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Elimination of Contamination in Plant Tissue Culture Laboratory

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ABSTRACT

Microbial contamination remains a persistent obstacle in the advancement of in vitro techniques, particularly in the context of plant tissue culture and micropropagation. Maintaining a sterile environment throughout the establishment, manipulation, growth, and storage of in vitro cultures is essential for research and commercial applications. Strategies and tactics for reducing contamination in plant tissue culture laboratories are examined in this review. These include identifying the sources of contamination, conducting routine monitoring, using aseptic procedures, properly sterilizing, handling plant materials safely, and disposing of them. By examining previous and current research, this study offers insights into optimizing these methods to effectively control contamination in various crops and explants, providing valuable guidance for researchers and practitioners seeking to establish robust contamination control protocols in plant tissue culture laboratories.

Keywords: *Plant Tissue Culture, Microorganism, Contamination, Sterilization*

Introduction

Plant tissue culture is useful for preserving and growing a wide variety of plant species; however, contamination is a major issue. In the laboratory, dust, microorganisms, and plant debris can cause plant cells or explants to die, which can also reduce the output yield [1]. The effectiveness of tissue culture depends on identifying the sources of contamination and implementing the required controls. Contamination in plant tissue culture laboratories is a major problem that causes a lot of time, effort, and money to be lost for researchers. Maintaining a sterile atmosphere and following established methods are crucial to prevent contamination in plant tissue culture facilities. Contamination can enter the laboratory environment through a variety of routes, including airborne microorganisms, contaminated equipment, media, reagents, and improper handling techniques [1] [2]. Microorganisms in the air can stick to open culture vessels and media, resulting in contamination. Microorganisms can enter culture media if tools such as pipettes, bottles, and culture jars are not adequately sanitized. A source of contamination might also come from media and reagents that are not sterilized before use. Contaminants may be introduced into the environment during culture operations if sterile conditions are not maintained or incorrect handling procedures are employed [2] [3]. Additionally, previous studies have demonstrated that endophytic and epiphytic fungi on plant surfaces can introduce contaminants into culture systems [4]. As these microbes are difficult to eliminate, effective control measures are needed to lessen their effects.

According to a previous study [5], bacterial and fungal spores are the most common forms of contamination in tissue culture laboratories. This study also found that the two most frequently isolated bacterial taxa were *Alternaria* and *Aspergillus*. In addition to using standard practices, disinfectants, and UV light can help reduce contamination rates in tissue culture laboratories [6]. According to [7], the layout and architecture of plant tissue culture labs should consider airflow and ventilation in addition to avoiding potential sources of contamination, such as water sources. Contamination in tissue culture laboratories allows microorganisms to proliferate and compete with plant cultures for nutrients and space, resulting in reduced growth and survival rates. Genetic changes and mutations also occur in plant crops and can affect their fidelity and integrity. To prevent contamination, strict aseptic techniques should be used, equipment and growth media should be properly sterilized, and cultures should be monitored regularly for signs of contamination. To minimize the risk of contamination, it is also important to maintain a clean and controlled laboratory environment and train personnel in proper handling and care of plant cultures.

Determining the possible sources of contamination is essential to keep the plant tissue culture lab free of contaminants. Strict adherence to aseptic practices and safety precautions are required to guarantee successful plant tissue culture and to avoid contamination. This entails utilizing only sterile tools and media, maintaining a dust-free workspace within a clean room or laminar flow hood, and preventing entry of extraneous pollutants. The problem of contamination in plant tissue culture laboratories has been the subject of numerous studies. The types, sources, and levels of contamination in plant tissue cultures, as well as suggested corrective actions [2]. In their investigation of the risk of cross-contamination resulting from microbial transfer across different plant species, [4] endophytes

and epiphytes of different plant species were also identified as potential contamination sources. The study concluded that rigorous separation and ongoing monitoring are necessary for lab benches, equipment, and media of different plant species to minimize the risk of cross-contamination.

