B.Sc.Microbiology

2019-2020

Semester – VI

18MBU611A MUSHROOM CULTIVATION - PRACTICAL
(4H – 2C)

Instruction Hours / week:L: 0 T: 0 P: 4 **Marks:** Internal: **40**
External: **60** Total: **100**

End Semester Exam: 6 Hours

COURSE OBJECTIVES

To teach on classification, cultivation, diseases and health benefits of mushrooms.

COURSE OUTCOME

To impart knowledge on various mushrooms and its cultivation techniques

EXPERIMENTS

1. Oyster cultivation and demonstration of Button mushroom cultivation
2. Tissue isolation and sub culturing
3. Spawn making using sorghum
4. Fruiting bags production – preparing beds (chopping and sterilization of straw)
5. Field trip to commercial mushroom farms and scientific institutions.

SUGGESTED READINGS

1. Alice, D., Muthusamy and Yesuraja, M. (1999). Mushroom Culture. Agricultural College, Research Institute Publications, Madurai.
2. Marimuthu, T. et al. (1991). Oyster Mushroom. Department of Plant Pathology. Tamil Nadu Agricultural University, Coimbatore.
3. Nita Bhal. (2000). Handbook on Mushrooms. 2nd ed. Vol. I and II. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.
4. Pathak, V. N. and Yadav, N. (1998). Mushroom Production and Processing Technology. Agrobios, Jodhpur.
5. Tewari Pankaj Kapoor, S. C. (1988). Mushroom Cultivation. Mittal Publication, New Delhi.
6. Tripathi, D. P. (2005). Mushroom Cultivation. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Expt 1:

OYSTER MUSHROOM CULTIVATION PROCESS

MATERIAL REQUIREMENT

Substrate :

A large number of agriculture, forest and agro-industrial byproducts are useful for growing oyster mushroom. Substrates should be fresh, dry and free from mould infestation. Oyster mushroom can utilize a large number of agro-wastes including straw of wheat, paddy and ragi, stalks and leaves of maize, jowar, bajra, and cotton, sugarcane bagasse, jute and cotton waste, peanut shells, dried grasses, sunflower stalks, used tea leaf and discarded waste paper. It can also be cultivated using industrial wastes like paper mill sludge, coffee by-products, tobacco waste etc. About 1.5 -2.0 kg of good substrate will be required per bag of 80 cm x 40 cm size.

Mushroom Spawn

Three to four week old non-contaminated spawn @ 10 % of dry weight of the substrate is required for the purpose. Spawn of good quality should be collected from a reliable source. Further, the species / variety should be chosen basing on the temperature and relative humidity of the cropping season of the locality. Just prior to use the 200 gm Spawn is extracted from the bottle with hooked iron rod and divided into four parts.

Substrate Supplementation

Some of the common supplements are wheat bran, rice bran, soybean cake, groundnut cake, maize meal, horse gram powder, cotton seed meal etc. wheat bran and rice bran should be used at the rate of 10%, while other should be tried @ 3-6% on dry weight basis of the substrate. Supplements after pasteurization are thoroughly mixed with straw while spawning. Addition of supplements increases the substrate temperature and hence, it is risky during the work period to supplement the substrate.

Polythene Bag

Transparent polythene tube of 125-150 gauge with a dimension of 80 cm x 40 cm is suitable for oyster cultivation. Bags of 60 cm x 40 cm may also be used for the purpose. The bags can be reused for the second crop after proper cleaning.

CULTIVATION PROCEDURE

Substrate Processing

Freshly procured good quality substrate is chopped to 4-5 cm size by a chaff cutter and steeped in a chemical solution of carbendazim 50 % WP(75ppm) and formal dehide (500 ppm) for a period of 6 hours. Then the straw is taken out and excess water is to be drained. Ninty liters of water mixed with 7.5 g carbendazim 50% WP and 125 ml formaldehyde (37-40%) will be appropriate for the purpose. However seam and hot water treatment methods are preferred as there are reports of phytotoxicity associated with chemical treatment. Here, the pre-wetted substrate after chopping is soaked in hot water (65-70^0 C) for one hour. In case of steam pasteurization, the pre-wetted straw is steam pasteurized at 60-70^0 C for one hour, cooled at room temperature and then seeded with spawn. Through pasteurization, the competitor moulds
are either killed or their growth is suppressed for 25-40 days after spawning.

