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Contamination Problems in the Micropropagation of *Passiflora* Species: Approaches for Control

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Review

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Abstract
Passiflora species are economically important plants widely cultivated in tropical and subtropical regions for their diverse nutritional, medicinal, ornamental, and economic uses. Although conventional propagation methods are commonly employed, these methods are associated with several limitations, including low multiplication rates, the risk of disease transmission, and genetic variability, particularly in seed-derived progeny. Therefore, plant tissue culture techniques have emerged as effective alternatives, enabling the rapid production of genetically uniform and healthy plant material. However, contamination is one of the most critical factors limiting the success of Passiflora micropropagation. When not effectively controlled, contamination can inhibit regeneration and shoot development, ultimately leading to the loss of explants and established cultures. Therefore, proper evaluation of contamination sources and the selection of appropriate sterilization and contamination-control strategies are essential for successful micropropagation. This review comprehensively examines contamination-related problems associated with the micropropagation of *Passiflora* species, the potential sources and types of contaminants, and the chemical and physical approaches used for their prevention and control, based on the available scientific literature.

Keywords: Contamination, Endophytic Microorganisms, *In vitro* Propagation, Sterilization

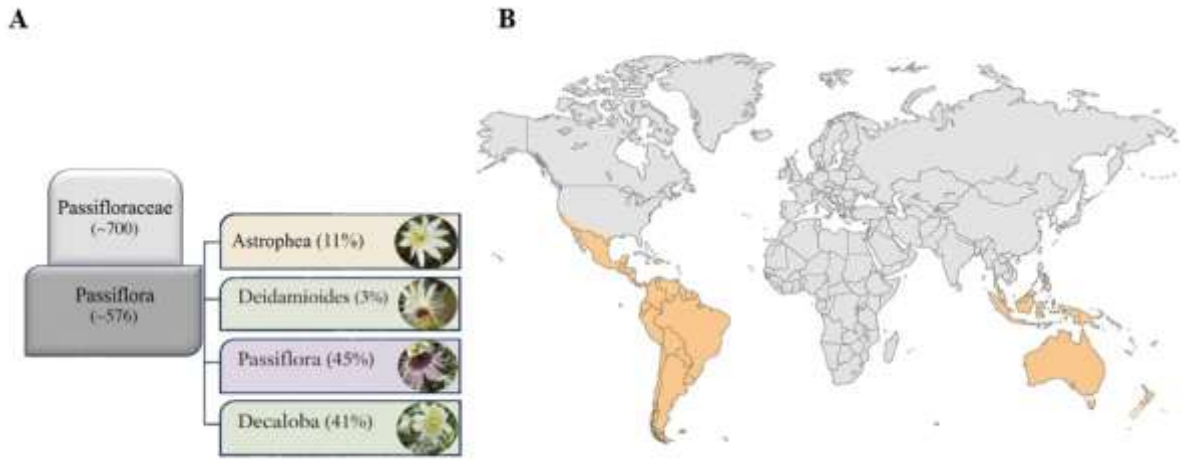
1. Introduction

The family Passifloraceae comprises 16 genera and approximately 700 species, including Passiflora edulis. The genus Passiflora represents the largest group within this family, encompassing 576 species, the majority of which are native to the tropical and subtropical regions of the Americas (Figueiredo et al., 2016). The cultivation of Passiflora species is concentrated mainly in Latin American countries, particularly Bolivia, Brazil, Colombia, Ecuador, Paraguay, and Peru. These species have been reported to occur naturally across a broad geographical range extending from the United States to Argentina, as well as in parts of Asia, Australia, including China (He et al., 2020; Mezzonato-Pires et al., 2022) (Figure 1).

Among Passiflora species, 12 are cultivated commercially. These include *P. edulis* Sims, *P. alata* Curtis, *P. setacea* DC., *P. ligularis* Juss., *P. tripartita* var. *mollissima, P. tarminiana, P. maliformis* L., *P. edulis* Sims f. *edulis* (Gulupa), *P. quadrangularis* L., *P. cincinnata* Mast.,

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P. nitida Kunth, and *P. incarnata* L. (Carlosama et al., 2020). These species are not only consumed as fresh fruits but also serve as important raw materials in the food industry, particularly in the production of juices, concentrates, and other processed products. In addition, several species are widely utilized as ornamental plants because of their attractive flowers, while others are valued for their pharmacological properties and have traditionally been used in herbal medicine. Recent studies have highlighted their potential as sources of bioactive compounds, including antioxidants, alkaloids, and flavonoids, which contribute to their increasing importance in nutraceutical and pharmaceutical applications (Santos-Tierno et al., 2021).



