

Comparative Analysis of Different Substrate Pasteurization Methods on the Growth Performance of *Pleurotus pulmonarius* (Oyster Mushroom)

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Abstract

Pasteurization of substrate is the process of reducing the number of microorganisms in a growing substrate. This process gives mycelium a competitive advantage over destructive microorganisms, allowing it to colonize the substrate and ultimately produce mushrooms. This study evaluated the effects of three different substrate pasteurization methods: boiling, hydrated lime, and sodium hypochlorite, on the growth performance of *Pleurotus pulmonarius*. Sawdust mixed with rice bran and calcium carbonate was treated with each method, then inoculated with prepared sorghum grain spawn. Several factors were monitored, including spawn run time, harvest time, primordial initiation, diameter of pileus, length of stipe, total yield, and biological efficiency. The results indicated that boiling significantly improved mushroom performance, resulting in the shortest colonization period of 14 days, the highest total yield of 550 grams, and the greatest biological efficiency of 11.22%. In comparison, the hydrated lime and sodium hypochlorite treatments produced lower yields of 425 grams and 420 grams, and biological efficiency of 8.5% and 8.4%, respectively. This study concludes that boiling is the most effective pasteurization method among the three, as it supports optimal mycelial development and fruiting. These findings are consistent with previous research that advocates for thermal pasteurization as a reliable strategy for enhancing mushroom cultivation outcomes in low-resource settings.

Keywords: *Pleurotus pulmonarius*, substrate, pasteurization, growth performance

Citation

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Introduction

Mushrooms are edible fungi and are fleshy, spore-bearing reproductive structures that are cultivated on organic substrates and have been used for healing purposes for thousands of years (Etich et al., 2013). Additionally, mushrooms can be used for dyeing wool and other natural fibres. Before the invention of synthetic dyes, they were a primary source of textile dyes (Mussak & Beehold, 2009). Mushrooms are rich in protein, chitin, minerals, and vitamins, containing all the essential amino acids required by humans (Reiset al., 2018).

Pleurotus pulmonarius is particularly effective at degrading lignin, enabling it to thrive on various lignocellulosic materials. The cultivation of this

mushroom is straightforward and employs low-cost production technology, which promotes consistent growth and high biological efficiency. It can grow well in varying temperature conditions, making it ideally suited for year-round cultivation in different regions, including countries like Nigeria (Akinyele & Adejumo, 2017).

Substrate pasteurization is the process of reducing the number of microorganisms in a growing substrate. This process gives mycelium a competitive advantage over harmful microorganisms, allowing it to colonize the substrate and ultimately produce mushrooms. While there are various techniques for pasteurization, boiling is the most common. Other methods include steaming, cold pasteurization, and the use of certain

chemicals. Pasteurizing straw helps create a suitable mushroom substrate and controls unwanted mushroom or fungal weeds due to its antimicrobial properties. Effective pasteurization is crucial for promoting the growth of *Pleurotus pulmonarius* mycelium, leading to a good yield and high-quality mushrooms. In their study on substrate pasteurization for growing *Pleurotus ostreatus*, Sánchez et al., (2011) noted that raw substrates are usually not clean enough for cultivating white oyster mushrooms. One common contaminant, *Trichoderma* spp. (green mold) causes significant losses in mushroom cultivation. This fungus is particularly prevalent during the early stages, especially during the spawn run, but can also appear during the cropping period. When properly prepared, oyster mushrooms grow very well on pasteurized substrates.

Azeez et al. (2019), in their study on the nutritional benefits of mushrooms and the challenges of mushroom production, noted that in Nigeria, there is a growing demand for alternative sources of food and medicine. According to Earnshaw et al., (2012), in their study on the growth and yield of oyster mushrooms cultivated on different substrates with varying levels of wheat bran, it was found that pre-treatment of substrates is essential to minimize contamination.

