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Toward improved spawn quality: Optimizing corn grain preparation for *Volvariella volvacea*

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Abstract

Volvariella volvacea is a commercially important mushroom in tropical agriculture, yet its cultivation efficiency is often limited by inconsistent grain spawn quality. Factors such as unsuitable soaking duration and suboptimal supplementation with calcium carbonate ($CaCO_3$) can hinder mycelial colonization, thereby reducing yield and productivity. Therefore, optimizing grain spawn preparation is essential to enhance the growth performance and productivity of this mushroom. This study investigates the effects of soaking duration and calcium carbonate ($CaCO_3$) concentration on the mycelial growth of *V. volvacea* grain spawn. Corn grains were soaked in boiling distilled water for 1 to 5 min, with soaking in room-temperature distilled water for 6 hours used as the control. Grains soaked for 3 to 5 min showed significantly improved mycelial extension (ranging from 2.171 to 2.343 cm) and reduced colonization duration (6 days), with visibly denser mycelial growth, as assessed through qualitative observation. These optimal soaking durations were then used to assess the effect of different concentrations of $CaCO_3$ (1, 3, and 5% w/w). Grains treated with 3% $CaCO_3$ exhibited highly favorable mycelial density. Thus, these findings recommend a 5-min soaking duration combined with 3% $CaCO_3$ for optimal grain spawn preparation, facilitating rapid and dense mycelial growth. This optimized method provides a practical framework for enhancing colonization speed and improving spawn quality in *V. volvacea*, thereby increasing the efficiency and reliability of corn grain spawn preparation.

Keywords: Calcium carbonate, Grain spawn, Mycelial growth, Soaking duration, *Volvariella volvacea*

1. Introduction

Mushrooms have existed for centuries, and many edible mushrooms are found growing in the wild and in non-controlled environments. With the advancement of modern society, the demand for this highly nutritious food has skyrocketed, particularly in efforts to fight against food scarcity in developing countries. To meet the ever-increasing demand for mushrooms, its production has become a highly technical process which requires expertise and specialized knowledge [1]. The role of biotechnology in the mushroom industry is crucial, as growers increasingly depend on *in-vitro* propagation methods using tissue culture. However, less knowledgeable and skillful growers often face challenges such as contaminated growth substrates, unavailability of high-quality mushroom spawn, and large variations in mushroom yields [2]. Mushroom grain spawns can be defined as pasteurized grains that have been fully colonized with mycelium. The preparation of mushroom grain spawn is regarded as the most important part in mushroom production as it serves as the initial inoculum for mushroom cultivation, impacting yield and growth rates [3].

Unlike oyster mushroom (*Pleurotus* spp.) and black jelly (*Auricularia auricula*), for which growers use grain spawn as commercial spawn, paddy straw mushroom (*Volvariella volvacea*) growers have traditionally used and sold substrate spawn as commercial spawn. It was not until 2016 that the use of grain spawn for *V. volvacea* was

locally introduced as part of efforts to increase production volume and reduce cost [4]. Various types of grains can be effectively utilized to produce mushroom grain spawns, including millet, sorghum, wheat, and corn [1,5]. Due to its availability and abundance, corn is widely used for grain spawn production. It also contains carbohydrates, proteins, sugars and vitamins to yield mushroom mycelia faster, thicker and denser [6].

Several previous studies have investigated the role of physical and chemical pretreatments in improving grain spawn quality. For example, boiling and pH adjustment of millet and corncob mixtures were reported to enhance colonization rates and substrate quality [7]. Similarly, sorghum grains boiled for 15–25 min and adjusted to pH 7.8–7.9 achieved rapid colonization within 10 days [8], while longer boiling durations of around 30 min were also shown to promote faster colonization compared to shorter treatments [9]. Together, these findings highlight the importance of both heat treatment and pH adjustment in influencing mycelial growth across different grain types.

However, despite evidence from related cereals, limited research has specifically addressed corn grain spawn preparation for *V. volvacea*, particularly in the combined context of short boiling treatments and $CaCO_3$ supplementation. As spawn quality is critical for reliable cultivation, there remains a need to refine preparation methods to enhance colonization efficiency and mycelial vigor. Therefore, this study evaluated the effects of soaking duration and $CaCO_3$ concentration on the mycelial growth of *V. volvacea* in corn grain, with the aim of improving colonization speed and overall spawn quality.

2. Materials and methods

2.1 Materials

The materials used included coarse corn grain (Grade B), which has minor imperfections such as slight discoloration or small cracks but is free from mold or major defects. Other materials included limestone ($CaCO_3$), distilled water and potato dextrose agar (PDA) (PDA; Oxoid, UK). The *V. volvacea* fruiting bodies were provided by the Malaysian Nuclear Agency.

