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EFFECT OF DIFFERENT SUBSTRATE STERILIZATION METHODS ON PERFORMANCE OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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ABSTRACT. Proper sterilization of substrates is an indispensable step in oyster mushroom cultivation. Oyster mushroom growers in Nepal usually follow three different substrate sterilization methods; however, their comparative effectiveness is vastly unexplored. Thus, these experiments were carried out at the Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Lamjung, Nepal from January to March, in the years 2017 and 2019. The objective of these experiments was to identify the most appropriate method of sterilization. Three different types of sterilization methods viz chemical sterilization (formaldehyde + carbendazim), steam sterilization, and hot-water sterilization were evaluated for the growth parameters and productivity of oyster mushroom cultivated on rice straw. The experiments were laid out on Completely Randomized Design (CRD) with ten replications. The results showed that the spawning rate was 3.2% of the wet substrate. Data were collected until the third flush. A significantly longer duration to colonize the substrate (29.7 days) was observed under chemical sterilization. The oyster mushroom performed best under steam sterilization as it took the shortest time for pinhead formation (34.30 days), fruiting body formation (43.60 days), cropping duration (89.30 days), and produced the highest mushroom yield (1401.9 g per 4 kg bag), and consequently, the highest biological efficiency (101.38%). Average pileus diameter and stipe length were statistically indifferent among the treatments suggesting the significant effect of sterilization methods on the yield of oyster mushroom but not on its morphological attributes.

Introduction

The use of mushrooms as food is probably as old as civilization itself. Over 200 mushroom species have long been used as functional foods around the world (Kalač, 2013), but only about 35 species have been commercially cultivated (Aida *et al.*, 2009). Over time, an increase in awareness about mushrooms nutritive and medicinal value has enhanced their consumption (Chang, 1999).

Among the cultivated mushrooms, oyster mushroom (*Pleurotus* species) is flourishing in the temperate and sub-tropical environment due to its excellent flavour and taste (Ganeshan *et al.,* 1989). *Pleurotus ostreatus* is the second-largest commercially cultivated mushroom species in the world (Royse, 2013). Oyster mushroom can be grown on various substrates due to their strong enzymatic features (Atila, 2016). *Pleurotus ostreatus* is easier to cultivate, favourable to eat, and grow economically on different kinds of organic waste raw materials and different climatic conditions (Kong, 2004; Sitaula, 2018; Tekeste *et al.*, 2020).

The extensive cultivation of oyster mushroom is also because of its simplistic cultivation, high biological efficiency, and greater nutritional significance (Singh *et al.,* 1990). Nutritionally, the oyster mushroom is an ideal food for humans. It is rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), and fibre (8.7) with

345 Kcal energy in 100 g of mushroom on a dry weight basis (Pandey, Ghosh, 1996).

Oyster mushrooms, having a considerable economic, medicinal and nutritional value, are most commonly and commercially cultivated on the non-composted lignocellulosic substrates (Savoie *et al.,* 2007) due to which the agriculture, industrial, and forest wastes can be efficiently utilized (Sanjel *et al.,* 2021). Various substrates, including paddy straw, maize stalks/cobs, vegetable plant residues, and bagasse, are extensively used in its cultivation (Sher *et al.,* 2011). Successful cultivation of oyster mushroom on the various substrates, including sawdust, chopped office papers, cardboard, and plant fibres, are also reported from multiple research works (Mandeel *et al*., 2005). Dubey *et al.* (2019) found that rice straw was found most favourable for mushroom cultivation rather than wheat straw, sugarcane bagasse and banana leaves.

Growing *P.ostreatus* requires sterilization of substrates, which means pre-treatment of the substrates to eliminate pathogenic and competitive micro-organisms, and to enhance the mycelial growth of mushroom. Sterilization of the substrates for the production of mushrooms is carried out mainly to avoid the presence of pathogens that appear to compete for the nutrients available in the unsterilized substrates. It is one of the crucial steps in oyster cultivation, which can determine the success of the cultivation (Ali *et al.*, 2007). Three different treatments, viz. steam sterilization, immersion in hot water, and chemical treatment, are explained in the literature (Mejía, Albertó, 2013). However, there are not enough works comparing the effectiveness of these sterilization methods influencing the quality and quantity of the mushroom produced. Therefore, the present research was undertaken to find out the most appropriate sterilization method, which will be helpful for further improvement in the yield of mushrooms.

