MUSHROOM CULTURE

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LECTURE 1

Introduction to Mushrooms

Importance & History

Importance:

- Mushrooms are being used as food since time immemorial. These have been considered as the delicacy. From the nutrition point of view mushrooms are placed between meat and vegetables.
- These are rich in protein, carbohydrate and vitamins. Mushrooms are low in caloric value and hence are recommended for heart and diabetic patients. They are rich in proteins as compared to cereals, fruits and vegetables. In addition to proteins (3.7 %), they also contain carbohydrate (2.4 %), fat (0.4%), minerals (0.6 %) and water (91%) on fresh weight basis. Mushrooms contain all the essential nine amino acids required for human growth. Mushrooms are excellent source of thiamine (vitamin-B1), riboflavin (B2), niacin, pantothenic acid, biotin, folic acid, vitamin C, D, A and K which are retained even after cooking. Since mushrooms possess low caloric value, high protein, high fibre content and high K: Na ratio, they are ideally suited for diabetic and hypertension patients. They are also reported to possess anticancer activities.
- India is primarily agriculture based country blessed with a varied agro-climate, abundance of agricultural waste and manpower, making it most suitable for cultivation of all types of temperate, subtropical and tropical mushrooms. It can profitably be started by landless farmers, unemployed youths and other entrepreneurs. It requires less land as compared to other agricultural crops and is basically an indoor activity. These are the ideal tools for recycling the agricultural wastes which otherwise may pose problem of disposal and atmospheric pollution.
- Therefore, mushroom cultivation is not only of economic importance but also has important role to play in integrated rural development programme by increasing income and self employment opportunities for village youths, woman folk and housewives to make them financially independent.

History:
A. Button mushroom

- **1630:** Cultivation of white button mushroom started first in France in the open on ridges made out of horse dung manure.
- **1707:**Tournefort at Royal Academy of Science, France, mentioned about compost preparation and mushroom cultivation.
- **1731:** French method of cultivation was introduced into England by Miller.
- **1779:** Abercrombie described a method of composting stable horse manure in stacks.
- **1831:** Callow grew mushroom in cropping houses warmed by fire heat and got fairly good yield (1.5 lbs/sq.ft)
- **1893:**Costanitin pointed out that the incidence of diseases made constant changing of growing area necessary.
- **1902:** Ferguson published details of spore germination and growing of mycelium.
- **1905:** Duggar succeeded in making mycelium cultures from the tissue of mushroom caps.
- **1929:** Lambert discovered that spawn could also be prepared from single spore cultures.
- **1937:**Sinden found that about one third of monospore cultures of *A.bisporus*he prepared were incapable of producing fruit bodies.
- **1950:**Sinden and Hauser introduced “Short Method ” of composting.
- **1973:** The first strain of *A.bitorquis* introduced commercially by a French firm Somycel as strain No. 2017 and later by Le Lion

B. Oyster mushroom:

- **1917:** Falck described the first successful cultivation of *Pleurotusostreatus.*
- **1951:**Lowhag was the first to grow *Pleurotus* on sawdust mixtures.
- **1962:** Bano and Srivastava reported mass production on straw-based substrates and their work paved the way for large scale commercial exploitation.

History of Mushroom Cultivation in India

Cultivation of edible mushrooms in India is of recent origin, though methods of cultivation for some were known for many years. The important historical developments in the cultivation of edible mushrooms are as below:

- **1886:** Some of specimens of mushrooms were grown by N.W. Newton and exhibited at the annual show of Agriculture, Horticulture Society of India.
- **1896-97:**Dr. B.C. Roy of the Calcutta Medical College carried out chemical analysis of the local mushrooms prevalent in caves or mines.
- **1908:** A thorough search of edible mushroom was initiated by Sir David Pain.
- **1921:** Bose was successful in culturing two agarics on a sterilized dung medium, details of which were published in the Indian Science Congress held at Nagpur during 1926.
- **1939-45:** Attempts on experimental cultivation of paddy straw mushroom (*Volvariella)* was first undertaken by the Department of Agriculture, Madras.
- **1941:**Padwick reported successful cultivation of *Agaricusbisporus*from various countries but without much success in India.
- **1943:** Thomas *et al*. gave the details of cultivation of paddy straw mushroom (*V. diplasia)* in Madras.
- **1947:**Asthana reported better yields of paddy straw mushroom by adding red powdered dal to the beds. He suggested April-June as the most suitable period for cultivating this mushroom in central Provinces and also carried out the chemical analysis of this mushroom.
- **1961:** A scheme entitled "Development of mushroom cultivation in Himachal Pradesh" was started at Solan by the H.P. State Govt. in collaboration with I.C.A.R. This was the first serious attempt on cultivation of *Agaricusbisporus*in the country.
- **1962:** Bano*et al*. obtained increased yield of *Pleurotus* on paddy straw.
- **1964:** Cultivation of *Agaricusbisporus* on experimental basis was started by CSIR and State Govt. at Srinagar in J&K.
- **1965:**Dr. E.F.K. Mantel, F.A.O., Mushroom Expert, guided and assisted Department of Agriculture for construction of modern spawn laboratory and a fully air conditioned mushroom house. Research on evaluation of different strains and use of various agricultural wastes, organic manures and fertilizers for preparing synthetic compost were undertaken. Dr. Mantel's consultancy concluded after a period of 7 years.
- **1974:**Dr.W.A. Hayes, F.A.O., Mushroom Expert, guided further in improving the method of compost preparation, pasteurization and management of important parameters in the mushroom house. New compost formulations, casing materials and important parameters like nitrogen content in the compost, moisture in the casing mixture, air movements and maintenance of proper environmental factors were also standardized which raised the mushroom yields from 7 to 14 kg/m^2.
- **1977:** A 1.27 crore, Mushroom Development Project was launched under U.N.D.P by the Department of Horticulture (H.P) wherein the services of Mr. James Tunney were made available. He got a bulk pasteurization chamber constructed and made available readymade compost and casing to the growers of H.P. The U.N.D.P. Project was concluded during 1982 and since then the Department of Horticulture (H.P) is running the project.
- **1982:** The Indian Council of Agricultural Research (ICAR) sanctioned the creation of National Centre for Mushroom Research and Training (NCMRT) during VIth plan on October 23, 1982 with the objectives of conducting research on mushroom production, preservation and

utilization and to impart training to scientists, teachers, extension workers and interested growers.

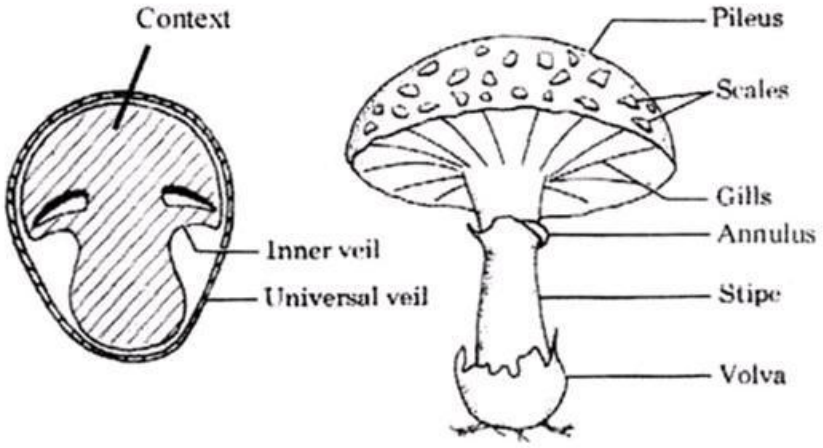
- **1983:** All India Coordinated Project on Mushroom (AICRPM) was initiated during VIth Five-Year Plan on 01.04.1983 with its headquarter at National Research Centre for Mushroom Presently known as Directorate of Mushrooms.
- Presently there are ten co-ordinating and one co-operating centres working under AICRPM located in 11 states. Of these,nine centres are based at State Agricultural Universities, while two at the ICAR institutes.

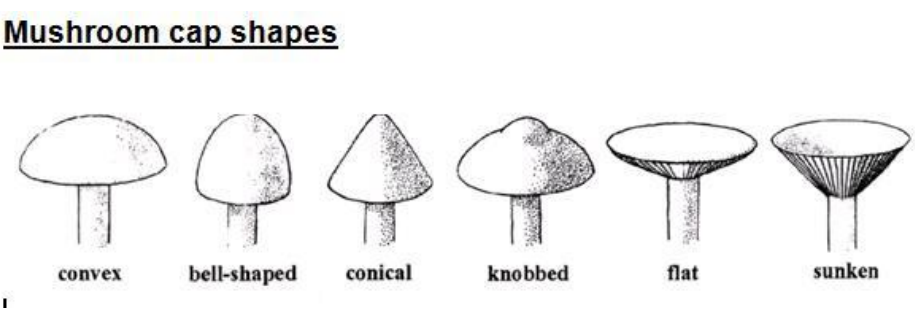
Classification of Mushrooms

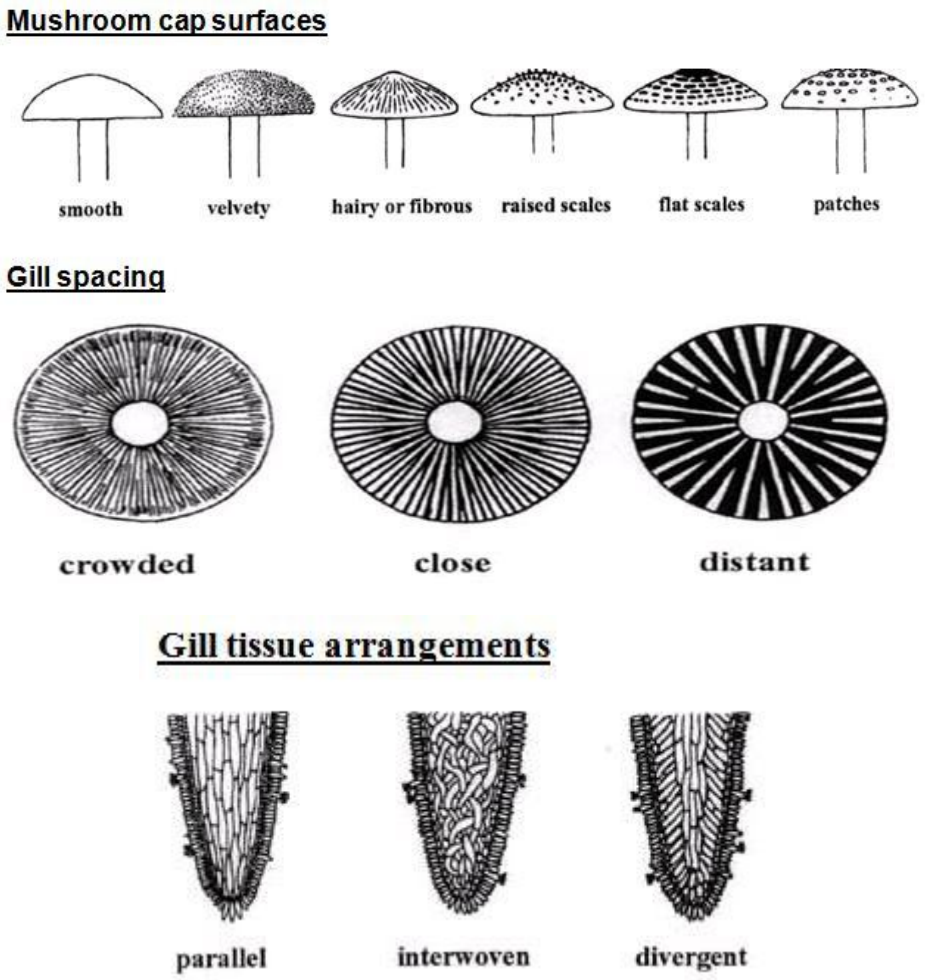
Classification of Mushrooms

- Mushroom is a fleshy fruiting body of some fungi arising from a group of mycelium buried in substratum. Most of the mushrooms belong to the Sub- Division: Basidiomycotina and a few belong to Ascomycotina of Kingdom-Fungi.
- It is reported that there are about 50,000 known species of fungi and about 10,000 are considered as edible ones. Of which, about one hundred and eighty mushrooms can be tried for artificial cultivation and seventy are widely accepted as food. The cultivation techniques were perfected for about twenty mushrooms and about dozen of them have been recommended for commercial cultivation. However, only six mushrooms are widely preferred for large-scale cultivation. They are :

1. Paddy straw mushroom - *Volvariella spp.*
2. Oyster mushroom - *Pleurotus spp.*
3. Button mushroom -*Agaricus spp.*
4. Milky mushroom - *Calocybe spp.*
5. Shiitake mushroom _ *Lentinulla spp.*
6. Jew's ear mushroom - *Auricularia sp.*







A .*Agaricusbisporus:* The Button Mushroom

- Cap: 3-16 cm, convex to broadly convex or nearly flat in age; dry; smooth or with pressed-down or small scales; white in some varieties, brown in others. Gills free from the stem; close; pinkish to pinkish brown at first, becoming dark brown to blackish. Stem 2-8 cm long; 1-3 cm. thick; sturdy; more or less equal; smooth or with small scales below the ring; white, often bruising brownish; with a ring that sometimes disappears in maturity. Flesh ¬white and firm; usually bruising and staining brownish (see top illustration).Odour and taste pleasant.Chemical Reactions -cap not yellow with KOH. Spore print brown .Microscopic Features -spores 5.5-8.5 x 4-6.5 µ; elliptical; smooth. Basidia 2-spored.

B.*Pleurotusspp.*:The Oyster mushroom

- The cap of oyster mushroom is tongue shaped , maturing to a shell shaped form , 50-150 mm in diameter , whitish to grey to blue grey in colour . Flesh is thin and white , margin is occasionally wavy, gills are white, decurrent , broadly spaced, stem attached in an off - centred fashion and is short at first and absent in age . Spores are whitish to lilac grey in mass, mycelium whitish , fast growing rhizomorphic to linear . Basidiatetrapoplar , producing 4 haploid spores, heterothallic, clamp connections present . Because of the allergic nature of spores , some sporeless strains have also been developed.

C. *Volvariellaspp.*:The Paddy straw mushroom

- Mushrooms are white initially, become dark tan in colour as the veil tease and then changes to a pale tan with age. Fruiting bodies are small when young enveloped by a sheath like universal veil, which soon breaks as fruit bodies mature, leaves an irregular cup-like sack at the base of the stem known as volva. Cap 5-15 cm broad, egg shaped and expands to campanulate or convex with slight umbo .

Gills are free, white first and soon pinkish, spores are pinkish to pinkish brown in mass, 7.5–9 x 4–6 µ in size. Stem 4-20 cm long, solid, smooth and white to yellowish in colour. Stem base is encased in a thick volva .Basidia are tetrapolar , producing four haploid spores , primary homothallic, clamp connections are present, form cheilocystidia, pleurocystidia and chalamydospores.

Mushroom Poisoning and treatments

Eating poisonous mushrooms may cause different types of reactions which can broadly be classified as follows :

1. **Gastric disorder:** The poison causes serious gastric disturbance, it chiefly acts by exciting and then paralysing the central nervous system as by *Amanita muscaria* or poison containing irritant which cause gastric enteritis by direct action on the mucous membrane of the digestive system.e.g *Gyromitraesculenta.*
2. **Nervous disorder:** It causes degeneration of cells , especially of the nervous system and grandularparenchymatous tissues like liver as in case of *Amanita phalloides.*
3. **Muscular disorder:** There may be exciting of the muscular system , especially the smooth muscular fibre as it is there in the uterus , vessels etc.
4. **Haemolytic disorder:** There can be destruction of blood or haemolysis as in case of *Amanita rubescens*

Treatments :

- All the collectors of wild mushrooms should be careful about mushroom poisoning and have some knowledge of the first –aid remedies in case of mushroom poisoning and then the patient should immediately be taken to a doctor.
- The patient should be made to cover his body with a blanket , lie down calmly and given the first –aid treatment till the arrival of the doctor.
- **Removal of poison from the stomach** : The patient may be made to vomit by putting his fingers inside the mouth or throat or by giving warm water with one tablespoonful of mustard seeds or apomorphine. The stomach should be completely washed by means of a stomach tube . One can also give some sedatives like warm water , 4--5 tablespoonful of warm milk , two tablespoonful of olive oil beaten with the yolk of an egg etc.
- **Elimination of the toxin**: The ingested poison in the stomach can be removed by putting charcoal powder in the stomach and if it has already been absorbed in blood then subcutaneous injections of atropine or other antidotes can help in removing the effect of poisoning.

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LECTURE 2

Equipments & Collection

The Equipments and Tools Required:
Ice boxes, Cutting knives, blades, rubber gloves, scissors, paper bags, polythene bags, paper napkin, old newspaper pieces, blotting paper pieces, field guide book on mushrooms, umbrella, torch, digital camera, an altimeter, a notebook and pen ,collecting baskets, loose wearing with a hat and hunter shoes.


Fig. 2.1 Wild forests as ideal places for Mushroom collection
Fig. 2.2 Use of a hand digging tool in the forest during the rainy season


Fig. 2.3 & 2.4 Persons engaged in collection of wild mushrooms

How to Collect Wild Fungi from forests?
Different types of mushrooms appear in meadows , fields or forests just after the first showers in the rainy season The following points should be taken care while collecting wild fungi during rainy season :

1. The colour, shape, size and the habitat of each collection should be noted .
2. Attempt should be made to compare the morphological characters with the ones given in the guide book.
3. Do not touch the fruiting body and never try to find out its taste in a hurry.Take photographs of the mushrooms when still in the soil.
4. Fruit body found should first be examined carefully.
5. The fungi should be carefully cut or dug up with the help of a knife or hand digging tool and arranged in a single layer at the bottom of the basket.
6. Collected fungi should be handled as little as possible and not bruised or crushed.
7. Though fungi such as many polypores and hydnums do not suffer much from handling , hence these should be wrapped in paper and packed more closely.

8. The locality and date, also other evanescent characters, such as a distinctive smell , change of colour when gently touched or bruised and so on , should be noted .
9. For any unknown species, especially of the gill- fungi, a spore-print should be obtained . This is done by gently removing the stem from cap, laying the cap , gills downwards on a sheet of white paper and leaving it for some hours or overnight.
10. The spore- powder deposited gives the colour of the spores , which is important for identification purposes.
11. Since fleshy fungi can not be preserved in their natural form and colour , students should make coloured drawings that will provide permanent records.

Identification of Fleshy Fungi

Points to be Observed for Identification of Fleshy fungi

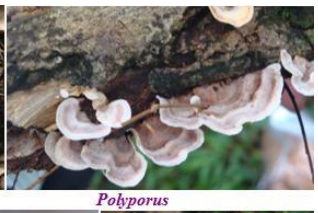
- **General Appearance:** Size, whether growing solitary or in groups, texture, colour, any change of colour with age or on drying, presence or absence of veils in the young stage.
- **CAP:** Size, shape, colour, nature of surface (whether smooth, slimy, scaly or fibrillose),kind of margin, whether easily separated from stem.
- **STEM:** Size, shape (whether equal in thickness throughout or thickened above or below), colour, nature of surface, presence of rings or volva, whether the flesh is continuous with that of the cap or distinct (cartilaginous).
- **TUBES:** Length, colour, shape of mouth, mode of attachment to stem.

Schizyophyllum

Tremella

Mycena

Volvariella



Polyporus

Pleurotus

Volvariella

Calocybe

- **GILLS:** Colour when young and later, texture, thickness , whether crowded or distant (spaced), whether all of the same length or of different lengths and method of attachment to the stem.
- **FLESH:** Thickness, colour, any change of colour or exudation of a milky or coloured juice when cut , texture , smell , taste .
- **SPORES:** For identification purposes, the microscopic characters of spores like colour and other anatomical details are necessary . The properly dried specimens are filled in air tight polythene or paper packets and labelled.

Preparation of media for raising of Pure culture

The pure cultures are raised on a convenient culture medium which are generally in solidified state due to the addition of Agar-agar , a sea weed. The composition of media and the methods of preparation are as given below :

1. Potato - dextrose Agar medium (PDA)
Peeled and sliced potato ---- 250g.
Dextrose ---- 20g
Agar –agar powder ---- 20g
Water ---- 1000 ml
About 250 gram potato are peeled, cut into small pieces, boiled in water for 25-30 minutes and filtered through a muslin cloth. The volume of the extract is raised to 1000 ml with water and boiled along with dextrose and agar-agar powder so as to get a thoroughly mixed solution. Before filling in the test tubes or narrow mouthed Erlenmeyer flasks (for pouring media in Petri plates sterilized in an oven at 180ºC for two hours) , the pH is adjusted to 7.0 and then after plugging with non-absorbent cotton, sterilized at 15 lbsp.s.i for 15 – 20 minutes in an autoclave or pressure cooker.