2.2 Experimental Design

This study adopted a sequential experimental design to optimize grain spawn preparation for *V. volvacea*. The approach was conducted in two distinct stages, consistent with widely applied optimization strategies in biological research [10-13].

Stage 1 – Single-factor screening: The first stage evaluated the effect of soaking duration on mycelial growth, with $CaCO_3$ concentration fixed at 1% w/w, calculated relative to the dry grain weight (e.g., 1 g $CaCO_3$ per 100 g grains). Six treatments were tested: a 6-hour soak in room-temperature distilled water (control) and boiling-water soaks for 1, 2, 3, 4, and 5 min. For each soaking duration, 3 replicates were prepared, each comprising 6 test tubes. Mycelial extension, colonization duration, and mycelial density were recorded to identify durations that promoted rapid and dense colonization.

Stage 2 – Targeted two-factor optimization: The soaking durations that performed best in Stage 1 (3, 4, and 5 min) were selected for further testing in combination with three $CaCO_3$ concentrations (1, 3, and 5% w/w). The same replication structure as Stage 1 was applied, 3 replicates per treatment, each with 6 test tubes. In addition to recording mycelial extension, colonization duration, and mycelial density, the pH of the grain substrate was measured prior to inoculation using MQuant® pH-indicator strips to assess the effect of $CaCO_3$ on substrate alkalinity. This targeted design allowed the assessment of pH adjustment effects within the most biologically relevant soaking time range, optimizing resource use while generating results applicable to practical spawn production.

2.3 Initiation of Mother Culture

The fruiting bodies of *V. volvacea* were carefully dissected to expose the fresh interior stem tissue. Alcohol-soaked cotton swab was used to remove any contaminants or damaged tissue. The stem tissue was then excised using a scalpel under aseptic conditions to prevent contamination. Subsequently, the fragment was placed in a petri dish containing PDA and labelled as S0. The dish was then sealed with parafilm and incubated at room temperature (RT 25°C). After incubation, the initial culture (S0) was observed for growth. Once mycelial growth was established, the mother culture was propagated to generate subsequent generations (S1 to S3). Each generation was transferred to fresh PDA plates to maintain growth. The S3 culture was then selected as the mother culture to prepare the grain spawn (Figure 1).

Figure 1 The mother culture (S3) of *Volvariella volvacea*.

2.4 Grain Spawn Preparation

The preparation of corn grain spawn aimed to optimize the soaking duration of the corn grains and the concentration of $CaCO_3$ to enhance mycelial growth. The corn grains were soaked for varying durations: the control group was soaked in distilled water for 6 hours, while other groups were soaked in boiling water for 1 to 5 min. After determining the optimal soaking duration, the next step was to optimize the $CaCO_3$ concentration by testing 1, 3, and 5% w/w concentrations. Following the soaking process, the grains were sterilized and inoculated with a *V. volvacea* culture under aseptic conditions. The inoculated grains were then incubated to allow mycelial colonization. This method was used to identify the optimal soaking duration and $CaCO_3$ concentration for promoting fungal growth and fruiting body production.

2.4.1 Optimization of Soaking Durations

Corn grains were thoroughly washed under clean running water to remove debris and dirt before soaking. The grains were then subjected to six different soaking durations: 6 hours in room-temperature distilled water (control) and 1 to 5 min in boiling distilled water. Distilled water was boiled to 100°C and subsequently used for soaking the grains. Excess water was drained from the soaked grains by gentle tossing. The grains were then mixed with 1% w/w $CaCO_3$. The treated corn grains were poured into 150 × 18 mm test tubes, filling them to approximately 5 cm in height as shown in Figure 2. For each soaking duration, the test tubes were divided into three replicates, with six test tubes in each replicate. The test tubes were autoclaved at 121°C and 1 atm for 20 min, then allowed to cool down (RT 25°C). Subsequently, a 1×1 cm^2 mycelium plug from S3 mother culture was transferred onto the surface of the grains in the test tube under aseptic conditions. The grain samples were then incubated in a well-ventilated room (RT 25°C). Observations focused on the duration of mycelium colonization. Treatments where grains exhibited faster mycelium growth were advanced to the next step: optimizing $CaCO_3$ concentration.

Figure 2 Grain spawn.

