AS-2980

B.Sc. (Rural Technology) Third Semester Examination - 2013

RT-307

Mushroom Production Techniques

Section - A

(i) (b) Pileus

(ii) (a) Agaricus

(iii) (b) Lentinula edodes

(iv) (a) Grifola frondosa

(v) (a) 20-30°C

(vi) (a) Spawn

(vii) (c) Pleurotus sajor caju

(viii) (c) White button mushroom

(ix) (a) Coprinus spp

(x) (c) Mycogone perniciosa

Section - B.

Short answer

2. Nematodes are small micro-organisms common in all soils and often observed to infest the compost or well casing soil used for mushroom production. They are capable of rapid growth and the population can range from a few hundred

to as many as ten thousand per 50g of compost.

A large number of mushroom farms in the country have suffered crop losses due to heavy infestation of nematodes in the compost or casing soil. In the compost heap they are killed where the temperature goes upto 71°c – 77°c. While they manage to survive in the outer part of the stack where it is cooler.

Mushroom mycelium is seriously damaged by nematodes. They are also known to feed on mushroom turning them brown.

Control:

Once an infestation has occurred it is very difficult to eradicate as it can be carried from crop to crop.

Rigorous sanitary measures are necessary to prevent the infestation from establishing itself in the house.

The bed boards and boxes must then be thoroughly cleaned and dipped for 4-5 min in boiling water and sprayed with 2% formaldehyde.

Where pasteurisation of compost is not possible, DBCP-EC (Nemagon 60%) 40 ml in 4 lit. of water should be added to 300kg of wheat straw at the first or second turning.

Treatment of casing soil above 65°c for 15-20 min. Thionazin at 80 ppm conc. is used during spawn running. It destroys nematodes without checking the growth of mushroom.

Answer: 3. White button mushroom or Agaricus bisporus can be grown anywhere under following essential condition.

(i) Temperature (ii) Moisture
(iii) Ventilation (iv) Spawn (v) Suitable growing space

(i) Temperature:
Temperature required for the spread of the mycelium is 22 – 25°C. For the fructification the temperature requirement is 14-18°C. The temp. should be uniform throughout the growth of the crop.

(ii) Moisture: Moisture is an important factor in the cultivation of the mushroom and needs careful application. The mushroom requires an atmosphere nearly saturated with moisture while the direct application of water on the beds is more or less injurious to the growing crop.

(iii) Ventilation: Good aeration is essential for a healthy crop, as ventilation is one of the important factors governing mushroom production. CO_2 level of 0.10 – 0.15 % volume is necessary during crop production and this can be acheived by giving 4 – 6 air changes/hour or introducing 10 cubic ft fresh air/sq. ft. bed area/hour.

(iv) Spawn: For better production a good spawn is required. Good spawn should be of a strain originating from a single specimen of a perfect crop. The substratum must be covered with white mycelium, it must be uniform, and should be absolutely free not only from mould but also from all other microorganism.

(v) Suitable growing space: Mushroom growing space should be easily accessible so that the raw material (manure/casing soil/straw) can be brought in and removed with ease. It should be well ventilated, and completely isolated from the surrounding in order to facilitate the fumigation and disinfection of the beds.

4. Culture preparation of mushroom:

Mushroom grow on a variety of culture media and on different agar formulation, both natural and synthetic.. e.g. PDA, MYA, PCA etc

Main steps are as follows:

(i) Medium preparation
(ii) Sterilization of mushroom of desired strain.
(iii) Inoculation
(iv) Subculture or multiplication
(v) Preservation

(i) Medium preparation: Synthetic media are often expensive, PDA (Potato dextrose agar) is the simplest medium for growing the mycelium of mushroom.

Ingredients are

Potato, diced	— 200 g
Dextros	— 20 g
Agar powder	— 20 g
Distilled water	— 1000 ml.

After preparation of broth agar is added and then filled in bottle / flask for sterilization. Autoclave / Pressure cooker is used for the sterilization of culture medium.

(ii) Sterilization of mushroom: The mushroom is thoroughly washed in water and surface sterilized with 70% alcohol and again washed with distilled water under aseptic environment (Laminar flow).

(iii) Inoculation: For the inoculation of mushroom contamination free petriplates are selected. surface sterilized mushroom's surface is removed with help of scalpel and the cut length wise from cap downwards. Small piece of mushroom is inserted with help of sterilized needle or forceps on the fo culture medium and then inoculated petriplates incubated for 3-5 days. If mycelial growth is yellowish, greenish or blackish it may be contaminants, in this case the petridish should be discarded.

