Practical Manual

MUSHROOM PRODUCTION TECHNOLOGY

Course Code: SEC-111 2(0+2)

For Undergraduate Agricultural Students

2024

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Syllabus: Mushroom & its importance, mushroom culture preparation, culture media preparation, isolation methods, inoculation methods, what is spawn: master and commercial spawn preparation, production technology of mushroom: material requirement, material used, substrate preparation, compost preparation, spawning, spawn running, crop room management, cultivation process of oyster, white button, milky etc., mushroom cultivation, harvesting, packaging etc., Mushroom produces and its by-product: Value additive food preparation, vermicompost preparation, marketing, Management of insects & diseases through sanitation, fumigation, chemical etc., crop room management, project report preparation. One week exposure visit to mushroom production unit, research laboratory, mushroom farms etc.

Name of Student .....

Roll No. .....

Batch .....

Session .....

Semester .....

Course Name : .....

Course No. : .....

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Date: Course Teacher

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Practical No. 1

Objective: To get familiar with general equipment and glassware used in Mushroom laboratory.

The students in batches will visit the laboratory to acquaint themselves with different appliances, tools, glass-wares, and other miscellaneous items, which they will be using in various exercises and experiments to be conducted.

Identify the laboratory equipment available in the Laboratory:

(a) Laboratory appliances/tools:

1.		10.	
2.		11.	
3.		12.	
4.		13.	
5.		14.	
6.		15.	
7.		16.	
8.		17.	
9.		18.	

(b) Glassware:

1.		10.	
2.		11.	
3.		12.	
4.		13.	
5.		14.	
6.		15.	
7.		16.	
8.		17.	
9.		18.	

2. Label the following laboratory instrument/equipments and state its principle and functions.

Auto Clave: ……..

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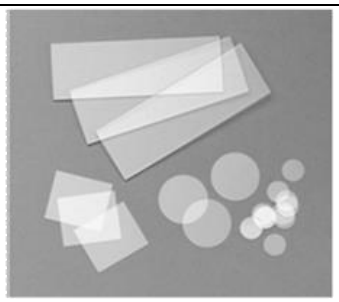
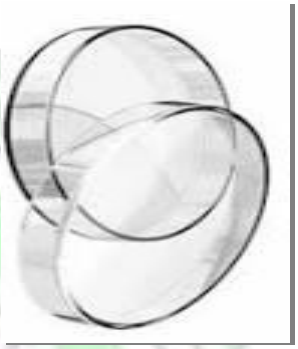
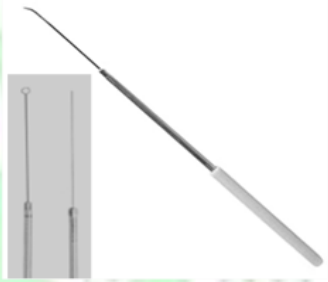
Laminar Air Flow: …… …… …… …… …… …… …… ……	
BOD Incubator: …… …… …… …… …… …… …… ……	
Hot Air Oven: …… …… …… …… …… …… …… ……	
Spirit Lamp: …… …… …… …… …… …… …… ……	
Dissecting Needle:…… …… ……	

Slide and Cover slip: …… …… …… …… …… …… ……	
Petri plate/ Petri dish:…… …… …… …… …… …… ……	
Inoculating Needle: …… …… …… …… …… …… ……	
Watch Glass: …… …… …… …… …… …… ……	
Haemocytometer/Hemocytometer: …… …… …… …… …… …… ……	

Practical No. 2

Objective: Preparation of Potato Dextrose Agar (PDA) and Broth medium

Prepare one litre of Potato dextrose Agar and broth medium. Describe the procedure and quantity of the components.

Materials required: ……

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Procedure……

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Practical No. 3

Objective: Isolation of Mushroom fungi

Materials required: ……

Procedure ……

Practical No. 4

Objective: Purification of Mushroom fungi

Materials required: ……………………………………………………………………………………………………

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Procedure………

Practical No. 5

Objective: General Morphological study of Mushroom fungi - I

Slide preparation of Mushroom and Microscopic observation

Materials required: ……

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Procedure……

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Observation

Practical No. 6

Objective: General Morphological study of Mushroom fungi - II

Slide preparation of Mushroom and Microscopic observation

Materials required: ……

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Procedure……

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Observation

Practical No. 7

Objective: General Morphological study of Button Mushroom

Slide preparation of Button Mushroom and Microscopic observation

Materials required: ……

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Procedure……

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Observation

Practical No. 8

Objective: Mother spawn preparation

Inoculation on grain and mass multiplication of mushroom

Materials required: ……

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Procedure……

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Observation

Practical No. 9

Objective: Commercial spawn production-I

Multiplication of mushroom

Materials required: ……

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Procedure……

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Observation

Practical No. 10

Objective: Commercial spawn production-II

Multiplication of mushroom

Materials required: ……

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Procedure……

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Observation

Practical No. 11

Objective: Compost preparation-I

Substrate preparation for Mushroom Cultivation

Materials required: ……

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Procedure……

Practical No. 12

Objective: Compost preparation-II

Substrate preparation for Mushroom Cultivation

Materials required: ……

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Procedure……

Practical No. 13

Objective: Spawning Preparation

Mushroom Spawn Preparation

Materials required: ……

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Procedure……

Practical No. 14

Objective: Preparation of Casing Soil-I

Casing Soil Preparation

Materials required: ……………………………………………………………………………………………

Procedure……………………………………………………………………………………………

Practical No. 15

Objective: Preparation of Casing Soil-II

Casing Soil Preparation

Materials required: ……………………………………………………………………………………………

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Procedure……………………………………………………………………………………………………

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Practical No. 16

Objective: Button Mushroom Cultivation-I

Button Mushroom Production

Materials required: ……

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Procedure………

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Practical No. 17

Objective: Button Mushroom Cultivation-II

Button Mushroom Production

Materials required: ……………………………………………

Procedure……………………………………………

Practical No. 18

Objective: Oyster Mushroom Cultivation-I

Oyster Mushroom Production

Materials required: ……

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Procedure……

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Practical No. 19

Objective: Oyster Mushroom Cultivation-II

Oyster Mushroom Production

Materials required: ……

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Procedure……

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Practical No. 20

Objective: Milky Mushroom Cultivation-I

Milky Mushroom Production

Materials required: ……………………………………………………………………………………………

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Procedure………………………………………………………………………………………………

Practical No. 21

Objective: Milky Mushroom Cultivation-II

Milky Mushroom Production

Materials required: ……

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Procedure……

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Practical No. 22

Objective: Paddy Straw Mushroom Cultivation-I

Paddy Straw Mushroom Production

Materials required: ……

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Procedure……

Practical No. 23

Objective: Paddy Straw Mushroom Cultivation-II

Paddy Straw Mushroom Production

Materials required: ……

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Procedure……

Practical No. 24

Objective: Shitake Mushroom Cultivation-I

Shitake Mushroom Production

Materials required: ……

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Procedure……

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Practical No. 25

Objective: Shitake Mushroom Cultivation-II

Shitake Mushroom Production

Materials required: ……

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Procedure……

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Practical No. 26

Objective: Reishi Mushroom Cultivation-I

Reishi Mushroom Production

Materials required: ……

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Procedure……

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Practical No. 27

Objective: Reishi Mushroom Cultivation-II

Reishi Mushroom Production

Materials required: ……

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Procedure……

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Practical No. 28

Objective: Diseases of Mushroom and Their Management-I

Studies of Fungal, Bacterial and Viral Disease of Mushroom

Materials required: ……

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Procedure……

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Observation

Practical No. 29

Objective: Diseases of Mushroom and Their Management-II

Studies of Fungal, Bacterial and Viral Disease of Mushroom

Materials required: ……

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Procedure……

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Observation

Practical No. 30

Objective: Insect Management of Mushroom-I

Studies of Insect and Their management in Mushroom

Materials required: ……

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Procedure……

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Observation

Practical No. 31

Objective: Insect Management of Mushroom-II

Studies of Insect and Their management in Mushroom

Materials required: ……

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Procedure……

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Observation

Practical No. 32

Objective: Mite Management of Mushroom

Studies of Mite and Their management in Mushroom

Materials required: ……

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Procedure……

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Observation

Practical No. 33

Objective: Preservation of mushroom

Growth and multiplication of Mushroom on Media

Materials required: ……

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Procedure……

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Observation

Practical No. 34

Objective: Storage of mushroom

Transfer of Mushroom Culture at low temperature

Materials required: ……

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Procedure……

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Observation

APPENDICES

GENERAL SAFETY RULES AND PROCEDURES

- Food or drinks are prohibited in the laboratory,
- Only closed-toe shoes are to be worn in the laboratory. Sandals are not permitted,
- Keep hands and other objects away from your face, nose, eyes, ears, and mouth. Application of cosmetics in laboratory is prohibited.
- Work areas/surfaces must be disinfected before and after use,
- Laboratory coats must be worn and buttoned while in the laboratory,
- Long hair should be secured behind your head,
- Hands must be washed before leaving the laboratory,
- All unnecessary books, purses, briefcases, etc., must be kept off the countertops,
- Label all materials with your name, date, and other applicable information (e.g., media, organism, etc.),
- Dispose of wastes in their proper containers,
- When handling chemicals, note the hazard code on the bottle and take the appropriate precautions indicated,
- Report any broken equipment,
- Do not pour chemicals down the sink,
- If you are injured in the laboratory, immediately contact your course instructor,
- Follow all instructors given by your course instructor in the lab,
- Always wipe and clean the lenses of your microscope before putting it away. Use the appropriate tissue paper and cleaning solution for this purpose. And make sure to carry the microscope carefully in the correct manner.
- Do not walk about the laboratory with transfer loops, wires, needles, or pipettes containing infectious material,
- Turn off the incinerators before leaving the laboratory,
- Flame (sterilize) transfer loops, wires, or needles before and immediately after use to transfer biological material,
- Return all chemicals, reagents, cultures, and glassware to appropriate places,

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GENERAL BIOCONTROL LABORATORY EQUIPMENT

(a) Laboratory appliances/tools:

1.	Autoclave	**6.**	Hot-air oven	**11.**	Scissor	**16.**	Sprit Lamp
2.	Freeze	**7.**	Incubator	**12.**	Cork-borer	**17.**	Forceps
3.	Hot Plate	**8.**	Pan (different sizes)	**13.**	Needle, Inoculating needle	**18.**	Rotary shaker
4.	Knife / Blade	**9.**	Scalpel	**14.**	Bearing Blander	**19.**	Glass marker
5.	Inoculating needles	**10.**	Laminar flow	**15.**	Gel electrophoresis	**20.**	Centrifuge

(b) Glass-wares:

1.	Conical flask (different sizes)	**5.**	Beaker (different sizes)	**9.**	Slides
2.	Measuring cylinder (different capacity)	**6.**	Pippet (different volume)	**10.**	Watch glass
3.	Petridishes	**7.**	Culture tubes	**11.**	Dropping bottle
4.	Cover-slip	**8.**	Nematode counting dish	**12.**	Bearman funnel

(c) Miscellaneous items:

1.	Cotton	**5.**	Blotting paper	**9.**	Washing brush
2.	Aluminium foil	**6.**	Wash bottle	**10.**	Washing powder
3.	Trays	**7.**	Thread	**11.**	Wire basket
4.	Sieve of different sizes	**8.**	Rubber bands	**12.**	Mortar and pestle

MICROSCOPE

Compound Microscope: A Compound microscope consists of more than one lenses fitted one above the other at a proper distance (160mm) in a cylindrical tube. An object magnified by one lens (Objective) is further magnified by another lens (eye piece).

