AV-9025

Department of Rural Technology and Social Development, GGU, Bilaspur (C.G.)

Odd Semester Examination 2015-16: B. Sc. (Hon's) III Semester

RT-307 (Mushroom Production Techniques)

Time : 03 hours **Max. Marks: 30**

Section – A

Q.1.Objective Type Questions (Attempt all): **1X10 = 10 Marks**

i. _____is known as temperature tolerant white button mushroom.

b. *Agaricus bitorquis*

ii. *Agaricus bisporus* belongs to family_____.

a. *Agaricaceae*

iii. Basidiospores are_____spores.

a. exogenous

iv. _____toxin is present in *Amanita muscaria*.

b. Ibotenic acid

v. _____ is known as 'king oyster mushroom'.

a. *Pleurotus eryngii*

vi. Formaldehyde is used as_____in mushroom cultivation.

d. Disinfectant

vii. Short method of button mushroom compost preparation requires_____ days.

c. 14-18 days

viii. 'Directorate of Mushroom Research' is located at _____.

d. Solan (H.P.)

ix. Primary mycelium of *Pleurotus* is_____and _____.

a. Clampless, Non- fertile

x._____is / are the poisonous mushrooms.

d. All of these

Section-B

Short Answer Type Questions (Attempt any four): **2.5×04 = 10 Marks**

Q.2. Explain 'scope of oyster mushroom cultivation'.

In India and other countries, the specialty edible mushrooms are mushroom that are not grown commonly and available commonly in the market. Any mushroom other than popularly grown White Button mushroom *Agaricus bisporus* falls under the specialty category, and these mushrooms are generally not available in departmental stores/with vegetable vendors in India. In India and EU countries. The oyster mushroom is one of the most suitable fungal organism for producing protein rich food from various agrowastes without composting. The other major oyster producing countries are South Korea, Japan, Italy, Taiwan, Thailand and Philippines. At present India produces annually 10,000 tons of this mushroom. It is popularly grown in the states of Orissa, Karnataka, Maharashtra, Andhra Pradesh, Madhya Pradesh, West Bengal and in the North-Eastern States of Meghalaya, Tripura Manipur, Mizoram and Assam.

Pleurotus mushroom can degrade and grow on any kind of agricultural or forest wastes, which contain lignin, cellulose and hemicellulose. Among all the cultivated mushrooms *Pleurotus* has maximum number of commercially cultivated species suitable for round the year cultivation. Moreover, variation in shape, colour, texture, and aroma are also available as per consumer's choice. *Pleurotus* mycelium can grow on fresh or fermented straw and it does not require composted substrate for growth. Substrate preparation for oyster mushroom is very simple. Further this mushroom does not require controlled environmental conditions like *A. bisporus* as most of the species have very wide temperature, relatively humidity and CO_2 tolerance. Unlike white button mushroom, the oyster mushroom fruit bodies can be easily dried and stored. Dried oyster mushrooms can be instantly used

after soaking in hot water for 5 to 10 minutes or it can be used in powdered form for several preparations. Fresh mushrooms have a shelf life of 24-48 h even at room temperature. The productivity of oyster mushroom per unit time is very high as compared to all other cultivated mushrooms. One can harvest minimum of about 500 to 700 kg of fresh oyster mushroom from one ton of dry wheat or paddy straw in 45-60 days, while with the same quantity of straw only about 400-500 kg of white button mushrooms are obtained in 80-100 days (including period needed for compost preparation). Yield of this mushroom can further be increased by supplementing the substrate with suitable nitrogen source viz., soybean and cottonseed meal or by introducing high yielding cultures/strains. The present day cultivation technology of oyster mushroom is a result of various successive steps evolved throughout the world during 20th century. A very primitive form of growing *Pleurotus* spp. was adopted by Lumberman in Europe during 19th century that involved collection of wood logs and stumps showing fructification in nature and keeping them in cool and moist places.

These medicinal mushrooms together with nutritionally rich mushroom are in great demand in USA, EU countries, India, Australia and other countries. While mushroom is the health food, medicinal mushrooms offer a big hope for cure of those human diseases where there is no known cure available in other systems of medicine in the world. Mushrooms are the health food and recommended by FAO-UN as a potent protein source along with soybean for all, especially in developing countries.

Q.3. Explain the medicinal values of mushrooms.