The pre-treatment for mushroom cultivation aims to exclude unwanted microorganisms while promoting the growth of mushroom mycelium. It was emphasized that the substrate should be cleaned and treated through pasteurization to suppress or inhibit competing fungi. Research conducted by Colavolpe et al., in 2014 examined various treatment methods, including immersion in hot water, steam sterilization, and immersion in alkaline water. They recommended two effective treatments for controlling *Trichoderma*: immersing the substrate in hot water at 60 °C for 30 minutes and in 5% alkaline water for 36 hours. The study aimed to compare the effectiveness of boiling, hydrated lime, and sodium hypochlorite solution as methods of substrate pasteurization, evaluating their impact on the performance of *Pleurotus pulmonarius*.

Materials and Methods

Pleurotus pulmonarius spawn cultures were obtained from the Biotechnology Department of the Federal Institute of Industrial Research, Oshodi, Lagos. Melina tree sawdust was obtained from the Sawmill, Ilaro, Ogun State. Heat-resistant plastics, sorghum, and rice bran were purchased from Sayedero market in Ilaro, Ogun State.

Preparation of Spawn Grain

Preparation of the spawn grain was done according to the method of Okwulehie et al., (2018). Sorghum grain was rinsed to remove impurities, and was soaked for 12 hours, and then boiled for 5 minutes in a muslin cloth. After draining and cooling, 250 grams of the sorghum was mixed with 2 grams of calcium carbonate and a vitamin B complex tablet in heat-resistant plastic bottles. The openings were cotton-plugged and covered with aluminium foil, then pasteurized for 3 hours in an oil drum and allowed to cool. In a sterile room, 25 grams of mycelia from *Pleurotus pulmonarius* were inoculated into the cooled sorghum, covered with tissue, and secured with a rubber band. The inoculated bottles were incubated at room temperature in a dark room at 27°C until fully colonized.

Preparation and Inoculation of Substrate

The substrate was prepared following the Onyeka & Okchie (2018) method with modifications. **Pasteurization by Boiling:** Sawdust was pressed for a smooth texture, mixed with water, and left to ferment for three days under polythene sheets. After fermentation, 4,350 grams of sawdust received 500 grams of rice bran, 100 grams of calcium carbonate, and 50 grams of sugar. These were mixed well, and water was added to achieve 72% moisture. The mixture was divided into five portions, placed in perforated 1-liter heat-resistant containers, covered, and pasteurized for three hours.

Pasteurization with Hydrated Lime: Another 4,400 grams of sawdust had 500 grams of rice bran and 100 grams of calcium carbonate added, achieving 72% moisture. This sawdust was packed into a permeable sack, submerged in a solution of 85 grams of hydrated

lime dissolved in 10 litres of water for 12 hours, drained on a sterile plastic sheet, and then divided into five portions in perforated heat-resistant bags.

Pasteurization with Sodium Hypochlorite: The third batch, also 4,400 grams, was treated similarly, with 500 grams of rice bran and 100 grams of calcium carbonate mixed in. After achieving 72% moisture, it was packed into a sack and submerged in a solution of 95 millilitres of 5% sodium hypochlorite and 10 litres of water for 12 hours. It was then drained and divided into five portions in perforated heat-resistant containers.

Inoculation: Seventy grams of 13-day-old spawn were added to the containers under sterile conditions. They were incubated in a dark room at room temperature with 70-80% relative humidity. Water was sprinkled on the floor to promote mycelium growth. After the spawn running, containers were moved to the fruiting room, maintained at 80-90°F, with moisture provided by regular watering. Mushrooms were harvested at peak development.

Data Collection

The yield of *Pleurotus pulmonarius* cultivated on various substrate pasteurization methods was evaluated by measuring the weight, diameter of the pileus, and size of the fruit bodies after primordia initiation. Measurements from the replicates were averaged. Fruit body height was measured in centimetres from the base of the stipe to the pileus, while pileus diameter was measured across its width. Fresh weight was recorded using an electronic balance (model APX 200, Denver Instrument, Arvada, Colorado). Biological efficiency was calculated as the ratio of the fresh weight of harvested mushrooms to the dry weight of the substrate. Additional data recorded included the time for mycelial growth after inoculation and the days until primordia initiation. Values represent the mean from four replicates per treatment.

Data Analysis

One-way Analysis of Variance (ANOVA) was used to analyse the collected data.