Parts of a Compound Microscope:

Eyepiece: It is a lens that fits into the top of body tube (drawtube). It is also called an ocular lens. It is usually marked with 6X, 10X or 15X which means it can magnify the object 6, 10 or 15 times.

Drawtube: This is a small cylindrical tube on the top of which eyepiece is fitted.

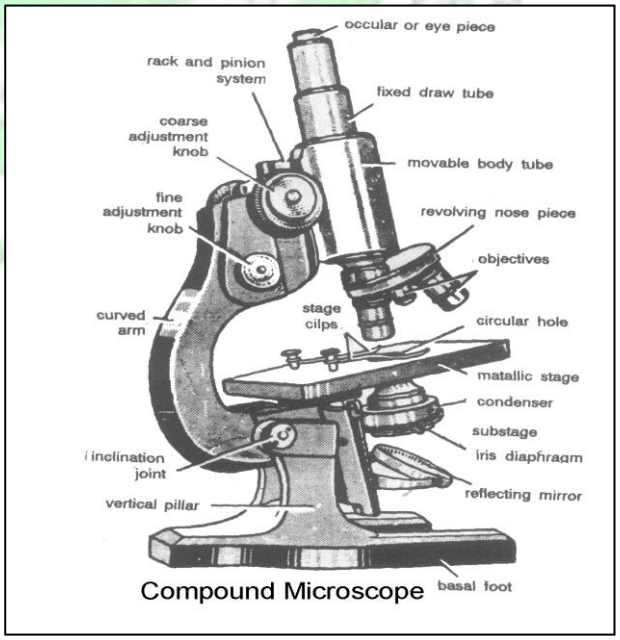
Body tube: It is a hollow cylindrical tube attached to an upper end of the arm on which it can be moved up and down with the help of a coarse adjustment knob.

Arm: It is a curved structure used for holding the microscope.

Coarse adjustment knob: This is used to locate the object by the objective.

Fine adjustment knob: Mostly fitted below the coarse adjustment knob. It is used when the object is viewed either under high power or under low power to get a sharp and distinct view.

Inclination Joint: It is the point where a microscope with a stage and body with two lenses can be bent to a



Compound Microscope

comfortable angle for smooth and strain free observation. This point lies close to the junction of stage and arm.
Nose piece: It is a disc like body fitted at lower end of the body tube. It has provisions for three lenses. It can be revolved also.
Objectives: They are the lenses of different magnifications, screwed in the nosepiece. Objective is also marked with 6X, 10X, 40X, 100X etc.
Stage: A flat rectangular or square plate with a round aperture in the centre of the stage.
Clip: Two clips on either side of the aperture on the stage for holding the slide.
Mechanical device: A slide is fitted in it, which can be moved forward, backwards, right and left to locate the object.
Diaphragm: It is a circular plate with several holes of different diameters and is attached underneath the stage. It can also be rotated so as to bring the hole of the diaphragm in front of the hole in the stage. It regulates the quantity of light towards body tube.
Condenser: It is used to regulate the intensity of light.
Pillars: These are the two vertical structures to give the support on the base of microscope.
Mirror: It is a spherical reflecting mirror, which can be adjusted to direct the light through diaphragm, stage and lenses. It's fitted in a mirror holder.
Foot or Base: It forms the base of the microscope.

GENERAL INTRODUCTION OF MUSHROOM

"Mushroom" is derived from Greek word "*Mykes"* means mushroom/fungus. A mushroom is the fleshy, spore bearing fruiting body of a fungus, typically produced above ground on soil or its food source. The fungi belong to class Basidiomycetes and some are Ascomycetes. They achrolophyll, and saprophytes and grow mostly on dead and decaying organic materials. People have harvested mushrooms from the wild for thousands of years for food and medicines. Of the estimated 1.5 million species of fungi, about 10,000 produce the fruiting bodies we call mushrooms. The Chinese first cultivated shiitake (*Lentinula edodes*) mushrooms around 1100 AD, with domestication efforts beginning centuries earlier. Roughly 300 mushroom species are edible, but only 30 have been domesticated and 10 are grown commercially. Button, oyster, and shiitake mushrooms make up about 70 per cent of the world's production. Today mushroom farming is being practiced in more than 100 countries and its production is increased at an annual rate of 6-7%. China is the leading mushroom producing country in the world. In India, Himachal Pradesh is premier state of commercial mushroom cultivators and known as a land of mushrooms. Punjab, Uttarakhand, Haryana, Uttar Pradesh, Orissa, Tamil Nadu and entire Northeastern parts are major contributors of mushroom production in the country

Types of Mushroom – They are classified into two categories *viz.*

Edible: Button (*Agaricus bisporus*), Oyster (*Pleurotus* spp), Milky (*Calocybe indica*), Paddy straw (*Ganoderma lucidum*), Reishi (*Volvariella volvacea*), Shiitake mushroom (*Lentinus edodes*), Maitake/Hen of the Woods (*Grifola frondosa*), Lion's Mane (*Hericium erinaceus*), Ears (*Auricularia* spp.), Shaggy Mane (*Coprinus comatus*)

Non-edible: Poisonous mushroom *Amanita phalloides, A. muscaria* (Mushroom poisoning is due to toxin like amanatin, Gyromitrin, muscarine, coprine)

The Directorate of Mushroom Research is located in Solan, Himachal Pradesh, popularly known as the "Mushroom City of India."

Year wise production schedule of commonly grown mushrooms –

Scientific name	Common name	Production month	Temp.
Agaricus bisporous	Button mushroom or Khumb mushroom	Mid November to mid-February	20-22^0 C
Pleurotus spp	Dhingri mushroom	Oct.- March	22-25^0 C
Calocybe indica	Milky mushroom or Dudhiya mushroom	June-August	25-30^0 C
Volvariella volvacea	Paddy straw mushroom	September to November	28-32^0 C

IMPORTANCE OF MUSHROOM

Mushrooms, also called **'white vegetables'** or '**boneless vegetarian meat'** contain ample amounts of proteins, vitamins and fibre apart from having certain medicinal properties. Mushroom contains 20-35% protein (dry weight) which is higher than those of vegetables and fruits and is of superior quality. Mushrooms are now getting significant importance due to their nutritional and medicinal value and today their cultivation is being done in about 100 countries. At present world production is estimated to be around 5 million tonnes and is ever increasing. Though 20 mushroom varieties are domesticated about half a dozen varieties viz; button, shitake, oyster, wood ear and paddy straw mushrooms contribute 99% of the total world production.

NUTRITIONAL IMPORTANCE: Mushrooms are a good source of quality proteins and contain 3-7% when fresh and 20 – 35% protein {dry weight basis} which is higher than vegetables and fruits. they have a high percentage of all 9 essential

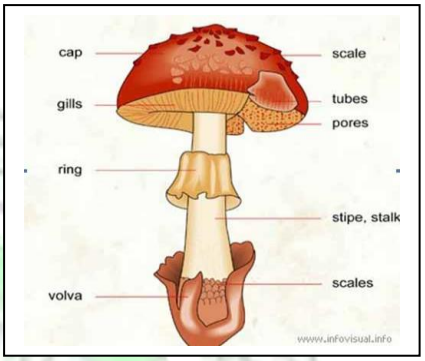
amino acids and are very high in lysine and tryptophan, the 2 essential amino acids deficient in cereals. Mushrooms are almost free from fat except for linoleic acid and are richer than most vegetables in water soluble vitamins (B1, B2, B 12) niacin and pantothenic acid. They are good sources of minerals i.e. Ca, Fe, P, K, Na and folic acid. Analysis of some common edible mushrooms showed that they contain 89-91% water, ash 0.97-1.26%, protein 20-35%, fat 0.25-0.65%, crude fibre 0.09-1.67%, carbohydrates 5.3-6.28% and energy value of 24.4-34.4 k.cal.

MEDICINAL IMPORTANCE: It has spectacular growth in and commercial activity associated with, dietary supplements, functional foods and other products *that are 'more than just food'*. Medicinal fungi have routinely been used in traditional Chinese medicine. Today, an estimated six per cent of edible mushrooms are known to have medicinal properties and can be found in health tonics, tinctures, teas, soups and herbal formulas. *Lentinula edodes (shiitake) and Volvariella volvacea (Chinese or* straw mushroom) are edible fungi with medicinal properties widely diffused and cultivated.

The medicinal properties of mushrooms depend on several bioactive compounds and *their bioactivity depends on how mushrooms are prepared and eaten.* Shiitake are said to have antitumour and antiviral properties and remove serum cholesterol from the bloodstream. Other species, such as *Pleurotus (oyster), Auricularia* (mu-er), *Flammulina (enokitake), Termella (yin-er) and Grifola* (maitake), all have varying degrees of immune system boosting, lipid lowering, anti-tumour, Microbial and viral properties, blood pressure regulating, and other therapeutic effects.

Mushrooms represent a vast source of yet undiscovered potent pharmaceutical products and their biochemistry would merit further investigation. The medicinal importance of mushrooms is given below.

- Anti-diabetic and anti-hypertensive properties
- Anti-Anaemic: reduce anaemia due to presence of folic acid.
- High blood pressure reducing potential:
- Anti-oxidative property: due to the presence of ergothionine.
- Cardio-vascular diseases preventive property: to avoid cardio-vascular diseases.
- Immune system enhancer:
- Hormone stimulator:
- Anti-obesity properties:
- Cell revival potential: contain significant amounts of riboflavin and niacin, and are also rich in selenium; an antioxidant that helps to protect against cell damage resulting from free radicals.



Income benefits: Mushroom cultivation activities can play an important role in supporting the local economy by contributing to subsistence generating additional employment and income through local, regional and national trade; and offering opportunities for processing enterprises (such as pickling and drying). *Income from mushrooms can supplement cash flow, providing either:* a safety net during critical times, preventing people from falling into greater poverty; a gap-filling activity that can help spread income and generally make poverty more bearable through improved nutrition and higher income; or a stepping stone activity to help make people less poor, or even permanently lift them out of poverty.

Livelihood opportunities: Trade in cultivated mushrooms can provide a readily available and important source of cash income – for men and women and the old, infirm and disabled alike. The role played by women in rural mushroom production can be very significant. Certain parts of the mushroom cultivation process, such as filling substrates in containers and harvesting, are ideally suited for women's participation. Several programmes have enhanced women's empowerment through mushroom production by allowing them to gain farming skills, financial independence and self-respect.

