooms remain in fresh form at room temperature for 3-4 days and one week in refrigerator.

5.7. EXERCISE

Procure spawn of button mushroom or any other mushroom mentioned above and prepare the beds. Write the materials required and method of cultivation.

5.8. SELF ASSESSMENT QUESTIONS

To assess your progress on your own, answer the following questions in the space provided below without referring the text above.

1. What is a spawn running room?

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2. What is spawning?

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3. What are the temperature and humidity requirements of spawn running and cropping room for oyster and button mushrooms.

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4. Define compost and composting.

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5. What does the presence of *Coprinus* in the compost indicates?

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6. What is Casing? Write the components of any Casing soil.

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Dr. I. Kunwar
Ms. K. Prasunamma

BLOCK - IV : PESTS AND DISEASES AND PRESERVATION

Cultivated mushrooms are attacked by many microorganisms like fungi, oacteria, viruses, insects and nematodes and cause serious diseases. In this laboratory manual an attempt is made to acquaint the students with various competitor moulds, fungal diseases, bacterial diseases, viral diseases, insect pests and nematode diseases of mushrooms and their control.

Due to their short shelf life, preservation of mushrooms is very much important. Hence, various preservation techniques like canning, steeping preservation, dehydration, freezing and pickling are included in this block.

The students can get additional information on this aspect from the second course viz, Cultivation of Mushrooms.

UNIT - 6 : WEED MOULDS, PESTS AND DISEASES

6.1. AIM

In this unit you will study the competitor moulds, various diseases of mushrooms, causal organisms, symptoms caused and their control measures.

6.2. OBJECTIVES

After completing this unit, you will be able to :

- list out the competitor moulds, fungal, bacterial and viral diseases and insect pests of mushrooms,
- describe the structural details of competitor moulds and symptoms of various diseases and
- identify the causal organisms and explain their control measures.

6.3. INTRODUCTION

Mushrooms are attacked by many fungi, bacteria, viruses, insects and nematodes which cause serious diseases. Mushrooms are grown on a substrate, which is specially prepared for it. Certain saprophytic weed moulds grow on the substrate and compete with the mushroom mycelium for food, water and nutrients. Such moulds are known as competitor moulds. Some of the competitor moulds, common diseases of mushrooms and their control are given below.

6.4. COMPETITOR MOULDS AND FUNGAL DISEASES

The mushrooms are usually attacked by fungi. Crop loss occurs in great amounts due to the fungal diseases.

6.4.1. Competitor moulds

Common competitor moulds that are seen during the cultivation of mushrooms are green moulds (Species of *Aspergillus, Penicillium* and *Trichoderma*), black moulds (Species of *Mucor* and *Rhizopus*), ink caps (*Coprinus* spp.), olive green moulds (*Chaetomium* spp.), *Fusarium* spp., *Alternaria alternata, Cladosporium* spp., *Verticillium* spp., *Sordaria* spp., *Arthrobotrys* spp. etc.

Aspergillus spp.

1. Vegetative mycelium consists of white, branched hyphae.
2. Conidiophores are erect or sub erect, hyaline, become dark brown with age.

3. Conidia vary greatly in size, shape and colour (dark green in *A. fumigatus*, yellow green to deep olive brown in *A. flavus*, black in *A. niger* etc.).

Penicillium spp.

1. Vegetative mycelium consists of creeping, branched, septate hyphae. Conidiophores are unbranched, erect with a verticil (whorl) of primary branches at the apex.
2. The primary verticil bears secondary and even tertiary branches sometimes or with a verticil of phialides (conidia bearing cells).
3. Conidia are borne in chains and form a brush-like head.
4. Conidia are globose, ovate or elliptical, rough or smooth, yellowish green to bluish grey green (*P. chrysogenum*) or meadow green with a fairy green growing margin (*P. notatum*) in colour.

Trichoderma spp.

1. Vegetative hyphae are septate, creeping and form a flat and firm turf. *Trichoderma viride* is the most common species.
2. The conidiphores of the species are not distinct from vegetative hyphae. They are indefinite in length and di or trichotomously branched.
3. Conidia occur in groups, smooth, thick walled, globose or ovoid and green in colour.

Mucor spp.

1. Mycelium is well developed, hyphae are coenocytic, producing septa only at the base of the reproductive organs like sporangia or gametangia.
2. Sporangiophores arise singly and form a thick turf.
3. Columella is globose or ovoid and spores are smooth and elliptic.
4. *Mucor racemosus* and *M. varians* are the two commonly occurring species.