(A) Classification of the family Passifloraceae, showing the four main subgenera of Passiflora (*Astrophea*, *Deidamioides*, *Passiflora*, and *Decaloba*) and their approximate proportions according to Feuillet and MacDougal (1999). (B) Major natural distribution areas of Passiflora species across the Americas, Asia, and Australia, highlighted in orange (Figueiredo et al., 2016).

Figure 1. Taxonomic classification and geographical distribution of Passiflora

Passiflora species can be propagated using both generative (seed) and vegetative methods, including cuttings, grafting, and layering (Khas et al., 2020). In commercial cultivation, seed-based propagation is widely used in many countries because of its low cost and ease of application in nurseries. However, plants derived from seeds exhibit high genetic variability, leading to non-uniform plantations (Khas et al., 2020). Such variability can significantly affect important agronomic traits, including growth vigor, flowering time, fruit set, yield, fruit size, and fruit quality. Consequently, orchard management, harvesting, and marketing can become more complex, ultimately reducing overall productivity and economic returns (Faleiro et al., 2019). Therefore, vegetative propagation methods are generally preferred for the production of uniform and healthy plant material, as they help maintain genetic uniformity, preserve desirable traits, and enable clonal multiplication of elite genotypes.

Vegetative propagation methods, particularly cuttings and grafting, are among the most widely used techniques for cultivating Passiflora. However, their large space requirements, relatively long production time, and high risk of viral contamination significantly limit their commercial applicability (Halkoglu et al., 2025). Therefore, micropropagation has emerged as an effective alternative to conventional vegetative propagation and is now widely used for many species, including fruit trees.

Micropropagation offers several important advantages, including the rapid production of virus-free and uniform plant material, large-scale clonal propagation of elite genotypes, maintenance of genetic fidelity, reduced risk of pathogen transmission associated with conventional propagation methods, and propagation under controlled conditions that are independent of seasonal constraints (Permadi et al., 2025). In addition, it facilitates the exchange of plant material in accordance with international phytosanitary standards (Khas et al., 2020; Oros et al., 2020). Micropropagation has been applied to Passifloraceae species since 1966 and has attracted increasing attention from researchers globally (Halkoglu et al., 2025). Most studies have focused on *P. edulis* Sims (Burbulis et al., 2021), *P. edulis* f. *flavicarpa* (Trevisan and Mendes, 2005), *P. alata* (Pinto et al., 2010), *P. foetida* (Shekhawat et al., 2015), *P. gibertii* (Faria et al., 2017), *P. quadrangularis* (Oros et al., 2022), and *P. caerulea* (Jafari et al., 2022). Despite these advances, the micropropagation of Passiflora species is still affected by several challenges, including contamination, low multiplication rates, necrosis, and poor rooting (Khas et al., 2020). Among these, contamination is considered one of the most common and critical constraints, particularly during the culture establishment stage (Sivanesan et al., 2021). If not effectively controlled, contamination at this stage can reduce the regenerative capacity of explants, suppress their growth, and ultimately result in their death (El-Banna et al., 2021; Permadi et al. 2023).

Numerous sterilization and disinfection approaches have been investigated to prevent and control contamination during the micropropagation of Passiflora species (Ożarowski and Thiem 2013; Mohammadi et al. 2023). However, only a few studies have provided a comprehensive comparative evaluation of these methods. Furthermore, the available findings have rarely been synthesized, making it difficult to develop an integrated understanding of contamination management during micropropagation of Passiflora species. Accordingly, this review examines the contamination problems encountered during the micropropagation of Passiflora species, their potential sources, and the methods used to control them *in vitro*. By synthesizing findings from different Passiflora species, this review aims to provide an integrated perspective on contamination management and support the development of more effective micropropagation protocols for Passiflora species.