Strict and ongoing environmental monitoring and maintenance are necessary to control contamination in plant tissue culture labs [8]. The application of aseptic procedures, including the use of sterile tools, media, containers, and workstations, is one of the most crucial steps in preventing contamination [9]. This aseptic method reduces the possibility of microbial contamination and minimizes cross-contamination across cultures. Furthermore, it is critical to sterilize the plant materials to eradicate surface bacteria. This can be achieved by applying solutions such as ethanol or bleaching to plant tissues and explants [8–10]. It is also important to handle plant material properly; if any contaminated or dead plant material is found, it must be removed immediately [11]. Regular monitoring and visual inspection of plant tissue cultures are essential to detect signs of contamination. Visual and microscopic examinations of cultures can help determine the presence of microbial contamination [8] [11]. It is also important to develop strategies to prevent contamination from spreading within the laboratory, including the decontamination of laboratory surfaces and equipment [12]. Numerous studies have investigated effective treatments to prevent the contamination of plant tissue cultures. For instance, [13] researched the use of cinnamon extract as an antimicrobial agent to control fungal contamination in plant tissue cultures.

Therefore, the goal of this review is to provide a comprehensive overview of the various methods and approaches that can be utilized to reduce contamination in laboratories working with plant tissue cultures. It covers topics such as aseptic procedures, contamination monitoring, and control methods as well as sources of contamination and techniques for isolation, identification, and sterilization. Ultimately, the primary objective was to offer valuable advice to practitioners and researchers in the field of plant tissue culture to enhance the quality and reliability of their research.

Plant Tissue Culture Sources of Contamination in the Laboratory

Contamination poses a significant risk to plant tissue culture and can have deleterious effects on crop output and quality. Numerous sources of contamination, such as airborne particles, contaminated equipment, incorrect handling of plant material, and improper sterilization processes, were identified in a detailed review conducted by [14] [15]. Introducing air pollutants into laboratories is not recommended because bacterial and fungal spores can stick to the medium, plant tissue, and equipment and contaminate them [16] [17]. Laboratory personnel bear primary responsibility. Maintaining a hygienic laboratory setting with sufficient ventilation and measures to reduce contamination from this source are important [16] [18].

Another frequent problem is contamination by plant materials. Bacteria, viruses, and fungi are the most common infectious agents. Application of surface-sterilized explants to shoots. [8] [19] [20] studied *Camellia japonica L.* shoot cultivation *in vitro*. According to this study, surface contaminants can enter culture vessels when shoot explants are not properly surface sterilized [21]. Therefore, surface disinfection is important for the production of clean crops. Contaminated plant materials can also be sources of infection [22] [23].

advocated the use of aseptic techniques to prevent the transfer of contaminants to culture vessels when handling plant materials. The use of contaminated water and media is another source of contamination for plant tissue culture laboratories. To avoid contamination with bacteria, fungi, and other contaminants, the media and solutions must be prepared and stored correctly. The use of media in plant micropropagation has been examined previously [10] [24]. Plant development can also be hampered by the use of tainted growth regulators in plant tissue culture. Growth regulators should be prepared and stored properly to prevent pollutants from entering the plant tissue culture environment [14] [25-26].

According to[27] [28] Inadequate sterilization of equipment, glassware, and media can also lead to the introduction of contaminants into the culture environment Autoclaving sterilization is the most commonly used sterilization method in plant tissue culture laboratories. However, its correct use is essential to ensure the removal of microbial contamination. Sterilization protocols and equipment should be regularly monitored to ensure proper functioning, and autoclaves should be verified and tested before use [29] [30]. According to a review by [31], various sterilization methods can be used for plant tissue cultures. Each method has advantages and disadvantages and should be selected based on the specific needs of the laboratory and plant tissue culture studies being performed. Additionally, Poor aseptic techniques in plant tissue handling, subculture, and culture maintenance can lead to contamination in plant tissue culture laboratories. Failure to operate in a sterile laminar flow hood or improper use of sterile equipment can introduce contaminants into the culture. To improve aseptic techniques, staff should be trained and laboratories should be designed and equipped with appropriate types of equipment [32] [33]. Automated systems in plant tissue culture have been proposed to improve aseptic processing and eliminate human errors [34] [35]. Better aseptic techniques are essential for preventing the introduction of contaminants into the culture environment.