The substrate is dried in shade for few hours in order to maintain the moisture level of 55-60%. The substrate necessary for raising one bag may be divided into four lots after drying.

Raising of bag

One end of the polythene tube is tied with a rubber band and the moistened substrate is put inside to a height of 15 cm. Substrate is then gently pressed and one part each of spawn(50g) and supplement(50 g) spread at the periphery close to polythene(See the Video). Likewise, four such layers are made and the bag is closed at the upper end after pressing the substrate. For a bag out of 2 kg of dry straw, 200 g each spawn and supplement will be used. 15-20 small holes (0.5 cm diameter) should be made on all sides to

facilitate gas exchange. Instead of layer spawning, mixed spawning may also be followed where the required quantity of spawn is mixed with the prepared substrate (soaked straw) and incorporated into the bag. The bags are then incubated in a well ventilated room at 250 C. During the mycelial growth bags should not be opened.

After Care

Once the mycelium has fully colonized the substrate forming a thick mycelial mat, it is ready for fruiting. Contaminated bags with mould may be discarded while bags with patchy mycelia growth may be left for few more days for completion of the mycelia growth. These bags are opened after 15-16 days. But in case of P. eous and P. djamore var. are opened after 12 days as fruiting comes out within these. The bundles after opening are arranged on shelves at a distance of 20 cm between each bag in the tier or hanged with plastic rope. Appropriate temperature (20-30^0 C), humidity(70-80%) and light(200 lux) with good ventilation should be maintained in the cropping room. Bags are watered twice daily depending upon the weather condition.

Harvesting

Primordia(small eggs) appears within 4-5 days of opening the bag that came to the harvestable stage 3-4 days later. The mushrooms should be harvested when the cap begins to fold inwards. Picking is done by twisting the mushroom gently without disturbing the surrounding fruit bodies. Crop should not be watered before harvesting. The second crop appears after 7-10 days. Hence within 45 days crop period, 3-4 crops are expected. With exception, under suitable growing condition, a biological efficiency of 100% is achieved in commercial farms.

Mushrooms Cultivation: procedure for mushrooms cultivation!

Direct utilization of fungi as food:

Many Agaricales and Helvellales are directly used as food. There is a non-poisonous edible toadstool, i.e., Coprinus sp. found in lawns in the rainy season. Agaricus campestris is edible mushroom and cultivated for its fructifications. The fruiting bodies are quite fleshy and eaten directly as vegetable or with rice as 'pulao'.

These mushrooms are being successfully cultivated in South India. Morchela esculenta is another important edible fungus. It is found in Kashmir and Punjab plains. Its local name is 'guchi' and sold very costly. Torulopsis utilis, is used for the large-scale production of yeast for food purposes. Saccharomyces cerevisiae is used in bread making industry.

Of the many mushrooms that can be cultivated, only three kinds namely button mushroom (Agaricus bisporus), straw mushroom (Volvariella volvacea) and oyster mushroom (Pleurotus sajorcaju) are suitable for growing in India where suitable environmental conditions exist. The following account deals with cultivation of Button Mushroom (Agaricus bisporus).

Growing Season:

Agaricus bisporus being a temperate mushroom grows best during winter throughout the plains of North India. It can however, be grown throughout the year in hills. The most suitable temperature for the spread of mycelium is 24-26°C. Temperature ranging from 16-18°C is essential for the formation of fruit bodies. Higher temperatures are harmful but the lower temperature retards the development of both mushroom mycelium and fruit bodies.

Mushroom House:

The mushroom house can be any available room, shed, basement, garage, etc. The growing house should be well ventilated and not stuffy.

Compost:

The cultivated mushroom is grown on special compost. Two types of composts, natural and synthetic are used for growing this mushroom.

Composting Yard:

The compost should be prepared on well cleaned concrete or pucca floor, which should be on a higher level so that the run-off water does not collect near the heap. Composting is usually done in the open, but it has to be protected from rain by covering it with polythene sheet. It can also be done in a shed with open sides or a large room to shelter it from rain.

Synthetic Compost:

The following ingredients are required:

Wheat straw (chopped 8-20 cm. long) – 250 kg.

Wheat/Rice bran – 20 kg.

Ammonium sulphate/calcium ammonium nitrate – 3 kg.

Urea – 3 kg.

Gypsum – 20 kg.