2. Microbial Contamination in Micropropagation of Passiflora

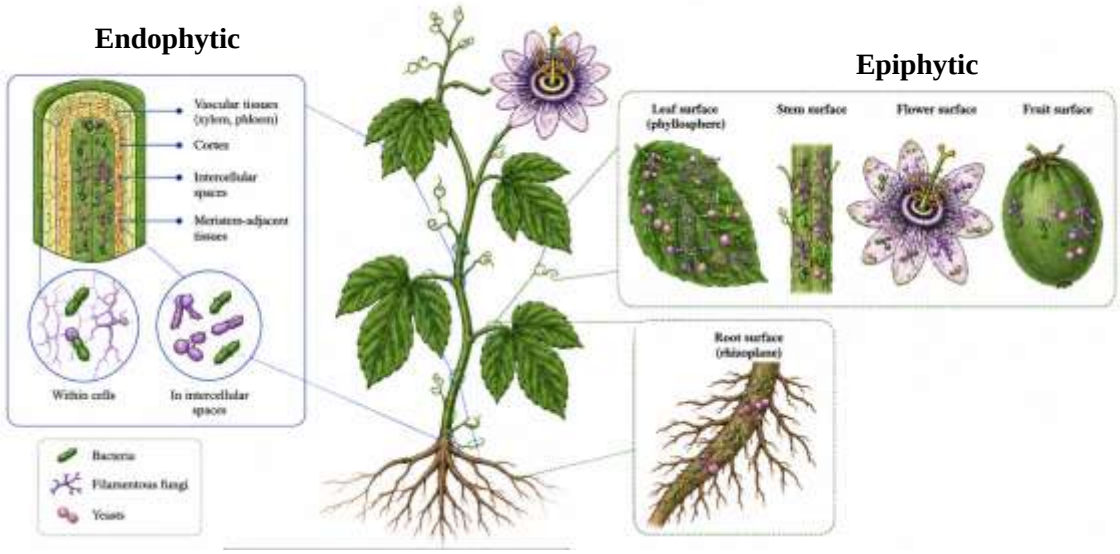
Microbial contamination remains a major constraint in the micropropagation of Passiflora species, limiting successful culture establishment and overall propagation success (Abdalla et al., 2022; Darwesh et al., 2024). Microbial contamination refers to the proliferation of microorganisms associated with *in vitro* cultured explants, either on their surface (epiphytic) or within their internal tissues (endophytic) (Leifert and Cassells, 2001). The favorable conditions of the *in vitro* culture environment promote the rapid proliferation of these microorganisms, thereby adversely affecting the establishment of the culture and subsequent regeneration of the plant.

Fungi and bacteria are the most common microbial contaminants reported in plant tissue cultures (Sriskandarajah, 2006). Similarly, these two groups are the predominant contaminants encountered during the micropropagation of Passiflor*a* species (Shekhawat et al., 2015; Khas et al., 2020; Oros et al., 2022). The high incidence of these microorganisms is closely associated with the biological characteristics of their genera. Species belonging to the Passifloraceae

family are generally semi-woody or climbing plants that are naturally exposed to diverse epiphytic and endophytic microorganisms under field and greenhouse conditions (Abdalla et al., 2022) (Figure 2). As a result, explants used for micropropagation frequently harbor these microorganisms, which can become a major source of contamination during culture establishment.

In plant tissue culture, fungal contaminants proliferate rapidly in culture media, where they compete with plant tissues for nutrients and produce phytotoxic compounds that adversely affect plant growth and development (Herman, 2017). In addition, their rapid sporulation and dispersal facilitate the spread of contamination within the culture vessels. The most commonly reported fungal genera in plant tissue cultures are *Aspergillus, Penicillium, Fusarium, Cladosporium, Trichoderma*, and *Rhizopus* (Permadi et al., 2025).