2. Potato -dextrose Yeast Agar Medium (PDYA)
Just like preparation of PDA , PDYA can be prepared by adding 2g Yeast extract in the solution for selected fungi .

3. Malt Extract Agar medium (MEA)
Malt extract ---- 25g
Agar- agar powder ---- 20g
Distilled water ---- 1000ml
(pH—7.0)
Malt extract and agar are mixed in 1 litre water and boiled by continuously stirring with a glass rod so as to avoid formation of clumps.

4. Compost Extract Agar medium (CEA)
Pasteurized compost ---- 150g
Agar –agar powder ---- 20g
Water ---- 1000ml
(pH ----7.0)
Compost is boiled in 1.5 to 2.0 litre water for few minutes till volume of the water is reduced to half and after filtering through muslin cloth, the volume is again made to 1 litre and autolclaved after mixing agar powder in it and filling in the test tubes.

5.Malt Peptone Grain Agar Medium (MPGA)
Malt extract ---- 20g
Rye or Wheat grains ---- 5g
Yeast (Optional) ---- 2g
Agar-agar powder ---- 20g
Peptone ---- 5g
(pH -7.0)

Wheat or rye grains are boiled in water for 1-1.5 hours, the filterate is mixed with other ingredients and continuously stirred while heating before filling and autoclaving.

Isolation techniques

Isolation techniques for getting pure cultures and their maintenance:

There are two methods to have a mushroom culture - the Spore Culture and Tissue Culture technique.

1. Spore Culture

a) Spore Print :

- In order to get a spore print or collection of spores , the cap from a healthy, disease free mushroom is removed , surface cleaned with a swab of cotton dipped in alcohol and placed on a clean sterilized white paper or on clean glass plate or on surface of the clean glass slides .The surface nearby should be thoroughly sterilized. To prevent air flow , place a glass jar or clean glass or cup over the cap surface . Spores will fall on the white paper or slide surface within 24-48 hours exactly like radial symmetry of the gills .The spore print on the paper can be preserved for a longer time by cutting and folding it into two halves.

Fig. 2.17 Healthy fruit body kept covered for getting spore print
Fig. 2.18 Spore print collected after 24 -72 hours

b) Spore transfer and germination:

- In order to get a pure culture , the scalpel is sterilized by keeping it on a burning flame for 8-10 seconds till it becomes hot red , cool it by dipping in a sterilized medium , scrap some spores from the spore print taken on a paper or glass slide and transfer them by gently streaking on the agar medium aseptically. Minimum, three agar dishes should be inoculated for each spore print and the culture developed after its incubation at appropriate temperature is known as multispore culture.

2.Tissue Culture

- A small bit from the pileal region is cut with the help of a sterilized blade or scalpel, washed several times in sterilized distilled water and dried in a clean tissue paper before inoculating aseptically on a Petri plate or tube containing suitable culture medium.The inoculated Petri plates are incubated at 25 ± I C for 6-12 days and observed at different intervals for the mycelial growth. All Petri plates / glass tubes showing contaminations should be discarded and only the ones with pure growth should be retained for further use after ascertaining the purity and true to type nature of the culture.

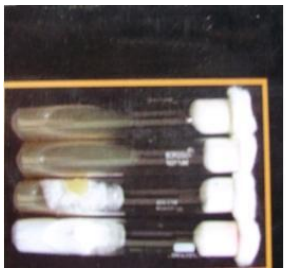


Fig. 2.19 & 2.20 Healthy fruit body picked for tissue isolation in an Air flow chamber

Fig. 2.21 Agar slants and pure culture of a mushroom in test tubes

Fig. 2.22 Pure cultures of a mushroom in agar medium in Petri dishes.

Fig. 2.23 The inoculated tubes and Petri dishes kept for incubation in B.O.D incubator

Fig. 2.24 Pure cultures in tubes and Petri dishes stored in refrigerator

Sub-culturing:

The pure culture of edible mushroom, once established either through spore culture or tissue culture technique , is maintained properly in cool atmosphere or a refrigerator. Sub-culturing is done from time to time by aseptically transferring a small piece of growing pure culture along with the culture medium on the test tube slants containing same or other suitable medium.

The pure culture of a mushroom can be used for preparing master cultures for large scale spawn production on commercial scale . It will be discussed in the next lesson in detail.

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LECTURE *3*

Spawn and its Production

What is Spawn?

- In dictionary term " spawn " actually refers to the fingerlings of fish, but here spawn means the vegetative mycelial network of a mushroom developed after the germination of one or more than one fungal spore (s) grown on a convenient medium. It comprises of the mycelial network along with a supporting medium which provides nutrition to the fungus for its growth and development.

Fig. 3.1 A close view of PP bag containing grain substrate kept for incubation just after inoculation from Master Culture.
Fig. 3.2 A close view of the completely spread spawn bag after 20 days of incubation – ready for use.

SPAWN PRODUCTION OF MUSHROOMS
Raising or procurement of Pure culture of mushroom.

- As already discussed in the earlier lesson,the pure culture of a fungus can be raised either by the spore print technique or the tissue culture technique. Once pure culture of a particular mushroom is established or procured from some reliable source, the process of production of mushroom spawn involves the following steps :

1. PREPARATION OF MASTER / STOCK CULTURE:

- Preparation of master culture or mother spawn is carried out under completely sterile conditions. Pure culture raised either from tissue or spores is inoculated in a suitable substratum (wheat, sorghum or rye) which provides food to the mycelium. Ten kg. of wheat grains are boiled in 15 litres of water for 20 minutes. Water is then drained off and the grains are put over the sieve or on a wire mesh tray for 8-10 hours to dry or remove excess of water. Grains are now mixed with gypsum (calcium sulphate) and chalk powder (calcium carbonate) at the rate of 2% and 0.5%, respectively on dry weight basis. 10 Kg of dry wheat grains will require about 200g gypsum and 50g chalk powder. This will help to check the pH of the medium and also prevent sticking of grains with one another. The grains are filled into half or one litre glucose bottles or PP bags which are plugged with non-absorbent cotton and sterilized at 22 lbp.s.ipressure for 1.5-2 hours. Sterilized bottles are allowed to cool down overnight. Next day bottles are inoculated with the bits of agar medium colonized with the mycelium of pure culture. Inoculated bottles are incubated at 25± 1ºC. After 7 days of inoculation, bottles are shaken vigorously so that mycelial threads are broken and become well

mixed with the grains. Two week after inoculation, the bottles are ready as stock culture for further multiplication of spawn. One bottle of stock culture or master culture or mother spawn is sufficient to multiply 30-40 grain bottles or ppbags .

2. MULTIPLICATION OF SPAWN FROM STOCK / MASTER CULTURE

- Master spawn or master culture bottles / bags are further used for inoculation of large number of other grain bags / bottles prepared by the same technique and resultant is the commercial spawn. Generally few mycelial coated grains from one master culture bottle / bag will be inoculated into 30 – 40 grain bags aseptically in front of the HEPA (High Efficiency Particulate Air) filters of a Laminar flow and then incubated in a room at 25 ± 1 ° C for12-15 days.The commercial spawn thus prepared is used for inoculating the compost beds as seed .

Precautions, Characters and Storage of Spawn

PRECAUTIONS TO BE OBSERVED :

- Avoid overcooking of grains as it may lead to splitting of grains.
- Don't dry the cooked grains on the floor. Always dry over hessian cloth spread on a raised platform or on a wire mesh tray .
- Use only recommended dose of $CaCO_3$ for mixing with the cooked grains. Mixing over dose reduces the fungal growth in the inoculated bags.
- Avoid further sub culturing of the second generation spawn. This leads to loss of vigour of the spawn which again leads to reduced yield. Repeated sub culturing leads to complete loss of vigour. In such cases the fungal growth may be noted in the compost beds but buttoning may be completely arrested.

CHARACTERS OF GOOD SPAWN :

- There should be proper coating of the mycelium around every grain used as substrate for spawn.
- The growth of the mycelium in the spawn bottles should not be cottony or fluffy type but it should be strandy .
- The growth of fresh spawn is more or less white. Brown coloration develops as spawn grows.
- There should not be any slimy growth in the spawn bottles which is an indication of bacterial contamination.
- There should not be any greenish or blackish spot in the spawn bottles. Such type of spots indicate that the spawn is contaminated with moulds.

Precautions during transit of spawn:

- Care must be taken during transit that spawn bottles are not exposed to bright sun light and a temperature higher than 30^{o}C. To avoid such risks , spawn bottles are packed in thermocol boxes containing ice cubes or should be transported during night hours when it is cool.

Storage of spawn:

- Fresh spawn should always be used for seeding and its long time storage should generally be avoided. However, the spawn can be stored at 4-6ºC for one month in case it is not used due to certain unavoidable circumstances.

Fig. 3.3 Workers busy in mixing and filling of substratum for spawn preparation

Fig. 3.4 & 3.5 Sterilized grain bags kept for incubation on shelves in an incubation room at 25 ± 1ºC after inoculation from the Master Culture bags

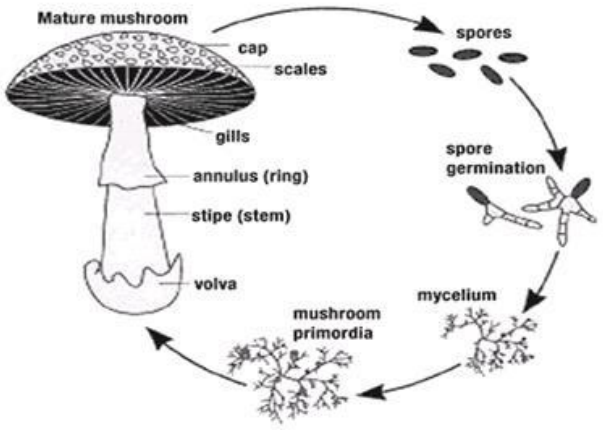
Fig. 3.6 A close view of PP bag containing grain substrate kept for incubation just after inoculation from Master Culture.

Fig. 3.7 A close view of the completely spread spawn bag after 20 days of incubation – ready for use.

LECTURE 4

Genetic Improvement of Mushrooms

Majority of the cultivated edible fungal species belong to Basidiomycotina group with small majority belonging to Ascomycotina group. One must know the biological behaviour of mushrooms so as to develop a programme for further improvement in qualities.



Life cycle of a typical mushroom

It is well understood that the yield and quality of a particular mushroom species depend on the genetic makeup of the mushroom variety and the environmental conditions in which it is growing.The interaction between genes and environmental conditions determines the overall performance of a mushroom variety, including its characters and behaviour. Following different methods have been adopted for the genetic improvement of button mushroom from time to time :

- Introduction
- Selection
- Anastomosis
- Hybridization
- Mutation
- Protoplast fusion
- Genetic Engineering.

1. INTRODUCTION :

It is the quickest and an easy method of crop improvement. Here a number of surveys are made , various isolates growing in different environment are collected and screened for their yield performance at a changed environment. This introduction proceeds for isolation and selection of superior type for their direct use and it builds up the genetic base for further improvement in performance under the breeding programme. Large scale adoption of strain S-11 and RRL-89 in Himachal Pradesh and Jammu & Kashmir, respectively in India are the results of the introduction of this species. Similarly K-32 and K-26 were found to be the best amongst five strains of *Agaricusbitorquis.*

Fig. 4.2 Field trials conducted for the evaluation of strains

2. SELECTION :

It is the process of retention of desired genotypes and elimination of undesirable ones within a strain, but its success depends upon the presence of high additive genetic variance and the least influence of genotype x environment interaction on the expression of trait to be selected. In button mushrooms, selection can be made from single spore, multispore or tissue culture techniques. Single monosporic culture technique is helpful only in homothallic species (species bearing the sex structures in the same thallus) like *Agaricusbisporus*and *A. subfloccosus*, but in case of heterothallic species like *A.bitorquis, A.campestris , A arvensisetc ;* selection is done using multispore or tissue culture technique.

Fig. 4.6 Field trials conducted for the evaluation of strains of milky mushroom

Genetic Improvement of Mushrooms (Contd..)

3.HYBRIDIZATION :

It consists of mating of self sterile and compatible homokaryotic lines that results in creation and selection of desired traits, but it is the assembling of the best combination of genes into one individual variety so as to produce higher yield with best quality mushroom which is the ultimate objective of a mushroom breeding programme. Application of biotechnology in the genetic improvement programme of mushroom has introduced new technique such as DNA based markers which has provided much needed boost to the on

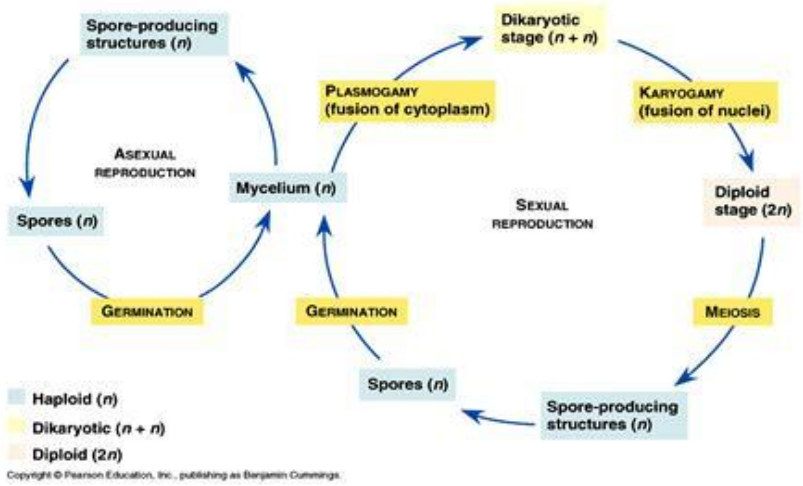
going breeding efforts in case of button mushroom. Rafalski and Tingey, 1993 have described the use of DNA based technology in breeding programme as Molecular Breeding. The DNA based marker like RFLP (Restriction Fragment Length Polymorphisms), Allozymes, RAPDS (Random Amplified Polymorphic DNAs) , ITS (Internal Transcribed Spacing) etc: are being utilized under Molecular Breeding programmes for isolation of homokaryons, confirmation of hybridization, assessment of diversity in *Agaricus* breeding programme. The most commercially cultivated mushrooms in the world . Horst-U1, Horst-U2 , S-11 , S-791 strains of *A.bisporus* are other examples of the breeding successes .The greatest advantage of hybridization programme is that the hybrids generated are known to give maximum yield performance and show phenotypic stability under stress.

Following are the steps involved in hybrid development in mushrooms :

- Selection of parent lines
- Isolation of homokaryons
- Crossing of compatible homokaryons
- Identification of hybrids.

A. Selection of parent lines :
Lines possessing wider adaptability, genes resistant to diseases and insect-pests, better yield performance and morphological characters, better shelf life, suitability of processing etc; are chosen as parent for hybridization. In India the high yielding and better quality germplasm lines like S-11 , S -44 , NCS-6 , ARP -215 , ARP-217 , 224 , 225 ; P-1 , ITCC-1924 , S-56 etc; have been identified as parents for single spore selection and hybridization. RAPD markers have successfully been used for assessment of diversity in *A.bisporus*germplasm at DNA nucleotide level and to fingerprint each genotype for strain protection.

B. Isolation of Homokaryons:
For hybrid breeding , isolation of homokaryons from heterokaryotic parental lines is required which is very difficult in case of *A.bisporus* due to its unusual life cycle termed as secondary homothallism or Intramixing where majority of spores are binucleate and self fertileheterokaryons as compared with the other heterothallic species such as *A bitorquis*., *A campestris* , *Aarvensis*etc ; where each basidiospore gives rise to self –sterile mycelium and hence single spore isolate in these mushrooms are homokaryotic and are cross fertile. Moreover, *A .bisporus*also lacks clamp connections, the morphological markers which facilitate easy distinction of homokaryons from heterokaryons. These factors have affected the mushroom improvement programme in *A.bisporus.*



The traditional method of homokaryon isolation technique involves the identification of naturally occurring monokaryon spores which are at a frequency of 0.1 to 40 per cent in *A.bisporus.* These homokaryons are isolated either by dilution plate technique or the micro manipulation technique. In dilution plate technique

the basidiospores are serially diluted in sterilized distilled water and plated on a suitable agar medium so that it may have 8-10 spores only in a plate.. The spores are stimulated for quick germination by placing growing mycelium in the lid of the Petri dish which are placed upside down and incubated at 25-28º C . The plates are critically examined after 4-5 days of incubation and the single spore colonies are marked under microscope and aseptically transferred to the slants containing suitable agar medium like MEA , PDA , Compost-agar medium etc. The micromanipulator method consists of picking the spores directly from aberrant three and four basidia aseptically. The germinating single spore can also be picked up and transferred separately by using this method.

The following criteria are used alone or in combination for the identification of homokaryons in *A.bisporus.*

- Colony morphology
- Slow mycelial growth
- Non- fruiting
- Source of spores (3 and 4 -sporedbasidia)

The mycelium derived from infertile single spore isolate (homokaryons) in *A.bisporus* is mostly of slow growing and appressed type, whereas the homokaryons of hetrerothallic spp. like *A .bitorquis* also grow slowly but it exhibits a dense matted type of mycelialgrowth.The criteria of colony morphology, slow mycelial growth and non-fruiting characters used for identification of homokaryons are cumbersome and affected by the environmental factors and also the frequent reports of homokaryotic fruiting in several cultivated mushrooms by different mushroom workers making these criteria unreliable and ambiguous. With the availability and use of modern tools of molecular biology viz; Allozymes , Restriction Fragmented Length Polymorphism (RFLP) , Random Amplified Polymorphic DNAs (RAPD) etc; for homokaryon isolation in *A.bisporus*, genetic improvement of button mushroom has become easy. These techniques have been found to be quick and reliable as these are based on DNA markers and are not influenced by the environmental factors.

C. Crossing of compatible homokaryons :
The known compatible homokaryons are anastomosed (mated) by growing them side by side on sterilized agar medium and incubation at favourable temperature as the method devised by Eliott (1978). The formation of fluffy growth at the hyphal confrontation or junction zone is indicative of development of a hybrid. A big piece of the mycelial growth from this junction zone is cut and shifted aseptically to a new agar medium for getting pure hybrid culture which is further used for preparing spawn for its testing.

D. Confirmation of hybrid testing :
In case of widely cultivated mushrooms , *A.bisporus and A.bitorquis* , the fertile heterokaryon or hybrid formed by mating of two compatible homokaryons forms no clamp connection and secondly the microscopic observation of only heavier growth at the zone of confrontation between the two compatible homokaryons. But following the latest development, formation of hybrids in these mushrooms can be confirmed by a number of tests like the fructification test, auxotrophic markers, resistance markers, allozyme markers, DNA markers – RFLP's and RAPDs as described below :

Evaluation: After the identification / confirmation of new hybrid formation, these are evaluated through Initial Evaluation Trial (IET) for the yield, quality, resistance against diseases and pests and for other traits. The hybrids found superior and better are further put to multilocational trials and those found successful are again put in On-farm trials in farmers' field and only then one with best performance on all aspects , is released for commercial production/cultivation.

Hybridization has only been found to be the most reliable and sustainable strain improvement method in case of mushrooms.The hybrid U-1 and U-3 of *A.bisporus* developed by Dr.GerdaFritsche in Holland are still performing good since 1981 which possess characters like high production with good canning quality. Similarly hybrids K- 32, K -26 and K -46 of *A.bitorquis* also performing very well .

4.MUTAGENESIS :

Abrupt change on the genes of DNA of some mushrooms may result in development of variability and a new strain, though it is very rare. The common white button mushroom is also the result of mutagenic change that occurred in some cream strain of *A.bisporus* during 1927 (Kligman,1950). Now-a-days mutagenesis is attempted to get new variable strains for selection and hybridization programmes. Here hyphal fragments. protoplast and basidiospores are used for mutagenesis to develop strains with desired traits like resistance against fungicides and diseases, higher yields, tolerance for high temperature, sporeless strain etc.

5. PROTOPLAST FUSION :

It is a non-conventional method of gene transfer as it involves breaking down of the natural barrier of gene exchange as found in conventional system of breeding. The following technology is involved in protoplast fusion:

- Use of cell wall digestive enzymes , Novozyme 234 with osmotic stabilizer (0.6 M Sucrose or 0.5 M MgSo4)
- Fusion of the protoplast with $CaCl_2$ and polyethylene glycol.
- The regeneration of evaluation of somatic hybrids.The technique of rising electrical pulsation (Electrofusion) for the protoplast fusion is also being used now-a-days.

6. GENETIC ENGINEERING:

- The technique is based on the systematic practice of isolation, cloning and insertion of desirable gene (s) onto the genome of target organism. For successful delivery of transferring DNA into mushroom genome , Electroporatin and Biolistic methods have been recently used (Moore *et al*,1995 and Mooi Brock *et al,* 1996)
- Other approaches like Marker Assisted Selection (MAS) , Back crossing and the use of Quantitative Trait Loci (QTLS) have also been considered new approaches for button mushroom breeding and recently *Agrobacterium tumifaciens* has successfully been used for the genetic transformation of gill tissue and germinating spores in *A.bisporus* .