Results and Discussion

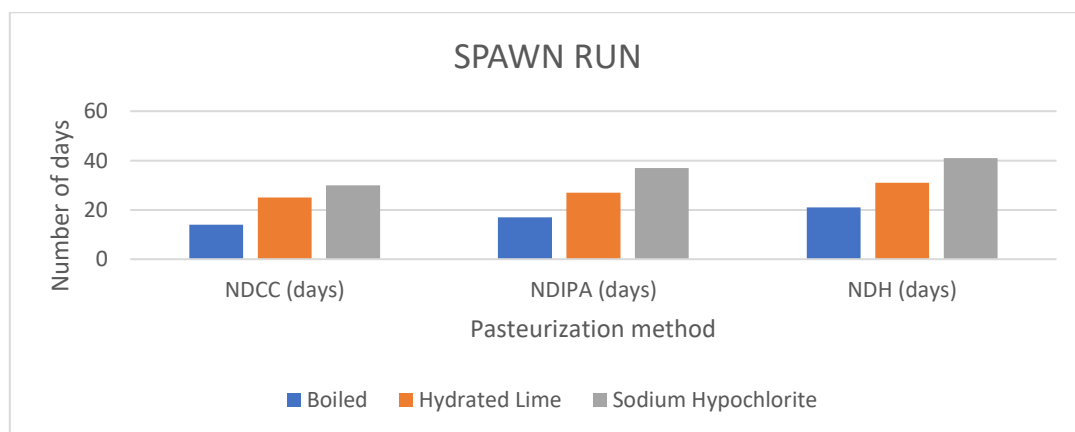


Figure 1: Spawn run time of the mushroom cultivated on substrates prepared with different pasteurization methods

NDCC: Number of days to completely colonize the substrate

NDIPA: Number of days to initiate primordial appearance

NDH: Number of days taken to harvest

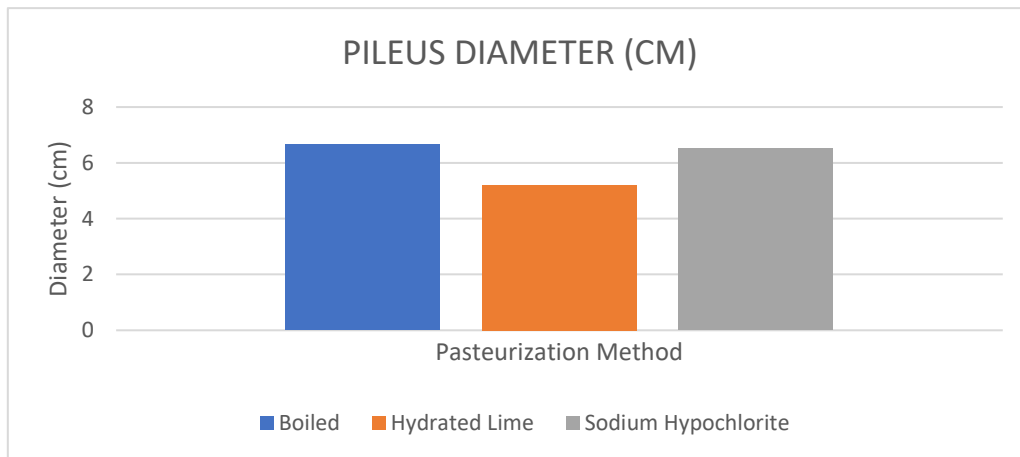


Figure 2: Pileus diameter (cm) of the mushroom cultivated on substrates prepared with different pasteurization methods

