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IMPACT OF DIFFERENT SUBSTRATE STERILIZATION METHODS IN PLEUROTUS SP. MUSHROOM CULTIVATION: A COMPREHENSIVE REVIEW

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ABSTRACT

Effective substrate sterilization is a cornerstone of successful mushroom cultivation, preventing contamination by competing microorganisms. This review comprehensively examines various sterilization methods, drawing insights from the scientific literature. We explore established techniques like composting, analyzing their role in creating a selective environment conducive to desired fungal growth while suppressing unwanted microbes. The review then evaluates chemical sterilization, considering its efficacy against contaminants alongside potential environmental concerns. Hot water treatments are subsequently dissected, revealing their ability to achieve a balance between contaminant control and substrate nutritional value preservation. Steaming techniques are then meticulously analyzed, evaluating their efficiency in achieving sterility at different pressure and time combinations. Finally, the innovative Ozone based sterilization and Cold plasma technology is introduced, highlighting its potential to optimize the balance between eliminating contaminants and preserving beneficial components for optimal mushroom growth. This critical appraisal of diverse sterilization techniques empowers researchers and cultivators to select the most appropriate method for their specific mushroom species and cultivation goals, ultimately promoting enhanced yields, improved crop quality, and the advancement of the mushroom cultivation industry.

Keywords: Substrate Sterilization, Mushroom Cultivation, Contamination Prevention, Sterilization Methods, Fungal Growth, Environmental Concerns.

I. INTRODUCTION

Since ancient times, mushrooms have been an essential part of human civilization and are loved for their nutritional and medicinal qualities everywhere in the world. Acknowledged as "The New Superfood" of the future, they are an essential part of a person's diet to improve their overall health and well-being. Mushrooms possess a wide range of clinical applications and a great therapeutic potential. Numerous kinds of mushrooms have been found to contain a broad variety of bioactive substances, such as phenolic compounds, peptides and proteins, polysaccharides, polysaccharides protein complexes, terpenoids, and peptides. Packed with an abundance of nutrients and numerous biomolecules with therapeutic value, they are recognized globally and considered the world's largest unexplored dietary supplement resource (Kour et al., 2022).

In the recent years, owing to significant healthier lifestyle changes, dietary practices of people and consumers opting for immunity enhancing supplements especially during the COVID-19 pandemic, the yearning for mushroom based nutraceuticals and functional foods is on rise (Kour et al., 2022). Worldwide, the inclusion of mushrooms in diets is growing (Samuel, Ajonina and Tatah, 2012), due to their high nutritional content and therapeutic qualities (Chang and Buswell, 1996; Miles and Chang, 1997; Chang and Miles, 2004). They are an excellent source of minerals, vitamins, and protein are significant in terms of nutrition as well due to their higher protein, dietary fiber, and mineral contents (Khan et al., 2009). The use of mushrooms as a source of biologically active compounds with potential medical benefits, such as hepatoprotective, immunopotentiating, anti-viral, anti-cancer, and hypocholesterolaemic agents, is now increasing prominence, known as **"Mushroom Nutriceuticals,"** this novel class of compounds can be extracted from the fruiting body or fungal mycelium and are a significant part of the mushroom biotechnology industry, that is rapidly growing (Chang and Buswell, 1996). Mushrooms are beneficial against lung diseases, diabetes, and ulcers (Quimio, 1980; Wasser, 2005). This

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is against the backdrop of the knowledge that mushrooms have a great potential for converting inexpensive celluloses into valuable protein The ability to grow quickly and adapt to different lignocellulosic substrates allows for the cultivation of different mushroom species in different regions of the world (Poppe, 2000).

About Pleurotus Species:

About 40 distinct species, collectively referred to as "**Oyster Mushrooms**," belong to the genus Pleurotus (Krishnamoorthy and Sankaran, 2014). Many Pleurotus species are now commercially grown due to their abundant mineral content, therapeutic qualities, short life cycle, reproducibility in the recycling of specific industrial and agricultural wastes, and minimal resource and technological requirements (Yildiz et al., 2002).

Pleurotus sp. is most well-known species and is consumed by people worldwide because of its flavor, taste, high nutritional content, and therapeutic qualities. P. ostreatus has been shown to possess antidiabetic, antibacterial, anticholestrolic, antiarthritic, antioxidant, anticancer, eye health, and antiviral properties due to its diverse active components and nutritional compositions (Krishnamoorthy and Sankaran, 2014).