Medicinal values of the some important mushroom are given below:

1. Good for heart

The edible mushrooms have little fat with higher proportion of unsaturated fatty acids and absence of cholesterol and consequently it is the relevant choice for heart patients and treating cardiovascular diseases.

2. Low calorie food

The diabetic patients choose mushroom as an ideal food due to its low calorific value, no starch, little fat and sugars. The lean proteins present in mushrooms help to burn cholesterol in the body. Thus it is most preferable food for people striving to shed their extra weight.

3. Prevents cancer

All forms of edible mushrooms, and white button mushrooms in particular, can prevent prostate and breast cancer. Fresh mushrooms are capable of arresting the action of 5-alpha-reductase and aromatase, chemicals responsible for growth of cancerous tumors. The drug known as Polysaccharide-K (Kresin), is isolated from *Trametes versicolor* (*Coriolus versicolor*), which is used as a leading cancer drug.

4. Anti-aging property

The polysaccharides from mushrooms are potent scavengers of super oxide free radicals. These antioxidants prevent the action of free radicals in the body, consequently reducing the aging process. Ergothioneine is a specific antioxidant found in *Flammulina velutipes* and *Agaricus bisporus* which is necessary for healthy eyes, kidney, bone marrow, liver and skin.

5. Regulates digestive system

The fermentable fiber as well as oligosaccharide from mushrooms acts as a prebiotics in intestine and therefore they anchor useful bacteria in the colon. This dietary fibre assists the digestion process and healthy functioning of bowel system.

6. Strengthens immunity

Mushrooms are capable of strengthening the immune system. A diverse collection of polysaccharides (beta-glucans) and minerals, isolated from mushroom is responsible for up-regulating the immune system.

Q.4. Differentiate between oyster and button mushroom.

Sr. No.	*Agaricus* (Button mushroom)	*Pleurotus* (Oyster mushroom)
1	Umbrella shaped fruiting body	Fan like fruiting body
2	Pink/ brown coloured gills	White/ gray coloured gills
3	Stipe- central and long	Stipe- lateral and undistinguishable
4	Annuals present on the stipe	Annuals absent
5	Vulva absent	Vulva absent
6	**e.g. *Agaricus bisporus***	**e.g. *Pleurotus sajor caju***

Q.5. Which raw materials and ingredients are required for compost preparation for white button mushroom?

Raw Material and Ingredients Required for Composting:

1. Agricultural base materials

These base materials form the bulk of compost and for this purpose wheat straw is favoured all over the world. However, quality compost can be prepared using variety of other materials including paddy straw, hay, barley, oat, maize stalks and leaves, sugarcane bagasse, sugarcane trashes and leaves, soybean stalks, mustard stalks, etc. These base materials act as a reservoir of cellulose, hemi-cellulose and lignin, which is utilized by *A. bisporus* during its growth as a carbon source.

2. Supplements

Above base materials do not have adequate amount of nitrogen and other nutrients required to start the fermentation process having required C/N ratio. The compounding mixture is supplemented with other materials having nitrogen and carbohydrate sources. These materials can be classified as follows.

a. Animal manure

Chicken manure has proved to be the best alternative of horse manure. Other manures viz., pig, cattle and sheep have also been tried for compost preparation but with limited success. All these manures provide nitrogen to the compounding mixture, little of carbohydrate is also provided. These materials are highly variable in composition and their N-content may vary from 1 - 5 percent and it is released slowly during composting process. If horse manure is used in composting then it should be used alongwith bedding and urine, as it will not require any further supplementation. If it is not having enough bedding and urine when collected from a clean stable, supplementation with inorganic nitrogen along with some wheat straw may prove useful.

b. Carbohydrate sources

These materials are essentially required to hasten the composting process, to balance the C/N ratio and also for the establishment of the bacterial flora in the compost. Molasses, wet brewer's grains, malt sprouts, potato wastes, apple and grape pomace can be employed as carbohydrate sources, since these materials provide readily available nutrients to microorganisms.

3. Nitrogen fertilizers

This category includes fertilizers like, urea, calcium ammonium nitrate, ammonium sulphate. Nitrogen content of these fertilizers is very high (24-46%), which is released quickly, resulting in quick establishment of microflora.