Coprinus spp.

1. Species of *Coprinus* usually appear on the substrate during spawn run or after casing.
2. *Coprinus* produces white to cream coloured mushrooms at first, later turning to bluish black slimy mass, due to autodigestion.
3. The pileus is 1.5-4cm wide, oval or elongated at first, later becoming conical.
4. The white surface is covered with whitish scales and they disappear after sometime.
5. As the cap expands, the margin splits and finally turns into umbrella shape at the time of dissolution.
6. Gills are white at first, upto 1mm wide, 6-10cm long, turn black at the time of liquefying.
7. Stipe is white, shining, fragile, hollow, bulbous at the base, tapering upwards and measures about 2-4" long and 2-3 mm thick.
8. Spores black in colour, elliptic and 8-12 x 3-5μ in size

Chaetomium spp.

1. Some vegetative hyphae form a mat in the substratum and some pale brown hyphae are aerial which later on become dark and abundant.
2. Perithecia may be olive brown, black, or dark grey in colour
3. They are oval, subglobose or barrel shaped with stiff or coiled branched or unbranched hairs. Asci cylindrical or club shaped.
4. Ascospores are lemon shaped.

Fusarium spp.

1. The mycelium is white to brownish
2. The conidiophores are solitary or aggregated into groups.
3. Conidia are hyaline and principally of two types. They are microconidia and macroconidia.
4. Macroconidia are several celled, slightly curved, boat shaped and are produced in slimy masses.
5. Microconidia non-septate or one septate, ovoid, smaller produced in slimy spore balls or chains.

Alternaria alternata

1. Colonies of *Alternaria alternata* are olive black with immersed mycelium.
2. Conidiophores are branched or unbranched, short and septate and brown in colour.
3. Conidia occur in chains and they are muriform with 3-5 septa.
4. Conidia are obclavate, ovoid with a short beak.

Cladosporium spp.

1. Species of *Cladosporium* consist of effuse colonies with olive or buff dark brown colour.
2. Mycelium is submerged.
3. Conidiophores are straight or flexuous and usually unbranched.
4. Conidia catenate, ellipsoidal or cylindrical with a distinct protuberant scar at each end.

Verticillium spp.

1. Mycelium is well developed with septate and branched hyphae.
2. Conidiophores which bear small, hyaline conidia have many branches and are characteristically arranged in whorls.
3. Conidia appear in clusters and are surrounded by a sticky mucilage.

Sordaria spp.

1. *Sordaria fimicola* is the mostly studied and widely occurring species.
2. The fungus does not produce conidia and reproduces only by ascospores. Asci develop within perithecium.
3. The ascospores are dark brown in colour and are surrounded by a gelatinous sheath.

Arthrobotrys spp.

1. The species *Arthrobotrys pleuroti* is commonly seen in the beds of oyster mushrooms in India.
2. Fluffy growth is seen on the substrate and fruit bodies.
3. The infected tissues become water-logged, yellow and rot.

Control

1. Proper sterilisation of the substrate and maintenance of strict hygiene are essential.
2. Avoid excessive watering.
3. Spray bavistin or benomyl (100ppm).

6.4.2. Fungal Diseases on White Button Mushroom

(i) Cobweb disease

Causal organism : *Cladobotryum dendroides*

Symptoms

1) Greyish white mycelium turning to red on maturity.
2) The mycelium grows on the casing soil and covers the fruit bodies also.
3) Conidiophores are usually erect, simple or branched.
4) Conidia are single, pointed at the base, 2-3 septate, slightly constricted at the septa.

Control measures

1) Spray dithiocarbamates.
2) Apply 70% calcium hypochlorite.
3) Reduce the humidity of cropping room.

(ii) Wet bubble

Causal organism : *Mycogone perniciosa*

Symptoms

1) Mushroom tissue turns into distorted mass. Stipes swollen; whitish growth on fruit bodies.
2) Brown to amber coloured liquid ooze out due to the decay of pinheads.

Control measures

1) Spray Dithiocarbamates.
2) Maintain hygiene.

(iii) Dry bubble

Causal organism : *Verticillium fungicola*

Symptoms

1) Light brown spots appear superficially on the cap; small and tilted cap.
2) Splitting of stipe occurs.