Isolation, Identification, and Characterization of Contaminants in Plant Tissue Culture Laboratories

In plant tissue culture laboratories, the process of isolating, identifying, and characterizing contaminants helps maintain the integrity and purity of plant materials while also halting the spread of undesirable microbes. It is crucial to remember that contamination can originate from a variety of sources, such as bacteria, fungi, viruses, and mycoplasmas, and that it can alter the morphology and physiology of plant tissues. Numerous techniques are needed to identify and characterize contamination, including plating, visual inspection, and molecular procedures like polymerase chain reaction (PCR) and sequencing [8] [36]. The focus of this research is to determine the type and level of microbiological contaminants in plant tissue culture laboratories and develop reliable methods for their control and prevention. Several studies have been conducted on the isolation, identification, and characterization of contaminants.

As per a previous report [37], it is possible to distinguish and recognize bacterial contaminants in plant tissue cultures. They integrated plating, 16S rRNA gene sequencing, morphological and biochemical assays, and other culture-dependent and independent techniques. According to this study, tissue cultures can be contaminated by several bacterial species, including *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Bacillus fluorescein*.

The authors emphasized the need to use these molecular techniques to accurately and rapidly determine bacterial contamination in plant tissue cultures.

[38] revealed in another investigation that they were able to isolate and identify fungal contamination from banana tissue cultures. This study employed molecular approaches, such as internal transcribed spacer region (ITS) DNA sequencing, in addition to culture-dependent techniques, including plate counting and morphology-based identification. To reduce contamination, this study identified several fungal species, including *Aspergillus, Alternaria, Penicillium*, and *Fusarium*. It also suggests that the plant materials, media, and disinfectant solutions were properly sterilized. The separation and detection of fungal contamination in diverse plant tissue cultures has also been reported [39]. The authors emphasized the significance of early detection and suitable management approaches by precisely identifying and characterizing fungal contamination using microscopy and molecular techniques. The investigation also focused on the molecular characterization and detection of bacterial contaminants in date palm tissue cultures [40]. In this study, bacterial species isolated from contaminated cultures were identified by 16S rRNA gene sequencing. Numerous bacterial species, such as *Pseudomonas Mendocino*, *Bacillus subtilis*, and *Enterobacter kowani*, were found in this study, emphasizing the necessity of routine tissue culture monitoring and aseptic procedures to avoid contamination.

A published study isolated and identified bacterial contamination in orchid tissue culture [41]. The species and culture-dependent molecular techniques were used to investigate antibiotic resistance. The investigation revealed high levels of antibiotic resistance in certain bacterial species, and identified several bacterial taxa, including *Bacillus*, *Pseudomonas*, and *Stenotrophomonas*. This emphasizes the importance of consistently monitoring and regulating tissue culture hygiene to avoid contamination. Furthermore,[42] studied the traits of bacterial pollutants in tissue-cultured potatoes. The authors identified specific bacterial species and evaluated their pathogenicity using phenotypic and genotypic analyses. They highlight the necessity of implementing efficient management techniques to stop the spread of bacterial contamination in plant tissue cultures.

Additionally [43] concentrated on the detection and description of fungal contamination in plant tissue cultures in a different investigation. Several fungal strains including *Aspergillus spp*., *Penicillium spp*., and *Fusarium spp*. were isolated and identified in this study. In addition, fungal strains were classified according to their morphology, pathogenicity, and molecular identity. This study emphasizes the necessity of using aseptic methods and appropriate sterilization to prevent fungal infections in plant tissue cultures. Researchers found and described viral contamination in plant tissue cultures in a study by [44]. In this study, several viral strains, including *tobacco mosaic virus* and *cucumber mosaic virus* (CMV), were isolated and identified (TMV). Researchers have also characterized the virus strains based on their morphology, molecular identification, and pathogenicity.

Handling Contamination in Plant Tissue Culture Laboratories

Creating efficient procedures for managing contamination in plant tissue culture laboratories is the subject of numerous studies.