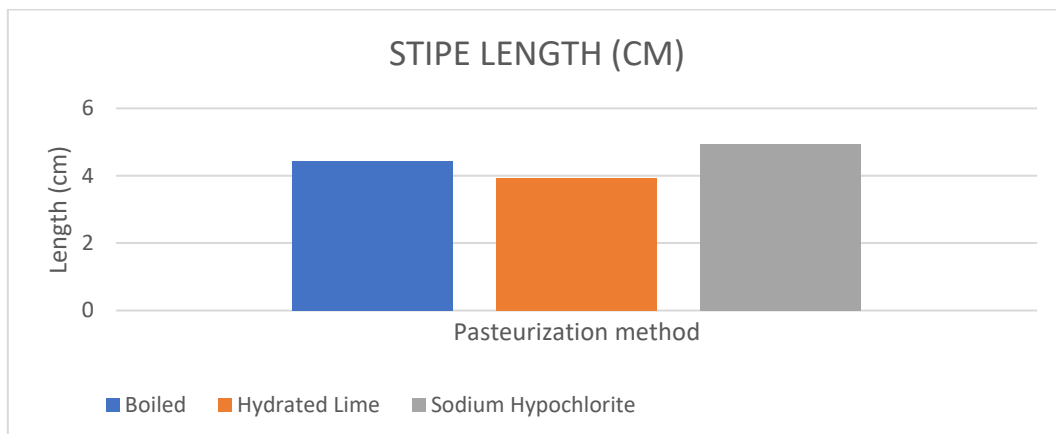


Figure 3: Stipe length (cm) of the mushroom cultivated on substrates prepared with different pasteurization methods

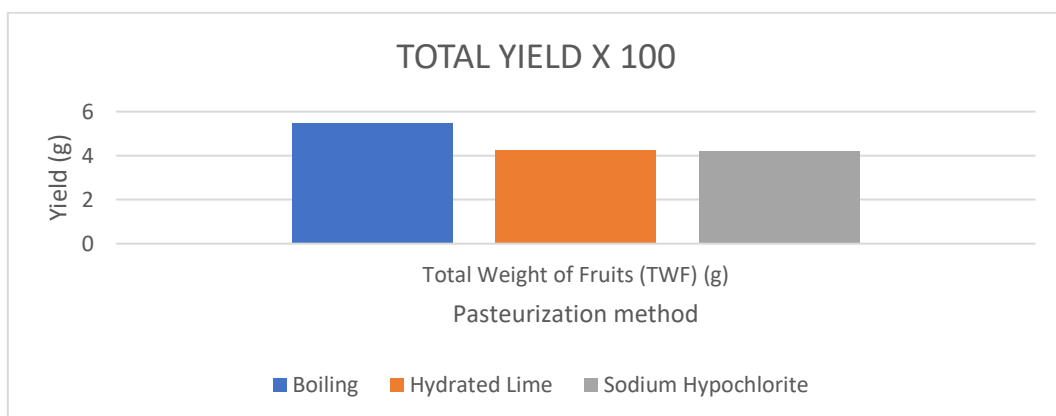


Figure 4: Total yield (g) of the mushroom cultivated on substrates prepared with different pasteurization methods

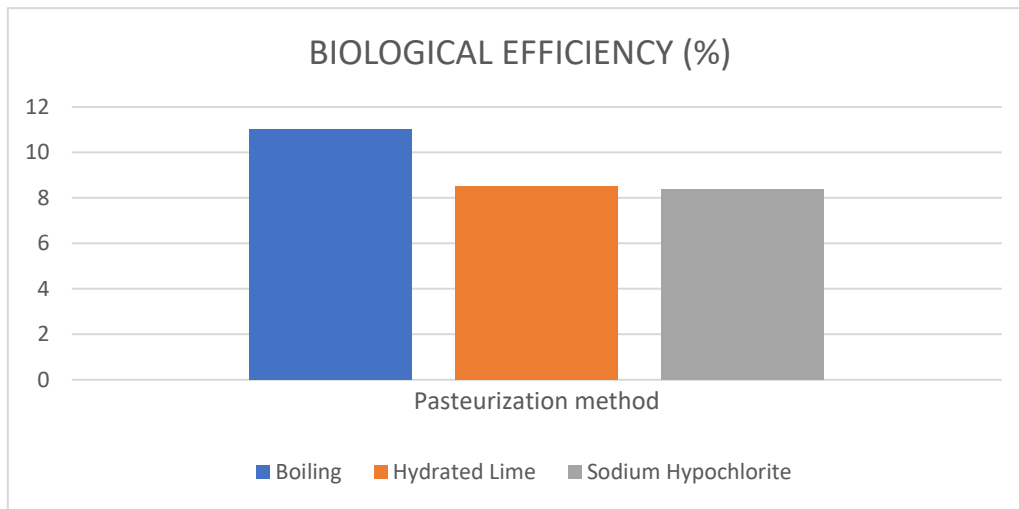


Figure 5: Biological efficiency (%) of the mushroom cultivated on substrates prepared with different pasteurization methods