Material and methods

Two independent experiments were conducted in Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Lamjung, Nepal, from January to March, in the year 2017 and 2019, to explore the best sterilization method, *i.e*., chemical, steam and hot-water sterilization in the production of oyster mushroom. Rice straw was taken as the substrate and subjected to hand-chopping to the required length of 5 cm.

Preparation of substrates

Chemical sterilization was carried out as explained by Siddhant *et al.* (2014)**.** The chopped straw was dipped into a 200 L metal drum containing 125 ml formalin mixture and 15 g Carbendazim per 100 L of water. The mixture was stirred with the help of a wooden pole. The drum was made airtight, and the substrate was allowed to soak for 18 hours in the solution. Then, the solution was drained out and the moisture content of about 65% was maintained in the wet substrate before spawning. For steam sterilization, the chopped substrate was soaked in water for 12 hours before sterilization. Presoaked substrate was exposed to steam for about 15–20 minutes, and cooled before spawning, as explained by (Gowda, Manvi, 2019).

We followed the Kalita (2015) method for hot water sterilization. The presoaked substrate was kept in the drum and boiled for 30 minutes, and the solution was drained before spawning. The experiment was set up using a complete randomized design (CRD) with three treatments and ten replications.

Spawning and incubation

Spawning was carried with fresh grain spawn on a 3.2% (weight/weight) rate based on the wet substrate. On every layer of around 4 inches of sterilized straw in polybags, spawn was placed near the periphery forming a circle, and bags were pressed to compact. Three layers of spawning were done to make balls of 4 kg, and perforation of the bag was done after completion of packing by making 8–10 holes of size 8 mm for aeration. Cotton was plugged into each hole to prevent contamination as well as entry of insects. Then, the bags were suspended on strings in a dark and pre-fumigated room with no ventilation. Thus, prepared each ball represented one replication. The temperature of the room was maintained at 13–14 °C, and relative humidity was 80%. The continuous observation was done till the full spawn run, and then the bags were cut from two sides for fructification. Ventilation with diffused light was provided with two gentle irrigations per day and other necessary crop management. Watering was stopped 24 hours before harvest.

Data collection and analysis

Harvesting was done when the majority of caps attained their maximum size. The experiment was terminated after three flushes from each replication. Data were recorded periodically on different parameters, including the number of days to full colonization, the number of days to first fruiting, harvesting duration (day), and mushroom yield in every flush (g), pileus diameter (cm), stipe length (cm), and biological efficiency (BE). The fresh mushroom yield on each harvest was measured, then total fresh yield and biological efficiency were calculated (Eq. 1).

$$BE = \frac{Yield\ of\ fresh\ mushrooms,\ g}{Total\ weight\ of\ dry\ substrate\ used,\ g} \times 100 \quad (1)$$

Statistical analysis

Data were analyzed using RStudio 1.2.5033. The treatment means were separated using Least Significant Difference (LSD) at the 5% level of significance.

Results and discussion

Vegetative and reproductive growth

The time required for full colonization of substrate, also known as spawn run duration, and was compared between three methods of sterilization. In our experiment, a significantly prolonged duration (29.70 days) for complete mycelial growth was observed in the chemically sterilized substrate (Fig. 1**)**.

Hot-water sterilization took the shortest duration (26.20 days), which was statistically at par with steam sterilization. The pattern was similar for days taken for the first pinhead formation and the cropping duration, reflecting the significant effect of the method of sterilization used. Chemical sterilization took the longest duration of 38.20 days for the appearance of the first pinhead, whereas steam sterilization took the shortest duration of 34.30 days. In all the treatments, the pinheads were formed between 8–9 days after the full colonization of the substrate. A significantly longer cropping duration of 96.1 days was recorded in chemical sterilization.

