Spawn Production Technology

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Introduction

Spawn is the vegetative mycelium from a selected mushroom grown on a convenient medium like wheat, pearl millet, sorghum, etc for raising mushroom crop. It essentially involves preparation of pure culture of mushroom from tissues/ spores that is evaluated for yield, quality and other desirable traits is generally maintained on any agar medium, followed by culturing on sterilized grains and further multiplied on grains. The spawn thus comprises of selected mycelium of the mushroom and a supporting medium which provides nutrition to the fungus during its growth. From 1652 to 1894 A.D. spawn was gathered from the wild rather than made. Before the advent of grain spawn, different kinds of spawn used were Natural or Virgin spawn (from the pastures & meadows), Flake spawn (breaking of beds through which mushroom mycelium has run), Mill-track spawn (bricks dried and made from mixture of horse dung, cow dung and loam soil) and manure spawn (on sterilized horse manure or compost manure).

The first pure culture spawn was produced in France in 1894 on horse manure compost. Costanin and Matruchot (1894), the two Frenchmen from Pasteur Institute, France germinated spores, made culture and used it for making spawn after sterilizing horse manure (Manure Spawn).

In 1902 Ferguson - an American, published method of spore germination to make pure culture and the technique was no more a secret. In 1905 Duggar, an American, made mycelial culture from tissue of mushroom caps. By 1907 Lambert’s American spawn company was marketing seven pure strains of button mushroom. In 1926 a single cluster of white mushroom in a bed of brown mushroom was observed by Mr. Downing from which culture was made by Mr. L.F. Lambert.

The process of making spawn on grain was introduced by the Pennsylvania State University, which held two patents on it. These patents were assigned to the university by the inventor, Professor J.W. Sinden in 1932. Grain spawn had an advantage over manure spawn as it could be mixed easily and provided many inoculum points. The grain spawn was further perfected by Stoller in 1962. Today most of the traditional spawn laboratories world over is using wheat, rye and millet grains as substrate for spawn making and are following the standard techniques of mother spawn from pure culture mycelium grown on synthetic medium. The spawn production technology is divided into following steps (Fig.1).
A. Pure culture preparation

Pure culture of fleshy fungi/mushrooms can be prepared either by multi-spore culture or tissue culture. Multi-spore culture is made from spore print that can be obtained by having a fresh fruit body after alcohol sterilization above a petriplate/sterilized paper. Serially diluted loop full of spores are then transferred to sterile Potato-dextrose-agar (PDA) or Malt-extract-agar culture slants. These slants are then incubated at 25°C ± 2°C for 2 weeks to obtain pure culture. For tissue culture, the basidiocarp after alcohol sterilization is cut longitudinally into 2 halves and bits from collar region are transferred to pre sterilized PDA or MEA culture medium. The Petri-plates are incubated at 25°C ± 2°C in BOD incubator for one week. Mycelium from growing edges is carefully transferred to MEA/PDA slants and again
incubated for 2-3 weeks to obtain pure cultures. Basic materials and equipment required for pure culture is given below in Fig.2.

![Fig.2: Preparation of pure culture](image)

### B. Substrate preparation

Mushroom spawn can be prepared on any kind of cereal grains like wheat, jowar, bajra or rye and agricultural waste like corn cobs, wooden sticks, rice straw, saw dust and used tea leaves, etc. Spawn substrate i.e. cereal grains should be free from diseases and cereal grains should not be broken, old, and damaged by pests. Most of the cereal grains are good substrate for spawn production of white button mushroom. The grains are thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains are then soaked in sufficient water for 20-30 minutes and boiled in a container for 20-25 minutes. Normally for soaking and boiling 20 kg of wheat grain, 35 liters of water is required. Excess water from the boiled grains is removed by spreading on sieve made of fine wire mesh or muslin cloth. The grains are left as such for few hours on the sieve so that the water on surface gets evaporated. Now the grains are mixed with Gypsum (Calcium sulphate) and chalk powder (Calcium carbonate) so that the pH of the grains is around 7 to 7.8 and they do not form clumps. Different people have given different ratios for mixing Gypsum and Calcium carbonate. The best results have been obtained by using 200 g Gypsum and 50 g chalk powder for 10 kg grains (dry weight basis). First Gypsum and chalk powder are separately mixed and then they are thoroughly
mixed with the grains. This mixing should be done on a smooth surface after wearing gloves. Different steps of substrate preparation are given in the Fig. 3.

