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Technological advances in spawn production for cultivation of (*Calocybe indica* P. & C.) milky mushroom: A critical review

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Abstract

Calocybe indica, commonly known as the milky mushroom, is a thermotolerant tropical edible mushroom belonging to the family Lyophyllaceae. It has gained considerable commercial importance in India and other warm, humid regions because of its ability to grow and fruit efficiently at temperatures ranging from 30–38 °C, where many temperate mushroom species perform poorly. Among the various factors influencing successful cultivation, the production of healthy, vigorous and contamination-free spawn is particularly important, as spawn quality directly affects substrate colonization, pinning, yield, biological efficiency (BE) and overall production profitability. Comparative results from published laboratory and field studies are compiled to evaluate the influence of different grain spawns, fruiting substrates, straw combinations, casing materials, spawn rates and spawn age on mycelial growth, yield and biological efficiency. Among the grain substrates evaluated, bajra (pearl millet) and sorghum consistently supported rapid mycelial colonization, with complete spawn run reported within approximately 13.7–13.8 days. The review further highlights the importance of temperature, pH, moisture content, substrate C: N ratio, sterilization and hygienic handling in maintaining spawn quality and minimizing contamination. A chronological assessment of studies conducted over more than four decades indicates the consistent suitability of sorghum and bajra as grain carriers and demonstrates the influence of spawn rate and spawn age on colonization and mushroom production. Despite considerable progress, standardized protocols for sawdust- and liquid-based spawn production in *C. indica* remain limited. Further research in these areas could contribute to the development of reliable, cost-effective and commercially scalable spawn-production systems for the expanding milky mushroom industry.

Keywords: *Calocybe indica*, milky mushroom, spawn production, grain spawn, biological efficiency, mushroom cultivation, casing material

1. Introduction

Mushroom cultivation provides an effective way of converting agricultural wastes, particularly lignocellulosic residues, into nutritious food [1]. Materials such as crop straw and other farm residues, which are often burned or left to decompose, can instead be used as substrates for mushroom production [2]. In this way, mushroom cultivation not only helps in waste management but also produces a food that is rich in protein and other nutrients [3]. This makes mushroom farming a highly efficient form of bio-recycling that can be adopted by both small-scale farmers and commercial growers [4].

Among the tropical mushrooms cultivated in India, *C. indica* (P. & C.), popularly known as the milky mushroom, Dudhia Chatta or Kuduk, holds a unique position. One of its most important characteristics is its ability to grow and produce fruiting bodies at relatively high temperatures, generally between 30 and 38 °C [5]. This heat tolerance makes it particularly suitable for cultivation during the hot and humid summer and monsoon seasons. In contrast, temperate mushrooms such as the button mushroom (*Agaricus bisporus*) require controlled environmental conditions during these periods, while oyster mushrooms (*Pleurotus* spp.) may also show reduced performance under very high temperatures [6]. *C. indica* was first described from West Bengal, India and its cultivation has gradually spread to several agro-climatic

regions of India and other parts of South and Southeast Asia [5, 7].

The genus *Calocybe* contains around 40 species, among which *C. carnea*, *C. ionides*, *C. gambosum* and *C. indica* are considered edible [5, 8]. The history of *C. indica* began with the collection of its wild fruiting bodies from markets in Calcutta [9]. Its identity was later confirmed at the Royal Botanic Garden, Edinburgh and its edibility was established soon afterward. The natural occurrence of the species was subsequently reported from the plains of Rajasthan and Tamil Nadu [10]. Over time, several Indian cultures of *C. indica* have also been studied and characterised using molecular techniques [5].

Although *C. indica* has a relatively limited natural distribution, its ability to tolerate high temperatures and its good nutritional and commercial qualities have attracted considerable interest [5]. For this reason, it is often regarded as a specialty mushroom, a species that may have limited natural availability but possesses significant potential for commercial cultivation [9, 10, 11, 12, 13].

Milky mushroom is valued not only for its attractive white, fleshy fruiting bodies and relatively long shelf life, but also for its nutritional and nutraceutical benefits [14]. It contains appreciable amounts of protein, dietary fibre and essential amino acids and has also been reported to possess anti-hyperglycaemic and antioxidant properties [15]. These

nutritional and functional qualities, together with its relatively simple cultivation requirements, have contributed to its increasing popularity among farmers. Unlike some other cultivated mushrooms, milky mushroom does not necessarily require highly sophisticated growing facilities, strictly pasteurised compost or a fully controlled cropping room. As a result, its cultivation has gradually expanded across several parts of India and neighbouring countries [5, 16, 17].

In recent years, a growing number of laboratory studies and reviews have provided further evidence of the biological activities associated with *C. indica*. Extracts and polysaccharides obtained from the mushroom have been investigated for their antimicrobial, anti-inflammatory, antioxidant and anti-genotoxic properties [18]. These findings have increased interest in the possible use of *C. indica* in functional foods and nutraceutical products, while also adding to its value as a commercially promising cultivated mushroom [15, 19].

Whatever substrate is selected for growing the mushroom, successful cultivation begins with good-quality spawn. Spawn is essentially the vegetative mycelium of the mushroom grown on a suitable nutrient-rich carrier, most commonly cereal grains, under clean and aseptic conditions [20]. Once fully developed, the spawn is introduced into the bulk growing substrate, where the mycelium spreads and begins the process of colonisation. For this reason, spawn is often referred to as the seed of mushroom cultivation [21].

The quality of spawn has a direct influence on almost every stage of mushroom production. Healthy and vigorous spawn promotes rapid and uniform colonisation, reduces the risk of contamination and ultimately contributes to better and more consistent yields. In contrast, poor-quality spawn can seriously affect crop performance and may even lead to complete crop failure. Such problems can arise from an unhealthy mother culture, unsuitable or poor-quality carrier material, inadequate sterilisation, contamination during preparation, or the use of spawn that has been stored beyond its viable shelf life [22]. Consequently, the preparation and maintenance of high-quality spawn are considered among the most critical steps in successful mushroom cultivation [5, 7].

Despite the importance of spawn production, information specifically dealing with spawn preparation in *C. indica* is still scattered among research publications, regional agricultural bulletins and extension materials. A clear and updated synthesis focusing specifically on the production of quality spawn for milky mushroom is therefore useful for researchers, growers and students. Bringing this information together can help improve understanding of the spawn-production process and support more reliable and efficient cultivation of *C. indica* [5, 23, 24].

This review aims focuses on the biological and physiological factors involved in spawn development and examines the major types of spawn, including grain, sawdust, straw-based and liquid spawn [25, 26, 27]. Particular attention is given to the different grain substrates that have been tested for *C. indica*. The review also brings together the published methods into a clear, step-by-step protocol for spawn production. In addition, yield and biological efficiency (YBE) data from independent studies are compared in both tabular and graphical forms [28, 29]. The progress of research on grain substrates and spawn density is also traced to highlight how

these practices have evolved over time. Finally, the review discusses the major challenges faced by the milky-mushroom spawn industry and suggests areas that deserve further research and development [5].