The significance of avoiding or managing contamination in plant tissue culture laboratories is a recurring theme in these studies. A study by [45] stressed the need to adhere to the correct processes and rules to prevent contamination. Similarly, [46] emphasized the significance of aseptic practices in avoiding contamination.[47] in contrast, concentrated on creating efficient remedies to stop contamination after it has already occurred. These studies highlight the significance of avoiding or managing contamination in plant tissue culture, although from various angles.

Different approaches to managing microbial contamination in plant tissue cultures have been suggested in several scientific publications. As an illustration, some advocate using natural items as a therapeutic approach. Essential oils and plant extracts are used as natural products. According to [48], contamination in plant tissue cultures can be managed using AgNPs made from plant extracts. Similarly, [49] reported the use of thyme and rosemary essential oils to manage fungal and bacterial contamination in tissue cultures. According to these studies, contaminated plant tissue cultures can be successfully treated with natural ingredients. According to other studies, chemical disinfectants should be used to treat contamination. According to [45], the surfaces and instruments should be disinfected using a 70% ethanol solution. Comparably, [46] described disinfecting culture equipment using a mixture of bleach and ethanol solutions. Similarly, [47] suggested sterilizing plant tissue cultures with a hydrogen peroxide solution containing 2%. Collectively, these studies imply that chemical disinfectants may be useful in limiting the growth of microorganisms in plant tissue cultures.

Physical therapy is another recommended strategy for the management of microbial contamination. For example, certain studies recommend the use of ultraviolet (UV) radiation. To sterilize plant tissues, [45] suggested subjecting them to UV light for 10 to 30 minutes. Furthermore, a few studies have recommended the use of filtration methods to eliminate contaminating microbes. The use of membrane filtration to eliminate contamination from culture media was suggested by [47]. Collectively, these investigations imply that physical interventions may be useful in limiting microbial contamination in plant tissue cultures. The significance of reducing the influence of the control and treatment methods on the growth and health of plants is another similarity between these studies.[45] emphasized the significance of limiting the quantity of physical and chemical treatments performed to avoid damaging plant tissues.[46] suggested the use of disinfectants at lower dosages to reduce their effects on plant growth.

Additionally, it has been reported by [50-53] that achieving successful plant growth and development requires controlling contamination in plant tissue cultures. All these articles identified various treatment strategies to combat contamination, such as the use of natural products, disinfectants, and plant extracts. These articles also emphasize the importance of developing safe and efficient treatments that do not damage plant cultures during administration.

Prevention of Contamination in Plant Tissue Culture Laboratory

For plant cultures and experiments to be effective, contamination must be prevented in plant tissue culture laboratories. Standard operating procedures (SOPs) and aseptic techniques are necessary to maintain aseptic conditions [1].

This can be accomplished by routinely checking cultures for pollutants, sterilizing equipment, and training personnel in appropriate clothing. To stop microbial growth in the lab, the authors advise adopting disinfection procedures and sterilizing explants, media, and lab equipment. To reduce the risk of contamination, laboratory surfaces, instruments, and equipment should be regularly cleaned and disinfected. According to [54] the presence of bacteria, fungi, and viruses poses a significant threat to plant tissue culture. The authors suggested several techniques to prevent contamination. These include maintaining a clean and sterile environment, proper use of personal protective equipment, aseptic techniques for handling cultures and equipment, regular monitoring of cultures for contamination, and use of sterile media and equipment.

Similarly, [55] found that plant tissue culture laboratories may be affected by many types of contaminants, such as bacteria, fungi, and viruses. The use of laminar flow hoods, positive pressure airflow, and disinfection techniques as physical barriers is among the suggested countermeasures. To prevent widespread culture contamination and the consequent loss of plant material, it is recommended to routinely check cultures for early detection of contamination. The authors also stated that the use of sterile, high-quality culture fluids, instruments, and reagents is necessary for the successful cultivation of plant tissues. Additionally, [56] reported that airborne contaminants can enter the laboratory and affect culture quality. To reduce contamination risks, the authors recommended the use of laminar flow hoods, air filters, and controlled airflow systems. They suggested that proper ventilation could significantly reduce the risk of contamination and contamination-related losses.

