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Evaluating the Economic Viability of *Pleurotus florida* and *P. eryngii* Mushroom spawn culture and production in Agribusiness

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Abstract:

Mushrooms are one of the most abundant fungi and have been used as a food source for years. A variety of them are cultivated across the country and have become an additional source of income for farmers. Mushroom cultivation and spawn production have the potential to ensure food security and provide employment opportunities, especially for rural and small-scale farmers. However, due to the inadequacy of knowledge and skills for mushroom farming in remote areas and the inaccessibility of quality spawn at affordable rates, large-scale production of this potential cash crop is hindered. A Project to prepare Spawn Culture was launched with the aim of inculcating the idea of agripreneurship among students. In this paper, we have discussed the materials and methods used in preparing mushroom spawn from *Pleurotus florida* and *Pleurotus eryngii*. In the following sections, we have also discussed the estimated cost of producing the commercially significant "1 Kg spawn bag". The present paper provides the procedure followed for Spawn Cultivation to inculcate the idea of agri-entrepreneurship among students and create a market interface so that the students can then reach out to encourage scaling up mushroom cultivation among small-scale farmers.

Article Highlights

- Our study aims to provide cheap and best mushroom spawns to the farmers or to the students engaging themselves in the same fields.
- We opted protocols and standardized that to get maximum yield at minimum costs.
- The research will show the complete details to the entrepreneurs to produce more spawns so that the farmer can get for more crops.

Introduction:

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Mushrooms have been cultivated and consumed around the globe for centuries and are an essential part of the cuisine of several countries. About 1.5 million species of mushrooms exist; however, only 70,000 species have been described [1, 2]. These organisms belonging to the kingdom Fungi and phylum Basidiomycota are not just excellent sources of dietary fiber, protein, essential vitamins, minerals, and bioactive compounds; several species are also known for their therapeutic and medicinal properties.

In present times, mushroom cultivation has emerged as a promising and profitable agribusiness as it is a landless crop and doesn't require traditional large farms, so it also contributes to the decline of many organic waste piles [5]. The practice of mushroom cultivation involves a series of steps that require maintenance and hygiene, and one of the most important steps is the seed culture or spawn culture of mushrooms [47].

Spawn is the vegetative mycelium impregnated onto a suitable growth substrate, like grains [4, 7]. Spawn is widely used as a seed for obtaining fruiting bodies of mushrooms by following suitable cultivation methods [13, 18]. The method of spawning was introduced by Pennsylvania State University in 1932. The method was further perfected by Stoller in 1962 [16]. Being the seed of the mushroom, the quality of the spawn plays an important role in the final product of cultivation [13]. Several seeds can be used to prepare spawn, like rice, wheat, soybean, millet, rye, and sorghum. Agricultural waste can be used as well [13]. However, most labs prefer to use wheat, rye, and millet due to their low cost and easy availability. Spawn is prepared from pure fungal cultures of desired mushroom tissue or spores on synthetic nutrient media like PDA, MEA, etc. [16]. As preparing cultures requires an aseptic environment, the right equipment and technology are needed in order to maintain the required conditions and avoid contamination [13]. Cultures can be prepared either in petri dishes or in test tubes as slants [14]. The first step in the preparation of a culture is the selection of the right synthetic nutrient media, like PDA. The preparation of media is done in a conical flask by correctly weighing the required amount of PDA and distilled water. The media is autoclaved, and then within a laminar flow hood, the media is poured into Petri plates or test tubes. Once the plates are settled, they are inoculated with the desired sample (either tissue, spore, or inoculum from the mother culture). Paul Stamets and J.S. Chilton, in their 1983 book, The Mushroom Cultivator: A Practical Guide to Growing Mushrooms at Home [10], recommend two ways of preparing the cultures of mushrooms.

- The first method involves using the spores of mushrooms. As these are viable even after months of decomposing fruiting bodies, one can effectively prepare cultures using them. Several strains can be obtained using spores as starter cultures.
- The second method is achieved by inoculating the growth medium with tissue taken from the freshly picked fruiting body with the desired traits. Clones are prepared, and there is negligible scope for variation. [10]

Tissue culture is often used in the propagation of edible and medicinal mushrooms. In this method, a young fruiting body is picked from self-cultivated mushrooms or purchased from the market. It is rigorously washed with distilled water and then surface sterilized using 70% ethanol. The fruiting body is longitudinally cut into two halves with a sterilized scalpel. As the fruiting body is a compressed mass of hyphae, tissue can be extracted from either the upper part of the stipe, from the cap, or from the junction of the gill plates and the cap. The tissue is immediately put on the nutrient media with minimum exposure to the surrounding environment. The plate is also immediately closed. Once sealed with

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parafilm, the plates containing the tissue are incubated in the conditions required for growth. The growth can be observed within 3-5 days of inoculation [7, 9, 10, 11, 12, 14].