The casing soil is spread over a plastic sheet and treated with formalin by sprinkling. The treated soil is piled up in a heap and covered with another plastic sheet for 48 hours. The soil is turned frequently for about a week to remove all traces of formalin which can be tested by smelling it. After casing, the temperature of the room is maintained for further three days, after which it must be lowered to below 18°C. At this stage lot of fresh air is needed and therefore, the growing room should be ventilated by opening windows, etc.

Cropping and Harvesting:

The first flush of the pin heads becomes visible 15-20 days after casing or say about 35-40 days after spawning. Small white buttons develop 5-6 days after pin head stage. The right stage of harvest is when the cap is still tight over the short stem. In case the buttons are allowed to mature, the membrane below the cap will rupture and the cap will open up in umbrella-like shape.

Such mushrooms are considered to be the inferior. Harvesting is done by holding the cap with forefingers slightly pressed against the soil. The soil particles and mycelial threads clinging to the base of the stalk are chopped off. Mushroom can also be harvested by cutting off with a sharp knife at soil level.

Yield:

The average yield of 3-4 kg per tray is considered normal. However, if compost is carefully prepared, spawn reliable and favourable termperature prevailing during the growing period, then a yield of 5-6 kg per tray is possible. Partial or complete failure may also happen due to negligence.

Storage:

The mushrooms are best consumed fresh. Storage in refrigerator for a few days is possible. The mushrooms should be placed between moist paper towel for storing in a refrigerator.

Introduction

Mushrooms belong to a separate group of organisms called Fungi. They lack the green matter (chrolophyll) present in plants and grow on dead and decaying organic materials. From these decaying substrates, they absorb their nutrition with the help of very fine thread like structures (mycelium) which penetrate into the substratum and are generally not visible on the surface. After the mycelium has grown profusely and absorbed sufficient food materials, it forms the reproductive structure which generally comes out of the substrate and forms fruiting body, commonly known as mushroom. The mushroom fruiting body may be umbrella like or of various other shapes, size and colour. Commonly, it consists of a cap or pileus and a stalk or stipe but others may have additional structures like a veil or annulus, a cup or volva, performing various functions in the life-cycle of the fungus.

Historical

Mushrooms have been devoured as food by mankind since time immemorial after collecting from the forests. However, mushrooms could not be domesticated due to their complex nature. Though Chinese were the first to do the artificial cultivation of the tropical and subtropical mushrooms about thousand years ago real commercial ventures started when Europeans started cultivation of button mushroom in green houses and caves during 16^{th} and 17^{th} century. The success to isolate pure culture through tissues and spores was the turning point in the process of commercial mushroom production in world. Mushrooms are now getting significant importance due to their nutritive and medicinal values and income generating venture in about 100 countries.

Mushroom being an indoor crop does not require arable land, except for some non-agricultural land to build the infrastructure for preparation of substrate, rising of crop, preparation of spawn and postharvest handling. White button mushrooms in India is grown seasonally and in environment controlled cropping houses and both require building of basic infrastructure. Seasonal growing is done for 5-6 months when outside temperatures are favourable for the crop, i.e., during winter months in N.W. plains and from September to April in the hills.

Components of Mushroom Farm

Low cost Thatched Huts / Mushroom growing Houses

Spawning

For spawn run air temperature of 23° ± 1C is maintained in the room, with corresponding bed temperature of 24-25°C (1-2°C higher than air temperature). The fresh air valve is closed and entire air is re-circulated, allowing the carbon dioxide to accumulate to the level of 15000 ppm, desirable for quick spawn run. Higher concentration of CO_2 accelerates the spawn run/vegetative growth of the mushroom fungus. During spawn run above temperature has to be maintained, till entire compost is impregnated with the mushroom mycelium, alongwith other parameters like high CO_2 concentration, high RH (will be discussed later). Increase or decrease in temperature effects the CO_2 production of the compost and the RH of the room. With increase in temperature, RH will tend to fall, and with decrease in tempt. RH will increase. The properly insulated room will ensure uniform temperature in the cropping room at every stage of crop growth. The air will go into the room at the will of the grower and as per requirement inside, suiting the crop stage. The heat from the cropping room is removed via cooling coils in the AHU.