2.4.2 Optimization of $CaCO_3$ Concentrations

The treatment of corn grains soaked in boiled distilled water for 3, 4, and 5 min were used to optimize $CaCO_3$ concentrations. Similar preparations were applied to these treatment groups, with varying $CaCO_3$ concentrations; 1% (control), 3%, and 5% w/w. For each treatment group, the test tubes were divided into 3 replicates, with 6 test tubes per replicate. The test tubes were autoclaved at 121°C and 1 atm for 20 min, then allowed to cool to room temperature. Subsequently, a 1×1 cm^2 of S3 mycelium plug was placed onto the upper surfaces of the corn grains in the test tubes under aseptic conditions. The grain spawns were then incubated in a well-ventilated room (RT 25°C).

2.5 Data Collection and Analysis

The mycelial growth was monitored every 2 days over a span of 2 weeks. Quantitative parameters, including growth performance, average growth rate, average mycelial extension, and colonization duration, were assessed. The average mycelial growth rate was determined using equation (1). Qualitative evaluations, such as mycelial density, were conducted visually. Mycelial density was scored using a three-level visual scale: (+) low density – sparse, uneven colonization with visible gaps between mycelial threads; (++) moderate density – partial surface coverage with moderate thickness; (+++) very dense – uniform and thick mycelial mat fully covering the grain surface. Data from each group of six test tubes were averaged into a single biological replicate, giving $n = 3$ per treatment. All values are presented as mean ± standard deviation (SD). The means of the quantitative data were analyzed using one-way ANOVA, and any significant differences between treatments were determined using Tukey's HSD test at $p \leq 0.05$. The statistical analysis in this study was performed using the Statistical Package for Social Sciences (SPSS), version 30 (IBM, USA).

$$Growth\ rate = \frac{Total\ average\ mycelial\ growth\ (current\ day) - Total\ average\ mycelial\ growth\ (previous\ day))}{2\ (days)} \quad (1)$$

3. Results and Discussion

3.1 Effects of Soaking Duration on Volvariella volvacea Mycelial Growth

The effect of soaking treatment on mycelial extension, colonization duration, and mycelial density is presented in Table 1. The mycelial extension of grains soaked in boiled distilled water for 3 min (2.300±1.980 cm), 4 min (2.171±2.046 cm), and 5 min (2.343±2.096 cm) showed significant improvement, with nearly double the mycelial extension compared to the control (1.212±1.188 cm). This improvement is also evident in the colonization duration, which was reduced by almost 50%, from 11.50±1.95 days (control group) to 6 days (3 to 5 min groups). The growth rate of *V. volvacea* is shown in Figure 3. The mycelial growth rate for all treatments exhibited an identical peak time at day 4. However, grains soaked in boiling distilled water for 3 to 5 min showed vigorous growth activity, recording the highest average growth rates (1.10, 1.35, and 1.50 cm/day, respectively) and resulting in the fastest colonization duration. In contrast, the control treatment displayed a relatively slow average growth rate throughout the observation period, with multiple peaks at days 4 and 8. Grains soaked in boiling distilled water for 1 minute showed a peak at day 4 but exhibited a decreasing growth pattern, thereafter, making it one of the slowest treatments to fully colonize the grain (11.47±1.34 days). Vigorous and rapid mycelial colonization is desirable since it increases the likelihood of outgrowing competitors, reduces cropping time, and allows for earlier harvesting [14-15].

The effect of boiling the corn grains may improve the morphology of the grains, which contributes positively to the compactness and aeration of the grain spawn. Poor aeration can lead to inefficiency in the mycelium's ability to fully utilize the nutrients provided by the grains, potentially stunting its ability to exhibit vigorous and rapid growth [16]. Additionally, a study by Kumar et al. [17] showed a positive interaction between boiling sorghum grains and the mycelial growth performance of *Agaricus bisporus*, further supporting the findings of this paper. The importance of high-quality grain spawn preparation is also highlighted in a study by Devi and Sumbali [18], which demonstrated that vigorous mycelial growth is positively linked to the duration of pinhead formation, yield, and biological efficiency (BE) of *Microcybe gigantea* (Massee). The mycelial density of *V.volvacea* grown on corn grains soaked in boiled distilled water for 2 to 5 min was notably high compared to control. High mycelial density is favorable as it enables faster colonization of solid waste during cultivation [19].

Furthermore, grain moisture is an important factor to get a successful spawn as it facilitates nutrient absorption and supports active mycelial development [6]. In this study, soaking corn grains in boiling distilled water for extended time (3-5 min) likely enhanced grain hydration, thereby creating more favorable internal environment for mycelial growth. It is also important to consider that physical size of the grain substrate. Previous studies have shown that smaller kernels fractions tend to facilitate better mycelial growth due to their increased surface area and ease of colonization [20]. However, the present study used Grade B coarse corn kernels, which likely have a

denser structure and lower initial permeability. As a result, a longer soaking duration was necessary to sufficiently hydrate the grains and enable optimal mycelial penetration and expansion. Among the treatments, soaking for 5 min produced the longest mycelial extension, fastest colonization duration and moderate mycelial density – factors that are collectively beneficial for producing robust and competitive spawn.