Mushroom as Decomposer of Agricultural Waste: Huge quantities of lingo-cellulolytic and other organic waste residues are generated annually through the activities of agricultural, forest and food processing industries. More than 300 million tonnes of agricultural waste is available annually in India and about half of this residue remains unused. Most of the mushrooms possess the enzyme complexes that enable them. The production systems use agricultural waste products, including straw, chaff, sugar beets, corncobs, waste paper, sawdust, coffee grounds, livestock manure, slaughterhouse wastes, and other materials. Once the substrate has been broken down during mushroom production, it can be sold for organic fertilizers and compost.

Potential use as Organic manure: Spent mushroom substrate (SMS) is a good nutrient source for agricultural land and has high cation exchange capacity, capable of holding nutrients in the soil and retaining slow mineralization rate quality as an organic matter. The addition of SMS in nutrient poor soil leads to improvement in soil texture. It has been also revealed recently that mushroom mycelia can play a significant role in the restoration of clean environments.

MORPHOLOGY OF WHITE BUTTON MUSHROOM

The fruiting bodies of mushrooms are either umbrella shaped or of various other shapes, sizes and colours. Commonly it consists of a cap or pileus and a stalk or stipe but other varieties have additional structures like veil or annulus, a cup or volva. Morphologically, fruiting body of a mushroom is divided into two parts

Underground part: This part of fruiting body remains beneath the soil which is made up of secondary mycelium.

Aboveground part: The aboveground part of fruiting body of mushrooms is made up of tertiary mycelium which is the advanced stage of secondary mycelium.

The fruiting body/basidia consist of following parts

Pileus or cap: The cap or pileus is the expanded portion of the carpophore (fruit body) which may be thick, fleshy, membranous or corky. It is a shape structure but the shape is different, depending on the species and the stage of growth. It can be conical flat or even spherical. The surface may be smooth, hairy or carry scab like fragments which usually have remnants or a universal veil. The cap supports and protects the gills or pores where the spores are produced.

Gills/Pore: Usually present on the lower surface of the cap and composed of many thin layers stacked side by side. Some mushrooms have pores instead of gills which belong to Order Polyporales. The gills are tiny tubes packed closely together forming a sponge layer or smooth, wrinkled or veined. The spores are produced within the gills.

Annuls/Ring: A partial veil grows from the edge of the cap to the stem, the **ring** is which is left on the stem as the cap grows and breaks the veil. The veil provided extra protection for the spores at young stages.

Stripe/stalk/stem: The stalk or stipe is negatively geotropic and helps to hold the cap or pileus above the ground level. The

stipe supports the pileus and increases the mushroom's height so spores can disperse in a wider radius. The stem may be solid and fleshy.

Volva: It is a cup-shaped structure surrounding the swollen basal portion of the stalk. It is found in a few species of mushroom especially in poisonous mushrooms such as Amanitas group etc. except in some cultivated mushrooms i.e. *Volvariella volvacea.*

Mycelium: The mycelium is the hidden 'body' of the fungus, which presents underground portions and grows saprophytically. It is the dikaryotic, secondary mycelium and absorbs food for the nutrition of the fruiting body.

MORPHOLOGICAL CHARACTERISTICS FOR IDENTIFICATION OF DIFFERENT MUSHROOM

Name of the Mushroom	Species	Morphological characteristics for identification
White button mushroom	*Agaricus* sp.	•Cap or pileus is fleshy in structure, which is convex to broadly convex or nearly flat with an increase in age, smooth or with pressed-down fibres or small scales, white in some varieties, brown in others. •Gills free from the stem, close, pinkish to pinkish brown at first and later become dark brown to blackish. •The Stem or stipe is 2-8 cm long, 1-3 cm thick, smooth, or with small scales.
Oyster mushroom	*Pleurotus*	•The fruiting body of an Oyster mushroom is characterized by having a broad, fan or oyster-shaped cap with different colours based on species. •The Cap is laterally attached (with no stem), white, firm, and varies in thickness due to the stipe arrangement. •The mushroom gills are white to cream and descend on the stalk if present. •The mushroom's stipe is often absent. When present, it is short and thick.
Paddy straw mushroom	*Volvariella*	•*Volvariella* are characterised by having deep salmon pink gills. •Do not have a ring, and have an *Amanita* like volva at the base of the stem.
Milky Mushroom	*Calocybe indica*	• Like pileus, resembling a button mushroom.
Shiitake Mushroom	*Lentinula edodus*	•Shiitake are tan to dark brown and have broad umbrella shaped caps, wide open veils, tan gills and curved stipe.
Reishi Mushroom	*Ganoderma lucidum*	•Cap is kidney-shaped with pores instead of gills on its underside, •The upper surface is shiny and dark red. •When it is young, it has a yellow, white edged and relatively smooth upper surface. As it matures, the entire upper surface gets reddish brown and more scalloped.
	Boletus sp.	•They don't have gills but 'spongy' pores which are white, cream or yellow.
Poisonous Mushroom	*Amanitas* sp.	•Mushroom of this group has white gills and spores and mostly grows from a sack-like or bulbous structure called a volva.
	Russulas sp.	•They have very brittle gills and stems.

STEPS OF MUSHROOM CULTIVATION

1. Identifying and cleaning a dedicated room or building in which temperature, moisture and sanitary conditions can be controlled to grow mushrooms,
2. Choosing a growing medium and storing the raw materials in a clean place under cover and protected from rain,
3. Pasteurising or sterilizing the medium and bags in which, or tables on which, mushrooms will be grown (to exclude other fungi that would compete for the same space – once the selected fungi have colonized the substrate can fight off the competition),
4. Spawning the beds with spawn (spores from mature mushrooms grown on sterile media),
5. Maintaining optimal temperature, moisture, hygiene and other conditions for mycelium growth and fruiting is the most challenging step; adding water to the substrate to raise the moisture content since it helps ensure efficient sterilization,
6. Harvesting and eating, or processing, packaging and selling the mushrooms, and
7. Cleaning the facility and beginning again.

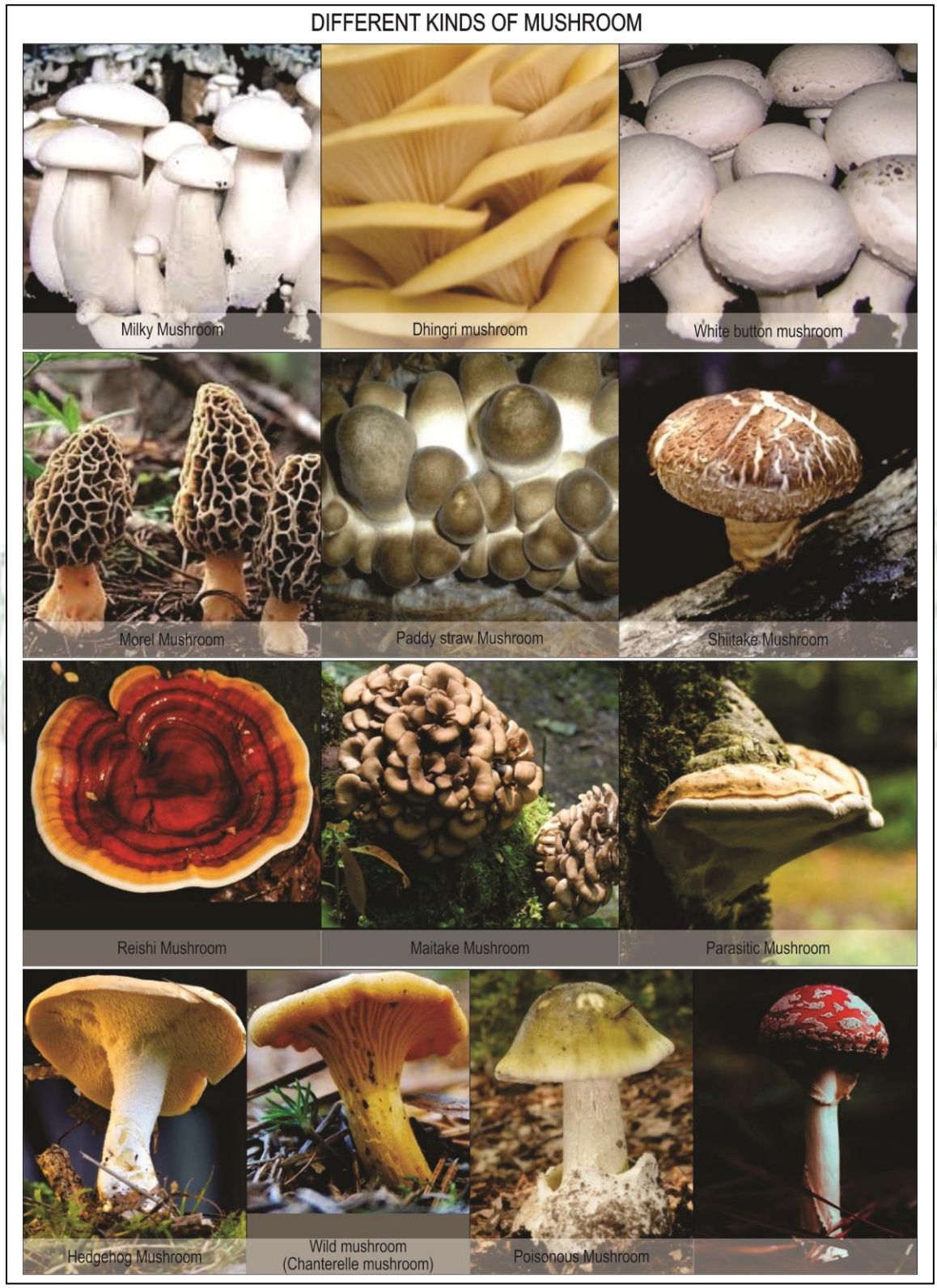
POTATO DEXTROSE AGAR AND BROTH MEDIUM

Materials required: Peeled potato slices (200g); Dextrose (20 g); Agar- agar (20 g); Distilled water (1000 ml)

Method:

- Take 200 g of peeled Potato slices and boil in 500 ml of distilled water.
- Then filter the liquid (potato infusion) with the help of a clean muslin cloth.
- Heat the potato infusion and add dextrose and agar-agar until it gets melted or without agar-agar known as broth
- Stir the media regularly with a clean glass rod.

- Make up the volume to 1000 ml by adding the required water.
- Fill the media in a conical flask and put on the cotton plug.
- Autoclave the media at 1.1kg/cm^2 pressure for 20-25 min. at 121.6°C temp. Thus, the medium is ready for use.



ISOLATION AND MAINTENANCE OF MUSHROOM FUNGI

Mushroom culture may be developed and maintained in two ways, one from its spores and the other from live tissue of a mushroom. Either type can produce a viable strain of mycelia. Usually, spores and mycelium from spores are used to develop mushroom culture. Spores have the advantage over live tissue that it can be stored for longer, in case of no use.

