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PREPARATION OF *PLEUROTUS* SPAWN ON DIFFERENT GRAIN SUBSTRATES

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Abstract: The cultivation of *Pleurotus* is gaining momentum due to its nutritional value and economic potential. The preparation of mushroom spawn on grain media involves inoculating them with pure cultures of certain mushroom species under sterile conditions, yet contamination of the spawn is a significant barrier to the growth and spread of mushroom farming in underdeveloped nations. In this study, the spawn growth of *Pleurotus* species on various grain substrates including maize, wheat, sorghum and bajra was explored. The results revealed significant differences in spawn growth duration among the substrates. Maize substrate exhibited the longest duration for spawn growth, requiring 19 days, while wheat, sorghum and bajra substrates displayed shorter duration of 15, 10 and 11 days respectively. Furthermore, each substrate demonstrated distinct characteristics influencing spawn growth, highlighting the importance of substrate selection in *Pleurotus* species cultivation. These finding provide valuable insights for optimizing spawn production process and enhancing the efficiency of *Pleurotus* species cultivation practices.

Keywords: Bajra, Maize, Oyster mushroom, Resource utilization, Sorghum, Spawn, Wheat grain.

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INTRODUCTION

Fungi are the diverse heterotrophic group of organisms and largest livings community on Earth after the insects (Verma and Prakash, 2020; Singh and Singh, 2022). The most common and known macrofungal group is mushroom. The mushroom mycoflora are characterized by their distinct fruiting bodies. These mushrooms are belonging to the class of Basidiomycetes and Ascomycetes (Singh and Singh, 2023a; 2023b). They have a variety of ecological habitat in terrestrial ecosystems such as saprophytic, parasitic and symbiotic or mycorrhizal association (Singh and Singh, 2023c).

Mushrooms are referring as an important nutritional and known to be as vegetarian meat due to enrich quality nutritional and mineral

constituents as well as their unique test and flavour with immunity booster property (Wasser, 2002; Singh and Singh, 2023d). Among all the qualities of mushrooms, become a reason of cultivation to fulfill the requirements and needs. The mushroom mycelium plays alternative applications in environmental sustainability (Yang *et al.,* 2021; Patel and Sharma, 2023).

Spores are used by mushrooms to reproduce, and in the right circumstances, they develop into hyphae, or mycelium. Hyphae are typically filamentous and tiny, invisible to the unaided eye. By fusing of hyphae, or plasmogamy, germinated hyphae produce primary mycelium and subsequently secondary mycelium (Singh and Singh, 2023c). They grow into colonies as they take up nutrients from the substrate. The temperature, humidity, and other environmental factors influence the mycelium's development of fruiting bodies, or sporocarp. A sporocarp, a young fruiting body, is referred to as a pin (bud) and develops into a fully formed body with identifiable sections (structure). The gills in the lower portion of the pileus (cap) contain spores, which are in charge of the creation of future generations (Jarial *et al.*, 2020; Singh and Singh, 2022).

The mushroom is achlorophyllous creatures and fleshy macrofungi can grow in different habitats (Bijla and Sharma, 2023). These are typically grown in fields, forests on manure, water channels, and hilly regions during their favorable environment (Tripathi *et al.*, 2017). The mushroom mycelium grown on a specific substrate of grains and utilized as a growing medium for mushrooms is known as mushroom spawn. Grain spawn is a medium that has been infected with mycelium from a pure culture of the selected strain of mushrooms, and spawn generation is a fermentation process in which the mushroom mycelium multiplies by passing through a dense organic matrix (Hoa *et al.*, 2015).

There are a number of compounds that are known to enhance fruiting, and the production of carpophores has been connected to spawn grains like wheat, millet, and maize. They are made out of rice bran, cassava peels, and soybean powder (Jarial *et al.*, 2020). Although grains spawn is currently made by inoculating sterilized grains with complete sterile precaution, small and medium spawn-laboratories still struggle with contamination problems, or the unwanted intrusion of harmful and competing organisms in the spawn, rendering them useless and dangerous for mushroom production due to their potential to infect the bed and the crop as a whole (Hoa *et al.*, 2015). It grows independent of sunlight, relatively fast growing does not require fertile soil since grown on composted or non-composted agro-wastes as wheat and paddy straw, banana leaves, sugarcane bagasse and leaves, wheat barn, rice husk, sawdust etc. and their culture can be concentrated within a relatively small space. No much research work on mushroom has yet been done to standardize the production practices of mushroom in most of the country while countries like India and Pakistan have done much works in this regard.