[57] revealed that the quality of culture media is affected by sterilization methods. The authors recommended several sterilization techniques for culture media, including gamma irradiation, autoclaving, and filtering. To avoid contamination, they advise employing verified sterilization procedures and keeping an eye on process efficacy. Similar procedures for medium preparation, aseptic techniques, and sterilization are covered in the Handbook of Plant Cell Culture: Techniques for Propagation and Breeding [58]. The authors emphasized the significance of maintaining a sanitary and hygienic work area, which includes routine hand washing, donning gloves, and sterilizing equipment surfaces. Aseptic laboratory conditions can be maintained using disinfectants and antimicrobial agents. To lower the danger of contamination, the manual suggests minimizing congested work areas and designating specific locations for waste collection and cleaning.

[8] reported about how crucial laboratory upkeep and design are to keeping plant tissue culture labs free of contamination. This entails keeping a clean, regulated space with the proper humidity, lighting, and temperature levels. To reduce the amount of contaminants that enter, the author stressed the usage of air curtains, filters, and laminar flow hoods. By keeping particles out of the working area, maintaining positive pressure airflow in the laboratory can help prevent contamination even more. [59] further emphasized how crucial proper laboratory layout and upkeep are to the success of plant tissue culture. They highlighted various tactics, such as the application of aseptic procedures, antibacterial agents, and physical barriers. The authors stressed that to guarantee good plant tissue culture, it is essential to use premium sterile culture material, tools, and reagents. To further reduce the entry of pollutants, the book suggests maintaining positive pressure airflow and utilizing laminar flow hoods to create sterile working spaces.

Control of Contamination in Plant Tissue Culture Laboratory

To generate clean and healthy cultures, control over contamination in plant tissue culture is essential. The growth and development of plant tissue culture can be hampered by contaminants such as bacteria, fungi, viruses, and other microorganisms, which can result in poor quality or loss of the intended plant material. Numerous investigations have looked into various approaches to contamination control.

Sterilization Procedures: To avoid contamination, it is essential to keep plant tissue culture laboratories sterile. The need for disinfecting lab supplies, media, and plant materials used in tissue culture was emphasized in a study conducted by [60]. To avoid bacterial and fungal contamination, the authors advise using chemical disinfectants such as alcohol and sodium hypochlorite. Furthermore, high-temperature settings, autoclaving, and filter sterilization are further techniques utilized to accomplish successful sterilization, according to[47] [61]. Since plant tissue culture techniques are widely used in many fields, such as biotechnology and plant breeding, it is crucial to follow the right sterilizing procedures to guarantee the repeatability and success of plant research.

Aseptic Procedures: In plant tissue culture laboratories, using aseptic techniques is a highly recommended way for contamination management. In an investigation by [62], the authors suggest using face masks, gloves, sterile culture trays, and laminar flow benches to successfully stop the entry and growth of undesirable bacteria. By lowering the possibility of contamination and maintaining the integrity of plant cultures, these procedures aid in the creation of a sterile and regulated environment. Additionally, [63] noted the significance of aseptic practices in maintaining settings free of contamination. They stress how important it is to limit the transmission of invasive microorganisms by using sterile tools and avoiding nonsterile surfaces. To mitigate the potential for contamination, [64] provides comprehensive guidelines for upholding aseptic culture conditions and handling protocols. By employing these aseptic techniques, plant tissue culture laboratories can significantly improve the accuracy and dependability of their research.

Standard Operating Procedures: To ensure contamination control in plant tissue culture laboratories, it is crucial to establish and follow Standard Operating Procedures (SOPs). The authors of research by [65] stress the value of SOPs in standardizing many facets of cultural approaches. This means developing efficient waste management techniques, establishing appropriate equipment cleaning and maintenance guidelines, and outlining exact handling protocols. SOPs ensure that experiment results are reliable and repeatable by minimizing human error and inconsistent practices that could lead to contamination. Moreover, to prevent cross-contamination within cultures, [66]. encourage the implementation of SOPs for handling media, transferring cultures, and other important processes. Plant tissue culture labs can create a strong system that lowers the possibility of contamination and preserves the integrity of their cultures by adhering to these standard operating procedures.