Isolation from the Tissue of the fruiting body: A newly flush mushroom with covered gills with membrane is selected for isolation of fungus. A small bit of membrane from gill portion is taken with the help of a sterilized needle and inoculated centrally on the surface of the pre-sterilized PDA or MEA medium. The Petri plates are incubated at 25±2°C in a BOD incubator for one week. Mycelium from growing edges is carefully transferred to MEA/PDA slants and again incubated for 2-3 weeks to obtain pure cultures.

Spore culture: Large-sized, healthy and intact mushroom with membrane (veil) is collected. It is surface sterilized and mounted on a wire stand over a Petri dish in a sterilized glass beaker. The spores get deposited as a spore print. The spores are stored under sterile conditions in a refrigerator for future use. The Spores are inoculated to the sterilized PDA and MEA culture slants. These slants are then incubated at 25±2°C for 2 weeks to obtain a pure culture.

Multi spore culture: Spore suspension is prepared in sterilized distilled water. One ml of spore suspension containing more than a hundred spores is mixed in each slant culture tube containing about 5-7 ml of sterilized PDA. The slants are incubated at 28°C. The mycelium threads become visible on the slant surface.

Cultures from another source: Cultures may also be grown from spawn obtained from another source. A piece of the spawn is aseptically transferred to agar slants. These slants are then incubated at 25±2°C for 2 weeks to obtain a pure culture.




MOTHER SPAWN PREPARATION

Spawn is the mycelium of mushrooms growing in its substratum and prepared for propagating mushroom production. In simpler language, it is defined as a medium impregnated with mushroom mycelium that serves as the “seed” for mushroom cultivation.
Spawn are two types: i) Mother/Master spawn, ii) Commercial spawn

Preparation of Mother/Master spawns: Mother spawn prepared using pure culture mycelium on agar medium in Petri plates as inoculants is referred to as mother spawn. It is also known as stock or master culture.

Requirements: The required materials are listed below for mother spawn production

Raw materials: Fresh mushroom culture, Grain substrate (wheat, jowar, bajra, rice etc.), Glucose/milk bottle, Aluminium foil, Chemicals ($CaCO_3$ and $CaSO_4$), Non-absorbent cotton, Rubber bands

Appliances/tools: Autoclave, Laminar airflow, BOD Incubator, Inoculation needle, Spirit lamp and Alcohol/Spirit, Thermometer and Hygrometer, LPG/electric stove (for boiling substrates), Fry pan (capacity 20-25 kg) for boiling substrate, Mesh (for filter water from substrate-3x6 fit

Procedure

- Take the required amount of healthy and cleaned cereal grains (wheat/jowar/bajra)
- Boil the grains in water for 15-20 minutes. Normally for soaking and boiling 10 kg of wheat grain requires 20 litre of water.
- Remove excess water by spreading it on a sieve.
- Dry the grains on polythene or bedsheet in the shade (4 hrs.) to remove extra moisture and add CaCO3 (0.5%) and CaSO4 (2%)
- Glucose/milk bottle up to 3/4 volume is filled with 500g dry grains. Normally 300g grains in 500 ml glucose/milk bottle.
- Plugged the bottle with non-absorbent cotton and covered it with aluminium foil and autoclave at 121^0 C at 15 psi for 15 minutes.
- Sterilized bottles are immediately brought out from the autoclave transferred to the inoculating chamber and allowed to cool down overnight.
- Next day, bottles are kept in a laminar airflow chamber under UV light for 20-30 minutes before inoculation.
- Bottles are inoculated with 2-3 bits of agar medium colonized with the mycelium of pure culture of desired strain under aseptic condition using laminar flow and shaken gently.
- Incubate in BOD at $23+2^0$ C for 20-25 days.
- Master spawn is ready.

- Mother spawn bottles must be labelled, firm name, species, quantity, and date of inoculation are marked to know the age and type of spawn.

Precautions to be taken during mother spawn production

- Always use fresh mushroom fungal strain.
- Always keep the inoculation chamber neat and clean.
- The working person should clean his/her hands using alcohol before using the inoculation chamber.
- Switch on UV light in the inoculation chamber for 30 minutes before inoculation by keeping sterilizing substrates like forceps, inoculation needle, culture media, spirit lamp etc. inside the chamber.
- Inoculation is always to be done near the spirit lamp to avoid contamination.
- Inspect the bottles regularly and discard contaminated ones immediately.
- Observe that within 15-25 days of inoculation, mycelium growth covers the entire substrate and the spawn is ready for use.

PRODUCTION OF COMMERCIAL SPAWN

Commercial spawn can be prepared in polypropylene bags which must be heat resistant. Commercial spawn is prepared from mother/master spawn.

Procedure

- Take the required amount of healthy and cleaned cereal grains (wheat/jowar/bajra)
- Boil the grains in water for 15-20 minutes. Normally for soaking and boiling 10 kg of wheat grain requires 20 litre of water.
- Remove excess water by spreading it on a sieve.
- Dry the grains on a polythene or bed sheet in shade (4 hrs.) to remove extra moisture then add $CaCO_3$ (0.5%) and $CaSO_4$ (2%)
- Heat resistant polypropylene bags are filled with 500g (35 x 17.5cm) or 1 kg (35 x 17.5cm) dry grains (PP bag).
- PP bags are plugged with non-absorbent cotton with the help of a PP neck plastic ring and are covered with aluminium foil and autoclave at 121^0 C at 15 psi. for 15 minutes.
- Sterilized PP bags are shaken well before inoculation so that the water droplets accumulated inside the bags can be absorbed by the grains.
- Sterilized PP bags are immediately brought out from autoclave transfer in the inoculating chamber and allowed to cool down overnight.
- The next day, PP bags are kept in a laminar airflow chamber under UV light for 20-30 minutes before inoculation.
- PP bags are inoculated with 10-15 gm of grains from master spawn bottle of desired strain under aseptic conditions using laminar flow or one bottle of master spawn which is sufficient for inoculating 25 to 30 commercial spawn bags. After inoculation, all the PP bags are shaken gently.
- Incubate in BOD at $25+1^0$ C for 20-25 days.
- Inoculated bags are gently shaken on the 5^{th} and 10^{th} days.
- Commercial spawn is ready in 20-22 days.
- PP bag must be labelled firm name, species, quantity, and date of inoculation to know the age and type of spawn.

Precautions to be taken

- Always keep the inoculation chamber neat and clean.
- The working person should clean his/her hands using alcohol before using the inoculation chamber.
- Switch on UV light in the inoculation chamber for 30 minutes before inoculation by keeping sterilizing substrates like forceps, inoculation needle, culture media, spirit lamp etc. inside the chamber.
- Inoculation is always to be done near the spirit lamp to avoid contamination.
- Use 15-20 days-old mother spawn for inoculation.
- Mother spawn beyond 3-4 generations should not be used as it starts degenerating.
- PP bag must be labelled with the firm name, species, quantity, and date of inoculation to know the age and type of spawn.


Boiling


Sterilization


Commercial spawn


other spawn production

COMPOST PREPARATION

The substrate on which button mushroom grows is mainly prepared by mixing plant wastes (cereal straw/ sugarcane bagasse etc.), fertilizers (urea, superphosphate/gypsum etc.), supplements (rice bran/ wheat bran), water etc. To produce 1 kg of mushroom, 220 g. of dry substrate materials are required. It is recommended that each ton of compost should contain 6.6 kg nitrogen, 2.0 kg phosphate and 5.0 kg of potassium (N: P: K- 33: 10: 25) which would get converted into 1.98% N, 0.62% P and 1.5% K on a dry weight basis. The ratio of C: N in a good substrate should be 25-30:1 at the time of staking and 16-17: 1 in the case of final compost. There are two methods of compost preparation.

SHORT METHOD OF COMPOSTING

Compost prepared by short method is superior in terms of quality production and is very less prone to infection and disease.

Materials required For Short Methods of composting: Plant wastes (cereal straw/ sugarcane bagasse etc.) - 1000 kg**,** Urea – 14.5 kg**,** Wheat Bran or Brewer's grain – 72 kg**,** Gypsum - 30 kg**,** Poultry manure 400 kg

Procedure

First phase of composting: During the first phase of compost preparation, paddy straw is placed in layers and sufficient water is added. Then poultry manure + Wheat bran (chokar) is mixed and then added to the straw as 50 – 60 cm thick**.** After 2 days mix all i.e. straw + poultry + wheat bran**.** After 2 days urea added and mixed + watering. The whole thing is mixed thoroughly with the straw and made into a stack (almost 5 feet high,5 feet wide and of any length can be made with the help of wooden boards). 2nd day 1st turning**.** 4th day 2nd turning**.** 6th day 3rd turning and add gypsum**.** 8th day fill in the tunnel.

Second phase of composting: This is the pasteurization procedure which is done in a closed environment using microbe microbe-mediated fermentation process. The whole process is carried out inside a steaming room where an air temperature of 60^{o}C is maintained for 4 hours. The second phase is the pasteurization phase. Fill in Tunel – kept for 7 days for pasteurization. Closed door, outside light should not be entered**.** Fan on/ Blower on to facilitate uniform distribution of temperature at 45.5^{0}C. After 7 days open the door and allow to come sunlight – kept for 2 days –to increase the temperature to 48-52^{0}C. Boiler on – send steam for 6 – 8 hrs for pasteurization. Door open & allow to enter the fresh air for 2 days – to decrease the temperature and allow to cool down by bringing the temperature to 25°C, which is considered as the favourable temperature for spawning. Compost ready for spawning should possess 70% moisture, pH 7.2-7.5, Ammonia below 0.006%, Nitrogen around 2.5%, dark brown colour and Fire fungus (*Actinomycetes)* for good growth.

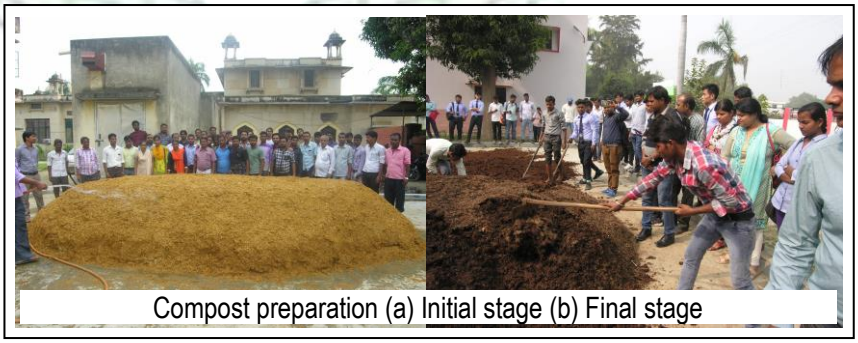
LONG METHOD OF COMPOSTING

It is a primitive method of composting and is usually practised in areas where facilities for steam pasteurization are not available. It takes 28 days to make it complete and requires seven turnings. The disadvantage of this method is that it gives low yield and invites several pests and diseases. The following materials are required for a long method of compost.