In oyster mushroom cultivation, the above parameters reflect the speed of vegetative and reproductive growth of the mushroom. Rapid growth and development are desirable for the profitable business; however, multiple factors, including ambient environmental condition, substrate physical and chemical properties, and the presence of competitive and pathogenic organisms, and antigrowth substances, will influence the rate of growth. Generally, the oyster mushroom takes up to three weeks for a full spawn run in its optimal environment (Shah *et al.*, 2004; Kalita, 2015). However, we observed a delay of 6–10 days, reflecting the suboptimal temperature (13–14 °C) for mycelia growth in our experiments. Atila (2016) reported that 7–12 days is taken from full spawn run to pinhead formation which is by our result. The fruiting bodies appeared on substrates sterilized by different methods 17–20 days later after a full spawn run. Our results are similar to Tan (1981), who reported that *P. ostreatus* took 14–21 days for fruiting body formation after the full spawn run.

The increased cropping duration in chemical sterilization is probably due to the anti-fungal effect of fungicides that hindered the mycelial growth of the mushroom, which subsequently delayed all the later developmental stage up to the third harvest. A similar delay in cropping duration was seen by Ali *et al.* (2007) where all the species of Pleurotus treated with formalin took long to complete the mycelial growth.

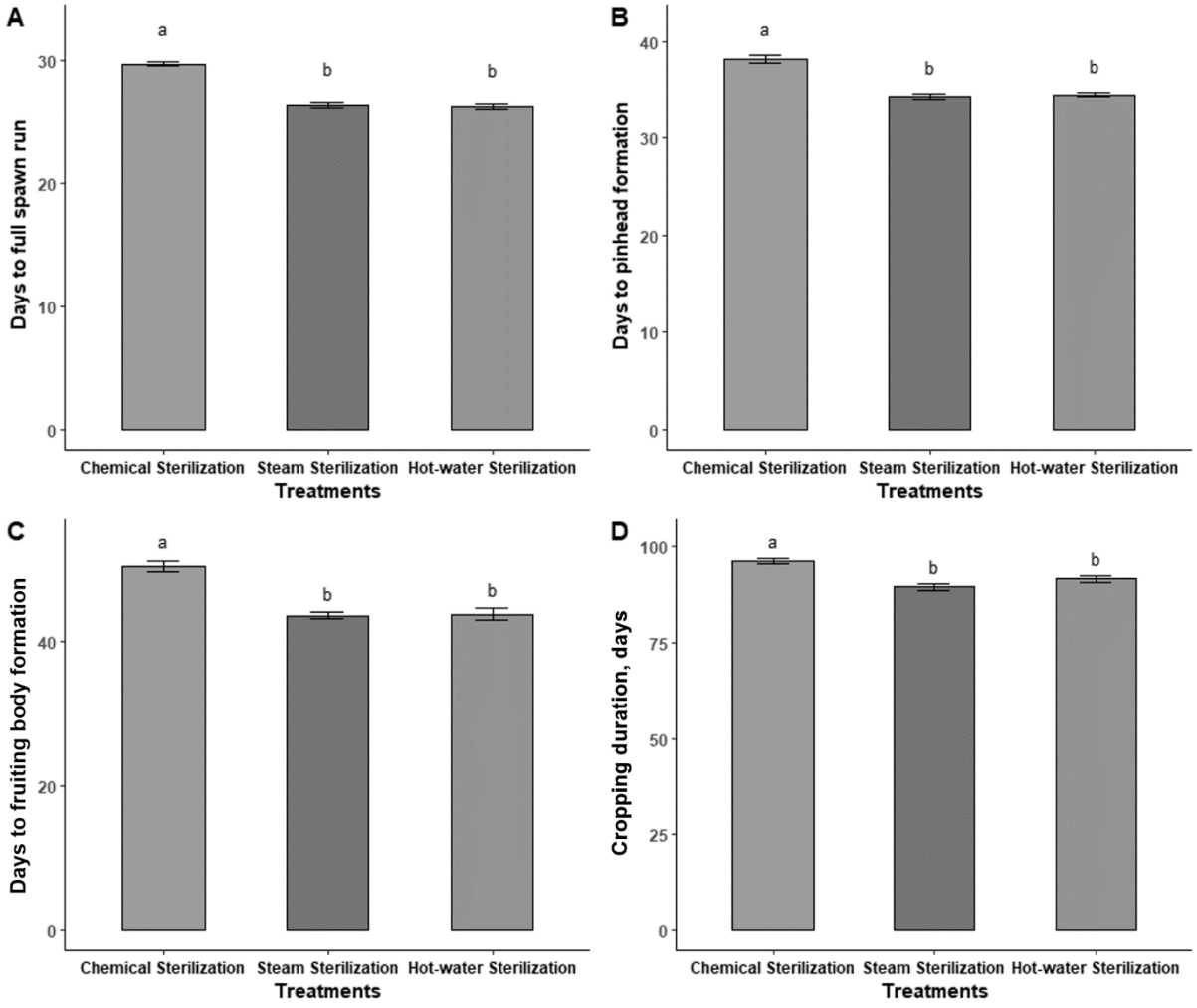

Figure 1. Bar plots showing the effect of sterilization methods on the days taken for full spawn run, pinhead formation, fruiting body formation, and cropping duration of *P. ostreatus*. Values in a bar with the same letter(s) are not significantly different at P = 0.05, according to LSD (least significant difference) test. The black bars in the plots indicate the standard error of the mean

Mushroom morphology

There was no significant difference between the treatments for pileus diameter and stipe length. The stipe lengths ranged from 7.0 to 7.1, and the diameters of pileus ranged from 9.3 to 9.6 (Table 1). It is reasonable that sterilization methods had no impact on the morphological properties of the mushroom. Such parameters highly depend on the mushroom strain and the nutrient factor of substrates. In our experiment, the substrate was the same in all treatments; thus, no variation was observed. The Pileus diameter of *P. ostreatus* was recorded in different growing substrates between 4.0–10 cm (Yildiz *et al.*, 2002). The variation in their work may be due to the variation in nutrient factors of multiple substrates.