LECTURE 5

Cultivation technique of button Mushrooms - I

White Button Mushroom

1. WHITE BUTTON MUSHROOM (*Agaricusbrunnescens* Peck.)

White variety -------A. *brunnescens* var. *albidus*
Brown variety ------A. *brunnescens* var. *bisporus*
Cream variety ------A. *brunnescens* var. *avellaneous*

This mushroom is commonly found growing in soil enriched with cow dung, horse dung or forest litters in temperate climate. A most widely cultivated mushroom in the world. The name *Agaricus*originated from the greek word Agaricon—with a Scythian people called Agari who were knowing the use of medicinal plants and employed a fungus called " agaricum ", probably a polypore in the genus *Fomes*. *Brunnescens*means brown in latin, as the colour changes to brownish after bruising . It is also called as *A. bisporus*because of the two sporedbasidium.

Description:
White button mushroom (A . *brunnescens*) is thick fleshed , robust with thin gills on the underside of the cap that are pinkish white in early age and darkening to chocolate brown at maturity. Cap is whitish, cream coloured or brown. Cap surface smooth to appressed and dry. The stem is short, thick adorned with a persistent membranous annulus from a well developed partial veil. Spores chocolate brown in mass, basidia bipolar (twospored) forming diploid spores, secondarily homothallic, clamp connection absent. Mating of compatible dikaryons typically results in development of strain which is more vigorous and high yielding. Mycelium is dingy white, moderately rhizomorphic.

White button mushroom (*A. bisporus)* cultivation

Nutritional Value : Button mushrooms contain 90-92 % water and only 8-9% dry matter. Also contains 3.92 % protein, 1.09 % crude fibre, 1.25 % ash, 0.19 % fat and 56 mg. niacin / 100 g weight.

Spawn production : The Master culture and spawn are produced on wheat or rye grains buffered with Calcium carbonate and Calcium sulphate.

Cultivation : Button mushrooms, including the high temperature species *A. bitorquis*(20 – 25° C) require

well decomposed manure for its cultivation which is prepared by long method or the pasteurization method of composting by mixing wheat or rye straw with supplements like chicken manure, cotton seed cake, wheat bran, urea, gypsum etc. The prepared compost is filled in polythene bags or wooden trays, spawned by through or layer spawning method and incubated in a closed room at 25 ± 1ºC and 90 % relative humidity with high concentration of carbon dioxide (5,000 to 10,000 ppm) in the absence of light . After 10 -15 days of incubation, when mycelium of spawn completely impregnates the compost, it is covered with 1-1.5 inch layer of sterilized wet casing mixture containing FYM alone or FYM + spent compost or FYM + forest soil or soil + sand + coco coir or sand + soil + paddy ash or peat soil . The mycelium of button mushroom will not fructify unless it is covered on the surface with a layer of fine casing mixture.

Composting

Composting: Compost can be prepared by two methods :

1. Long method of composting
2. Short or pasteurization method of composting

1. Long Method of Composting:

A) Formula developed by Mushroom Research Laboratory, Solan

Wheat straw ------ 1,000Kg or
Paddy straw ------- 1,250Kg
CAN ------- 30Kg
Super phosphate ------- 25Kg
Urea -------- 12Kg
Muriate of Potash --------- 10Kg
Wheat bran ---------- 100Kg
Molasses ---------- 16.6litres
Gypsum ---------- 100Kg
Folidol dust ---------- 750 g

B) Formula developed by IIHR, Bangalore

Paddy straw ---------- 150Kg
Maize stalks ---------- 150Kg
Ammonium sulphate ---- 9Kg
Super phosphate ------ 9Kg
Urea ------- 4Kg
Rice bran ------ 50Kg
Cotton seed meal ------ 15Kg
Gypsum ------ 12Kg
Calcium carbonate ----- 10 Kg

Long method of composting was first advocated in India by Mantel *et al.* (1972). To begin with the composting process, clean the composting yard thoroughly and wash it with 2% formalin solution. Wheat straw or any other base material to be used is spread in a thin layer of 8-10 inches thickness over the floor of composting yard. Sprinkle water over the straw with a hose pipe and wetting of straw is done repeatedly at least 2-3 times a day for 2 days with the help of forks. Before mixing with the wet wheat straw, the ingredients like urea, CAN, super phosphate,wheat bran etc. (except insecticides and gypsum) are thoroughly mixed , wetted with water and then covered with damp gunny bags 14-16 hours before use.

Fig. 5.2 & 5.3 Fresh Wheat straw and Paddy straw stored for compost preparation
Fig 5.4 Chicken manure stored for substrate preparation

Preparation :

- **Day 0:** On this day fertilizer mixtures are spread evenly on the pre- wetted straw. This mixture is made into a stack with the help of wooden boards or pile formers . Dimensions of pile should be 5x5x adjustable length. Height and width of the pile should not be more than this otherwise pile may become too hot due to high temperature and the anaerobic conditions may prevail in the centre which may not yield good quality compost.
- **Day 1-5:** Start monitoring the temperature of the heap. Temperature should start rising after 24-48 hours of stacking and reach 65-70°C in central core. If the moisture of the mixture is less, than water can be sprayed. Watering should be stopped as soon as leaching starts from the bottom of pile. If water starts leaching in large quantity then it should be collected in a guddy pit and put on the top of the pile.

Long method of composting - stacking the heap on Day-0 with the help of pile formers (a & b) and a rectangular shaped compost heap raised after completion of the pile forming process (c)

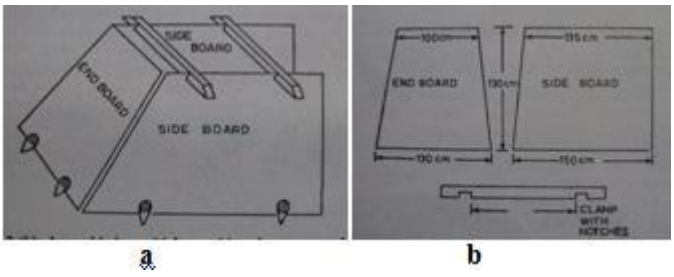


Diagram of a Pile Forming Board for stacking heap of compost during compost making in mushroom cultivation (a& b)

- **Day 6: First turning:** On this day first turning is given to the stack. The aim of turning is that every portion of the pile gets equal aeration and water for proper decomposition of the base material. The correct method of turning is as follows :
- Remove about 1 feet compost from top and side of pile, shake thoroughly so that excess of ammonia is released and it is exposed to the air properly, and keep this portion on one side. Now remove the central and bottom portion of the pile, shake these with the forks and keep them separately. Now the new pile is made with the help of boards keeping the central portion at the bottom. Top and sides portion should be placed at the centre while bottom part comes on the top and sides. During pile formation watering is done ,if required.
- **Day- 10: 2nd turning.** Break open the stack, remove it as indicated above, water may be added if required and restack it .
- **Day-13: 3rd turning:** Restack and add required quantity of gypsum
- **Day-16: 4th turning**
- **Day-19:5th turning**
- **Day-22:6th turning**
- **Day-25:7th turning** : add required quantity of Folidol dust
- **Day 28: Filling day.**Break open the pile and check for the smell of ammonia , if it still persists, give an additional turning after 3 days. This way compost is prepared by long method in 28-30 days.

2. Short or Pasteurization Method of Composting :

Formula given by Mushroom Research Laboratory , Solan

Wheat straw (chopped) ------ 1000 Kg
Chicken manure ---------- 400 Kg
Brewer's grain or wheat bran -- 72 Kg
Urea ---------- 14.5 Kg
Gypsum ---------- 30 Kg.

- This is done in two phases. **Phase- I** is done in the composting yard while **phase II**, inside a closed chamber called pasteurization chamber or tunnel (bulk chamber) with the help of aerated steam for pasteurization and conditioning of compost.
- **Phase I:** Phase - I involves pre-wetting of straw and mixing of ingredients in the straw as in long method. But in this case turning is given after every 48 hours (2nd day). During third turning or on 6th day total amount of gypsum is added in the compost. After 4th turning on 8th day, the compost is filled in pasteurization tunnel on 10th day.

Phase –I of composting ------ first turning after mixing urea and pile being formed with the help of a Pile Former (1) third turning being given by breaking the heap and adding water (2), picture of a front loader tractor (3) and compost turning machine (4) for mechanical composting

Phase II: (Pasteurization)

- After filling partially decomposed compost in pasteurization chamber or tunnel, a temperature of 48-50 ° C is maintained for next 2-3 days by circulating the inside air. Then with the introduction of steam, temperature of the tunnel is raised to 58-60ºC for 6 hours.
- Fresh air is then allowed to enter the room so as to bring down the temperature to 50-52°C which is maintained for 3-4 days for conditioning. When ammonia smell gets eliminated, then fresh air is introduced in the tunnel to cool down the temperature of the compost to 25-28°C. By pasteurization method, compost is prepared within 18-20 days.

Qualities of a good Compost

Qualities of a good Compost:

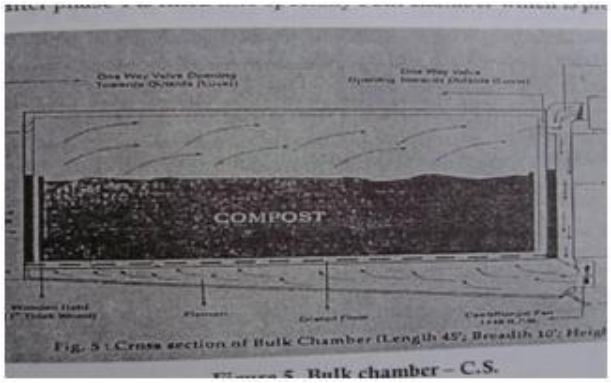
- Compost should be dark brown in colour with profuse fire fangs.
- Compost should have moisture percentage of about 68-70 percent.
- pH of the compost should be in the range of 7.2-7.8.
- There should not be any smell of ammonia.
- It should not be sticky or greasy.
- It should be free from insects and nematodes.

This method has got certain advantages over long method of composting as detailed below :

- More compost per unit weight of ingredients is produced .
- Total period of composting is reduced
- The yield is almost doubled
- All the harmful fungi, competitor moulds, insects, nematodes and other pathogens get killed during pasteurization which otherwise cause reduction in yield
- Most part of Ammonia liberated is converted into microbial protein which otherwise go waste in long method of composting.
- Conditions inside a pasteurization chamber favour proper temperature and aeration resulting in the preparation of good quality compost free from all types of harmful microorganisms .

3. Bulk pasteurization method:

It is similar to the short or pasteurization method of composting but in a modified form of technology. Here after phase -1 of composting, compost is treated / pasteurized in bulk inside a specially built chamber known as the chamber or tunnel in Phase –II .



Bulk chamber – C.S.

The diagram of a Bulk Pasteurization Chamber (Tunnel) showing the compost after Phase-1, filled in the chamber having ducts for fresh air entry and the slanting floor with grated plenum and blower fitted underground

1 2

Pasteurization chamber/Tunnel of a Bulk Pasteurization Chamber showing grated panel (1) and compost after phase-I being filled in the chamber for pasteurization process or Phase II (2)

3 4

Showing the outer part of a bulk pasteurization chamber with its door closed and air handling unit (3) and the blowers fitted outside the pasteurization chamber

The bulk pasteurization method is again having some advantages over the short or pasteurization method of composting :

- More compost per unit size of the room can be treated at a time.
- Facilitates the preparation of best quality compost.
- The cost of pasteurization is reduced.
- Yield per unit weight of compost is much higher
- Labour cost is reduced.
- The heat generated by compost is utilized for its further pasteurization, hence cost of diesel, electricity or fuel is reduced.
- Spawn running can also be done in the tunnel itself thus reducing the cost and saving of time
- Environmental pollution is very much reduced

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LECTURE 6

Cultivation technique of button Mushrooms - II

Cultivation technique

A . Filling:
The compost is filled in wooden trays or shelves or in polythene bags at different rates. The hard-compressed bags / beds attain more compost as compared to slightly compressed bags. The dry weight of substrate per square foot of cropping surface largely determines total yield. During summer the compost is slightly pressed while filling so that due to the metabolism of the growing mycelium, bed temperature may not rise as enough of heat is generated during that period. Similarly it is hard-pressed during winter season. Nutrients from the farthest point of the compost bed are transported to the growing mushroom mycelium. Filling of the trays / bags 6-8 inches deep with compost, stacking them closely, with their upper end covered with polythene or newspaper in a closed room, has been found to provide conditions for efficient spawn run and the heat generated can be managed easily. Moreover, it will add to the ideal temperature (25±1° C) required for rapid colonization of the compost with mycelium.

B .SPAWNING AND SPAWN RUNNING :
a) Spawning: Mixing the mushroom seed or spawn in the compost is called as spawning. There are different methods of spawning which are as follows:

1. **Surface spawning:** Grain spawn is scattered all over the surface of the compost in trays or racks which is then covered with 2 cm thin layer of compost.
2. **Double layer spawning:** Usually done under unfavourable environmental conditions at low temperature. The trays are half filled with compost, spawn is scattered over it, then trays are filled completely with compost and again spawned in the same manner. Finally a thin layer of compost is spread on the spawn covering it completely.
3. **Through spawning:** The desired quantity of spawn is mixed thoroughly in the required quantity of compost which is then filled in racks, trays or bags. This type of spawning is done mainly in bag cultivation.
4. **Spot spawning:** Trays are filled with compost. Spawning is done in 1-2 inches deep hole made in the compost about 4-5 inches apart in rows. A tea spoonful spawn is filled in the holes which are later covered with compost. After spawning, trays or racks are covered with old newspaper sheets and watered lightly with the help of water sprayer. In Polythene bag cultivation, its mouth is tied with the help of thread.
5. **Active spawning:** Here in place of grain spawn, fresh compost after complete colonization by mushroom mycelium is used as spawn. In this method spawn run is very quick but care should be taken to avoid use of contaminated compost.

b)Spawn running:
The temperature of the mushroom house, where trays or bags are kept for incubation should be maintained between 22-25°C. The humidity should remain at 80-85% RH level. This can be maintained by frequently spraying water on walls and floor of the mushroom house. During spawn running , fresh air is not required, hence room should be kept closed to create darkness. Higher CO_2 concentration than the normal level in the air favours mycelial growth of the mushroom. Under favourable environmental conditions within 14-15 days of spawning, the compost surface is covered with the cottony growth of the white mycelium. This condition is called spawn run. If temperature is lower than optimum level, it prolongs the spawn run period even up to 22 days while higher temperature retards mycelial growth.

Fig. 6.1 Pasteurized compost filled in polythene bags after spawning and kept in the spawn running room on the shelves for spawn run.

c) Supplementation at spawning:
In order to get additional increase in yield, some selected nutrients are added in the compost at the time of spawning. They are designed to become available to the mushroom mycelium during the early flushes. These supplements are specially formulated nutrients encapsulated in a denatured protein coat.The application rate is 5-7 % of the dry weight of the substrate. One has to be careful as these materials enrich the substrate, making it more suitable to contaminants, if factors predisposing to their growth are present.These type of supplements may cause 5-10 per cent increase in yield.

Cultivation Technique (Contd..)

C.CASING
What is casing?

Covering the top of mushroom beds after completion of spawn run with a layer of appropriate soil mixture is known as casing. Mushroom growers in different countries use different types of casing materials depending upon their availability . Different materials used in India as casing mixture are:

1) Loam soil + Sand (4:1)
2) Two year old farm yard manure + loam soil (1:1)
3) Two year old spent compost + sand + lime (4:1:1)
4) Two year old spent compost + loam soil + FYM (2:1:1)
5) Paper mulch + 2 year old spent compost
6) Two- three year old spent compost + FYM (1:1)

Why Casing is necessary?
Casing of mushroom beds or spawn run compost is necessary because:

- Casing soil is a nutrient deficient medium, which helps in converting the vegetative phase into fruiting.
- Fruit bodies are formed in abundance and thus production is economical.
- It helps in conserving the environment in mushroom beds

Characteristics of a good casing :

- Good water holding capacity and more pore space percentage.

- Capable to release harmful gases during cropping.
- Free from harmful microorganisms.
- pH should be slightly alkaline.
- Should be properly decomposed.
- Free from heavy metals and ions.

Treatment of casing soil:

For killing various pests and disease propagules present in casing mixture, casing soil is treated with chemicals or pasteurized with steam.

1. Chemical treatment of casing mixture :

- Casing can be disinfected with formaldehyde treatment.The formaldehyde solution is prepared by mixing 2 litre of formalin (4O% a .i) in 40 litres of water to obtain 2% solution . Casing mixture, made up into a rectangular pile, is drenched thoroughly with this solution and then covered with a polythene sheet or tarpaulin sheet. The treatment should be given at least 2 week before casing is to be done. In other words, casing should be prepared and treated immediately after compost has been spawned. It should be ensured that casing mixture should not have traces of formalin when applied on the beds.

2. Pasteurization of casing mixture:

- In farms where facilities for pasteurization of compost with steam are available, casing can also be pasteurized. For pasteurization of casing mixture, casing soil is filled in trays and trays in turn are stacked in the pasteurization room. Steam is introduced to bring the temperature of casing mixture to 65-70°C and which is maintained for 6-8 hours. All the harmful microorganisms, including mushroom nematodes are killed at this temperature. Useful bacteria like *Pseudomonas* which play a positive role in introduction of fruit bodies are not killed and survive at this temperature for 7-8 hours. Casing soil pasteurized in this manner gives best result.

Fig. 6.5 Heap of loam soil used as one of the casing ingredients

CASING APPLICATION AND MYCELIAL COLONIZATION :

Fig. 6.6 to 6.9 Mycelium threads from grain spawn spreading in the compost filled in polythene bags.

Application of Casing:

- When spawn run is completed, the casing is done over spawn run compost after removing newspaper sheet from the trays on racks or after opening mouth of the poly bags. Spawn run compost is slightly pressed and covered on the surface with 4-5cm thick layer of casing soil. After casing, the temperature of the mushroom house is maintained at 24-25 °C for another 8- 10 days and water is sprayed over casing soil. Within 8-10 days,white mycelium spreads in the casing soil. Thereafter temperature of the mushroom house is lowered down to 18 °C and maintained between 14-18°C during rest of the fruiting period. Whenever required ,watering is done with the help of sprayer and RH is maintained at 80-85% throughout the cropping period.

D) CROP MANAGEMENT:

- The casing medium harbours some beneficial bacteria and activated charcoal like material which help in initiation of fruiting bodies on the casing surface. Casing mixture also helps in conserving moisture in the beds and gives support to the fruiting bodies .
- As soon as the white cottony growth of the mycelium appears on the casing surface, fresh air should be introduced inside the cropping room and bed temperature lowered to 16-18 °C which is to be maintained throughout the cropping period . The CO_2 level is also lowered to below 1000 ppm. Under such conditions, the initiation of fruiting bodies i.e.pinning takes place within 6-7 days of aeration which reaches to the harvesting stage within next 4-5 days. The individual fruit bodies are harvested carefully without disturbing the adjoining pinnings and before the cap opens. The cropping period lasts for 40-60 days. Mushrooms appear in flushes provided optimum conditions like bed temperature (16-18º C) , relative humidity (80-90 %) by spraying water with misty nozzle, about 4-5 air changes every hour resulting into less than 1000 ppm in the cropping room with no light at all , are maintained.
- The environmental factors like temperature, relative humidity, light , air flow in the cropping room etc; all play vital roles which together determine the nature of further mushroom development. The mushroom crop grows in cycle called “Flushes” or " Breaks ". Depending on the species being grown, day intervals with each successive flush bearing fewer mushrooms. These flushes normally appear in 7-10 days.