In contrast, bacterial contamination is often more difficult to detect, particularly when caused by endophytic bacteria (Permadi et al., 2023). These bacteria are typically located in intercellular spaces and cortical parenchyma tissues (Gunson and Spencer-Phillips, 1994) and may persist through multiple subcultures without producing visible symptoms. This latent nature makes their early detection and elimination particularly challenging during *in vitro* culture. The bacterial genera most frequently reported in plant tissue culture include *Agrobacterium, Bacillus, Corynebacterium, Propionibacterium, Proteus, Pseudomonas,* and *Staphylococcus* (Nsofor 2021). However, studies identifying fungal and bacterial contaminants associated with Passiflora species at the genus and species levels using morphological and molecular approaches are limited (Oros et al., 2022).



Endophytic microorganisms colonize internal plant tissues, whereas epiphytic microorganisms colonize plant surfaces. Both groups can occur in different plant organs and can serve as potential sources of contamination during *in vitro* culture establishment.

Figure 2. Schematic illustration of the localization of endophytic and epiphytic microorganisms in the roots, stems, leaves, flowers, and fruits of Passiflora plants

In most studies, contamination was reported as fungal or bacterial without further identification of the causative microorganisms. Consequently, knowledge of the diversity and distribution of microbial contaminants associated with Passiflora spp. is limited. A more detailed identification of these contaminants could improve our understanding of contamination

sources and support the development of more effective sterilization and contamination control strategies for micropropagation of Passiflora species.

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3. Strategies for Controlling Microbial Contamination in Micropropagation of Passiflora

Numerous strategies have been developed to prevent and control microbial contamination during micropropagation (Bunn and Tan, 2002). These strategies are generally classified into two main categories: *in vivo* (pre-treatment) approaches and *in vitro* (laboratory-based) techniques. While *in vivo* approaches primarily focus on reducing the initial microbial load and improving explant quality, *in vitro* strategies aim to directly eliminate contaminants using physical and chemical methods.

3.1. Pre-treatment (*In vivo*) approaches

The success of *in vitro* culture depends not only on procedures carried out under laboratory conditions but also on the preparatory steps applied *in vivo* prior to culture initiation (Leifert and Cassells, 2001). The first step in this process involves cultivating donor plants in controlled environments such as greenhouses or growth chambers. These conditions can reduce the epiphytic microbial load on plant surfaces, thereby improving the success of culture establishment (Leifert et al., 1994; Niedz and Bausher 2002). Additionally, maintaining stable environmental conditions limits the ability of pathogens to adhere to and proliferate on the plant surface. Regular monitoring, along with appropriate cultivation practices, helps minimize latent contamination within plant tissues and supports the selection of high-quality explants (Leifert et al., 1994). In this context, fungicidal and antibacterial pre-treatments applied to explants should be carefully optimized according to the plant developmental stage (juvenile or mature) and explant type (e.g., shoot tip, node, or leaf) (George et al., 2008). This integrated approach

plays a key role in reducing initial contamination levels and enhancing the effectiveness of subsequent *in vitro* sterilization procedures.

In the micropropagation of *Passiflora* species, various pre-treatment strategies have been employed to reduce the microbial load on explants prior to surface sterilization and minimize contamination during the establishment of *in vitro* cultures. These approaches include washing explants under running tap water, pre-treatment with 70% ethanol (Jafari et al., 2016; Oros et al., 2020), and the application of systemic fungicides, particularly 0.1% Bavistin solution, prior to surface sterilization (Shekhawat et al., 2015). These procedures primarily target epiphytic microorganisms on explant surfaces, thereby enhancing the effectiveness of subsequent sterilization.

3.2. *In vitro* contamination control strategies

Sterilization strategies applied under *in vitro* conditions play a key role in the direct control of microbial contamination during the micropropagation of plants. These strategies are generally classified into two main categories: physical and chemical methods (Babu et al., 2022). Physical methods, such as thermotherapy and sonication (ultrasound treatment), can facilitate the removal of surface-associated contaminants and, in some cases, reduce the initial pathogen load (Panattoni et al., 2013). However, because their effectiveness when used alone is often limited, microbial contamination in micropropagation is primarily controlled using chemical approaches (Misra and Misra, 2012). Chemical methods mainly include sterilizing agents used for surface disinfection and antibiotic-based approaches employed to control microbial contamination in culture media.