2. Taxonomy, biology and cultural requirements

Calocybe indica belongs to the family Lyophyllaceae under the order Agaricales. Unlike wood-degrading mushrooms such as *Pleurotus* and *Lentinula*, *C. indica* is mainly associated with the decomposition of plant litter, straw and composted organic materials. This explains why it is commonly cultivated on wheat straw, other agricultural residues and nutrient-enriched compost rather than on hardwood logs or sawdust-based blocks [30]. The fungus produces white to creamy, cottony mycelium and grows relatively rapidly on commonly used laboratory media, including potato dextrose agar (PDA) and malt extract agar (MEA) [31]. Mycelial growth is generally favoured by temperatures between 25 and 32 °C, with many strains showing particularly good growth around 28–30 °C. A near neutral to slightly alkaline pH, generally around 7.0–7.5, also supports healthy mycelial development [5, 9, 16, 32].

C. indica is well adapted to warm conditions and is considered a highly thermotolerant mushroom. Its mycelium grows actively across a broad temperature range, from around 25 °C to above 32 °C, while the formation and development of fruiting bodies are most favourable at temperatures between 30 and 38 °C, with relative humidity maintained at about 70–85%. Temperatures considerably below 25 °C can restrict or delay pin formation [33]. Under suitable environmental conditions, the mushrooms develop in compact clusters and produce thick, creamy-white, fleshy caps that may reach 8–10 cm in diameter. Generally, two to three flushes can be obtained from a spawned substrate bag or bed, although the yield tends to decrease with successive flushes.

Unlike *Agaricus*, *C. indica* does not strictly require a casing layer for pinning. However, applying a thin layer of moist soil, sand, compost, or vermicompost over the fully colonised substrate can be beneficial [34]. A properly prepared casing layer helps maintain moisture around the developing pins, promotes more uniform pin formation and can improve the overall yield [5, 16, 35, 36].

Because *C. indica* can grow and fruit well under relatively high temperatures, it has an important advantage over many other cultivated mushroom species. In tropical regions, both spawn production and mushroom cropping can often be carried out without refrigeration, which helps reduce energy costs and makes cultivation more accessible to small-scale growers [5]. However, during spawn multiplication, it is important to maintain clean, hygienic and reasonably draft-free conditions to avoid contamination and ensure healthy spawn development [5, 35].

3. Importance and classification of spawn

In mushroom cultivation, spawn can be considered the equivalent of seed in conventional agriculture. It is a carrier material such as grain, sawdust, straw, or a liquid medium that is first sterilised and then thoroughly colonised by a pure culture of mushroom mycelium under aseptic conditions [37]. Good-quality spawn should be true to the desired mushroom strain, vigorous, free from bacterial and fungal contamination

and physiologically mature enough to establish itself quickly after inoculation. When added to a prepared bulk substrate, usually at around 2-5% (w/w) for grain spawn, the actively growing mycelium spreads rapidly throughout the material. This quick colonisation helps the mushroom mycelium compete with unwanted microorganisms and leads to the spawn run stage, during which the substrate becomes progressively colonised by the mushroom fungus [5, 7].

3.1. Spawn quality

Spawn vigour plays an important role in determining how quickly the substrate becomes fully colonised. Strong and uniformly growing spawn allows the mushroom mycelium to establish itself faster, thereby shortening the period during which the substrate is vulnerable to green mould (*Trichoderma* spp.), bacterial blotch and other competing microorganisms [7, 38, 39].

Spawn purity is one of the most important factors for successful mushroom production. Spawn contaminated with unwanted fungi or bacteria can seriously affect crop performance. Even a small number of contaminated bottles or bags may introduce problems into the production system,

so spawn should be carefully examined and unsuitable units discarded before use [7] [38].

The choice of spawn substrate also has a significant influence on mushroom production. Different substrates can affect the rate at which mycelium spreads and can ultimately influence yield and biological efficiency. Comparative studies further highlight these differences [24, 38, 39, 40].

The age and storage conditions of spawn can greatly influence its quality and performance. Spawn that is kept for too long after complete colonisation, or stored at temperatures that are too high, tends to lose its vigour and becomes more vulnerable to contamination [5, 7].

3.2. Classification of spawn types

Spawn used in mushroom cultivation can generally be classified into four main types based on the material used as the carrier, as summarised in Table 1. Among these, grain spawn is the most commonly used for *C. indica*. Cereal grains and millets provide a suitable nutritional base for mycelial growth and offer a large surface area for colonisation [41]. They are also relatively easy to prepare, handle and mix uniformly with the cultivation substrate.

Table 1: Classification of spawn types used for milky mushroom cultivation [5, 7].

Spawn type	Typical carrier material	Relative colonisation speed	Relative cost	Suitability for <i>C. indica</i>
Grain spawn	Wheat, paddy, sorghum, bajra (pearl millet), maize, cotton seed, pulses	Fast (13–20 days)	Low–moderate	Most widely used and best-documented; industry standard
Sawdust spawn	Hardwood sawdust, often supplemented with bran	Moderate–slow	Low	Limited use; species is not primarily lignicolous
Straw-based spawn	Chopped, sterilised cereal straw	Slow–moderate	Very low	Occasional use where grain is scarce; larger particle size slows colonisation
Liquid spawn	Liquid nutrient broth (sugar/malt/yeast-extract based)	Very fast propagation, but requires careful transfer	Moderate	Emerging technique; scope for further validation in <i>C. indica</i>

Sawdust spawn is commonly associated with wood-decaying mushrooms, but its use in *C. indica* is comparatively limited because the species is not primarily lignicolous. Nevertheless, some experimental studies have investigated supplemented sawdust as a possible material for spawn preparation or as a growth substrate [42].

Straw-based spawn, prepared by using chopped and sterilised cereal straw colonised with mushroom mycelium, is another option. It may be useful in situations where grain is expensive or not readily available. However, colonisation is generally slower than with grain spawn because straw contains fewer readily available nutrients and has a relatively coarse structure [43, 44].

The fourth type is liquid spawn, in which mushroom mycelium is multiplied in a liquid nutrient medium under either static or agitated conditions. This method is gaining attention because it can produce large quantities of mycelial biomass in a relatively short period. For *C. indica*, however, liquid spawn technology is still less extensively documented than conventional grain spawn [45, 46]. Even so, it has considerable potential for future cultivation systems, particularly because it may help reduce the time required to multiply healthy mycelium from the mother culture to the final spawn [5, 7, 38].

3.3. Historical development of spawn technology

The term **spawn** comes from the Old French word *espandre*, meaning to expand. In mushroom cultivation, the term has

been defined in different ways over time. Today, mycelium refers to the vegetative growth of the fungus, whereas spawn generally refers to the nutrient-rich material carrying the mycelium that is used to inoculate the cultivation beds.

The development of pure-culture spawn production marked an important advancement in mushroom cultivation. Duggar [47] was among the first to develop a method for producing spawn by growing fungal tissue taken from fruiting bodies on suitable nutrient media. Later, Sinden [48] developed and patented the use of cereal grains as a substrate for spawn production. His technique involved colonizing sterilized grains with a pure fungal culture and became the foundation of modern commercial spawn production. The same basic principle continues to be widely used today, including in the grain-spawn preparation method used for *C. indica* described [47, 48].