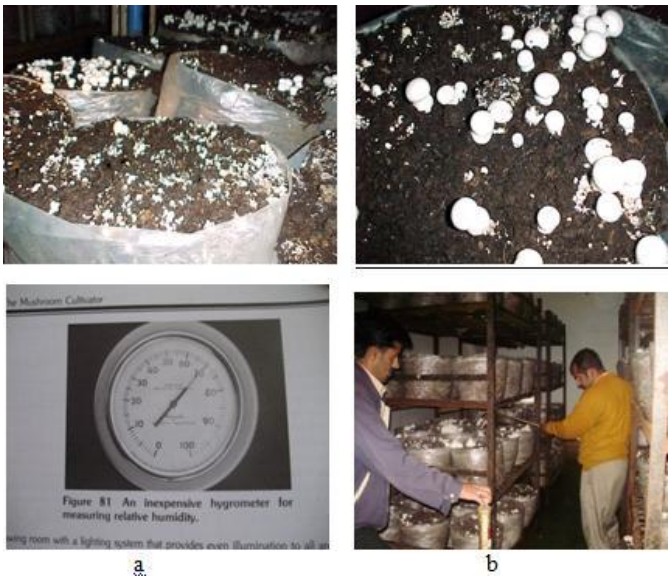

Fig. 6.10 & 6.11 Button mushroom beds in cropping stage

Fig. 6.12 & 6.13 Photograph of a Hygrometer for measuring humidity (a) and water being sprayed on the cropping beds with the help of a foot sprayer pump having a fine spray nozzle (b)

Fig. 6.14 & 6.15 Showing the compost bags kept on shelves for spawn run, the air duct and the Air Handling Unit in the cropping room (a)
the ventilator for entry of fresh air and the controlling board fitted outside a cropping room (b)

E) HARVESTING:

- Timing is the most important factor in button mushroom harvesting. Mushrooms should be picked before the veil breaks and the stem elongates. Damage to pinheads and disturbance of the casing soil must be minimized during picking. The standard harvesting technique consists of grasping the base of the stem, pull it with a twisting motion being careful not to disturb adjacent pinheads. The stem base, with mycelia and casing particles adhered to it, is trimmed with the help of a short bladed knife. All trimmings should be kept in a plastic bag and removed from the cropping area. Mushrooms growing in clusters should be broken apart and harvested individually. Immature mushrooms should be left attached to the casing for further development.

Fig. 6.16 - 6.18 Harvesting of mushrooms with the help of two fingers and a thumb and cutting of stem root with a knife

F) Yield :

- The cropping stage lasts for 40-60 days and production comes to 12-25 Kg / 100 Kg compost depending upon the quality of spawn, compost, casing mixture and prevailing environmental conditions in the mushroom house.

*****☺*****

LECTURE 7

Cultivation Technology Oyster Mushrooms

Introduction to Cultivation Technology

This mushroom is also known as Oyster mushroom . Word “Pleurotus” comes from the Greek word “Pleuro” which means formed laterally or in sideways position, referring to the lateral position of the stem relative to the cap. The species epithet “ostreatus” refers to its oyster shell like appearance and colour.

Natural Habitat:

- It is a wood decomposing, saprophytic or parasitic fungus which grows abundantly on standing and fallen forest plants like alder, cottonwood, maple etc; Found abundantly in river valleys and the fruit bodies appear in the falls, early winter and spring.

Nutritional Value:

- It contains 91% water and 9% dry weight; 30.4 % crude protein and 109 mg niacin/100 g dry weight. The spores of oyster mushrooms may be allergic causing breathing problem to some and sometimes difficult to digest for some people. It contains more protein than found in button mushroom.

Cultivation:

- Oyster mushroom (***Pleurotus spp.***) is commonly called Dhingri in India. It has oyster like shape because of which it is popularly known as oyster mushroom. Its cultivation can be done on number of agricultural wastes and organic waste materials. The important substrates include straw of different cereals, sugarcane bagasse, cotton waste, jute, groundnut pod shells, small wood pieces, saw dust, maize cobs, banana pseudostems, etc. depending upon the widespread availability of these materials
- Commonly cultivated species of ***Pleurotus*** includes P. ***sajor-caju***(Fr.) Singer, P. ***ostreatus*****,P. *florida***, **P. *cornucopiae***, **P. *eryngii*****, P. *flabellatus*****, P. *opuntiae*****, P. *platypus*****, P. *cystidiosus*** and **P. *columbinus***. Different species are grown under different agroclimatic conditions.

a. Substrate preparation:

It is commonly cultivated on wheat or rice straw, due to their easy availability in large quantities . The straw of 4-6cm size is taken and dipped in cold water for 10-12 hours. Straw can be sterilized by various methods as given below:

- **Hot water treatment:** The soaked straw is dipped in hot water at 80^{o}c for 2 hours. Hot water treatment makes hard substrate soft so that growth of the mycelium takes place very easily. This method is not suitable for large scale commercial cultivation.

- **Steam pasteurization:** In this method pre-wetted straw is pasteurized by passing steam through the straw for 2-3 hours. This method is used for commercial cultivation.
- **Chemical sterilization technique:** In this method 7.5g bavistin and 125 ml formalin are dissolved in 100 litre water and slowly poured on the heap of wheat straw. Soaked straw is covered with a polythene sheet. After about 18 hours the straw is taken out and excess water drained off.

b. Spawning:

- The process of spawn making is the same as in ***Agaricus***. The normal rate of spawning in pasteurized substrate is 1.5-2.0 % of the wet substrate, however it is slightly higher (2.0-2.5%) in unpasteurized material. The spawning is usually done in layers or even in thorough spawning care should be taken that the spawn gets uniformly mixed with the substrate, while in layer method the spawn is mixed after each layer of 3-4 cm thickness of straw.
- Polythene bags (50X75cm) have been found to be the best and cheap container for ***Pleurotus*** cultivation. Before filling the substrate in polythene bags, holes of about 1cm diameter should be made at 10-15 cm distance all over the surface for diffusion of gases and heat generated inside. After filling the substrate in the bags, the mouth of the bag should be tied with thread and kept at 22-26ºC temperature on shelves in a mushroom house for spawn run. R.H. of mushroom house should be maintained between 80-85%.

Fig. 7.3 Mixing spawn of oyster mushroom in the sterilized substrate containingstraw, wood chips and bran
Fig. 7.4 Spawning and filling the substrate in polythene bags

C. Cropping and management:

- Within 15-18 days of filling and spawning, white cottony growth of the mycelium spreads in these bags which can be noticed easily. These bags are cut open and kept in mushroom house on racks, 25-30cm apart from one another or these may also be hanged on nylon ropes keeping some distance between them. Water is sprayed over them in the morning and evening hours to maintain 80-85 % RH in mushroom house and also temperature between 22-26ºC. Pinning starts in next 4-5 days and fruit bodies become fully grown within a week of pinning.

Fig. 7.5 Straw bags showing completion of spawn run within 12-14 days of spawning

Cultivation Technology (Contd..)

d. Harvesting:

- The cropping stage lasts for 30-45 days at 20 – 25ºC , 85 – 92 % humidity and less than 600 ppm CO_2 . Approximately 4-6 air changes per hour and light 200 Lux / hour to 12 hour per day are most stimulatory. Regular misting is recommended to prevent cracking of caps and resting primordia. The mature mushrooms are harvested individually before incurved margin expands to plane by slightly twisting and lifting the fruit bodies with the help of two fingers and a thumb . The lower root portion is removed with the help of a knife.

Fig. 7.6 Fruit bodies of *Pleurotussajor-caju* grown on wheat straw as substratum

e. Yield:

- The average yield comes around 100-125 kg mushrooms / 100 kg dry straw or substratum.

f. Marketing and preservation:

- The Oyster mushrooms are packed in perforated polythene bags in different packings after proper cleaning. These are either sold fresh in the market or stored in a refrigerator / deep freeze for 4-6 days. Canning can also be done for long term storage but it is not recommended as these can easily be dried in the sun or in a mechanical dehydrator and kept for a longer period when packed in air tight packing. For cooking the dried mushrooms , these have to be dipped in lukewarm water for 15-20 minutes. Pickle making is also an easy and economic method of their preservation.

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LECTURE 8

Cultivation Technology of Paddy Straw Mushroom (*Volvariellavolvacea, V.diplasia*)

a) Nutritional value:

- Paddy straw mushrooms are very tasty and good flavoured. These are known to be very nutritious having 26-30 % protein, 9-12 % fibre , 9-13 % ash , 45- 50 % carbohydrate and rich in minerals , vitamins C and B.

b) Spawn production:

- Spawn is produced either in rice straw or rye , sorghum , millet or wheat grains . The mycelium in spawn bottle is fast growing,rhizomorphic to cottony, colour is typically whitish to greyish white.

c) Cultivation:

- Commonly cultivated varieties of paddy straw mushroom (***Volvariella***) are ***V. volvacea*****(Bull ex Fr.**) Singer, *V. diplasia* (Berk and Br.) Singer and *V. esculenta*(Mass) Singer . This mushroom is commonly cultivated on paddy straw in the open as well as inside a mushroom house. Open cultivation method is very common among marginal and small growers.

1. Open air cultivation:
a) Preparation of beds and spawning:

- In this method 100X60cm size foundation beds of 15-20 cm height are made with the help of bricks or mud under the shade, to save them from rains or direct sunlight. Paddy straw bundles of 7-8 cm diameter are made by tying them at one end. The length of these bundles is kept between 70-80 cm. These bundles are soaked in water for 16-18 hours in a water tank. For chemical sterilization of the straw, bavistin 7g and formalin 125 ml can be added in 100 litre of water. After dipping bundles in water, cover the water tank with the polythene sheet. Later ,bundles are taken out and excess water allowed to drain off on a cemented floor.

Fig. 8.2 Outdoor cultivation: Bed formation by lining wet paddy straw bundles on wooden platforms

- A bamboo frame exactly of the size of the bed on foundation is kept on the floor. Now place four bundles of paddy straw (water soaked) side by side over bamboo frame, keeping tied end in one direction. Place another set of four bundles over it but this time tied end in opposite direction. In this way 8 bundles make the first layer of bundles. Scatter the grain spawn about

8-12cm from the edges of the layer bundles. Spread the spawn along with powdered arhar pulse or gram flour. Wheat bran or rice bran can also be added. Place the second row of the bundles and spawn on it as described earlier. Likewise third and fourth layer of bundles are also placed and spawned. Finally, the square shaped bed is covered with a transparent polythene sheet and bed temperature of 32 ± 1 ° C is maintained . Within 7-8 days mushroom mycelium permeates the straw completely and at this stage the plastic cover is removed. If the surface of the bed appears to be dry, spray water with the help of water sprayer at least once in a day.

Fig. 8.3 Young pinheads appearing from the beds

b) Fruiting and harvesting:

- Mushroom fruiting occurs nearly 18-20 days after spawning at favourable moisture and temperature conditions .Fruiting continues for another 10-12 days. In paddy straw alone , yield of 12-14 kg /100 kg of wet substrate can be obtained.
- Harvesting of mushroom is done when volva just breaks and mushroom exposes from inside. In any case mushroom should be harvested before it opens. Paddy straw mushrooms are very delicate in nature and can be stored under refrigerated condition for 2-3 days only. Drying of mushroom can be done under shade or in sunlight.

Indoor Cultivation

Indoor Cultivation:

- The principal of indoor cultivation is the same as that of white button mushroom. Therefore, indoor cultivation of paddy straw mushroom is done inside the mushroom house on pasteurized compost.

a) Substrate:

- Suitable substrates for paddy straw mushroom cultivation are banana leaves, paddy straw , cotton waste etc. For indoor cultivation, rice straw and cotton wastes in 50:50 ratio is preferred which gives more consistent yield.

b) Composting:

- The composting process involves two phases: Phase I is an outdoor process while phase II involves pasteurization and conditioning of the compost.

Phase I (Outdoor composting):

- This mushroom requires very little nitrogen for its growth. Paddy straw and cotton wastes when used in 50 : 50 ratio, will provide 1.4% nitrogen, while some nitrogen is generated by the microorganisms during composting and spawn running processes. The pre-wetted straw and cotton waste are mixed thoroughly and then piled up. Pile raised is narrow with a height of 1.5cm. After 2 days, first turning is given to this pile. During this turning, rice bran @ 50% (w/w basis) is added. Watering is done if required. Remake the pile and leave it for another 2-3 days and only then the compost becomes ready for phase II.

Phase II (Indoor composting):

- After phase-I, compost is taken inside the mushroom house, placed on the shelves and preheated at 40-45 ºC. Now steam is introduced in the mushroom house for 2-3 hours so as to raise the temperature of the house to 60-65 ºC. This temperature is maintained for another 2-3 hours. The steam supply is then cut off and fresh air given. In next 8 hours temperature of the mushroom house goes to 50-52 ºC, which is maintained for another 12 hours or till the smell of ammonia persists in the compost. This process is completed in 4-5 days.

Spawning and Cropping:

- When treated beds do not have the smell of ammonia and temperature of the compost cools down to 34-38 ºC, spawning is done @ 2% of the compost (w/w). After spawning, doors of the mushroom house are closed for 3-4 days. Temperature during this period remains between 34-38 ºC (but should not be less than 30 ºC). R.H is to be maintained between 80-85 % by spraying water daily . Little aeration is also provided. Within 4-5 days, mushroom mycelium spreads in the compost. Then temperature of the mushroom house is lowered to 28-30 ºC by opening ventilators. If bed surface appears dry, water is again sprayed. During next 2-3 days, doors are kept open to allow some light.. This condition is maintained till sufficient amount of fruit bodies are formed. When primordia formation is completed, air of the room is circulated for at least 5 minutes for 5-6 times a day. Bed temperature is kept below 32 ºC and RH between 85-90%. In next 4-5 days mushrooms become large enough for harvesting.

Fig. 8.4 Mature pinheads formed in Clusters
Fig. 8.5 Fruit bodies nearing to their maturity

d) Harvesting:

- Fruit bodies are harvested when they become mature and before the cap opens completely, mainly in its egg form. The fruit bodies have got very low keeping quality and hence consumed immediately or they can be canned or dried and packed in sealed polythene bags so that these may be kept for a longer period. Cropping cycle lasts for 7 – 12 days in two flushes

Fig. 8.6 & 8.7 Mature fruit bodies ready for the harvest

e) Yield and Marketing:

- Yield varies from 22-28 kg to 25 – 45 kg per 100 kg straw. Due to very low keeping quality, these mushrooms can not be stored even in the refrigerator for more than 15-24 hours. Generally mushrooms are sold fresh or in canned form but rarely in dried form in the market.

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LECTURE 9

Cultivation Technology of Milky Mushroom

1. Substrate Preparation:

- *Calocybeindica*Purkayastha and Chandra, can be grown either on composted or fresh straw. For compost preparation, the soil, sand and maize meal are mixed in 12:6:1 proportion and sprinkled with sterile water. It is then autoclaved at 15 lbs. p.s.i. for one hour. Fresh straw (paddy / wheat) is chopped and soaked in clean water for 8-16 hours and subsequently soaked in hot water (80-90ºC) for 40 minutes to achieve pasteurization. This method is popular among small growers.

a) Steam pasteurization:

- Wet straw is filled inside insulated rooms either in perforated shelves or in wooden trays. Steam is released under pressure from boiler so that temperature inside the substrate is raised to 65ºC which is maintained for 5-6 hours.

b) Sterilization:

- Substrate is filled in polypropylene bags and sterilized in an autoclave at 15lbs.p.s.i for one hour. Once pasteurization / sterilization is over, bags containing straw are shifted to spawning room for cooling, bag filling and spawning.

2. Spawning and spawn running:

- At the time of spawning moisture content of the substrate should be 62-65 % and a higher spawn dose (4-5%) of wet substrate is used. After spawning, bags are shifted to spawn running room and kept in the dark where temperature of 35ºC and relative humidity above 80 % are maintained. It takes about 15-20 days when the substrate is fully colonized and bags are ready for casing. Bags are shifted to cropping rooms for casing and cropping.

Fig. Bags showing substratum fully colonized with the mycelium

a) Casing:

- Casing means covering the top surface of the bags after spawn run is over with 2-3 cm thick pasteurized casing mixture. Casing mixture (soil 75 % + sand 25%) with its pH adjusted to 7.8-7.9 is pasteurized in an autoclave at 15 lbsp.s.i for one hour or chemically treated with formaldehyde solution (4%) about one week before casing. Casing soil so treated should be covered with polythene sheet for about a week for proper fumigation and to avoid escape of chemical . After a week the sheet is removed and the mixture is turned at an interval of 2 days so that at the time of casing the mixture becomes free from formalin fumes. Casing mixture is spread on the straw surface in uniform layer of 2-3 cm thickness and bed temperature of 30-35ºC and 80-90% relative humidity are maintained.

Fig. 9.4 Cased beds

b) Cropping:

- When the temperature of cropping room is maintained at 30-35ºC along with 80-90 % RH and sufficient light during the day time, it results in the initiation of fruit bodies within 3-5 days in the form of needle shape which matures in about a week. Mushrooms with 7-8 cm diameter caps are harvested by twisting which are then cleaned and packed in polythene bags for marketing.

c) Yield:

- It is a crop of 40 – 45 days cycle and the yield varies from 12-15 Kg per 100 Kg compost. The mushrooms are either sold fresh in the market or canned for long time preservation.

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LECTURE 10

Post Technology- Preservation of Mushrooms Harvest

PHT - Preservation of Mushrooms

A. Harvesting:

- Proper stage of harvesting have been worked out in case of most of the cultivated mushrooms.Generally they should be harvested while the partial veil are still intact or before they open.

Fig. 10.1 Harvesting button mushrooms gently with two fingers and a thumb
Fig. 10.2 Harvested mushrooms along with their rootbuts

- The time and stage of harvesting matters a lot from the marketing point of view. Button mushrooms are harvested with delicate hands with the help of two fingers and a thumb.
- Harvesting is the most labour intensive activity in mushroom cultivation as individual mushroom has to be handpicked at the proper stage as in case of tea industry.

Fig. 10.3 Paddy straw mushrooms- ready for the harvest
Fig. 10.4 Heavy flush of milky mushrooms

B. Grading, packing and storage:

- Soon after harvest, mushrooms have to be cleaned and graded before sending to the market or storage in a cool atmosphere. The grading and sorting is done according to their colour (pure white , slightly brown , damaged), size , stage of the cap or partial veil (Intact , slightly open , open), length of the stem etc. Grading is generally done on the basis of size of the button, shape of pileus and opening of gills , also known as buttons, cups and umbrellas , respectively . The mushrooms for

fresh market are packed in plastic containers, perforated polythene bags of 100 gauge thickness or loose bags at varying packages.

Fig. 10.5 & 10.6 Button mushrooms kept in the container after removal of rootbuts and packed in in plastic trays after grading

Fig. 10.7 & 10.8 The milky mushrooms and button mushrooms packed in perforated polythene bags after harvesting and cleaning.

- Mushrooms can be stored in cold storage at 1-2ºC for a number of days or in deep freezers at below 0ºC or in vacuum freezers where water in cell walls and interhyphal spaces is evaporated with a vacuum that brings temperature from ambient to 2-3ºC in 15-20 minutes. Mushrooms should be packed generally in polythene bags (perforated) and placed gently inside the cardboard boxes with some paper packings so that they do not get pressed or jerked during transportation.

C. Transportation:

- Mushrooms can be stored at low temperature (4-5ºC) for 3-4 days only. These are to be transported in cool environment, either in ice boxes or in refrigerated vans and once they reach in the market , they must be immediately transferred to the deep freezer.

D. Preservation of mushrooms:

a) Canning and Freeze drying:

- For canning, mushrooms are harvested at the appropriate stage i.e. before opening, cleaned properly in cold water, blanched by dipping in boiling water for 4-5 minutes, graded and filled in standard canning jars which are then filled up to the brim with hot and boiling citric acid or vinegar solution and cooked by passing through a seamer for 4-5 minutes before sealing with a cap or lid . These sealed can jars are then pasteurized in an autoclave at 10 lbs. psi for 30-40 minutes, cooled and kept under observation for sometime before labeling.
- In freezing method, ninety percent water content of mushroom fruit bodies become crystallized and mushrooms are held together by ice crystals rather than by their own cellular structure. The ideal method of drying and freezing is the freeze-drying technique. Here every part and content of mushroom is preserved, including the flavour, form , nutritional and medicinal contents. But it is a very costly method and only few can afford it.

b) Drying:

- When mushroom production increases manifold, it becomes difficult to market fresh ones, especially in favourable peak season. During that period prices also come down and it is not affordable for small growers from hilly areas to send their produce in the market. The only alternative for them is either to dry their produce or go for preservation. Most of the mushrooms have 90-95 % water, hence very difficult to dry them and some like *A .bisporus, Calocybeindica , Stropharias*etc; can not be dried as they possess poor rehydration quality and become brown and black due to heat. Many other mushrooms like species of *Pleurotus, Auricularia, Morchella, Lentinulla, Ganoderma, Flammulina, Sparassis, Pholiotae*tc; can be dried and packed for long duration storage.

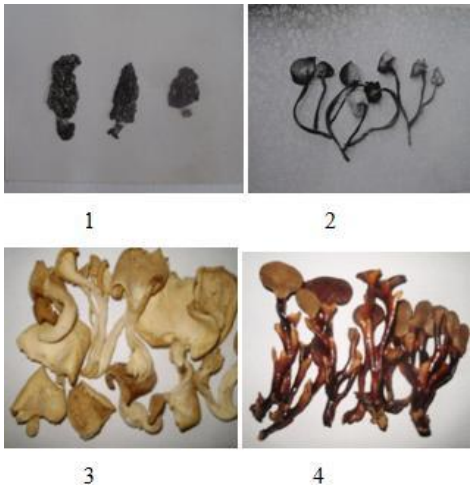

Fig: 1) Sun dried fruit bodies of *Morchella,* 2) *Flammulina,* 3) *Pleurotus* and 4) *Ganoderma*

- In hilly areas people generally stitch garlands of freshly picked mushrooms in steel wire or thread and keep them hanging in the sun or near their kitchen for 6-7 days for drying and thus preserve them. Now-a-days apart from sun drying, bigger and sophisticated Mechanical Dehydrators are available in the market. A good dryer should have the capacity to dry mushrooms within 24-48 hours by passing warm air not hotter than 110 ºF. The damaged and insect infested fruiting bodies should never be dried but discarded. Once properly dried (0.1 - o .5 % moisture content) , these should be carefully packed in airtight polythene bags and hermatically sealed so that air does not pass through it.