(iv) Subculture or multiplication:

Petridish having white thread-like mycelial growth is selected for subculture. A small portion of mycelium is transferred on culture medium for subculture under aseptic condition.

(v) Preservation: After inoculation of 3-5 days, the mycelium will spread on to the agar surface. These petridish must be sealed with parafilm and kept in a refrigerator.

For long term preservation cultures are prepared in slants and add previously sterilized mineral oil to the fully grown culture. Culture stored in mineral oil can be kept for 1-3 years. Viability may be checked after 2 years of storage.

Answer: 5

Shiitake mushroom: It is native to Asia, mainly China and Japan. It is Japanese name, where 'Shii' is the name of tree that usually host the mushroom and 'Take' mean the mushroom. Scientific name of this mushroom is Lentinula edodes and it is very adaptable and can grow anywhere e.g. in simple greenhouse, under tree shade.

It is fleshy mushroom and rich in nutrients. It contains protein (18%), potassium, niacin, calcium, magnesium, phosphorus and Vitamin-B. It flavour is 4 to 10 times more intense than that of ordinary button mushroom.

Shiitake is low in sodium and glucose but rich in fibre, hence it is ideal for diabetics. It is also recommended for lowering serum cholesterol.

It is rich in Lentinan, which is used against cancer by Japanese. It is also a source of selenium, an antioxidant that is said to prevent cancer.

shiitake healing properties are also reflected in its anti-viral strengths. The Japanese pharmaceutical company, Ajinomoto, is already using Lentinan from shiitake mushrooms to treat stomach cancers. Other countries are also using it as injectable medication to fight cancer.

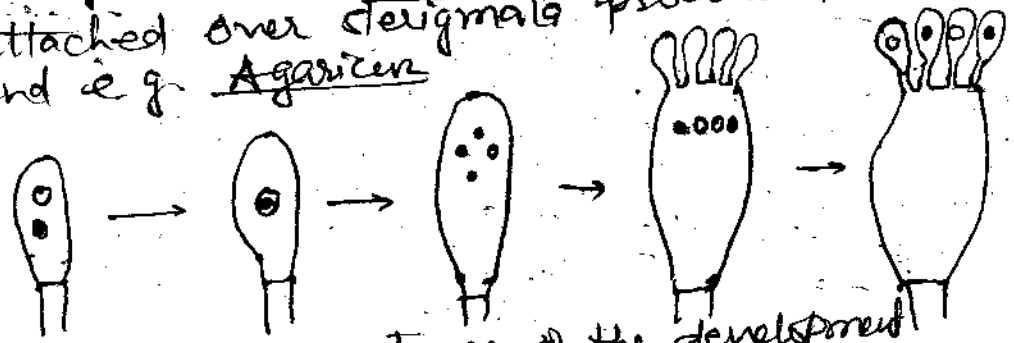
6.

Answer: Basidium formation is an essential and very important process of Basidiomycetes. First both nucleus of penultimate cell fused to form a diploid nucleus. The cell with diploid nucleus increases in size to form basidium. The fusion of nuclei may take place directly in basidium e.g. Mushroom or in teleutospores which produce basidium e.g. Puccinia

Basidia may be of two types:

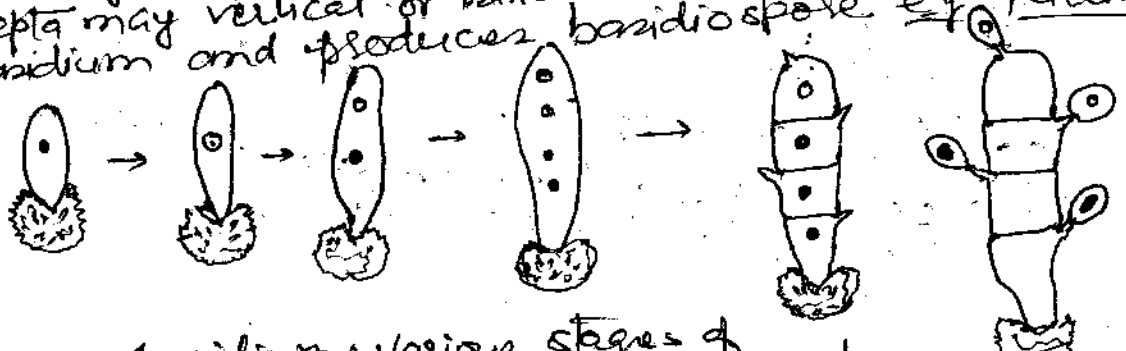
1. Holobasidia 2. Phragmobasidia

1 Holobasidia: It is unicellular and club-shaped structure in which basidiospores are attached over sterigmata produced on its upper end e.g. Agaricus



Holobasidium: Various stages of the development

2. Phragmobasidium: It is septate and four celled. septa may vertical or transverse. Each cell function as basidium and produces basidiospore e.g. Puccinia



Phragmbasidium: Varian stages of development.

