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Milky Mushroom (*Calocybe indica*) Grain Spawn Production: A Practical Guide for Tropical Farmers

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Milky mushroom, Calocybe indica P. Purkayastha & A. Chandra, is an edible basidiomycete first described from West Bengal, India, and subsequently commercialised in Tamil Nadu during the late 1990s. Unlike the more familiar button mushroom (*Agaricus bisporus*) or oyster mushroom (*Pleurotus ostreatus*), milky mushroom thrives at temperatures of 30–38 °C, rendering it the preferred choice for year-round cultivation in the humid tropics where other commercially important species either fail to fruit or perform sub-optimally.

The crop possesses several characteristics that make it especially attractive to resource-constrained smallholders. Its fruitbodies are large, uniformly white, firm-fleshed, and nutritionally rich in protein, dietary fibre, and essential minerals. Critically, harvested mushrooms retain their marketable quality for five to seven days without mechanical refrigeration, a property that substantially reduces post-harvest losses in regions with underdeveloped cold-chain infrastructure. The species grows productively on a wide range of lignocellulosic agricultural residues, including rice straw, wheat straw, cotton gin trash, and banana pseudo-stems, all of which are abundant and inexpensive across South and South-East Asia.

Despite these favourable attributes, widespread adoption of milky mushroom cultivation has been constrained by inconsistent access to quality spawn. Spawn — grain fully colonised by vigorous mushroom mycelium — is the functional equivalent of certified seed in conventional crop production. Sub-standard spawn, whether contaminated, aged, or derived from degenerated cultures, invariably leads to poor colonisation, low yields, and economic loss. This article provides an evidence-based, practically oriented account of grain spawn production for milky mushroom, drawing on published research to guide grain selection, preparation, sterilisation, inoculation, and storage.

Biology and Cultivation Requirements

Calocybe indica belongs to the family Lyophyllaceae. Its mycelium is white, cottony, and relatively fast-growing on standard agar media such as potato dextrose agar (PDA) at 28–32 °C. The species is thermophilic: mycelial growth and substrate colonisation proceed optimally near 30 °C, and fruiting requires sustained temperatures within the 30–38 °C band. Exposure to temperatures below approximately 25 °C inhibits pin formation. Fruiting bodies develop as dense clusters, with individual caps reaching 8–10 cm in diameter under optimal conditions. Two to three productive flushes can be harvested from a single substrate bag before biological efficiency declines. Unlike many gilled fungi, milky mushroom does not strictly require a casing layer to initiate fruiting, although the application of a thin (2–3 cm) moistened loam-and-coir casing after full substrate colonisation consistently improves pin density and total yield.

Spawn Production: Principles and Procedure

Spawn production is a sequential process encompassing culture maintenance, grain preparation, sterilisation, aseptic inoculation, and incubation. Rigorous adherence to each stage is essential; a lapse at any point propagates forward, compromising the entire batch.

Maintenance of the Mother Culture

All spawn production must originate from a certified, pathogen-free stock culture. The preferred starting material is a pure mycelial culture maintained on PDA slants or in sterile liquid medium. Mother cultures should be sub-cultured at intervals not exceeding 30 days to prevent phenotypic degeneration and senescence. Farmers without the capacity to maintain agar cultures should procure fresh cultures exclusively from accredited government mycology laboratories or reputable commercial suppliers, verifying provenance before each spawn cycle.

Grain Selection and Comparative Assessment

The grain substrate exerts a measurable influence on colonisation rate, contamination susceptibility, and ultimately, crop yield. Four grains are routinely evaluated in the literature for milky mushroom spawn production:

- Sorghum (**Sorghum bicolor** L. Moench; jowar): Colonisation is rapid, typically completed within 13–15 days at 30 °C. Sorghum exhibits the lowest contamination failure rate among commonly evaluated grains (approximately 12.7% in controlled trials) and is generally the most cost-effective option in Indian and sub-Saharan African markets.
- Wheat (**Triticum aestivum** L.): An intermediate performer in terms of colonisation speed (16–18 days). Wheat absorbs water more readily than sorghum, which increases the risk of bacterial contamination if surface moisture is not adequately removed prior to sterilisation. Failure rates of 18–20% have been reported.
- Maize (**Zea mays** L.): The slowest-colonising grain evaluated (18–19 days). Large kernel size results in significant water absorption and a tendency toward uneven sterilisation if pressure is not maintained for the full recommended duration. Contamination rates are moderate (approximately 14.3%).
- Pearl millet (**Pennisetum glaucum** (L.) R.Br.; bajra): Pearl millet spawn colonises at a rate comparable to sorghum (13–14 days) and, in yield trials, has produced the highest total fresh mushroom weight of any grain examined. However, it also exhibits the highest

contamination susceptibility (up to 20.7%), necessitating meticulous sterilisation and inoculation technique.

