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Mother Spawn Preparation in Oyster Mushroom Cultivation

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Oyster mushrooms (*Pleurotus* spp.) rank among the most extensively cultivated edible fungi worldwide, a status attributable to their rapid growth cycle, nutritional density, and relatively uncomplicated production requirements. Within the multistep framework of commercial mushroom cultivation, spawn preparation constitutes a foundational and technically critical operation, as the physiological quality and microbiological purity of the spawn material directly determine colonisation efficiency, crop uniformity, and overall yield potential.

Mother spawn, also referred to as primary or stock spawn, is defined as the first-generation mycelial inoculum produced directly from authenticated pure culture under strictly controlled laboratory conditions. It functions as the master inoculum from which secondary (planting) spawn is subsequently multiplied for large-scale application. Given its pivotal role in safeguarding genetic integrity and cultural vigour across successive spawn generations, the preparation of mother spawn demands precision at every stage, from substrate selection through to post-inoculation incubation.

Cereal grains — principally wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), maize (*Zea mays*), and pearl millet (*Pennisetum glaucum*, commonly known as bajra) — are the most widely employed substrates for grain spawn production. These grains offer an optimal balance of soluble carbohydrates, nitrogenous compounds, and physical surface area to support vigorous mycelial colonisation. The present review systematically describes the materials, methodological procedures, quality criteria, and contamination management strategies associated with mother spawn preparation in Pleurotus cultivation.

Significance of High-Quality Mother Spawn

The production of superior-quality mother spawn is indispensable for the following interconnected reasons:

- Genetic fidelity: Mother spawn preserves an uncontaminated, authenticated mycelial lineage, preventing phenotypic and physiological degeneration across inoculum generations.
- Colonisation efficiency: High-vigour spawn promotes rapid substrate penetration, reducing the window of vulnerability to competitive contamination.
- Yield and quality improvement: Robust mycelial networks established from pure spawn underpin higher fruiting body density, uniformity, and nutritional quality.
- Disease suppression: Microbiologically clean spawn substantially lowers the incidence of pathogenic mould and bacterial infection during cropping.
- Scalable inoculum production: Mother spawn serves as the primary template for commercial spawn multiplication, making its quality directly consequential for large-scale operations.

Materials Required

Grain Substrates

Selection of the grain substrate is governed by local availability, cost, and the nutritional and physical suitability for mycelial growth. The following cereal grains are commonly employed:

- Wheat grains (*T. aestivum*)
- Sorghum grains (*S. bicolor*)
- Maize grains (*Z. mays*)
- Pearl millet grains (*P. glaucum* / bajra)

Wheat grain is most frequently preferred owing to its uniform size, moderate starch content, and consistent water absorption properties, which collectively facilitate even colonisation.

Chemical Amendments

Two inorganic amendments are incorporated into the prepared grain substrate to optimise physicochemical conditions for mycelial growth:

- Calcium carbonate ($CaCO_3$, 2% w/w): Functions as a pH buffer, counteracting the production of organic acids during fungal metabolism and preventing acidification of the substrate.
- Gypsum ($CaSO_4 \cdot 2H_2O$, 4% w/w): Acts as a surface conditioning agent that reduces intergranular adhesion, thereby maintaining grain individuality and improving gas exchange within the spawn bottle.

Equipment and Consumables

The following equipment is required for aseptic and efficient mother spawn production:

- Autoclave or pressure cooker (capable of 121°C / 15 psi)
- Borosilicate glass bottles or polypropylene spawn bags
- Laminar airflow (LAF) cabinet or a purpose-built clean room
- Spirit lamp or Bunsen burner for point-of-use surface sterilisation
- Inoculation loop, needle, or scalpel (autoclavable)
- Non-absorbent cotton wool plugs and aluminium foil or craft paper for closure
- Rubber bands or autoclave tape

Procedural Methodology

Grain Selection and Pre-cleaning

Only morphologically intact, pathogen-free grains should be used. Prior to processing, grains are visually inspected to remove foreign matter, stones, and damaged or discoloured kernels. The selected grains are then rinsed repeatedly with clean potable water until the wash water runs clear, removing surface dust, residual agrochemicals, and superficial microbial load.

Hydration and Boiling

The cleaned grains are boiled in an excess volume of water for approximately 15 to 20 minutes. The objective of this step is controlled hydration: grains must absorb sufficient moisture to support fungal metabolism while retaining structural integrity. The endpoint is reached when grains are fully softened but have not burst or become mushy, as structural damage significantly impairs colonisation by promoting anaerobic pockets and bacterial rot. A final equilibrated moisture content of 50 to 55% (wet weight basis) is optimal.

