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Practical 1

ORIENTATION TO A MUSHROOM FARM

OBJECTIVES

After completing this practical you will be:

- Orientated to a mushroom farm.
- Identify different types of edible and non edible mushrooms.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Disposable mask and shoes, Disinfectants, Soap

PROCEDURE

1. Visit any nearby site undertaking seasonal cultivation of button mushroom.
2. Visit a nearby integrated mushroom farm or public sector mushroom institute having spawn lab, compost unit, cropping rooms, analysis lab and museum.
3. Identify different types of mushrooms during visit to farm and museum.

OBSERVATIONS AND RESULT

1. Photograph and record names of main equipments in spawn lab and how much spawn can be produced per day in the lab visited by you.
2. Record different components of compost yard (like pre-wetting area, compost pile making area, bunker, tunnel, spawning area, etc).
3. Record the size of tunnels and its production capacity.
4. List the different mushrooms observed by you in farm or museum and record their characteristics.

Notes

Common name	Scientific name	Colour	shape	Remarks

PRECAUTIONS

1. Follow the hygiene related instructions and wear the mask and disposable shoes wherever instructed.
2. Do not pick up mushrooms from the bed without permission.
3. Take permission before photographing any part of the unit or crop.

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Practical 2

PREPERATION OF PURE CULTURE AND MAINTAINANCE OF CULTURES

OBJECTIVES

After completing this practical you will be able to:

- Prepare pure culture of mushroom by different methods.
- Use pure culture for preparation of spawn.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Boiling Pan, Heater/Gas Stove, Autoclave Vertical Small, Laminar Air Flow, Incubator, Refrigerator, pH Meter, Lab Coats, Gloves, Caps, Footwear, Masks, Disposable Coats, Gas/LPG Cylinder, Burner/Spirit Lamps, Weighing Balance, Flask, Culture Tubes, Petri Plate (Pre-Sterilized), Non Absorbent Cotton/Polyfill, Forcep, Inoculation Needle, Spirit, Sanitizer, Alcohol, Agar-Agar, Glucose, Malt Extract, Potato, Sodium Hydroxide, Hydrochloric Acid, Yeast Extract

PROCEDURE

A. Preparation of Potato Dextrose Agar /Malt Extract Agar Medium

1. Take 20 g dextrose, 20 g peeled potato and 20 g agar-agar.
2. In a pan take one litre of distilled water and boil peeled potato. After boiling, sieve the solution and mix dextrose and agar-agar in it and stir the medium properly.
3. Fill above solution in test tubes up to 1/3rd of size or half in conical flask as per requirement.
4. Plug the mouth of tube/ conical flask with non-absorbent cotton.

Notes

5. Autoclave the material by keeping in autoclave for 15 minutes at 121°C and 15 psi pressure.
6. Take out the test tubes and conical flasks.
7. Put the test tubes in standing position to make slants or pour 20 ml above medium in Petri dishes in Laminar Air flow and leave here to solidify.

B. Preparation of Culture

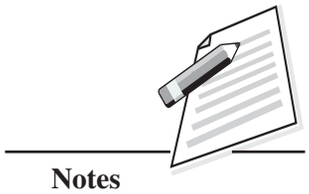
1. Bring test tube of pure culture of any mushroom from recognised source or take fresh fruit body of mushroom. Clean it with alcohol. Cut the mushroom vertically and with the help of sterilized knife, take small piece out of it
2. Take inoculating needle, heat it to red, cool in alcohol by dipping in it.
3. Open the pure culture tube and take out small bit of mycelium with inoculating needle and inoculate in other test tube or petri dish having autoclaved medium or inoculate tissue of above cleaned mushroom. Inoculate the medium (PDA/MEA) with tissue piece of mushroom/spore/a bit from culture tube.
4. Keep the inoculated test tubes or petri dishes in BOD incubator or 24±1°C. Put in BOD Incubator at 24±1°C for mycelial growth.
5. Fresh culture will become ready within 25-30 days. Pure culture of mushroom is ready for further use to prepare mother spawn.
6. Retrieve the pure culture periodically.

OBSERVATIONS AND RESULT

1. Colour of medium becomes off white when potato is used whereas when Malt Extract is used, it becomes dark brown in colour.
2. Medium solidify at cooling.

PRECAUTIONS

1. Mix the ingredients of medium thoroughly
2. Do autoclaving at prescribed temperature and pressure.
3. Pour optimum quantity of medium in petri dishes or test tubes.



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Practical 3

PREPERATION OF MOTHER SPAWN, COMMERCIAL SPAWN AND ITS STORAGE

OBJECTIVES

After completing this practical you will be able to:

- Prepare mother spawn.
- Multiply spawn for commercial use.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Boiling pans/boiling kettle, Stove or steam, pH meter, Autoclave, BOD incubator, Laminar air flow, Refrigerator, Glassware, chemicals for medium preparation, non-absorbent cotton, polypropylene bags (and bottles), disinfectant (formaldehyde), calcium carbonate, calcium sulphate, Wheat/Sorghum/Bajra, Steel racks, working tables, troughs, sieves, inoculating needles, scalpels, test tubes, petri plates.