4. Mother culture (stock culture) maintenance

Successful spawn production starts with a healthy, vigorous and genetically stable mother culture. For *C. indica*, the stock culture is generally maintained on Potato Dextrose Agar (PDA) or Malt Extract Agar (MEA) in the form of slants or plates. Proper maintenance of the mother culture is important because it helps ensure consistent growth, good spawn quality and reliable mushroom production [5, 7].

Obtaining a reliable culture

The initial culture should preferably be obtained from an

authenticated mushroom culture collection, recognised repository, or research institute. Using a verified culture helps prevent strain misidentification and provides confidence in its growth and yield characteristics [7].

The culture can be transferred to fresh PDA or MEA and incubated at around 25–30 °C. Only healthy, actively growing, contamination free colonies showing the typical creamy white, fluffy mycelial growth should be selected for further use [5, 38].

The mother culture should be renewed periodically, preferably by isolating tissue from healthy and freshly harvested fruiting bodies. This helps maintain the vigour of the mycelium and reduces the possibility of losing growth and yield potential due to prolonged and repeated sub-culturing [5, 7].

Working cultures may be stored under refrigeration at approximately 4–8 °C for short-term maintenance. It is also advisable to keep duplicate cultures as backups so that the stock can be recovered if the primary culture becomes contaminated or is accidentally lost [7].

Maintaining the mother culture carefully is therefore an essential step in mushroom cultivation, as the quality and vigour of the stock culture directly influence the quality of the spawn and, ultimately, the productivity of the crop.

4.1 Strain selection, molecular characterisation and breeding advances

The identity, genetic stability and vigour of the mother culture play an important role in determining the overall quality of spawn. For this reason, recent research on *C. indica* has increasingly focused not only on improving spawn substrates but also on identifying and characterising superior strains. Nath *et al.* [49] evaluated 33 wild collected *C. indica* strains from different parts of India. The strains were first confirmed through ITS-based PCR analysis and were then assessed for genetic diversity using ten SRAP marker combinations. The study detected 211 alleles, with 93.84% of the loci being polymorphic, highlighting the considerable genetic diversity present in natural populations of *C. indica*. This diversity provides valuable opportunities for selecting strains with desirable traits for cultivation and future breeding programmes [49].

The selected strains were further evaluated through multi-

location cultivation trials. Eight strains produced yields that were at least 10% higher than the released check strain DMRO-302. Among them, DMRO-299 recorded the highest BE of 64.08%. Some strains also showed advantages beyond yield; DMRO-54, DMRO-202 and DMRO-299 combined good productivity with desirable nutritional and functional characteristics, including higher antioxidant activity, phenolic content or protein concentration. These findings demonstrate the importance of combining molecular characterisation with field-level performance when selecting promising strains for spawn production and commercial cultivation of *C. indica* [49].

Earlier multilocation trials by Sharma *et al.* [50], carried out over three years at several agricultural universities in India, also demonstrated the importance of selecting better-performing strains. The study identified a new strain, DMR Milky-985, which performed better than the standard check strain DMRO-334, recording a BE of 55.7% compared with 52.6% and an average fruit-body weight of 47 g compared with 38 g. These findings suggest that replacing or improving strains can be an effective strategy for increasing spawn performance, alongside the careful selection of grains and substrates. More recently, the development of a near chromosome-scale genome assembly of *C. indica*, based on a combination of Nanopore long-read and Illumina short-read sequencing and deposited in the NCBI BioProject database (accession PRJNA927116), offers an important genomic resource for future strain improvement. This reference genome could support the development of marker-assisted selection, rapid strain identification and authentication and contaminant-screening tools for commercial *C. indica* spawn laboratories-areas that also represent important research gaps highlighted in [50, 51].

4.2. Protocol for grain spawn production

The following step-by-step protocol provides a consolidated approach to grain spawn production in *C. indica*, based on procedures reported in earlier studies. It brings together the key stages involved in preparing, sterilising, inoculating and incubating the grain substrate to obtain healthy and vigorous spawn. A simplified production timeline is presented in Table 2, for easy reference [7, 24, 38].

Table 2: Indicative timeline for grain-spawn production of *C. indica* [7, 38].

Stage	Typical activity	Approximate duration
Day 0	Mother-culture verification; work-area disinfection; equipment preparation	0.5–1 day
Day 0–1	Grain washing, boiling/soaking and surface-drying; CaCO ₃ amendment	0.5–1 day
Day 1	Bottling/bagging of grain substrate; plugging	0.5 day
Day 1–2	Autoclave sterilisation (121 °C, 15 psi) and overnight cooling	1–1.5 days
Day 2–3	Aseptic inoculation with mother culture in laminar flow / still-air box	0.5 day
Day 3–17 to 24	Incubation / spawn run at 25–30 °C in the dark	14–21 days
Day ~18–24 onward	Quality screening and use of spawn for bulk-substrate inoculation (at 3–5% w/w)	as required

5. Grain selection and pre-treatment

Good-quality, healthy grains that are free from insects, mould and other visible damage should be selected for spawn preparation. Grains such as wheat, paddy, sorghum, bajra, or other suitable cereals and millets can be used. The selected grains are first cleaned thoroughly to remove dust, chaff, stones and other unwanted materials and then washed properly. They are boiled in water for about 20–30 minutes,

or until they become adequately soft while still retaining their shape [20, 52]. The grains should not be overcooked, split, or become mushy. After boiling, the excess water is drained off and the grains are spread evenly on a clean surface or perforated tray to cool and dry. They should be allowed to dry until the surface moisture is removed and the individual grains remain separate and non-sticky. This pre-treatment helps produce grains with a suitable moisture level and

texture for subsequent spawn inoculation and mycelial growth [7, 24, 38, 53].

5.1. pH adjustment and bottling

After draining, the grains are mixed with calcium carbonate (CaCO_3) at about 2% (w/w). This helps maintain a suitable pH and also reduces excessive stickiness on the grain surface. In some protocols, calcium sulphate (gypsum) may also be added for a similar purpose. The treated grain is then transferred into heat-resistant glass bottles or polypropylene bags, filling them to roughly three-quarters of their capacity. The remaining space allows sufficient air exchange and provides room for mycelial growth during colonisation [54]. The containers are closed with non-absorbent cotton plugs to prevent contamination while allowing gas exchange. When glass bottles are used, the neck may additionally be covered with paper and secured with a rubber band to provide extra protection during handling and sterilisation [7, 38].

5.2. Sterilisation

The filled containers are sterilised in an autoclave at 121 °C and 15 psi for about 60-120 minutes, with the exact duration depending on the size of the load and the type of container used. Where required, empty glassware can be sterilised separately in a hot-air oven at 160 °C for approximately 2 hours. After autoclaving, the containers are left undisturbed to cool, usually overnight, in a clean area. Allowing them to cool gradually helps minimise condensation and reduces the risk of contamination before inoculation [7, 38].

