



Sodium hypochlorite effectively revives slightly contaminated stage II and IV tissue-cultured ‘Cavendish’ banana

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Abstract

Contamination in plant tissue culture reduces explant survival and leads to significant losses in propagation efficiency. This study identified the dominant microbial contaminant in tissue-cultured ‘Cavendish’ banana and evaluated the effectiveness of sodium hypochlorite (NaOCl) in rescuing slightly contaminated cultures during multiplication (Stage II) and acclimatization (Stage IV). Fungal contaminants were isolated, purified, and identified, with *Chrysosporium* sp. emerging as the predominant species (53.85%), followed by *Aspergillus* sp. and *Rhizopus* sp. Slightly contaminated explants were treated with NaOCl at 0.60%, 0.80%, 1.00%, and 1.20% (v/v). NaOCl treatments improved explant survival and allowed recovery within three days. At Stage II, all tested concentrations enhanced callus formation and survivability, while at Stage IV, 1.0–1.2% (v/v) NaOCl resulted in better plant growth, including higher meristem height, increased leaf production, and improved rooting. Among the treatments, 1.0% (v/v) NaOCl was identified as the most consistent balance between contamination control and explant viability. Unlike most studies that focus on preventive sterilization at the initial stage (Stage I), this study demonstrates the potential of NaOCl as a curative approach for managing contamination at later stages of micropropagation. This offers a practical strategy to reduce losses and improve efficiency in commercial banana tissue culture systems.

Keywords: Callus formation, *Chrysosporium* sp., Decontamination, Explant survival, Totipotency

1. Introduction

The surge in global demand for bananas has spurred banana-growing countries to augment their production volume and expand cultivation areas. In key exporters like the Philippines, maintaining market competitiveness is essential [1]. However, increased production has also heightened the spread of fungal and viral diseases through non-indexed planting materials, which leads to declining yields and higher cultivation costs. This demands the need for disease-free seedlings [2] and careful selection of planting materials, including eliminating somaclonal variants, sourcing healthy explants, and ensuring true-to-type cultivars [3]. Plant tissue culture is a micropropagation technique that grows explants in an artificial medium under sterile conditions [4]. It relies on cellular totipotency and plasticity that enables explants to regenerate into whole plants genetically identical to the mother plant. This technique helps meet the increasing demand for food while improving crop yield and quality [5]. However, microbial contamination remains a challenge due to the nutrient-rich culture environment favorable for microorganisms to thrive [1]. This recurring challenge often stems from fungal, bacterial, and viral pathogens, unsuitable explants, and poor laboratory practices [3].

Biotic contaminants, including endophytic microbes, can invade plant tissue through natural openings or wounds, leading to cross-contamination and reduced production efficiency [6]. Strict aseptic techniques, proper

sterilization, and careful selection of uncontaminated explants are essential to minimizing contamination and ensuring successful tissue culture outcomes [3; 7]. NaOCl is a common chlorine source used for disinfection purposes. Its potent oxidizing properties make it highly effective in killing pathogens, including bacteria and viruses, by disrupting chemical bonds within their molecules [8]. Chlorine-containing disinfectants interact with compounds such as enzymes in bacterial cells, causing the molecules to destabilize or change shape, ultimately impairing the proper function of enzymes and leading to cell death [9]. When NaOCl is diluted in water, it forms hypochlorous acid (HOCl), the primary antimicrobial species responsible for its disinfecting activity. HOCl penetrates microbial cells and causes oxidative damage to proteins, enzymes, and nucleic acids, ultimately leading to cell death [10].

Numerous studies have quantified the preventive efficacy of sodium hypochlorite (NaOCl) during the initial explant establishment phase in banana tissue culture. For instance, Keshari et al. [11] demonstrated that standard sterilization protocols utilizing 1% NaOCl alongside 70% ethanol yielded an aseptic culture establishment rate of $\geq 96\%$ for various banana cultivars. Similarly, Hassen et al. [12] reported that treating *Musa paradisiaca* suckers with 20% (v/v) NaOCl for 20 minutes effectively eliminated surface contaminants, particularly exogenous bacteria. These findings are supported by earlier reports emphasizing that surface sterilization using chlorine-based disinfectants is a critical step in establishing contamination-free cultures [6; 7]. More broadly, plant tissue culture studies have consistently shown that NaOCl is widely used due to its strong oxidizing capacity and broad-spectrum antimicrobial activity, which enables it to inactivate a wide range of microorganisms present on explant surfaces [2; 4; 10]. Although they were found effective, the success of NaOCl is highly dependent on concentration and exposure time. Goswami and Handique [13] highlighted that while NaOCl is effective in reducing contamination, excessive concentrations or prolonged exposure can lead to tissue damage, reduced explant viability, and poor regeneration. Similar observations have been reported in other tissue culture systems, where improper sterilization can either fail to eliminate contaminants or cause phytotoxic effects that compromise plant development [2; 7; 10]. In addition, contamination remains a persistent problem in banana tissue culture, with studies reporting the presence of fungal and bacterial contaminants even under controlled laboratory conditions [5]. These findings suggest that although NaOCl is an effective preventive disinfectant, its application requires careful optimization to balance antimicrobial activity and tissue survival.

While these past studies firmly establish NaOCl as a potent decontaminant, they almost exclusively focus on the preventative surface sterilization of fresh explants at Stage I. They rarely address the critical issue of rescuing cultures that develop latent contamination later in the micropropagation cycle. While preventative sterilization is well-documented, much less attention has been given to managing contamination that emerges later in the cycle, specifically during Stage II (multiplication) and Stage IV (acclimatization). This gap is particularly relevant in Stage II and Stage IV, where contamination can lead to substantial losses. Stage II is a critical phase characterized by rapid shoot proliferation, and the emergence of latent contaminants during this stage can significantly affect the survival and multiplication of shoots [2]. Furthermore, Stage IV represents the transition of in vitro plantlets to ex vitro greenhouse conditions [4]. At this stage, plantlets are more exposed to environmental microbes and residual contaminants from in vitro culture [7]. Successfully rescuing plantlets at these advanced stages is economically important, as considerable time, labor, and resources have already been invested in their development [6].