Subcultures can be prepared from this mother culture to obtain pure cultures for utilization in spawn production [8].

Once the plates are fully covered and pure, they can be used as an inoculum for spawn. For preparing spawn, grains are first selected, soaked overnight, and then simmered. The grains thus become soft but do not burst due to partial boiling. Once the excess water is sieved out, the grains are mixed well with CaCO3 and autoclaved. Bags are allowed to cool and, in a sterilized laminar-flow hood, inoculated with the culture. The bags are then incubated at the desired temperature for 2–3 weeks to achieve full colonization of the grains. This bag can be used to inoculate other spawn bags, and this is called the master spawn or the mother spawn. The fully colonized bags can be stored at refrigerator temperature, i.e., 4°C. [13,16] According to data, the spawn demand in India as of 2016–2017 was 8000–10,000 tons per year. So far, 90% of the supply to the mushroom cultivators is through private companies, while only 10% is through public sector suppliers. [17]

We selected the *P. florida* and *P. eryngii* species as they are highly edible and nutritious, rank second among the commercially cultivated mushrooms in the world, and are demonstrated to possess antioxidant, anti-inflammatory, and antitumor activities. Oyster mushrooms possess bioactive compounds with hypocholesterolemia activities, such as polysaccharides, mevinolin, and other statins. [21, 22, 23]

Materials and Methods:

Glassware: Autoclavable Petri plates, beakers, conical flasks, and pipettes of 10 ml Chemicals: PDA

(potato dextrose agar), ethanol, $CaCO_3$, distilled water

Machinery: BOD incubator, laminar flow hood, oven, refrigerator, hot plate, autoclave

Miscellaneous: Muslin cloth, polypropylene bags, foil paper, cotton, sanitizer, tissue paper, spirit lamps, Matchbox, wheat grains, scalpel, inoculating loops, forceps, needle, scissors, markers, parafilm tape, adhesive tape

PRE-REQUIREMENTS AND PREPARATION

The lab was sanitized and fumigated one day prior to the culturing, or transfer of culture into bags. All necessary precautions were taken to maintain aseptic conditions. The fruiting bodies of King oyster (*Pleurotus eryngii*) mushrooms were purchased from the market. The fruiting bodies were washed first in running tap water, then in distilled water. The surface was sterilized using ethanol. The nutrient medium required for the preparation of mushroom culture is PDA, or potato dextrose agar media [24]. With the help of commercial PDA media,the petri plates for the culture were prepared. In the Laminar flow hood, the PDA was poured into Petri plates and allowed to set.

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MOTHER CULTURE PREPARATION:

Once set, the fruiting body, after being washed and dipped in alcohol (shown in figure 1), was cut longitudinally (figure 2) using an autoclaved scalpel into two halves, and the exposed tissue of the stalk was sliced into small sections. With autoclaved forceps, the inner tissues were carefully picked, and the PDA plates were inoculated. The inoculated plates were sealed using parafilm and normal transparent adhesive tape and appropriately labeled. These were then incubated at 25°C±2°C in the BOD incubator for one week. The culture was obtained within 10–13 days. This Mother culture was then used to prepare subsequent subcultures and grain spawn.

Figure 1 represents the fruiting body of *Pleurotus sp.*

Figure 2 represents the longitudinal section of the mushroom.

Mycelium culture from prepared Spawn:

The spawn of *Pleurotus florida* was purchased from the market. Mother cultures (Figure 9) were prepared by using seeds impregnated with Mycelium as inoculum. Aseptic conditions were strictly maintained during the entire protocol. The cultures were incubated at 25°C±2°C, temperature.

SUBCULTURE PREPARATION:

The fungal Mycelium covered the entire plate within 4-5 days of inoculation (Figure 3). Subcultures and spawn were prepared thereafter (Figure 4, 10).

Fig. 3 Shows the mycelium growth on the PDA media.

Fig. 4 shows the subculture grown from the mother plate

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COMMERCIAL SPAWN BAG PREPARATION:

Wheat grains were used as a nutritional substrate medium for the growth of mycelium [25]. The grains were soaked overnight for about 12–20 hours and then simmered at 65–70 degrees Celsius for 20–25 minutes. They were wrapped in a muslin cloth and autoclaved for 1.5 hours at 15 psi pressure and 210 °C. The cooked grains were then mixed with 10 g/kg of calcium carbonate ($CaCO_3$). The dried grains were filled into bags (Figure 7) and then kept in a laminar flow hood. The bags were inoculated one by one with the culture (Figure 5). One entire plate was used in preparing a 1-kg bag of spawn (Figure 6). The bags were then sealed using a cotton plug, and the top was covered with foil. The inoculated bags were then kept in a BOD incubator maintained at a temperature of 25 degrees Celsius (Figure 8). The initiation of mycelial growth on the grains was observed in the first 5 days (Figure 11), and the entire colonization took about 15 days (about 2 weeks).