C. Mother spawn preparation

About 350 g prepared substrate is filled in glucose/milk bottles up to 2/3 volume and plugged with non-absorbent cotton. The plugs are covered with aluminum foil. These bottles are then autoclaved at 22 psi pressure (126°C) for 2 hr. These autoclaved bottles are left in the room for 24 hours for cooling and are kept on laminar flow under U.V. tube for 20-30 minutes before inoculation. A piece of mycelium (pure culture) grown in Petri plates is aseptically transferred to these bottles and inoculated bottles are incubated at 25°C. Inoculated bottles are gently shaken on 5th and 10th day. This spawn prepared using pure culture mycelium on agar medium in Petri plates as inoculant is referred as mother spawn. Fully colonized mother spawn bottles can be used for inoculating commercial spawn bags after two to three weeks. Inoculated bottles are incubated at 22-25°C.

Fig.3: Steps of substrate preparation

Fig.4: Mature master culture
D. **Commercial spawn preparation**

Commercial spawn can be prepared in polypropylene bags (heat resistant). Normally for half and one kg spawn the bags should be of 35 x 17.5cm and 40 x 20cm size, respectively. Polypropylene bags should have double sealing at the bottom and after filling the grains these are plugged with the help of a PP neck and non-absorbent cotton. The bags are then sterilized at 22 lb psi. pressure for 1.5 to 2 hours. Autoclaved bags are shaken well before inoculation so that the grains absorb the water droplets accumulated inside the bags. The sterilized bags are kept on the laminar flow under U.V. tube for 20-30 minutes. Ten to fifteen g of grains from master spawn bottle is inoculated per bottle under aseptic condition or one bottle of master spawn is sufficient for inoculating 25 to 30 commercial spawn bags.

Inoculated bags are again shaken so that the inoculum is well mixed with other grains. Then the bags are kept in incubation room for mycelium spread. During incubation the bags are regularly examined for mould infestation. Contaminated bags should be immediately removed to avoid build-up of contamination in the vicinity. Normally it takes 15-20 days for complete spread of mycelium on the grains. Fully colonized bags should be kept in cold room (+4°C) for future use. The contaminated bottles/bags/tubes etc. are autoclaved before emptying and discarding.

![Fig. 5: Mature spawn](image-url)
Table 1: Temperature requirement for storage of button mushroom spawn

<table>
<thead>
<tr>
<th></th>
<th><em>Agaricus bisporus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Days for complete colonization of mother spawn</td>
<td>20-21</td>
</tr>
<tr>
<td>Days for complete colonization in commercial spawn</td>
<td>12-14</td>
</tr>
<tr>
<td>Incubation temperature (°C) during colonization</td>
<td>25</td>
</tr>
<tr>
<td>Storage temperature (°C)</td>
<td>4</td>
</tr>
<tr>
<td>Shelf life of spawn</td>
<td>Two months</td>
</tr>
</tbody>
</table>

E. Spawn storage and its transport

Freshly prepared spawn should be used because the mycelium is in the state of active growth. The spawn bag after completion of growth can be stored for 2-3 months.

Earlier spawn was prepared in milk or glucose bottles, which was difficult to transport from one place to another. Heat resistant polypropylene bags have revolutionized the spawn industry. High-tech multinational spawn labs now use polypropylene bags with microfilm windows for aeration. Though polypropylene translucent bottles of 5-10 litres capacity are also used in Europe and USA for spawn production, but it has not been introduced in India due to high cost of the material. Ready spawn in polypropylene bags should be packed in well ventilated cardboard cartons and maintained at 2-4°C in storage. The spawn is transported from one place to another in refrigerated vans or during night when temperature does not rise above 32°C.

Spawn production cycle

1. Preparation of Mother Spawn

   - Step-1: Selected healthy and cleaned cereal grains
   - Step-2: Boil Grains in water (15-20 min.)
   - Step-3: Remove excess water on sieve
   - Step-4: Dry grains in shade (4 hrs.)
   - Step-5: Mix CaCO$_3$ (0.5%) and CaSO$_4$ (2%)
   - Step-6: Fill 300g grains in glucose/milk bottle
Step-7  Plug and autoclave at 22 psi. for 2 hr
Step-8  Inoculate growing mycelium of desired strain using laminar flow
Step-9  Incubate in BOD at 23±2°C for 20-25 days
Step-10 Master spawn is ready

2. **Preparation of Commercial Spawn**

Step-1  Use polypropylene bags instead of bottle
Step-2  Upto autoclaving (Step 1 to 7) is same as of mother spawn
Step-8  Inoculate 15-20 grains from mother spawn to polypropylene bags containing grains.
Step-9  Shake bags after 7-8 days
Step-10 Incubate at 23±2°C in incubation room
Step-11 Commercial spawn is ready in 2-3 weeks

Problems faced during pure culture/spawn preparation and their solution are given in Table 2.