Despite the use of standard preventive protocols, contamination remains a common challenge in plant tissue culture, often arising from inadequate sterilization or improper handling procedures [2; 7]. Only a limited number of studies have explored curative approaches for already contaminated cultures. Chang et al. [14] reported that NaOCl treatment can reduce contamination and explant mortality in banana tissue culture. Building on this approach, the present study develops a targeted curative strategy for Stage II and Stage IV ‘Cavendish’ cultures. Instead of discarding contaminated explants, this study evaluates specific NaOCl concentrations to suppress active fungal growth while preserving the regenerative capacity of the tissues.

Hence, this study aimed to evaluate the potential of different NaOCl concentrations to rescue slightly contaminated tissue-cultured ‘Cavendish’ banana cultures at different stages of micropropagation. Specifically, the objectives were to: (1) isolate, purify, and identify the dominant microbial contaminant affecting the cultures; (2) evaluate the effects of different NaOCl concentrations (0.60%, 0.80%, 1.00%, and 1.20%) on the survival and recovery of slightly contaminated tissue-cultured ‘Cavendish’ banana at the multiplication (Stage II) and acclimatization (Stage IV) stages; and (3) determine the most effective NaOCl concentration for improving explant survival, callus formation, and growth recovery.

2. Materials and methods

The study was conducted in a 12 m² modified tissue culture laboratory at Dole-Stanfilco, Luna Zone, Sampao, Kapalong, Davao del Norte, Philippines. The facility, equipped with modern tools and adequate lighting, maintained strict aseptic conditions to prevent contamination. Clorox® Concentrated Germicidal Bleach (The Clorox Company, Oakland, CA, USA), containing sodium hypochlorite as the active ingredient (6.15% w/v), was

used in the experiment. The product was purchased from a local retail store in Tagum City, Davao del Norte, Philippines.

2.1 Identification, isolation, and purification of contaminant

Explant culture follows five stages [3; 15]: donor plant preparation (Stage 0), initiation (I), multiplication (II), rooting (III), and acclimatization (IV). This study focused on contamination occurring at stages II and IV to synchronize contamination events. Microorganisms associated with tissue culture contamination were identified, isolated, and purified following Magbalot-Fernandez et al. [16]. The workflow for contaminant identification, isolation, and purification is illustrated in Figure 1. Healthy ‘Cavendish’ banana suckers (50–100 cm) were collected, washed, air-dried, and prepared for explant culture. Corm cubes (6–8 cm) were excised by removing outer bracts and excess tissue, then surface-sterilized by soaking in a bleach solution for 20 min. The explants were subsequently rinsed three times with sterile distilled water to remove residual disinfectant. Under aseptic conditions, shoot tips were excised, meristematic tissues were exposed, and trimmed into approximately 1.5 cm explants. These were inoculated onto multiplication medium and incubated at 26 °C under a 16 h light/8 h dark photoperiod for four weeks. Out of 50 culture bottles established, 13 exhibiting browning or blackening symptoms were selected for contaminant isolation (Table 1). The predominant fungal contaminant was isolated by plating infected tissues onto Potato Dextrose Agar (PDA) and purified through successive subculturing onto fresh medium. Pure cultures were incubated for seven days prior to morphological characterization using microscopic examination.

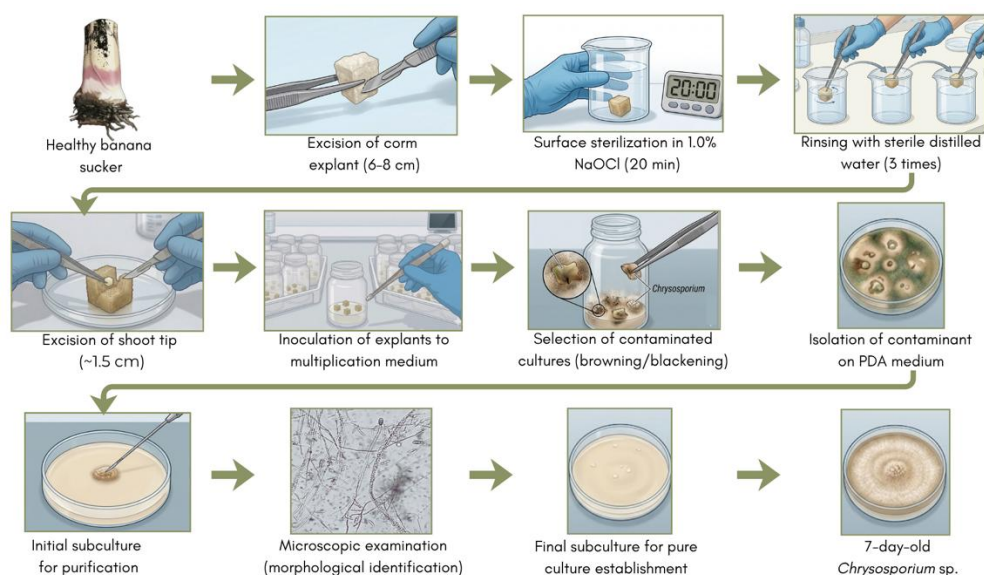


Figure 1 Schematic workflow of contaminant identification, isolation, and purification from tissue-cultured banana explants.

Table 1 Incidence and contamination rate of putative fungal contaminants isolated from banana tissue culture bottles (n = 50).