However, according to scientific classification, Pleurotus sp. mushrooms were classified as (Krishnamoorthy and Sankaran, 2014).

Taxonomic Description

Kingdom	:	Fungi
Phylum	:	Basidiomycota
Class	:	Agaricomycetes
Order	:	Agaricales
Family	:	Pleurotaceae
Genus	:	Pleurotus

Source: (Krishnamoorthy and Sankaran, 2014).

What is mushroom cultivation?

A reliable biotechnological method for converting different lignocellulosic wastes is said to be **'Mushroom Cultivation'**. In order to create simpler compounds for nutrition, it breaks down the complex organic materials (the substrate) on which it grows (Chang and Miles, 1992). Certain mushrooms are useful as dietary supplements, others are nutritious foods, and some offer both medical and nutritional benefits. Natural foods used as dietary supplements are becoming more and more commercialized as a result of the public's growing concern about nutrition and health issues in recent years. In this sense, mushrooms might be categorized as functional foods (medicinal or nutritional foods). Medicinal or functional foods should not make any claims to be cures for illnesses, but a growing body of research indicates that certain foods, like mushrooms, can help prevent illnesses and, in certain situations, even reverse or reverse the effects of existing illnesses (Chang and Miles, 1989).

Mushroom cultivation and industry can produce zero emissions and provide new employment opportunities. The global value of mushroom cultivation and mushroom-derived medicinal goods was estimated at 14 billion US dollars in 1994, similar to the value of coffee output in 1997. The growth rate of commerce in mushrooms and related goods may surpass that of coffee products in the future. Mushrooms cannot convert carbon dioxide and water into complex organic matter due to their absence of chlorophyll. However, they can generate enzyme complexes that transform waste materials into various products, including food, feed, fertilizers, health supplements, and medicines. These products also have positive benefits on human welfare and environmental regeneration. Research and production of edible macrofungi and therapeutic mushrooms can contribute to fair economic growth, as over 70% of agricultural and forest products are unproductive and squandered during processing. Thus, the cultivation of mushrooms and their derivatives, along with sustainable Research and Development, have the potential to fuel a **"Nongreen Revolution"** (Chang, 1999).

What is substrate?

Organic materials that facilitate the growth, development, and fruiting of mycelia in mushrooms are commonly referred to as **'Mushroom Substrates'.** All of the components or foods required for the growth of mushrooms are combined to create these materials (Kadhila et al., 2008).

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What is Sterilization?

Sterilization is a technique used to prepare mushroom substrates that includes the use of steam, pressure, time, and temperature to kill spores and living organisms.

Is Sterilization of substrate in Mushroom cultivation necessary?

For mushroom cultivation to avoid contamination and to create the best conditions for the growth of mushroom mycelium, the substrate must be sterilized. A mycelium of fungus can be outcompeted by contaminants such as mold, bacteria, and other organisms, which stunts growth and reduces yields. Sterilization, according to science, eliminates both dormant and living organisms, such as fungal spores, leaving the mushroom mycelium free to grow.

Depending on the nutritional value and kind of mushroom being grown, different substrates may require different levels of sterilization.

Factors affecting Substrate sterilization in Mushroom cultivation:

- Temperature
- Pressure
- Time
- Substrate type
- Type of Mushroom Cultivated
- Moisture content
- Nutrient content of Substrate
- Sterilization Equipments
- Importance of understanding factors

Source:**https://nublumemushroom.com/blogs/blogs/the-ultimate-guide-to-sterilizing-mushroom-substrate-for-beginners**

Significance of Substrate sterilization in Mushroom cultivation:

Substrate sterilization plays a pivotal part in oyster mushroom development by killing contaminants that can restrain mycelial development and guaranteeing effective mushroom generation. Legitimate sterilization strategies are fundamental for keeping up a clean and ideal environment for mushroom development. In a study by (Patel et al., 2023), the authors emphasize the significance of Substrate Sterilization in Oyster mushroom cultivation to eliminate potential pollutants that could obstruct mycelium growth. Various sterilization methods are discussed, each with its benefits and drawbacks, highlighting the importance of choosing the right sterilization technique for optimal mushroom production.

