AGRI MAGAZINE
(International E-Magazine for Agricultural Articles)
Volume: 03, Issue: 05 (May, 2026)
Available online at http://www.agrimagazine.in

Sterilization Techniques Used in Mushroom Cultivation

*Jothika M S and Dr. K. Vignesh
Palar Agricultural College, Melpatti, Vellore, Tamil Nadu, India
*Corresponding Author's email: selvamdpi111@gmail.com

Mushroom cultivation is a globally significant agricultural enterprise requiring strict hygienic conditions to ensure productive and high-quality yields. Contamination by pathogenic bacteria, fungi, and competing microorganisms poses a persistent challenge that can lead to considerable crop and economic losses. This article provides a comprehensive review of the major sterilization and pasteurization techniques employed in modern mushroom cultivation—including steam sterilization, pasteurization, hot water treatment, chemical disinfection, ultraviolet (UV) irradiation, flame sterilization, and membrane filtration. The mechanisms, procedural guidelines, comparative advantages, limitations, and commercial relevance of each method are examined. The article further discusses hygienic management practices and the integration of advanced technologies in commercial production. By adopting appropriate sterilization strategies, growers can substantially reduce contamination, improve colonization efficiency, and maximize profitability.

Keywords: mushroom cultivation, sterilization, pasteurization, contamination control, substrate preparation, mycelial growth

Introduction

Mushroom cultivation has evolved into a globally significant agricultural enterprise, providing nutritious food, supplementary income, and economic opportunities to farmers and agri-entrepreneurs across diverse production scales. However, maintaining the hygienic conditions necessary for successful mushroom production remains one of the most critical operational challenges. Contamination by bacteria, molds, viruses, insects, and other competing microorganisms can rapidly compromise substrate integrity, suppress mycelial growth and resulting in significant yield losses.

Sterilization—defined as the complete elimination of all viable microorganisms, including resistant endospores—is therefore a cornerstone practice in mushroom cultivation. It creates a biologically clean slate within the substrate, enabling mushroom mycelium to colonize the growing medium without competition. Pasteurization, a related but distinct process involving partial microbial reduction through controlled heat, offers a cost-effective alternative particularly suited to oyster mushroom cultivation on lignocellulosic substrates.

The selection of an appropriate sterilization method depends on multiple factors including substrate composition, cultivation species, production scale, equipment availability, and economic constraints. This article presents a systematic review of the principal sterilization techniques employed in mushroom cultivation, analyzing their underlying mechanisms, procedural requirements, practical advantages, and limitations in the context of contemporary commercial production.

Importance of Sterilization in Mushroom Cultivation

Mushroom mycelium exhibits relatively slow growth compared to many saprophytic and pathogenic microorganisms commonly found in organic substrates. In the absence of sterilization, competing organisms such as Trichoderma spp., Aspergillus spp., Penicillium spp., and various bacterial species can rapidly colonize the substrate, excluding mushroom

mycelium from the available nutrient pool and causing partial or complete crop failure. Effective sterilization neutralizes this competitive pressure, establishing conditions conducive to rapid and uniform mycelial colonization.

Sterilization Techniques in Mushroom Cultivation

Multiple sterilization and pasteurization approaches are employed in mushroom cultivation, each suited to specific substrates, species, and production contexts. The following sections examine each method comprehensively.

Steam Sterilization (Autoclaving)

Steam sterilization under pressure—commonly referred to as autoclaving—is considered the gold standard for substrate and spawn sterilization in mushroom cultivation. The method operates on the principle that pressurized steam raises the effective temperature of water above its atmospheric boiling point, achieving sustained temperatures sufficient to denature cellular proteins and inactivate all microbial life forms, including heat-resistant endospores. The standard autoclave protocol involves loading substrate-filled polypropylene bags or glass bottles into the chamber and applying saturated steam at 121°C (250°F) under a gauge pressure of 15 psi (103 kPa) for 15–30 minutes. Dense or large-volume substrates may require extended cycles of 45–90 minutes to ensure uniform heat penetration throughout the load. Following sterilization, substrates must be cooled to below 30°C in a clean, low-contamination environment before inoculation. Steam sterilization is routinely employed in the production of grain spawn (wheat, sorghum, rye), enriched sawdust substrates for Shiitake and Oyster mushrooms, and tissue culture media. Its principal advantage is the reliable, complete elimination of microbial contaminants across all substrate types, ensuring consistent colonization outcomes.