7.

Answer: Various substrate used for the preparation of spawn e.g. Wheat grain, Cotton waste, Rice straw, lotus seed shell and manure etc.

1. Grain: This is the substrate which is universally used for the production of spawn. The larger grains carry a greater reserve of food material per grain for mushroom mycelium. Different workers reported different grains for making grain spawn. e.g. rye grain, sorghum, jowari grain

Grain spawn run is faster and growers can save few days for starting production.

2. Rice straw: The rice straw chopped into pieces about 2-2 cm long then soaked in water for 15-2 hrs. The excess water is drained off and the straw pieces transferred to 1 liter glass bottle and a solution of 2% sucrose 2% peptone is added (10ml/bottle) to each bottle

3. Lotus seed shell: The lotus seed shell along with stable manure is used as substrate for spawn production. Overnight soaked lotus seed shells and manure (1:3) are mixed and allow to age for 15 days. The pile is turned every 4 days. The fermented substrate is then filled in bottle for sterilization for atleast 1 hours. They are then allowed to cool for inoculation.

4. Cotton waste: Cotton waste is preferred as a spawn substrate. This grade usually contains cotton seeds and other waste material from local textile factories. This material provide more nutrients and air space for mycelial growth. Sometimes used tea leaves are also mixed with this material.

Section C

Long answers

8. The mother spawn is used to inoculate the final spawn container in which the planting spawn will be produced. Various substrates are used for the preparation of mother spawn but grain spawn is preffered. Vaious steps of mother spawn production are as follows:

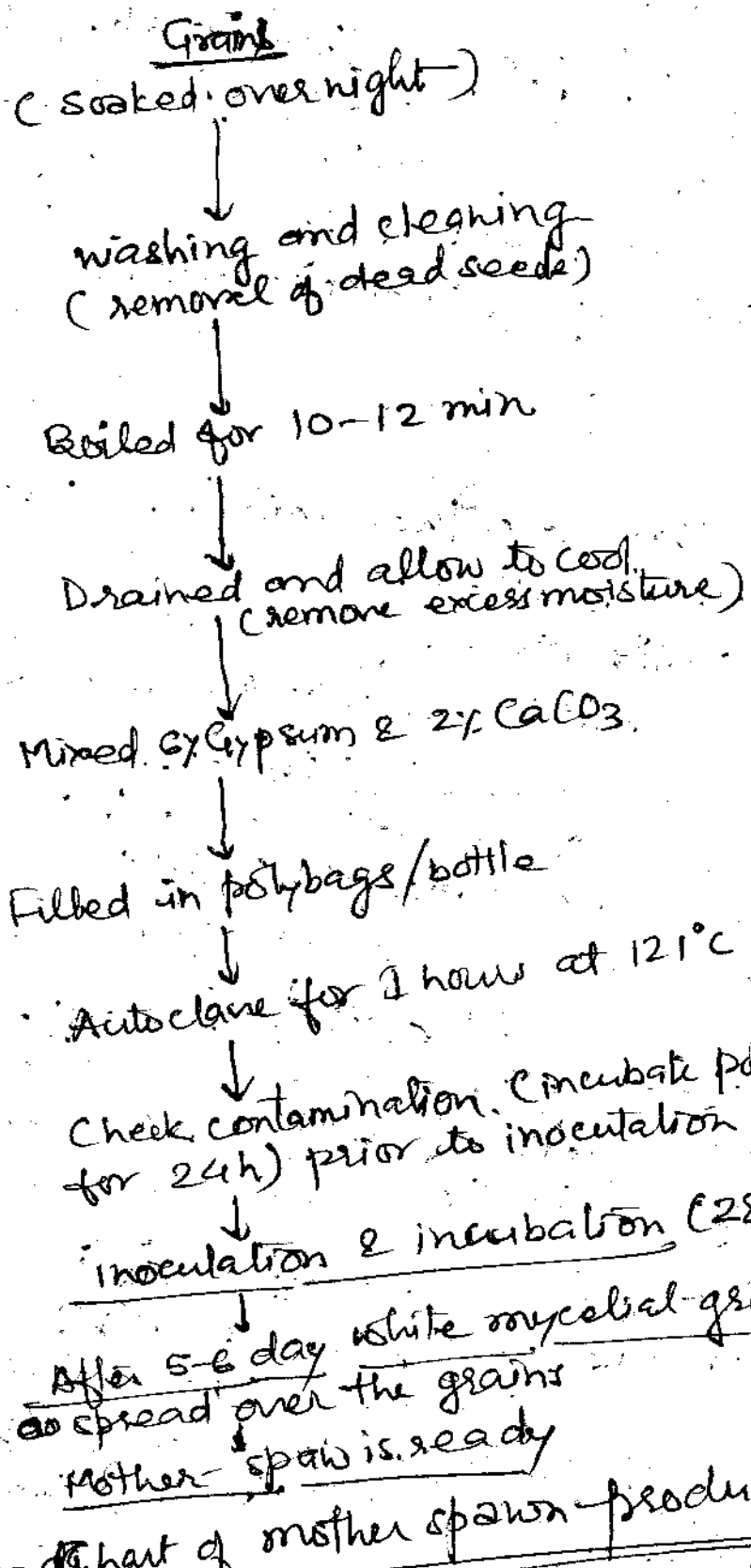
(i) Preparation of substrate
(ii) Inoculation of sterilized spawn substrate
(iii) Incubation.