On the basis of available evidence, sorghum is the recommended default grain for farmers new to spawn production, offering the best balance of colonisation speed, contamination resistance, and cost. Pearl millet may be adopted by experienced cultivators who can guarantee strict aseptic technique.

Grain Preparation

1. Cleaning and selection: Procure clean, dry grain free from visible mould, insect damage, or impurities. Rinse thoroughly with clean water, removing shrivelled kernels and foreign matter by flotation.
2. Soaking: Immerse the rinsed grain in clean water at ambient temperature for 12 hours. This pre-hydration ensures uniform moisture penetration to the kernel core, which is essential for even sterilisation.
3. Partial cooking: Drain the soaked grain and transfer to a cooking vessel. Simmer in fresh water for 15–30 minutes until grains are fully swollen but structurally intact. The outer pericarp should remain unbroken; burst grains create an overly moist, nutrient-rich environment that promotes bacterial growth.
4. Surface drying: Drain the cooked grain and spread it in a single layer on a clean cloth, wire mesh, or plastic sheet for 30–60 minutes. The objective is to remove free surface moisture while retaining internal hydration. Grains should feel firm and damp, not wet or sticky to the touch.
5. Addition of calcium carbonate: Thoroughly mix approximately 2% calcium carbonate (agricultural lime, $CaCO_2$) by dry weight into the prepared grain. This buffering agent moderates pH, retards bacterial proliferation during sterilisation, and improves grain flowability within spawn containers.

Container Selection and Loading

Suitable containers include wide-mouth borosilicate glass jars (500 mL to 2 L), aluminium screw-top tins, or autoclavable polypropylene bags. Each container should be filled to approximately two-thirds to three-quarters of its capacity to allow adequate steam penetration. An air-filtration port — a 4–5 mm aperture covered with non-absorbent cotton wool, polyester fibre, or a commercially produced hydrophobic filter disc — must be incorporated to permit gas exchange during incubation while excluding contaminants. All containers should be clearly labelled with the grain type, culture strain, and date of preparation.

Sterilisation

Effective sterilisation is the single most critical determinant of spawn quality. Loaded containers must be processed in a pressure cooker or autoclave at 15–20 psi (103–138 kPa) for 90–120 minutes. Smaller loads (up to 10 one-litre jars) generally reach adequate sterilisation within 90 minutes; larger or denser loads require the full 120-minute cycle. Following sterilisation, containers must be allowed to cool completely within the sealed vessel before removal. Transferring hot containers to a non-sterile environment risks thermal convection currents drawing in contaminated air through filter ports.

Where a pressure cooker is unavailable, a steam pasteurisation approach (sustained steam at 95–100 °C for three to four hours) may be employed as a less reliable alternative. This method does not achieve true sterilisation and is associated with higher contamination rates; it is not recommended for critical seed spawn production.

Aseptic Inoculation

Inoculation must be conducted in the cleanest environment achievable. Preferred facilities, in descending order of reliability, are: a laminar-airflow cabinet, a purpose-built glove box, a self-constructed still-air box, or a wiped-down, enclosed room. All work surfaces should be disinfected with 70% isopropyl alcohol immediately before use. The operator should wear sterile gloves and a face mask.

The inoculation needle, scalpel, or transfer loop must be flame-sterilised and allowed to cool before each transfer. A small fragment of actively growing mycelium on agar, or an appropriate volume of liquid culture inoculum, is introduced through the filter port. The port is then re-sealed with fresh cotton or tape. Each container should be inoculated rapidly and minimised in its time of exposure to the open environment.

Incubation and Monitoring

Inoculated spawn containers are incubated in darkness at approximately 30 °C. A stable temperature within the 28–32 °C range is adequate; wide diurnal fluctuations slow colonisation and may favour competing organisms. Ambient humidity in the incubation room should be maintained at 40–60% to minimise condensation on container walls without desiccating filter ports.

Containers should be inspected daily. Every three to five days, jars should be gently agitated or inverted to redistribute the advancing mycelial front, accelerating homogeneous colonisation. Full colonisation — defined as 100% grain coverage by dense white mycelium with no visible uncolonised zones — is typically achieved within 15–20 days for sorghum and pearl millet, and 18–20 days for wheat and maize.