Furthermore, research by (Mistry et al., 2023) delves into different Substrate sterilization methods and their impact on the performance of Oyster mushrooms. This study underscores the indispensable nature of proper Substrate sterilization in Oyster mushroom Cultivation, emphasizing the need for growers to follow effective sterilization practices to ensure successful mushroom production. Here is a Clarification of the Significance of Substrate sterilization.

Contamination Avoidance:

Substrate sterilization is vital to avoid the development of annoyance microorganisms that can prevent mushroom development. Successful sterilization strategies help eliminate contaminants and make a favourable environment for solid mycelial colonization (Gowda and Manvi, 2019).

Enhanced Growth and Yield:

Appropriate Substrate sterilization advances solid mycelial development, quicker colonization, and higher yields of mushrooms. By disposing of competition from undesirable life forms, Sterilization guarantees ideal conditions for Oyster mushroom development (Shrestha et al., 2021).

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Quality Assurance:

Sterilizing the substrate is basic for guaranteeing reliable quality in mushroom generation. Sullied substrates can lead to poor-quality mushrooms with compromised taste, texture, and nutritional esteem. Sterilization makes a difference keep up the required quality benchmarks (Shrestha et al., 2021).

Research on Sterilization strategies:

Studies have investigated different Substrate Sterilization procedures, such as Chemical Sterilization, Hot Water Treatment, and Steam Sterilization, to decide their viability in clam mushroom development. Investigate points to recognize the foremost reasonable Sterilization strategies for maximizing yield and quality (Patel et al., 2023).

Financial Affect:

Productive Substrate Sterilization practices contribute to the financial reasonability of Oyster mushroom development by lessening contamination dangers, expanding efficiency, and guaranteeing steady edit quality. Legitimate Sterilization Strategies are basic for feasible and beneficial mushroom cultivation (Soldatenko, Devochkina and Ivanova, 2019).

II. REVIEW OF LITERATURE

Traditional sterilization methods:

Traditional sterilization methods are commonly used in mushroom cultivation to eliminate contaminants and ensure successful growth. These methods involve techniques like Steam Sterilization, Hot Water Immersion (scalding), Chemical Sterilization, Composting and Pasteurization (Gowda and Manvi, 2019).

Steam Sterilization (Autoclaving):

Steam sterilization, moreover known as autoclaving, could be a broadly utilized strategy in mushroom development. It includes exposing substrates to high-pressure immersed steam at temperatures over 121°C. Steam sterilization viably kills microorganisms, including spores, guaranteeing a sterile environment for mushroom development (Patel et al., 2023).

Hot Water Immersion or Scalding:

Hot water treatment is another conventional strategy where substrates are soaked in hot, boiling water to dispose of contaminants. This strategy includes soaking the substrate in water at temperatures extending from 70 to 80°C some time recently bringing forth. Hot water at 80°C is used in both techniques. The length of time separates the two of them. Usually, the substrate is submerged in Hot water for an hour in order to scald. It takes 1.5 hours for Immersion.

In this method, after soaking in tap water for the entire night, the Cellulosic materials were decanted to remove the excess water. Once more, the excess water was extracted by decantation after two hours of cooling after the addition of hot water (at 70 to 80°C) (Gowda and Manvi, 2019).

Composting:

Button mushroom cultivation usually involves composting (Agaricus). However, it was also incorporated into the culture of oyster mushrooms (Pleurotus).

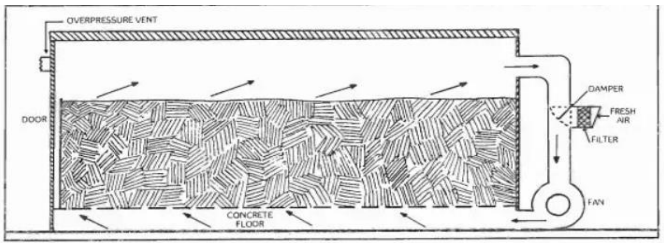

Fig 1: Sketch of a tunnel

Source:https://improvemushroomcultivation.com/sterilization-method-will-impact-your-mushroom-yield/#_ftn1

Compositing is done in two steps:

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Phase 1- Is the initial phase in breaking down the mixed raw materials and is usually carried out outside using Chemical and Biological processes. The substrate itself heats up to 80°C during this phase.