**Table 2: Problems, causes and solution faced during pure culture/spawn preparation**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar medium very soft or hardly solidifies</td>
<td>Quantity of agar insufficient i.e. too low</td>
<td>Use proper quantity of agar in medium</td>
</tr>
<tr>
<td>Agar surface in the plates not smooth or lumpy</td>
<td>Agar medium partially solid when poured</td>
<td>Pour agar medium when it is still hot</td>
</tr>
<tr>
<td>Contaminants appear after 2-3 days on the surface of the medium after sterilization and before inoculation</td>
<td>Medium not sufficiently sterilized Medium not aseptically poured</td>
<td>Sterilization should be carried for the recommended period and temperature/pressure Medium should be poured aseptically</td>
</tr>
<tr>
<td>Transferred</td>
<td>Non viable</td>
<td>Use viable culture</td>
</tr>
<tr>
<td>Issue</td>
<td>Possible Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mycelial bit/ tissue resume no growth</td>
<td>Inoculum/culture</td>
<td>Actively growing culture</td>
</tr>
<tr>
<td></td>
<td>Wrong type of medium</td>
<td>Use correct medium</td>
</tr>
<tr>
<td></td>
<td>Incorrect formulation or pH</td>
<td>Properly check the formulation and pH of the medium</td>
</tr>
<tr>
<td></td>
<td>Needle or scalpel used to transfer the culture bit too hot</td>
<td>Cool the flamed needle before picking the inoculum</td>
</tr>
<tr>
<td>Contamination develops on the plugs after 2-3 days</td>
<td>Culture used already contaminated</td>
<td>Use fresh/ disease free cultures</td>
</tr>
<tr>
<td></td>
<td>Filters of the laminar flow damaged</td>
<td>Filters should be checked or replaced as per recommendation</td>
</tr>
<tr>
<td></td>
<td>Incubation room too much loaded with air born inoculum</td>
<td>Sterilize incubation rooms from time to time</td>
</tr>
<tr>
<td>Resulting mycelial growth slow and fluffy</td>
<td>Strain degenerated</td>
<td>Obtain another culture or retrieve stock culture</td>
</tr>
<tr>
<td>Grains contaminated after sterilization and before inoculation</td>
<td>Highly infected seeds</td>
<td>Use fresh and clean seed</td>
</tr>
<tr>
<td></td>
<td>Grains not fully sterilized</td>
<td>Prolong sterilization period</td>
</tr>
<tr>
<td>Mycelial growth very thin and hardly penetrates the grains</td>
<td>Grains too dry</td>
<td>Boil the grains sufficiently</td>
</tr>
<tr>
<td></td>
<td></td>
<td>And adjust proper moisture levels</td>
</tr>
<tr>
<td>Mycelial growth does not continue to the bottom</td>
<td>Excessive grain moisture</td>
<td>Adjust proper moisture level</td>
</tr>
<tr>
<td>Mycelia growth very thin, hardly penetrate the grains</td>
<td>Grain too dry</td>
<td>Adjust proper moisture level</td>
</tr>
<tr>
<td>Mycelia do not grow through substrate or patchy growth</td>
<td>Grains contaminated with bacteria due to improper sterilization Less vigorous strain</td>
<td>Use recommended sterilization time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use vigorous strain</td>
</tr>
<tr>
<td>Contamination appears on the surface of the grain or on the mycelia plug which was</td>
<td>Contamination occurred during inoculation Mycelia plug or culture contaminated</td>
<td>Inoculation should be performed in a more aseptic way and observe</td>
</tr>
<tr>
<td>inoculated</td>
<td>complete cleanliness</td>
<td></td>
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<tr>
<td>--------------------------------</td>
<td>----------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Mycelia growing very slowly</td>
<td>Unsuitable substrate</td>
<td></td>
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<tr>
<td></td>
<td>Incubation temperature not suitable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Culture have degenerated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use recommended substrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Check the temperature requirement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use vigorous culture</td>
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</tbody>
</table>