4. Concentrate meals

Animal feeds are generally kept in this category, which include, wheat or rice bran, dried brewer's grain, soybean meal, cotton seed meal, castor meal, sunflower meal, etc. These materials supply both nitrogen and carbohydrates, which as in case of animal manures are released slowly. Nitrogen content may vary from 3-12% depending upon the source.

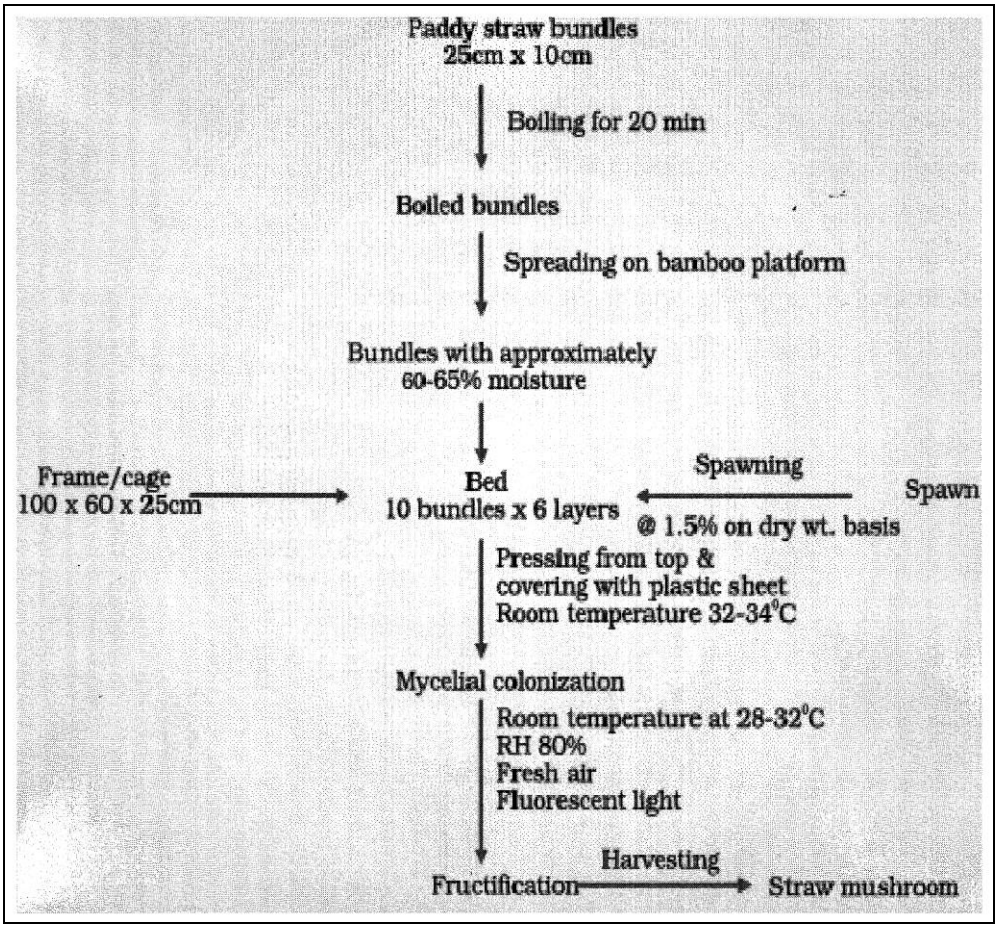
5. Supplements to rectify mineral deficiencies

In addition to carbon and nitrogen, *A. bisporus* also requires little quantities of potash, phosphorous, calcium and magnesium for its growth. Fertilizers viz., MOP and superphosphate can be kept in this category. Besides this, gypsum and calcium carbonate can also be kept here. Gypsum also has stabilizing effect on ammonium content. Furthermore, gypsum serves as a calcium source for the mushroom and also for the oxalic acid produced by the mushroom mycelium, which gets converted into calcium oxalate.

Q.6. Write down procedure of 'Cage method of paddy straw cultivation'.