Phase 2- Is the biological process that completes the Decomposition and is carried out inside. The temperature is not regulated in phase 1, but it is carefully regulated for a predetermined period of time in phase 2. The temperature is maintained at 56–60°C for eight hours throughout the Initial half of this process, known as the 'Pasteurization Phase'. The so-called conditioning period begins after that, when the temperature is lowered to 45°C for a maximum of seven days. Following the removal of the volatile ammonium from the process air, the conditioning phase comes to a conclusion.

Source:https://improvemushroomcultivation.com/sterilization-method-will-impact-your-mushroom-yield/#_ftn1

Pasteurization:

Pasteurization could be a strategy commonly utilized for less nutritious substrates like straw. This procedure includes heating the substrate at temperatures extending from 60 to 80°C to kill harmful microorganisms whereas protecting useful microbes. The pathogen type contained inside the substrate will determine the pasteurization time and temperature. Temperature influences the pasteurization time (Abe et al., 1992; Mansur et al., 1992). Therefore, choosing the ideal Pasteurization temperature and time is a difficult operation that must be done in order to preserve the beneficial organism in the disinfected substrate (Gowda and Manvi, 2019). Pasteurization is viable in diminishing contamination dangers and planning substrates for mushroom development (Patel et al., 2023).

Steam Pasteurization:

For this treatment, a significantly altered version of the (Khare et al., 2010) approach was used. The steam pot was a metallic drum with 210 liters capacity. After cleaning, the steam pot was set over the firewood burner and 60 centimeters of water were added. Inside the steam pot was a cage that was 25 cm high above the water's surface. The cage was filled with the substrate. The water was kept at boiling point for two hours while the drum was sealed with two layers of synthetic (nylon) bags and fastened with strings (Khonga et al., 2013).

Chemical Sterilization:

Chemical sterilization includes the utilize of fungicides like benzimidazole compounds (e.g., benomyl, carbendazim) to treat substrates for mushroom development. Whereas chemical sterilization is prevalent due to its low cost, it raises concerns approximately residue discovery in mushrooms and potential wellbeing dangers. Inquire about focuses on distinguishing secure fungicide concentrations for successful substrate sterilization (Patel et al., 2023).

The most attention is drawn to chemical sterilization since it eliminates all of the complications associated with other techniques. This technique does, however, have a number of disadvantages, such as the use of carcinogenic chemicals that can pollute the environment, harm the health of workers who inhale the fumes, cause chronic diseases in humans due to chemical residues in mushrooms, and increase the risk of competitor molds developing resistance to the chemicals over time (Gowda and Manvi, 2019).

Table 1: Compiles and displays the various Steam (hot) Sterilization and Pasteurization methods used over the past few decades.

Pre treatment	Inference	Referance
Paddy straw is steam sterilized for 1 hour at 121°C.	Inequitable industrial technique that has a catastrophic impact on competitor molds and infections	(Rajarathnum and Bano, 1987)
Hot water pasteurization at 60°C for two hours, and hot water pasteurization at 60°C for three hours, at 121°C for four hours. (using sugarcane bagasse and horse manure compost)	Due to cost and capacity concerns, hot water pasteurized bagasse (60°C for 3 hours) proved to be a feasible method, even though autoclaved bagasse produced the best yield (410.4 g/1kg substrate with BE of 82.10%).	(Oseni et al., 2012)

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Hot water treatment for five hours at 90°C	Pleurotus growing at its best in polyethylene bags	(Dravininkas, 1997)
Immersion in hot water at 65 ± 5°C for 10 to 1 hour	keeps competitor organisms away and creates an ideal environment for Pleurotus species growth of mycelium	(Gowda and Manvi, 2019; Kurtzman and Zadrazil, 1982)

Table 1 Non-chemical sterilization of various substrates used in mushroom cultivation (Gowda and Manvi, 2019). Due to the high cost of the equipment required for Autoclaving, Hot water, and Steam Pasteurization, Mushroom growers prefer Chemical Sterilization as a means of decontaminating the substrate. Since Chemical Sterilization doesn't require additional equipment, it can be done quickly and affordably for large quantities of straw. Several chemicals are used to sterilize straw, but only a few have proven to work better than others. The most widely used chemicals are formaldehyde and hydrogen peroxide. Table 2 compiles the various chemicals used to sterilize the substrate.