Table 1 Effects of different soaking durations on the mycelial extension, colonization duration, and mycelial density of *Volvariella volvacea* on corn grain spawn.

Treatments	Mycelial extension (cm)	Colonization duration (days)	Mycelial density
Control	1.212 ± 1.188[a]	11.50 ± 1.95[a]	+
1 min	1.092 ± 1.081[a]	11.47 ± 1.34[a]	+
2 min	2.249 ± 1.959[b]	6.11 ± 0.46[b]	++
3 min	2.300 ± 1.980[b]	6.00[b]	++
4 min	2.171 ± 2.046[b]	6.00[b]	++
5 min	2.343 ± 2.096[b]	6.00[b]	++

Note: Values are means ± SD. Means with the same letters are not significantly different at $p < 0.05$. Mycelial density was assessed qualitatively: + = low, ++ = moderate, +++ = very dense.

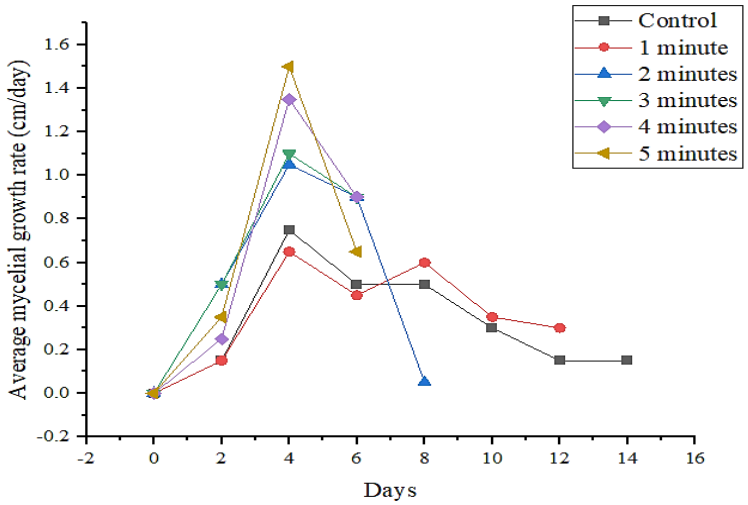

Figure 3 Mycelial growth rate of *Volvariella volvacea* on corn grain spawn subjected to different soaking durations (1–5 min in boiling distilled water and 6-hour room temperature control).

3.2 Effects of $CaCO_3$ Concentrations on Volvariella volvacea Mycelial Growth

Corn grains soaked in boiling distilled water for 3 to 5 min were selected based on superior mycelial extension, colonization duration, and mycelial density. Grains treated with 1% $CaCO_3$ (control) were compared to those treated with 3 and 5% $CaCO_3$. The pH of treated grains, measured using MQuant® pH-indicator strips, is shown in Table 2.

Table 2 The pH of grain treated with different concentrations of $CaCO_3$.

$CaCO_3$ Concentration (%)	pH
1	5.0
3	5.5
5	6.0

Table 3 and Figure 4 provide a comprehensive understanding of the effects of soaking duration and $CaCO_3$ concentration on the growth performance of *V. volvacea*. Across all treatments, there were no statistically significant differences in final mycelial extension (Table 3), however, Figure 4 reveal that certain treatments supported faster early-stage development, especially up to day 4, which is crucial for rapid substrate colonization. Even though the 4 min, 5% $CaCO_3$ treatment showed the highest peak growth rate (~2.0 cm/day on day 4), this treatment however did not record the shortest colonization time (5.67±0.75 days) and had only moderate mycelial density (++). Despite having only moderate mycelial density (++), the colonization speed of 5 min, 5% $CaCO_3$ treatment offers a distinct cultivation advantage with significantly faster colonization (4.76±0.98 days) along with respectable growth rate (~1.85 cm/day on day 4).

