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Research Article

OPTIMIZING SURFACE STERILIZATION OF *Lansium domesticum* LEAF LAMINA EXPLANTS USING SODIUM HYPOCHLORITE

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Abstract

Lansium domesticum Corr. is a fruit crop with high consumer demand; however, its conventional propagation is relatively slow. In vitro culture offers a potential alternative to accelerate plant propagation. This study aimed to evaluate sodium hypochlorite (NaOCl)-based sterilization protocols and identify the most effective treatment for in vitro establishment of leaf lamina explants. The experiment was conducted at the Tissue Culture Laboratory, Faculty of Agriculture, Universitas Sriwijaya, from July to September 2024 using five sterilization treatments with different NaOCl concentrations and exposure times. Data were analyzed descriptively to determine the percentages of bacterial contamination, fungal contamination, surviving explants, and browning incidence. The results showed that the treatment consisting of liquid detergent (3 min), 0.2% streptomycin sulfate (10 min), 0.2% benomyl (10 min), 1% NaOCl (15 min), and 70% ethanol (5 min) was the most effective protocol. This treatment resulted in 63% explant survival, 0% browning, 33% bacterial contamination, and 3% fungal contamination. This study provides a practical sterilization protocol that balances antimicrobial effectiveness and tissue viability, representing a novel contribution for in vitro propagation of *Lansium domesticum*. The findings have important implications for improving large-scale propagation efficiency of this economically important tropical fruit species. These results indicate that moderate NaOCl exposure is essential to achieve an optimal balance between contamination control and explant viability in in vitro culture systems.

Keywords: Bacteria, Browning, Fungi, Sodium-hypochlorite

INTRODUCTION

Lansium domesticum Corr. is a highly favored tropical fruit with considerable commercial value in Southeast Asia, including Indonesia. Despite its strong market demand, large-scale production of *Lansium* is constrained by its slow propagation rate (Metalisa et al., 2022). Conventional propagation methods, such as using seed and vegetative propagation, present significant limitations. Seed-derived plants require approximately 15–20 years to reach fruiting stage and often exhibit genetic variability, resulting in inconsistent fruit quality. Similarly, vegetative propagation techniques do not always guarantee uniformity or rapid multiplication.

To overcome these limitations, in vitro propagation through tissue culture offers a promising alternative. Tissue culture enables the rapid production of genetically uniform plantlets identical to the mother plant within a shorter time frame (Agustiningrum et al., 2023; Twaij et al., 2020). This technique involves culturing plant cells, tissues, or organs on a nutrient medium supplemented with amino acids, sugars, vitamins, inorganic salts, plant growth regulators, water, and a solidifying agent such as agar (Pepe et al., 2025; Renugaadevi R et al., 2025; Sudheer et al., 2022). However, the nutrient-rich composition of culture media also creates a favorable environment for the growth of bacteria and fungi (Cahyono & Ningsih, 2023), making contamination one of the major challenges in tissue culture (Leelavathy & Deepa Sankar, 2016; Odutayo et al., 2007; Wang et al., 2021).

Surface sterilization of explants is a critical step in minimizing contamination and ensuring successful culture establishment (Rahmadi et al., 2020). Different plant species respond variably to sterilizing agents and immersion times. Common disinfectants used in tissue culture include sodium hypochlorite (NaOCl), ethanol, bactericides, and fungicides (Irmawati et al., 2023; Pratiwi et al., 2021; Röhner et al., 2020). Sodium hypochlorite is widely used due to its strong antimicrobial activity, primarily through disruption of microbial cell membranes and removal of surface contaminants such as soil particles and debris (Bello et al., 2018; Jaiswal et al., 2017).

Excessive sterilization may damage plant tissues and induce browning, whereas insufficient sterilization leads to contamination. Previous studies have reported inconsistent results depending on species and sterilization conditions (Hesami et al., 2019; Kaya & Özatay, 2024; Liu et al., 2024; Setamam & Sidik, 2024). Despite previous studies on sterilization techniques, there is limited information on the optimal NaOCl concentration and exposure time for leaf lamina explants of *Lansium domesticum*. This represents a critical gap, particularly for improving in vitro propagation efficiency of this species. Therefore, this study aimed to evaluate different NaOCl-based sterilization treatments and determine the most effective protocol for reducing contamination while maintaining explant viability.

RESEARCH METHOD

The study was conducted from June to September 2024 at the Tissue Culture Laboratory, Faculty of Agriculture, Universitas Sriwijaya, Palembang, Indonesia (2°59′23.4″S; 104°43′53.4″E). Leaf lamina explants were collected from healthy young seedlings of *Lansium domesticum*, selected based on uniform size and physiological condition to ensure experimental consistency. The explants were cultured on Murashige and Skoog (MS) basal medium (Nugrahani et al., 2024; Shelepova et al., 2025) solidified with agar.