Control measures

1) Spray benomyl (0.05%) or dithiocarbamates.
2) Strict hygiene.

6.4.3. Fungal Diseases on Oyster Mushroom

(i) Disease caused by Cladobotryum spp.

Cladobotryum verticillatum, C. apiculatum and *C. variospermum* attack oyster mushrooms.

Symptoms

1) White cottony growth on the substrate; Brown irregular sunken spots; fluffy growth on fruit bodies; decay of fruit bodies emitting foul smell are the important symptoms.

Control measures

1) Spray bavistin (500 ppm).

(ii) Diseases caused by Gliocladium spp.

Gliocladium deliquescence and *G. virens* attack oyster mushrooms and show the following symptoms.

Symptoms

1) Oyster mushroom fruit bodies are covered by the mycelium; green spots appear; young pin heads turn brown, soft and decay.
2) Mature ones show spots surrounded by an yellow halo.

Control measures

1) Spray benomyl or bavistin (100 ppm).

(iii) Disease caused by Arthrobotrys pleuroti

Symptoms

1) Fluffy growth observed on the substrate and fruit bodies.
2) The tissue turns yellow, rot and become water logged.

Control measures

1) Spray bavistin (50 ppm).

(iv) Disease caused by Sibirina fungicola

Symptoms

1) Stipe, gills and primordia show powdery white growth.
2) Brownish discolouration and soft rot on young pin heads mature ones turn fragile.

Control measures

1) Spray benomyl.
2) Maintain proper relative humidity and aeration.

6.4.4. Fungal Diseases on Paddy Straw Mushroom

Button rot diseases

Causal organism : *Sclerotium* spp.

Symptoms : The young pinheads show rotting.

Control measures : Spray bavistin (50 ppm).

6.5. BACTERIAL DISEASES

Common bacterial diseases on various mushrooms that are prevalent in India are as follows.

1. ***Bacterial blotch or brown blotch or bacterial spot***

Causal organism : The disease is caused by *Pseudomonas flourescence* in white button mushroom whereas in oyster mushroom it is caused by *P. tolaasii.*

Symptoms

1) The pathogens produce yellow to chocolate brown lesions on mushroom tissues.
2) Superficial discolouration upto 2 to 3mm. deep, water soaked areal blotches appear; spots appear at or near the edge of mushroom cap; severe infection causes splits in the caps.
3) Mushrooms become distorted.

Control measures

1) Maintain proper relative humidity (85%), temperature (20°C) and air movement.
2) Spray 0.015% chlorine solution
3) Proper pasteurization of casing soil.

(ii) 'Yellowing' of Oyster mushroom or Yellow blotch.

Causal organism : 'Yellowing' in oyster mushroom is caused by *Pseudomonas alcaligens* and *P. agarici.*

Symptoms

1) Mushrooms turn yellow.
2) Slimy growth on the fruit bodies appear.

Control measures

1) Spray 0.015% chlorine solution.
2) Maintain strict hygiene.

(iii) ***Bacterial rot of Paddy Straw Mushroom***

Causal organism : *Pseudomonas* spp.

Symptoms : Rotting occurs in mushroom tissue.

Control measures : Spray 0.015% chlorine solution.

6.6. VIRAL DISEASES

Viruses or virus like particles (VLP) have been observed in different mushrooms.

(i) *Agaricus bisporus (White button mushroom)* is affected by spherical (25nm-50nm) bacilliform (18 x 50nm), club-shaped (60 x 120nm) particles of viruses causing X-disease or die-back disease.

(ii) *Pleurotus* spp. (oyster mushroom) is affected by spherical (26±2nm) or flexuous rods (40-60nm long).

(iii) *Volvariella volvacea* (paddy straw mushroom) is infected by spherical particles of 20nm size.

Symptoms

1) Mushrooms appear in clusters; delayed appearance of pin heads; half-white colour of caps, early maturity; watery stipes; abnormal veils and hard gills.
2) Specific musty (stinking) smell in diseased mushrooms

Control measures

1) Maintain strict hygiene.
2) As a precautionary measure steam the substrate for 12 hrs. at a temperature of 70°C.
3) Disinfect little holes in the floor, racks, walls, doors with formaldehyde (0.2%).
4) When the disease is already present, pick the mushrooms when still closed.
5) Use separate instruments for each room.
6) Allow few visitors into the diseased rooms
7) Eliminate pests by spraying pesticides.