Cage method of paddy straw cultivation:

- Select dry, fresh and hand threshed paddy straw free from moulds and leafy portion. Make 25 cm long and 10 cm thick bundles @ 60 bundles for each cage (Bed).
- Soak the bundles in boiling water for 20-30 minutes and allow cooling and draining of excess water.
- Disinfect the cage and polythene sheet with 2% Formalin or Dettol solution.
- Arrange ten bundles uniformly in the cage as the bottom layer and put some spawn grains over and inside the bundles. Put a second layer of ten bundles over the first and spawn as before. Repeat this till six layers of bundles are achieved or till filling of the cage.
- Spray solutions of 0.1% Malathion and 0.2% Dithane Z-78 all over the bed. Cover the whole bed with polythene sheet and bind securely with a binding thread.
- Keep the spawned cage in a room or a shed for mycelial run. A warm place with temperature around 30˚C is helpful for better mycelia growth.
- Remove the polythene sheet after the mycelial run is completed. Maintain high humidity in and around the bed till pinheads appear.
- Pinheads appear within 10-15 days after spawning. Harvest at the egg stage.
- Continue spraying water for the next flush of mushroom to appear within a week or so.



Cage method of paddy straw cultivation

Q.7. Describe the procedure for canning of mushrooms.

Canning is the technique by which the mushrooms can be stored for longer periods up to a year and most of the international trade in mushrooms is done in this form. The caning process can be divided into various unit operations namely cleaning, blanching, filling, sterilization, cooling, labelling and packaging. In order to produce good quality canned mushrooms, these should be processed as soon as possible after the harvest. In case a delay is inevitable; mushrooms should be stored at 4 to 5°C till processed.

The mushrooms with a stem length of one cm are preferred and are canned whole, sliced and stems and pieces as per demand. Well graded fresh mushrooms white in color, without dark marks on either caps or stems are preferred for canning. Whole mushrooms are washed 3-4 times in cold running water to remove adhering substances. Use of iron free water with 0.1% citric acid prevents discoloration. Thereafter blanching is normally done to inhibit polyphenol oxidase enzymes activity and to inactivate microorganisms. It also removes the gases from the mushroom tissue and reduces bacterial counts. The mushrooms are blanched in stainless steel kettles filled with a boiling solution of 0.1% citric acid and 1% common salt. The blanching time ranges from 5-6 minutes at 95-100°C. The mushrooms after blanching are filled in sterilized tin cans (A-2½ and A-1 tall can sizes containing approximately 440 and 220 g drained mushroom weight, respectively). Brine solution (2% salt with 0.1% citric acid or 100 ppm ascorbic acid) is added to the mushroom-filled cans after bringing its temperature to 90°C.

After filling, the cans are exhausted by passing them in exhaust box for 10-15 minutes, so that temperature in the centre of cans reaches up to 85°C. Then the cans are sealed hermetically with double seamer and kept in upside down position. After exhausting of cans, sterilization of cans is needed. Sterilization is the process of heating the cans up to 118°C to prevent the spoilage by microorganisms during storage. The cans cooled immediately after sterilization process to stop the over-cooking and to prevent stack burning. Cooling can be done by placing the cans in a cold-water tank. Thereafter the clean and dry cans are labeled manually or mechanically and packed in strong wooden crates or corrugated cardboard cartons. The cans are stored in cool and dry place before dispatching to market.

Section-C

Long Answer Type Questions (Attempt any two): 5×02 = 10 Marks

Q.8. Enlist and explain different methods of substrate preparation for oyster mushroom.

Methods of substrate preparation

i. Steam pasteurization
ii. Hot water treatment
iii. Chemical sterilization technique
iv. Sterile technique
v. Fermentation or composting

i. Steam pasteurization

In this method prewetted straw is packed in wooden trays or boxes and then kept in a pasteurization room at 58-620C for four hours. Temperature of the pasteurization room is manipulated with the help of steam through a boiler. Substrate after cooling at room temperature is seeded with spawn. The entire process takes around 3-5 days.

ii. Hot water treatment

The substrate (wheat straw) after chopping (5-10 cm) is soaked in hot water (65 to 70°C) for one hour or 60 to 120 minutes at 80°C or in case of paddy straw at 85°C for 30-45 minutes. After draining excess water and cooling, spawn is added. Hot water treatment makes the hard substrate like

maize cobs, stems, etc. soft so that the growth of mycelia takes place easily. This method is not suitable for large-scale commercial cultivation.

iii. Chemical sterilization technique

Various species of *Trichoderma, Gliocladium, Penicillium, Aspergillus* and *Doratomycs* spp. are the common competitor fungi on the straw during oyster mushroom cultivation. If present on the straw during spawn run, they do not allow the growth of mushroom mycelium resulting in yield loss or complete crop failure. When wheat straw or paddy straw is treated by steeping in a chemical solution of carbendenzim 50% WP (37.5 ppm) and formaldehyde (500 ppm) for a period of 16-18 h, most of the competitor moulds are either killed or their growth is suppressed for 25-40 days after spawning.