PROCEDURE

1. Take wheat/sorghum/pearl millet or any other cereal grain for making spawn.
2. Boil wheat grains partly so that the grains become slightly soft but do not burst.
3. Put grains on a sieve to drain out the excess water and allowed to dry for evaporation of surface water
4. Add chalk/calcium carbonate (0.5%) and gypsum (2%), mix thoroughly and fill in bottles/ polypropylene bags.

Notes

5. Plug the bottles using plugs made of non-absorbent cotton/polyfill.
6. Sterilize bottles/bags in autoclave for 1.5 hour at pressure of 22 pounds per square inch (psi). The temperature inside the autoclave should reach to about 126.5°C.
7. Take out bottles/bags, cool and inoculate with mushroom culture in laminar flow.
8. Incubate bottles/bags at 25°C for 2-3 weeks during which the mushroom mycelium fully colonizes (covers) the wheat grains.
9. Mother spawn becomes ready.
10. Commercial spawn is made in polypropylene bags and the readymade spawn from bottles (commonly called mother spawn) is used as inoculum for spawn to spawn multiplication. Few grains of readymade spawn are added in each bag. Normally it takes 15-20 days for complete spread of mycelium on the grains.

OBSERVATIONS AND RESULT

1. Boiled wheat grains becomes white at complete colonization by mushroom mycelium
2. Sweet smell of mushroom emits from fully colonized bags.
3. Record consumption of raw material, pressure and temperature of autoclave and temperature of incubation.

S. No.	Name of Strain	Name of mushroom	Date of Inoculation	Temperature (°C)	Date of shifting to Cold Store

PRECAUTIONS

1. Always keep the inoculation chamber and its surroundings very clean.
2. Use clean grains for spawn.

Notes

3. Take utmost caution in autoclaving.
4. Switch on UV tube in the inoculation chamber for 30 minutes before inoculation by keeping sterilized substrate, forceps, cultures inside the chamber. Switch off UV tube 15 minutes before you enter the inoculation room.
5. Inoculation is always done near the spirit lamp flame to avoid contamination.
6. Swab your hands and clean inoculation area using alcohol.
7. Shake bottles and bags after 10 days or so to ensure uniform white silky growth and also to remove contaminated bottle.
8. All the bottles must be labelled indicating firms name, species, variety, date of inoculation to know the age and type of spawn.

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Practical 4

COMPOSITING METHODS

OBJECTIVES

After completing this practical you will be able to prepare selective compost for cultivation of button mushroom following appropriate steps.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Source of water, Loader, Phase-I and Phase-II tunnels/Bunkers, Blowers, Temperature meter, Dragger Pump and tube, pH meter, Wheat Straw, Chicken Manure, Gypsum, Wheat Bran, Urea.

PROCEDURE

1. Wetting of ingredients. Wet wheat straw and chicken manure thoroughly till they absorb sufficient water (around 75%). Collect leached water in a gudy pit constructed for the purpose and use it regularly for wetting the materials. After thorough wetting of the substrates make a simple heap/stack of such materials.
2. Shift the material to bunkers after pre-wetting for two days and thorough mixing of all the ingredients.
3. Inject air regularly or in pulses and shift material to next bunker after mixing after 2-3 days.
4. Shift to Phase-II for pasteurization after three to four such changes/turnings in tunnels.
5. After few hours of filling, raise temperature inside the tunnel to around 57-60° C for 6-8 hours to pasteurize the compost.

Notes

6. Drop this temperature slowly in the range of 55-45°C and maintain this condition for 4-5 days for conditioning as number of heat loving (thermophillic) fungi grow and these give a conspicuous white colour to the compost.
7. Cool the compost.
8. Ready compost must free from ammonia.

OBSERVATIONS AND RESULT

Characteristics of the compost at the end of phase-I

- Brownish throughout. Pieces of straw gleaming and wet.
- Straw rather long but can be broken with some force.
- Properly hydrated, around 72-75% moisture; when squeezed drops of water .appears between the fingers.
- Very heavy smell of ammonia. pH approximately around 8.2-8.5.
- Still sticky and slimy, hands get dirty and wet.
- Actinomycetes (fire fangs) not so apparent.
- Nitrogen content between 1.5-2.0%; ammonia concentration around 800-1000 ppm.

Characteristics of the compost after Phase-II

- Dark brown in colour, full of thermophilic fungi and actinomycetes.
- It is soft, straw breaks rather easily.
- Moisture around 64-66%. No liquid oozes when squeezed firmly.
- Pleasant sweet smell.
- No stickiness. Hands stay clean and dry.
- N content > 2%.
- Ammonia below 10 ppm.