E. Marketing:

- Since mushrooms are highly sensitive and early perishable products, these should reach to the market as early as possible, immediately after the harvest. In most of the farms, workers get up at about 1or 2 AM in the morning and operations like harvesting, cutting, cleaning and packaging are completed by 4 AM so that they may reach to the market along with their produce by 6 AM. The white colour is preferred by the consumers, hence to increase the whiteness and shelf life, most of the growers in Asian countries treat or wash their button mushroom produce in 0.05 per cent KMS or Potassium metabisulphite solution for 1 minute (5 g in 10 litre water) .
- In India mushrooms are sent to the market either in loose packing or in poly packets of different weights and sold through auction in vegetable markets or through vegetable vendors. Mushrooms packed in attractive boxes and covered with attractive papers are known to fetch higher price as compared with the mushrooms in ordinary packings.
- Individual farmers going to the markets for selling their produce are generally exploited by the traders. The growers must form a cooperative society and members should abide by its rules and regulations which will work for the production as well as marketing of their mushrooms in

a joint manner. Society can also get the latest market trend through internet and inform its members regularly so that they may be able to sell their produce in the market where higher prices are being offered.

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LECTURE 11

Acquaintance with infrastructure, equipments and machineries required in the mushroom cultivation process

Infrastructure Required

Infrastructure Required:

1. Spawn production laboratory:

- Basically minimum 4 to 5 rooms with clean and tile fitted floors and walls are required for a spawn production laboratory. The rooms will be required for storing raw materials; boiling of grains, mixing filling in PP bags / glass bottles and their autoclaving; one inoculation chamber fitted with glass doors containing Laminar flow; one or two insulated rooms for incubation of inoculated bags and one cold room required for storing completely spawn run bags ready for distribution.

2. Compost preparation and cropping unit:

- A big cemented and well covered composting yard for preparation of compost and other substrates.
- One or more Insulated Pasteurization Chambers for the pasteurization of compost.
- A cemented, well insulated, small chamber for sterilization of casing mixture
- One well built, clean and fly proof mushroom production unit with more than six insulated cropping rooms fitted with air handling units and steel racks for production of mushrooms.

Fig. 11.1 **View of a clean and well built mushroom** production unit
Fig. 11.2 view of a well covered composting yard

Machinery &Equipments required

B. Machinery and Equipments Required:

- Various types of machineries and equipments having different uses will be required in a mushroom production unit.

a) For Spawn production unit:

1. Big Autoclave (Horizontal type)

- It is required for the sterilization of grain bottles and substrates filled in polypropylene bags for producing spawn and also the non-composted substrates for production of speciality mushrooms.

2. Small Autoclave (Standing type):

- It is for the sterilization of culture media in tubes / flasks and the substrates, including grains for production of Master culture and spawn in glass bottles / PP bags on a small scale.

3. Pressure Cooker:

- A big size pressure cooker (5-10 litre capacity) will be required for sterilization of media for routine laboratory work.

4. Boiler:

- Baby boiler run by wood fuel, electricity or diesel will be required for production of pressure steam for boiling , sterilization of grains and pasteurization of compost and casing mixture.


5. Laminar Flow:

- This is the machine on or in front of which isolations are taken or inoculation of grain bags / bottles with master cultures is done under aseptical conditions. The positive air pressure passed through the HEPA filters (High Efficiency Particulate Air) retains most of the fine bacterial and fungal spores so as to have minimum contamination problem.


6. Boiling Vessels:

- Steam operated Stainless Steel boiling vessels are required for boiling of grains.

7. Weighing Machine:

- Weighing machines is necessarily required for the exact measurement of raw materials for producing spawn and compost

8. Steel or cemented racks:

- Racks are required in the incubation and storage rooms on which the inoculated bags are to be kept at a particular temperature for mycelial run and their storage at different temperatures. Steel or iron racks will also be required for keeping large number of compost bags at required temperatures during spawn run and cropping stages .

9. Steel Trolleys:

- About 5-6 pushing type steel trolleys will be required for easy movement and carriage of grain bags, spawn bottles, compost bags and other materials from one room to another room

10. BOD Incubators:

- These are required to incubate cultures inoculated or transferred in tubes, Petri dishes, flasks and Master culture bottles for their speedy growth at a fixed temperature .

11. Oven:

- The oven is required for the sterilization of glasswares, including Petri plates, pippetes, beakers, glass tubes etc.

12. Refrigerators:

- In order to maintain purity of the fungal cultures for a considerable period, these are to be kept in the refrigerators in a cool environment.

13. Wire mesh Tray:

- One or two wire mesh trays will be required for removing excess water from boiled cereal grains or the boiled substrates like straw or sawdust used for mushroom production.

Machinery and Equipments required (Contd..)

b) For composting and cropping unit:

14. Blowers: Blowers of different capacities are required in the pasteurization room and cropping rooms for supply of fresh air and steam.

Fig. 11.24 The blowers fitted near the pasteurization chambers for circulation of fresh air and steam

15. Air Handling Unit: Air Handling unit is required for supply of filtered fresh / hot / cool air inside the cropping as well as the pasteurization rooms .

Fig. 11.26 Air handling unit inside the cropping room

16. Spray Pumps: For maintaining humidity (80-90%) inside the cropping rooms, the floors, walls and mushrooms beds are to be daily sprayed with clean water with the help of fine nozzle spray pump so as to get misty sprays.

Fig. Water being sprayed on the mushroom beds with a foot spray pump

17. Pile formers or Boards: It consists of three wooden planks or steel boards of desired size (4-5 feet height, 4-4.5 feet width and 5-6 feet length) for making the compost pile. It is used every time the compost is turned so as to give support to the pile while stacking.

Fig. The set of a Pile former consisting of three steel boards

18. Long handled pitchfork: These are used for compost turning , handling and filling of compost

19. Shovel or belcha: Used for handling and filling of materials.

20. Hose with nozzle: It is for the watering and quick wetting of basic materials in bulk like straw, chicken manure, horse dung etc, when used on a large scale .

21. Small tractor with a front loader: This tractor will be helpful in turning, transferring and handling of heavy materials like compost, casing and other materials.

Fig A Front loader tractor

22. Turning machine: It is a machine fitted with rotators which helps in rapid and efficient turning of compost using negligible number of labourers .

Fig. Turning machines fitted with rotators for turning of composts and filling inside the tunnel.

23. Conveyer Belts: These are long and moving belt like structures which help in transferring compost or other materials mechanically from one place to other in a very speedy manner that saves labour and time.

24. Thermometers: These are required for measuring temperature of the compost heaps on the platform, compost beds in the spawn running, cropping rooms, pasteurization rooms so as to know temperature at

every stage of composting and mushroom growing.

25. Hygrometer: For measuring humidity inside the cropping and spawn running rooms.

Fig.The Hygrometer for humidity measurement.

26. The pH meter: An instrument for measuring pH of the materials like compost, casing mixture, media etc.

Fig. The electronic pH meter

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LECTURE 12

Problems in Mushroom cultivation - I

Important Pests of mushrooms and their management

In mushroom cultivation, one commonly comes across certain undesirable microorganisms which appear in spawn bags, spawned composts and cropping beds resulting into spoilage of spawn, hinderance in spread of mycelium during spawn- run period as well as the fruit body formation during cropping period causing reduction in yield and sometimes crop failures. These are also known as contaminants because they are undesirable ones. The contaminants can be divided into three well defined groups :

1. Insects, mites , nematodes and animal pests: Majority of these contaminants are big enough to be seen with naked eyes, whereas some like nematodes are microscopic also .

2. Pathogens: These are the microscopic contaminants that directly attack mushroom fruit bodies and cause economic damage like viruses, bacteria (*Pseudomonastolassii , P. spp.*), and fungi (*Verticilliumfungicola , Mycogoneperniciosa, Dactyliumdendroides, Trichodermaviride*).

3. Competitor or indicator moulds: Those contaminants, mostly fungi, which compete for food in the substratum along with the mushrooms.

- Following are some of the established vectors or the sources of contamination:

1. Air 2. The mycelium or spawn 3.The substrate or the compost 4.Casing materials 5.Grower or workers. 6. Equipments, containers and tools. 7. Water 8. Insects and animals.

Familiarization with the Insect-Pests, Nematodes and Animal Pests of Mushrooms

1) MUSHROOM FLIES: Mushroom flies and midges are present in nature wherever fungi are found. Attracted by the odour of the decomposing manure and vegetable matter as well as smell of the growing mycelium, the adult female enters the composting yard or the mushroom farm and lay eggs on the compost, near the mycelium or fruiting bodies. Mainly three types of flies are known to infest mushroom beds:

1. Phorid fly or dung fly (*Megaselianigra , M. halterata*)
2. Sciarid fly or big fly (*Lycoriellasolani , L. mali , L. auripila*)
3. Cecids or gall midges (*Heteropezapygmiae , Mycophilaspeyeri*)

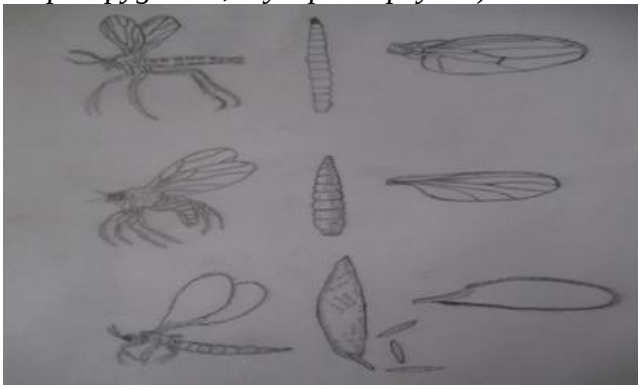
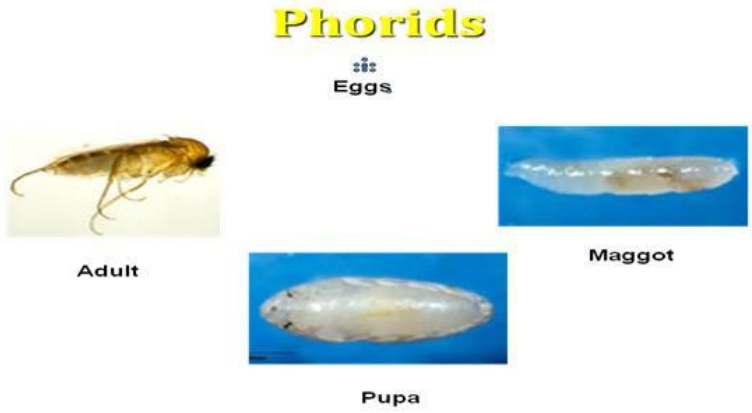


Fig. The adult, larva and wing venation of a phorid fly (Top row), Sciarid fly (Middle row) and a cecid fly (Lower row). The structure of a mother larva is also shown in case of cecid fly.

Nature of damage: The larvae of flies that emerge from the eggs laid in the mushroom beds, mainly cause the damage as they directly feed on the white mycelium spread in the compost and casing layer and also feed on the mushroom fruit bodies making tunnels through the stems. Mushrooms from the infested mushroom beds are found blackened from inside and infested with white larvae. Mushrooms infested at the pinhead stage become brown and remain stunted. Infested oyster mushrooms remain stunted, wrinkled and bent downwards with a large number of larvae and pupae lying embedded inside the tissues. Adult flies are the carriers of mites and mushroom pathogens such as spores of *Verticillium*, *Trichoderma*, *Mycogone* etc. attached to their hairy body parts.

Lifecycle: The adult female fly lays about 150-170 eggs in the compost or mushroom beds which hatch into larvae. After feeding for some time, each larva secretes from the mouth and forms a pupa. As a result of the metamorphological changes inside, larva turns into an adult fly and comes out of the pupal cell for causing further damage and breeding. In case of cecid flies, the reproduction takes place paedogenetically. Here a larva becomes mother larva and instead of forming a pupa, a mother larva carries about 14 – 16 larvae in its body which hatch out after few days. Thus they multiply in a very rapid manner and so the damage also increases.



Life cycle of a Phorid fly

Control measures:

- Strict hygiene in the mushroom house .
- Proper turnings during composting process.
- All the doors, windows, exhaust vents and fresh air intake openings should be fitted with fine wire mesh / mosquito netting.
- All the implements and tools should be cleaned and disinfected.
- Proper pasteurization of the compost at Phase –II with aerated steam at 58-59 ºC for 3-4 hours and the conditioning at 50-55º C till ammonia is eliminated.
- Drymixing of the casing materials, proper prewetting and its sterilization with steam at 65± 1ºC for 3-4 hours or with 5 % formalin solution .
- Use of light traps and sticking bands .
- Storage of raw materials in dry and ventilated rooms .
- The spent compost, after the end of the crop, should always be thrown away at a distant place.
- Growing rooms, all containers and equipments / implements should be cleaned with water and disinfected every time before and after the crop is over.
- Spraying beds with safe insecticides like malathion(0.05 %) or DDVP (0.025 %) one week before harvest .

2) MUSHROOM MITES: Mites are very small, spider like in appearance that live and breed in decomposing vegetable matter feeding on moulds present therein. They differ from the insects in that the mites have four pair of legs instead of three pairs. The environmental factors like moist and warm

atmosphere (20 – 30 ºC) and closed area support their exponential growth and a rapid succession of generation. Under adverse conditions, certain mites have the ability to change into an intermediate stage called a " Hypopus " which have flattened body, short stubby legs and a sucker plate with which they become attached to moving objects and thus are dispersed or carried away to distant places, mainly through the mushroom flies and human beings .

Fig. 12.3 Mushroom mites on the fruit bodies staying in groups
Fig. 12.4 An adult mushroom mite with four pair of legs

Nature of damage: Mites have the chewing type of mouth part with which they eat mycelia and the mushrooms. They devalue the crop causing certain spots on the surface and crawl into the pickers' body causing discomfort.

LIFE CYCLE: The mites complete their life cycle within 13 days at 75 º F and 36 days at 60 º F as the high temperature facilitates rapid reproduction. They lay eggs which hatch into larva ,protonymph and tritonymph stages before reaching the adult reproductive stage

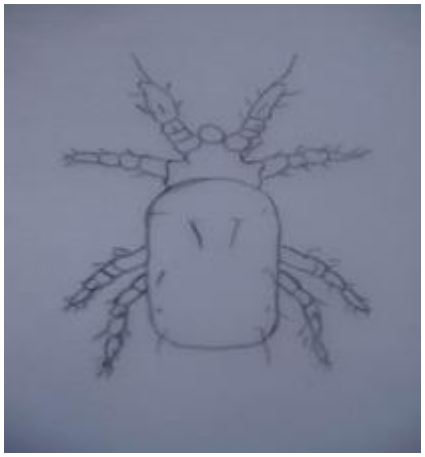


Fig. 12.5 An adult mushroom mite

Control methods:

- Complete hygiene and sanitation
- Proper pasteurization of compost and casing materials
- Drenching mushroom houses and premises with endosulfan, diazinon or dicofol(0.1%) .
- Use of fresh polythene bags and chemical sterilization of empty trays and trolleys.
- Burning sulphur in the empty rooms @ 2-3 lbs /1000 cu.ft.
- Cook out of the exhausted compost with live steam at 71ºC for 8–10 hours
- Disposal of spent compost at a distant place

- Spraying beds with safe insecticides like chlorfenvinphos, fenitrothion(1ga.i / m² bed area) or malathion (0.05 %) .

Familiarization with the Insect-Pests, Nematodes and Animal Pests of Mushrooms (Contd..)

3) SPRINGTAILS:

- Adults are silver grey to ground colour with light violet band along the sides of the body and black cellular fields present on the head. Body length is 0.7 to 2.25 mm and abdomen 4-6 segmented. Antennae are 3-6 segmented.*Lepidocyrtus sp., L. cyaneus, Seirairicolor, Achorutesarmatus*etc. are the main species damaging mushrooms.

Life cycle:

- Springtails enter the mushroom house mainly through organic matter. .A female lays about 10-40 eggs which are smooth, spherical, white and measure 0.19 mm .The eggs hatch in 30 days at 30 ºC. Life cycle ranges from 70 – 78 days at 26 ºC.
-

Fig. Showing the morphology of spring tails

Nature of damage:

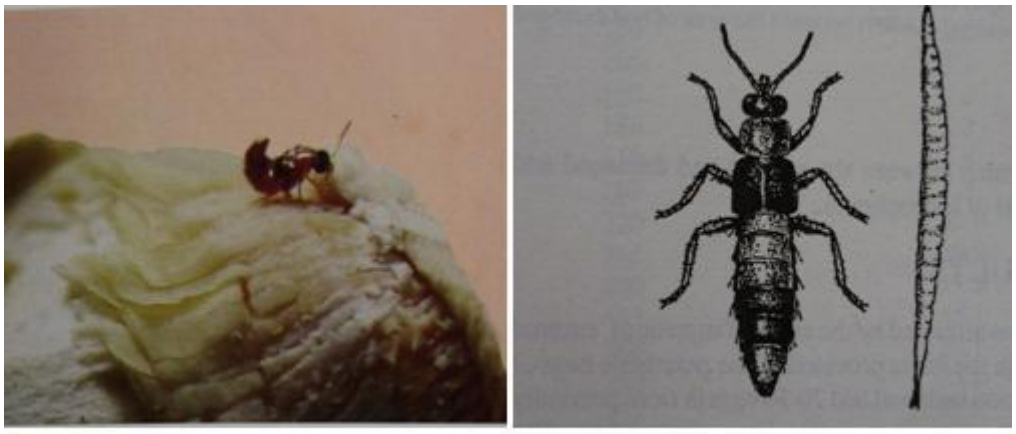
- Springtails cause damage to the oyster, button and shiitake mushrooms. Staying in groups in the dark , they feed on mycelium in the compost resulting in disappearance of mycelium from spawn – run compost. Fruiting bodies of button mushrooms are also attacked causing slight pitting or browning at feeding sites. In oyster and shiitake, they feed on gills destroying the linings and also eat out the mycelial strands at base of the stipes.

Control methods:

- Preventive measures like clean cultivation, proper pasteurization of compost and casing materials, proper disposal of spent compost, raising the crop above floor level etc; should be followed.
- Use of 0.05 % malathion as spray for disinfection, mixing diazinon 30 ppm in compost at the time of filling and spray of insecticides like malathion or dichlorovos at 0.025 – 0.05 % conc. during spawn run and cropping have been recommended for their control.

4. BEETLES:

- Some beetles (*Staphylinussp , Scaphisomanigrofasciatum*) have also been found to cause serious damage to the oyster mushroom crop. These tiny insects are dark brown in colour with short elytra and large membranous hindwing and tip of the back culled over its body. The beetle *Scaphisomanigrofasciatum* is deep amber coloured, with its head hypognatus and top of the abdomen not fully covered with elytra.



a b

Fig.Photographs of beetles as mushroom pests. (a) Beetle feeding the mycelial layer of mushroom (b) an adult beetle with its larva

Nature of damage:

- The grubs are found to feed voraciously on the mycelium and spawn, making tunnels in the stipe, pileus and gills of mushrooms. The infested fruiting bodies turn into abnormal shape and rotten masses. Grubs are seen hiding in between the gills of oyster mushrooms. The insect has been found to complete its life cycle within three weeks.

Control methods:

- Strict hygiene
- Proper pasteurization of straw.
- Application of chlorinated water or bleaching powder on cropping beds .

5) MUSHROOM NEMATODES:

- Nematodes, especially the myceliophagous nematodes are the most numerous and harmful creatures. Also known as eelworms, these are microscopic, thread like roundworms which live in soil, decomposing organic matter, fresh or salt water, also living on host plants, fungi, insects and animals.

Sources of infestation:

- Compost ingredients like wheat straw, chicken manure, horse manure, saw dust, pig manure, cotton cake; farm soil, air, water; casing materials like FYM, spent compost, moss pea , forest soi ; wooden trays, shelves and other containers etc; can be the primary source of infestation.

Spread:

- Once these nematodes get entry into the mushroom house, they further spread through air, faulty spray of water, workers' hands, implements, mushroom flies, mites etc.