iv. Sterile technique

It is also known as 'Till method'. The chopped substrate after soaking in cold water is put in heat resistant polypropylene bags and sterilized in an autoclave at 20 p.s.i. for 1-2 hours followed by spawning under aseptic conditions. This method is more suitable for research work rather than on large-scale commercial production.

v. Fermentation or composting

This method is a modification of composting technique used for white button mushroom. It is most suitable for hard substrates like cotton stalks, maize stalks, leguminous stubbles, etc. Both aerobic and anaerobic fermentation of the substrate is suitable for *Pleurotus* cultivation. Composting should be done on a covered area or shed. Chop the substrate into 5-6 cm long pieces. Add ammonium sulphate or urea (0.5-1%) and lime (1%) on dry weight basis of the ingredients. Horse manure or chicken manure (10% dry weight basis) can also be used instead of nitrogenous fertilizers. Addition of lime improves the physical structure of the compost. After mixing all the ingredients sprinkle water till it is completely wet. Prepare a triangular heap of 75-90 cm but not more than 1 meter height. After 2 days of fermentation, turning of pile is done adding 1% superphosphate and 0.5% lime. The compost will be ready after 2 days of this turning.

Q.9. Which steps should be adopted as routine practice to keep away pest and diseases during mushroom production?

The following preventive and/or eradicative control measures are adopted for the management of diseases:

Ecological-by manipulations of environmental factors such as temperature, humidity and ventilation

Biological-by incorporation of biocontrol agents and organic amendments

Chemical-by use of safe and minimum doses of specific fungicides, antibiotic, etc

Sanitation and hygienic measures are most essential to manage the disease particularly under Indian conditions although under certain situations use of chemicals is inevitable.

Sanitation and hygiene

Hygiene covers all the measures, which are necessary to minimize the possible incidence of the pests and pathogens. Thus, hygiene and sanitation go hand in hand at all stages of mushroom growing. Farm hygiene is the best defense for a mushroom grower against mushroom pests and diseases particularly during the present time, when use of chemicals on food crops is being discouraged. Based on the critical observations during all the stages of mushroom production, the following steps should be adopted as a routine practice for successful mushroom cultivation.

- The location of mushroom unit should be away from chemical industries and should be free from toxic fumes or gases
- Floor for the preparation of compost should be cemented /tiled and covered with a roof.
- Substrates used for compost preparation should be fresh, protected from rain and mixed in exact proportion.

- Pasteurization and conditioning of the compost should be for optimum duration at right temperatures as over/under pasteurization may produce poor quality compost and invite disease problems.
- Do not allow free access of persons working in composting yards to spawning and other cleaner areas without changing the dress and foot-dip.
- Spawn should be fresh and free from all contaminants.
- Spawning area must be washed and disinfected with 2% formalin.
- The fresh air should be filtered before it enters the growing rooms to exclude all particles of 2 micron and above.
- Casing mixture should be properly sterilized (65°C for 5-6 hours).
- Casing mixture should be stored in a clean and disinfected place. All the containers, equipments and machinery used for casing should be thoroughly washed and disinfected.
- Picking should start from new or cleaner crop towards older crops.
- Waste from picking, trash, stems mushrooms should be carefully collected not allowing to fall on the floor and be disposed off carefully.
- Avoid surface condensation of water on developing mushrooms.
- Add bleaching powder (150ppm) at every watering to manage bacterial disease.
- Remove heavily infected bags from the cropping rooms or treat the patches by spot application of 2% formalin or 0.1% Bavistin.
- Maintain optimum environmental conditions in the cropping rooms to avoid abiotic disorders.
- Control insect-pests well in time to avoid the spread of pathogen by them.
- At the end of crop, cook out at 70°C for 12 hours is very essential to eliminate all pests and pathogens.