3.2.1. Surface sterilization agents

Chemical sterilization is the most widely used method for controlling microbial contamination during the micropropagation process. Various sterilizing agents, including sodium hypochlorite (NaOCl), calcium hypochlorite [$Ca(OCl)_2$], mercuric chloride ($HgCl_2$), hydrogen peroxide (H_2O_2), ethanol (C_2H_5OH), and silver nitrate ($AgNO_3$), are commonly employed to eliminate microorganisms present on the surface of explants and, to a lesser extent, within internal tissues (Mihaljević et al., 2013; Sessou et al., 2020). These sterilizing agents exert antimicrobial effects by inducing oxidative damage, disrupting membrane integrity, and altering proteins and nucleic acids.

Among these agents, NaOCl, owing to its strong oxidizing capacity, reacts rapidly with amino acids, nucleic acids, and various nitrogen-containing compounds (such as amines and amides), resulting in their molecular degradation. As a result of these reactions, byproducts such as aldehyde derivatives, ammonium chloride (NH_4Cl), and carbon dioxide (CO_2) are produced. These processes ultimately impair the essential cellular functions of microorganisms, resulting in cell inactivation (Hesami et al., 2017).

In addition to NaOCl, other hypochlorite derivatives, particularly calcium hypochlorite [$Ca(OCl)_2$], exhibit strong antimicrobial activity through the formation of hypochlorous acid (HOCl) in aqueous solution. Their effectiveness is generally associated with concentration, with higher concentrations generally resulting in lower contamination levels, likely because of the chemical alterations in microbial nucleic acids (Dukan et al., 1999; Mbah and Wakil, 2012).

In addition to hypochlorite-based agents, $HgCl_2$ is another potent sterilizing agent that is widely used in micropropagation studies. $HgCl_2$ is a heavy metal compound with strong antimicrobial activity that is effectively used for contamination control by exerting cytotoxic effects on microorganisms. Its mechanism of action involves disruption of membrane integrity,

inhibition of enzymatic activities, induction of DNA damage, and enhancement of oxidative stress (Rabbani et al., 2009). However, despite its high sterilization efficacy, the phytotoxic effects of $HgCl_2$ on plant tissues cannot be ignored. At higher concentrations or with prolonged exposure, it may cause cellular toxicity, adversely affecting explant viability and regenerative capacity (Rodboot et al., 2024).

The success of sterilization is not determined solely by the type of agent used but is also strongly influenced by the application conditions of the agent. The concentration of the sterilant and duration of exposure vary considerably depending on factors such as plant species, explant source, and tissue type (Waheeda and Shyam, 2017). Together, these factors determine the balance between effective microbial elimination and minimal tissue damage (i.e., phytotoxicity) in plants. In delicate tissues with high meristematic activity, such as shoot tips and nodal segments, lower concentrations and shorter exposure times are generally preferred to prevent necrosis. In contrast, more resistant materials, such as lignified tissues or dormant seeds, may require longer exposure periods to ensure effective sterilization (Waheeda and Shyam, 2017; Okoroafor, 2022). However, excessive exposure can increase oxidative stress and disrupt cellular integrity, ultimately reducing regeneration capacity. Although the general principles of chemical sterilization are similar across plant species, the effectiveness of sterilizing agents may vary considerably among Passiflora species, depending on the explant type, tissue characteristics, and treatment conditions.

Studies on surface sterilization in Passiflor*a* species have indicated that NaOCl is one of the most commonly used agents used (Oros et al., 2022). However, other sterilizing agents, such as $HgCl_2$, $Ca(OCI)_2$, and H_2O_2, have also been successfully applied to different Passiflora species (Feuillet and MacDougal, 1999; Rathod et al., 2014; Prithviraj et al., 2015; Batool et al., 2024). The limited number of studies directly comparing these agents under similar conditions suggests that the current findings are primarily based on species-specific investigations. Therefore, sterilization efficiency appears to depend on the type of sterilizing agent used, its concentration, and duration of exposure. For instance, the application of 15% NaOCl for 10 min has been reported to be sufficient for the sterilization of *P. caerulea* seeds, whereas *P. incarnata* seeds require a higher concentration and longer exposure time, with 20% NaOCl applied for 20 min due to greater tissue resistance (Jafari et al., 2016; Ożarowski et al.2022). In contrast, lower concentrations are generally preferred for delicate tissues such as leaves and nodal segments. In *P. caerulea* leaf explants, 5% NaOCl applied for 15 min has been shown to be more effective than 10% NaOCl (Prithviraj et al., 2015). Similarly, in Passiflora ('Tainung') nodal segments, 2% NaOCl for 20 min effectively reduced contamination (Chen et al., 2020), whereas 1% NaOCl applied for 8 min was sufficient for P. xishuangbannaensis explants (Meng et al., 2021).