The Alkaline Immersion Technique (AIT), which is inexpensive and non-thermal, is a simple Chemical disinfection method that involves soaking the substrate in lime water for 12 to 48 hours. Table 2 illustrates this technique. The AIT methodology was suggested by Contreras et al., (2004). Later, many workers followed the example. However, according to Hernandez and Sanchez, (2013), the disadvantages of this approach included the potential for bacterial contamination and fly egg survival, the requirement to soak straw overnight before use, a longer preparation time, lower mushroom yields, and a higher risk of B.E (Biological efficiency).

Table 2: Chemical sterilization of Different substrates used in mushroom cultivation (Gowda and Manvi, 2019).

Chemical	Inference	Referance
In a solution (10 L of water + 12.5 mL formaldehyde + 0.7 g carbendazim per kg substrate) for 18 to 24 hours.	The seven cultivated species of Pleurotus showed varying average biological efficiencies, ranging from 35% to 85.2%.	(Chandravanshi et al., 2012)
After being soaked in water for 12 hours, paddy straw was treated with 500 ppm of formaldehyde and 75 ppm of carbendazim. The solutions of hydrogen peroxide at 1.5%, 3.0%, 6.0%, and 8.0% (v/v) • A 33% chlorine bleaching powder solution at 2%, 4%, 6%, 8%, and 10% (w/v)	Total inhibition of competitor molds, with the maximum yield of 129.5 g/1 kg of substrate treated with formaldehyde and bavistin. However, up to 39.33% of contamination was noted, which is twice as common as hot water treatment.	(Saritha and Pandey, 2010)
Sterilized paddy straw using 500 ppm of formalin and 75 ppm of carbendazim	Maximum Pleurotus sp. yield (1700 g/kg, 85.9% B.E.)	(Rajak et al., 2011)

COMPARISON OF METHODS

Each conventional sterilization strategy has its benefits and downsides. Steam sterilization ensures thorough sterilization but may harm supplements within the substrate. Hot water treatment is compelling for lessening microbial stack, whereas chemical sterilization raises concerns almost residue detection. Pasteurization balances contamination control with supplement conservation, making it appropriate for different substrates (Patel et al., 2023).

To get the best results, it's essential to select the right sterilization technique based on the nutrients in the substrate and the kind of mushrooms being grown.

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Summary:

Boiling, pasteurization, Sterilization, and Fermentation are some of the substrate disinfection techniques used in mushroom cultivation that are covered in this review. The most common method is Chemical Sterilization because it is simple to use, but it has disadvantages like worker carcinogenicity, environmental pollution, and possible resistance to competing molds. However, since Pasteurization produces organically rich waste that can be used as fertilizer for the growth of cash crops, it is more effective in eliminating competitors and lowering yield. Extended cooling times during Pasteurization may result in contamination issues. Moreover, it eliminates helpful organisms that hinder the growth of contaminants, increasing its cost and rendering it unaffordable for growers of commercial mushrooms.

Consequently, it is advised to pasteurize using steam or hot water, which is better suited for small-scale growers. Due to a variety of factors, including spawn quality, pasteurization effectiveness, substrate quality, moisture, availability of nutrients, hygienic conditions, cross-contamination, and environmental factors during growth, mushroom growth and yield results are still inconsistent.

III. FUTURE DIRECTIONS AND ADVANCEMENTS IN STERILIZATION METHODS

Innovative Sterilization Techniques:

a) Ozone-Based Sterilization

Ozone-based sterilization may be a novel strategy that appears guarantee in oyster mushroom development due to its effectiveness and proficiency. This strategy includes infusing ozone into the mushroom substrate at particular concentrations and treatment times to attain sterilization. The utilize of ozone in mushroom cultivation has been found to be more compelling in terms of generation and time compared to conventional steam sterilization procedures. The infusion of ozone into the substrate allows for the end of contaminants, counting microscopic organisms and spores, without the required for energy consumption related with steam sterilization. Investigate has focused on determining the ideal ozone concentration and treatment time required to sterilize the whole volume of the substrate successfully. Comes about have appeared that ozone treatment is less time-consuming than conventional strategies, driving to expanded generation productivity and decreased costs within the long term (Sidik et al., 2015).