Putative Contaminant	Frequency	Contamination Rate (%)
<i>Chrysosporium</i> sp.	7	53.85
<i>Aspergillus</i> sp.	3	23.08
<i>Rhizopus</i> sp.	1	7.69
Unidentified microorganisms	2	15.38
Total contaminated bottles	13 (26%)	100%

2.2 Degree of contamination

Contamination levels were assessed based on a modified tissue culture protocol adapted from Reina [17]. The degree of contamination was classified into four categories according to the visible extent of microbial growth on the culture medium and explant tissues (Figure 2). Cultures were considered free from contamination when no visible microbial growth (e.g., mycelial structures, colonies, or discoloration) was observed on both the medium and explants. Slight contamination (minor) was defined by the presence of small, localized microbial growth, typically confined to a limited area of the culture medium and not yet affecting the explant. Moderate

contamination (major) was characterized by more extensive microbial growth, with visible spread across the medium and partial contact or early impact on the explant tissues. Severe contamination (potentially disastrous) was assigned when dense and widespread microbial growth covered a large portion of the medium, often accompanied by tissue browning, necrosis, or complete deterioration of the explant. Pre-trial observations were used to standardize these categories and ensure consistency in scoring as illustrated in Figure 2. Consequently, the most frequently occurring contaminant in the laboratory was isolated, propagated, and uniformly introduced into all culture bottles to establish a consistent level of contamination prior to treatment application.

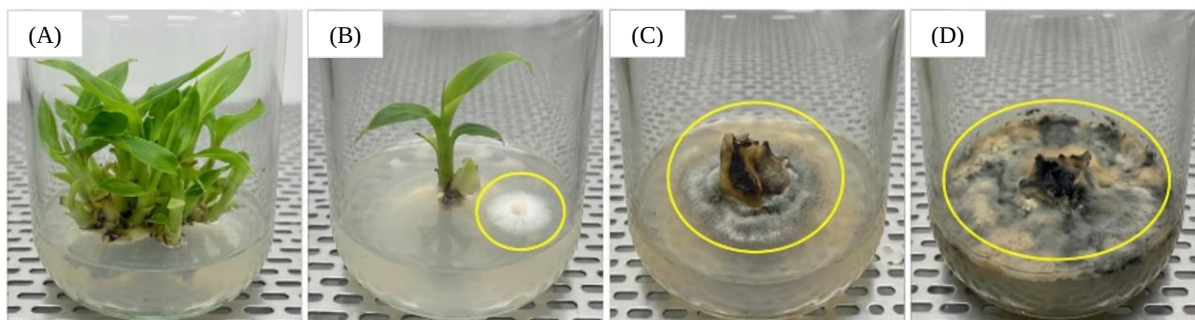


Figure 2 Standardized contamination severity scale in banana cultures as adapted in this study. (A) absence of contamination; (B) slightly contaminated; (C) moderately contaminated; and (D) severely contaminated. Yellow circles indicate the visible signs of microbial contamination on the culture medium and explant tissues.

2.3 Pre-trial

A preliminary experiment was conducted to evaluate the effectiveness and suitable range of NaOCl concentrations for rescuing contaminated banana tissue cultures. Eight concentrations (0.20%, 0.40%, 0.60%, 0.80%, 1.00%, 1.20%, 1.40%, and 1.60% v/v NaOCl) were initially tested using slightly contaminated explants under controlled laboratory conditions. Observations were recorded daily based on visible reduction of microbial growth, explant survival, and early signs of tissue recovery. While all treatments showed some degree of decontamination within seven days, clear differences were observed in both the rate of contaminant suppression and the condition of the explants. Lower concentrations (0.20% and 0.40%) were less effective in suppressing microbial growth, as residual contamination persisted beyond the early observation period. In contrast, higher concentrations (1.40% and 1.60%) showed rapid microbial inactivation but were frequently associated with signs of tissue stress, including slight browning and reduced vigor. Intermediate concentrations (0.60%, 0.80%, 1.00%, and 1.20%) provided a more favorable balance between antimicrobial efficacy and explant viability. These treatments consistently reduced visible contamination within three days while maintaining acceptable tissue condition and recovery potential. Based on these observations, the four intermediate concentrations were selected for further evaluation in the main experiment.

2.4 Experimental design and treatments

The study followed a Completely Randomized Design (CRD) with five treatments, each replicated four times using 10 tissue-cultured ‘Cavendish’ banana samples per replicate. Two experimental setups were conducted at different tissue culture stages (Stages II and IV). Each replicate consisted of 10 glass jars (400 mL) with plastic lids. The treatments were: T1 – control (distilled water); T2 – 0.60% NaOCl (v/v); T3 – 0.80% NaOCl (v/v); T4 – 1.00% NaOCl (v/v); and T5 – 1.20% NaOCl (v/v).

2.5 Inoculation of contaminant, treatment application and storage of treated cultures

The workflow for contaminant inoculation, treatment application, and post-treatment handling is illustrated in Figure 3. Contaminant-free explants at Stages II and IV were inoculated with the identified dominant fungus (*Chrysosporium* sp.) by applying 0.5 mL of a diluted liquid suspension onto the cultures. The inoculated explants were maintained for two days to allow the development of slight contamination. Slightly contaminated cultures were then selected, and rescue was initiated by removing visibly infected tissues under aseptic conditions. The explants were subsequently dipped in NaOCl solutions at treatment-specific concentrations for 1 min, followed by rinsing with sterile distilled water to remove residual disinfectant. Treated explants were placed on sterilized paper and allowed to air-dry. After drying, explants were transferred to fresh culture medium, labeled, and stored

on a separate rack under controlled conditions. Cultures were maintained at 26 °C under a 16 h light/8 h dark photoperiod, with temperature monitored using a digital gauge.