MATERIALS AND METHODS

Source of Materials

The mushrooms used in this study were taken from the Department of Botany, C.M.P. Degree College, Prayagraj, India. The pure culture of the oyster mushroom (*Pleurotus* spp.) was developed in the Microbiology and Plant Pathology Laboratory. The grains used such as wheat, maize, bajra and sorghum were purchased from market.

Spawn Production Technology

A selected mushroom's spawn is the vegetative mycelium that has grown on an accessible substrate, such as wheat, pearl millet, sorghum, etc. for the production of mushroom crops. It simply requires starting with a pure culture of mushrooms from tissues or spores, maintaining it on any form of agar medium, cultivating the mushrooms on sterile grains, and then growing them further on grains. The spawn is thus made up of the mycelium of the mushroom and a medium that provides a support for the growth of the fungus.

Before grain spawn gained popularity, many different types of spawn were utilized, including natural or virgin spawn (from pastures and meadows), flake spawn (from cracked beds

through which mushroom mycelium has run), and mill track spawn (bricks). In France in 1894, the first pure culture spawn was produced on compost manufactured from horse manure. Most traditional spawn facilities now create mother spawn using pure culture mycelium that has been produced on synthetic medium using wheat, rye, and millet grains as the substrate. There have been a few minor adjustments to mechanization and container over time (Hoa *et al.*, 2015; Jarial *et al.*, 2020).

Substrate Preparation

Any type of cereal grain, including wheat, jowar, bajra, or rye, as well as agricultural waste, including maize cobs, wooden sticks, rice straw, sawdust, spent tea leaves, etc., can be used to make mushroom spawn. The spawn substrate, cereal grains, must be free of disease and must not be broken, old, or insect-damaged. However, wood-rotting fungi like shiitake (*Lentinula edodes*) and black ear mushroom (*Auricularia* spp.) perform better on sawdust-based substrates (Singh and Singh, 2023d). White button mushroom (*Agaricus bisporus* and *A. bitorquis*), oyster mushroom (*Pleurotus* spp.), and paddy straw mushroom (*Volvariella volvacea*) can all produce their spawn on most cereal grains.

The grains are thoroughly washed in sufficient water three to four times to get rid of undesired grass, weed, and other seed as well as soil and straw pieces. A pot of washed grains is cooked for 20 to 25 minutes after the seeds have been soaked overnight in enough water (Aditya *et al.*, 2022). Extra water from the grains can be removed by spreading the boiling grains out on a sieve made of fine wire mesh or muslin fabric. The grains are left on the sieve in this configuration for a few hours to allow the surface water to evaporate. Now, in order to maintain the grains' pH between 7.8 and 7.9 and avoid clumping, gypsum (calcium sulphate) and chalk powder (calcium carbonate) are added to the mixture (Pandey *et al.*, 2017).

There are numerous ratios that have been suggested for the combination of gypsum and calcium carbonate. The best results were obtained with 20g of gypsum and 5g of chalk powder for 1 kg. of grains (dry weight basis). Before being properly blended with the grains, gypsum and chalk powder are first combined separately. This mixing was done on a smooth surface while wearing gloves.

Spawn Preparation

Spawning can be prepared in bags made of heat-resistant polypropylene. Typically, the bags for half and one kilogram of spawn should be 35 x 17.5 cm and 40 x 20 cm, respectively. Polypropylene bags should have a double-sealed bottom, and after the grains are inside, the bags are sealed using a PP neck and non-absorbent cotton (Akter *et al.*, 2019). The bags are then sterilized at 22 lb psi pressure for 1.5 to 2 hours. Autoclaved bags are aggressively shaken before inoculation to make sure the grains are soaking up the water droplets that have formed inside the bags (Pandey *et al.*, 2017). The sterilized bags are left on the ultraviolet-lit laminar flow system for 20 to 30 minutes. Per bottle, ten to fifteen grammes of grains from the master spawn are inoculated under (Narh *et al.*, 2011).