6.7. INSECT PESTS AND NEMATODES

A pest may be defined as a creature which is undesirable in a particular situation, for reasons of health and hygiene, comfort and acceptability.

Some insects and nematodes that cause damage to mushroom beds are given below. The nematodes and insect pests on the basis of occurrence are classified into two categories.

1. Major pests 2. Minor pests

6.7.1. Major pests

Mushroom flies, mites and nematodes (eelworms) are grouped under major pests.

(i) *Mushroom flies*

Sciarids, Phorids and Cecids are the three types of flies that cause damage to a considerable extent to mushroom industry, of which, sciarids cause a major loss to mushroom beds.

Sciarids

1) These are small (3-4mm) gnat like flies with long antennae.
2) They usually grow on rotting vegetables, wild fungi, leaf moulds and migrate to mushroom houses.
3) They produce white, legless larvae (1-8mm. long) with a shiny black head bearing large, powerful chewing mouth parts.
4) Larvae attack the pin heads and buttons; buttons become brown and leathery; they make tunnels in the stipe.
5) The adults serve as vectors of *Verticillium fungicola* and bacteria.

Phorid flies

1) Phorid flies are small (2-3mm) hump-backed with inconspicuous antennae and brownish black in colour. They are stouter than sciarid flies.
2) Larvae are creamy-white, legless maggots with a pointed head.
3) Larvae feed on mushroom mycelium.
4) The stipe and cap are tunnelled by many larvae and eventually leads to bacterial decay.
5) The adults spread the fungal pathogen, *Verticillium fungicola* and active near the lights causing nuisance to mushroom pickers.

Cecid flies

1) Adult cecid flies are very small and never seen by a naked eye.
2) The larvae are small, legless maggots.

3) White or orange in colour with two 'eye spots' on the head end, that give 'x' appearance.

4) The larvae feed on the mycelium outside the stipes or at the junction of stipe and gills.

5) Secondary infection by bacteria occurs resulting in brown, discoloured stripes on gills, stipes and formation of tiny pustules with black liquid.

Control of Mushroom flies

1) Maintain strict hygiene.
2) Spray the trays and racks with 2% sodium pentachlorophenate solution.
3) At the time of spawning add diazonin (0.003%) to the compost.
4) Use sticky traps to control flies in spawn running room.
5) Throw the spent compost in the pits far away from the farm.

(ii) Mites

1. Mites are small, four legged creatures which are red, pale, brown, pale orange to dark red in colour.
2. All mites may not be harmful to mushroom bed, but a few make small pits in the stipes and caps leading to secondary infection by bacteria.

Control measures

1) Strict hygiene.
2) Proper pasteurization of compost and casing soil.
3) Spray 0.01-0.02% Kelthane (Dicofol) in the rooms.
4) Bury the spent compost in deep pits.

(iii) Nematodes (Eel worms)

1. Nematodes or eelworms are thread like, minute (upto 1mm. long) organisms that swim in the surface films of water in compost and casing soil.
2. Nematodes that encounter in mushroom beds are of three types.
3. They are myceliophagus, saprophagous and predatory nematodes.
4. Early infection causes complete crop failure.
5. Large number of eelworms adhere together in whitish clumps.
6. They puncture the mycelium with the help of a needle like mouthpart (stylet) and suck the mycelial contents.

Control measures

1) Strict hygiene.
2) Disinfect water.
3) Keep mushrooms house fly proof.
4) Spray insecticides.

6.7.2. Minor pests

1. Spring tails, millipedes, beetles, slugs, noctuid moths come under minor pests and these cause less damage to mushroom beds.

2. Spring tails are small insects (1mm long) with stout antennae, silvery, reddish brown or dark grey in colour. They are active in the dark and move by springing several centimetres with the help of their tails and present a silvery appearance. They feed on the mycelium as well as fruit bodies.

3. Millipedes, slugs and noctuid moths feed on caps, stipes of mushrooms and make cavities in the fruit bodies.

4. Beetles have been reported to damage the crops of *Pleurotus* spp. in India. They feed upon tissues of stipes, gills, pileus giving a fringed look.

Control measures

1) Strict hygiene; spraying malathion or nuvan (0.05%).

2) Grow mushrooms in shelves away from the floor of the room.

6.8. EXERCISE

You are supplied with some diseased mushrooms. Identify the diseases, write the causal organisms, symptoms and control of the diseases.