Five surface sterilization treatments were applied to the lamina explants. Each treatment consisted of 30 explants distributed across culture bottles, resulting in a total of 150 experimental units. Observations were conducted throughout the culture period for all the culture bottles. The sterilization procedure involved sequential immersion in liquid detergent (3 min), 0.2% streptomycin sulfate (10 min), 0.2% benomyl (10 min), sodium hypochlorite (NaOCl) at varying concentrations and exposure times, followed by 70% ethanol (5 min). The NaOCl treatments were: 1% for 25 min, 1% for 20 min, 1% for 15 min, 1.5% for 10 min, and 1.5% for 5 min.

The independent variable was the NaOCl concentration and exposure time, while the dependent variables included percentage of surviving explants, bacterial contamination, fungal contamination, and browning incidence. Surviving explants were defined as explants that remained green, free from contamination, and without browning (Inaya et al., 2024; Lestari et al., 2025). Bacterial contamination was identified by slimy exudates (Amiri et al., 2023; Sucahyo et al., 2023; Wulandari et al., 2022), while fungal contamination was indicated by mycelial growth (Darwesh et al., 2024; Li et al., 2022; Nisa et al., 2022; Sutarsih et al., 2022). Browning was defined as visible tissue discoloration due to phenolic oxidation (Oros et al., 2022; Zhao et al., 2021).

Data were analyzed descriptively using percentage-based comparison among treatments, as the objective of this study was to identify the most effective sterilization protocol rather than to test statistical differences. This study employed a laboratory-based experimental design using a completely randomized design (CRD); therefore, instrument-based validation, questionnaire-based data collection, and statistical modeling approaches (e.g., SEM or PLS) were not applicable. The experimental units consisted of individual explants, and treatment effectiveness was evaluated based on observable biological responses.

RESULTS AND DISCUSSION

Percentage of Surviving Explants (%)

Explants were classified as surviving when they remained free from contamination, did not exhibit desiccation, and maintained a visually fresh green appearance (Rodinah et al., 2016), indicating successful adaptation to the MS medium. Survival was assessed from the initial culture stage, including explants that remained viable without visible growth, up to those showing active growth responses. Observations were conducted for 28 days after inoculation (DAI).

The results showed that all explants across treatments remained viable at one week after inoculation (WAI), indicating that initial sterilization procedures were effective in suppressing immediate microbial growth. However, contamination increased at three weeks after inoculation, suggesting the presence of latent or endophytic microorganisms that were not eliminated during surface sterilization.

Among the treatments, S3 resulted in the highest survival rate (63%), followed by S2 and S4 (57%), while S5 showed the lowest survival (47%). This indicates that a moderate NaOCl concentration (1%) combined with an appropriate exposure time (15 min) provided the optimal balance between antimicrobial effectiveness and tissue tolerance. The survival percentage of explants at 28 days after inoculation is presented in Figure 1.

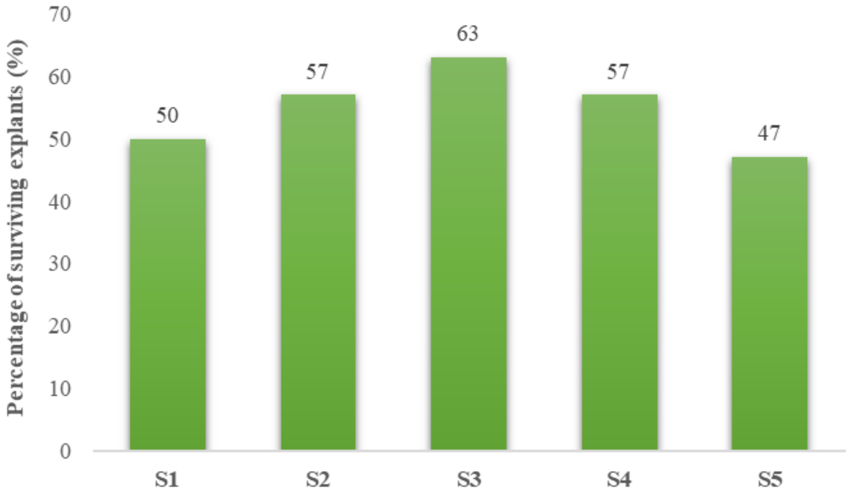

Figure 1. Percentage of surviving explants at 28 days after inoculation. S1 = NaOCl 1% for 25 min; S2 = NaOCl 1% for 20 min; S3 = NaOCl 1% for 15 min; S4 = NaOCl 1.5% for 10 min; and S5 = NaOCl 1.5% for 5 min

These findings directly answer the research objective, demonstrating that sterilization effectiveness is not solely determined by disinfectant strength but also by the interaction between concentration and exposure duration. Excessive sterilization may induce tissue damage (Dagne et al., 2023), while insufficient sterilization allows microbial survival (Eliwa et al., 2024). This result is consistent with previous studies (Chika et al., 2022), which reported that stepwise or moderate sterilization treatments are more effective in maintaining explant viability compared to extreme conditions. Similarly, Setiani et al. (2018) found that higher NaOCl concentrations can eliminate contamination but often induce browning and tissue damage.