5.3. Aseptic inoculation

Once the containers have cooled, they are moved into a laminar air-flow cabinet or a still-air inoculation box that has been thoroughly cleaned and disinfected with 70% alcohol. Where available, the workspace may also be treated with UV light before inoculation. A small piece of actively growing mother culture, or an initial batch of mother spawn, is then carefully transferred into each bottle under aseptic conditions. A flamed inoculation loop or sterile forceps is used for the transfer to minimize the risk of contamination and ensure healthy mushroom growth [7, 38].

5.4. Incubation (Spawn Run)

After inoculation, the bottles are kept in a dark or dim light, draft-free room at a temperature of around 25-30 °C. The mycelium usually starts to appear within 3-5 days and gradually spreads throughout the substrate. Depending on the type of grain used, complete colonisation of the bottles generally takes about 14-21 days (Fig. 1).

During incubation, the bottles may be gently shaken or rolled once or twice, if the type of container and substrate allows it. This helps distribute the growing mycelium evenly and reduces compaction of the grain, which can otherwise slow down colonisation. Proper temperature, darkness and minimal disturbance during this stage are important for healthy and uniform mycelial growth [24, 38, 55].

5.5. Quality assessment before use

Before using the spawn, it should be carefully checked to make sure it is healthy, active and free from contamination. Good-quality spawn should have a uniform and dense growth of white mycelium throughout the grains. Any unusual

patches or changes in colour, such as green, black, or pink growth, may indicate mould or bacterial contamination. Such spawn should not be used [7, 38]. Healthy spawn generally has a pleasant, fresh, earthy smell typical of mushrooms. A sour, unpleasant, or rotten smell is usually a sign of bacterial contamination and the affected spawn should be discarded [7]. The grains should remain separate and loose rather than becoming sticky, gummy, or excessively wet. Friable grains are easier to mix evenly with the bulk substrate and help in achieving uniform spawn distribution [7, 38]. Spawn should ideally be used soon after the grains are fully colonised. If immediate use is not possible, short-term storage at around 15-20 °C is recommended to slow down ageing of the spawn while avoiding excessive cold stress [5, 7].

6. Historical evolution of grain-substrate trials for *C. indica*

Research on grain substrates for *C. indica* spawn production has developed over more than four decades, with studies showing a gradual shift in preference from wheat grain towards sorghum and pearl millet (bajra). One of the earliest reports, by Purkayastha and Nayak [16], demonstrated that wheat grain could be successfully used for spawn production. Later, Doshi *et al.* [10] reported that sorghum was a more suitable substrate under the conditions of Rajasthan. Pandey *et al.* [56] compared six different grains and found that wheat, bajra and jowar (sorghum) were all suitable for spawn preparation. Their study also showed that the addition of chalk powder or gypsum did not significantly influence mycelial growth, although these amendments helped in reducing grain clumping.

Similar findings were reported by Tandon *et al.* [57], who observed the fastest mycelial growth on jowar grain, followed by bajra. They further reported that a combination of calcium carbonate and calcium sulphate in a 1:3 ratio promoted good mycelial growth while also helping to prevent clumping. The suitability of sorghum was subsequently supported by Kamugisha and Sharan [58] and Das [59]. In contrast, Sarmah *et al.* [60] explored the use of sterilised, pre-boiled rice grain as a spawn substrate. Pani [61] also used wheat grain for spawn preparation, whereas Senthilnambi *et al.* [55] evaluated a wider range of substrates, including maize, cotton seed, sorghum, horse gram, black gram, cowpea and ragi. Among these, sorghum supported the fastest colonisation, with complete mycelial growth occurring in 13.7 days (Fig.1).

Further evidence came from Raja and Ganesh [62], who incorporated sorghum and paddy grains into their cultivation studies. Overall, this chronological body of research indicates a consistent preference for sorghum and bajra as grain substrates for *C. indica*, particularly because they support faster and more vigorous spawn colonisation than wheat and paddy. These findings highlight the growing importance of sorghum-based substrates in *C. indica* spawn production [10, 16, 55, 56, 57, 58, 60, 61, 62].

6.1. Comparative performance of spawn and cultivation substrates

A considerable amount of research has been carried out to evaluate different grain substrates for spawn preparation, as well as various bulk substrates and casing materials for the cultivation of *C. indica*. Since these studies were conducted using different strains, environmental conditions, locations

and experimental approaches, their findings are discussed individually rather than combined into a single pooled analysis. In particular, pearl millet (bajra) and sorghum grains have generally shown good potential for spawn production, while paddy straw has consistently performed well as a bulk substrate for fruiting. These findings highlight the importance of selecting substrates that are not only nutritionally suitable for mycelial growth but also readily available and practical for cultivation under local conditions [24, 38, 55, 63].

6.2. Cereal-grain and millet substrates for spawn preparation

Several studies have examined cereal grains and millets as substrates for spawn preparation in *C. indica*, with noticeable differences in mycelial growth and subsequent mushroom

production. Panda *et al.* [24] compared wheat, paddy, bajra and sorghum grains under controlled laboratory conditions at Visva-Bharati, West Bengal, India. The study showed that the type of grain used for spawn significantly influenced spawn-run time, yield and biological efficiency (Table 3). Among the grains tested, bajra (pearl millet) supported the fastest complete colonisation of the spawn bottle, which took only 13.80 days. Bajra spawn, when used with the most suitable bulk substrate, also resulted in the highest YBE in the study. Wheat grain performed particularly well in other aspects, producing a BE of 95.50%, the earliest primordial initiation (17.3 days) and the longest stalks. In contrast, paddy grain showed relatively slower primordial formation, taking about 18.7 days.

Table 3: Performance of cereal-grain spawn substrates for *C. indica* [24].

Grain spawn substrate	Days to full mycelial colonisation	Reported yield / BE	Notable remarks
Bajra (pearl millet) grain	13.80 (fastest of the four grains tested)	Highest YBE among the grains tested (in the best-performing bed combination)	Best pileus diameter (8.5 cm); recommended as most efficient overall
Wheat grain	Slower than bajra	BE 95.50%; yield ~ 955 g/bed	Fastest primordial initiation (17.3 days); longest stalk length (17.3 cm)
Paddy grain	Slower than bajra	Lower than bajra/wheat in this trial	Latest primordial initiation recorded (18.7 days)
Sorghum grain	Fast	Lower yield than bajra in this trial	Fastest spawn run on bed in combination with paddy straw (22.3 days)

A similar advantage of sorghum grain was reported by Senthilnambi *et al.* [55]. In their study, sorghum-based spawn resulted in the quickest complete colonisation of the production substrate, requiring only 13.7 days. This was considerably faster than the other substrates tested, including corn, cotton seed, horse gram and cowpea, which

generally required around 19 days for complete colonisation. These findings indicate that the choice of grain substrate can have a substantial effect on the rate of mycelial establishment and may therefore influence the overall efficiency of *C. indica* spawn production (Fig. 1) [55].