6.9. SELF ASSESSMENT QUESTIONS

To check your progress on your own, answer the following questions in the space given below without referring the text above.

1. Write about the 'dry bubble' disease.

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2. Name the causal organisms of cobweb and wet bubble diseases.

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3. Write about any two viral diseases of mushrooms.

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4. Write about any two bacterial diseases of mushrooms and their control.

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5. How do you control mites of mushrooms?

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Ms. K. Prasunamma

UNIT - 7 : PRESERVATION OF MUSHROOMS

7.1. AIM

In this unit you will study the preservation and packgaing methods of mushrooms.

7.2. OBJECTIVES

After studying this unit, you will be able to :

- list out various methods of processing to preserve mushrooms,
- describe the method of processing suitable for different varieties of mushrooms,
- explain the role of preservation techniques in extending shelf life of mushrooms in an alternative form,
- describe the use of the processed mushroom and
- list out and describe different packing methods of fresh and dried mushrooms.

7.3. INTRODUCTION

Mushroom production has been increasing rapidly in the country in the recent years. In 1970 it was estimated to be only 100 tonnes. It increased to 400 tonnes in 1975; 1000 tonnes in 1986; 7000 tonnes in 1990; 12000 tonnes in 1992; approximately 25,000 tonnes in 1993 and over 30,000 tonnes in 1994. There is a huge gap in demand and supply possibly because of unorganised production and lack of proper marketing channels for this highly perishable product.

7.4. METHODS OF PRESERVATION

Fresh mushrooms can not be retained at room temperature for more than 24 hours as they lack the protective covering and hence are delicate with a tendency to deteriorate at higher temperatures and in long term storage. Because of the biochemical changes occurring within the tissue, the high moisture content (90% moisture) and higher respiratory rate deterioration occurs very fast. This makes them slimy in texture with off flavour. The product ultimately becomes unacceptable in the market as it looses the texture and the flavour.

To avoid wastage by spoilage, mushrooms can be conserved by preservation; thus extend their shelf life to reach a distant market and widen the scope of marketing. Mushrooms can be processed as canned, dried and frozen mushrooms. They can also be preserved by preparing ketchup, soup powders and pickles.

The most popularly grown cultivated mushrooms which can be preserved by the above said methods are :

1. *Agaricus bisporus* - White button mushroom
2. *Pleurotus florida* - Oyster mushroom
3. *Volvariella volvacea* - Paddy straw mushroom

7.4.1. Canning

Button mushrooms are more suitable for this purpose. The mushrooms, after cleaning and grading are blanched and preserved in brine (salt solution).

Materials required : Mushrooms, citric acid, common salt, ascorbic acid, water.

Procedure :

1. Take small button mushrooms with 0.5 to 1.0cm. stalk and cut the stalk close to the button with stainless steel knife to avoid discolouration of mushroom due to traces of iron. Wash them thoroughly in running water to remove soil sticking to fruit bodies. To bring extra whiteness to mushrooms 0.1% citric acid and 0.025% potassium metabisulphite can be added to the water used for cleaning.

2. Then blanch them. Blanching is nothing but the treatment of mushrooms (or fruits & vegetables) with boiling water or steam for short periods, followed by immediate cooling.

   Blanching is done due to the following reasons.

   a) to inactivate the polyphenol oxidase enzyme,
   b) to remove gases from the inner tissues,
   c) to reduce the bacterial count,
   d) to improve texture and
   e) to improve flavour and quality

   Blanch the cleaned mushrooms for 2-3 minutes in hot boiling water followed by prompt cooling in cold water containing 0.1% to 0.2% citric acid (1g or 2g in 1000ml water) and 1% sodium chloride (common salt). Blanching causes 25-30% loss in weight which is unavoidable.

3. Fill the blanched buttons in plain cans. The cans should be washed with a jet of water or sterilised by steam before use. Fill the blanched buttons at the rate of 200g. in one lb. jam size can having about 250ml brine gives at least 4-5% drain weight.

   Brine : Prepare brine solution by adding 1-2% common salt and 0.1% citric acid to water and heat till the brine solution reaches the boiling stage.

   For filling a can of 1 lb. jam size with 200g. blanched mushrooms, take 5g. common salt and 0.25g. citric acid in 250ml. of water and boil the solution.