The inoculated bags are shaken once more to make sure the inoculum is dispersed equally throughout the grains. After that, the bags are kept in the incubator area so that mycelium can grow. While incubation, the bags are routinely inspected for mold growth. Contaminated bags need to be taken out and disposed away right away in order to stop the spread of contamination in the region. Normally, it takes 15 to 20 days for the mycelium to completely cover the grains. Fully colonized bags should be kept in a cool location for subsequent use. The spawn of the button mushroom, (*Pleurotus* spp.) can be stored at this temperature. However, spawn from *Volvariella*, *Ganoderma*, and *Calocybe* cannot be kept in culture or stored at temperatures lower than 150°C (Baghel *et al.*, 2020).

Different steps involved in Spawn preparation:

Step 1: Clean wholesome cereal grains.
Step 2: Cook grains in water for 15 to 20 minutes.
Step 3: Squeeze extra water through a strainer.
Step 4: Dry grains in the shade for four hours.
Step 5: Combine 0.5g CaCO3 and 20g CaSO4.
Step 6: Use polypropylene bags.

Step 7: Inoculate 15-20 grains from mother spawn to polypropylene bags containing grains.
Step 8: Shake bags after 7-8 days.
Step 9: Incubate at 23+20 ^{0}C in incubation room.
Step 10: Commercial spawn is ready within 20-22 days

RESULTS AND DISCUSSION

The effects of various substrate grains on oyster mushroom (*Pleurotus* spp.) spawn growth were investigated and spawn developed very differently on various grains. Of the grains tested, sorghum (10 days) showed the shortest time needed for oyster mushroom (*Pleurotus* spp.) spawn growth. Bajra and Maize trailed and reported by 11 days and by 19 days respectively. It took 15 days for the spawn on wheat grain to grow (Table-1). The wheat grains were entirely covered in a dense, white mycelial growth that was tightly packed together. On the other hand, insufficient grain covering and low mycelial development were seen in maize oyster mushrooms (*Pleurotus* spp.).

Table 1: Effect of different grains substrate on spawn development of Oyster mushroom.

S. No.	Treatment	Spawn development (in days)	Growth Characteristic
1.	Maize	19	All grains have white colony mycelium growth, but the grains are not tightly packed together.
2.	Wheat	15	All grains were covered in a white cottony thread like mycelium, but they were not tightly held together.
3.	Sorghum	10	Tiny white mycelial development All grains were tightly bound together and mycelium-covered completely.
4.	Bajra	11	White compact mycelial growth all grains were completely covered by mycelium and tightly held with each other.

CONCLUSION

The study on the preparation of spawn on different grain substrates revealed significant variations in oyster mushroom (*Pleurotus* spp.) spawn growth across different grains. Sorghum emerged as the most conducive substrate, with the shortest growth period of 10 days. Bajra followed closely at 11 days, while maize exhibited a longer growth period of 19 days. Wheat grain, despite taking 15 days, demonstrated robust mycelial growth, forming a dense white coating on the grains. In contrast, maize exhibited poor mycelial growth and incomplete coverage. These findings underscore the importance of substrate selection in optimizing spawn growth for oyster mushroom cultivation, with sorghum and wheat grains presenting as favorable options.

Further research could explore the underlying mechanisms driving these substrate-specific responses to enhance mushroom cultivation efficiency. Sorghum grain was the substrate where oyster mushroom mycelium grew the fastest. Sorghum grains made the finest substrate for spawn preparation. These results underscore the importance of selecting the appropriate grain substrate based on factors such as availability, cost-effectiveness, and desired spawn development time. Researchers and practitioners in mushroom cultivation can utilize this information to streamline their production processes and optimize resource utilization.

REFERENCES

1. **Aditya, Jarial R.S. and Jarial K.** (2022). Evaluation of Different Grain Substrates for Spawn Production and Yield Performance of Blue Oyster Mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead]. *Journal of Eco-Friendly Agriculture*. 17(2): 393-398. https://doi.org/10.5958/2582-2683.2022.00076.4.

2. **Akter F., Ahmed K.U. and Miah N.** (2019). Effect of Different Spawn Seed on Growth and Varieties of the Oyster Mushrooms (*Pleurotus* spp.). *Research in Agriculture, Livestock and Fisheries*. 6(2): 181-192.