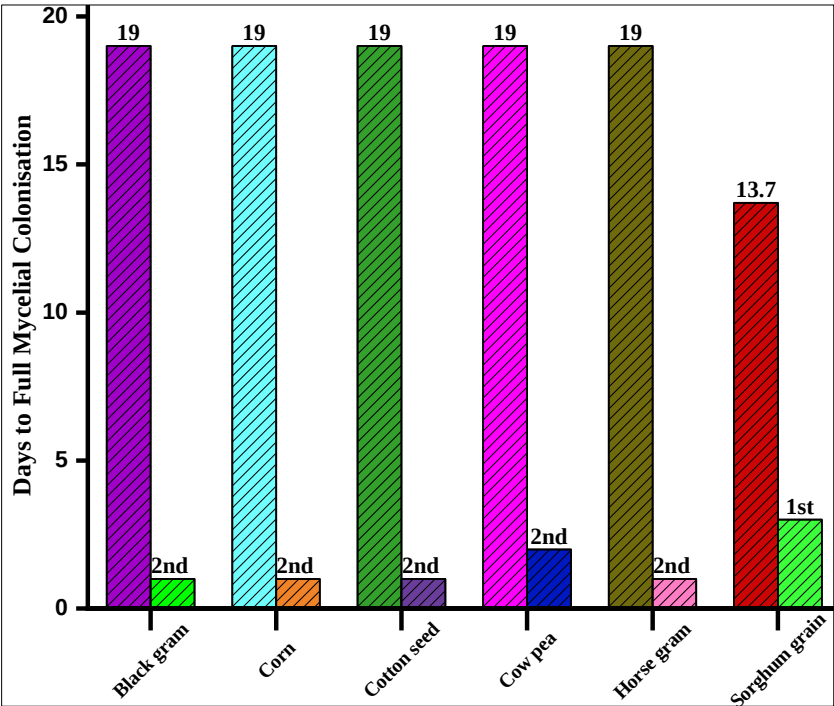


Fig 1: Spawn-run performance on grains and substrates [55].

6.3. Bulk substrates for fruiting

After the spawn has completely colonised the grain, it is transferred to a bulk substrate to support fruiting. For *C.*

indica, these substrates are generally prepared from cereal straws and other readily available lignocellulosic agricultural wastes. Among the materials evaluated by Patel and Trivedi

^[63], paddy straw proved to be the most suitable substrate. It supported the quickest spawn run (18.4 days), earliest primordial formation (25.2 days) and first harvest (32.4 days). It also produced the highest YBE(134.86%). Wheat

straw and sugarcane trash showed comparatively similar performance, with biological efficiencies of 85.07% and 85.02%, respectively (Fig. 2).

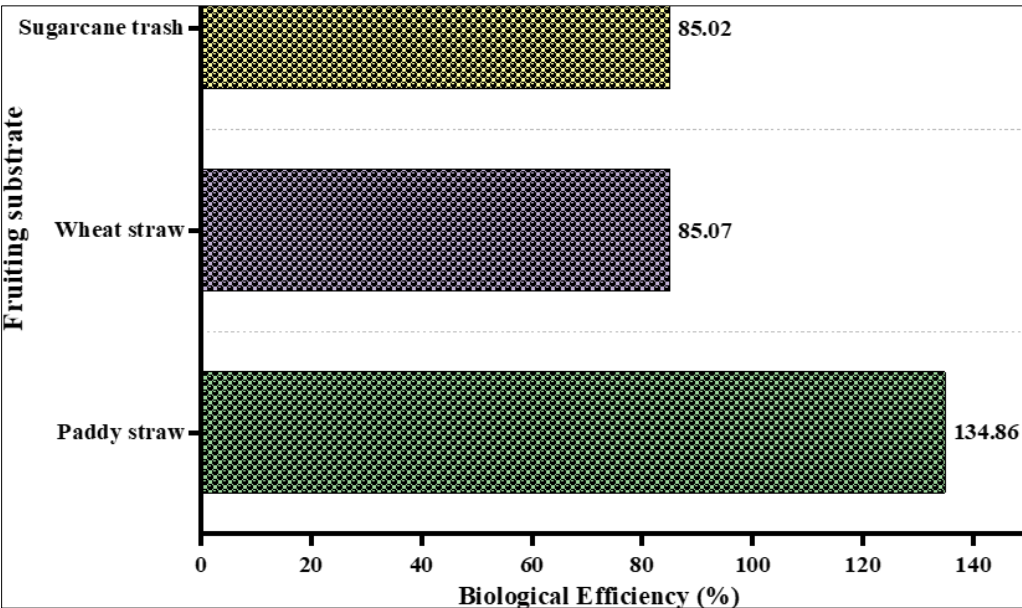


Fig 2: BE of sporophore on substrates ^[63].

Similar results were reported from a trial conducted in the Konkan region of Maharashtra, where paddy straw again performed better than the other substrates tested, recording a BE of 81.05%. Horse-gram waste and bamboo leaves followed, with biological efficiencies of 50.0% and 40.62%,

respectively, whereas the locally available wild grass gave the lowest performance (Fig. 3). These findings indicate that paddy straw is not only widely available but also provides a favourable substrate for the growth and fruiting of *C. indica* ^[64].

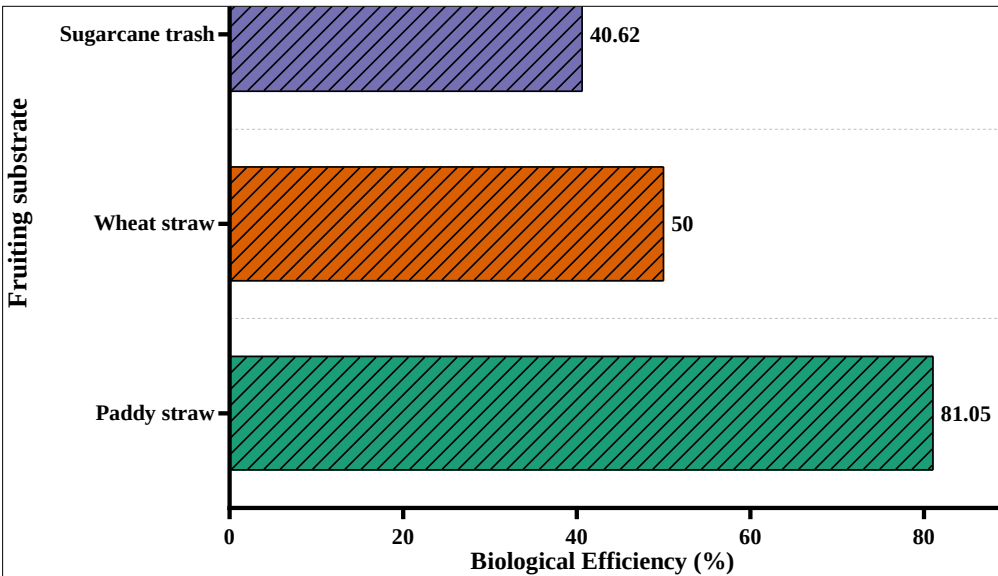


Fig 3: BE of *C. indica* on locally available substrates in a Konkan-region regional trial

Other studies have also highlighted the potential of paddy straw, either alone or supplemented with nitrogen-rich residues such as *Lathyrus* straw. Such combinations can improve mushroom production, whereas the addition of mustard residue has been associated with a substantial reduction in yield. This poor performance may be related to allelopathic or antimicrobial compounds naturally present in

Brassica residues (Fig. 4) ^[38], which can interfere with mycelial growth and fruiting ^[7, 35, 63]. Overall, the available evidence suggests that paddy straw is one of the most reliable bulk substrates for *C. indica*, although the suitability of alternative agricultural residues can vary with their nutritional composition and local availability.