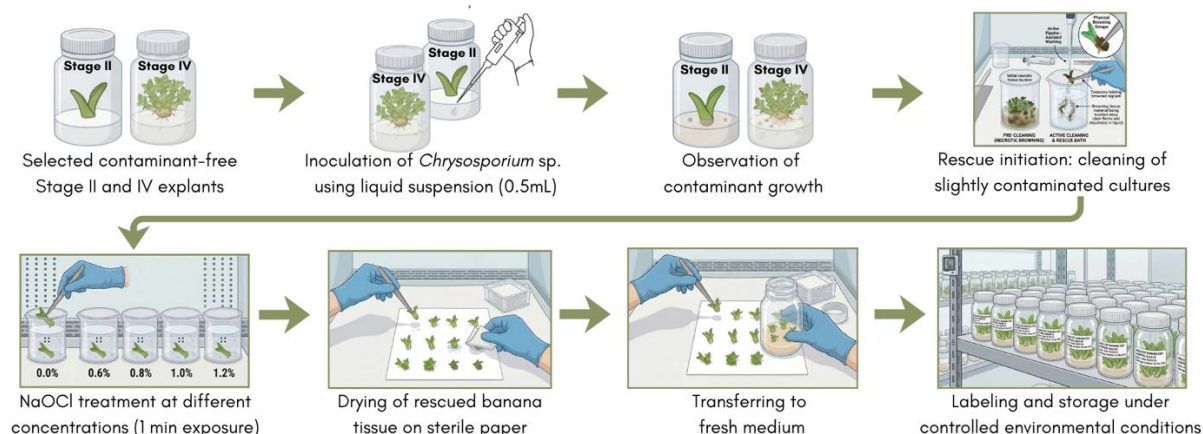


Figure 3 Schematic of contaminant inoculation, treatment application, and storage of treated banana explants.

2.6 Data gathering and analysis

For stage II cultures, the percentage of rescued bottles was measured by counting the number of bottles showing no signs of contamination and the percentage were determined using the formula: $\text{Rescue \%} = (\text{Rescued bottles} / \text{Total bottles}) \times 100$. Callus formation data were recorded at the termination of the experiment and was done by counting the callus formed per replicate. The number of days the contaminated culture recovered were counted and recorded accordingly. In stage IV cultures, the same method was applied to determine the percentage of rescued bottles. The height of the meristem was measured using a measuring stick, and leaf formation was counted from the mother tissue culture. The number of cultures (rooting) produced was determined by counting cultures that had 3–4 leaves with a height of 2.0–3.0. New subcultures emerging during the study were counted, and recovery times were recorded. The number of days it took for the contaminated cultures to recover was also recorded, as previously described. These parameters were gathered at the end of the experiment, or seven days after treatment application. Data were analyzed using Analysis of Variance (ANOVA) with significant differences tested using the Tukey Honest Significant Difference (HSD) at a 1% significance level ($p \leq 0.01$).

3. Results and Discussion

3.1 Contaminants causing contamination in banana tissue culture

Table 1 and Figure 4 present the incidence and relative proportions of fungal contaminants observed in banana tissue culture bottles. A total of 13 bottles (26%) showed contamination. Among these, *Chrysosporium* sp. was the most frequently isolated fungus, accounting for 53.85% of the contamination cases, followed by *Aspergillus* sp. (23.08%), *Rhizopus* sp. (7.69%), and unidentified microorganisms (15.38%). The high occurrence of *Chrysosporium* sp. indicates that it is a major contaminant under the present culture conditions and justifies its selection for further investigation.

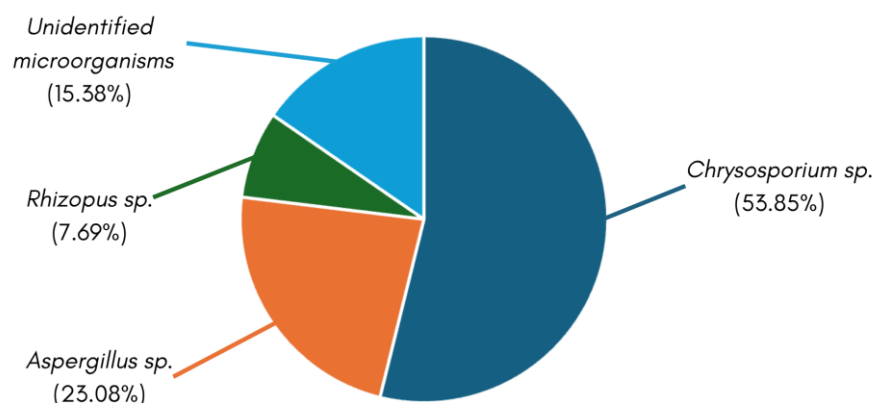


Figure 4 Relative proportions of fungal contaminants isolated from contaminated banana tissue culture bottles based on frequency of occurrence (n = 13).

Colony morphology of *Chrysosporium sp.* was distinct as it appeared to be semi-elevated, circular, smooth, and rapidly spreading, with white to off-white pigmentation. Microscopically, it displayed hyaline, septate hyphae, and simple or branched conidiophores producing conidia in tree-like arrangements (Figure 5). This was also described by Cobrado and Fernandez [18] and Wang et al. [19], who reported similar characteristics in abaca tissue culture and environmental isolates, respectively. The genus *Chrysosporium sp.* which belongs to the Onygenaceae family, is keratinophilic and commonly found in soil, decomposing organic matter, and even animal substrates [20]. Its frequent detection in culture systems is attributed to its thermotolerance, resistance to sterilization, and ability to release secondary metabolites that inhibit competing microbes [19; 21]. Additionally, some species may act as endophytes which becomes active during tissue culture, which likely explains their persistence despite standard hygiene protocols [22].