4. Pour this hot boiling brine (250ml) to fill the can up to brim leaving 1.5cm head-space. Brine with 2% common salt, 2% sugar and 0.3% citric acid has also been recommended for this purpose. Tomato juice as a new canning medium has also been tried successfully at the Indian Institute of Horticultural Research, Bangalore.

5. **Exhausting** : After filling the can, exhaust the can by heating it till the centre of the can attains 80°C. This helps in removing the extra fill and also the air trapped in the mushroom tissue and liquid media.

6. **Sealing** : After exhausting, seal the cans hermetically and process in an autoclave for about 25-30 min. at 10 lbs/sq. inch of steam pressure or at 15 lbs/per sq. inch for 15-20 min.

7. After processing, cool it promptly to prevent over-cooking and also to prevent stack burning. Cooling can be done by placing the cans in galvanized iron tank fitted with water connection for constant flow of water. Now store the cans in a cool and dry place.

End Product : After the entire procedure, canned button mushroom in brine is obtained which can be stored for 1 to 2 years.

Use of the product : Use it in any preparation just like fresh mushrooms after necessary washing and taking into consideration the salts in it.

7.4.2. Steeping Preservation

Generally button mushrooms are preserved by steeping, but even oyster mushrooms can be tried at higher salt concentration (20-22%). The mushrooms after cleaning, washing and blanching are steeped in salt solution.

Materials required

Mushrooms, citric acid, common salt, sulphurdioxide, water.

Procedure

1. Clean the mushrooms and grade them. After washing, blanch it for 5 minutes.
2. **Blanching** - Take 1g of citric acid and mix in 1000ml water and boil the mushrooms for 5 minutes in this solution.
3. After blanching, wash the mushrooms in cool plain water.
4. Then put the blanched mushrooms in 5% common salt solution (50g salt in 1000ml of water) containing 0.5g citric acid and 0.6g of potassium metabisulphite.
5. Now fill them in cans of suitable size and close tightly.

End Product : Mushrooms steeped in salt solution in the can are finally obtained. This procedure/product has a limited utility as it can be preserved only for a short period of about 2 months. However, it is very difficult to get customers for such a produce.

Use of the product : After thorough washing, the canned mushrooms can be used in many culinary preparations just like fresh mushrooms.

7.4.3. Dehydration

Dehydration of mushrooms can be done by the following methods.

a) Sun drying and

b) Mechanical drying in ovens.

A. Sun Drying

It is the oldest method of preserving the mushrooms and is still popular due to its practicability. Generally oyster, paddy straw, shiitake and milky white mushrooms are dried in this way during the days with high temperature (above 25°C), low humidity (less than 50% RH) and high wind velocity.

Procedure

1) Take the fruit bodies of any of the above mentioned mushrooms and bead them in a thin thread and hang in the air under direct sun light for efficient dehydration.

2) Otherwise spread the mushrooms on a clean cloth and dry them under sun light. This product after complete drying is reduced to about 10-12% of its original weight. It is better to oven dry them at 55-60°C for 4-6 hours before packing in air-tight bags. This method, gives a product with dark colour. However, to retain the colour and to reduce the bacterial count, blanching can be done.

Materials required

For blanching and after treatment : Potassium metabisulphite, sodium chloride, mushrooms, water.

Procedure

1) Harvest mushrooms at full mature stage with full stalk. Early break (i.e., first flush) gives better product due to low tyrosine content. Keep it as whole or dice it.

2) Wash the mushrooms thoroughly and blanch for 3-5 minutes in live steam or boiling water.

3) After blanching, dip the mushrooms for 5 minutes in 1000ml of water containing 0.6g potassium meta-bi-sulphite and 0.4g sodium chloride.

4) Now dry in sunlight. Weight of the dried mushrooms will become to 1/8 to 1/12th of their original weight depending on the species used.

5) The moisture content in the dried product should not be more than 5-6%.

6) Pack the dried mushrooms in hermetically sealed air tight tins for quality retention and then store at a cool dry place.

End Product : Dried whole/diced mushrooms with 5-6% moisture are obtained and can be stored upto 6 months.

Use of the product : Put the dry mushrooms in luke warm water for 10-20 minutes for rehydration and use it like fresh ones; or can be used after powdering for preparation of soup mixes or to blend with some other dishes.

B. Mechanical Drying

1) When the atmospheric temperatures are low or the humidity is high, mushrooms can be dried in ovens.

2) Conventional ovens are not suitable as they result in dark brown coloured product with tough texture.