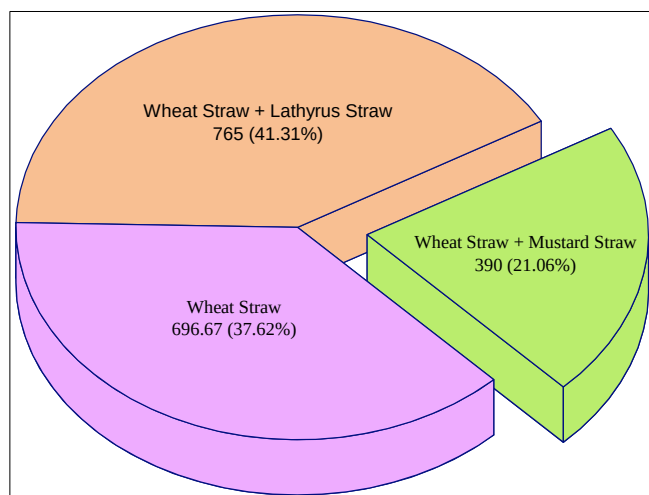


Fig 4: YBE of substrates.

Evidence for suitable fruiting substrates for *C. indica* is also emerging from countries outside India as its cultivation expands into different agro-climatic regions. In an exploratory study from the Philippines, Alvarez *et al.* [65] evaluated rice straw, banana leaves, corn husks and corn cobs, using rice hulls as the control substrate. Among the substrates tested, 100% corn husk supported the fastest mycelial colonisation, with the substrate being fully colonised within 20 days. In contrast, 100% corn cob produced the highest yield, along with larger caps, thicker stalks and the highest biological efficiency. The combination of 25% corn cob and 75% rice hulls showed the poorest overall performance. Although the study was conducted using a single strain at one location, its findings suggest that maize residues could be promising low-cost and locally available materials for *C. indica* cultivation. Since corn husk and cob had not been evaluated in the earlier Indian studies discussed above, these materials deserve further investigation, particularly in maize-producing regions where such agricultural residues are readily available [65].

7. Casing materials

Although *C. indica* is capable of producing fruiting bodies without a casing layer, the use of a suitable casing material generally improves pin formation and overall yield. Maurya *et al.* [38] evaluated different casing materials and found that a mixture of compost and vermicompost performed best, producing the highest yield (813.33 g/bed) and BE (81.33%). Vermicompost alone also performed well, with a yield of 748.33 g/bed and BE of 74.83%. In contrast, the casing prepared from garden soil and farmyard manure in a 1:1 ratio recorded the lowest yield (690 g/bed) and BE (69.0%) among the treatments tested [38].

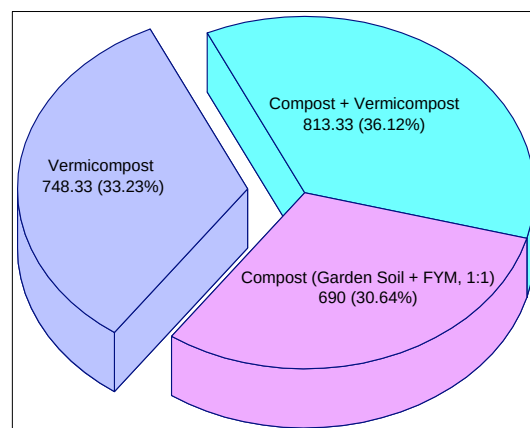


Fig 5: YBE effect of casing material [38].

Similar results have been reported from the Konkan region, where enriched casing materials such as a mixture of sand, soil and dried biogas spent slurry, as well as vermicompost alone, performed considerably better than simple soil-based casings. In these trials, the use of enriched casing materials resulted in more than twice the biological efficiency obtained from beds without an enriched casing. Taken together, these studies suggest that casing materials enriched with organic matter, particularly vermicompost, can provide a more favourable moisture and nutrient environment for fruiting and thereby improve the productivity of *C. indica* beds [17, 38]. A recent large-scale study explored the potential of spent mushroom substrate (SMS), the exhausted growing medium left after a mushroom crop has been harvested, as a casing material. This approach has considerable sustainability potential, particularly because the growing mushroom industry produces large amounts of SMS as a waste by-product. Bashir *et al.* [66] compared SMS casing with conventional loamy soil for *C. indica* cultivation. The study found that loamy soil produced a slightly higher yield and biological efficiency, with 326.2 g/bed and 78.99% BE, compared with 295.7 g/bed and 71.59% BE obtained using SMS. However, mushrooms grown with SMS casing contained considerably higher levels of copper, zinc and phosphorus. These improvements were associated with a distinct microbial community in the SMS casing, particularly bacteria belonging to the genera *Bacillus*, *Priestia* and *Lysinibacillus*. The researchers suggested that these microorganisms may contribute to phosphorus solubilisation and enhance nutrient uptake by the mushrooms. Therefore, although SMS cannot yet be considered a direct replacement for the vermicompost-enriched casing materials, it offers a promising and environmentally friendly way to reuse mushroom waste, especially when improving the mineral and nutritional quality of mushrooms is a priority rather than achieving the highest possible yield [66].

7.1. Spawn rate, age and run

In addition to the differences observed among substrates, several studies have examined how spawn rate, spawn age and spawn-run conditions influence the growth and yield of *C. indica*. Purkayastha and Nayak^[16] reported that increasing the spawn rate from 0.8 to 1.6% resulted in higher yields, indicating that spawn quantity can have a significant effect on production, independent of the substrate used. Pandey *et al.*^[56, 67] found that 21-day-old spawn, when applied at a rate of 4%, resulted in faster substrate colonisation as well as the highest number and weight of sporophores. They also observed a decline in yield as the age of the spawn increased beyond 21 days. Similarly, Tandon *et al.*^[57] identified 4% as the optimum spawn rate for achieving rapid spawn run and good sporophore production.

The material used during the spawn-run stage can also influence the speed of colonisation. Raina and Gupta^[68] compared several carriers, including paddy straw, wheat straw, maize straw, sugarcane bagasse, maize core and their combinations. They observed the fastest spawn run on paddy straw, whereas sugarcane bagasse and maize core showed slower colonisation. Bhatt *et al.*^[69], in a comparison of wheat straw, paddy straw and their different combinations, reported that wheat straw supported the quickest spawn run.

Other factors related to spawn-run management have also been shown to affect growth. Pani^[61] observed that transparent polythene bags promoted the earliest spawn run compared with other bag colours and light conditions. Kumar *et al.*^[70], while evaluating different strains of *C. indica*, found that strain CI-6 completed both spawn run and pinhead formation in the shortest time.