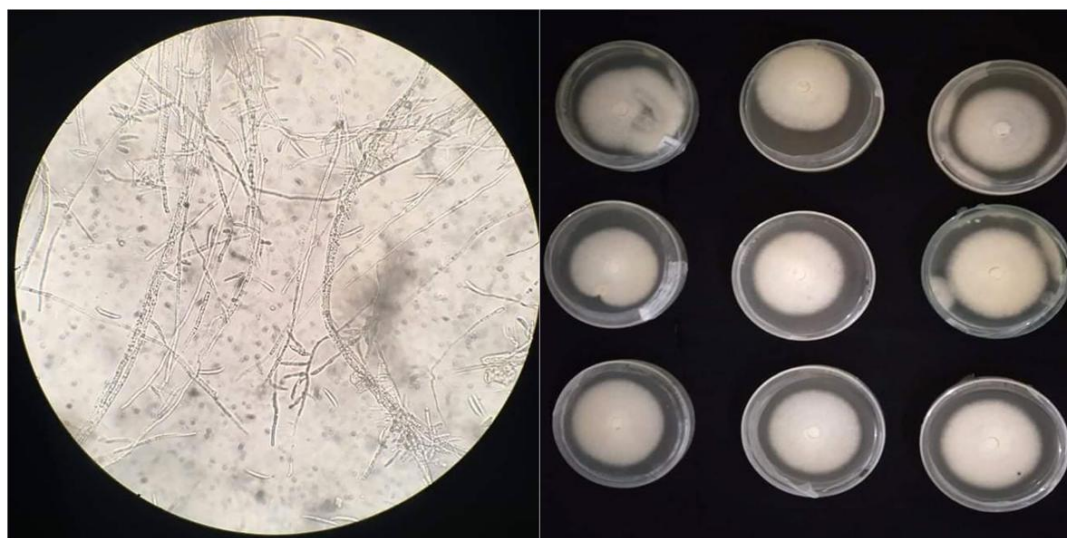


Figure 5 Identified contaminant isolated, purified and mass produced in vitro. (left) Microscopic view of *Chrysosporium sp.* at 80x magnification showing branched and septated hyphae; (right) Five-day-old *Chrysosporium sp.* cultures in a petri dish showing white mycelia.

On the other hand, *Aspergillus sp.* colonies appeared flat, smooth, and cottony, maturing from bluish-gray to dark green. Microscopically, it had septate hyphae and globose vesicles bearing conidia at the tips of conidiophores which was also characterized by Cobrado and Fernandez [18]. This genus is a notorious airborne contaminant in laboratory environments, particularly tissue culture incubators, where spores persist in microenvironments such as rubber seals or inadequately sanitized surfaces [23]. Moreover, *Rhizopus sp.* formed dense, aerial, cottony mycelia, with colonies turning gray upon sporulation. Microscopically, its coenocytic

hyphae supported sporangia at the tips of erect sporangiophores. Although generally considered a saprophytic postharvest pathogen [24], *Rhizopus* can compete in culture media by rapidly reproducing through prolific spore formation [16].

The isolation of these contaminants reveals the inherent vulnerability of in vitro tissue culture systems to environmental contamination. This is particularly evident in laboratories where sterilization protocols are insufficient or hygiene practices are not strictly enforced [25; 26] *Aspergillus* and *Rhizopus spp.* can secrete pectinolytic enzymes, notably pectinases, which degrade plant cell walls, leading to tissue necrosis, inhibited shoot proliferation, and increased mortality of cultured explants [26].

3.2 Survival of stage II contaminated tissue-cultured banana using NaOCl

NaOCl treatment effectively rescued contaminated stage II banana tissue cultures, with significant improvements in the percentage of experimental bottles rescued, callus formation, and recovery time compared to untreated samples (Figure 6). Statistical analyses showed that NaOCl-treated cultures (T2, T3, T4, T5) performed better than untreated cultures (T1), with recovery occurring within three days. The treatments also significantly influenced the number of calluses formed.

NaOCl, a widely used chlorine-based disinfectant, acts as a powerful antimicrobial agent. The active compound, hypochlorous acid (HOCl), rapidly penetrates microbial cell walls and causes cellular damage by interacting with amino acids, lipids, nucleic acids, and membrane components [8]. The oxidizing properties of NaOCl also disrupt cellular functions by reacting with amino acids and producing aldehydes, NH_4Cl , and CO_2 [10]. This disinfectant is particularly effective in mitigating contamination during tissue culture, where maintaining sterility is crucial for successful callus and organ development. The study confirmed that NaOCl treatment enhanced callus formation and reduced recovery time in contaminated banana cultures. Prior research supports these findings which elucidates NaOCl's role in maintaining aseptic conditions and promoting tissue culture success by preventing microbial growth [10].