Material required: Plant wastes (cereal straw/ sugarcane bagasse etc.) - 1000 kg**,** Urea – 10 kg**,** Calcium Ammonium Nitrate - 30 kg**,** Wheat Bran – 50 kg**,** Murate of potash – 10 kg**,** SSP – 10 kg**,** Gypsum 40 -50 kg **and** FYM

Procedure

- First straw spread on cemented floor and water spay and mixed it properly. It should continue for 2 days to moisten straw uniformly.
- Then one day before to prepare heap, CAN + SSP + MOP +Urea + Wheat bran are mixed properly and then covered with wet gunny bag or other to prevent release of ammonia gas.
- Next day, all of these are mixed with wet straw and make a heap as 1 meter height and 1 meter wide and kept it for 5 days. Initially inside temperature is equal to outside but after 3 days temperature will raise as 65 -70^{0} C.
- In this method, the first turning is given about six days after preparation of the substrate as heap.
- The second turning is given on the tenth day.
- The third turning on the thirteenth day when gypsum is added.
- The fourth, fifth and sixth turnings are given on the sixteenth, nineteenth and twenty-second day. Here mol aces can be used @ 2kg/1 quintal of wheat straw. Insect population will increase, therefore systemic insecticide may be applied.
- On the twenty-fifth day, the seventh turning is given by adding another spraying of systemic insecticide (i.e. Nuvan@ 1ml/litre of water).
- The eighth turning is given on the twenty-eighth day after which it is checked whether there is any smell of ammonia present in the compost.
- The compost is ready for spawning only if it doesn't have any smell of ammonia (Ammonia should not be more than 8-10 ppm at the spawning stage.); otherwise a few more turnings are given at an interval of three days till there is no smell of ammonia.



Compost preparation (a) Initial stage (b) Final stage

METHODS OF MUSHROOM SPAWNING

Spawning: The process of mixing spawn with substrate or compost is called spawning. It is the actual planting of the spawn and therefore, it requires care depending on the species of mushroom and methods being followed. Spawn runs after the spawning to give rise to mycelium and fruit bodies.

Spawning of Oyster Mushroom: The sterilized substrate is opened either on cemented floor or on the polythene sheet which are also previously sterilized with formaldehyde solution @ 2%. The moisture content of substrate should be 70-72%. Polythene bags (35 x 50 cm, 150 gauges) are used for the filling of a substrate. Freshly prepared 15-20 days old grain spawn is used for spawning. Spawning can be done either by layer or mixed method.

Layer spawning: Substrate is filled in polybag to a depth of 8-10 cm followed by press gently with hand and broadcasted with a handful of spawn above it. Similarly, 2nd, 3rd, 4th etc. layers of substrate were added and spawned before closing of bag. The bags are then transferred to crop room for spawn running.

Mixed spawning: In case of mixed methods, the spawn is mixed thoroughly with a sterilized substrate. Seems, the straw after hot water treatment or chemical treatment becomes heavy and spawning is done @ 2.5% of wet straw weight (25g/kg of straw in polybag method). The polybags are then filled with a mixture of spawn and substrate before closing of bag. The bags are then sealed and 10 to 15 small holes (0.5-1.0 cm dia) should be made on all sides, especially in the bottom for leaching excess water. The bags are then transferred to crop room for spawn running.

Layer spawning and Mixed spawning

Spawning of Button Mushroom: The well prepared compost is filled in polythene bags (90 x 90 cm, 150 gauge thick having a capacity of 20-25 kg. per bag) or trays (mostly wooden trays 1m x 1/2 m x 15-20cm accommodating 20-30 kg. compost) or shelves which are either covered with a newspaper sheet or polythene. Normally, three methods of spawning are used in case of button mushrooms.

1. **Surface/broadcast spawning:** Compost is filled in formalin sterilized wooden boxes of size 60 x 90 x 15 – 23 cm, accommodating 6-10 kg compost or 1m x 1/2 m x 15-20cm accommodating 20-30 kg compost , the spawn is evenly spread over the surface of the box and allowed to grow into the compost. The top portion is covered with a thin layer of compost and covered with formalin sterilized newspaper or polythene sheet to prevent loss of moisture content in mushroom beds and allow for spawn running.
2. **Layer spawning:** Spawning is done by scattering the spawn on boxes beds once when half-filled with compost and secondly, after the complete filling of the boxes. The spawn is gently pressed down with the forefinger uniformly each time and trays are covered with formalin sterilized newspaper or polyethene sheets.
3. **Mixed spawning:** The spawn is mixed throughout the compost @ 1.5-2.0kg/100 kg of compost and filled in formalin sterilized wooden boxes and covered with formalin sterilized newspaper or polythene sheet. Advantages to mixed spawning are it reduces the spawn growing period, spawn is evenly distributed in the compost and increases mushroom yield.

Spawn run: The process of colonization of compost from grain inoculum is called spawn-run. These mushroom boxes are placed in a growing chamber, where temperature ranges between 22-28°C and 90-95% RH (relative humidity). The paper over the beds is sprayed regularly with water to prevent drying out and humidity is built up by frequently watering the floor and walls. The room was kept closed as only a small amount of fresh air recirculation within the crop room for maintaining the carbon dioxide levels. Mushroom boxes are completely colonized by mushroom mycelium within 20-31 days. The compost becomes lighter in colour and the mycelium is seen as thin white-threads.

PREPARATION OF CASING SOIL

Casing soil: The compost beds after complete spawn run should be covered with a layer of soil (casing) about 3-4 cm. thick to induce pinning and fruiting of mushrooms. Normally, casing is applied @ 2kg casing for 1m x 1/2m wide. The casing material should have high porosity, water holding capacity and the pH should range between 7-7.5. The casing layer keeps the compost surface free from drying and works as a water reservoir for the mature mushroom. Thick casing layer of 3-4 cm is usually applied on the surface. The casing provides physical support, moisture and exchange of gases within the surface of the compost which helps in proper growth of the mycelium.

Material of casing: Various materials have been tested as casing substrate throughout the world by different workers. Peat moss is considered to be the best casing material but normally is not used in India, as such. In India, casing soil is prepared by using the following ingredients:

Mixture of substrates	Ratio	Mixture of substrates	Ratio
Two years old manure + garden soil	3:1	Two year old manure + spent compost	2:1
Two year old manure + garden soil	2:1	Two year old manure + spent compost	1:2
Two year old manure + spent compost	1:1	Garden soil + sand mixture	4:1

Preparation of casing soil

- Select the casing materials easily available in the area
- Selected materials should be broken down into small granular form
- Mixed all the materials uniformly and as per need, water spray may be given.
- All the material should be spread on cemented floor.
- Prepared a solution of formaldehyde at a concentration of 2%
- Spray the casing materials with freshly prepared formaldehyde solution followed by making it heaps.
- Cover the heap with a polythene sheath in an air-tight condition.
- Kept it for 7-10 days.
- Casing material is ready for used.

Vermi-compost

Farm yard manure

Garden soil

Sterilization of casing material

BUTTON MUSHROOM CULTIVATION

Button Mushroom (*Agaricus* spp.) is the most popular mushroom variety grown and consumed all over the world. It is extensively cultivated among all mushrooms throughout the world and contributes about 40 % of the total world production of mushrooms. This is the first mushroom to be commercially cultivated on an industrial scale. The genus *Agaricus* has two cultivated species namely *A. bisporus* (temperate button mushroom) and *A. bitorquis* (tropical or high temperature tolerant white button mushroom) *belonging to Class Basidiomycetes and Family Agaricaceae.*

Agro-climatic requirements:

- The best period for cultivation of button mushrooms in Indian plain region is winter months (October to March).
- Required optimum temperature: *A. bisporus* requires 20-22^{o}C for spawn run and 16-18^{o}C for fruiting. However another species *A. bitorquis* needs a higher temperature, 28-30^{o}C for spawn run and 25^{o}C for fruiting.
- Relative humidity of 80-90%.
- The major growing states are Himachal Pradesh, Uttar Pradesh, Punjab, Haryana, J&K, Maharashtra, Andhra Pradesh, Tamil Nadu and Karnataka.

Materials required

Raw materials: Fresh spawn (15 days old), Substrate (wheat/maize/pulse straw/sawdust), Polypropylene bag (PP bag)/trays (mostly wooden trays 1x1/2 m., Compost, Casing soil, Rubber band, Chemicals (Bavistin and Formalin), Molasses.

Equipment/ tools: Crop room, Water tank, Cemented floor, Polythene sheet, LPG / Electric stove (for boiling substrates), Pasteurization chamber, Mesh (for filter water from substrate-3x6 fit, Rubber pipe, Sprayer

CULTIVATION TECHNIQUE

1. Compost preparation (detail see compost preparation.).
2. Spawning (detail see spawn preparation).

Spawn running: After spawning, temperature and humidity of crop room should be maintained at 18-22^{0}C and 85-90%, respectively. The room should be closed and dark. Water should be sprayed over the covered newspapers, walls and floors of the crop room. If the spawning materials is covered with polythene sheet, there is no used of water spray over the spawning materials except walls and floors of the crop room. Conditions of the room should be maintained for 12-14 days.

Spawn running stage and Full fruiting growth of button mushroom

Casing: After 12-14 days, the compost beds surface will cover by white mycelium of the fungus. The white mycelia mat surface should be covered with 3-4 cm thick layer of casing soil to induce pin head formation and fruiting.

Fruiting: Under favorable environmental conditions viz. temperature (initially 23 ± 2^{0}C for about a week and then 16 ±

20^0C), moisture (2-3 light sprays per day for moistening the casing layer), humidity (above 85%), proper ventilation and CO_2 concentration (0.08-0.15 %) the fruit body initials which appear in the form of pin heads start growing after 7 days after casing and gradually develop into button stage take time 12- 15 days.

Harvesting and Yield: The crops should be harvested before the gills open as this may decrease their quality and market value. Harvesting is done at button stage and caps measuring 2.5 - 4 cm across and closed are ideal for the purpose. About 10-14 kg fresh mushrooms per 100 kg fresh compost can be obtained in two months crop. The short method used for preparation of compost under natural conditions gives more yield (15-20 kg/100 kg compost). About 40 -50 days can harvest fruit 7 days interval you can harvest mushrooms. After harvesting mushrooms treated with 0.05% potassium meta-bisulphite solution.

OYSTER MUSHROOM CULTIVATION

The *Pleurotus* mushroom is generally referred to as 'Oyster Mushroom' or 'Dhingri' in India. It is a ligno-cellulolytic fungus and grows naturally in temperate and tropical forests on dead and decaying wooden logs or sometimes on dryings in the trunks of deciduous or coniferous woods. It ranks second after button mushroom in terms of production. The fruit bodies of this mushroom are ear, fan or spatula shaped with different shades of white, cream, grey, yellow pink or light brown depending upon the species. There are 38 species of the genus recorded throughout the world (Singer). In recent 25 species have been commercially cultivated in parts of the world. This mushroom is cultivated in about 25 countries in far East Asia, Europe and America. The major producing countries are South Korea, Italy, Taiwan, Thailand and the Philippines. China alone contributes 90% of the total word production.