Notes

Note down the observations as follows:

S. No.	Date of Stack	Date of shifting in Phase-I	Date of shifting in Phase-II	Temperature (^{o}C)	Moisture (%)

PRECAUTIONS

1. Compost ingredients must be thoroughly mixed.
2. Maintain optimum level of moisture and aeration on compost
3. Must smell sweet, no ammonical smell.

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Practical 5

SPAWNING, CASING, CROPPING AND POST HARVEST HANDLING

OBJECTIVES

After completing this practical you will be able to

- Use different methods of spawning, casing, cropping, management of temperature and carbon dioxide at different steps for cultivation of button mushroom.
- Perform post harvest storage of button mushroom.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Thermometer, CO_2 Meter, Hygrometer, Spawn, Pasteurized Compost and Casing Soil

PROCEDURE

1. Take ready pasteurized compost and mix spawn in compost @ 0.5-1%.
2. Fill 10-12kg spawned compost in bags up to a feet OR spread the compost on beds upto a depth of 6".
3. Keep bags at a temperature of 23-24°C and maintain the temperature inside the bags at around 25°C.
4. Keep the rooms closed during spawn run to allow carbon dioxide content to go up to 10,000 ppm.
5. Complete spawn run takes about two weeks.

Notes

6. Do casing by covering the spawned compost by 1.5 inch thick layer of casing soil. Casing soil can be mixture of coir pith, farm yard manure, burnt rice husk and soil (Fig. 5.1).

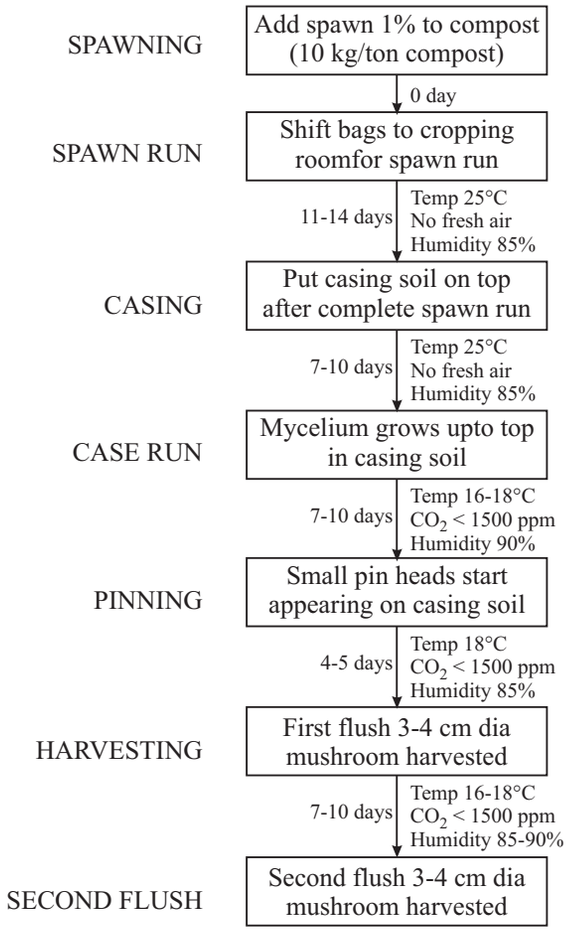

Fig. 5.1: Casing

7. After casing, keep temperature around 25°C for a week or so when mycelium travels into casing soil.

8. Lower down temperature to 16-18°C at this stage, gradually and introduce fresh air to lower the carbon-dioxide concentration to 1000-1500 parts per million (ppm). This induces pinning (small pin head size fruit bodies), which mature into fruit bodies within 3-4 days.

9. Harvest the mushroom by twisting.

10. To get next flush (crop) of mushrooms, maintain temperature (16-18°C), humidity (85-95%) and carbon-dioxide (1000-1500 ppm).

Notes

11. Keep mushroom in low temperature for marketing.
12. Do packaging in punnets (small boxes) instead of poly-bags.
13. Transport in refrigerated vans.
14. Mushrooms can be canned, freeze dried, or can be used for making pickles and number of other products.

OBSERVATIONS AND RESULT

1. Note down moisture content of substrate.
2. Do record temperature of growing rooms.
3. Monitor and record temperature of pasteurization chamber.
4. Record your observations as follows:

S. No.	Moisture (%)	pH	Temperature (°C)	Humidity (%)	CO_2 (ppm)

PRECAUTIONS

1. Moisture content of ready compost should not exceed 65-66%.
2. Variation in temperature and relative humidity should be minimum.
3. Maintain proper ventilation and provide optimum climate.