(i) Preparation of substrate: To prepare mother spawn substrate, seeds (wheat, rye, sorghum or rice) are washed thoroughly, then soaked overnight. Dead seeds or those that float on water surface are carefully removed. The following day, the seeds are washed again and boiled in water for at least 10-15 min. until they expand but not quite broken (burst). The grains are drained and allow to cool. Precipitated chalk ($CaCO_3$) and Gypsum are added to the grain. The seeds are then loosly packed in flat or round bottles that can withstand intense heat (Polybags are also used for packing) The bottles are filled 2/3 full then pluged the cotton plug. The grains are sterilized in autoclave/Pressure cooker for about 1 hour at 121°C. The bottles/Polybags are then cooled and checked contamination (after 24h) prior to inoculation.

(ii) Inoculation of sterilized spawn substrate

A vigorously growing mycelial culture, either in a flat bottle or on a petridish, is

used to inoculate the substrate used for producing the mother spawn. Inoculation should be done either within laminar air flow cabinet or previously cleaned inoculation room or inside a transfer chamber.



Flow-chart of mother spawn production

(iii) Incubation: Inoculated bottles/polybags are incubated at the appropriate temperature (28±2°C). After 4-5 days white mycelial growth spread over the grains if growth is not uniformed throughout the bottle or polybags then shake the bottle or break the grains by lemps for uniform growth. In this case, the individual grains are separated when fully covered with the mycelia and ready for inoculation to the next bottle.

9.

Answer: There are two main methods of composting

(1) Long method
(2) Short Method.

(1) Long method: The long method takes about 3-4 weeks. The compost should be prepared on a well cleaned, cemented floor. It may be done either in the open or under a shed whose sides are open. If composting is done outside, then the heap should be protected from rains by covering it with a plastic sheet. If composting done inside the room, then the room should be well ventilated.

There are two types of compost:

(a) Natural compost (b) Synthetic compost.

(a) Natural Compost: It includes Horse dung, Wheat straw (1/3 of the weight) Chicken manure (100-110 kg/tonne), Urea (3 kg/tonne).

(b) Synthetic compost:

Wheat straw (chopped) 250kg, wheat bran (25kg) Ammonium sulphate or calcium ammonium nitrate (4kg) urea (3kg), Gypsum (20kg)

(Various formula have been given by different worker. to prepare synthetic compost)

Method: Wheat straw is spread over a pucca floor and wetted thoroughly by sprinkling water. Wheat bran and other ingredients except gypsum are mixed thoroughly in wet straw, which is finally stacked into a pile about 1 metre high and 1 metre wide. The heap is compressed by applying light pressure. The heap with mixed ingredients can also be made with the help of the wooden mould. The pile should not be pressed tightly otherwise anaerobic condition will set in. It is essential to open the entire pile and re-do it a number of times according to the following schedule.

Stack the heap	0 day
1st turning	5th day
2nd turning	10th day
3rd turning	14th day, add 10kg gypsum
4th turning	18th day, add 10 kg gypsum
5th turning	22nd day, spray with 40ml nemagon
Final turning	26th day, spray 10ml melathion in 5 litre water

At each turning water should be sprinkled to make up the loss of water dare to evaporation. The compost, when ready for spawning, is dark brown/black in colour without any smell of ammonia and sufficient moisture.

