**SEC016503: MUSHROOM CULTURE TECHNOLOGY**

**PRACTICAL**

**EXPERIMENT-1**

**AIM**: TO PREPARE MEDIA FOR MUSHROOM CULTURE

**THEORY**: *All types of mushrooms use dead organic matter as a nutrient source, made available through decomposition. Thus, for the growth of mushroom mycelium, the nutritional requirement of mycelium must be met out by the media or substrate.*

*The media is a mixture of essential nutrients required for the growth and development of mushrooms in the laboratory. A typical media contains C, H, O, N, P, K, S, Ca, Cu, Co, Mg, Mn, Mo, Fe, and Zn.*

*On the basis of chemical composition, media are of the following types:*

1. *Natural media: It comprises of entirely natural products of unknown composition.*
2. *Semisynthetic media: It comprises of some known chemical components mixed with some unknown natural chemical components.*
3. *Synthetic media: It comprises completely of known chemical components.*

**MATERIALS REQUIRED**:

*Potato-250g*

*Dextrose-20g*

*Agar agar- 20g*

*Distilled water-1000ml*

*Conical Flask*

*Measuring Cylinder*

*Funnel*

*Glass rod*

*Muslin cloth*

*Beaker*

**PROCEDURE**:

*Potato dextrose agar medium is very commonly used for the isolation of fungus as well as for its maintenance.*

1. *We have to wash about 250 g of potato, peel off the skin, and slice them into small pieces.*
2. *We then cook the sliced potato in 500 ml. of water for 30 minutes in an open vessel or pressure cooker for 20 minutes.*
3. *We simultaneously mix 20 g agar with 500 ml. of water and boil in a cooker for 30 minutes.*
4. *Then we collect the potato extract by filtering through a muslin cloth or net filter.*
5. *We then add 20 g dextrose to the potato extract*
6. *We mix the molten agar thoroughly with the potato-agar mixture and make the volume to 1 liter with distilled water.*
7. *Then we check the pH of the medium using pH papers.*
8. *We then pour the medium into cleaned boiling tubes at 15 ml/tube and plug with non-absorbent cotton wool.*
9. *Then we arrange the tubes in a wire basket, cover them with a waste paper sheet, and tie them tightly with a cotton thread.*
10. *Then we sterilize them in an autoclave or a pressure cooker at 15 lbs pressure for 20 minutes.*
11. *We take out the sterilized tubes after releasing the steam and keep them in a slanting position to get agar slants.*
12. *After solidification of the media, the tubes are arranged in a wire basket and stored in a clean room for further use.*

**OBSERVATION**:

*Describe about the colonies appeared.*

**PRECAUTIONS**:

1. *Care must be taken while handling glassware.*
2. *All the beakers and test tubes are to the washed thoroughly and sterilized*
3. *Care must be taken while mixing the ingredients.*
4. *One must be careful while heating the mixture.*
5. *Proper timing must be maintained while preparing the mixture.*

**EXPERIMENT-2**

**AIM**: TO PREPARE A PURE CULTURE

**THEORY:** *A pure culture of an organism is a culture that is obtained from a single strain having no contamination of other strains of organisms. Pure cultures of fungi are obtained from a single spore of a strain having no contamination of other fungi.*

*Derivation of a pure culture is important for segregating a particular species of interest from a mixture of microorganisms in the environment. Pure culture facilitates various research work and extensive study of a particular species and its growth.*

**PROCEDURE**:

1. *We first scrap the spore mass from a fresh spore print or basidiocarp and suspend it in 100 ml of sterilized distilled water in flasks and we shake it well to obtain uniform spore suspension.*
2. *The spore suspension is serially diluted to obtain 15-20 spores per plate.*
3. *For serial dilution 9 test tubes each with 9ml distilled water are taken and are named as test tube 1, test tube 2, and so on. In the test tube 1 1ml of the spore suspension is added to make the total volume up to 10ml. Now 1ml of the solution is taken from test tube1 and added to test tube2 to make its total volume up to 10ml, this process is repeated with all the test tubes to obtain the desired dilutions.*
4. *Then we transfer the solutions of the test tubes in the solidified culture media and incubated at about 25°C for 10 to 14 days.*
5. *The Petri plate with the least number of colony is selected.*

**OBSERVATION:**

*Each of the petri plate incubated with different concentration of the spore suspension shows appearance of different number of colonies. The one with the highest dilution displayed a limited number of colonies indication the successful development of pure culture.*

**PRECAUTIONS**:

1. *Care must be taken while handling glassware.*
2. *Each suspensions must be labelled properly to avoid confusion.*
3. *All the suspensions must be dealt with separate apparatus to avoid contamination.*

**EXPERIMENT-3**

**AIM**: TO PREPARE SPAWN SEED FOR MUSHROOM CULTURE

**THEORY:** *Spawn is used as inoculum or seed for growing mushroom. Spawn has been defined as the vegetative mycelium or fungus growing on a convenient medium. Spawn comprises the mycelium of the mushroom and a supporting medium, which gives nutrition to the fungus during its growth. The spawn preparation involve mainly 3 steps – Pure Culture Preparation, Substrate Preparation & Inoculation*

**PROCEDURE**:

1. *PURE CULTURE*
2. *We first scrap the spore mass from a fresh spore print or basidiocarp and suspend it in 100 ml of sterilized distilled water in flasks and we shake it well to obtain uniform spore suspension.*
3. *The spore suspension is serially diluted to obtain 15-20 spores per plate.*
4. *For serial dilution 9 test tubes each with 9ml distilled water are taken and are named as test tube 1, test tube 2, and so on. In the test tube 1, 1ml of the spore suspension is added to make the total volume up to 10ml. Now 1ml of the solution is taken from test tube1 and added to test tube2 to make its total volume up to 10ml, this process is repeated with all the test tubes to obtain the desired dilutions.*
5. *Then we transfer the solutions of the test tubes in the solidified culture media and incubate at about 25°C for 10 to 14 days.*
6. *The Petri plate with the least number of colony is selected.*
7. *SUBSTRATE PREPARATION*
8. *We take wheat, jowar, rice or bajra grains and remove plant debris or soil particles.*
9. *Then we Soak it in water for 2 hours and boil the grains, till the grains are soft.*
10. *We then remove excess water and spread grains on cloth or wire mesh for 4-8 hours.*
11. *We mix gypsum (Calcium sulphate) 200g and calcium carbonate 50g per 10 kg of boiled grains.*
12. *We fill in any bottle with the prepared mother spawn.*
13. *We then autoclave or sterilize at 22 lb pressure for 2 hrs.*
14. *After cooling bottles or bags can be used for spawn.*
15. *INOCULATION*
16. *The mother spawn can be prepared by inoculating a small bit of tissue (5mm) from a culture tube.*
17. *Inoculated bottles are incubated at 25 or 30 C for 15 days.*

**OBSERVATION:**

*The bottles with the mother spawn changes its colour from light brown to white. This change indicates the healthy development of the required mycelium around the substrate. These spawns can later be directly used for the purpose of mushroom cultivaton.*

 **PRECAUTIONS**:

1. *Care must be taken while handling glassware.*
2. *Each suspensions must be labelled properly to avoid confusion.*
3. *All the suspensions must be dealt with separate apparatus to avoid contamination.*
4. *Care must be taken while preparing the substrate for the Spawn.*
5. *Packaging must be done carefully avoiding any kind of contamination.*

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