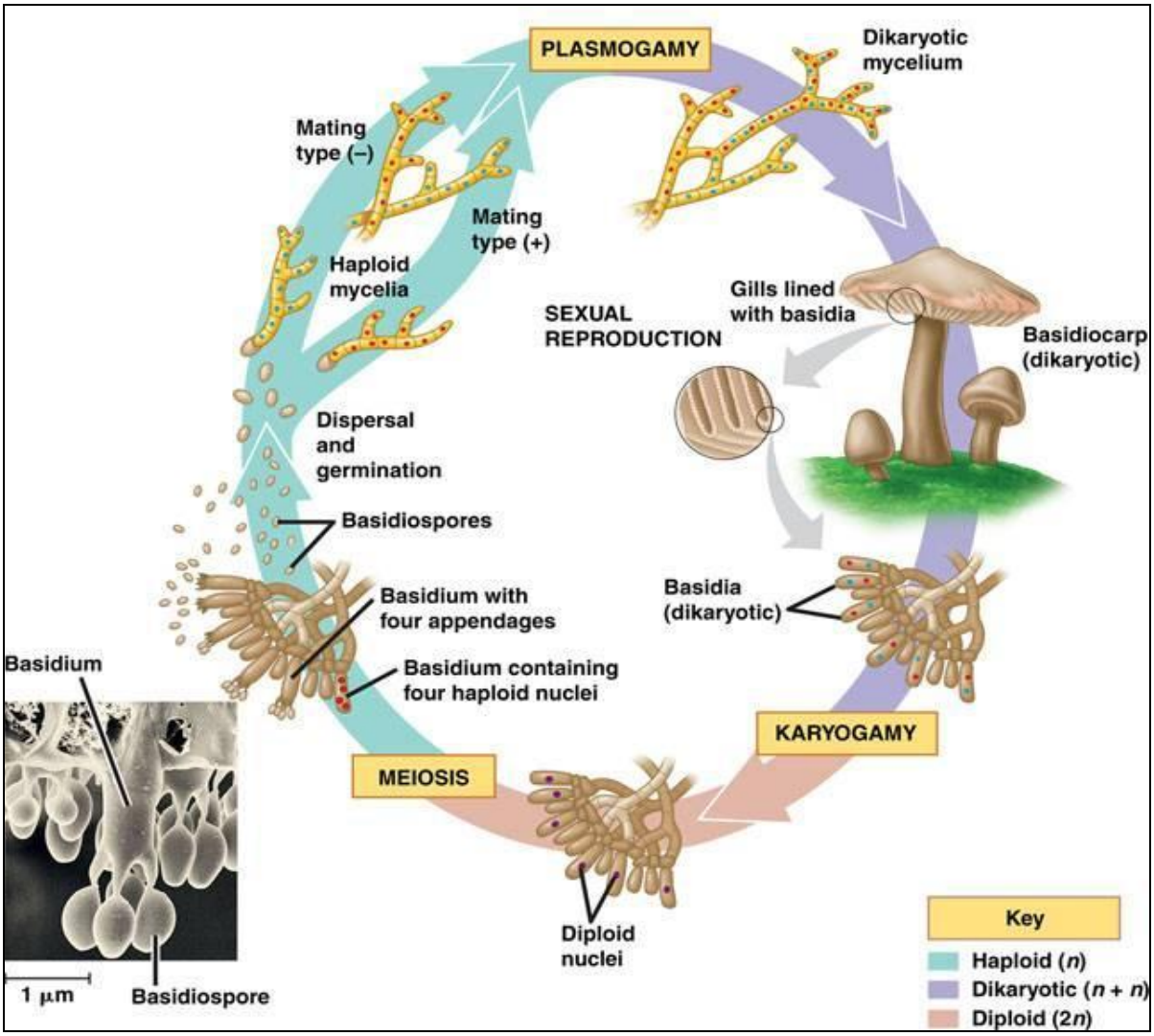
Use of chemicals

Some of the most common fungicides recommended for the control of major fungal pathogens of mushrooms and used in mushroom industry are:

- Benomyl (Benlate 50 wp)- For control of *Cladobotryum, Mycogone, Trichoderma, Verticillium,* mix 240 g/100 m² with casing or dissolve in water at 240 g/200 litres/100 m² during first watering.
- Carbendazim (Bavistin) same as for benomyl.
- Chlorothalonil (Bravo or Repulse)- to control *Mycogone* and *Verticillium*. Apply as spray 2 week after casing and repeat after 2 weeks later @ 200 ml in 100-200 litre water/100 m^2.
- Prochloroz Manganese (Sporgon)- to control *Mycogone, Verticillium, Cladobotryum*, give a single application of 300g/100litres/100m², 7-9 days after casing. For double application, use 113g/100litres/100m², 7-9 days after casing and repeat again between second and third flushes. For triple application, use 57g/100litres/100m², 7-9 days after casing and after first and third flushes (presently sporogon is not available in India).
- Thiabendazole(Tecto)- to control *Cladobotryum, Mycogone, Verticillium*, Apply at the same rate as Benomyl.
- Zineb- to control *Dactylium, Mycogone, Geotrichum* and *Verticillium,* Use 350 g/ 100 m² every week after casing. For wettable powder, 1 kg/1000 litres @ 5 litre/ 100 m² after casing and between flushes.

As a general practice, cook out of compost, fumigation of cropping rooms after cropping with formaldehyde and spray with copper fungicides helps in removing primary inoculum. Similarly it may be appropriate to spray 0.5% formalin or 0.1% bavistin just after casing to check the primary inoculum. The chances of infection are much higher at these stages as there is lot of movement of air, materials and persons and all are potential carrier of diseases.

Q. 10. Draw schematic representation of 'life cycle of *Agaricus*' and Explain.



Life cycle of *Agaricus bisporus*:

In the life cycle of mushroom fruiting stage is the formation of the visible mushroom, formed from an aggregation of hyphae or mycelium. The mushroom starts as small pinheads or primordial that rapidly enlarge into the button stage. The mushroom them differentiate into an umbrella like structure and ultimately forms gills underneath. At the edges of the gills, special cells, eventually fuse, doubling their chromosomes number. These cells are called as basidia and are the focal point in the reproductive phase of the mushroom.

The fusion of the two haploid nuclei, called karyogamy, results in the formation of a diploid nucleus in the basidium. Soon after, this diploid nucleus undergoes meiosis or reduction division and produces four meiotic nuclei. These four haploid nuclei will eventually migrate outside the basidial cell, through projections called sterigmata, into four basidiospores. These sporce continue to develop until they are forcefully librated from the basidia and propelled into free space. After only few hours of spore production and release, the mushrooms are in the last hours of life. After germination, the spore produces positive (+) and negative (-) haploid mycelia. The crossing over will takes place between different types of haploid mycelia in next stage. During mating plasmogamy occurs which resulted into dikaryotic mycelium.

Q. 11. Enlist different steps for spawn production and Explain 'Pure culture preparation' and 'Mother spawn preparation' in detail.

Steps for Spawn Production:

a. Pure culture preparation

b. Substrate Preparation

c. Mother Spawn Preparation
d. Commercial Spawn Preparation

Pure Culture Preparation

Pure culture of mushrooms can be prepared either by multi-spore or by tissue culture. Multi-spore culture is made from spore print that can be obtained by hanging a alcohol sterilized fresh fruit body on a loop of wire above a petriplate/sterilized paper. Spores are serially diluted and transferred to sterile potato-dextrose-agar (PDA) or malt-extract-agar (MEA) culture slants. These slants are then incubated at 25°C ± 2°C for 2 weeks to obtain pure culture. For tissue culture, mushroom after alcohol sterilization is cut longitudinally into 2 halves and bits from collar region (i.e. junction of cap and stalk) are transferred to pre-sterilized PDA or MEA culture medium, which is, incubated at 25°C ± 2°C in BOD incubator for one week. Mycelium from growing edges is carefully transferred to MEA/PDA slants and again incubated for 2-3 weeks to obtain pure cultures.

Mother Spawn Preparation:

About 300 g prepared substrate (boiled grains mixed with gypsum and chalk) is filled in glucose/milk/glass bottles upto 2/3 volume and plugged with non-absorbent cotton. The plugs are covered with aluminum foil. These bottles are then autoclaved at 22 p.s.i. pressure for 1.5 to 2 h. Autoclaved bottles are left in the room for 24 hours for cooling and are kept on laminar flow under U.V. tube for 20-30 minutes before inoculation. A piece of mycelium (pure culture) grown in Petri plates is aseptically transferred to these bottles and inoculated bottles are incubated. These bottles are gently shaked on 5th and 10th day for distributing the inoculum evenly in the bottles. This spawn prepared using pure culture mycelium as inoculant is referred as mother spawn. Fully colonized mother spawn bottles can be used for inoculating commercial spawn bags after two to three weeks.