In some cases, NaOCl may be less effective, particularly when the microbial load is high or when endophytic microorganisms are present. In comparison, $HgCl_2$ tends to be more effective, likely due to its stronger oxidative action and ability to penetrate tissues, allowing it to act on microorganisms not only on the surface but also within the internal tissues. In the nodal segments of *P. foetida*, treatment with 0.1% $HgCl_2$ for 5 min produced satisfactory results, whereas a lower concentration (0.05%) was sufficient for *P. quadrangularis* (Rathour et al., 2025). Similarly, in *P. edulis* seeds and *P. caerulea* leaf explants, 0.1% $HgCl_2$ was associated with lower contamination rates than NaOCl (Prithviraj et al., 2015; Huang et al., 2021). However, residual $HgCl_2$ can remain in the culture medium and negatively affect plant growth and morphogenesis. Therefore, careful rinsing after treatment is essential to minimize tissue damage and residual toxicity (Li et al., 2005).

Overall, NaOCl remains the most frequently used sterilizing agent in Passiflora micropropagation because of its low cost, wide availability, and relatively low phytotoxicity when applied at the appropriate concentrations. Although $HgCl_2$ often provides lower contamination rates, particularly in highly contaminated explants and tissues harboring endophytic microorganisms, its phytotoxic effects and environmental concerns considerably limit its routine application in plant-tissue culture. Therefore, the selection of an appropriate sterilizing agent should be based on the explant type, contamination level, and the balance between sterilization efficiency and phytotoxicity.

3.2.2. Antibiotic-based approaches

The use of antibiotics is widely recognized as an effective approach for controlling systemic (endophytic) contaminants that cannot be eliminated through surface sterilization alone. This strategy is particularly useful when sterilizing agents cannot sufficiently penetrate plant tissues, allowing endogenous bacterial contamination to persist in the explants. Commonly used antibiotics include tetracycline, cephalothin, gentamicin, polymyxin, rifampicin, streptomycin, and timentin (Mbah and Wakil, 2012).

These antibiotics exert antimicrobial effects through various mechanisms, including inhibition of protein synthesis, disruption of cell wall formation, and interference with nucleic acid metabolism in bacterial cells. However, their effectiveness may vary considerably depending on factors such as plant species, explant type, contaminant type, and the concentration, and the duration of treatment (Tewelde et al., 2020). In addition, prolonged or high-dose applications may lead to phytotoxic effects, the development of antibiotic resistance, and alterations in cytoplasmic DNA (Abdi et al., 2008).

These limitations have led researchers to seek safer and more sustainable alternatives. In this context, the Plant Preservative Mixture (PPM™), a commercial biocide, has gained attention as an effective antimicrobial agent in plant tissue culture systems (Plant Cell Technologies, 2019). It has shown considerable potential in reducing endophytic contamination, particularly in woody and fruit species, thereby improving overall culture success rate (Moreno-Peña et al., 2025). Compared to conventional antibiotic treatments, PPM™ offers the advantage of broad-spectrum antimicrobial activity and can be directly incorporated into culture media. However, its effectiveness may vary depending on the plant species and contaminant type, indicating that it should be considered a complementary, rather than a universally applicable, solution.

In Passiflora species, antibiotics have been used to reduce endophytic bacterial contamination, both as pre-treatments and by incorporation into the culture medium. For example, pre-treatment with rifampicin has been reported to improve the establishment of an aseptic culture (Oros et al., 2022; Rathour et al., 2025). In addition, the application of 1000 mg L^{-1} agrimicine to *P. edulis* f. *flavicarpa* explants significantly reduced bacterial contamination (Biasi et al., 2000). Similarly, the addition of antibiotics, such as rifampicin (20 mg L^{-1}) and kanamycin (50–150 mg L^{-1}) to the culture medium, has also been shown to be effective (Oros et al., 2022; Rathour et al., 2025).