Fig. 5 Shows a mycelium covered plate

Fig. 6 Shows inoculation of grains with mushroom mycelium

Fig 7 Shows grains weighed in polypropylene bags

Fig 8 Shows inoculated bags kept in BOD incubator

Results:

Table-1 comprises the total cost for production of 1Kg Spawn

Material used	Amount of material used	Cost of per unit	Total cost (Amount of material*cost per unit)
Ethanol	10ml	0.472₹ per ml	4.72₹
PDA	1.2gm	10.915₹per gm	13.098₹
Wheat	1kg	39.61₹ per kg	39.61₹
Parafilm	10cm	1.956 ₹ per cm	19.56₹
Polypropylene	1	10₹ per piece	10₹

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Fig. 9 Mother culture of Oyster sp.

Fig. 10 Subculture for Oyster sp.

Subcultures (T1 and T2 generations) for *oyster sp*.

PDA used in making 1 petri plate = 1.2 gm

Cost for 1 petri plate: 1.2 gm * 10.915 = 13.09

Fig. 11 1Kg spawn bags

The total cost of production of 1 kg of spawn is 86.988 INR, of which approximately 87 INR are used for producing 1 kg of spawn.

LIMITATIONS IN CURRENT MUSHROOM SPAWN INDUSTRY

Mushroom cultivation in the past decade has become a vital part of the integrated farming system (IFS) and practices across the globe, but Indian farmers are still not accustomed to the not-so-usual cultivating crop and its different method of production.

Indian Farmers are quite well adapted to the basic field work and farming techniques required to grow a crop on their farmland, but they lack the knowledge and skill of the technicalities and science involved to produce viable and efficient spawn and mushrooms.

Spawn production requires a lot of laboratory (Wet lab) work, which involves new and better scientific equipment and technologies. Not only for the continuation of spawn production but also to produce better-

quality strains (in terms of yield, nutritional value, medicinal value, disease and pest resistance, etc.), efficient labs with advanced technology are required.

But due to the lack of knowledge of the actual potential of the mushroom industry, this industry faces high cases of non-availability of funds from the regional to the central level. This becomes one of the reasons why the agribusiness industry has not been fully established in the agricultural sector and is lacking national as well as global connectivity in terms of agrientrepreneurship.

This industry requires a good number of skilled workers with resilience and expertise. As this is not in the mainstream agriculture sector, it faces a scarcity of affordable skilled labor. The inability to get a cheap labor force is one of the major backlogs in this sector.

Due to the unskilled working force, post-harvest management and marketing become extremely difficult as they do not possess the right amount of knowledge and experience for the management and maintenance of this crop.

The transportation of spawn and the mushrooms produced is not cheap. During pre- and post-harvest management, since storing the mushroom crop has a short shelf-life and requires time-critical transportation, the overall cost of the produce becomes higher, making it less affordable for the average-earning section of society to include it in their daily diet.

Optimal growth of mushrooms requires low temperatures and very high relative humidity. This makes their production a task that requires regular monitoring and maintenance. This itself becomes hugely labor-intensive, so to decrease such high monitoring, identifying suitable agro-climatic conditions is as vital and necessary as any other step in the production of the crop.

India, being a culturally rich country, inherits a lot of ethical beliefs and laws. Some of these orthodox thoughts also proved to be hurdles in accepting mushrooms as a food and as a crop.

Mushrooms, occurring naturally in the wild and being fungi, have often been presumed to be poisonous foods. Additionally, due to its unconventional cultivation methodology, it has been stigmatized as a food that is not vegetarian, particularly in India, which has the world's largest vegetarian population, thereby hindering its widespread adoption as a staple within Indian families. Therefore, the industry is working hard in a righteous way towards product promotion in the country. [43, 44]

CONCLUSION:

Mushroom cultivation and production have massive potential in our country because of the diversification in agriculture, topography, climate, and vegetation. Mushroom cultivation can be the best IFS model too, but the issue arises due to the non-availability of funds, the lack of harvest management, and the lack of proper knowledge among the farmers regarding mushroom farming. These disadvantages hinder the availability of raw materials and a cheap labor force. Although there have been many schemes like ATMA (Agricultural Technology Management Agency) of the state department of agriculture or NABARD (National Bank For Agriculture And Rural Development) and ICAR, research institutes have organized training and workshops to increase knowledge among farmers regarding mushroom cultivation to improve their living standards. The availability of quality spawn at cheaper rates, processing facilities, and marketing aspects are the major fields to be worked upon.

Conflict of Interest statement: On behalf of all authors, the corresponding author states that there is no conflict of interest.

DATA AVAILABILITY STATEMENT:

All the data given by us is complete in this manuscript and it can be shared with public domain. We have no objection in sharing our data.

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