Agro-climatic requirements

- March to October in mid hills and September to March is considered as the suitable periods for best cultivation.
- The optimum temperature for growth of most of the species of *Pleurotus* is 20-28^{o}C. However, *P. sajor-caju* can grow up to 30^{o}C temperature.
- Relative humidity (RH) of 80-90%.
- The major producing states are Odisha, Karnataka, Maharashtra, Andhra Pradesh, Madhya Pradesh, West Bengal and in the North-Eastern States of Meghalaya, Tripura, Manipur, Mizoram and Assam.

Materials required

Raw materials: Substrate (wheat/maize/pulse straw/mustard etc), Fresh spawn (15-20 days old), Poly-propylene bag (size-35 x 50 cm, 80 gauges), Supplement (rice/wheat bran), Rubber band, Chemicals (Bavistin and formalin).
Equipment/ tools: Crop room may be permanent or temporary, Water tank, Water bath, Cemented flour for drying, LPG / Electric stove (for boiling substrates), Pasteurization chamber, Fry pan (20-25 kg) for boiling substrate, Mesh (for filter water from substrate-3x6 fit.

Cultivation Technique:

- Fresh, golden yellow colour wheat straw is filled into gunny bags.
- The bag containing wheat straw is soaked in fresh water in a water tank containing 2.0% formaldehyde and left 10-12 hours.
- The bags were then brought out from water and kept on a cemented floor to remove excess water from straw.
- The straws are then spread on the floor of a composting yard or polyethene sheet.
- Spawning is done either layer or mixed methods @ 2.5% of the wet straw weight or 25g/kg of straw in polybag method.
- Resulting mixture is filled in a polypropylene bag (size 35 x 50 cm, 80 gauges)
- After spawning the bags are tied on the top with the help of rubber bands and holes of about 1 cm diameter are made at 10-15 cm distance across the surface facilitating free diffusion of gases and heat generated inside.
- The spawned bag is shifted to the closed crop room on racks 20 cm apart at 50-60 cm distanced shelves and maintained the temperature at 24-25^{0}C, relative humidity 70-85%, and darkness for about 12-14 days. RH is maintained by spraying water twice a day on walls and floors. The spawn running will take about 12-14 days at 24 ±1^{0}C.

Spawn running stage of oyster mushroom

Full growth of oyster mushroom fruit

- When bags are fully impregnated with white mycelium, the polybag is opened and maintained the room temperature at 22 ±1^{0}C, with relative humidity 85% by spraying water twice a day on the walls and floor of the room, for 4-6 hours ventilation per day and provide frequent light. This condition should be maintained for 7-10 days.
- Mushroom primordial begin to form, usually after 7 to 10 days of opening of the bags.
- Matured mushrooms are ready to harvest in another 2 to 3 days. The fruit of mushroom should be harvested by twisting before release

of spores,

- Depending upon the room conditions, the crop can be harvested up to 50-60 days.
- Fresh mushrooms should be packed in perforated polythene bags for marketing. The care should be taken that the fruit bodies are stacked in trays or baskets during transportation. The tray is should be covered with thin polythene sheet with perforation
- Freshly harvested mushrooms can be stored at low temperature (0-5^{0}C) for 6-7 days without losing quality

MILKY MUSHROOM CULTIVATION

Calocybe indica, commonly known as the milky white mushroom, is a tropical species of edible mushroom native to India. It appears in summer after rainfall in fields and on road verges. This variety has an excellent shelf life. Milky mushrooms are also known as Dudh chat in West Bengal. They can be grown on a wide range of substrates, as in the case of oyster mushrooms, which are made up of lignin, cellulose, and hemicelluloses. The substrate should be fresh and dry. They can grow in grasslands, fields, and road verges in Tamil Nadu and Rajasthan, generally on substrates rich in organic material. The mushrooms appear between May and August after spells of rainfall.

Agro-climatic requirements

- Best period for cultivation- summer season between May and August after spells of rainfall.
- Temperature for vegetative growth (spawn run) - 25-30^{o}C and for cropping 28-32^{o}C.
- Relative humidity of 60-90%.
- The major producing states are Tamil Nadu, Kerala, Karnataka, Odisha, Haryana, West Bengal, Rajasthan etc.

Materials required:

Raw Materials: Substrate (dried wheat/paddy straw), Fresh spawn (15-20 days old), Wheat bran, PP bag (60 x 30 cm size), Rubber band, Chemicals (Bavistin and formalin).

Equipment: Crop room/Hut- bamboo platform, Hot air oven, Cemented flour for straw drying, Casing preparation floor, Fire stove and Water tank

Cultivation Technique

- Fresh dried substrate with original colour (wheat straw/paddy straw) is most suitable for its cultivation.
- Substrate is soaked in fresh water for 24 hours and is treated with hot water at 80^{o}C for 1.5 to 2 hours or soaked in water containing formaldehyde @2% for 10-12 hours.
- Excess water is to be removed from the straw and added with wheat bran at 5% of the substrate's wet weight.
- Layer methods do spawning @ 2.5% of wet weight of the substrate.
- Bags after spawning are maintained in a cropping room at 25-30^{o}C, relative humidity 80-85% and darkness for 10-15 days.
- Casing soil of about 2 cm height is applied on the top of the open bed surface when the substrate gets fully colonized by the fungus mycelium, normally after 10 to 15 days.
- After application of casing, everyday water spraying should be done for at least 2-3 times to maintain casing in wet condition.
- After application of casing layer, the room temperature to be lowered to 25-26^{o}C and the crop begins to appear in another 7-10 days after complete case run.
- The primordial are developed into fully grown harvestable mushrooms in 4-5 days.
- Mushrooms are to be harvested after attaining the height of 7-8cm by twisting.
- The complete cycle takes about 45-50 days on synthetic logs.
- One kg of mushroom come from 10-12 fruiting bodies, yield capacity: 500-600 g/kg dry weight substrate.

Harvested mushroom can be cleaned and packed in perforated polythene / polypropylene bags for marketing. Mushrooms can also be wrapped in kiln film for longer storage.

PADDY STRAW MUSHROOM CULTIVATION

Paddy straw mushroom (*Volvariella volvacea*) is also known as Chinese mushroom or tropical paddy straw mushroom. Paddy straw mushroom is also called a "Warm mushroom" as it grows at relatively high temperatures. In India, it is mostly grown in Orissa, Andhra Pradesh, West Bengal, Tamil Nadu and Kerala. It is a very delicious and nutritious mushroom amongst the edible group and is a quickly growing mushroom that can be harvested on 12th or 13th day only.

Agro-climatic requirements

- The best period for cultivation are warmer months.

- Temperature for spawn run and cropping 30-35°C.
- Relative humidity of 85-90%.
- Growing areas: Odisha, Andhra Pradesh, West Bengal, Tamil Nadu and Kerala.

Materials required:

Raw materials: Fresh spawn (15 days old), Substrate (dried paddy straw), Supplement (besan), Rope, Chemicals (Bavistin and formalin), Binding thread

Equipment/Tools: Mushroom house/ hut-bamboo platform, Water tank, Cemented flour for straw drying, Fire stove, Sprayer and Thermometer

Cultivation technique

- Select dry, fresh and hand threshed paddy straw free from moulds.
- The straw is tied into bundles of 1.2 m long x 25 cm diameter (tie end) size followed by steeping of bundles in water in a cemented tank for 24 to 48 hrs.
- The bundles are taken out from tank and put on a cemented floor for a few hours to drain out excess water.
- The mushroom beds (cage) are prepared on a raised bamboo platform inside a thatched hut.
- Four wetted bundles are placed side by side on this platform, facing all the loose ends on one side.
- Then another four bundles are placed with their tied ends on the opposite side so that loose ends of all 8 bundles meet and overlap each other in the middle.
- Spawning is done on this first layer about 15cm away from the outer edge. The amount of spawn to be used is calculated at 1.5-2.0% of wet weight basis.
- On top of the spawned first layer, a little quantity of Besan (about 200 g per bed) is applied along with the spawn.
- Similarly, a second layer of 8 bundles is placed similarly and spawned on top of the spawned first layer of eight bundles. A third layer is again laid on top of the second layer and followed by 4th layer of bundles and spawning. The total 32 bundles make a single bed which is now pressed to remove the entrapped air and make it compact for effective spawn run.
- The beds are covered with polythene sheets or gunny sheets to avoid rapid water loss.
- The individual beds are watered in 2-3 times daily without opening them. The total dry weight of straw/bed is 25 to 32 kg. At least 18 to 22 litre water/ bed is sprayed to maintain a moisture level of 65 to 70%.
- After, 10 to 15 days, the bed will cover with mycelial growth under optimum conditions of 28 to 35°C with 85 to 90% RH. Pinheads will appear after 4-5 days of spawning.
- The mushroom is to be harvested in egg stage (2 inches in diameter), not allowing it to open like an umbrella. First harvesting is done after 9-10 days of spawning and the first flush lasts for 3 days accounting for around 75% of the total mushroom yield.
- The second flush appears after a few days and this flush accounts for the remaining 25% of the total mushroom yield.

This mushroom is not recommended to be stored in refrigerated because low temperature storage causes frost injury and deterioration in quality but can be stored afresh at a cold temperature of 10 to 15°C for 3 days in polythene bags with perforations. Wooden cases and bamboo baskets are also used for packaging and transport in long distance markets.

Spawning in paddy straw bundle

Pin head formation

Full grow of Paddy straw mushroom

SHIITAKE MUSHROOM CULTIVATION

Shiitake mushroom (*Lentinula edodes)* has delicious taste and medicinal attributes. It can be considered as king of mushroom and so known as miracle mushroom. Shitake mushroom sold at high price around the world due to its medicinal properties and richest sources of protein.

Agro-climatic requirements

- The best period for cultivation is summer season.
- Temperature for cropping: 16-22°C.
- Relative humidity of 85-90%.

- The major areas of production: Uttar Pradesh, Odisha, Punjab, Haryana, Maharashtra, Andhra Pradesh, Tamil Nadu and Karnataka.

Materials required

Raw materials: Fresh spawn (15 days old), Substrate (sawdust), Supplement (rice/wheat bran), Polythene bag (60 x 30 cm size) Rope, Non-absorbent cotton, Plastic neck and rubber bands, Chemicals (Bavistin and formalin)

Equipment/Tools: Crop room, Autoclave, Water tank, Cemented flour for straw drying, Fire stove, Hot air oven.

Cultivation technique: Shiitake mushroom grows on wheat straw/sawdust substrates, as Sawdust substrate gives better fruiting. Sawdust is supplemented with 6% rice/wheat bran and a small quantity of gypsum (about 1% dry w/w).