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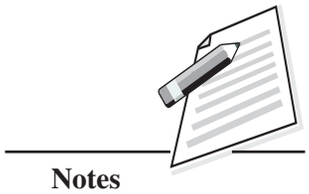

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Practical 6

CULTIVATION OF OYSTER MUSHROOM

OBJECTIVES

After completing this practical you will be able to

- Prepare substrate and perform cropping for oyster mushroom.
- Practice post harvest storage of oyster mushroom.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Autoclave, Boiler, Pasteurization Chamber, Tank, Polythene bags, Spawn, Straw, Plastic/Gunny bags

PROCEDURE

Preparation of substrate:

A. Use of straw as such without any treatment

1. Soak the straw in water as such overnight and use for cultivation. To make the pH slightly basic, add lime powder (1%) in the water.
2. Drain the excess water and air dry before spawning.
3. Add about 2.5% spawn on wet weight basis.

OR

B. Pasteurisation using chemicals

1. Sterilize straw using chemicals by soaking it in water containing formalin and carbendazim. For 10 kg straw, take 100 liters water, add 7.5 g carbendazim (50WP) and 125 ml formalin.

Notes

2. Soak straw in this solution for 18 hours and then take out to air dry for 2-4 hours depending upon the season.
3. Material is ready for spawning.
4. Do spawning @ 2.5% spawn on wet weight basis of straw.

OR

C. Hot water treatment after wetting the straw

1. Wet straw overnight.
2. Take out and soak in hot boiling water. Allow straw to stay in this water for few hours and then cool it before spawning.
3. Material is ready for spawning @ 2.5 of wet weight basis.

OR

D. Pasteurization in the tunnels

1. Soak straw outside just like wetting in button mushroom and later on fill in tunnels.
2. Introduce steam to raise the temperature to 60-62 °C and maintain it for 4-6 hours, followed by conditioning for 30-36 hours at 44-48 °C and then cool down.
3. Material is ready for spawning @ 2.5% on wet weight basis.

OR

E. Autoclaving

1. Soak straw in water and then fill in polypropylene bags.
2. Sterilize these bags at 22psi for 2 hours.
3. Bags are ready for inoculation with oyster spawn @ 2.5 on wet weight basis.

OR

F. Cultivation of Oyster

1. Do spawning in wetted/pasteurized straw @2-5% on wet weight basis. (one kg dry straw will require about 100 g of spawn). Mix Spawn thoroughly or put in layers inside the bag.

Notes

2. Keep bags inside room or any hut or on ground, or in tiers or hang from the roof or rack with the help of nylon rope.
3. Make small perforations in the bags for aeration.
4. Keep spawned bags at temperature of 24±2 °C for two weeks, whole bag becomes white.
5. For induction of fruiting, give diffused light and fresh air for 3-4 hours daily for production of normal fruit bodies on the bags. Large holes can be made in the bag or whole of polythene can be removed.
6. Keep temperature during cropping around below 20 °C or around 25 °C (depending upon the species) and humidity around 85 %.
7. Harvest mushrooms manually by twisting.
8. Do not water before harvesting.
9. Dry oyster mushroom in sun or in dry air cabinet.
10. Drying temperature should not be high (>60°C).

OBSERVATIONS AND RESULT

1. Note down moisture content and pH of substrate.
2. Record temperature and humidity during substrate preparation as well as during cropping.

S. No.	Moisture (%)	pH	Colour of mushroom	No. of days taken for growing

PRECAUTIONS

1. Moisture content of substrate must not exceed 62%.
2. Straw should be completely pasteurized/ sterilized.

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Practical 7

CULTIVATION OF PADDY STRAW MUSHROOM

OBJECTIVES

After completing this practical you will be able to follow appropriate steps for preparation of substrate and cropping of paddy straw mushroom under indoor conditions.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Chaff Cutter, Sutli, Paddy Straw, Besan, Spawn, Polythene, Ginning Mill waste cotton, $CaCO_3$

PROCEDURE

A. Compost preparation

1. Wet the Substrate (cotton waste or paddy straw + Cotton ginning mill waste in 1:1, w/w ratio) for first 2 days with sufficient treading of the cotton waste so that it absorbs sufficient water.
2. Add poultry manure @ 5.0% to the wetted substrate.
3. Raise Pile of substrate wetted for two days and pile should be is 1.5 m high × 1.5 m wide.
4. Give three turnings at an interval of one day each and add calcium carbonate @ 1.5% (dry wt basis) at third turning and left substrate for fermentation for next one day.