Pasteurization

Pasteurization constitutes a partial sterilization process designed to eliminate pathogenic and competing microorganisms while intentionally preserving a population of beneficial thermophilic organisms—including specific bacteria and actinomycetes—that contribute to substrate conditioning and mycelial development. Three principal pasteurization methods are employed in mushroom cultivation: (i) hot water pasteurization, in which the substrate is submerged in water heated to 60–80°C for 1–2 hours; (ii) steam pasteurization, in which low-pressure steam is circulated through the substrate mass; and (iii) bulk chamber pasteurization, a controlled industrial process in which large quantities of compost or straw substrate are conditioned in sealed rooms at 57–62°C for 4–8 hours, followed by a conditioning phase that promotes beneficial microbial activity. Pasteurization is the preferred sterilization strategy for the cultivation of Oyster mushrooms (*Pleurotus spp*.) on straw substrates and for the preparation of Phase II compost for Button mushrooms (*Agaricus bisporus*). It is considerably more economical and energy-efficient than autoclaving and is accessible to small and medium-scale producers without sophisticated equipment. The limitation is that pasteurization does not eliminate all microorganisms; persistent or heat-resistant contaminants may subsequently proliferate if post-treatment hygiene is not maintained rigorously.

Hot Water Treatment

Hot water treatment represents the simplest and most economically accessible sterilization approach available to small-scale and rural mushroom growers. The substrate - most chopped paddy straw, wheat straw, or sugarcane bagasse—is fully immersed in water heated to 65–80°C and maintained at that temperature for approximately one hour. Excess water is subsequently drained and the substrate is allowed to cool before spawning. While hot water treatment is effective in reducing the total microbial load and most vegetative bacterial and fungal cells, it does not reliably inactivate heat-resistant spores. It is, therefore, best suited for Robust mushroom species such as *Pleurotus ostreatus* that demonstrate strong competitive mycelial growth. The method requires no capital equipment investment and can be implemented with basic agricultural infrastructure, making it valuable in contexts where resource constraints preclude the use of autoclaving.

Chemical Sterilization and Disinfection

Chemical sterilization employs biocidal compounds to inactivate microorganisms on surfaces, equipment, and within cultivation spaces. It is important to distinguish between sterilant agents capable of eliminating all microbial life and disinfectants, which achieve significant but not necessarily complete microbial reduction. In mushroom cultivation, chemical agents are predominantly used for facility sanitation rather than substrate sterilization.

Chemical Agent	Primary Application
Formaldehyde (2–4% solution)	Mushroom house fumigation and room sterilization
Sodium hypochlorite / Bleach (1–2%)	Surface disinfection of walls, shelves, and floors
Hydrogen peroxide (3–6%)	Equipment and tool sterilization; surface disinfection
Isopropyl alcohol (70%)	Hand sanitization and instrument surface sterilization
Quaternary ammonium compounds	Broad-spectrum surface disinfectant for growing rooms
Lime (calcium hydroxide)	Substrate pH adjustment with partial disinfection effect

Procedures involve diluting chemicals to recommended concentrations, applying them by spray or surface wipe, and maintaining treated areas sealed for the prescribed contact time before ventilation. Safety precautions are paramount: personnel must use appropriate personal protective equipment including gloves, goggles, and respirators, particularly when handling formaldehyde due to its classification as a probable human carcinogen. Residual chemical levels in the cultivation environment must be allowed to dissipate fully before mushroom substrate or mycelium is introduced.

Ultraviolet (UV) Irradiation

Ultraviolet radiation in the germicidal range (UV-C, 200–280 nm) is employed to disinfect air, exposed surfaces, and working areas in tissue culture facilities, inoculation chambers, and laminar flow hoods. UV-C radiation acts by inducing the formation of thymine dimers in microbial DNA, causing lethal replication errors and inactivating airborne spores, bacteria, and viruses that would otherwise settle onto inoculation surfaces. UV sterilization offers significant advantages including the absence of chemical residues, effectiveness against a wide spectrum of airborne contaminants, and environmental compatibility. However, UV-C radiation has limited penetration capability and is effective only on directly exposed surfaces; it cannot sterilize substrates or materials with shading or physical obstructions. Personnel must never be exposed to UV-C radiation during operation, as it causes severe ocular and dermal injury. UV lamps also require regular replacement as germicidal efficacy declines with cumulative usage hours.

Flame Sterilization

Flame sterilization—the direct incineration of surface contaminants by passing metallic instruments through an open flame until red-hot—is a fundamental laboratory technique used during spawn preparation, tissue culture operations, and inoculation procedures. Instruments including scalpels, forceps, transfer loops, and inoculation needles are flamed in a Bunsen burner or alcohol lamp flame, then allowed to cool momentarily on a sterile surface or in agar before contact with culture material. This method achieves instantaneous, highly effective sterilization of small metallic tools and represents an indispensable technique in laboratory settings. Its application is, however, strictly limited to non-combustible, thermally resistant metallic instruments and cannot be used for substrate sterilization, plastic items, or large equipment surfaces.