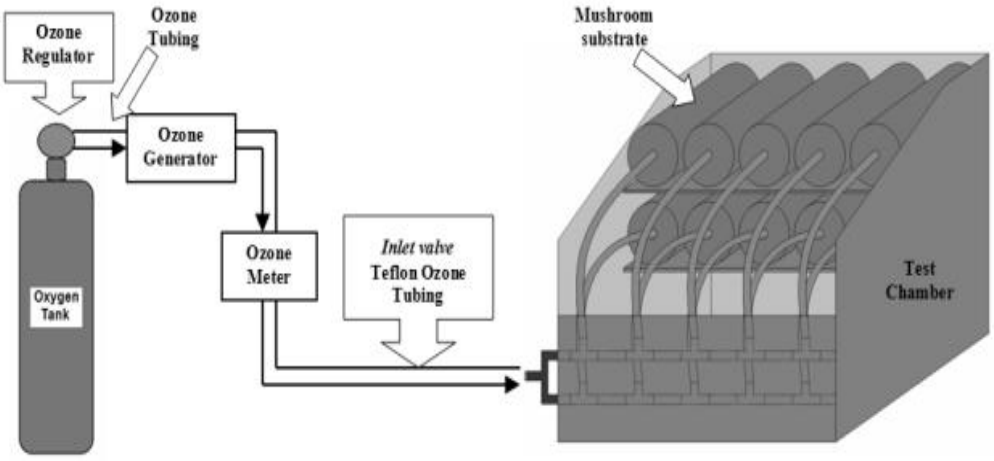

Fig 2: Ozone sterilizing system

Source: (Sidik et al., 2015).

The lengthy sanitization process system and the sharp rise in energy costs have a significant impact on mushroom farms' operating margins and expenses. Consequently, this has a significant effect on mushroom farms' capacity to meet consumer demands. In the industrial sector, ozone treatment is frequently used to treat wastewater, water, and odors (Omer and Walker, 2011). Applications of ozone for food processing and agriculture have drawn more attention recently. According to (Smilanick, 2003), the FDA classified ozone as a GRAS (generally recognized as safe) substance in 2001. Ozone is an extremely reactive form of oxygen that is made up of three bonded molecules. Due to its weak bond, one of the oxygen atoms is easily accessible for

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electron transfer. These cell walls are attacked by Ozone, which destroys the membranes and the organism's ultrastructural elements (Bocci, 2009; O'Donnell et al., 2012). Over time, Ozone treatment will prove to be more economical than traditional disinfection methods due to its shorter duration. An estimated six times more substrate can be sterilised per day using an ozone sterilising process than with a conventional technique. Depending on the Ozone concentration, the time required to kill the molds and bacteria in the substrate can be as little as 30 minutes, with a ten-minute ozone settle-down period.

Case study:

The ozone sterilizing instruments used in the experiment has been shown in Figure 2. For 30 minutes, ozone was injected into ten substrate samples that were placed inside black PVC bags at varying concentrations (50 ppm to 500 ppm). After adding roughly 2 g of oyster mushroom spawn to the treated sawdust substrate in the bags, the mixture was incubated for 60 days at a temperature of 28 to 30° C in a dark chamber, until full mycelium colonization was achieved. Using a measuring tape, the mycelium's growth (linear length) in each bag was recorded every six days. The spawn run rate (cm/day) for each spawn type was calculated using these data. When the mycelium had completely covered the substrate bag (meaning the spawn run had finished), and the recording of the spawn run in the substrate bag had finished. After that, the bags were moved to the mushroom cropping room, where fructification was started by opening them. The lowest concentration of ozone that can eliminate every bacteria and mold in the substrate in a given amount of time is considered the ideal concentration. The growth rate of the mycelium in each sawdust substrate was used to track and report the effectiveness of the ozone treatment at various concentrations. The ideal concentration and treatment duration were established, and the treatment time and ozone concentration were used to assess the efficacy of the ozone therapy (Sidik et al., 2015).