Table 1. Pileus diameter and stipe length of *P. ostreatus* ± standard error of the mean, as affected by different sterilization methods

Treatment	Diameter of pileus, cm	Stipe length, cm
Chemical sterilization (T1)	9.408 ± 0.36	7.187 ± 0.19
Steam sterilization (T2)	9.638 ± 0.40	7.07 ± 0.25
Hot-water sterilization (T3)	9.303 ± 0.53	7.027 ± 0.27
F test	NS	NS
LSD (0.05)	0.943	0.711
CV (%)	10.877	10.915

Values in a column with the same letter(s) are not significantly different at P = 0.05 according to LSD (least significant difference). CV: coefficient of variation. NS: not significantly different at P < 0.05

Mushroom yield

There were significant effects of the methods of sterilization on the fresh mushroom yield of *P. ostreatus*. The highest total yield of 1.40 kg ball^{-1} was obtained in steam sterilization, which was statistically at par with hot-water sterilization (Fig. 2). The lowest total yield (1.02 kg ball^{-1}) was obtained in chemical sterilization. Ali *et al.* (2007) found that steam sterilization produced the highest total yield than all other methods which is by our results. The superiority of the steam sterilization might be due to the generation of optimal physical and chemical property of substrate for the mycelium growth. The biological efficiency also varied significantly among the methods of sterilization. The highest biological efficiency (BE) of 101.38% was observed in steam sterilization, and the least BE (79.75%) was observed in chemical sterilization (Table 2).

Two different dominant contaminants viz *Trichoderma* spp. and *Coprinus* spp. were found during the cultivation period in the experiments. The microbial contamination was observed the highest in hot water sterilization (16.00%) and the lowest in chemical sterilization (11.75%) (Table 2). Ashraf *et al.* (2007) found that the fungus associated with oyster mushroom growing media (compost) such as *Aspergillus, Trichoderma, Mucor, Penicillium, Alternaria, Cladosporium, Monilia, Helminthosporium, Coccidioides* and *Scedosporium*. Sharma *et al.* (2007) in the results of his research in India showed that the fungus *Aspergillus* spp., *Aspergillus niger*, *Fusarium* spp., *Mucor* spp. and *Trichoderma* spp. were competitor fungus or may cause disease in cultivated mushrooms such as oyster mushrooms. Lopez-Arevalo *et al.* (1996) also found that *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp. and *Cunninghamella* sp. a fungal contaminant in the tropical country of Mexico.