Types of nematodes :

- The mushroom nematodes are of following two types:
  - Mycophagous or myceliophagousnaematodes
  - Saprophagous nematodes

I. Myceliophagous nematodes (*Aphelenchoidescomposticola, Aphelenchoidesagarici, A. neocomposticola, Ditylenchusmyceliophagous*) :

- These nematodes feed directly on mushroom mycelium and the fruit bodies. They are provided with a special type of mouth part i.e. stylet or needle with which these parasites puncture the hypha, inject digestive juices and suck the cellular contents leaving hyphal cell damaged which soon dies as it is drained of its cytoplasm. Since they have the capacity to multiply rapidly, these tiny pests millions in number, attack the mycelium moving from cell to cell and destroy the whole mycelial network in the compost within no time. The nematodes can reproduce 30 – 100 fold in about two weeks at 70 – 75 º F.
-

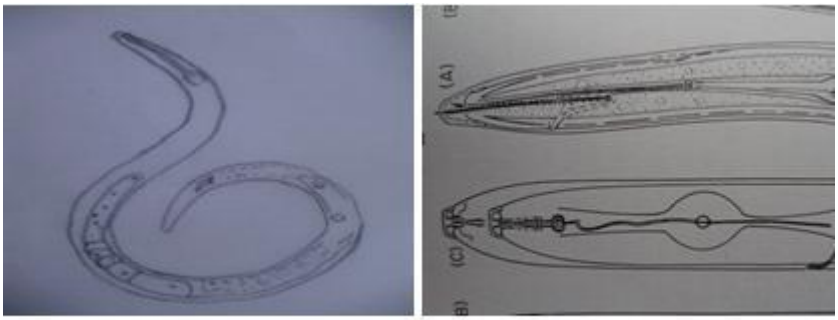


Fig. Showing: (1) the morphology of an adult myceliophagous nematode and its anterior body part with stylet, the needle like sucking mouth part and (2) bulbous oesophagous

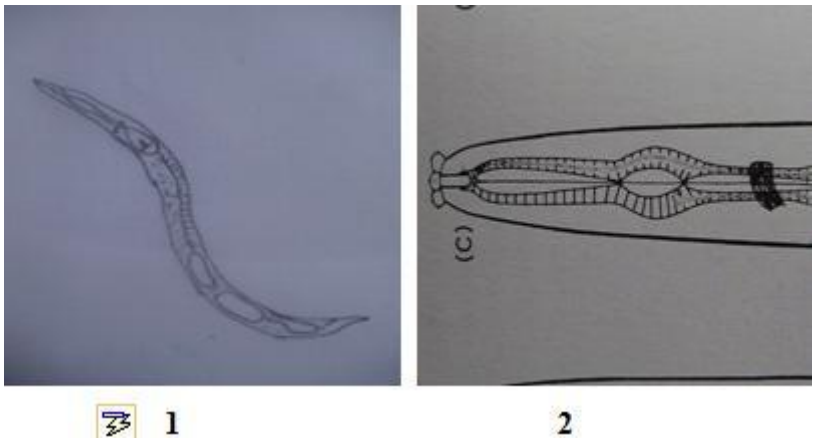


Fig. Showing (1) the morphology of a saprophagous nematode (2) with its tubular mouth part and bulbous oesophagous

Symptoms of nematode infestation:

- The compost surface sinks
- Mycelium grows sparsely in patches and turns stingy
- The white mycelium starts disappearing from the infected mushroom compost leaving only the coarse strands showing black compost mass .
- Because of the build -up of high population of bacteria, compost becomes soggy and foul smelling.
- The pinheads turn brown, watery and remain stunted.
- The fruit bodies appear in patches in the beds
- Due to reduction in flush pattern and crop duration, the yield is drastically reduced.

Life cycle: The female generally lays eggs which hatch into small larvae. These larvae feed on the substratum and change into L-1 , L-2 , L-3 stages until they become adults and enter the reproductive stage. These have a life span of 7 – 12 days or more which again depends on the prevailing temperature.

II Saprophagous nematodes (*Rhabditis spp. ,Panagrolaimus spp. Diplogaster spp.*):

- These are having a tube –like mouthpart instead of a stylet through which they suck the nutrient particles of the substrate, including mushroom compost, suspended in fine films of water. Since bacteria are present in large number in mushroom compost as well as in the casing , these materials provide excellent breeding grounds for saprophagous eelworms. Presence of saprophytic nematodes indicates improper hygiene, faulty pasteurization of compost or the casing mixture and imbalanced growing conditions.

Nature of damage :

- With their tube like mouthparts, they are structurally incapable of causing any direct damage to mushroom mycelium . Due to their faecal materials, the Rhabditids not only spoil the structure and quality of composts in cropping beds emitting foul smell, but also cause inhibition of mycelial growth, reduction in yield due to disturbed flush pattern, reduction in crop duration and quantitative loss of the sporophores etc.

Control methods:

- Complete hygiene
- Proper pasteurization of compost and casing materials
- Drenching mushroom houses and premises with some disinfectants
- Use of fresh polythene bags and sterilization of empty trays or trolleys with formalin or other disinfectants
- Use of nematode free spray water
- Workers should wear clean overalls, including hand gloves and first harvest the healthy sporophores carefully and only then the older infected ones
- Cook out of the exhausted compost at 71± 1 º C for 8 – 10 hours
- Disposal of spent compost at a distant place
- Growing resistant mushroom varieties like *Agaricusbitorquis ,Pleurotussajor-caju , Stropharia rugoso-annulata*etc.
- Nematode trapping fungi like *Arthrobotrysoligospora , A. superba , A. robusta* and several species of *Pleurotus*can be used as bio- control agents against mushroom nematodes .
- Mixing of plant extracts of neem , castor, groundnu , karanj etc. in compost at the time of spawning or cropping.

5) ANIMAL PESTS

1. Rats: Apart from the Insect-pests and nematodes, some animal pests like rats also cause the damage. In fact they feed on the cereal grains used as substratum for spawn production, but they disturb and damage the beds a lot.

Control methods:

- The rooms should be rat proof and
- Mouse traps should be used

LECTURE 13

Problems in Mushroom cultivation - II

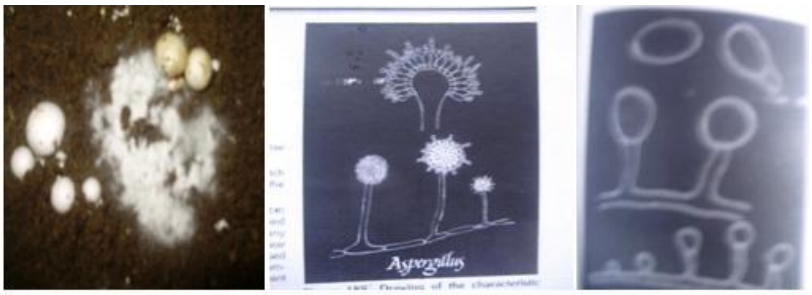
Diseases and mould problems in mushroom cultivation and their management

A. FUNGAL COMPETITORS OR INDICATOR MOULDS OR WEED FUNGI:

- While some fungi, bacteria and viruses directly attack mushroom fruit bodies causing pathogenic diseases, a large number of harmful fungi are encountered in compost and casing which may not be directly pathogenic , but may cause harm to the crop during spawn run and cropping stages. These are known as Competitor moulds as they compete for food with mushroom mycelium or " Indicator moulds " as presence of each mould indicates some deficiency or fault in compost or casing and also called as " Weed fungi " because of their undesirable occurrence.

The following are some of the established vectors of contamination :

- Air
- The mycelium or spawn
- The substrate or the compost
- Casing materials
- Growers or workers hands
- Equipments, containers and tools
- Water
- Insects and animals.



1. 2. 3.

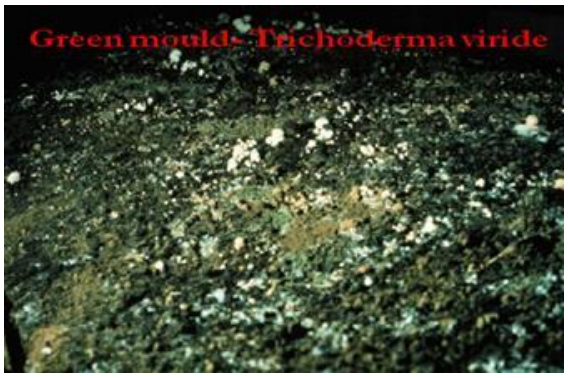
Fig. Occurrence of competitor moulds on mushroom bed:
(1) Conidiophores and conidia of *Aspergillussp*
(2) *Torulasp*
(3) that occurs frequently in mushroom compost

1) GREENMOULD:

- It is the most common mould and found in beds of every type of mushroom cultivated. Mainly three types of fungi *Trichoderma ,Penicillium and Aspergillus* have been found to be associated.

Symptomatology:

- Green patches appear in compost , spawn , on casing surface and also sometime on the mushroom surface, engulfing the fruit bodies with its white and greenish mycelium causing *Trichoderma*blotch disease.. The pathogenic species of *Trichoderma* like *Trichodermaharzianum*, infect the fruit body, otherwise green moulds try to spread rapidly and cover entire compost structure depending upon the quality of compost and environmental conditions .The appearance of green mould indicates poor quality compost, unhygienic cropping conditions and low compost pH .



Causal organism:

- The most common species of *Trichoderma* appearing in mushroom beds are *Trichodermaviride* , *T. koningi* , *T. harzianum* . *T. hamatum* and several species of *Aspergillus*and*Penicillium.*Rifai in 1969 revised and has proposed nine different species of *Trichoderma* .Trichodermaviride is the most commonly occurring weed mould whereas ,*T. koningi* and *T. harzianum* have been reported to be competitors as well as pathogenic to button mushroom producing blotch symptoms on fruit bodies.

1 2 3

Fig. Showing the (1) Green mould infection in mushroom bags
(2) Petri dish culture of *Trichodermaviride*, (3) microscopic observation of fungus *Trichodermasp*

Epidemiology:

- The fungus mainly enters spawn laboratory or the cropping room through air, dust particles, contaminated overall or hands, infected spawn, contaminated equipments and machinery; vectors like mites , mushroom flies etc. The compost quality mainly determines the establishment and growth of this mould. Poor quality compost prepared under unhygienic conditions, high moisture conten , use of straw having short texture for composting, highly pressed compost heap during composting, low pH of compost, high humidity etc; are the predisposing factors for the growth and development of the fungus.

Control methods or management:

- There should be complete hygiene inside and around the mushroom farm, compost ingredients should never come in contact with the soil particles; proper turnings, conditioning and pasteurization of compost is a must, use of foot dips at the doors of cropping rooms, lesser use of formalin sprays, proper cleaning of equipments and tools, use of clean and washed clothes, early removal of infected bags etc; are some of the recommended methods of control.Spray of some fungicides like 0.1 % carbendazim, thiabendazole ,mancozeb (0.2 %) etc. on cropping beds have been found effective in controlling the mould.

2) OLIVE GREEN MOULD:

- During spawn run stage, small military green coloured cockle burrs appear sometimes in the compost which is easily recognizable and that affect the yield . The occurrence of these moulds were first reported in India by Gupta *et al* ,1975 and Thapa*et al*, 1979.

Symptoms:

- The initial signs of fungus consists of appearance of greyish - white aerial mycelial growth in the compost just after spawning confused with the growth of mushroom mycelium . These mycelial structures later on give rise to small, round , military green or grey green cockle burr (1 / 16 inch diameter) structure in the compost strictly adhering to the straw.

a b.

Fig. 13.10 & 13.11 *Chaetomium*infection in straw of compost, (b) Ascus and ascospores of *Chaetomiumolivaceum*

Causal Organism:

- Mainly two fungi *Chaetomiumolivaceum* and *C. globosum* have been observed occurring in mushroom beds.

Epidemiology:

- The spores of *Chaetomium*are already present in the compost or they may come through air and casing materials . It has been found that during compost pasteurization process , mainly at peak heat or kill stage (59-60 º c) , it should never be processed in the absence of fresh air or Oxygen . Absence of aeration during peak heat or kill may lead to compost damage which favours the appearance and development of these fungi.

Control Methods:

- In case of pasteurization process , the peak heat or kill should be done at 58 – 59 ºC for 3-4 hours in the presence of fresh air or aerated steam . Carbendazim(0,05 %) and Dithane Z-78 (.2 %) have been found to be effective in controlling the mould only in case of minor damage of the compost.

3) BROWN PLASTER MOULD:

- The mould appears as white mycelial growth on the surface of compost during spawn run stage and also on the casing surface slowly changing colour from white to light brown to cinnamon brown and finally changed to rusty in appearance.

Causal Organism:

- *Papulosporabyssina* is the fungus responsible for causing brown plaster mould . The mycelium is initially white which later turns brownish ,septate , producing clusters of brown coloured , spherical bulbils.

a. b.

Fig. 13.12 & 13.13 Shows (a) Brown plaster mould on the mushroom bed surface, (b) the mycelial structure and bulbils of *Papulosporabyssina*, the causal fungus

Epidemiology:

- The fungus mainly enters through air, spent compost, casing material or the containers as well as the workers' hands. But a wet, soggy and improperly pasteurized , bad quality compost favours its rapid growth . It commonly occurs on compost prepared by long method of composting .A greasy and wet compost is vulnerable to infection.

Control methods:

- Good hygiene and preparation of good quality compost removes the chances of its appearance and further development . Addition of good quality gypsum is recommended and proper

turning of compost with attentive pasteurization procedures help in preventing this mould. Sometimes spray of some fungicides like carbendazim , TPM , TBZ (0.05 %) and Dithane Z-78 or Dithane M-45 (.025 %) have been recommended for its control .

Diseases and mould problems in mushroom cultivation and their management (Contd..)

4) WHITE PLASTER MOULD:

- The mould appears as white patches in between or on the compost surface during spawn run stage or also in the casing layer . It inhibits the growth of mushroom mycelium causing yield loss to the extent of 5 – 30 per cent.

Causal Organism:

- Scopulariopsisfimicola is the fungus responsible for the contamination.

Favourable factors:

- Under or over -composted conditions having high pH (above 8.0) favour the growth of this mould .

Control methods:

- Mixing of compost ingredients in recommended quantities, proper wetting and turning of compost under hygienic conditions have been highly recommended . Removal of mould from the compost layer and spray of benomyl or carbendazim(0.05 %) are recommended for its control . In case of high pH and moisture content of compost, delayed turning or conditioning and addition of gypsum is recommended.

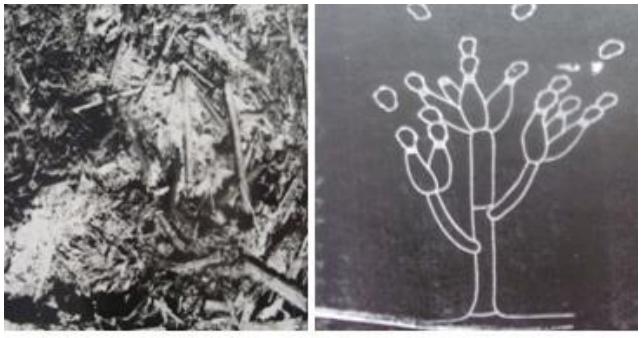
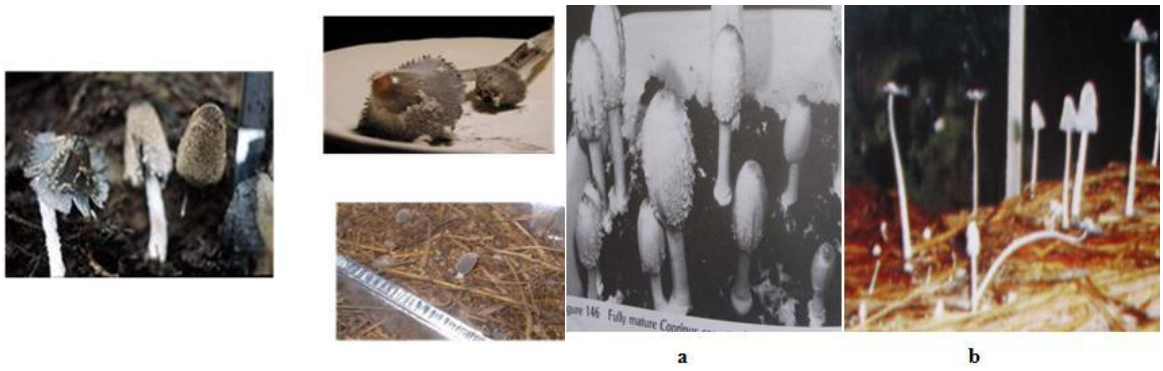


Fig. 13.14 & 13.15 shows: (a) Patches of white plaster mould in compost bed, (b) the conidiophore and conidial spores of *Scopulariopsisfimicola*

5) INKCAP OR *COPRINUS:*

- Long stemmed mushrooms with small caps are often seen coming out of the compost which soon turn black , collapse and get decomposed.



a b

Fig. 13.16 to 13.18 Inky Cap weed, Fig. 13.19 & 13.20 shows: Appearance of fruit bodies of *Coprinus spp.*as weed fungi or competitor moulds in compost and straw beds during mushroom cultivation process

Causal Organism:

- Several species of *Coprinus* like *Coprinuscomatus , C. logopus , C. atramentarius , C. fimetorius*etc; have been observed appearing in mushroom beds .

Favourable Factors:

- *Coprinus*spores generally enter through compost ingredients, chicken manure, improperly pasteurized compost, casing material and also through air. Their appearance indicates ammonia still present in the compost, a sign of improper pasteurization and turning or higher quantity of nitrogenous materials, including chicken manure added to the compost .

Control measures:

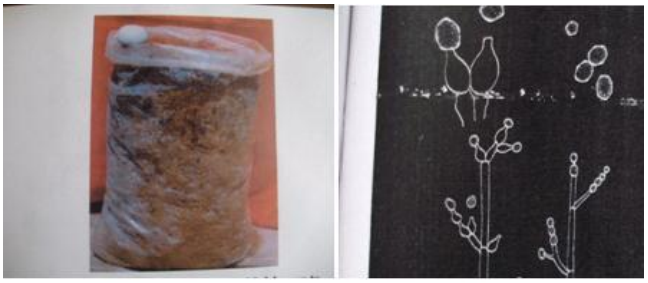
- Maintenance of hygienic conditions, mixing of quality ingredients while preparing compost at proper ratio, proper turning and pasteurization of compost is necessary Addition of too much nitrogenous material and water should be avoided.

6) YELLOW MOULD (*CONFETTI* , *VERT-DE-GRIS* , *MAT DISEASE*):

- Since a number of fungi produce yellow mycelial growth in the compost (yellow Mould) or beneath the compost in the form of yellow layer (mat) or in the form of circular colonies (confetti) or distributed all over the compost (Vert – de – gris) , these are known by different names .

Causal Organism:

- The mycelium of *Chrysosporiumluteum*is white at the initial stage that turns yellow to dark tan with dull white sporulation .



a b

Fig. 13.21 & 13.22 shows: (a) Occurrence of mat disease in mushroom bed, (b) the mycelial structure of fungus*Chrysosporium*sp. with conidiophores and spores

Epidemiology:

- The sources of inoculum are mainly the compost ingredients, chicken manure, air, spent compost and wooden trays . It further spreads through workers' hands or clothes, mushroom flies, mites, faulty technique of water spray (splash) and the picking tools .

Control measures:

- Proper hygiene, removal and burial of mould affected spent compost at a distant place, proper turning and pasteurization of the compost and casing mixture, use of light and misty water spray technique, covering the windows and ventilators with fine wiremesh, use of filtered air and spray of Benomyl(400- 500 ppm) and Blitox (0.25 %) have been found effective in controlling the disease.

7) SEPEDONIUM YELLOW MOULD:

- This mould is found growing in between the compost layer or at the bottom layer. The fungus is initially white but turns yellow or tan coloured at maturity .

Fig. 23 & 13.24 shows: (a) Mushroom beds infected with *Sepedonium*yellow mould, (b) the microscopic structure of *Sepedonium*sp producing two types of conidia

Causal Organism:

- S*epedoniumchrysospermum* Bull (Fries) and *S. maheshwarianum*Muker. have been found mainly responsible for the occurrence of the mould .

Epidemiology:

- Spent compost, soil , air, improperly pasteurized compost / casing soil, wooden trays etc; are the primary sources of inoculum as the thick walled chlamydospores are resistant to peak heat temperature, if not pasteurized properly. The compost prepared by long method of composting have more chances to have this mould.

Control methods:

- Strict hygiene followed by proper pasteurization of compost at 59 – 60 º C for minimum four hours is recommended. Use of filtered air with high efficiency filters in the cropping rooms and cook out of compost at the end of the crop with steam at 70 º C for 10 – 12 hours are recommended. Sterilization of chicken manure with 2 % formalin and 0.5 % Carbendazim prior to composting has been found to give good result (Vijay*et al* ,1993).