(ii) Short Method:

This composting has two phases.

Phase-I. outdoor Composting. Prestacking - four days.

All ingredients are added to wheat straw. except gypsum. Sufficient water is added by there should be no leaching. The stack should be 3.3 x 2.5 x 1.0 metre. Give turning and make the stack of slightly smaller size (3m x 2m x 1m). straw is trampled with feet and watered if necessary.

Day 0 - Prepare heap, add full quantity of urea and spray water if necessary

Day 2 - Give ~~second~~ first turning

Day 4 - Give second Turning and add full quantity of gypsum.

Day 6 - Give third turning

Day 8 - Fill in tray for steam pasteurisation

Phase-II It has two main purpose

(i) Conversion of ammonia into microbial protein

(ii) Pastourization - Killing of micro organism which are competitors and to make the substrate suitable only for Agaricus bisporus or white button mushroom.

Phase-II can be completed either by steam heat or fumigation with methyl bromide.

10.

Answer: The most important use of mushroom is as an article of food and its value as such is beyond the reach of the chemist or physiologist. In addition, mushrooms possess great value as condiments of food accesseries. Mushrooms are among the most appetising of table delicacies and add great flavour to food when cooked with them. Besides being an important food article, mushrooms are variously exploited by man. Various uses of mushrooms are as follows:

- As medicine: There is a long list of mushroom which have been used as medicine or medicinal purposes.

Polyporus officinalis ——→ tuberculosis, rheumatism, gout, jaundice

Auricularia auricula ——→ for inflamed eyes (as poultice) inflamation of the throat.

Fomes ignarius ——→ rapid coagulation of blood

Clavatia gigantia ——→ anaesthesia

Amnita muscaria ——→ as a powder or tincture.

Cordyceps ——→ to control blood sugar, Heal Hepatitis B. detoxification. Rectified kidney disorder

Lentinula edodes (Shiitake) ——→ for lowering serum control

Ganoderma lucidum (Reishi) ——→ for healing of tumours lowering of blood sugar & Cholesterol levels.

Maitake (Grifola frondosa) ⟶ Against cancer.
Boosting immune system
for Regulating blood sugar & cholesterol.
for Type 2 diabetes.

- As forest friend: Mushrooms are beneficial to the forest. The forestry mushrooms are nature's most active agents in the disposal of the forests waste material. There are few species of mushroom which attack the living trees whereas a large number of them grow on fallen timer, bark, sap wood etc. The mycelium of mushroom grows in a few years and the complete disintegration of the wood takes place. It gradually mixes with forest soil and provides food for the living trees.
- Tinder mushrooms: Polyporus fomentarius
- Dyeing: Polyporus bispidus: brown dye for colouring silk cotton and wool
  P. sulphureus ⟶ yellow colour
  F. ignitarius ⟶ brown black colour
- Writing material: Inky cap mushroom - Coprinus comatus ⟶ black liquid.
- Luminosity: Armillaria mellea
  Pleurotus Japonicus
- Hallucination: Amanita muscaria, Stropharia spp.
  Psilocybe spp. etc
- Flower pot: Polyporus spp.

⟶ ✕ ⟵

Answer:

Identification of mushroom requires a basic knowledge of the structure of the fungi and their life cycle. Identification of mushroom includes:

(i) Morphological observation
(ii) Chemical test
(iii) Molecular characterization

(i) Morphological observation:

To identify a given mushroom, the fruiting bodies should be carefully examined. A fresh fruiting body is better than a preserved or a dried one. Good reference material with colour pictures of the different mushrooms, is a basic requirement. A key is usually provided to simplify identification in the most reference texts.

Following morphological feature should be considered for the morphological observation.

A. Size, colour, and consistency of the cap and the stalk
B. Mode of attachment of gills to the stalk, colour of gills.
C. Spore prints or spore colour in mass
D. Smell, habitate and environmental conditions in which mushroom is growing

(ii) Chemical test: Some mushrooms are very palatable, due to their exotic taste but there are many mushrooms which are very poisonous. Unfortunately, there are no general guidelines for distinguishing between the poisonous and edible mushroom species. Such species can be identified by the

characterization of their extract for the presence of specific chemical substance. e.g. Amatoxin → Amanita.

(iii) Molecular characterization: This the final examination to rectify the taxonomic position of particular species. Isolation of DNA and their analysis by gel electrophoresis technique is used for molecular characterization of various closely related & distinctly related mushroom species.

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