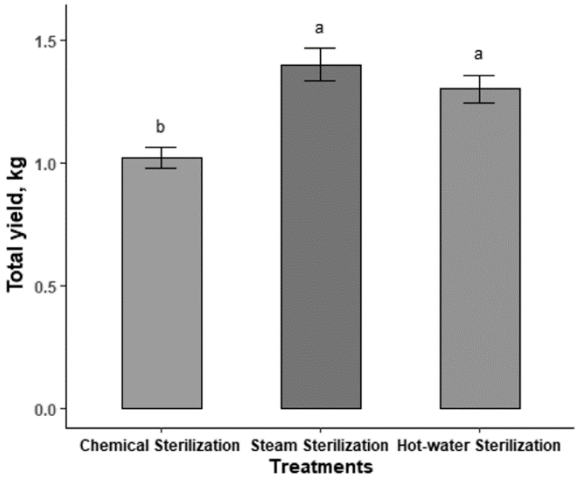

Figure 2. Bar plot showing the effect of sterilization methods on the total yield of *P. ostreatus*. Values in the bar with the same letter(s) are not significantly different at P = 0.05, according to LSD (least significant difference) test. The black bars in the plots indicate the standard error of the mean.

BE measures the ratio of the weight of fresh mushroom to the weight of dry substrate used in its production. In our laboratory, we have observed the BE for oyster mushroom up to 140%. BE in oyster mushroom production varies from 75.5 to 128.8% (Zhang *et al.*, 2002). In the real ground, the BE may be affected by several factors as substrate composition, ambient environment, mushroom strain, disease pest and other multiple management factors. BE of around 90% in hot water sterilization and 80% in chemical sterilization is an acceptable level. However, with the increase in room temperature, competitive moulds and microbes may reduce the BE of the mushroom crop (Biswas, 2014). Thus, we suggest all oyster mushroom growers wisely choose the sterilization techniques to obtain the economically profitable level of BE under suboptimal growing conditions.

Three substrate sterilization methods were evaluated for their effect on the growth and productivity parameters of oyster mushroom. There were significant differences in all the growth and yield parameters, but the morphological attributes were indifferent. Steam sterilization produced the highest fresh mushroom yield of 1409.1 g per bag of 4 kg wet substrate resulting in the highest biological efficiency of 101.3%. Chemical and hot water sterilization resulted in an acceptable production level with BE of around 80 and 90%, respectively. A Significantly longer cropping duration of 96.1 days was seen in chemical sterilization than hot water and steam sterilization. The same pattern was seen for other growth parameters, including days taken for full spawn run and fruiting body formation.

Table 2. Effect of the sterilization methods on the quantity harvests and biological efficiency of *P. ostreatus*.

Treatment	Fresh weight of mushrooms by flushes (g)			BE (%)	Incidence of microbial contamination (%)		
	First	Second	Third		*Trichoderma* spp.	*Coprinus* spp.	Average
Chemical sterilization	729.8 ± 0.03^{b}	214.9 ± 0.01^{c}	76.2 ± 0.02^{b}	79.75	13.75	9.75	11.75
Steam sterilization	890.8 ± 0.02^{a}	412.0 ± 0.03^{a}	160.1 ± 0.01^{a}	101.38	14.50	11.50	13.00
Hot-water sterilization	879.2 ± 0.09^{a}	309.9 ± 0.02^{b}	130.6 ± 0.02^{a}	90.39	16.50	15.50	16.00
F-test	***	***	**	***	**	**	**
LSD (0.05)	0.062	0.074	0.048				
CV (%)	7.9	25.1	42.5				

Values in a column with the same letter(s) are not significantly different at P = 0.05, according to LSD test.
CV: coefficient of variation; *** and ** significant different at P < 0.001 and P < 0.01, respectively

Conclusions

Proper sterilization of substrates is important for the effective and smooth cultivation of mushrooms. In our study, the different methods of sterilization have influenced the vegetative growth, morphology, cropping duration, mushroom yield and biological yield of oyster mushroom, however, the steam sterilization method was found more efficient with a shorter cropping duration, higher yield and higher biological efficiency than other methods. Thus, stem sterilization was found the best method of sterilization for the cultivation of oyster mushroom.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

SS – lead investigator and responsible for literature search and write-up.
SB, RKS, JS – responsible for the literature review and provided critical feedback on the manuscript.
All the authors read and approved the final manuscript.

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