8) FALSE TRUFFLE:

- It is the most serious competitor mould found during *Abisporus* cultivation apart from its appearance in *A.bitorquis*beds. It is commonly found occurring in compost prepared by long method of composting , especially during summer months .

Symptoms:

- The mycelial colour is initially white at the start and hence difficult to differentiate with the growth of the mushroom mycelium, but soon turns creamy yellow at later stage . It appears as small, wefts of white cream coloured mycelium in compost and casing soil, mainly below the casing. The mycelium becomes thick and develops into whitish, solid , round toirregular, wrinkled fungal masses resembling calve's brain which are the ascocarps of the fungus.


Fig. 13.25 & 13.26 shows: The calve's brain shaped ascocarps of *Diehliomycesmicrosporus*, the false truffle fungus

Causal Organism:

- The ascocarps of *Diehliomycesmicrosporus* are fleshy white initially which turn brown and reddish brown at a later stage.

Epidemiology:

- The fungus enters the cropping room through spent compost, chicken manure, casing material, old infected wooden trays and already infected rooms as the ascocarps can survive for a period of five years in soil and spent compost and for six months in the form of mycelium.

Control methods:

- The compost should never come in contact with the soil, hence it is always better to have a cemented composting yard , covered with a roof with slight gradient. Proper pasteurization of compost (59 º C for 3-4 hours), systematic turning and conditioning is very much essential for complete elimination of the fungus. The casing soil should be sterilized at 65±1º C for 6-8 hours. The bed temperature during spawn run and cropping should be maintained below 18 º C as it is a very critical situation . Cook out at 70 º C for 10-12 hours will eradicate the fungus as the thermal death point of the fungus has been reported to be 70 º C for 1 hour (ascospore)and 45 º C for 30 minutes (mycelium) .

9) LIPSTICK MOULD (*Sporendonemapurpurescens*):

- The mould is noticed as pink mycelial growth on the casing at several crackings or in loose areas of casing . Because of its pink coloured spores, it is known as Lipstick mould . It first appears as a white crystalline mould not differing from white mushroom mycelium in the spawned compost.

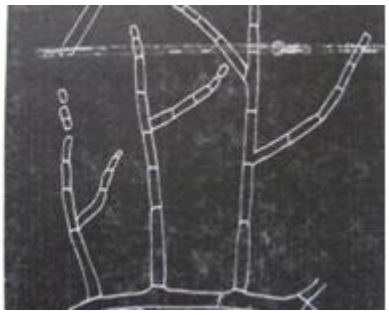


Fig. 13.27 & 13.28 shows: The mycelium , conidiophores and conidia of *S. purpurescens*

Epidemiology:

- The fungus enters mainly through soil, casing material and spent compost which is further disseminated through splashed water sprays and contact with workers' hands.

Control methods:

- Proper hygiene and pasteurization of compost at proper temperature eliminates the fungus.

10) OEDOCEPHALUM MOULD(*Oedocephalumfimetorium* , *O. spp.*):

- The mould appears as irregular, light silver grey patches on the compost surface during conditioning and at the time of filling or spawning . During spawn run the mould appears light grey in colour which soon changes to dark tan or light brown with the maturation of spores . It also appears on the casing surface .

Control methods:

- Hygienic measures and proper pasteurization of compost has been found to eliminate the mould .

11) CINNAMON MOULD (*Chromelosporiumfulva* ,*Ostracodermafulva, C . ollare* with their perfect stage as *Pezizaostracoderma*):

- Due to its cinnamon brown colour in the compost or casing layers in the form of circular white mycelial patches, it is known as Cinnamon Brown Mould. It appears as circular white patches of white mycelium which changes its colour to light brown, then light golden brown and ultimately to cinnamon with granular appearance.

Control methods:

- Proper hygiene and sterilization of casing avoiding bed temperature above 65 ºC, proper composting and pasteurization will eliminate the fungus. Dithane Z-78 and Dithane M-45 sprays (0.2 %) have also been found to control the mould .

Pathogenic Diseases of Mushrooms

B) PATHOGENIC DISEASES OF MUSHROOMS

1. DRY BUBBLE DISEASE OF MUSHROOMS: (Pathogen :*Verticilliumfungicola*Preuss.Hassebr)

Common Name: Brown spot , fungus spot , *Verticillium*disease , La mole , Dry bubble

- It is a most common and serious fungal disease of mushrooms. Sometime it may cause complete failure of the crop within 3-4 weeks.

Symptomatology:

- Numerous localized,light brown depressed spots appear on the mature sporophores. After coalition, these spots form irregular brown blotches with white fungal spore mass or grey mouldy fuzz covering the surface giving a dirty look.

Fig. 13.30 to 13.32 shows: (a&b) The brown spot symptoms of dry bubble disease on fruit bodies, (c) the microscopic structure of the pathogen with verticillate branching and conidia at the tips

Epidemiology:

- The fungus is soil borne and spores can survive in the soil for one year.It also perpetuates through resting mycelium from dried bulbils and spent compost.

Causal Organism:

- Numerous one celled, thin walled, hyaline, oblong to cylinderical conidia (3.5 – 15.9 x1.5-5 µ) are produced on lateral or terminal , verticillately branched , relatively slender and tall conidiophores (200 – 800 x 1.5 – 5.0 µ). Conidia accumulate in round clusters surrounded by a sticky mucilage. The fungus remains live in the soil for a long time.

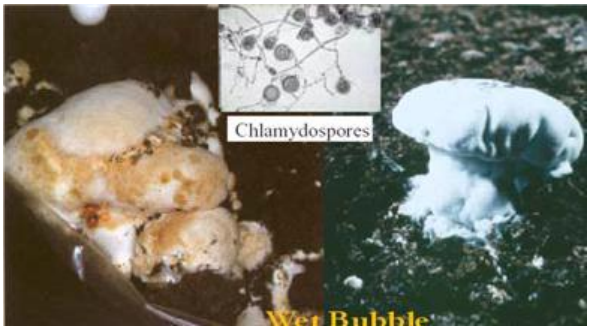
Control methods:

- Use of properly sterilized casing mixture, cook out of spent compost with steam at 71 º C for 8-10 hours and its disposal at a distant place, isolation and removal of infected sporophores from the cropping room, spray of fungicides like Dithane M-45 (0.2%) or Carbendazim (0,05 %) on cropping beds at 10 days interval, complete hygiene, proper pasteurization of compost etc, have been recommended.

2. WET BUBBLE DISEASE OF MUSHROOM: (Pathogen: *Mycogoneperniciosa*Magn.)

Common name of the disease: The disease is also known as wet bubble, La mole, bubble, Mycogonedisease or white mould

- It is a serious and devastating disease of white button mushroom all over the world .



Symptoms:

- The pathogen appears as a white mould attacking primordia and turning them into a soft whitish ball of mycelia. Early infection causes formation of sclerodermoid masses or forms whereas late infection causes production of mushrooms with thickened stipes and deformation of gills. At the later stage amber coloured fluid containing spores and bacteria ooze out from the brown and rotting interior of these bubbles sometime giving bad odour.
-

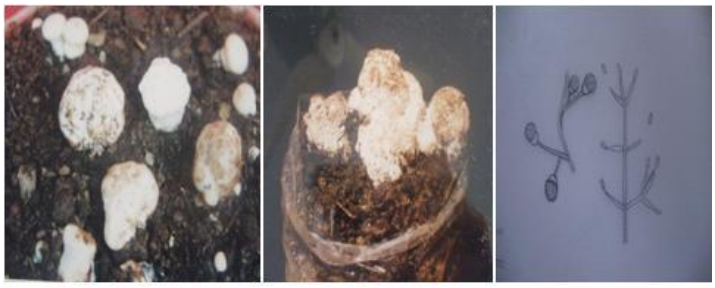


Fig. 13.34 to 13.36 Shows: (a&b) Bubble structures of *Mycogoneperniciosa*formed on mushroom beds (c) warty chlamydospore and single celled conidia borne on the conidiophores

Etiology:

- The disease is caused by a fungus *Mycogoneperniciosa*Magn which is having white, compact, felt like mycelium; hyphae branched, interwoven, septate, hyaline, 3.5 µm broad . Conidiophores short, slender, branched, hyaline measuring 200 x 3-5 µm having sub-verticillate to verticillate branches bearing thin walled, one celled conidia measuring 5-10 x 4-5 µm . It also forms large, dark, 2 celled chlamydospores with its upper cell warty, thick walled, globose, bright coloured measuring 15-30 x 10-20 µm; lower cells hyaline, smooth measuring 5-10 x 4-5 µm . It is the imperfect form of *Hypomycesperniciosa* .
-

Fig. 13.37 **shows: Dry bubble in Oyster mushrooms**

Sources of Infection:

- *Mycogoneperniciosa* is a soil borne fungus and enters the mushroom house through casing material, spent compost, infected trashes which are air as well as water borne or mechanically transmitted through men, mites, flies ,tools and containers . The aleurospores produced cause secondary infection but since chlamydospores survive for a considerable period in casing soil (more than 3 years), it may serve as the primary source of infection . A bed temperature of 25 º C and pH range of 6.0 to 8.4 are favourable for the pathogen.

Control methods:

- Proper sterilization of casing soil with live steam or formalin, use of plastic pots or common salt for early covering of the infected fruit bodies so as to prevent further spread of the disease, complete hygiene, cook out of the cropping beds / bags at the end of the crop with live steam at 71º C for 10 – 12 hours, fumigation of the cropping room with formaldehyde and spray of fungicides like Bavistin or Mertect (0.5%) immediately after casing etc; are the measures recommended for controlling this disease.

3. COBWEB DISEASE OF MUSHROOMS: (Pathogen- *Cladobotryumdendroides* (Bull : Merat)

Common names of the disease : Mildew, soft decay, Dactylium disease, *Hypomyces*mildew disease

Symptoms:

- It is cobweb like in appearance which appears as a small, white patches on the casing soil and then spreads to the nearest mushroom by a fine grey white mycelium . A floccose white mycelium covers the stipe, pileus and gills eventually resulting in decomposition of entire fruit bodies and change to slightly pinkish cover .at a later stage.

Etiology:

- *Cladobotryumdendroides(Dactyliumdendroides*) is the imperefect stage of *Hypomycesrosellus* . The hyphae are prostrate, branched, septate, hyaline with approximately opposite branches which divide above into usually those pointed branchlets; conidia multicelled, usually three or more connected cells which occur singly or in clustered form, terminally positioned at the end of branches often seen in a *Verticillium* like fashion; conidiophores are erect, similar or branched; conidia measuring 20-30x 5-12.5 µ in size.

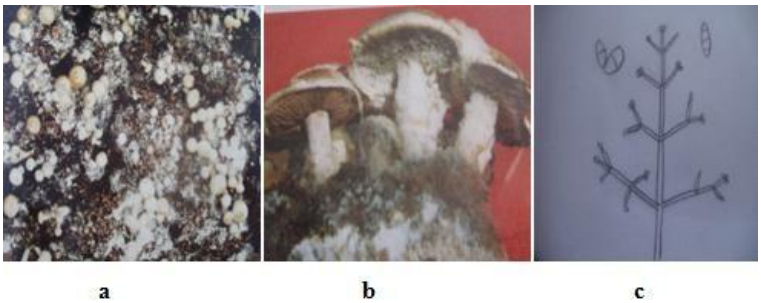


Fig. 13.39 to 13.41 shows: (a&b) Mouldy growth of mycelium of *Cladobotryumdendroide*s covering casing surface and engulfing the fruit bodies,
(c) Conidiophore bearing 2-3 celled conidia at the tips

Sources of infection and spread:

- It is a soil inhabiting fungus and introduced through casing ingredients, worker's hands, spores or mycelia surviving in the spent compost. It further disseminates through workers' hands, equipments and tools, air, water splash, mushroom flies etc. A bed temperature of 20 ° C and above with high relative humidity have been found to be favourable for rapid development of the disease and maximum damage.

Control methods:

- Complete hygiene, careful removal of cut mushroom trashes and young dried mushrooms; proper sterilization of casing mixtur , covering of infected pinheads with plastic cups or common salt are recommended.

4. *TRICHODERMA* BLOTCH OF MUSHROOM:

- The most common green mould fungus *Trichodermaviride* also infects the fruiting bodies by engulfing them and producing brown spots or blotch symptoms causing considerable loss . The details have already been described under the head green mould.

Bacterial Diseases of Mushrooms

C) BACTERIAL DISEASES OF MUSHROOMS
1. Bacterial blotch and bacterial pit diseases of white button mushroom:
Symptoms:

- Circular but irregular, yellowish spots appear superficially on or near margin of the cap of a wet mushroom which enlarge rapidly under high humidity conditions and coalesce to form bigger rich chocolate brown spots that are slightly depressed and slimy.

Causal organism:

- The pathogen *Pseudomonas tolaasii* can devastate the crop of button mushroom and*Psilocybe sp*. The bacterium has cylinderical(Bacilli) and spherical forms (Cocci) with its cells measuring 0.4-0.5x1.0-1.7 µ in size, with either one or more flagella (motile hairs) attached at one or both the ends for locomotion. The bacterium is gram negative in character
-

Fruit bodies showing blotch symptoms

Epidemiology:

- The casing ingredients and air borne dust particles are the primary sources of infection .Under high humidity and damp conditions, bacterial population increases on cap surfaces and cause the disease. The bacterium remains suppressed in the compost, casing , tools and debris under dry conditions, but it becomes active under high humidity conditions and further spreads through worker's hands, tools, mushroom spores, debris, water splash, flies, mites etc.

Control methods:

- Avoid heavy water sprays during rainy season, introduce fresh air immediately for about one hour after water spray and ensure that water droplets do not remain on the cap surface, remove all the diseased fruit bodies and spray bleaching powder (0.015 %) on the cropping beds at 7 days interval.

II. Bacterial disease of Oyster mushroom:

Yellow Blotch:

- The yellow blotch disease of *Pleurotus spp.* is caused by *Pseudomonas agarici.*

Symptoms:

- Disease appears as blotches of various sizes in pilei, yellow hazel brown or organic in colour. The infected fruit bodies turn yellow and remain stunted, turn slimy and start giving foul smell.

Fig. 13.43 shows: Symptoms of yellow blotch disease of *Pleurotussp.*Caused by Pseudomonas agarici

Control methods:

- Same as suggested for controlling bacterial blotch disease of button mushroom.

3) VIRAL DISEASES OF MUSHROOMS:

- Diseases due to mushroom viruses are also known as La France, Die back disease and Mummy disease .

Symptoms:

- The viral diseases are not detectable during spawn – run stage; the initiation of pinheads is inhibited and vigour of mycelium severely reduced; yield is drastically reduced, mushrooms appear with distorted shape, delay occurs in appearance of first flush, sporophores with elongated stem and small caps giving drum stick like appearance and tilted towards one side appear, mushrooms appear in patches, prematrure opening of veils, watery stipe and streaking in the stipe. In case of oyster

mushroom, dwarfing or elongation of stem has been observed whereas, no detectable symptoms appear in infected *Volvariella sp.*

Sources of Infection:

- Infected mycelium and spores released from infected mushrooms are the primary sources of infection. These viruses further disseminate through worker's hands, equipments, infected spawn / mycelium present in the trays / bags and spent compost etc.

Control methods:

- Complete hygiene, use of disease free spawn, frequent disinfection with formaldehyde, aeration strictly through high efficiency filters, cook out of exhausted compost at the end of the crop with live steam at 70-71 º C for 10-12 hours, regular disinfection of equipments, wearing clean and changed clothes everytime while entering a mushroom house, harvesting of mushrooms before opening when the veil is intact, visitors to be discouraged, wooden trays and shelves to be washed regularly with 4 % sodium pentachlorophenate solution, growing of resistant strains like *A. arvensis* and *A . bitorquis*have been recommended.

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LECTURE 14

Nutritional value of mushrooms and the mushroom recipe

Nutritional value of Mushrooms

1. Water:

- Almost all the mushrooms, barring few, are known to contain about 90 % moisture.

2. PROTEIN AND AMINO ACIDS:

- Mushrooms are known to produce high protein food per unit area as compared with other protein sources like cereal crops, animal and fish proteins. Mushroom protein is found to contain almost all the essential amino acids like Leucine, Isoleucine, Valine, Tryptophan, Hystidine, Threonine, Phenylamine, Methionine, Lysine and also the Alanine, Arginine, Cystine, Glycine, Glutamic acid, Proline, Aspartic acid, Serine etc. Protein content in mushrooms has been found to vary from 1.8 to 5.9 per cent as reported by different analysts. Efforts are also going on for the production of mushroom mycelium in huge quantity and to make them easily acceptable to the people as a substitute of mushroom protein.
- The relative proportion of amino acids in a mushroom is also equally important along with the total protein content. Certain amino acids are essential in a balanced diet because they can not be synthesized in the human body and a diet lacking even one of the essential amino acids will produce deficiency symptoms. Mushroom protein is believed to be less nutritionally complete than meat protein due to its relatively low content of certain amino acids. Although mushroom protein contains threonine, valine and phenylalanine in similar amounts to meat protein, it may be slightly inferior in isoleucine, leucine, lysine and histidine . Methionine and cysteine are somewhat lower in mushroom protein than meat protein and similar to vegetable protein. The protein of mushroom has more lysine and tryptophan than most vegetable proteins .Thus mushroom protein lies intermediate in nutritional quality between meat and vegetable proteins .

3. FIBRE:

- High fibre content (3-32 %) in mushrooms helps in digestibility and prevention of constipation and acidity problems . Mushrooms are also known to contain fibre with chitin as its main component .

4. CARBOHYDRATES:

- Carbohydrates are the main component of mushroom apart from water and account for an average of 4.2 per cent of the fresh weight . The edible mushrooms are quite low in carbohydrate and fat content . They contain 4-5 % carbohydrate, including chitin, hemicellulose and glycogen . Absence of starch in mushrooms makes them an ideal food for diabetic patients and also to those who want to shed excess fat from their bodies to remain slim.

5. FATS:

- Fat content is low (about 0.3 %) but it is rich in essential fatty acids like Linoleic, Palmitic, Stearic and Oleic acids. Cholesterol is absent but ergosterol is present (0.2 – 270 mg. / 100 gm . dry weight) which can be converted into Vitamin–D.

6. VITAMINS:

- Mushrooms have been known to be the good sources of almost all types of vitamins like thiamine (B 1), riboflavin (B 2), niacin, biotin, ascorbic acid, X - carotene (Vitamin – A) and ergesterol (Vitamin –D) are also active and Folic acid and vitamin B-12 are present in mushroom, although absent in vegetables . Presence of Vitamin – C (4-8 mg / 100 gm. dry weight), vitamin –K and vitamin –E have also been reported. Mushrooms have been reported to be an excellent source of riboflavin, niacin and pentothenic acid.

7. MINERALS:

- Mushrooms are good source of almost all the minerals. Calcium content is high, iron is in low amount but it is in available form . Good for hypertension patients as the Potassium / Sodium ratio is very high. Also contains Cadmium (0.002 ppm), Lead (0.03 -13.5 ppm) and traces of Selenium . Potassium, a mineral that is evenly distributed throughout the sporophore is present in such quantity that 200 g mushroom could provide the full daily requirement of this element. Copper is accumulated by members of the *Agaricus*family and is most abundant in the outer layers and in cap and gills .
- Apart from the high nutritional content, mushrooms are also known to have the medicinal value. With high protein, low calories, no sugar and starch , they are considered to be the " delight of the diabetic ” . Doctors generally recommend the sugar as well as heart patients to eat mushrooms because of low calories , low fat (rich in linoleic acid , lacking in cholesterol and the high Potassium – Sodium ratio) content mushrooms are the dieticians choice for those patients with hypertension, heart diseases and obesity. High fibre content and the alkaline ash content help those patients with constipation and hyperacidity problems . Diseases like scurby, beri- beri , cardiac discomfort etc, can be cured by consuming mushrooms regularly as these are rich in thiamine and other vitamin contents.

Mushrooms Recipe

MUSHROOM RECIPE:

- Mushrooms are liked by both vegetarians as well as the non-vegetarians as these are not only tasty but also very nutritious with some medicinal properties . Some of the favourite recipes are being given below:

1. MUSHROOM SOUP

Ingredients:	Quantity
1. Fresh Mushrooms	250 gm (pieces)
2. Milk	2 cup
3. Corn flour	20 gm
4. Butter	20 gm
5. Ginger	15 gm
6. Garlic	15 gm

7. Sugar, Salt & Pepper	to taste

Method:

- Put mushrooms in a pan, add ginger and garlic with three cups of water and boil. Allow it to cool, then grind in a Mixie and sieve. Put corn flour in milk, melt butter in a heavy pan, slightly heat and then add sieved material and milk to it and boil for ten minutes until it becomes thick .Add spices to taste. Season with sliced mushrooms or bread pieces and fresh cream and serve hot to four persons.