Overall, these studies indicate that successful *C. indica* spawn production and cultivation depend on more than simply selecting a suitable grain substrate. Spawn rate and age, the material used during spawn run, strain characteristics and container conditions can all influence colonisation speed and subsequent yield. Thus, these factors should be considered together when developing an efficient spawn-production and cultivation system for *C. indica*^[16, 56, 67, 68, 69, 70].

8. Factors affecting spawn quality and performance

8.1. Temperature and pH

Temperature plays an important role in determining how quickly and uniformly the spawn colonises the substrate. In *C. indica*, most studies have reported that a temperature range of about 25-30 °C is suitable for vigorous spawn growth, although the exact optimum may vary among strains. The pH of the substrate also affects mycelial development, with values close to neutral or slightly alkaline (approximately 7.0-7.5) generally considered favourable. This is why calcium carbonate (CaCO₃) is commonly added during grain preparation to help maintain a suitable pH for healthy mycelial growth^[5, 24, 38].

8.2. Moisture content

Moisture content is another important factor in producing good-quality spawn. If the grain does not contain enough moisture, mycelial growth can become slow and irregular. On the other hand, excessive moisture can make the grains waterlogged and sticky, creating conditions that encourage bacterial contamination and make the spawn difficult to

handle. Proper boiling of the grains, followed by sufficient surface drying before filling and sterilising the containers, is therefore essential for obtaining clean, well-colonised and easily manageable^[71, 72].

8.3. Sterilisation adequacy

Since grain is rich in nutrients, it provides an ideal environment not only for mushroom mycelium but also for many unwanted microorganisms. For this reason, inadequate sterilisation is one of the major causes of contamination during spawn preparation. Proper autoclaving is therefore essential for obtaining clean and vigorous spawn. In general, grain is sterilised at 121 °C and 15 psi for about 60-120 minutes, although the exact duration may need to be adjusted depending on the quantity of material and the size and shape of the containers used. In commercial spawn production, regularly checking the efficiency of the sterilisation process using chemical or biological indicators can further reduce the risk of contamination^[7, 38, 71, 73].

8.4. Substrate selection and particle size

The type and size of grain used for spawn can also influence how quickly and uniformly the mycelium grows. Grains such as bajra and sorghum, which are relatively small and more uniform in size, have generally been associated with faster and more even colonisation than larger or irregularly sized grains. This may be because smaller grains provide a greater surface area relative to their volume and allow the mycelium to access nutrients more readily. Thus, selecting an appropriate grain size, in addition to choosing a suitable grain type, can contribute to the production of healthy and well-colonised spawn^[7, 24, 38, 55].

8.5. Spawn rate and age

The amount and age of spawn used can have a noticeable effect on mushroom production. In general, spawn is added to the bulk substrate at around 3-5% (w/w). Using too little spawn can slow down substrate colonisation and give contaminants more opportunity to establish, whereas excessively high spawn rates may provide little additional benefit while increasing production costs. For best results, spawn should be used when it is fully colonised and still vigorous. Spawn that has been stored for too long may gradually lose its vigour and perform less effectively. These observations are consistent with the spawn-density and spawn-age experiments. Those studies showed that yield increased with increasing spawn rate up to approximately 1.6%, while spawn older than about 21 days showed a decline in yield potential. Together, these findings highlight the importance of using an appropriate spawn rate and fresh, active spawn for efficient cultivation of *C. indica*^[7, 16, 38, 56, 67].

8.6. Common contaminants

Contamination is one of the major problems encountered during mushroom cultivation, particularly when sanitation, sterilisation, or environmental conditions are not properly maintained. Some of the contaminants commonly encountered in mushroom-growing units include:

Green mould (*Trichoderma* spp.)- This is one of the most common contaminants of spawn and substrate, particularly under warm and humid conditions. Poor sterilisation and

inadequate hygiene can further encourage its development [7, 38, 74].

Bacterial wet spot or sour rot-This contamination is often associated with excessively moist grain or insufficient sterilisation. It may be recognised by a sour or unpleasant smell and a wet, slimy appearance of the affected spawn [7, 38]. Competitor moulds (*Aspergillus* and *Penicillium* spp.)-These moulds may become established when air quality and hygiene are poor during inoculation or when containers and substrates have not been adequately sterilised. Their presence can interfere with spawn growth and reduce the chances of successful colonisation [7, 38].

Beyond simply identifying these contaminants, field-level studies have also examined practical ways to control them. Kumar *et al.* [74] evaluated the management of green mould caused by *Trichoderma harzianum* in milky mushroom beds. In their *in vitro* study, three fungicides were tested and the carbendazim-based product Bavistin was found to be comparatively less harmful to *C. indica* mycelium, while still providing 67% inhibition of the pathogen at 200 ppm. The botanical product Nimbicidin performed even better, giving 90.68% inhibition at a 4% concentration.

Under cultivation conditions, both Bavistin (150 ppm) and Nimbicidin (4%) improved mushroom production compared with untreated beds infected with *Trichoderma*. Bavistin-treated beds recorded a yield of 351 g per bag, compared with only 107 g per bag in the untreated infected control and also showed a shorter spawn-run period. Nimbicidin produced comparable results and was statistically at par with Bavistin. These findings are particularly useful for growers and spawn producers because they demonstrate that *Trichoderma*

contamination can be managed effectively and that a botanical treatment such as Nimbicidin may provide a useful alternative to conventional fungicides [74].

9. Recent developments and emerging research directions

Over the past five years, research on *C. indica* has expanded well beyond the grain and substrate comparisons that characterised much of the earlier literature discussed. Recent studies are increasingly exploring several interconnected areas that could shape the future of milky mushroom cultivation and utilisation. Four major research directions are particularly evident: (i) molecular characterisation and breeding of high-yielding strains, including the development of the first whole-genome assembly for the species; (ii) the use of alternative casing and fruiting-substrate materials, particularly those that make productive use of agricultural and other locally available wastes; (iii) improved understanding and management of the contaminants through biological and chemical control approaches; and (iv) growing research into the bioactive, nutritional and nutraceutical properties of the fruiting body.

Together, these developments indicate a gradual shift in *C. indica* research from conventional production optimisation towards a broader approach that combines improved strains, sustainable cultivation practices, crop protection and value addition. The increasing attention given to nutritional and bioactive properties is also important because these characteristics may contribute directly to consumer interest and market demand, rather than being viewed simply as additional benefits of cultivation. Table 4, summarises the major quantitative findings from the recent literature.