According to Figure 1, the duration of substrate colonization by *Pleurotus pulmonarius* mycelium varied significantly with different pasteurization methods. Boiling resulted in the shortest spawn run time of 14 days, indicating rapid mycelial establishment due to effective elimination of competing microorganisms. In contrast, substrates treated with hydrated lime and sodium hypochlorite took longer, at 25 and 30 days, respectively. This aligns with Colavolpe et al. (2014), who noted that hot water pasteurization promotes faster mycelial spread. Boiling also led to earlier pinhead formation, averaging 17 days, compared to 27 and 37 days for lime and hypochlorite treatments. Early primordia formation indicates substrate readiness and mycelial growth, as emphasized by Sánchez et al. (2011), who linked delays in pin formation to microbial interference reduced through heat treatment. Chemical treatments may cause delays due to residual alkalinity, according to Earnshaw et al. (2012). The earliest harvest occurred after 21 days with boiling, while sodium hypochlorite and hydrated lime required 31 and 41 days, respectively. This progression from colonization to fruiting maturity illustrates the efficiency of boiling, which promotes quicker harvest cycles and reduces contamination risks, supporting Akinyele and Adejumo's (2017) findings on increased productivity with thermally treated substrates.

Figure 2 showed that boiling-treated substrates produced mushrooms with the largest pileus diameter, measuring 6.65 cm, while sodium hypochlorite resulted in a diameter of 6.51 cm. In contrast, substrates treated with hydrated lime yielded smaller caps, averaging 5.21 cm. The larger cap sizes associated with boiling and sodium hypochlorite suggest enhanced nutrient availability and hydration in the substrates. However, the benefits of sodium hypochlorite were not as apparent in other measured parameters. The diameter of mushroom caps is often linked to how easily the substrate can be digested and how active the mycelium is, as highlighted by Reis et al. (2018). Hydrated lime is known to buffer pH levels. However, in this study, its lower effectiveness could be attributed to inadequate detoxification or unfavourable environmental conditions.

As shown in Figure 3, the length of the stipes followed a similar development, with boiling resulting in the longest stipes at 4.44 cm, followed by sodium hypochlorite at 4.95 cm, and hydrated lime at 3.92 cm. Although sodium hypochlorite produced slightly longer stipes, the biological significance may be less important than factors such as cap size and yield. Elongated stipes that are not balanced with cap development can indicate light stress or inadequate aeration; however, all treatments in this study were subjected to the same environmental conditions. This

suggests that the method of pasteurization was the primary factor influencing the morphological outcomes. This finding aligns with Azeez et al. (2019), who observed that the morphology of fruit bodies is significantly affected by substrate processing techniques.

Figures 4 and 5 showed that the process of boiling produced the highest total yield of mushrooms (550 g) and biological efficiency (11.22%) when compared to 425 g (8.59%) and 420 g (8.48%) from hydrated lime and sodium hypochlorite, respectively. These figures demonstrate that boiling creates ideal substrate conditions for maximizing fruiting potential. Even though it requires significant time and energy, the resulting biological yield is considerable, making it a beneficial option for small to medium-sized cultivation. The relatively low biological efficiency of the chemical treatments may be due to inconsistent decontamination or residual compounds that hindered mycelial performance. This reinforces previous assertions by Okwulehie et al. (2018) that thermal methods generally produce superior outcomes in low-resource mushroom farming, where achieving chemical precision can be challenging.

Conclusion

This study emphasizes that substrate pasteurization methods greatly impact the growth and yield of *Pleurotus pulmonarius*. Among the three methods tested, boiling proved to be the most effective, resulting in the fastest colonization, largest fruit bodies (550 g total yield), and highest biological efficiency (11.22%). Hydrated lime and sodium hypochlorite were less effective due to inconsistent microbial suppression and chemical interference. The study concludes that boiling is a reliable pasteurization method for mushroom cultivation, especially in small-scale settings. Future research could explore hybrid methods or improve chemical treatments for better efficiency and consistency.

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