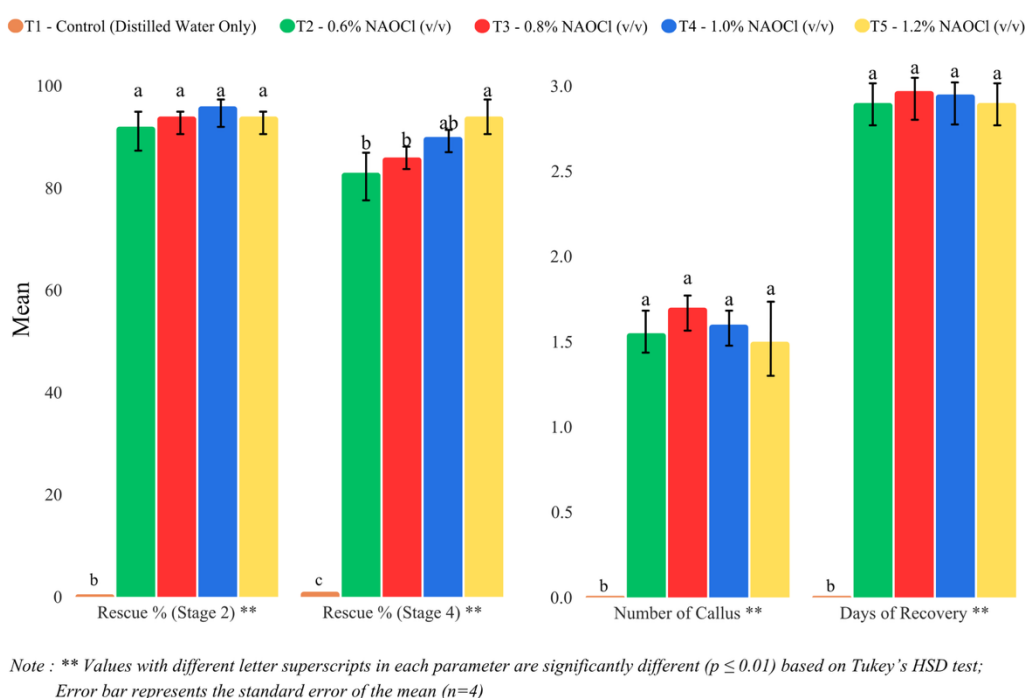


Figure 6 Rescue percentage (Stage II and IV), and number of calluses, and days to recovery of Stage II tissue-cultured banana as influenced by varying NaOCl levels.

3.3 Rescuing slightly contaminated stage IV tissue-cultured bananas using different NaOCl concentrations

Statistical analysis revealed that different concentrations of NaOCl significantly affected the rescue percentage of slightly contaminated stage IV tissue-cultured bananas (Figure 6). Treatments with NaOCl concentrations (T2, T3, T4, and T5) were more effective than the control (T1), particularly the 1.2% NaOCl (v/v) treatment (T5), which was comparable to the 1.0% NaOCl (v/v) treatment (T4). As shown in Figure 7, the stage IV tissue-cultured

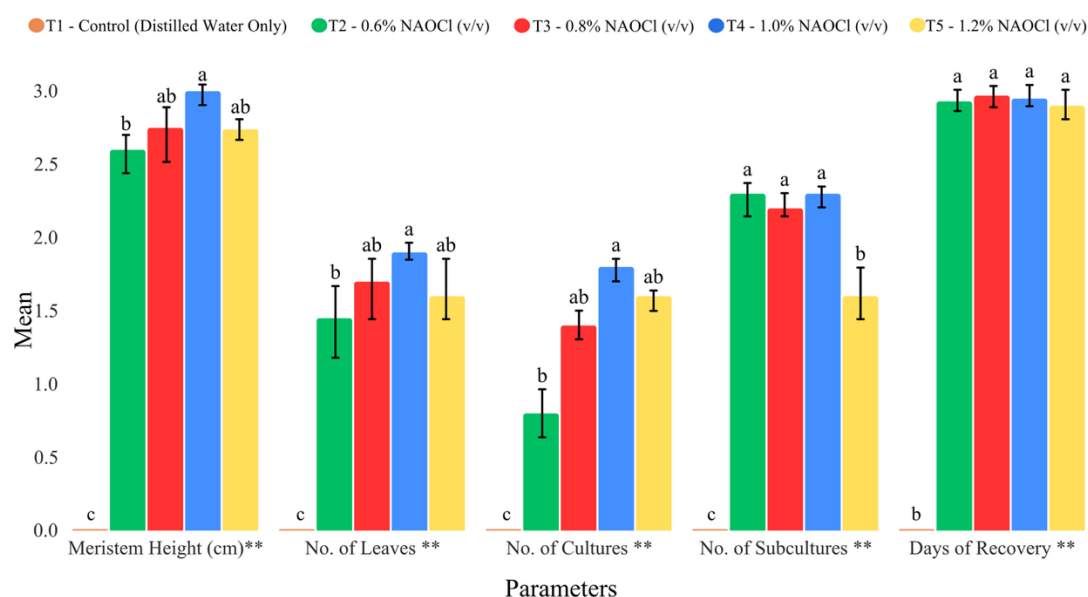
banana plantlets exhibited healthy growth seven days after being successfully rescued from slight contamination using 1.0% (v/v) NaOCl treatment.



Figure 7 Tissue-cultured banana explants recovered from slight contamination seven days after treatment with 1.0% (v/v) NaOCl, showing healthy and actively growing plantlets under contaminant-free conditions.

The meristem height, a key indicator of plant growth, also showed significant differences among treatments (Figure 8). The highest meristem height was observed in cultures treated with 1.0% NaOCl (T4), followed by comparable results for the 0.8% (T3) and 1.2% (T5) treatments. This suggests that 1.0% NaOCl is most effective in promoting plant growth, but other concentrations (0.6–1.2%) also significantly improved growth parameters, including increased meristem height (≈ 2.6 – 3.0 cm), higher leaf number (≈ 1.5 – 1.9 leaves), and enhanced rooting compared to the control. Moreover, leaf production was significantly influenced by NaOCl concentrations (Figure 8), with the highest leaf number observed in cultures treated with 1.0% NaOCl (T4). ANOVA showed no significant difference between the leaf production in cultures treated with 0.8% (T3) and 1.2% (T5) NaOCl, which were comparable to T4. Similarly, rooting showed significant improvement in cultures treated with 1.0% NaOCl (T4). The number of subcultures was also influenced by NaOCl levels, with T2, T3, and T4 showing a significant increase compared to T5 (Figure 8). Moreover, NaOCl-treated cultures required more time for recovery compared to the control, with subcultures developing in all NaOCl-treated groups within three days. This further indicates that decontamination did not delay tissue regeneration among NaOCl-treated cultures.