Steps

- Saw dust is soaked for 16-18 hours and wheat bran for three hours. All ingredients are thoroughly mixed.
- These mixtures are wetted on the composting platform and filled into polypropylene (heat resistant) bags (1.5 to 2 kg), pressed hard to drive out air and a hole.
- The substrate bags are plugged with non-absorbent cotton with the help of a plastic neck on the open side of the bag.
- The substrate bags are autoclaved at 121^{o}C at 15 psi for 90 minutes.
- After cooling the substrates are spawned (using grain spawn) @ 3% (dry weight basis) under aseptic conditions (on laminar flow).
- After inoculation bags, the fungus mycelium grows in two phases, spawn run and browning.
- The vegetative growth/spawn run is done at 24±1^{o}C, which takes 30-35 days.
- The bags after spawn run are opened and maintained in cropping room at 17-19^{o}C, 90% RH and 1000 ppm CO_2 concentration for browning.
- After browning of 30 days bags are shifted to the cropping room for maintenance at 22±1^{o}C.
- The complete cycle takes about four months on synthetic logs. The yield potential on synthetic logs is about 80-100% of the dry weight of the substrate.

REISHI MUSHROOM CULTIVATION

Reishi mushroom (*Ganoderma lucidum*), also known by various names like *Reishi*, *Ling Zhi*, and *Mannentake mushroom* is one of the pharmacologically and commercially important mushrooms. This mushroom has a great value in preparation of medicine rather than as food because it is bitter and corky hard. The market value of this mushroom is basically as herbal medicine and food supplements (nutraceuticals). *Reishi* mushrooms can be grown in both ways; seasonally under low cost growing rooms preferably polyhouses and around the year under environmentally controlled cropping rooms. Sawdust is considered as best substrate for cultivation of Reishi mushrooms.

Agro-climatic requirements

- Best period for cultivation is summer season in tropical areas.
- Temperature requirement for vegetative growth (spawn run) is 20-25^{0}C and 25-28^{0}C for fruiting.
- Relative humidity of 85-95%.
- The major Reishi mushroom producing states are Uttar Pradesh, Odisha, Punjab, Haryana, Maharashtra, Andhra Pradesh, Tamil Nadu and Karnataka.

Materials required

Raw materials: Fresh spawn (15 days old), Substrate (sawdust), Supplement (rice/wheat bran), Calcium sulphate and calcium carbonate, Polythene bag (60 x 30 cm size) Plastic neck and rubber bands, Chemicals (Bavistin and formalin)

Equipment/Tools: Autoclave, Water tank, Cemented flour for straw drying, Fire stove, Hot air oven.

Cultivation technique

- Sawdust supplements are amended with 20% wheat bran and wetted to a level of 65% moisture. Calcium sulphate (gypsum) and calcium carbonate (chalk powder) are also added to get a pH of 5.5-6.5.
- Mixed all the substrates and filled in heat resistant polypropylene bags (700 g dry wt; 2.1 kg wet).
- The substrate bags are plugged with non-absorbent cotton with the help of a plastic ring on the open side of the bag.
- The bags are then autoclaved at 121.6^{o}C at 15 psi for 15 minutes.
- After cooling the substrate is spawned @ 5% wet wt. basis under aseptic conditions under laminar flow.
- Spawned bags are kept in the closed rooms (high carbon dioxide), maintaining temperature 28-30^{o}C, relative humidity 80-90% under dark conditions for 25-35 days for incubation.
- The spawned bag will turn white all over within 25-35 days, thereafter top of the

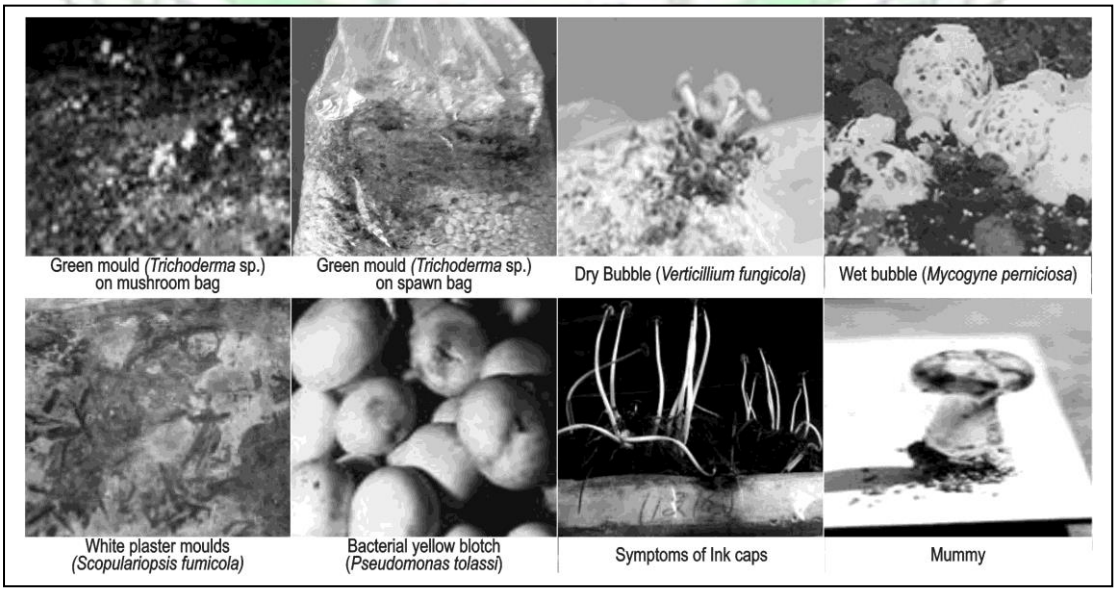
- The polythene bag is opened and in proper condition at temperature 28°C, 800 lux light, 1500 ppm CO_2, 80-85% RH are maintained for fruiting or pinning.
- Once the cap is fully formed, indicated by yellowing of the cap margin. The crop room temperature reduced to 25°C and RH to 60% for cap thickening, reddening and maturation of the fruit bodies. Full maturity is indicated by a completely reddish brown cap and spores are shed on the top of the cap.
- Harvesting is done by plucking, holding the root with one hand and pulling up with another. Scissors and knives can also be used but no residual bud should be left after harvesting. One cycle of the growing takes for 10-15 days.
- After harvesting of the first flush, the crop room condition is again to be maintained as temperature, 28°C, 95% RH, for initiation and completion of the second flush.
- Depending upon the conditions, 3-4 flushes come out and a total of 250 g fresh mushroom from one Kg dry substrate can be obtained.
- One crop takes about four months.

DISEASES OF MUSHROOM AND THEIR MANAGEMENT

Important diseases of mushrooms and their management

Name of disease	Causal organism	Symptoms	Management
Fungal diseases			
Dry Bubble in white button mushroom. It is also known as Verticillium disease, brown spot or La mole.	*Verticillium fungicola*	The infection occurs at the pin head stage (before differentiation of cap and stalk) the whole tissue will look like bubbles of 2 to 25 um in diameter. If the infection occurs at a later stage, the diseased mushroom becomes cracked and deformed	1. Pick and destroy infected mushrooms. 2. Use sterilized casing soil and pasteurized compost. 3. Affected patches may be sprayed with 2 % formalin. 4. Proper environmental conditions like humidity 80-85%, and temperature up to 14^0C help to reduce the disease.
Dry Bubble in Oyster mushroom.	*Diehliomyces microsporus*	Cottony weft of mycelium on bed surface. Wefts turn to dense small reddish brown, wrinkled, stromatic bodies resemble a truffle. Infected beds have peculiar disagreeable odour.	1. Good sanitation 2. Proper Pasteurization of casing material 3. Low temperature during spawn run
Wet bubble It is also called Mycogone disease, white mould or bubble disease	*Mycogyne perniciosa*	Developed white mouldy growth on the mushrooms, leading to their putrefaction (giving foul odour) with exudates of golden brown liquid. Mushrooms malformed, reduced or deformed caps with swollen stipes.	1. Clean environment around cultivation area. 2. Use of only sterilized casing soil with 1 per cent formalin before 5-7 days of its application followed by an immediate spray of carbendazim or benomyl or thiabendazole @ 0.1% after casing. 2. Heating and fumigation of mushroom houses is a good laboratory practice.
Cobweb It is also known as soft decay, dactylium disease and mildew disease	*Hypomyces rosellus* *Cladobotryum dendroides*	First appearance of small circular patches of white silky mycelial growth on the casing surface. As the disease progresses, a fluffy white mould grows over the mushrooms which look like cotton balls. Eventually mushroom turn turns brown and rots.	1. Sterilization of casing mixture at 50^0 c for 4 hrs. or with 1 percent formalin before 2-3 days of its application is the best practice to control the disease. 2. The RH and temperature during picking should not be exceed 90 % and 65^0F.
Green mould	*Trichoderma koningii,* *T. viride* *T.aggressivum f.sp.aggressivum*	Dark green mould patches appeared on casing and then spread to the stems. However, it is the most common disease of oyster mushroom where green coloured patches are appeared on mushroom bags and spawn bags.	1. Proper pasteurization and conditioning of compost check green mould. 2. Dipping a cotton swab in formalin solution (2%) and scrapping off the affected area might control. 3. If the cube attacks more than half, then the entire cube should be discarded. 4. Contaminated cube must be burnt or buried in a place far from the cropping room to avoid re-infection.
Cinnamon Mould Common name : Cinnamon brown mould, brown mould	*Chromelosporium fulva (Peziza ostrachoderma)*	Rapidly grow, circular, yellow-gold to golden brown patches are developed which later turn to cinnamon brown in circular patches. It is very common in areas where compost overheated during spawn run may be colonized.	1. Casing soil should not be made completely sterile by steam or formaldehyde. 2. Newly cased beds should be sprayed with Dithane Z-78 and maintain proper moisture content in casing layer.
Olive green Mould	*Chaetomium spp. (C. globosum)*	Grayish white mycelium is noticed on the compost soon after spawning. Spawn growth becomes delayed and reduced. Olive green to brown pin head size perithecia are developed in the infected areas.	1. The fermentation period of the compost should not be too short. 2. It is essential to use active compost that is not too wet and has a good structure. 3. Should not be added nitrogen, ammonium sulphate, urea, chicken manure or similar materials just before filling in tray or bag.