2. MUSHROOM PAKORA

Ingredients:	Quantity
1. Fresh Mushrooms	500 gm
2. Onion	1 big sized
3. Ginger	2 tbs chopped
4. Anardana powder	1 tbs
5. Gram flour (Besan)	150 gm
6. Garam masala	10 gm
7. Cooking oil	100 gm

Method:

- Boil washed mushrooms in salted water for 5 minutes and drain excess water . Spread mushrooms on a dry cloth for 10 minutes to dry. Mix dried and half cut mushrooms with all other ingredients, including gram flour and make a thick paste with little water. Deep fry in a pan without oil on medium heat . Serve hot with pudina (mint) chutney.

3. MUSHROOM OMELET

Ingredients: Eggs - Four
Oil - 20gm.
Onion - One
Fresh mushrooms - 100gm.
Salt and Pepper - To taste

Method:

- Beat broken eggs in a bowl with salt and black pepper added to it until egg yolk and white are mixed well. Pour eggs in the heated oil in a fry pan and allow the mixture to cover the base. Stir it continuously until all the liquid is set but still soft .Spread mushrooms and chopped onion on it .Lift the pan, fold omelet in half with the help of a pallet knife and slide slowly into a plate. Serve with toast and tomato sauce.

4. **MUSHROOM MUTTER (PEAS)**
   Ingredients:
   Fresh peas ----- 250g
   Mushrooms (cut into pieces) ----- 300g
   Onion (chopped) ----- 3(medium size)
   Garlic ---- 5cloves (crushed)
   Ginger ----- - 50g (grated)
   Oil ------ Half cup
   Tomato puree ----- -- One cup

Coriander leaves --- - -- 50g
Cumin seed ------- 1/2tbs
Coriander powder ------ - - 1tbs
Garam masala ------ 2tbs
Turmeric powder -- ---- ½tbs
Red chillies ----- - to taste
Salt ------ to taste

5. **Method:**

- Heat oil in a pressure cooker, add cumin seeds and when spluttering, add onion . Fry till golden brown and add coriander powder, turmeric, salt and other spices according to taste for one minute .Add tomato puree in the fried contents and fry until it starts leaving oil, add cut mushrooms and peas, fry for five minutes .

5. MUSHROOM BIRYANI

Ingredients: Cut Mushrooms - 500g (cut into two pieces)
Basmati rice ------ 2cups
Pure ghee ------- 50g
Onion ------- One sliced
Garlic & Ginger paste -------50g
Black Cardamom --------- 2pieces
Cinnamon powder --------- 5g
Garam masala --------- 1tbs
Cumin seeds ------- - 1tbs
Clove --------- 5g
Cassia -------- - 4-5pieces
Red chillies and salt ---------- to taste

Method: Basmati rice may be soaked in water for 15 minutes. Fry mushrooms in pure ghee for few minutes until golden brown and keep aside. Take pure ghee in a deep pan and put cumin seeds when it is hot. Add onion and when it turns golden brown, add garlic and ginger paste. Cook for two to three minutes and add all the remaining ingredients in it, including mushrooms. Stir well, cook for 2-3 minutes and add three and a half cups of water to it. Add rice when it starts boiling and keep it covered with lid.. Cook for 10-15 minutes on simmer heat. Put off the gas and let it be kept as such for 15 minutes until rice settles down and properly cooked . Serve hot with curd or pickle or chutney.

6. **MUSHROOM CUTLET**
   Ingredients:
   Mushroom sliced and cooked ---2cups
   Potato boiled and mashed -------3Nos.
   Onion chopped ------- 2Nos.
   Carrots chopped ------- - 2Nos.
   Shelled green peas --------- 2table spoon
   Garam masala powder ---------- ½ tea spoon
   Green chillies chopped --------- 4Nos.
   Ginger chopped --------- One small piece
   Egg --------- One
   Bread crumbs --------- - ½cup
   Salt ---------- to taste

Method:

- Fry chopped onion and chillies, add vegetables and cook. Mix garam masala powder with cooked mushrooms, mashed potatoes and salt. Remove from fire, shape into round cutlets .Dip these cutlets in beaten egg , roll in powdered bread crumbs and deep fry .

7. MUSHROOM CURRY

Ingredients:

Mushroom ---------- 2 cup
Onion ---------- One
Green chillies ---------- two
Tomato ---------- One
Turmeric powder ---------- pinch
Chilli powder ---------- One table spoon
Coriander powder --------- One table spoon
Pepper ---------- ½ tea spoon
Garam masala ---------- ½ tea spoon
Grated Coconut ---------- ½ cup
Vinegar ---------- One table spoon
Salt ---------- To taste

Method:

- Clean mushrooms, cut into two big pieces .Marinate mushrooms with little quantity of turmeric powder, chilli powder, salt and vinegar and keep for some time and then fry in oil . All the remaining masala powders are mixed and ground to form a thick paste .Cut onion and green chillies into small pieces, add masala paste to it and roast again .Add the coconut milk to the masala paste, allow it to boil for sometime, add mushrooms and again boil for some time.

8. MUSHROOM PICKLE
Ingredients:

Mushroom ------ - 1Kg
Onion ------- 30g
Salt ------- 80g
Garlic ------- 5g
Ginger ------- 20g
Redchilli powder --- 10g
Cumin ------ 5g
Coriander powder ----10g
Fenugreek ------- 10g
Ajwaain ----- 5g
Daalchini ------ 1g
Citric acid ------ 2g
Vinegar -------- 10ml
Mustard oil ------ 500g

Method:

- Clean mushrooms and cut into small pieces, fry them in hot mustard oil to brown colour. Remove mushroom pieces and let oil boil for sometime till the water content evaporates and

then fry mixture of garlic, onion, ginger till brown, add all other ground materials in it. Add fried mushrooms also in it and mix thoroughly while still on fire. Remove the mixture from fire and add vinegar and citric acid to it . Fill in the jars when cool and add remaining part of mustard oil to it .Cover tightly with a lid and place in a cool place .The materials should remain dipped in oil or add 250 mg Sodium Benzoate per Kg mushroom to it for its preservation .Daily keep them in the sun for some time .

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LECTURE 15

Medicinal mushrooms and their use in industries

Medicinal Mushrooms

- Mushrooms were earlier collected and eaten for their good taste, but later these came to be known as special kind of food because of their medicinal and dietery values. Liu (1993) and others have stated that *Ganoderma* has been valued in China for its medicinal properties. Many fungi have been discovered for their anti-fungal, anti-bacterial, anti- viral, anti-tumour and other properties of pharmacological values

- For centuries, the Japanese have hailed the shiitake mushroom (*Lentinullaedodes*) as an " elixir of life ", a cure-all, revitalizing both body and soul, a cure for cancer, impotency, senility and a host of other ailments .Chinese have also described them as " Elixir of life ". Romans considered them as " Foods of God ", whereas Greeks regarded them as "Providing strength to soldiers in the war ". FAO has also considered mushrooms as a food for underdeveloped countries where the protein malnutrition is very common.
- Chinese have roughly sorted out 107 kinds of medicinal mushrooms. Most of them are edible but few poisonous species are also in their list. Chinese are cultivating about 20 mushrooms which are anti cancerous (polysaccharide –peptide of *Coriolusversicolor*) , liver protective agent (Polysaccharide of shiitake mushroom), recuperating medicines for the stomach and intestine, stimulating the secretion of bile, cure for dizziness and headache (*Armillariamelea*, the honey mushroom) , sedative (*Ganodermalucidum*) and antiradiation drug (*Tremellafuciformis*) .Similar types of antitumour , immunopotentiator and interferon stimulating polysaccharides have been found in *Flammulinavelutipes, Ganodermaapplanatum, G.lucidum, Boletus edulis, Coriolusveriscolor, Calvatiagigantea, Tricholomamatsutake, Phelinuslinteus, Pholiotanameko, Lentinullaedodes* etc.

Fig. Shows: Medicinal mushrooms at their cropping stage with fruiting bodies

Recognition of nutritional and medicinal value of mushrooms

The important aspect of the mushroom conferences held in August ,1993 in Hongkong and another in 1994 in Beijing was the recognition of the nutritional, nutriceutical and medicinal values and studies on mushrooms.

Medicinal ingredients:

- The prominent medicinal ingredients of mushrooms are mostly the polysaccharides which are known to strongly inhibit the development of transplanted tumours in mice .Lentinan , from *Lentinullaedodes*has specially potent, anti-tumour activity (chihara*et al* , 1970), stimulating the immune response of the host against cancer. Another compound believed to have antitumouractivity ,retine has been reported in *Agaricuscampestris* which is the simplest member of a group of compounds known as a keto-aldehyde and present in animal tissues .

Medicines from poisonous mushrooms:

- *Amanita muscaria*, a deadly poisonous mushroom, commonly called " fly agaric ", has been used therapeutically as a powder, tincture for swollen glands, nervous troubles and epilepsy .Muscinol and ibotenic acid prepared from A.*muscaria*can cure the malfunction of " GABA" system of brain which is termed as " Schizophorenia ". GABA is Gama Amino Butyric Acid – identified as " Brain break "." Psilocybin " and " Psilosin " are the two other drugs used in medical science to treat mental disorders and which are extracted from the mushrooms *Psilocybemexicana .Amanita phalloides* is used against Cholera and intermittent fever.

Medicinal components --Medicines from edible mushrooms:

- Mushrooms have been considered a very good food for the maintenance of health as :
- Mushrooms are relatively high in good quality protein, containing all the amino acids and rich in lycine and leucine.
- Mushrooms are relatively low in total fat.
- Mushrooms have relatively large amount of carbohydrates and most of the species possess nutritionally valuable amount of fibre
- These are known to contain significant amount of the water soluble vitamins (thiamine, riboflavin , niacin and ascorbic acid) , as well as minerals.

Amongst edible mushrooms, *Agaricuscampestris ,Flammulinamellae, F. odilpis* etc. are known to have antibacterial actions against *Staphyllococcusaureus, Salmoniellatyphii* and *Escherchia coli*. The terpinoides namely, Illudin –M and Illudin –S extacted from *Clitocybeilludens*are very effective against *Plasmodium gallinaceum* .

Grouping of medicinal mushrooms:

- Based upon their utility and availability conditions, mushrooms can be rouped into following three different forms :
  - Nutraceuticals
  - Nutriceuticals and
  - Pharmaceuticals

a) Nutraceuticals:

- The term nutraceutical refers to functional foods that are consumed as a part of the normal diet. Mushrooms have nutritional values and health benefits, hence they can be referred to as nutraceuticals.

b) Nutriceuticals:

- The term mushroom nutriceutical is used for a new class of compounds that have been extracted from either the mushroom or the vegetative mycelium of mushroom species. Nutriceuticals have medicinal as well as nutritional attributes. It has been found that metabolites from the nutriceuticals may exhibit such features as anti-tumour, immunomodulating and hypocholesterolemic properties.
- The compounds involved in *Ganoderma* are triterpinoides and polysaccharides.Withnutriceuticals as with nutraceuticalsals, there is little chance of toxicity effects since the nutriceuticals are derived from edible mushrooms. Beneficial treatment of diseases can be obtained by consumption of mushrooms as a functional food or the use of extracted biologically active compounds as a dietery supplement in order to enhance the immune response of the human body, thereby increasing resistance against diseases and causing regression of a diseased state.

c) Pharmaceuticals: When a chemically defined preparation with medicinal properties is obtained from a natural product such as mushroom, this can be referred to as a Pharmaceutical.The pharmaceuticals prescribed by the physicians as a therapeutic treatment for a specific medical may be administered in a variety of ways e.g. orally, by inhalation , topically or by injection.

Application in mushroom biotechnology

- There has been a great upsurge in activities concerned with the use of mushroom products for medicinal purposes. Nutraceutical is important as a first step in the determination of the validity for use of the mushroom product as a prophylactic or potentially therapeutic substance.
- Mushroom nutriceutical is a new class of compounds extractable from either the mycelium or fruiting body of mushroom which may possess both nutritional and medicinal properties. When testing shows that a particular mushroom nutriceutical of known chemical structure has a recognized role in the treatment of a specific medical condition (e.g. lentinan in the treatment of stomach cancer), it then achieves the status of a pharmaceutical . Pharmaceuticals are used therapeutically for the treatment of a specific disease

LECTURE 16

Project cost - analysis for mushroom cultivation

Cost Analysis for Mushroom Cultivation

Mushroom growing is a highly profitable activity and can be taken up on a smaller or larger scale depending upon the capacity of an individual or organization. The spawn production, compost preparation and mushroom cropping are the components of mushroom farming which yield significant profit. The economics of commonly cultivated mushrooms is given as under :

A. Spawn Production Project

Economic of spawn production (100 spawn bags per day)

S.No	Item	Quantity	Rate(Rs.)	Total(Rs.)
A.	**Capital Investment**			
1.	Autoclave	2	20,000	40,000
2.	Boiler (GI drum 100 lit. Capacity)	2	2,000	4,000
3.	Culture room with work table	1	10,000	10,000
4.	UV lamp with fittings	1	1500	1500
5.	Tube light fittings	1	200	200
6.	Advance for LPG gas	2	2,000	4,000
7.	Spawn storage room	1	20,000	20,000
8.	Bunsen burner	1	150	150
9	Heat efficient chulah	1	600	600
	Total			80,450
B.	Fixed Cost			
1.	Interest on capital investment @ 12%			9,654
2.	Depreciation (Item 3 &7 @ 5%)			1,500
3.	Depreciation (Item 1, 2, 4, 5, 8&9 @10%)			4,645
	Total			15,799
C.	Recurring cost (100 spawn x 300 days)			
1.	Polypropylene (3%damage)	160 kg	80	12,800
2.	Cholam grain (3%damage)	9,300 kg	7	65,100
3.	Calcium carbonate (commercial grade)	185 kg	17	3,145
4.	Non- Absorbent Cotton (400 g Rolls)	775	60	46,500
5.	Fungicides & fumigants	-	-	1,000
6.	Electricity &fuel	-	-	25,000

7.	Labour @ 2 men per day for 300 days	600	50/heads	30,000
8.	Sundry items	-	-	2,000
9.	Glasswares and chemicals	-	-	5,000
10.	Miscellaneous	-	-	2,000
	Total			1,92,545

Cost of production / Year:

1.	Working expenditure	1,92,545
2.	Interest and depreciation on fixed cost	15,799
3.	Total Cost	2,08,344
Income		
1.	By sale of 30,000 spawn @ RS. 12per bag	3,60,000
2.	Total income	2,08,344
3.	Net income per year	1,51,656

Mushroom Production Project

B. Mushroom Production Project

Economics of Oyster mushroom production (5 kg/day / 300 days)

S.No	Item	Quantity	Rate (Rs.)	Total (Rs.)
A.	**Capital Investment**			
1.	Mushroom growing room (thatched)	1	7,500	7,500
2.	Chaff cutter (leaver type)	1	1,200	1,200
3.	Boiler	1	2,000	2,000
4.	Cement tub	1	1,000	1,000
5.	Sprayer	1	500	500
6.	Biomass stove	1	300	300
	Total			12,500
B.	**Fixed Cost**			
1.	Interest on A @ 12%			1,500
2.	Depreciation (Item 1 @ 30%)			2,250
3.	Depreciation (Item 2,3,4,5 &6 @ 10%)			500
	Total			4,250
C.	**Recurring Cost**			
1.	Paddy straw	3t	1,500/t	4,500

2.	Spawn bags	1,500	12	18,000
3.	Polythene bags for bed & packing	60 kg	80	3,600
4.	Fungicides, fumigants & chemicals	-	-	1,000
5.	Labour @ 1 per day	300	50 / head	15,000
6.	Others	-	-	5,000
	Total			47,100

Cost of production / Year:

1.	Working expenditure	47,100
2.	Interest and depreciation on fixed cost	4,250
3.	Total Cost	51,350
Income		
1.	By sale of 5 kg of mushroom daily @ Rs. 60 per kg	90,000
2.	Cost of spent mushroom compost	10,000
3.	Total income	1,00,000
4.	Net income per year	48,650

Mushroom Production Project (Contd..)

B. Mushroom Production Project

Economics of Milky mushroom production (5 kg /day /300 days)

S.No	Item	Quantity	Rate (Rs.)	Total (Rs.)
A.	**Capital Investment**			
1.	Mushroom growing room (poly houses)	1	12,000	12,000
2.	Chaff cutter (leaver type)	1	400	400
3.	Boiler (one for paddy straw& one for casing soil sterilization)	1	2,000	2,000
4.	Cement tub	1	1,000	1,000
5.	Sprayer	1	500	500
6.	Biomass stove	1	300	300
	Total			19,800
B.	**Fixed Cost**			
1.	Interest on A @ 12%			2,376
2.	Depreciation (Item 1 @ 10%)			1,200
3.	Depreciation (Item 2,3,4,5 & 6 @ 10 %)			780
	Total			4,356

C.	Recurring Cost			
1.	Paddy straw	1.5t	1,500/t	2,250
2.	Spawn bags	1,200	12	14,400
3.	Polythene bags for bed & packing	35kg	80	2,800
4.	Fungicides, fumigants & chemicals	-	-	1,000
5.	Labour @ 1 per day	300	50/head	15,000
6.	Others	-	-	5,000
	Total			40,450

Cost of production / year

1. Working expenditure : 40,450
2. Interest and depreciation on fixed cost : 4,356
3. Total cost : 44,806

Income
1. By sale of 5 kg of mushrooms daily @ Rs. 65 per kg : 97,500
2. Cost of spent mushroom compost : 10,000
3. Total income : 1,07,500
4. Net income per year : 62,694

Mushroom Production Project (Contd..)

B. Mushroom Production Project

Economics of Pady Straw mushroom production (5 kg /day /300 days)

S.No	Item	Quantity	Rate (Rs.)	Total (Rs.)
A.	**Capital Investment**			
1.	Mushroom growing room (poly house)	1	7,500	7,500
2.	Cement tub	1	2,500	1,200
3.	Sprayer	1	500	500
4.	Wooden planks /cement platform for mushroom bed preparation	1	8,000	8,000
	Total			18,500
B.	**Fixed Cost**			
1.	Interest on A @ 12%	-	-	2,220
2.	Depreciation (Item 1 @ 30%)			2,250
3.	Depreciation (Item 2,3,4,5 & 6 @ 10%)			1,100
	Total			5,570
C.	**Recurring Cost**			
1.	Paddy straw	10t	1,500/t	15,000
2.	Paddy straw spawn	1,800		27,000

3.	Horse gram powder	75 kg	15	1,875
4.	Polythene bags for mushroom packing	35	25	2,800
5.	Fungicides, fumigants & chemicals	-	80	2,500
6.	Labour 1 @ per day	300	-	15,000
7.	Others	-	50 / head	3,000
	Total			67,175

Cost of production / year

1. Working expenditure : 67,175
2. Interest and depreciation on fixed cost : 5,570
3. Total cost : 72,745

Income

1.By sale of 5 kg of mushrooms daily @ Rs. 65 per kg : 90,000
2.Cost of spent mushroom compost : 15,000
3.Total income : 1,02,000
4.Net income per year : 37,825

Mushroom Production Project

B. **Economics of production of button mushroom**(*Agaricusbisporus*)

Production of button mushroom on a small scale is not economical.However, if good compost is readily available or by creating facilities for LMC, button mushroom production can be taken up, especially at high elevation where suitable climatic condition normally exist.

Approximate cost estimated is given below (650 bags / crop having 10 kg of compost in each bag x 4 crops / year)

S.No	Item	Quantity	Rate (Rs.)	Total (Rs.)
A.	**Capital Investment**			
1.	Composting yard with cutting and soaking	-	-	2,00,000
2.	Spawn running & cropping rooms	6	25,000	1,50,000
3.	Casing soil preparation unit	1	20,000	20,000
4.	Steam generator, boiler & fittings	-	1,20,000	1,20,000
5.	Air cooler and humidifiers	6	25,000	1,50,000
6.	Water tank	-	-	50,000
	Total			6,90,900
B.	**Fixed Cost**			
1.	Interest on @ 12%			82,800
2.	Depreciation @ 10%			69,000

	Total			1,51,800
C.	**Recurring Cost**			
1.	Compost preparation by LMC	30t	-	90,000
2.	Fungicides, fumigants & chemicals	1,200	-	10,000
3.	Spawn bags	40	15	18,000
4.	Polythene bags	1,095	80	3,200
5.	Labour @ 3 per day	-	50	54,750
6.	Electricity, fuel etc.,	-	-	50,000
7.	Miscellaneous cost	-	-	10,000
	Total			2,35,950

Cost of production/year
1. Working expenditure : 2,35,950
2. Total fixed cost : 1,51,800
Total cost : 3,87,750

Income
1. By sale of 9,000kg mushrooms @ Rs.70 /kg : 6,30,000
2. Cost of spent mushroom compost : 20,000
3. Total income : 6,50,000
4. Net income per year : 2,62,250

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