Any container exhibiting green, black, orange, or yellow discoloration, or emitting a sour, putrid, or atypical odour, must be removed from the incubation room immediately and disposed of well away from cultivation areas. Contaminated spawn should under no circumstances be used.

Spawn Storage and Shelf Life

Freshly colonised spawn should be used at the earliest opportunity. Research on Calocybe indica demonstrates a statistically significant negative correlation between spawn age and final crop yield: spawn used at 21 days post-inoculation consistently delivers superior biological efficiency relative to older material. For temporary storage, sealed spawn containers may be held at 10–15 °C (a cool, dark cupboard or a domestic refrigerator set to its warmest setting) for up to three weeks without appreciable loss of viability. Storage at temperatures below 4 °C or above 20 °C is detrimental to mycelial vitality and should be avoided.

Comparative Assessment of Grain Spawn Substrates

Grain	Approx. Cost (INR/kg)	Colonisation Time (days)	Contamination Risk (% failure)	Notable Characteristics
Sorghum	~30	13–15 (fast)	~12.7 (lowest)	Best overall for beginners; lowest cost and risk
Wheat	~35	16–18 (medium)	~18–20 (high)	High water absorption requires thorough surface drying
Maize	~20–25	18–19 (slow)	~14.3 (moderate)	Large kernels prone to clumping; extended sterilisation required
Pearl Millet	~25	13–14 (fast)	~20.7 (highest)	Highest crop yields; requires expert aseptic technique

Table 1. Comparative performance of cereal grains as spawn substrates for Calocybe indica under tropical conditions. Cost data are indicative Indian market figures (2025). Contamination rates are drawn from controlled experimental trials.

Substrate Compatibility and Application

Milky mushroom demonstrates broad lignocellulosic substrate versatility. The most commonly employed and best-characterised bulk substrates are as follows:

- Rice straw and wheat straw (chopped to 3–5 cm) remain the standard substrates in India and South-East Asia, offering good water retention, adequate carbon-to-nitrogen ratio, and reliable availability. Straw is most efficiently treated by hot-water immersion pasteurisation (60–80 °C for one to two hours) or steam pasteurisation before spawn inoculation.
- Cotton gin trash, banana pseudo-stems, and maize stalks constitute viable alternative substrates where straw is scarce or costly. These materials may require longer pasteurisation cycles to achieve adequate decontamination.
- Supplemented sawdust formulations (hardwood sawdust with 10–20% wheat bran or rice bran by dry weight) support mycelial growth and fruiting when fully sterilised, though biological efficiency is generally inferior to straw-based systems.

Spawn is incorporated at a rate of 2–5% (w/w, spawn weight relative to dry substrate weight) by layering or thorough mixing. Following inoculation, substrate bags are incubated at 30 °C until fully colonised (typically 10–14 days). A moistened casing layer applied after colonisation and before fruiting induction reliably increases pin density and total yield.

Hygiene, Contamination Control, and Troubleshooting

Contamination by competing fungi (particularly Trichoderma spp., Aspergillus spp., and Penicillium spp.) and bacteria (Bacillus spp., Pseudomonas spp.) represents the most significant technical challenge in grain spawn production. The following measures collectively reduce failure rates to below 5–10% in well-managed operations:

- Maintain a dedicated spawn preparation area, physically separated from fruiting rooms and composting areas.
- Disinfect all work surfaces, tools, and container exteriors with 70% isopropyl alcohol immediately before use.
- Ensure grain is neither under-hydrated (impeding sterilisation) nor over-hydrated (creating a nutrient-rich liquid medium for bacteria).
- Adhere strictly to recommended sterilisation times and pressures; do not open the pressure cooker until pressure has fully equilibrated.
- Inoculate only from actively growing, visually clean mother cultures; discard any culture showing atypical coloration or odour.
- Segregate and destroy contaminated spawn promptly; never allow it to remain in proximity to clean spawn or substrate.

Common problems and their probable causes are summarised below:

- Patchy or slow colonisation: Excess surface moisture on grain; temperature below 28 °C; overly dense packing of containers.
- Green or black discoloration (Trichoderma or Aspergillus): Inadequate sterilisation or a breach of aseptic technique during inoculation.
- Slimy, malodorous grain (bacterial contamination): Excess moisture, insufficient $CaCO_2$ buffering, or incomplete sterilisation.
- Yellowing or browning of mycelium: Bacterial contamination or physiological stress due to temperature fluctuation or ageing.
- Failure to pin on colonised substrate: Fruiting room temperature below 25 °C; relative humidity below 80%; inadequate fresh-air exchange.