The superior performance of 1.0% (v/v) NaOCl can be explained by the balance between antimicrobial efficacy and phytotoxic effects. At lower concentrations (0.6–0.8%), the availability of hypochlorous acid (HOCl) may be insufficient to completely eliminate fungal contaminants, allowing residual microbial activity that can still interfere with nutrient uptake and cellular development. In contrast, higher concentrations ($\geq 1.2\%$) may generate excessive oxidative stress, leading to damage of plant cell membranes, protein denaturation, and reduced metabolic activity. This concentration-dependent response has been reported in previous studies, where NaOCl above optimal levels reduced explant viability despite effective disinfection [10; 13]. Therefore, 1.0% NaOCl represents an optimal threshold where microbial inactivation is maximized while minimizing oxidative injury to plant tissues, resulting in improved growth parameters such as meristem elongation, leaf production, and rooting.



Note : ** Values with different letter superscripts in each parameter are significantly different ($p \leq 0.01$) based on Tukey's HSD test; Error bar represents the standard error of the mean ($n=4$)

Figure 8 Survival performance of stage IV tissue-cultured banana as influenced by varying NaOCl levels.

NaOCl's effectiveness in rescuing contaminated tissue cultures is attributed to its broad-spectrum antimicrobial activity, which rapidly kills various contaminants through the oxidation of sulphhydryl groups in bacterial enzymes [27]. This action disrupts the metabolic functions of microbial cells, ensuring the cleanliness of tissue cultures. The meristem height and subsequent plant growth are critical indicators of the effectiveness of NaOCl in promoting healthy tissue development. Sawa [28] highlighted the importance of the meristem in plant development, and NaOCl's ability to remove contaminants ensures a conducive environment for tissue growth. Additionally, NaOCl's disinfecting action likely aids in enhancing enzymatic activities involved in plant development [29]. Leaf and root development in tissue culture are critical for successful plantlet propagation. The increased leaf production observed in NaOCl-treated cultures, particularly at 1.0% (v/v) as shown in Figure 7, suggests that effective removal of contaminants supports better plant growth. Effective suppression of microbial contaminants improves tissue condition and reduces disease development in banana systems [30], thereby contributing to a more favorable environment for normal physiological growth. This likely creates a more suitable environment for the action of growth regulators in the medium. In this study, the improved leaf formation may also be linked to the presence of 6-benzylaminopurine (BAP), which is known to promote shoot and leaf development. Similar observations were reported by Hossain et al. [31], who showed that BAP enhances leaf formation, while proper disinfection can help maintain conditions that favor organogenesis.

The high rescue efficiency observed in this study (≈ 90 – 96% at 0.6–1.2% NaOCl) is comparable to or slightly lower than the aseptic establishment rates reported by Keshari et al. [11], who achieved $\geq 96\%$ contamination-free cultures during Stage I using 1% NaOCl combined with ethanol. However, unlike these studies that focused on preventive sterilization, the present work demonstrates that similar levels of success can be achieved even under curative conditions at later stages of micropropagation. This highlights the effectiveness of NaOCl not only in preventing contamination but also in rescuing already affected cultures. Moreover, NaOCl's strong oxidizing properties make it an effective disinfectant for in vitro cultures, disrupting microbial cell walls and aiding in successful rooting and shoot development. Netravati et al. [9] demonstrated that NaOCl-treated banana tissues can root successfully, supporting this study's findings. The number of subcultures produced was influenced by NaOCl concentration, with T2, T3, and T4 showing better performance than T5. Buah et al. [32] noted that optimal growth conditions, including the right NaOCl concentration, facilitate subculture development, though excessive disinfectant concentrations can hinder tissue regeneration. Hesami et al. [10] highlighted that while NaOCl is effective in eliminating contaminants, higher concentrations can cause tissue damage, leading to reduced viability. Significantly, the recovery time for cultures treated with NaOCl was significantly longer than that of the control, with all NaOCl-treated subcultures developing within three days. Joy et al. [33] and Goswami and Handique [13] emphasized that disinfectants play a crucial role in enhancing micropropagation by preventing contamination and promoting the health of plantlets.

A clear concentration-dependent trend was observed in this study. Lower NaOCl concentrations (0.6–0.8%) were sufficient to improve survival but may not fully suppress microbial contamination, leading to slightly reduced growth performance. Conversely, increasing the concentration to 1.2% maintained high decontamination

efficiency but showed a tendency toward reduced subculture formation and slightly lower growth consistency, likely due to increased oxidative stress on plant tissues. These findings indicate that excessive NaOCl exposure can negatively affect tissue regeneration, supporting previous reports that highlight the narrow threshold between effective disinfection and phytotoxicity [10; 13].

3.4 Mechanism of NaOCl-mediated rescue of Stage II and Stage IV cultures

Figure 9 illustrates the putative mechanism by which NaOCl treatment rescued slightly contaminated banana cultures in this study. When dissolved in water, NaOCl forms hypochlorous acid (HOCl), which is widely recognized as the primary antimicrobial agent responsible for its disinfecting activity [8; 10]. HOCl is a highly reactive oxidizing species capable of penetrating microbial cells and inducing cellular damage through protein oxidation, enzyme inactivation, and oxidative stress, ultimately leading to microbial inactivation [8]. Although these mechanisms have been extensively described in microbial systems, they are considered applicable to fungal contaminants in tissue culture, consistent with its known efficacy against fungal spores and hyphal structures, including *Chrysosporium* sp.