3) When the fruit bodies are large, it is not suitable at all.

4) In the conventional oven, due to little air movement, the evaporated water condenses on fruit bodies making them mucilaginous leading to deterioration.

5) As mushrooms consist of 85-90% of moisture, such large moisture content after evaporation damages the equipment. Hence to get a better product, dry the mushrooms in an oven with forced air circulation.

Procedure

1) Take the freshly harvested mushrooms, clean and grade them.

2) After this, either put them directly in the oven for drying at 55°C or pretreat them with 0.06% potassium meta-bi-sulphite solution and 0.04% sodium chloride solution as done for sun drying.

3) The drying temperature of 55°C in special type of ovens gives better product regarding texture, colour and rehydration. Do not dry at higher temperatures, as drying is faster and the end product would be darker.

4) Commercial dehydration vary considerably from home made drier to the large scale units with automatic controls.

Other large commercial dehydrators for drying vegetables and fruits can also be used with minor process variations.

Advantages of mechanical drying

1. Faster and uniform drying is possible.
2. Drying is possible even in rainy season or when temperature is very low or in high humid conditions.
3. End product has the desired qualities like good texture, colour and rehydration capacity.
4. The dried product is hygienic when compared to sun dried product.

Packing : Pack the dried product in air tight containers for prolonged storage.

End product : A hygienically dried product with better colour and texture is obtained and can be stored upto 6-8 months in a cool dry place.

Use of the product : It can be powdered and used for soup mix or to blend with other dishes. It can also be rehydrated by soaking in luke warm water for 10-20 minutes.

7.4.4. Freezing

Button mushroom is used at early break stage for freezing. Freezing is done by pre-cooling followed by washing, blanching and freezing for storing at -20°C.

Materials required

Button mushrooms, sodium chloride, citric acid, Freon-12.

Procedure

1. Clean the mushrooms and pre-cool at 2 to 4°C and then wash with water containing 0.01% sodium chloride followed by 2 to 3 minutes blanching.
2. Blanch them in water containing 0.1% citric acid (1g in 1 litre water).
3. Now freeze them as such or after packing in pouches. For quick freezing use Freon-12.
4. **Storage** : These frozen mushrooms should be stored at -20°C till they are consumed.

End Product : Frozen mushrooms have great demand in the world market since they could be stored upto one year in storage chamber of the deep-freezer without any loss of flavour.

7.4.5. Pickling

Pickling is an important, simple, suitable and effective long term preservation method and also economically viable one. The process of preservation of food materials in salt solution or in acid solution or in vinegar with or without spices, sugar, permissible preservatives, edible oils etc. is called pickling. The shelf life of pickles is about one year after which it deteriorates due to rancidity of oil, due to loss of colour, flavour and spicyness.

Different kinds of pickles are made, but basically the following 3 types are important.

1. Prepared in oil
2. Prepared in lime juice
3. Prepared in vinegar

It is essential to standardise the quantities of spices used when prepared on large scale. Main problem in pickling is spoilage due to activity of micro-organisms (yeast and moulds) which may be present in the product or in the atmosphere. Hence it is essential to use preservatives.

Preservatives : Preservatives are chemical agents which retard, hinder or suppress the undesirable changes brought about in the food. They are either natural preservatives or chemical preservatives.

Salt, acetic acid (vinegar), lemon juice and oil are the important ingredients used in pickles which act as preservatives. The role of preservatives is given below.

Natural Preservatives

1. **Salt** : 10-20% salt is generally found to be ideal, because at this high salt concentration moulds or bacteria do not multiply.
2. **Vinegar** : This is also a preservative and is best at 4% concentration. It should not be less than 2%. In order to avoid dilution of vinegar due to the water coming out from the tissues, the mushrooms are kept in strong vinegar of about 10% acidity

for several days. This helps in expelling the gases present in the intercellular spaces of the mushroom tissue and also prevent subsequent dilution of the vinegar in the pickle.

3. **Sugar** : A concentration of 70% or more of sugar prevents spoilage of food material.

Chemical preservatives

For longer shelf life of the preparations, potassium meta-bi-sulphite (0.035%) or sodium benzoate at the rate of 0.075% while a maximum of 0.1% (1g/kg. i.e., 1000ppm) is added for pickles and sauces.