B. Bedding and Pasteurisation

5. After four days of outdoor composting, spread 5 cm to 10 cm thick compost on shelves.

Notes

6. Inject live steam after 8-12 hours of compost filling in the room. Maintain a temperature of 60-62 °C for 4-5 hours for cotton waste compost and 65 °C for 6 hrs for paddy straw compost.
7. After pasteurisation, keep compost at a temperature of 50 °C for next 24-36 hrs and followed by its natural cooling.
8. Do spawning with fresh spawn @ 1.5% (dry weight) or 0.4% (wet weight) basis of the compost at a depth of 2 to 2.5 cm at a distance of 12 to 15 cm apart.
9. Cover spawn with displaced compost and cover over bed with thin plastic sheet.
10. Maintain room temperature at 32 to 34 °C during spawn run for 4-6 days.
11. Give light along with little more ventilation in the rooms at end of 4-6 days of spawn run.
12. Remove plastic sheets after 4-6 days of spawning and spray little water on the beds.
13. Pinhead starts appearing on 5^{th} – 6^{th} day of spawning and after another 4 to 5 days, the first flush of mushroom will be ready for harvest.

OBSERVATIONS AND RESULT

1. Substrate seems completely fermented.
2. Monitor and record temperature and humidity of growing room.
3. Record the date of spawning and date of start of crop.
4. Substrate got colonized completely after 4-6 days.
5. Record your observations as follows:

S. No.	Temperature (°C)	Humidity (%)	Appearance of Compost

Notes

PRECAUTIONS

1. Substrate must be completely pasteurized after fermentation.
2. Maintain proper temperature, humidity and ventilation at all steps.

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Practical 8

CULTIVATION OF MILKY MUSHROOM

OBJECTIVES

After completing this practical you will be able to follow different steps for preparation of substrate and cropping of milky mushroom.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Polythene bags, Spawn, Straw, Plastic/Gunny bags

PROCEDURE

A. Preparation of Substrate:

Take straw, clean it and wet it and fill in plastic gunny bags.

(a) Hot water treatment

1. Boil water and submerge chopped wet straw filled in gunny bags in hot water for 40 minutes to achieve pasteurization.

OR

(b) Steam pasteurization

1. Fill wet straw inside insulated room on perforated shelves.
2. Release steam from a boiler, raise temperature inside substrate to 65 °C and maintain it for 5-6 hours.
3. Circulate air inside the room to achieve uniform temperature in the substrate.

OR

Notes

(c) Autoclaving

1. Fill substrate in polypropylene bags (35 x 45 cm, holding 2-3 kg wet substrate)
2. Sterilize bags at 15 psi for 1 hour.

(d) Spawning

1. Shift these bags for cooling and spawning.
2. Do layer spawning
3. Shift spawned bags to spawn running room and keep in dark.
4. Maintain temperature between 25-35 °C with 80% RH, to get substrate colonised by spawn.

(e) Casing

1. Prepare Casing mixture of coir pith and FYM and autoclave at 22psi for two hours.
2. Cut open bags from top and do casing.
3. Keep casing thickness between 3-4 cm. pH of casing material be adjusted to 7.8-7.9 with chalk powder.
4. Maintain temperature of 30- 35°C and RH 80-90% for entire cropping cycle.

(f) Cropping

1. Introduce fresh air after mycelium appears on casing soil.
2. Do minimum 3-4 air changes per hour.
3. Do watering regularly as good moisture and humidity are important.
4. Mushrooms starts appearing on the top of bag within two weeks.
5. Harvest mushrooms by twisting, clean and pack in perforated/ polypropylene bags for marketing.

OBSERVATIONS AND RESULT

1. Note down moisture content of substrate.
2. Do record temperature of hot water.

Notes

3. Monitor temperature of pasteurization chamber.

4. Write down your observations as follows:

S. No.	Moisture (%)	pH	Temperature (°C)	Humidity (%)

PRECAUTIONS

1. Moisture content of substrate must not exceed 60-62%.
2. Straw should be completely pasteurized.
3. Do casing after complete colonization of substrate.
4. Remove bags which are not colonized/ affected by moulds.

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Practical 9

CULTIVATION OF SHIITAKE

OBJECTIVES

After completing this practical you will be able to follow appropriate steps for preparation of substrate and cropping of shiitake mushroom.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Culture Tube, Weighing Machine, Autoclave, Laminar Air Flow, Air Conditioner, Saw Dust, PP Bags, $CaSO_4$, $CaCO_3$, Spawn, Wheat Bran.

PROCEDURE

Substrate preparation

1. Take 80% sawdust of poplar or saw dust of any other broad leaf tree; 19-20% wheat bran; $CaCO_3$ (1.5%), gypsum (0.5%), Sugar (1%) and citric acid (0.2%).
2. Mix the substrate thoroughly.
3. Add water to wet the substrate and maintain 60-65% moisture and pH 5.5-6.0 using gypsum and lime.
4. Dissolve soluble ingredients (citric acid, sugar and sulphate) in water before mixing in saw dust.
5. Soak the sawdust for 14-16 hours.
6. Mix all the ingredients thoroughly.

Bag filling and autoclaving

1. Fill the Polypropylene (heat resistant) bags (1.5 kg) immediately after mixing and wetting the substrate.

Notes

2. Give cylindrical shape by putting pressure to the bags.
3. Make vertical hole in the centre of the bag for later inoculation.
4. Push through plastic ring and plug with non-absorbent cotton.
5. Do sterilization of substrate in autoclave at 22 psi for 2 hours.