A comparative summary of sterilization protocols, antibiotic-based approaches, and their effects on culture establishment and contamination control in different Passiflora species is provided in Table 1.

Table 1. Summary of sterilization treatments and culture responses in different Passiflora species

Passiflora species	Explant type	Sterilization agent (concentration and duration)	Culture Response	Reference
P. quadrangularis	Nodal segment	0.05% $HgCl_2$, 4–5 min	Successful establishment of aseptic cultures	Rathour et al., 2025
P. edulis f. flavicarpa cv. Qinmei#9	Nodal segment	6.00% H_2O_2, 8 min + 8.00% NaOCl, 6 min	Successful culture establishment	Batool et al., 2024
P. edulis f. flavicarpa	Leaf disc	10.00% NaOCl, 12 min	100.0% survival rate	Malavipathirana et al., 2024
P. quadrangularis	Nodal segment	50.00% NaOCl, 10 min	0.0% contamination rate and 61.7% survival rate	Oros et al., 2022
P. incarnata	Seed	20.00% NaOCl, 20 min	Successful culture establishment	Ożarowski et al., 2022
P. xishuangbannaensis	Internode, petiole, tendril	1.00% NaOCl, 8 min	Successful culture establishment	Meng et al., 2021
P. xishuangbannaensis	Leaf	1.00% NaOCl, 8 min	Culture establishment was unsuccessful due to persistent endophytic contamination	Meng et al., 2021
P. edulis ('Tainong')	Stem segments and shoot tips	0.10% $HgCl_2$, 15 min	10.0% contamination rate and 83.3% survival rate	Huang et al., 2021
P. edulis 'Tainung No.1'	Nodal segment	2.00% NaOCl, 20 min	Aseptic cultures were established	Chen et al., 2020
P. edulis Sims	Nodal segment	0.10% AgNPs, 15 min	20.0% contamination rate and 68.3% survival rate	Hieu et al., 2018
P. edulis f. flavicarpa	Nodal segment	0.10% AgNPs, 15 min	21.7% contamination rate and 66.7% survival rate	Hieu et al., 2018
P. edulis	Seed	2.50% NaOCl, 25 min	Successful culture establishment	Antoniazzi et al., 2018
P. caerulea	Seed	10.00% NaOCl, 10 min	3.33% contamination rate and 86.66% germination rate	Jafari et al., 2016
P. caerulea	Leaf explant	0.10% $HgCl_2$, 3–5 min	Successful surface sterilization	Prithviraj et al., 2015
P. foetida	Nodal segment	0.10% $HgCl_2$, 5 min	Successful culture establishment	Shekhawat et al., 2015
P. alata	Hypocotyl segment	0.70% NaOCl, 20 min	Successful culture establishment	Pinto et al., 2010
P. edulis f. flavicarpa	Internodal segment	0.70% NaOCl, 20 min	Successful establishment of aseptic cultures	Biasi et al., 2000

The observed differences in sterilization requirements among Passiflora species are likely associated with variations in explant type, tissue characteristics, developmental stage, and microbial contamination level. In general, seeds require higher sterilant concentrations and

longer exposure times because their highly lignified seed coat and thick cuticular layer provide greater protection against the penetration of sterilizing agents. In contrast, more delicate explants, such as leaves, nodal segments, and shoot tips, are more susceptible to phytotoxic effects; therefore, they generally respond better to lower sterilant concentrations and shorter exposure times. Even when the same explant type is used, optimal sterilization protocols may vary among species because of species-specific differences in tissue sensitivities. Moreover, surface sterilization alone may be insufficient to eliminate endophytic microorganisms residing within internal tissues, highlighting the need for complementary approaches, such as antibiotic treatments or other antimicrobial compounds in specific cases. Overall, these findings indicate that no single sterilization protocol is universally applicable to all Passiflora species or explant types, emphasizing the importance of species-specific optimization to maximize aseptic culture establishment and micropropagation success in this genus.