Yellow mould	*Myceliophthora lutea, Chrysosporium luteum, C. sulphureum*	Brownish yellow corky layer of mycelium (stroma) with a white fluffy edge is generally observed at the junction of compost and casing layer.	1. Moisture and temperature level must be controlled to check the disease. 2. Proper pasteurization of the casing mixture either exposure for 6h at 51^{0}C or 4h at 54^{0}C.
White plaster moulds	*Scopulariopsis fumicola*	Dense white patches of mycelium on compost and casing soil can be seen, giving flour like appearance.	1. If the compost retains smell of ammonia and has a pH more than 8.0, white plaster moulds become common. Ammonia free compost must be used.
Bacterial diseases			
Bacterial blotch Bacterial blotch of mushrooms is also known as brown blotch and bacterial spot.	*Pseudomonas tolassi* Tolaas (1915) described it as *P.seudomonas fluorescens*, but Paine (1919) proposed *P. tolaasii* Paine.	Circular or irregular yellowish spots develop on or near the margins of the cap which enlarges rapidly under favourable conditions and coalesce to form rich chocolate brown blotches that are slightly depressed. As the disease progress, the symptom spread from pilei to stipes.	1. Manipulation of relative humidity, temperature, air velocity and air movement are significant in the control of this disease. 2. Temperature above 20^{0}C and relative humidity of more than 85 per cent should be avoided. 3. Additional ventilation and air circulation after watering can ensure the quick drying of mushrooms.
Yellow blotch	*Pseudomonas agarici*	Infected fruit bodies turn yellow and stunted. Slimy appearance of infected fruit bodies is a common symptom under high relative humidity (more than 90%). If RH is < 75%, the blotched fruit bodies give burnt ulcers appearance.	1. Proper ventilation and careful watering coupled with monitoring of temperature in the mushroom unit help in limiting the disease incidence.
Mummy Disease The disease was first described in 1942 by CM Tucker and JB Routien in the United States	*P. aeruginosa*	Stalk is bent and the cap tilted. There is a dense growth of mycelium around the base of the stalk on the surface of the casing layer. Mushrooms often fail to mature and remain in the "button" stage with unopened veil.	1. Strict hygienic condition in mushroom house. 2. Disinfecting the casing layer can help to reduce the infection. 3. Dig a trench to separate the diseased area from the healthy. 4. Compost & casing has to be removed from trench
Viral diseases			
La France, Watery stipe, X disease, Die back	Virus Rod shape Spherical, Club shape Bacilliform shape virus	A specific musty smell come out in a growing room. Mushroom fruits appear in dense clusters, maturing too early. The delayed appearance of pinheads in the first flush is an important indication of disease. Formation of fruiting primordia below surface of the casing layer and as soon as these mushrooms appear above the casing soil, their pilei are already opened.	1. Hygienic measures should be followed strictly. Virus free mushroom fungus culture and spawn should be used. 2. Steam the compost for 12 hours at a temperature of 70°C.
Weed			
Ink caps Common names: Ink weed, wild mushrooms	*Coprinus* sp.	It is a weed of mushroom. It is slender, bell-shaped mushrooms. Initially, cream coloured then blueish black and is usually covered with scales.	1. Use properly pasteurized compost and casing soil. 2. Before filling the trays the compost should be free from ammonia. 3. Excessive watering is avoided. 4. Rogue out young fruit bodies of the weed fungus



Green mould (*Trichoderma* sp.) on mushroom bag | Green mould (*Trichoderma* sp.) on spawn bag | Dry Bubble (*Verticillium fungicola*) | Wet bubble (*Mycogyne perniciosa*)

White plaster moulds (*Scopulariopsis fumicola*) | Bacterial yellow blotch (*Pseudomonas tolassi*) | Symptoms of Ink caps | Mummy

MAJOR INSECT, MITES AND NEMATODES OF MUSHROOM AND THEIR MANAGEMENT

Insect pest is another important biotic factors that hamper mushroom cultivation. Mushrooms can be infested by phorid flies, sciarid flies, cecids, springtails, mites, nematodes etc. The major insect pests and their management have been narrated in the table given below:

Name of Insects	Scientific Name	Characteristics of Insect	Damaging characters	Management
Insects-Flies				
Sciarid fly	*Lycoriella mali*	Small black insect about 0.25 inch long, with long antennae and gray wings folded over the back.	The larvae of the sciarid attack compost, spawn, mycelia, pins, and mushroom stems and caps.	1. Nylon or wire net (not less than 35mesh) should be placed at window to prevent the entry of flies into the crop rooms. 2. Mixing thoroughly fipronil 3% GR @ 100g in the 100kg to casing soil. 3. During composting, mix thoroughly fipronil 3% GR @ 50g/q of wheat straw during 7th turning. 4. Spray the affected patch with 2% formalin.
Phorid fly	*Megaselia halterata*	Flies are small, light to dark brown colour, 1.9-2.0 mm long, with a humpback appearance and very small antennae. They are stockier than sciarids and are very active, running and hopping erratically.	The flies feed on mycelia, and restricted spawn run. The infested mushrooms turn brown and leathery with rotting tissues.	
Cecid flies	*Mycophila speyeri*	The Cecid fly is a mosquito-like insect that lays its eggs. First daughter larva emerges from the mother. Final instar larvae are characterized by the presence of a "sternal spatula," a darkly pigmented, cuticular structure found on the anterior-ventral surface of the animal. Larvae which have this structure are destined for metamorphosis.	Cecids feed on the mushroom stems or gills, reducing marketable yield.	
Springtails	*Seira iricolor*	Adults are observed to be ground colour with light violet alongside of the body without forming a definite pattern. The adults measured 2.9 mm longs.	Adults & nymphs feed on mycelium by scraping from the spawn grains and cutting the mycelial strands.	1. Crop beds should be raised-off from the floor. 2. Compost & casing soil should be properly sterilized 3. Surrounding areas should be sprayed with fipronil 5% SC @ 1ml/lit of water.
Slugs and Snails			The pests chew up portion of the mushroom which may later get infected with bacteria, nematodes virus etc. and affect the quality of the crop.	1. Pests from the cubes should be removed and killed. 2. Hygienic conditions must be maintained.
Mites:				
Sporophagous mites	*Tryophagus putrescentiae*		It is feed on mycelium and damage sporophores by causing shrunk caps and brown rusted spot on buttons	1. Cleaning of the mushroom house and disposal of all organic debris. 2. Disinfestations of the mushroom house by spraying floor, wall with sulphur dust @ 2g/lit of water. 3. If mites present in the compost, spray of sulphur dust @ 2 g/lit water is advised.
Mycophagus mites (Red pepper mites)	*Pygmephorus* sp.	They are tiny, yellowish-brown in colour.	Mycophagus mites swarm in vast numbers on the surface of the casing and mushroom fruit. They cling to the body of mushroom flies and are spread from infested to uninfested mushroom farms.	
Nematode	*Aphelenchoides composticola, Ditylenchus myceliophagus* and *Rhabditis lambdiensis.*		Nematode attack compost become devoid of spawn smells fouls and sunken areas appear on the mushroom bed surface. Nematodes feed the contents of the hyphae, destroying them and turning the compost sudden. They also eat the mushroom and turn them brown/watery.	1. Casing soil should be sterilized by steam (70-75^{0}C for 6 hrs) or formaldehyde 40% (5% solution). 2. Adding and mixing of nemagon @ 40ml/30 kg in wheat straw compost.
Rodents	-		The attack by rodents is found mostly in low cost mushroom house (mud house). They eat the grain spawn and make holes inside the cubes.	1. Application of rat trap or sticky trap. 2. Burrow of rats should be close down with glass pieces and plaster.
Snake	-		They make hole in the mushroom bag for habitat.	1. Broadcasting of fipronil and 3% GR as borderline outside the hut is advised.

Insect pest is another important biotic factors that hamper mushroom cultivation. Mushrooms can be infested by phorid flies, sciarid flies, cecids, springtails, mites, nematodes etc. The major insect pests and their management have been narrated in the table given below:

PRESERVATION AND STORAGE OF MUSHROOM CULTURE

Cultural preservation is indispensable to have a stock of mycelium and for smooth spawn operation. The main objective of culture preservation is to store cultures in a viable and stable form for longer periods without losing genotypic, phenotypic and physiological traits.

1. **Frequent Transfer:** The most common method of short-term storage of mushroom culture is the storage of culture tubes either at room temperature (25–30^{0}C) for a period of 1–2 months or in a refrigerator (5–8^{0}C) for an average period of 3–4 months. More ordinary refrigeration of cultures at -10^{0}C and preferably lower, will allow storage of mycelium for a year or two with little loss in quality.
2. **Silica gel:** Silica gel is sterilized at 180^{0}c for 3h and stored in a tight container. Separately a spore or bacterial suspension is prepared in 5% skim milk at 4^0 c. Chill the silica to get to 4^{0}c and place it in a water bath. Add the suspension to the silica gel (@ 0.5 ml/4 gm) and leave the bottle in the bath for 30 minutes. Store at room temperature for 1 or 2 weeks with cap loose. Check the visibility of the culture is viable tighten caps and store at 4^{0}c for 4-5 yrs longevity.
4. **Soil Preservation: -** Small amount of garden soil with 20% moisture sterilized at 15 lb for 15 minutes for continuous 3 days. Culture suspension was prepared in sterilized water and added into soil, preserved at 4^{0}c.refrigerator.
5. **Freeze Drying -70^{0}c (Lyophilization):** Preservation of culture by drying (-70^{0}c) in a vacuum from the frozen state by the process known as sublimation for 5-10 yrs longevity.

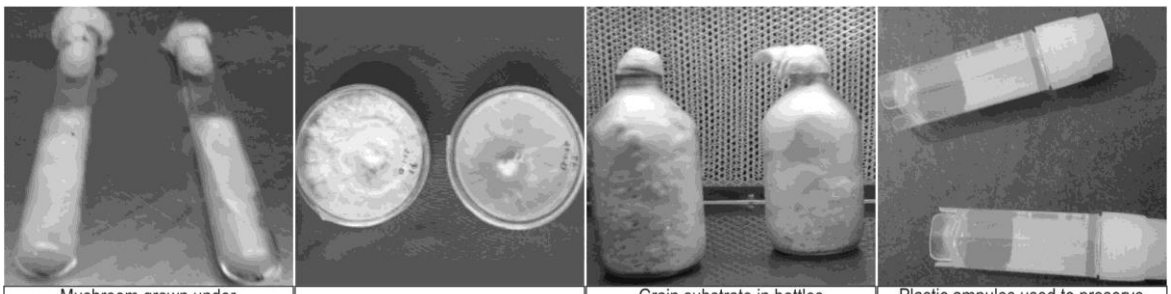
   Steps of lyophilization:

   i) Refreezing of cell suspension in 10% skim milk,
   ii) Vacuum drying in frozen state at low temp,
   iii) Continued vacuum drying at room temperature, and
   iv) Sealing of ampules.

   Freeze-drying and freezing are the most economical and effective methods under which the spores of mushroom fungi remain dormant and viable for a longer period.

Liquid N_2: when culture store at –196^{0}c, Metabolic activities is completely stopped, liquid N_2 = -196^{0}C, Liquid air = -184^{0}C (rust). Liquid nitrogen refrigeration is now considered the only adequate method to preserve mushroom cultures. The only long-storage method of *Volvariella* cultures is liquid nitrogen storage.

Mineral Oil/paraffin wax: Healthy culture covered by mineral oil up to 1 cm and stored at a cool place. Cultures stored in mineral oil can be kept either at room temperature or in a refrigerator for 1 to 3 years.



Mushroom grown under slant culture for storage | Mushroom mycelium in culture plate | Grain substrate in bottles for storage of mycelium | Plastic ampules used to preserve mycelium in liquid nitrogen refrigerator