Draining and Surface Drying

Following boiling, excess water is removed by draining through a wire-mesh sieve or muslin cloth. The moist grains are then spread in a single layer on a sanitised surface and allowed to air-dry until surface moisture has fully evaporated. Residual free water on grain surfaces must be eliminated before chemical amendment, as excess moisture promotes inter-grain adhesion and, more critically, creates conditions conducive to anaerobic bacterial proliferation during sterilisation.

Incorporation of Chemical Amendments

Once surface-dry, the grains are transferred to a clean mixing vessel, and the chemical amendments are incorporated by thorough hand mixing to ensure uniform distribution. As

indicated in Table 1, $CaCO_3$ is added at 2% and $CaSO_4$ at 4% of the dry grain weight. The combined amendments serve to neutralise pH drift, improve the physical free-flowing character of the grain mass, and supply trace mineral nutrients that enhance mycelial vigour.

Container Filling and Closure

The amended grain substrate is filled into borosilicate glass bottles (typically 500–750 mL capacity) or autoclavable polypropylene bags to approximately two-thirds of the container's total volume. Underfilling avoids compaction, while overfilling restricts headspace gas exchange and impedes proper mycelial development. Containers are sealed with non-absorbent cotton plugs, which are then covered with a layer of aluminium foil or brown craft paper and secured with rubber bands or autoclave tape to prevent plug wetting and contamination during sterilisation.

Sterilisation

Sterilisation is the most critical step in preventing microbial contamination. Filled containers are processed in an autoclave or pressure cooker at a temperature of 121°C under a pressure of 15 psi (103 kPa) for a minimum duration of 1.5 to 2 hours. These parameters are sufficient to achieve a sterility assurance level adequate for commercial spawn production by ensuring the destruction of all vegetative microorganisms as well as heat-resistant endospores. The sterilisation duration should be increased proportionally for larger batch volumes to account for heat penetration lag at the centre of the load.

Cooling Prior to Inoculation

Sterilised containers must be cooled to ambient temperature (below 30°C) before inoculation. Inoculating hot substrate causes thermal inactivation of the mycelial inoculum and leads to moisture condensation within the container, both of which promote contamination. Cooling is carried out within the LAF cabinet or a clean room to minimise exposure to airborne contaminants during this vulnerable period.

Aseptic Inoculation

Inoculation is performed under strictly aseptic conditions, either within a LAF cabinet providing ISO Class 5 (Class 100) air quality or in proximity to a lit spirit lamp or Bunsen burner that creates an upward convective air current to deflect airborne particles. Small, actively growing plugs of mycelium — typically 0.5 to 1.0 cm^2 — are excised from a healthy Pleurotus culture maintained on potato dextrose agar (PDA) and transferred aseptically into each spawn container. All instruments (scalpel, forceps, or inoculation needle) must be flamed and cooled before use, and the operator should wear sterile gloves and a face mask to minimise contamination risks.

Incubation

Inoculated spawn containers are incubated under conditions that optimise mycelial growth while suppressing competitor organisms. The recommended incubation parameters for Pleurotus spp. are as follows: temperature 22–28°C, relative humidity 70–80%, and ambient light conditions ranging from complete darkness to dim illumination, as phototropic responses are negligible during the vegetative colonisation phase. Under these conditions, complete colonisation of the grain substrate is typically achieved within 10 to 15 days, manifested as a dense, uniformly white, cottony mycelial network permeating the grain mass.

Quality Assessment Criteria for Mother Spawn

The acceptance or rejection of a batch of mother spawn should be based on the following observable quality indicators:

- Mycelial morphology: Dense, uniformly white, cottony growth is indicative of vigorous, uncontaminated Pleurotus mycelium. Any discolouration (green, black, yellow, or pink) is diagnostic of contamination.
- Colonisation uniformity: Even penetration throughout the grain mass, with no visible clear or poorly colonised zones, confirms effective inoculation and appropriate substrate preparation.

- Olfactory assessment: High-quality spawn exhibits a characteristic mild, pleasant mushroom-like aroma. Sour, putrid, or otherwise abnormal odours indicate bacterial or yeast contamination.
- Absence of visible contaminants: No mould colonies or bacterial slime should be detectable.
- Mycelial vitality: Active, fast-growing mycelium that has produced a uniformly colonised substrate within the expected timeframe reflects high biological vigour and inoculum potential.