Table 4: Recent research findings relevant to *C. indica* spawn production and cultivation

Recent research focus	Key quantitative finding	Reference
Molecular strain screening (33 wild strains, SRAP markers)	8 strains ≥10% higher-yielding than check; top strain DMRO-299: 64.08% BE	Nath <i>et al.</i> (2023) [49]
Multilocation strain trial (new strain DMR Milky-985)	55.7% BE vs 52.6% (check strain DMRO-334); +5.6% yield	Sharma <i>et al.</i> (2020) [50]
Spent mushroom substrate (SMS) as casing	71.59% BE (SMS) vs 78.99% (loamy soil); SMS casing enriched Cu, Zn and P in fruit bodies	Bashir <i>et al.</i> (2025) [66]
Alternative fruiting substrates (Philippines trial)	100% corn cob gave highest yield, cap size and BE; 100% corn husk fastest colonisation (20 days)	Alvarez <i>et al.</i> (2025) [65]
Chemical/botanical control of green mould (<i>Trichoderma harzianum</i>)	Nimbicidin (4%): 90.68% pathogen inhibition <i>in vitro</i> ; Bavistin-treated beds 351 g/bag vs 107 g/bag (untreated)	Kumar <i>et al.</i> (2017) [74]
Whole-genome sequencing	Near chromosome-scale assembly (Nanopore + Illumina)	NCBI BioProject (2024) [51]

Kumar *et al.* [75] broadly highlight several advantages of *C. indica*, including its relatively simple cultivation requirements, longer post-harvest shelf life of around 5-7 days compared with many other cultivated mushrooms and its promising nutraceutical properties. At the same time, they identify the choice of casing material and the maintenance of suitable environmental conditions during cropping as two of the major practical challenges faced by growers. These observations are consistent with the problems related to spawn and casing quality identified throughout the primary studies reviewed in this work.

Taken together, the recent research indicates that the basic grain-spawn production method established by earlier studies continues to be reliable and widely applicable. However, research is increasingly moving beyond the basic cultivation protocol towards strain-level improvement, the use of more sustainable production inputs and the development of new

value-added products and market opportunities [75].

10. Constraints and research gaps

Although research on *C. indica* cultivation has expanded considerably, several important gaps still need to be addressed. Most published studies comparing spawn and substrate treatments in *C. indica* have been conducted at a single location and during a single season, often using only a small number of strains. This makes it difficult to determine whether the reported results can be consistently applied across different agro-climatic regions [24, 38, 55]. Grain spawn remains the most widely studied system, while sawdust-based spawn and liquid spawn have received comparatively little attention in *C. indica*. Further research on these alternatives could help diversify raw-material sources and, particularly in the case of liquid spawn, potentially reduce production time [5, 38].

Reliable, strain-specific information on how long *C. indica* grain spawn can be stored under different temperature conditions is still limited. This creates difficulties for commercial spawn producers who need clear and evidence-based recommendations on storage periods and use-by dates [5, 7]. There are relatively few detailed economic studies comparing different spawn and substrate options specifically for *C. indica*. Factors such as grain cost, labour requirements and losses caused by contamination can substantially influence production costs, particularly when grain prices fluctuate [7, 38]. Molecular approaches could provide faster and more reliable ways of verifying strains and detecting contaminants in spawn production laboratories. However, such tools still need to be adapted, validated and made practical for routine use in *C. indica* cultivation [5]. Some progress has already been made in addressing these gaps. A genomic resource for *C. indica* is now publicly available, while recent multilocation strain trials have provided useful benchmarks for comparing strain performance. The next challenge is to turn these advances into practical breeding methods and routine laboratory-level quality-control tools that can be readily adopted by commercial spawn producers [49, 50, 51].

Overall, future research should move beyond short-term, small-scale cultivation trials and place greater emphasis on multilocation validation, strain-specific optimisation, alternative spawn technologies, storage stability, production economics and molecular quality control. Addressing these areas would help make *C. indica* spawn production more consistent, efficient and suitable for large-scale commercial cultivation.

11. Future prospects

The consistent performance of pearl millet (bajra) and sorghum grains in supporting rapid and efficient spawn run suggests several promising areas for future research. One possibility is to develop grain-blend spawn, for example by combining bajra with a slower-growing but nutrient-rich grain. Such combinations may help maintain the rapid colonisation associated with bajra while improving yield consistency under different seasonal conditions. Liquid-spawn technology also deserves greater attention in *C. indica*. Studies examining suitable nutrient media, agitation conditions and transfer methods could help reduce the time required to produce vigorous, field-ready spawn. This would be particularly useful for commercial nurseries, where faster spawn production could increase overall production efficiency [28].

Further work is also needed to test enriched casing materials, especially vermicompost-based formulations, under different locations and growing conditions. Repeated validation across seasons and regions could help develop more reliable casing recommendations for growers. At the production level, the introduction of simple, field-friendly tests for checking spawn purity and vigour could also be valuable. Such quality-control measures may help growers identify poor-quality spawn before it is used, thereby reducing avoidable crop losses [24, 38, 55].

The availability of a genome assembly for *C. indica* creates another important opportunity for future research. Marker-assisted selection could potentially be used to identify high-yielding and rapidly colonising strains at an earlier stage,

including strains similar to those reported by Nath *et al.* [49] and Sharma *et al.* [50]. This could make strain improvement more efficient and reduce the dependence on lengthy multilocation phenotypic screening alone. At the same time, the growing evidence for the antimicrobial, antioxidant and anti-genotoxic properties of *C. indica* [15, 19] points towards opportunities beyond fresh-mushroom production. Further research could support the development of value-added and nutraceutical-oriented products, potentially create additional sources of income and improve the overall economic potential of *C. indica* cultivation. Together, advances in spawn technology, strain improvement, quality control and value addition could make the cultivation system more efficient, reliable and commercially attractive for growers.

12. Conclusion

Spawn is one of the most important factors in the successful cultivation of *C. indica*, as the type of spawn substrate can influence how quickly the mushroom colonises the growing medium, as well as the final yield and biological efficiency. The studies reviewed in this work suggest that pearl millet (bajra) and sorghum grains are particularly suitable for producing healthy and vigorous spawn. Among the different materials tested for bulk cultivation, paddy straw has consistently shown better results than wheat straw, sugarcane trash and several other agricultural residues. The use of improved casing materials, especially those containing vermicompost or compost-vermicompost mixtures, can also increase mushroom production compared with simple soil-based casing [28].

However, good results do not depend on the choice of substrate alone. Maintaining proper hygiene and aseptic conditions, ensuring adequate sterilisation, controlling moisture and using fresh, vigorous spawn at the right time are equally important for achieving reliable and consistent production. These basic practices form the foundation of successful milky mushroom cultivation.

There is still considerable scope for further research. Spawn production systems such as liquid spawn and combinations of different grains need more detailed investigation, while substrate and casing recommendations should be tested under different climatic and growing conditions. Such studies could help make cultivation practices more dependable and economically viable for farmers and growers in tropical and subtropical regions.

Recent research has further expanded our understanding of *C. indica*. Differences among strains at the genetic level have been identified, creating opportunities for selecting and developing better-performing strains [49, 50]. The availability of genomic information is another important step forward, as it can support future research and breeding programmes [51]. Studies on green-mould contamination have also provided useful information for improving contamination management during spawn production and cropping [74]. At the same time, growing evidence of the nutritional and nutraceutical value of milky mushroom adds to its importance as a potential food and health-promoting crop [15, 19].

Overall, *C. indica* has considerable potential as a commercially important mushroom because of its ability to grow under warm conditions and its nutritional value. By combining suitable spawn, locally available substrates, improved casing materials and careful cultivation practices,

growers can achieve better and more consistent production. Continued research, particularly on strain improvement, spawn technology, contamination control and cultivation under different environments, will help unlock the full potential of this promising mushroom and contribute to more sustainable and profitable mushroom farming.

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