Filtration Sterilization

Membrane filtration achieves sterilization by the physical removal of microorganisms through semi-permeable membranes with defined pore sizes, typically 0.22 µm for bacterial sterilization. This technique is indispensable for processing heat-sensitive liquid media,

culture additives, and nutrient supplements that would be degraded by thermal sterilization. High-efficiency particulate air (HEPA) filtration systems, which remove particles ≥0.3 µm with 99.97% efficiency, are employed in clean room facilities and laminar flow hoods to maintain sterile air environments during inoculation. Filtration sterilization preserves the biological activity of heat-labile compounds, making it essential for specialty culture media preparation. The associated equipment costs are considerably higher than thermal methods, and its utility is confined to laboratory and specialized research applications rather than field-scale substrate sterilization.

Hygienic Management Practices:

Sterilization procedures, irrespective of their efficacy, must be complemented by comprehensive hygienic management practices throughout the cultivation cycle. Re-contamination of sterilized substrates can occur rapidly if post-sterilization handling and environmental controls are inadequate. The following practices are recommended as integral components of a contamination management system:

- Rigorous hand washing and the use of disposable gloves before handling spawn or inoculating substrate
- Use of pathogen-free, microbiologically tested water in substrate preparation and facility cleaning
- Regular cleaning, disinfection, and positive-pressure ventilation of growing rooms and incubation chambers
- Periodic sterilization and alcohol flame treatment of all tools used during inoculation and harvesting
- Prompt identification and removal of contaminated bags or logs to prevent spore dispersal
- Integrated pest management protocols to control insect and rodent vectors within the production facility
- Worker training in aseptic technique and contamination identification

Conclusion:

Sterilization constitutes an indispensable pillar of mushroom cultivation science, directly determining the microbial ecology of the growing substrate and, by extension, the productivity and economic viability of the entire cultivation operation. The methods reviewed in this article—steam sterilization, pasteurization, hot water treatment, chemical disinfection, UV irradiation, flame sterilization, and membrane filtration—represent a complementary toolkit from which practitioners can draw according to their specific cultivation requirements, species selection, resource availability, and production scale. Steam sterilization remains the definitive method for achieving complete substrate sterility in commercial spawn production and high-value species cultivation. Pasteurization and hot water treatment offer accessible, cost-effective alternatives for small and medium-scale producers, particularly those cultivating Oyster mushrooms on straw substrates. Chemical and UV methods serve critical supplementary roles in maintaining environmental hygiene within cultivation facilities.

References

1. Chang, S. T., & Miles, P. G. (2004). Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact (2nd ed.). CRC Press.
2. Stamets, P. (2000). Growing Gourmet and Medicinal Mushrooms (3rd ed.). Ten Speed Press.
3. Tripathi, D. P. (2011). Mushroom Cultivation. Oxford and IBH Publishing Co. Pvt. Ltd.
4. Pathak, V. N., Yadav, N., & Gaur, M. (2010). Mushroom Production and Processing Technology. Agrobios (India) Publications.
5. Sharma, V. P. (2013). Techniques of Mushroom Cultivation. ICAR Publications, Indian Council of Agricultural Research.
6. Sánchez, C. (2010). Cultivation of Pleurotus ostreatus and other edible mushrooms. Applied Microbiology and Biotechnology, 85(5), 1321–1337.

7. Vignesh, K., Rajamohan, K., Balabaskar, P., & Anandan, R. (2021). In vitro efficacy of PGPR Pseudomonas fluorescens against Fusarium wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici. *Plant Archives (09725210)*, *21*(1).
8. Royse, D. J., Baars, J., & Tan, Q. (2017). Current overview of mushroom production in the world. In D. Zied & A. Pardo-Giménez (Eds.), Edible and Medicinal Mushrooms: Technology and Applications. Wiley-Blackwell.
9. Philippoussis, A. (2009). Production of mushrooms using agro-industrial residues as substrates. In E. Nigam & A. Pandey (Eds.), Biotechnology for Agro-Industrial Residues Utilization. Springer.
10. Bano, Z., & Rajarathnam, S. (1988). Pleurotus mushrooms. Part II: Chemical composition, nutritional value, post-harvest physiology, preservation, and role as human food. Critical Reviews in Food Science and Nutrition, 27(2), 87–158.
11. Vignesh, K., Vengadeskumar, L., Sanjaygandhi, S., & Sabesan, T. (2023). Prevalence of maydis leaf blight of maize in Tamil Nadu and assess the morphological character and virulence of Bipolaris maydis (NISIK.) shoemaker. *The Pharma Innovation Journal*, (12), 6.
12. Wasser, S. P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology, 89(5), 1323–1332.
13. Block, S. S., Tsao, G., & Han, L. (1958). Experiments in the composting of mushroom substrate. Mushroom Science, 4, 175–195.
14. Deacon, J. W. (2005). Fungal Biology (4th ed.). Blackwell Publishing.