Spawning and spawn run

1. Do spawning of substrate @ 3% under aseptic conditions.
2. Place bags in cropping room (incubate) in a 4 h / 20 h light/ dark cycles at 23-25°C.
3. Incubate the bags for spawn run for 60-80 days. During the period it goes through mycelial growth, mycelail coat, mycelial bump and browning stages.
4. Provide aeration for development of pigmentation to turn brown.
5. Remove the plastic when bags partially (half or one third) turned brown. The coat will gradually become hard. While outside of the substrate should be hard, the inside should be softer and moist.

Fruiting

1. Provide suitable temperature, high RH, good ventilation and cold water shock treatment for induction of fruiting.

Various cropping parameter

Stages/Activity	Days	Temperature (°C)	Light intensity (Lux)	Humidity (%)
Incubation	50-120	25±2	500-1000	65-70
Induction	2-4	10-20	500-1000	85-95
Fruiting	7-14	12-18	500-1000	60-80
Rest	7-21	20-30	None	65-70
Induction	2-4	10-20	500-1000	85-95

2. Harvest the mushroom by holding the stalk of the mushroom and break them from the substrate.
3. Don’t tear mushroom from the surface.

Notes

4. Harvest mushrooms at an early stage.
5. Don't water scars left after harvesting for 3-4 days.

OBSERVATIONS AND RESULT

1. Sawdust first becomes white, and then started browning.
2. Record date of spawning, time of incubation, date of cold treatment.
3. Record temperature, humidity and light intensity periodically.
4. Coat becomes hard, formation of mycelia bump at growing stage.
5. Note down your observations as follows:

Sr. No.	Date of Spawning	Date of Cold treatment	Temperature (°C)	Humidity (%)	Light intensity

PRECAUTIONS

1. Moisture content of saw dust based substrate must not exceed 60-65%.
2. Substrate must be sterilized immediately after bag filling to avoid fermentation.
3. pH of substrate should range between 5-6.
4. Optimum conditions for growth and fruiting should be provided.

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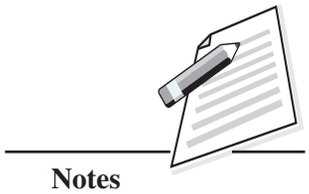

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Practical 10

CULTIVATION OF *GANODERMA*

OBJECTIVES

After completing this practical you will be able to follow appropriate steps for preparation of substrate and cropping of *Ganoderma.*

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Culture Tube, Weighing Machine, Autoclave, Laminar Air Flow, Air Conditioner, Saw Dust, PP Bags, $CaSO_4$, $CaCO_3$, Spawn, Wheat Bran

PROCEDURE

1. Take the sawdust of broad-leaf trees (Poplar, Sheesham) or mixture of trees.
2. Add 20% wheat bran to sawdust, and wet it to a level of 60-62% moisture.
3. Add one per cent of calcium sulphate (gypsum) and one percent of calcium carbonate (chalk powder) per kg sawdust to get a pH of 5.5.
4. Fill mixed substrate (700 g dry wt; 2.1 kg wet wt) in polypropylene bags and plug the mouth with non-absorbant cotton after putting a plastic ring on mouth of PP bag just like grain spawn pack of mushrooms in polybags.
5. Sterilize the bags in autoclave at 22 psi. for 2 hours.
6. Spawn the substrate with wheat grain spawn @ 3% on the dry weight basis under sterile conditions after cooling.
7. Incubate for Spawn-run at 28-35°C in closed rooms (high carbon dioxide) and darkness.
8. After complete spawn run (bags become white all over), which takes about 25 days, cut polythene at top level of the substrate totally exposing the top side.

Notes

9. Provide proper conditions for fruiting or pinning (temp. 28-32°C, 1500 ppm CO_2, 800 lux light, 95% RH).

10. Once the pins have grown up enough to form the cap reduce relative humidity to 80% and introduce fresh air to achieve around 1000 ppm CO_2.

11. Once the cap is fully formed as indicated by yellowing of the cap margin, lower the temperature to 25°C and RH to 60% for cap thickening, reddening and maturation of the fruit-bodies.

12. Do harvesting by tight plucking, holding the root with one hand and pulling up with another; or use scissors and knives to harvest the mushroom but no residual bud should be left after harvesting.

13. After harvesting the first flush, provide conditions for pinning again i.e. 28°C, 95% RH, 1500 ppm CO_2, 800 lux light for staring and completing the second flush.

14. One cycle of the growing takes 10-15 days. Depending upon the conditions, 2-3 flushes appear and a total 25% B.E. can be achieved. One crop cycle takes about four months.