3. **Baghel D., Shukla C.S., Singh H.K., Banvasi P. and Kerketta V.** (2022). Effect of Cereal Grains on Spawn Development and Different Substrates on Growth and Yield of *Hypsizygus ulmarius*. *International Journal of Current Microbiology and Applied Sciences*. 9(5): 2175-2181. https://doi.org/10.20546/ijcmas.2020.905.248.

4. **Bijla S. and Sharma V.P.** (2023). Status of mushroom production: Global and national scenario. (2023). *Mushroom Research*. 32(2):91-98.https://doi.org/10.36036/MR.32.2.2023.146647.

5. **Hoa H.T., Wang C.L. and Wang C.H.** (2015). The effects of different substrates on the growth, yield and nutritional composition of two Oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*. 43(4): 423-434. http://dx.doi.org/10.5941/MYCO.2015.43.4.423.

6. **Jarial R.S., Sharma A.K., Jarial K. and Jandaik S.** (2020). Evaluation of Different Grain Substrates for the Spawn Production of *Pleurotus cornucopiae*. *International Journal of Current Microbiology and Applied Sciences*. 9(6): 1689-1700. https://doi.org/10.20546/ijcmas.2020.906.209.

7. **Narh D.L., Obodai M., Baka D. and Dzomeku M.** (2011). The efficacy of sorghum and millet grains in spawn production and carpophore formation of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer. *International Food Research Journal*. 18(3):1092-1097.

8. **Pandey N., Kerketta A., Sahu B. and Singh H.K.** (2017). Screening of Suitable Grains Substrates for Spawn Development, Growth and Yield of *Pleurotus flabellatu*. *Research Journal of Agricultural Sciences*. 8(2): 536-541.

9. **Patel J.Y. and Sharma J.** (2023). Alternative applications of mushroom mycelium for environmental sustainability: opportunities, challenges and future perspective. *Mushroom Research*. 32 (2): 99-113. https://doi.org/10.36036/MR.32.2.2023.141696.

10. **Singh B. and Singh V.K.** (2022). Macrofungal (mushroom) diversity of Uttar Pradesh, India. *International Research Journal of Modernization in Engineering Technology and Science*. 4(8): 208-217.

11. **Singh B. and Singh V.K.** (2023a). Molecular Characterization and Biochemical Analysis of *Schizophyllum commune* from Ayodhya, India. *Universe International Journal of Interdisciplinary Research*. 3(11): 11-19. https://www.doi-ds.org/doilink/05.2023-43444842/UIJIR.

12. **Singh B. and Singh V.K.** (2023b). Characterization and nutritional analysis of cultivable wild edible Mushrooms collected from District Ayodhya (U.P.), India. *International Journal of Biological Innovations*. 5(1): 170-175. https://doi.org/10.46505/IJBI.2023.5115.

13. **Singh B. and Singh V.K.** (2023c). Nutritional analysis of some wild collected macrofungi from Ayodhya, Uttar Pradesh, India. *International Journal of Current Research in Biosciences and Plant Biology*. 10(6): 1-6. https://doi.org/10.20546/ijcrbp.2023.1006.001.

14. **Singh B. and Singh V.K.** (2023d). Diversity of Wood-Inhabiting Macrofungi from District Ayodhya, Uttar Pradesh, India. *Kavak* 59(3): 1-11. https://doi.org/10.36460/Kavaka/59/3/2023/51-61.

15. **Tripathi N.N., Singh P. and Vishwakarma P.** (2017). Biodiversity of macrofungi with special reference to edible forms: a review. *Journal of Indian Botanical Society*. 96(3): 144-187.

16. **Verma A.K. and Prakash S.** (2020). Status of Animal Phyla in different Kingdom Systems of Biological Classification. *International Journal of Biological Innovations*. 2 (2): 149-154. https://doi.org/10.46505/IJBI.2020.2211.

17. **Wasser S.P.** (2002). Medicinal mushrooms as source of antitumor and immune-modulating polysaccharides. *Appl. Microbiol. Biotechnol*. 60(3): 258-274. 10.1007/s00253-002-1076-7.

18. **Yang L., Park D. and Qin Z.** (2021). Material function of mycelium-based bio-composite: A review. *Frontiers in Materials*. 8:737377. 10.3389/fmats.2021.737377.