Common Contaminants, Characteristics, and Causative Factors

Table 1 summarises the principal microbiological contaminants encountered during grain spawn production, their macroscopic characteristics, and the primary operational failures that predispose batches to each type of contamination.

Contaminant	Observable Characteristic	Primary Cause
Trichoderma spp. (Green Mould)	Greenish sporulation on grain surface	Inadequate heat penetration during sterilization
Aspergillus niger (Black Mould)	Dark black spore masses	Contaminated environment; poor aseptic technique
Bacterial Rot	Slimy, malodorous grain with discolouration	Excess residual moisture post-boiling
Yeast Contamination	Creamy, mucilaginous colonies	Suboptimal handling during inoculation

Trichoderma spp. represent the most frequently encountered and economically damaging group of contaminants in grain spawn production, given their rapid sporulation, enzymatic cellulolytic capacity, and competitive exclusion of Pleurotus mycelium (Chang & Miles, 2004). Preventive measures include rigorous sterilisation validation, maintenance of a clean working environment, and use of pre-authenticated pure cultures.

Critical Precautions During Spawn Preparation

Adherence to the following operational precautions is essential for achieving consistently contamination-free mother spawn:

- Maintain rigorous environmental cleanliness in all laboratory and preparation areas by regular disinfection with 70% ethanol or 0.5% sodium hypochlorite solution.
- Validate autoclave performance regularly using biological indicators (Geobacillus stearothermophilus spore strips) to confirm adequate sterilisation.
- Eliminate residual free moisture from grains prior to filling and sterilisation, as excess water generates anaerobic microenvironments conducive to bacterial growth.
- Use authenticated, contaminant-free pure cultures that have been maintained under appropriate storage conditions (4°C on PDA slants or cryopreserved).
- Completed spawn should be stored at 4°C in a refrigerator if not used immediately, and used within four to eight weeks to preserve mycelial viability.
- All inoculation steps should be executed with minimal exposure time to open-laboratory air; inoculated containers should be resealed promptly.

Agronomic and Economic Advantages of High-Quality Spawn

The production and use of high-quality mother spawn generates measurable benefits across the cultivation system:

- Significantly elevated mushroom yield per unit substrate weight, translating directly to higher farm revenue.
- Accelerated spawn run resulting in a shortened crop cycle and increased production frequency per growing season.
- Enhanced disease resistance during cropping, reducing dependency on chemical inputs and improving sustainability credentials.

- Uniform fruiting body development facilitating mechanised or standardised harvesting operations.
- Improved profitability margins for small and large-scale cultivators alike through reduced crop losses attributable to contamination.

Sequential Workflow for Mother Spawn Preparation

Table 2 presents the integrated sequential workflow for mother spawn preparation, summarising each operational stage along with its critical technical parameters.

Step	Stage	Key Details
1	Pure Culture	Authenticated Pleurotus isolate on PDA
2	Grain Selection	Sound, unbroken cereal grains (wheat / sorghum / maize / bajra)
3	Washing & Boiling	Hydration boil 15–20 min; moisture ~50–55%
4	Chemical Treatment	$CaCO_3$ (2%) + $CaSO_4$ (4%) for pH & texture
5	Container Filling	Fill to two-thirds capacity; cotton-plug closure
6	Sterilization	Autoclave at 121°C / 15 psi for 1.5–2 h
7	Aseptic Inoculation	Transfer active mycelium under LAF conditions
8	Incubation	22–28°C, 70–80% RH, dim light, 10–15 days
9	Mother Spawn Ready	Complete colonisation; pure white cottony growth

Conclusion

Mother spawn preparation constitutes a scientifically precise and technically demanding component of the oyster mushroom production system. The biological performance of every subsequent inoculum generation, and ultimately the commercial viability of the cultivation enterprise, is fundamentally contingent upon the purity, vigour, and quality of the mother spawn. The integration of rigorously standardised substrate preparation protocols, validated sterilisation procedures, and strict aseptic inoculation technique is therefore non-negotiable for sustainable, high-yield Pleurotus cultivation. As global demand for edible fungi continues to grow, driven by increasing recognition of their nutritional, medicinal, and environmental value, the adoption of evidence-based spawn production methodologies will be essential for securing food security outcomes and expanding the economic viability of mushroom cultivation across both developed and developing agricultural contexts (Pathak et al., 2010; Singh & Chaube, 2009).

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