Higher mycelial densities (+++) were recorded under 3% $CaCO_3$ treatments for grains soaked for 3, 4, and 5 min. These findings suggest that moderate $CaCO_3$ concentrations enhance colonization speed and mycelial vigor, likely by optimizing substrate pH and improving nutrient availability which is in line with the findings of Akinyele and Adetuyi [21]. Fast and dense colonization is beneficial for commercial mushroom production by reducing the risk of contamination and shortening the cultivation period. While 5% $CaCO_3$ treatment at 5 min showed the fastest colonization time, the lower mycelial density might affect subsequent stages like pinhead formation and yield. Therefore, the 3% $CaCO_3$ treatment at 5 min offers a better balance with significantly fast colonization time (5.64±0.78 days), high and sustained growth rate (~1.85 cm / day) and very dense mycelium which are desirable for substrate colonization and subsequent fruiting body formation. The role of limestone ($CaCO_3$) is not limited to regulating the pH of the grains. Calcium is also essential as it is believed to stimulate and induce various intracellular effects that influence growth, sporulation and differentiation. It was shown in a previous study that shiitake (*Lentinula edodes*) treated with $CaCO_3$ exhibited improved yield and biological efficiency [15].

Table 3 Effects of different $CaCO_3$ concentrations on mycelial extension, colonization duration, and mycelial density of *Volvariella volvacea* on corn grain spawn soaked for 3, 4, and 5 min.

Duration (min)	$CaCO_3$ Concentration (%)	Mycelial extension (cm)	Colonization duration (Days)	Mycelial density
	1	2.300 ± 1.980^a	6.00^a	++
3	3	2.557 ± 2.019^a	5.89 ± 0.46^b	+++
	5	2.540 ± 2.040^a	6.00^a	++
	1	2.171 ± 2.046^a	6.00^a	++
4	3	2.278 ± 2.071^a	5.97 ± 0.24^a	+++
	5	2.501 ± 2.106^a	5.67 ± 0.75^b	++
	1	2.343 ± 2.096^a	6.00^a	++
5	3	2.400 ± 2.135^a	5.64 ± 0.78^b	+++
	5	2.355 ± 2.227^a	4.76 ± 0.98^c	++

Note: Values are means ± SD. Means with the same letters are not significantly different at $p < 0.05$. Mycelial density was assessed qualitatively: + = low, ++ = moderate, +++ = very dense.

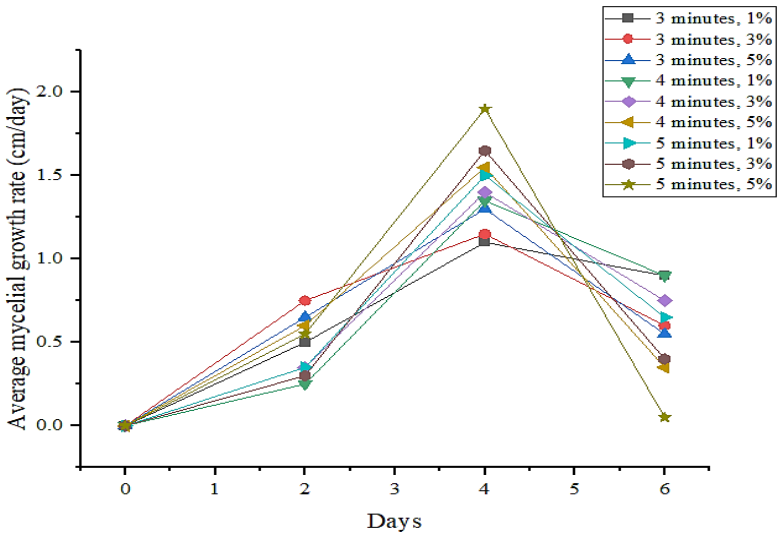

Figure 4 Growth rate of *Volvariella volvacea* on corn grain spawn treated with varying $CaCO_3$ concentrations (1%, 3%, and 5%) following different soaking durations.

4. Conclusions

This study highlights the significant effects of soaking duration and calcium carbonate ($CaCO_3$) concentration on the mycelial growth performance of *V. volvacea* on corn grains substrate. Soaking the grains in boiling distilled water for 3 to 5 min markedly improved mycelial extension, enhanced mycelial density and reduced colonization duration compared to control group which required over 11 days to achieve full colonization with sparse mycelial density.

Further enhancement was observed when treated with 3% to 5% of $CaCO_3$. Among all treatments, soaking corn for 5 min with 3% $CaCO_3$ resulted in the best overall performance: high mycelial extension, reduced colonization time and very dense mycelial growth. This treatment also consistently maintained high growth rates and reached peak performance around day 4 – indicating early and vigorous colonization.

These findings underscore the importance of both physical and chemical pretreatments of grain substrates in improving the quality of mushroom spawn. The combination of boiling and appropriate pH adjustment resulted in faster colonization and denser mycelial growth of the grain spawn. This optimized method provides a practical framework for enhancing colonization speed and improving spawn quality in *V. volvacea*, thereby increasing the efficiency and reliability of grain spawn production.

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