Summary:

The study's findings have an impact on mushroom cultivation on a large scale generally. According to the experimental findings, injecting Ozone as a treatment system to sterilize mushroom substrate works better, faster, and cheaper than the existing techniques. The findings also indicate the ideal ozone concentration, the shortest treatment duration, and the system's possible application in extensive mushroom farming (Sidik et al., 2015).

b) Cold Plasma Technology:

The crop residue into a biomass that is higher in protein and affect the yields of mushrooms when used as a substrate for mushrooms. Agribusiness as a whole as well as farmers may find these substrates' great potential for mushroom growth to be both economically inviting and advantageous (Rosmiza et al.,2016) (Letti et al.,2018). Aspergillus (Bellettini et al., 2019) or Trichoderma species (Sudirman and Solihin, 2014) residues, however, are present on the substrate when using crop residue, A long-standing knowledge base exists regarding Trichoderma green mold infection (Bellettini et al., 2018). Many techniques are used to get rid of microorganisms, like using fungicides or combining 10% alcohol, 70% clorox, and 0.5% formalin during the sterilizing process. Inactivating microorganisms also requires the traditional 48-hour steaming method. These approaches are not without their drawbacks, though; some are labor-intensive, time-consuming, potentially fatal, and harmful to the environment like chemical sterilization (Li et al.,2016).

Consequently, Cold Plasma (CP) Technology has gained prominence in the agriculture sector as a means of overcoming these barriers (Sivachandiran and Khacef, 2017). For Surface Decontamination, CP is an effective for microbial inactivation (Bourke et al., 2018). CP is an ionized gas that can be produced at atmospheric pressure using a dielectric barrier discharge (DBD) system (Sivachandiran and Khacef, 2017). The electron temperature in atmospheric pressure cold plasma is significantly higher than the neutral and ion temperatures, which are normally similar to each other (Misra et al., 2014). Since the industry does not require extreme processing conditions like high temperature and pressure, the design and control of plasma sources operating at or near atmospheric pressure is of interest, both technically and commercially (Misra, 2016).

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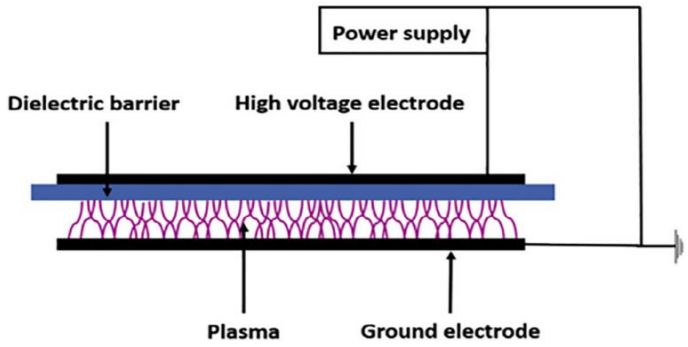

Fig 3: Dielectric Barrier Discharge (DBD) Plasma System

Source: (Agun et al., 2021)

One of the most popular techniques for producing cold plasma is Dielectric Barrier Discharge (DBD), in which plasma is produced between two metal electrodes, at least one of which has a dielectric layer covering it. In order to prevent arc transition and to provide a more uniform treatment, the dielectric layer distributes streamers randomly across the electrode surface (Wan et al., 2017) The mushroom industries are interested in CP generation for mushroom cultivation due to its ability to be applied in ambient conditions, speed up the cultivation period, increase treatment speed, and enable industrial applicability. This is true both technically and commercially (Bourke et al., 2018).

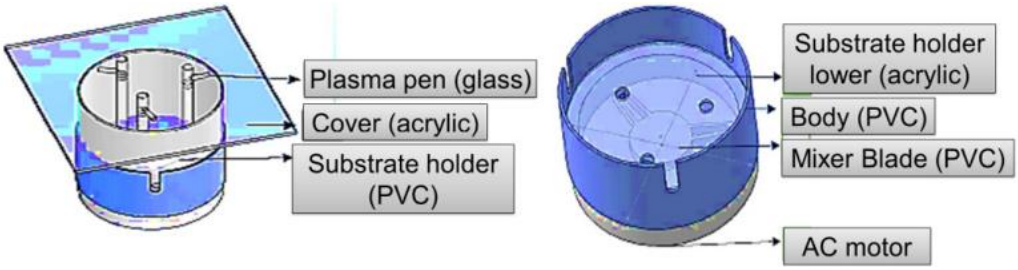

Fig 4: (a) DBD-CP pen mixer system; (b) DBD-CP pen

Source: (Agun et al., 2021)