Casing

The environmental conditions suitable for spawn run, are suitable for case run as well. The same conditions will be provided for 7 days for case run, as for spawn run, i.e., temperature of 23°C in the air and 24°C in the bed. The RH/CO_2 will be same as required for spawn run. Within one week the case run will be completed, and case run is completed the moment the mycelium is observed in the valleys. Valleys are areas between the peaks as can be seen on top of casing. Casing is applied uniformly and the material used should not be a finely ground casing soil but in the form of small clods, which form valleys/peaks on surface of casing. The CO_2 conc. and RH should also be within the optimum range for effective/quick case run.

Crop Management

After completion of case run, the cooling of the room is enhanced to bring the air tempt down to 15-17°C in the room within 2-3 days time. Simultaneously, the fresh air vent is opened to 30% and rest of the air is re-circulated (70%). This brings down the CO_2 conc. in the room to 300 ppm to 1000 ppm, desired for pinhead formation. Likewise, the RH is also reduced to 85% from 95%. This facilitates pinhead formation on the casing within a week's time. The pinheads grow into full button sized mushrooms in another 3-4 days. The environment parameters are maintained as above during entire period of cropping. Temperature has influence on RH and CO_2 conc. and hence should be maintained/manipulated, keeping in mind its effect on other two factors. All the three parameters work in synergy with each other to induce pinning on casing surface.

Harvesting

Mushrooms are harvested by gently holding a mushroom body and twisting it. Washing becomes necessary to remove soil particles if non-peat casing soil is used but washed mushrooms generally deteriorate rapidly than mushrooms packed dry, due to the increased water content that results in greater growth rate of spoilage by bacteria. Small growers wash in solution of reducing agents to retard the browning caused by polyphenoloxidase.

Processing

Sun-drying of mushrooms is one of the simplest and oldest methods followed by the growers from the time immemorial. Due to the difficulties in drying of some of the mushrooms, new preservation technologies like cabinet drying, canning, pickling, freeze-drying and irradiation treatment of mushrooms have developed to improve the shelf life and consumption of mushrooms. A variety of products are being prepared from mushrooms. These are mushroom pickle, mushroom powder for preparing mushroom soup, mushroom sauce, mushroom candy etc. Farmers can prepare these products when there is surplus.

Expt :2

Tissue Culture

Tissue culture technique is used to bring the edible mushroom to pure culture so that the mushroom fungus can further be used to prepare spawn, which is an essential material for mushroom cultivation. This nucleus culture is grown on Potato Dextrose Agar medium in test tubes. A small tissue from a well-grown mushroom is aseptically transferred to agar medium in a test tube in a culture room.

The test tubes are incubated under room temperature for 10 days for full white growth of fungal culture. This is further used for preparation of mother spawn.

Procedure

1. Select well grown, disease free button mushroom early in the morning and keep it on a clean paper for 2-3 hr, to get certain amount of moisture present in the mushroom to get evaporated.
2. Clean the culture room/ laminar flow chamber with antiseptic solution.
3. Keep the sterilized PDA slants, razor blades, forceps etc. inside the chamber and put on he UV

light.

4. After 20 minutes put off the UV light and start working after 5 minutes.
5. Sterilize all the instruments to be used by exposing to Bunsen burner.
6. Take in the mushroom and split open the mushroom longitudinally into two halves.
7. Using a blade cut a small piece of tissue from the centre of the spilt mushroom at the junction of pileus and stipe.
8. Remove the cotton plug of the agar slant and the tissue is aseptically placed inside the slant by using a sterilized forceps and closes it immediately.
9. After transferring tissues from the mushroom , the tube are arranged in a wire basket and kept in a clean room at room temperature for the growth of the fungus
10. Observe the tube at periodical intervals and remove the contaminated ones. The tubes will be ready for further use within another ten days. The base spawn is used for preparation of mother spawns.

Precautions to be observed:

- Wash the hands with antiseptic lotion before start working inside the chamber. If possible, it is better to use hand gloves while operation.
- It is better that the maximum of two persons may work inside the room at a time. Avoid unnecessary talking while working inside the room.
- While separating the tissue from the centre of the mushroom it should not touch the bottom or sides of the mushroom.

Expt:3 Spawn making using sorghum

MotherSpawn:

Mother spawn is nothing but the mushroom fungus grown on a grain based medium. Among the several substrate materials tested by TNAU, Coimbatore, sorghum grains are the best substrate for excellent growth of the fungus. Well-filled, disease- free sorghum grains are used as substrate for growing the spawn materials. The various steps involving in preparation of mother spawn are listed below here under.