15. Provide following conditions at various stages:

Stage	Activity/Conditions
Ingredients	Saw dust (I kg) + Wheat bran (0.2 kg) + $CaCO_3$ (10g) + $CaSO_4$ (10g)
Substrate preparation	Wetting (65%), bag filling (2.1 kg), autoclaving at 22 psi for 2 hr
Spawning	Saw dust or wheat grain spawn @ 3% on dry weight basis
Spawn run	28-32°C, high CO_2, dark
Pinning	28-32°C, RH 95%, 1500 ppm CO_2, light > 800 lux
Cap formation and growth	28-32°C, RH 80%, 1000 ppm CO_2
Cap maturation	25 °C, RH 60%
Harvesting	Fully reddish brown cap, tight plucking

Notes

16. Burn the used substrate as fuel.

17. Wash harvested mushrooms with water and dry at low temperature (<50°C) in the cabinet driers, preferably at 35°C in the dehumidifying cabinet drier. Freeze drying is, however, the best.

OBSERVATIONS

1. Note down that saw dust has been fully colonized by mycelium and become white.

2. Sawdust bags have become hard.

3. Record temperature, humidity during incubation and fruiting.

4. In starting, white with yellowish flat fan shaped mushroom starts appearing turns into red.

5. Bags become light weighted at completion of crop.

6. Record your observations in the table given below.

S. No.	Temperature (°C)	Humidity (%)	Days in Spawn Run	Fruiting days

PRECAUTIONS

1. Moisture content of saw dust based substrate must not exceed 60-62%.

2. pH of substrate should range between 5-6.

3. Optimum conditions for growth and fruiting should be provided.

4. SMS should be discarded after cooking out.

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Practical 11

CULTIVATION OF *CORDYCEPS MILITARIS*

OBJECTIVES

After completing this practical you will be able to practice different steps for preparation of substrate and cropping of *Cordyceps militaris* mushroom

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Autoclave, BOD incubator, Weighing Balance, Shaker, Laminar Air Flow, Flask, Measuring Cylinder, Jars, Glucose, Peptone, $MgSO_4$, KH_2PO_4

PROCEDURE

A. Liquid Spawn

1. Preparation of Liquid Medium and Liquid Spawn

   (i) To begin with, mycelium is cultured on a liquid medium.

   (ii) Take glucose 30 g; peptone 10 g; yeast extract 5 g; KH_2PO_4 1 g; $MgSO_4$ 0.5 g and dissolve these in one litre of distilled water.

   (iii) Pour medium in flasks (50 ml in 250 ml flask), plug with non-absorbent cotton autoclave it, cool and culture it from test tubes and keep on shaker for 4-5 days for preparation of liquid culture.

B. Substrate preparation

To cultivate this mushroom, brown rice is supplemented with nutrient medium.

1. Soak brown rice in water for 30 minutes, clean and wash thoroughly.
2. Keep on a sieve or cotton cloth and allow to dry for 30 minutes.

Notes

3. For preparation of nutrient solution, add Glucose 30 g, peptone 5 g, yeast extract 3 g and KH_2PO_4 2g, MgSO4 0.5 g, Vit. B_{12} 10 mg, Multivitamin vitamin 10 g in 1000 ml water.

4. Take 20- 25 g rice per jar, add about 40 ml nutrient solution to it and cover the jar with autoclavable cap or polypropylene bag and autoclave at 15 psi for 40-50 minutes.

5. Cool the jars and add 5-10 ml liquid spawn in substrate prepared at point no. 4, Laminar Air Flow cabinet to ensure that there is no contamination. Move the Jars left to right to spread the liquid medium over the solid medium.

C. Cultivation

1. After inoculation with liquid spawn, keep the jars in dark for 8-10 days at 20-22°C, RH 65-70%.

2. Cover Jars with dark polythene or cloth to create dark conditions.

3. After complete colonization of the substrate, keep the jars in light for a week when the colour of mycelium turns orange.

4. Thereafter, provide 1000 lux light for 12 hours daily. It may take 7 days for yellowing, another 2 weeks for bubble and then pinheads formation.

5. Reduce the light to 8-12 hours when distinct pinheads are formed, it may take another 5-6 weeks till mushrooms attain harvestable size of 4-6 cm. It can take up to 72 days after expose the colonize substrate and the total time required for one crop from inoculation to harvest can be up to 3 months.

6. Fresh air at regular intervals to avoid high carbon dioxide build up in the incubation room.

7. Harvest mushroom when head of mushroom turns club shaped.

8. Pluck out mushrooms out of jars and dry. It can be sold as such or used for making different types of neutraceuticals/dietary supplements.