Standard Operating Procedure and Production Timeline

The following timeline assumes a single batch of 20 one-litre jars of sorghum grain spawn inoculated from a liquid culture.

Day 0 — Culture Preparation: Verify mother culture viability. Prepare inoculation tools. Disinfect work area. Prepare sterile liquid culture or confirm agar slant is actively colonised.

Day 1 — Grain Soaking: Weigh and rinse sorghum grain. Immerse in clean water (grain-to-water ratio 1:3) for 12 hours at ambient temperature.

Day 2 — Grain Cooking and Loading: Drain soaked grain; simmer for 20 minutes; drain and surface-dry for 45 minutes on a clean cloth. Mix 2% $CaCO_3$ by weight. Fill jars to three-quarters capacity; fit cotton-wool filter plugs; seal lids. Load into pressure cooker.
Day 2–3 — Sterilisation: Sterilise at 15 psi for 90 minutes. Allow to cool overnight within the sealed cooker.
Day 3 — Inoculation: Transfer cooled jars to still-air box. Flame-sterilise inoculation needle; allow to cool. Inoculate each jar with liquid culture through filter port. Re-seal. Label with grain, strain, and date. Transfer to incubation area.
Days 4–20 — Incubation (Spawn Run): Maintain 30 °C in darkness. Inspect daily. Gently agitate jars every three to four days. Remove any contaminated jars immediately.
Days 15–21 — Spawn Ready: Jars showing 100% white colonisation are ready for use. Deploy spawn at once or store at 10–15 °C for up to three weeks.
Parallel — Substrate Preparation: While spawn is colonising, chop and pasteurise straw (60–80 °C for one to two hours). Cool to below 30 °C before inoculation.
Day 18–21 — Substrate Inoculation: Mix spawn into cooled substrate at 3–5% by weight. Pack into polypropylene bags; seal. Incubate at 30 °C.
Days 28–35 — Colonised Substrate: Substrate bags should be fully white. Apply casing layer if desired. Transfer to fruiting room (30–32 °C; relative humidity 85–90%; indirect light; fresh-air exchange).
Days 35–42 — Pin Initiation and Harvest: Pins appear within three to seven days of fruiting induction. Harvest individual clusters as caps reach full size but before margin begins to curl upward. Expect two to three flushes at seven- to ten-day intervals.

Quality Indicators and Yield Benchmarks

Growers should evaluate spawn quality against the following objective criteria:

- Visual appearance: spawn grain should be uniformly and densely covered with snow-white mycelium throughout the jar. No uncolonised zones, no green, black, or orange patches.
- Odour: pleasant, mild, earthy, or fungal scent. Any sour, ammoniacal, or putrid odour indicates contamination.
- Texture: individual grains should be bound together by mycelial strands into a firm, coherent mass that fractures cleanly when shaken. Slimy or liquid-filled jars indicate bacterial spoilage.

Under optimised conditions with fresh sorghum or pearl millet spawn applied to well-pasteurised rice straw, biological efficiency values of 80–120% (fresh mushroom weight relative to dry substrate weight) are routinely achievable. Cumulative yields of 300–500 g per kg of dry substrate over two to three flushes represent a reasonable commercial benchmark. Pearl millet spawn has produced total yields of approximately 1,340 g per experimental block in published trials, surpassing yields from other grain substrates tested under the same conditions.

Conclusion

Milky mushroom occupies a distinctive and commercially valuable niche in tropical mushroom cultivation. Its heat tolerance, robust shelf life, and adaptability to low-cost agricultural substrates position it as an important livelihood crop for smallholder farmers across tropical Asia and Africa. The quality of grain spawn is the single most controllable determinant of cultivation success, and investment in rigorous spawn production protocols yields disproportionate returns in crop reliability and profitability. Sorghum grain is recommended as the primary spawn substrate for most producers, offering the optimal combination of colonisation speed, contamination resistance, and affordability. Pearl millet spawn may be adopted by advanced practitioners to maximise biological efficiency, provided that stringent aseptic standards can be maintained. In all cases, the use of fresh, certified mother cultures, correct grain preparation, full-pressure sterilisation, and disciplined inoculation technique are non-negotiable prerequisites. Continued research into liquid culture inoculation methods, alternative spawn carrier materials, and optimised substrate formulations offers promising avenues for further improving the productivity and accessibility of milky mushroom cultivation in resource-constrained contexts.

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