3.2.3. Next-generation approaches for contamination control

In recent years, several next-generation approaches have been investigated to improve microbial contamination control in plant tissue cultures. These approaches aim to enhance sterilization efficiency, facilitate the early detection of latent contaminants, and improveoverall contamination control. Among these, nanotechnology-based antimicrobial compounds, molecular diagnostic techniques, and artificial intelligence-assisted monitoring systems have shown considerable promise. Among these emerging technologies, nanotechnology-based antimicrobial compounds, particularly silver nanoparticles (AgNPs), have attracted considerable attention because of their broad-spectrum antimicrobial activity, relatively low phytotoxicity, and potential to serve as alternatives to conventional chemical sterilant for controlling contamination in plant tissue cultures. For example, silver nitrate ($AgNO_3$) has been reported to provide higher sterilization efficiency and improved explant viability than NaOCl in *P. edulis* f. *flavicarpa* leaf explants (Malavipathirana et al., 2024). Similarly, the application of AgNPs at a concentration of 0.1% for 15 min has been reported to be more effective than NaOCl and $HgCl_2$ in nodal explants of *P. edulis* and *P. edulis* f. *flavicarpa* (Hieu et al. 2018). Silver-based compounds, particularly AgNPs, have demonstrated promising antimicrobial activity while maintaining explant viability in vitro. This advantage may be attributed to the multiple antimicrobial mechanisms associated with silver, including disruption of microbial cell membranes, interference with cellular proteins and enzymes, and induction of oxidative stress**.**

In addition to improving sterilization efficiency, recent advances have focused on the early detection of latent microbial contamination. Molecular diagnostic techniques have emerged as valuable tools for identifying microorganisms that remain undetected after surface sterilization. Techniques such as 16S rDNA gene-based PCR and quantitative PCR (qPCR) enable the identification of bacterial contaminants that may remain undetected after surface sterilization and before visible contamination symptoms appear (Tsoktouridis et al., 2014; El-Banna et al., 2021; Tekielska et al., 2024). These approaches have the potential to reduce culture loss and improve contamination management by facilitating the early detection of latent contamination.

Beyond molecular diagnostics, artificial intelligence (AI)-assisted monitoring systems based on image processing and machine learning algorithms have recently emerged as promising tools for the automated detection of microbial contamination in plant tissue culture systems (Hettiarachchi et al., 2025). By enabling rapid and non-destructive assessment of *in*

vitro cultures, these technologies may facilitate the early detection of contamination events and support more efficient decision-making during micropropagation. Although their application in Passiflora tissue culture remains largely unexplored, they offer promising opportunities to complement conventional contamination control strategies and improve future contamination management during Passiflora micropropagation.

4. Conclusion

Microbial contamination remains one of the most critical constraints limiting the success of micropropagation in Passiflora species, particularly during the culture establishment stage. This review demonstrates that contamination originates from multiple sources, including epiphytic and endophytic microorganisms, and that effective control requires a multifaceted approach. Although pre-treatments (*in vivo)* help reduce the initial microbial load, they are generally insufficient when used alone. Therefore, *in vitro* sterilization methods, particularly chemical approaches, are essential for achieving aseptic culture. However, while antibiotics can be effective in controlling bacterial contamination, their use is associated with important limitations, including phytotoxicity and the development of antimicrobial resistance. Future contamination management strategies for the micropropagation of Passiflora species are expected to increasingly incorporate nanotechnology-based antimicrobial agents, molecular diagnostic tools, and artificial intelligence-assisted monitoring systems. Although the application of these technologies in Passiflora tissue culture remains limited, they offer promising opportunities for the early detection and more effective management of microbial contamination.

Overall, improving the micropropagation of Passiflora requires the development of integrated sterilization and contamination-control protocols that enhance contamination control while maintaining the viability and regeneration potential of explants.

Ethics Statement

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Author Contribution Statement

The author confirms sole responsibility for the conception, literature review, analysis, preparation, and writing of the manuscript and has read and approved the final version for publication.

Conflict of Interest Statement

The author declares that there is no conflict of interest regarding this study.

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