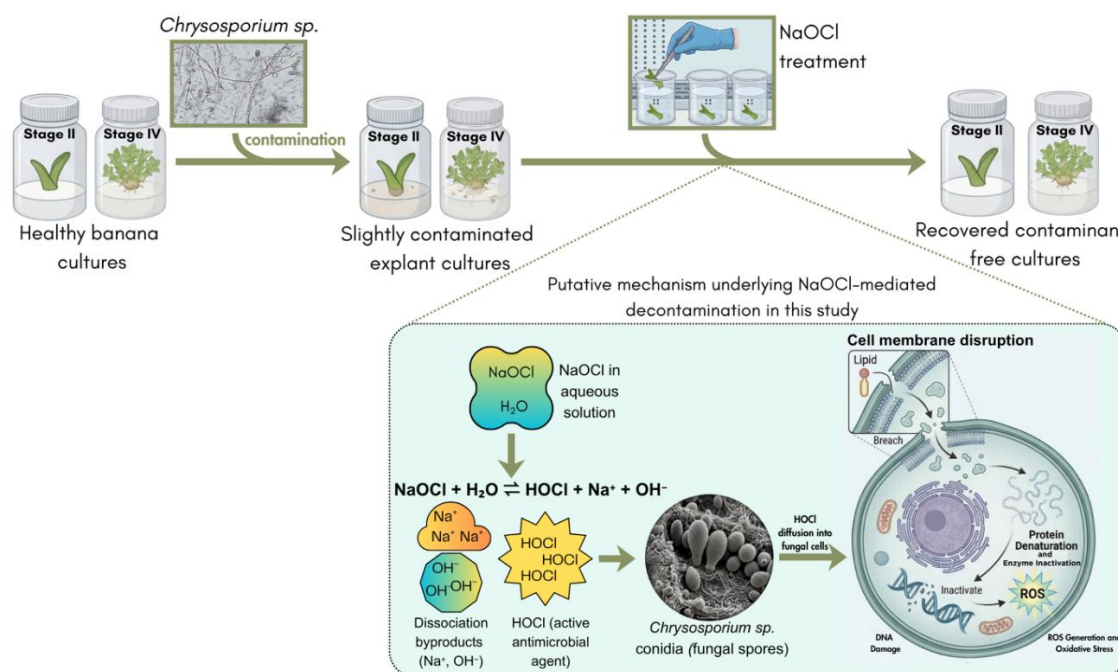


Figure 9 Schematic representation of NaOCl-mediated decontamination of *Chrysosporium* sp. in banana tissue culture.

In Stage II cultures, NaOCl treatment (0.6–1.2%) significantly improved explant survival and promoted callus formation compared with untreated controls. The removal of fungal contaminants likely reduced competition for nutrients and minimized the accumulation of toxic microbial metabolites, thereby facilitating explant recovery. Tissue culture systems are highly sensitive to contamination, and early suppression of microbial growth is essential for maintaining cellular proliferation and differentiation [2; 22]. The rapid antimicrobial action of HOCl therefore restored a favorable microenvironment for callus initiation and growth, which explains the enhanced survivability and faster recovery observed in treated explants. In Stage IV cultures, higher NaOCl concentrations (1.0–1.2%) were more effective, resulting in greater meristem height, increased leaf production, and improved rooting success. At this stage, the benefit of NaOCl treatment is reflected in improved plantlet development rather than callus formation. Once contamination was suppressed, plantlets were able to resume normal physiological processes, including shoot elongation and root formation. Meristematic tissues play a central role in plant growth and development [28], and successful micropropagation depends on maintaining healthy, contamination-free explants [29]. The improved growth parameters observed in Stage IV therefore indicate that NaOCl treatment effectively restored conditions necessary for normal plantlet development.

Although both 1.0% and 1.2% NaOCl showed strong decontamination and growth responses, the 1.0% concentration appears to provide the most practical balance between antimicrobial efficacy and phytotoxicity. At lower concentrations, the disinfectant effect may be insufficient to fully inactivate fungal contaminants, allowing residual microbial growth to persist. In contrast, higher concentrations may induce oxidative stress that can

damage plant tissues and impair regeneration capacity [10; 27]. While 1.2% NaOCl showed comparable performance in some parameters, the use of 1.0% is strategically advantageous, as it achieves effective decontamination and favorable growth responses with reduced risk of tissue damage and lower chemical input. The mechanism illustrated in Figure 9 supports the conclusion that NaOCl-mediated rescue of banana tissue cultures is driven by the formation of HOCl, which rapidly inactivates fungal contaminants. At an optimal concentration of 1.0%, this antimicrobial action is sufficient to suppress contamination while preserving tissue integrity, allowing explants to recover and resume normal growth.

4. Conclusion

This study identified *Chrysosporium sp.* as the dominant contaminant in tissue-cultured ‘Cavendish’ banana under the present laboratory conditions. It demonstrated that NaOCl can be effectively used to rescue slightly contaminated cultures at both the multiplication (Stage II) and acclimatization (Stage IV) stages. Among the concentrations tested, 1.0% (v/v) NaOCl produced the most consistent results, improving survival, callus formation, and plant growth, as indicated by increased meristem height, leaf number, and rooting. Subculture production was also enhanced at 0.60% and 1.0% (v/v), suggesting that these concentrations are suitable for continued propagation. Notably, contaminated cultures were able to recover within three days, indicating rapid suppression of microbial growth and restoration of normal development. Unlike previous studies that focus primarily on preventive sterilization during culture establishment (Stage I), this study provides evidence that NaOCl can be applied as a curative strategy to recover contaminated cultures at later stages of micropropagation. This finding highlights a practical and cost-effective approach to reduce losses and improve the efficiency of tissue culture operations, particularly in commercial banana production.

5. Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Google Gemini to generate conceptual illustrations to enhance visual clarity, which subsequently refined and enhanced using Canva. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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7. Author Contributions

Villanueva, M.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft; Manigo, B.: Conceptualization, Methodology, Supervision, Visualization, Writing – review & editing; Besas, U.: Conceptualization, Supervision, Visualization and Validation; Hagonos, A.: Methodology, Writing – review & editing.

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