Mushroom Pickle

Ingredients

Fresh mushrooms	1 kg
Cumin seeds	15 g
Fenugreek seeds	15 g
Coriander seeds	15 g
Mustard powder	15 g
Turmeric powder	20 g
Chilli powder	20 g
Ready made pickle masala salt	100 g
Refined oil	250 g

Procedure

Clean the mushrooms and chop them. Then simmer it for 10 minutes in 150g oil in a steel pan and keep them aside. Roast cumin, fenugreek and coriander seeds and powder them.

Then mix it with mustard, turmeric and chilli powders. Put these spices and fry lightly in the same oil used for cooking mushrooms. Now add the cooked mushrooms to this and mix thoroughly. Add vinegar and boil it for five minutes and then cool.

Heat the remaining oil and add to the mixture after cooling. Pack the pickle in air tight glass bottles or polythene bags.

End product : Mushroom pickle using oil and vinegar can be stored for more than six months.

Mushroom pickle in Vinegar

Ingredients

Fresh mushrooms	50 g
Salt	30 g
Mace	2 blades
Onion (ground)	4 g

Onion chopped	15 g
Pepper white	2 g
Nutmeg grated	1 g
Vinegar	500 ml

Procedure

1. Immerse mushrooms in water for a few minutes and then drain.
2. Now cover them with vinegar containing spices.
3. Boil this mixture slowly for 10 minutes and then pour the contents in dry jars.
4. Leave for 1 day and then seal them air-tight.

End product : This pickle in vinegar is for short term storage.

Mushroom Ketchup

Ingredients

Mushrooms	500 g
Salt	12 g
Ground spice mix (Cardamom, pepper, dry ginger, cloves, mace & cinnamon) (2g each)	12 g
Vinegar	500 ml

Procedure

1. After cleaning, keep the mushrooms in salt for 12 hours and then blend them to a fine slurry.
2. Now add vinegar and spice mix and boil to get ketchup consistency.
3. Add small amount of monosodium glutamate to enhance meat like flavour.
4. Fill while hot and sterilize the bottles for 30 minutes in boiling water. After cooling store them in cool dry place. Ketchup with tomato paste can also be prepared.

End product : Tasty, nutritive ketchup, which can be stored upto 8 months is ready.

Food Product Order

Food products which are exported are required to pass FPO specifications (Food Products Order 1955). For domestic consumption, FPO specification is not required unless demanded by the consumers. However, it is advisable to market processed food products having FPO license.

FPO 1955 is applicable to edible products of animal, poultry and vegetable or agricultural origin. Mushrooms as such are not covered under FPO. However, FPO specification laid down for mushroom pickles are applicable, which are as follows.

A. Pickles in Oil

The substances that may be added are spices, salt, oil, sugar, jaggery, onions, garlic, acetic acid, turmeric, condiments and permitted preservatives. The pickles shall be of pleasant taste and flavour and be free from added copper, alum and mineral acid.

B. Pickles in vinegar

The fluid portion of the pickles which shall be vinegar, shall constitute not more than 1/3rd of the total content and shall not contain any other ingredient excepting spices, salt and sugar. The pickle will be free from added copper, mineral acids, alum, harmful colours and will not show any signs of fermentation. The product will be reasonably free from sediment. Minimum percentage of acidity of fluid portion as acetic acid is 2%.

C. Pickles in citrus juice or in brine

Substances that may be added are spices, salt, sugar, jaggery, onions, garlic, sodium benzoate and soluble calcium salt in 12% salt solution or citrus juice. Pickles shall be free from added copper, alum, mineral acids.

For all the above products the kind of mushrooms shall be declared on the label. When more than one type of mushroom is used, the product shall be labelled as mixed pickle.

7.5. EXERCISE

Procure fresh mushrooms and dry them by any of the methods you have studied. Describe the procedure of the same.

7.6. SELF ASSESSMENT QUESTIONS

To check your progress answer the following questions without referring to the text above.

1. Why do you process or preserve mushrooms?

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2. List out various preservation methods?

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3. What is blanching and why is it done?

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4. What are the chemicals used and the concentration of each one of them in the preparation of brine for steeping preservation?

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5. What is the treatment given to mushrooms before drying to get a good coloured dried product?

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6. What is the approximate quantity of fresh mushrooms required for obtaining 5kgs. of dried product?

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7. Conventional ovens are not suitable for drying mushrooms. Explain.

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8. What are the advantages of mechanical drying?

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9. What is freeze drying?

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