Case Study:

(a) The DBD-CP pen mixer setup

A new and scalable dielectric barrier discharge cold plasma (DBD-CP) pen mixer system will be used for the experiments. The apparatus will be placed inside a fume chamber. The DBD-CP pen mixer system will receive atmospheric pressure at a flow rate of 2 L/min while accounting for varying exposure times of 0, 5, 10, 15, 20, and 25 minutes. Using power stainless steel electrodes, a 6 kV AC high voltage (KVM500 Adjustable Power Supply; 1–40 kV–70 kHz 10–300w) was used to create the DBD plasma shown in Figure 4(b). The ground electrode was made of copper. A Picoscope (TDS2014, American Tektronix Co.) was used to measure the discharge voltage and current (Agun et al., 2021).

(b) The development of DBD-CP pen mixer

This plasma mixer is made up of three plasma pens that are symmetrically placed 120 degrees apart from one another. In order to ensure that every substrate was treated equally and received an equal amount of plasma, this system was made. The following are the design configurations: (i) The height of the plasma pen is 15 cm; (ii) The mixer body measures 22 cm in height and 15 cm in diameter; (iii) The substrate capacity is 50 g after sterilization; and (iv) The AC motor runs at 220–240 Watts at 3 rpm (Agun et al., 2021).

Therefore, the goal was to investigate the effectiveness of the Dielectric Barrier Discharges Cold Plasma (DBD-CP) pen mixer system on the sterilization of agricultural residue substrate used in Oyster mushroom production. The electric field created by voltage breakdown is used to discharge the plasma and initiate this renewal process. The plasma will discharge onto the crop residue substrate after varying sterilizing times of 0 minutes, 5 minutes, 10 minutes, 15 minutes, 20 minutes, and 25 minutes. The approach of examining soil-borne

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pathogen inactivation on agro-waste substrate will involve the use of bacteria colony forming units (CFUs) (Agun et al., 2021).

Summary:

A viable and effective technique for sterilizing crop residue used as mushroom substrate is cold plasma technology. It can take the place of conventional steaming techniques, sanitizing bacterial colonies and accelerating the growth of mushrooms. While standard steaming takes 48 hours, the ideal sterilizing duration is only 25 minutes. To maximize processing conditions and promote mushroom development, more research is required (Agun et al., 2021).

Pros and Cons of Substrate sterilization

Pros:

The removal of exceed species.

Higher yields obtained by supplementing with nutrients; and the capacity to cultivate a greater range of mushrooms on diverse nutrient-rich substrates.

Cons:

High energy usage.

Expensive pressure equipment.

Need for close observation while the heating process goes on.

A laborious procedure that needs diligence and dedication.

IV. CONCLUSION

In Oyster mushroom cultivation, sterilizing the substrates is an essential step that eliminates contaminants and creates an optimal environment for mycelial growth and mushroom production. The efficacy and impact of several sterilization techniques, including chemical sterilization, composting, steam sterilization, hot water immersion, pasteurization, and composting, have been thoroughly investigated. Every approach has benefits and cons, with influencing elements like financial viability, avoiding contamination, and preserving nutrients playing a role.

Furthermore, advanced sterilization techniques like cold plasma technology and ozone-based sterilization have emerged as potential substitutes, providing quicker, more economical, and efficient results than conventional techniques. Ozone-based sterilization exhibits promise for promoting yield and cutting expenses in the mushroom industry because of its capacity to penetrate substrates and get eliminate of contaminants without using as much energy as steam sterilization. Likewise, agricultural residues can be sterilized with cold plasma technology, which speeds up the cultivation process without compromising the integrity of microbes.

The development of sterilization techniques not only increases the productivity of mushroom farming but also supports sustainable agricultural practices by lowering energy use and waste production. To fully realize the potential of these methods in commercial mushroom production, more research is necessary to optimize them. Substrate sterilization is still, in general, a crucial component of mushroom cultivation. It assures high-quality yields, reduces the risk of contamination, and moves the sector closer to greater sustainability and profitability.

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