Procedure

1. Wash the sorghum grains in water thoroughly to remove chaffy and damaged grains.
2. Cook the grains in an autoclave / vessel for 30 minutes just to soften them.
3. Take out the cooked grains and spread evenly over a Hessian cloth on a platform to remove the excess water.
4. Mix Calcium carbonate (CaCO3) thoroughly with the cooked, dried grains @ 20 g / kg.
5. Fill the grains in polypropylene bags up to ¾ th height (approximately 300-330 g/bag), insert a PVC ring , bold the edges of the bag down and plug the mouth tightly with non-absorbent cotton wool.
6. Cover the cotton plug with a piece of waste paper and tie tightly around the neck with a jute thread.
7. Arrange the bags inside an autoclave and sterilize under 20 lbs. pressure for 2 hours.
8. Take out the bags after cooling and keep them inside the culture room and put on the UV light.
9. After 20 minutes put off the UV light and start working in the culture room. Cut the fungal culture into two equal halves using a inoculation needle and transfer one half portion to a bag. Similarly, transfer another half portion of the culture to an another bag.
10. Incubate the inoculated bags in a clean room under room temperature for 10 days for further use to prepare bed spawn.

Expt:4

BedSpawn:

The method of preparation of bed spawn was same as that of mother spawn. The cooking, filling and sterilization were similar to that of mother spawn. After sterilization , the bags are taken for inoculation.

Precautions to be observed:

- Avoid over cooking of sorghum grains on the floor. Always dry over hessian cloth spread on a raised platform.
- Don't dry the cooked grains on the floor. Always dry over hessian cloth spread on a raised platform
- Use only recommended dose of CaCo3 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 bed spawns. This leads to lose of virulence of the spawn lead to reduced yield and repeated sub culturing lead to complete lose of virulence wherein the fungal growth may be noted in the beds but no buttoning is completely arrested.

Procedure:

1. The sterilized bags are placed inside the culture room and put on the UV light.
2. After 20 minutes put off the UV light and take in the well-grown mother spawn.
3. Transfer spawn from the mother spawn to sterilized bags @ 10 g per bag.
4. After inoculation the spawn bags are kept in a clean room for fungal growth. (This is first generation of bed spawn).
5. Use the bed spawn after 10 days of inoculation for bed preparation.

Sub culture the first generation bed spawn as mother spawn to produce one more generation, which is second generation bed spawn. It is quite true that happiness lies on exploring many things. We can extremely happy to let you know that our trip to the mushroom farm was quite interesting and innovative. The place was really awesome surrounded by hills.The mushroom farm we visited named as Western Ghats mushroom farm is located in kanuvai, Coimbatore. It was true that we were eagerly waiting to visit the place, since this is the first all together gathered outing that took place in our class. The way that leads to the farm is surrounded by rocky hills and cropland fields, the weather was really sunny.

Expt:5 Field trip to commercial mushroom farms and scientific institutions.

Amidst of the woods and fields, is present the mushroom farm, that is being maintained in a small area. The farm is being maintained by Mr. Nivish ram and family for nearly 6 months.

When we entered the farm it was clearly observed that the place is an elephant zone. The people working in the mushroom farm warmly greeted us pausing all their works. As soon as we entered the farm, Mr. Nivish whose qualification is M.Com, started explaining to us about the cultivation of mushroom. They cultivate OYSTER MUSHROOMS in small scale and sell them in the market and nearby local trade centres. With all the terms that should be clearly highlighted,Mr. Nivish explained to us how the mushrooms are being cultivated using the spawn that is made using white corn. The spores are inoculated in the white corn and these spawns are collected from kurinji mushroom farm located in TVS Nagar. The

oyster mushrooms are cultivated using air-dried, moisturized straw. The tightly packed bag containing layer of spawn and straw are kept hanging inside the hut that is used for incubation purpose. The cultivation days cycle is around 60days. They earn approximately 30,000 per month. The investment is ₹3,00,000.

The cultivation mushrooms are packed and sold. Mr. Nivish ram obtained training from TNAU Coimbatore, specifically to cultivate mushrooms. There was a clear understanding of how the mushrooms are being cultivated and the issues that are being faced during cultivation.

We have attached some images of the visit. Hereby we all conclude that the trip was entirely useful and innovative. Apart from this, the day was full of fun and laughter. Thanking the institution for we have been provided with this opportunity.