Manage Medium Components: One of the most important ways to lower the risk of contamination in plant tissue culture laboratories is to control the media components. [67]

emphasize the need to obtain media components from reliable vendors, confirm batch consistency, routinely calibrate pH meters, and uphold appropriate storage conditions. By taking such precautions, the laboratory can lessen the likelihood of introducing contaminants through medium components. Culturists are urged by [62] to properly prepare and disinfect the culture media to prevent contamination. The authors emphasize how important it is to adhere to strict sterilization protocols to ensure the medium's sterility and prevent the growth of microorganisms. Moreover, several substitute techniques have been proposed by [68], to manage insect pests in plant tissue culture labs while preserving lab safety. These techniques, which range from mechanical to biological, are all meant to eradicate pests without running the danger of contaminating the area. Plant tissue culture labs can efficiently handle medium components and reduce the chance of contamination by putting certain precautions in place.

Regular Culture Monitoring: Regular culture monitoring at different stages is essential to guarantee early detection of contamination in tissue culture propagation. To successfully monitor cultures, [61] reported the value of visual inspections, microbiological assays, and molecular biology approaches like DNA fingerprinting. With the use of these monitoring tools, researchers can identify any indications of contamination, such as anomalies in vision or the presence of microbiological contaminants. [47] [60] further emphasizes how important it is to remove infected cultures as soon as possible to stop contamination from spreading throughout the laboratory and maybe into the surrounding area. Plant tissue culture laboratories can protect the integrity and purity of their cultures by establishing routine monitoring procedures and acting quickly in cases of contamination.

Managing Insect Pests: Reducing contamination in plant tissue culture labs requires effective management of insect pests. According to [69], controlling insect pests with insecticides is a successful strategy. However, because strong insecticides can be harmful to lab personnel as well as plant materials, the authors advise against using them. When using such chemicals, care must be taken to avoid compromising the quality of the cultures. However, to maintain laboratory safety, [68] offer substitutes including mechanical, biological, and physical approaches of insect control. These techniques try to get rid of bugs without putting your health in danger. Researchers can avoid contamination in plant tissue culture labs by putting safe handling procedures for insect pests in place and maintaining tissue culture quality.

Quality Control: Upholding the standard of cultures in plant tissue culture labs is crucial to preventing contamination [70]. It is important to implement quality control methods to assess genetic stability and cultural competency. The authors discussed the value of employing bioassays to identify desired traits in cultures, such as resistance to herbicides or salt. It is also advised to conduct morphological studies to confirm genetic stability and uniformity. These two methods provide an effective way to evaluate cultures and ensure their quality before using them in further research. By aiding in the detection and elimination of noncompetitive cultures, efficient quality control processes can guarantee the preservation of the genetic stability of the culture and lessen the risk of contamination.

Plant Tissue Culture Environment: The key to preventing contamination in plant tissue culture is to establish and maintain an ideal laboratory environment. To stop contaminants from entering [71] emphasize how important it is to make sure that adequate air filtration is in place. The writers reported that to protect against undesirable microbes, the environment must be meticulously maintained. Similarly, [72] emphasized how crucial it is to precisely regulate humidity and temperature to meet the needs of various plant species. In tissue culture, different plants could need different humidity and temperature conditions to flourish. Furthermore, to encourage healthy growth and reduce the chance of contamination, lighting schedules that are customized to each plant species' unique requirements are necessary. In plant tissue culture laboratories, establishing an optimal laboratory environment with careful air filtration, temperature and humidity management, and tailored illumination can greatly aid in the control of contamination.

Conclusion

This review aimed to systematically analyze previous research on controlling contamination in plant tissue culture. It is evident from the research that contamination is a significant issue that hinders successful in vitro plant propagation. Various authors have explored different methods for contamination control, including identifying potential sources of contamination, regular monitoring of cultures, implementing aseptic techniques, proper sterilization procedures, effective handling, and disposal of plant materials using various methods to eliminate contamination, continuous monitoring, and the use of disinfectants and antimicrobial agents. It is important to minimize the impact of these methods on plant growth and health. A collective approach of staff training, sterilization protocols, monitoring, and quality control measures can establish contamination control in plant tissue culture laboratories and contribute to advancements in agricultural and biomedical research.

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