OBSERVATIONS AND RESULTS

Observe the following:

1. Mycelial balls formation in liquid media on shaker starts.

2. Whitish on surface of solid substrate which turn into orange colour.

3. Orange colour pins start at top of medium.

Notes

Record your observation in the Table given below:

S. No.	Date of inoculation of liquid media	Date of inoculation of solid media	Date and day of dark period start and end	Date and days of exposure to light	Days for pinhead formation	Date of harvesting of mushroom

PRECAUTIONS

1. Autoclave the solutions precisely.
2. Maintain the conditions as mentioned in Lesson no. 7 of Self Learning Material.

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Practical 12

IDENTIFICATION OF INSECTS AFFECTING BUTTON MUSHROOM

OBJECTIVES

After completing this practical you will be able to identify insect pests of mushrooms and their management using appropriate method.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Sample of insect affected mushroom sample, Notebook and Pen, Insect collection box, Insect collection Net

PROCEDURE

1. Collect the sample of affected mushroom.
2. Collect the sample insect dead or alive.
3. Match the symptoms of affected mushroom with the symptoms given in chapter 8 and 9 of Self Learning Material.
4. Identify the insect with the details given in lesson-9 of study material and note down control measures.

OBSERVATIONS

S. No	Mushroom Insect/Sample	Symptoms	Suggested control measures

Notes

PRECAUTIONS

1. Wear gloves while picking diseased mushroom and insects.
2. Burry samples deep in soil after practical is done.
3. Use of pesticide after harvesting of mushroom.

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Practical 13

IDENTIFICATION OF INDICATOR MOULDS IN BUTTON MUSHROOM CROP

OBJECTIVES

After completing this practical you will be able to identify different indicator moulds and defects in the compositing and crop management practices.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Infected mushroom samples, Infected part of compost and casing sample, Collect Brown, Black, Green patches on compost and casing.

PROCEDURE

1. Collect the mushroom which are abnormal in shape, colour etc.
2. Record the symptoms, site of infection, etc.
3. Match the symptoms with the details given in Lesson 9 and Table 9.1 of Self Learning Material.

OBSERVATIONS AND RESULT

Note down your observations in the Table given below:

S. No.	Mould	Indication	Suggested control measures

Notes

PRECAUTIONS

1. Collect sample by wearing gloves.
2. Dispose infected sample properly after practical is done.

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Practical 14

DISEASES CAUSED BY FUNGI, BACTERIA AND ABIOTIC FACTORS

OBJECTIVES

After completing this practical you will be able to identify different diseases caused by fungi, baceteria and abiotic factors causing disorders in button mushroom.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Diseased mushroom samples, Notebook, Pen

PROCEDURE

1. Bring diseased mushroom samples from the mushroom farm.
2. Record the data from where the sample was brought.
3. Match the symptoms of diseased mushroom with the symptoms given in chapter 9 of Self Learning Material.
4. Identify the disease and note down the control measures.

OBSERVATIONS AND RESULT

Record your observations in the Table given below:

S. No.	Name of Disease	Observed symptoms	Stage of Incidence	Suggested control measure

Notes

PRECAUTIONS

1. Choose the sample of disease mushroom cautiously wearing gloves on hand.
2. Bury the sample in soil after noting down details.

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Practical 15

MARKET SURVEY AND COST BENEFIT ANALYSIS

OBJECTIVES

After completing this practical you will be able to understand the market trends and use the collected information for cost benefit analysis.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Information of market from various places, Details of entrepreneurs and receipts, Calculator, Pen and notebook

PROCEDURE

1. Study the market survey details and analyze.
2. Collect the details on expenses, receipts and workout the cost of production.
3. Do the analysis including depreciation on cost of fixed assets and interest on total investment.
4. Calculate cost benefit ratio on above data with the help of Lesson 12 of Self Learning Material.

OBSERVATIONS AND RESULT

1. Analyze the data.
2. Extrapolate the results.
3. Conclude with recommendation.

Notes

PRECAUTIONS

1. Do the calculation correctly.
2. Increase the sample size to reduce the errors.

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Practical 16

MANAGEMENT OF SPENT MUSHROOM SUBSTRATE (SMS)

OBJECTIVES

After completing this practical you will be able to manage spent substrates and waste disposal of mushrooms generated from its cultivation.

TOOLS/EQUIPMENT/MATERIAL REQUIRED

Spent mushroom substrate (SMS), Hand gloves, Water, Loader

PROCEDURE

1. Take out SMS from mushroom farm.
2. Dump it at faraway place for weathering down using loader.
3. Put it in stack, water it and leave earthworms for vermin composting.
4. Ready to use in nursery, field, lawns.
5. Make further uses as per Lesson-13 of Self Learning Material.

OBSERVATIONS AND RESULT

Record your observations in the Table given below:

S. No.	Date of Emptying of SMS from growing room	Date of turning and watering in SMS	Physical Characteristic of material	Nutrient status after vermi composting	Change in colour and smell

Notes

PRECAUTIONS

1. Wear hand gloves while working in farm.
2. Handle mushrooms carefully.
3. Wash your hands properly after practical is done.

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