The culture medium was supplemented with auxin and cytokinin at a concentration of 1 ppm each. Nevertheless, this hormonal combination was not effective in inducing shoot or callus formation in the tested lamina explants. According to Sarianti et al. (2022), shoot induction requires a balanced ratio between auxin and cytokinin, and inappropriate concentrations may inhibit morphogenic responses. Therefore, the lack of growth observed in surviving explants may be attributed to suboptimal hormonal balance rather than to sterilization effects alone.

Percentage of Contaminated Explants (%)

Contamination observed during the culture period consisted of two main types, namely bacterial and fungal contamination. Both types of microbial infection represent major constraints in plant tissue culture, as they compete with explant tissues for nutrients and may release toxic metabolites that inhibit growth or cause tissue necrosis. The incidence and severity of contamination varied among sterilization treatments, indicating differences in the effectiveness of the applied surface sterilization protocols.

Bacterial contamination was characterized by the appearance of slimy exudates on the surface of the culture medium or surrounding the explants, which gradually spread across the entire medium (Ali et al., 2018). Media contaminated by bacteria typically appeared wilted and eventually led to tissue deterioration and explant death (Dunaeva & Osledkin, 2015). Fungal contamination was assessed based on the appearance of fungal growth on the Murashige and Skoog (MS) medium and on the explant tissues. Fungal infection was indicated by the emergence of fine white mycelial threads that progressively expanded and eventually covered both the explants and the culture medium (Budiarni et al., 2024; Rawal & Keharia, 2019).

The results showed that bacterial contamination was highest in S1 (47%) and lowest in S5 (3%), whereas fungal contamination was highest in S5 (17%), as given in Figure 2. This contrasting pattern indicates that higher NaOCl concentration with shorter exposure time may be more effective against bacteria but less effective in controlling fungal spores.

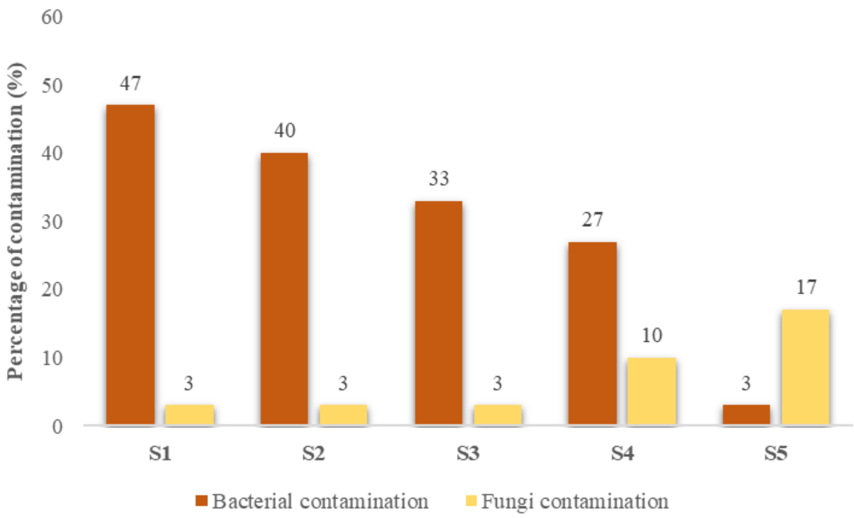

Figure 2. Percentage of contamination at 28 days after inoculation. S1 = NaOCl 1% for 25 min; S2 = NaOCl 1% for 20 min; S3 = NaOCl 1% for 15 min; S4 = NaOCl 1.5% for 10 min; and S5 = NaOCl 1.5% for 5 min.

These results highlight a trade-off between sterilization strength and spectrum of antimicrobial activity, where different microorganisms respond differently to sterilization treatments. Bacteria, which are generally more sensitive to chemical disinfectants, were effectively reduced at higher NaOCl concentrations. However, fungal spores, which are more resistant, may require longer exposure time for complete elimination.

The absence of contamination at one week after inoculation further supports the hypothesis that contamination observed later originated from latent or endophytic microorganisms rather than external surface contaminants. This finding is consistent with Cassells & Doyle-Prestwich (2009), who reported that endophytic microbes often remain undetected during early incubation stages but proliferate under favorable in vitro conditions. Thus, the findings emphasize that surface sterilization alone may not fully eliminate internal contaminants, and additional strategies such as antibiotic supplementation or explant pretreatment may be required.

Percentage of Explant Browning (%)

Browning was identified by progressive discoloration of the explants, beginning with yellowing and eventually developing into brown pigmentation (Amente & Chimdessa, 2021; Chai et al., 2018). Observations were conducted over a four-week period after inoculation until browning symptoms were fully expressed.

Browning was observed only in treatments S4 (6%) and S5 (33%), while no browning occurred in S1, S2, and S3, as shown in Figure 3. This indicates that increased NaOCl concentration may induce oxidative stress in plant tissues, leading to phenolic oxidation. Browning is commonly associated with the enzymatic oxidation of phenolic compounds catalyzed by polyphenol oxidase (PPO), resulting in quinone formation that is toxic to plant cells (Cai et al., 2020; Sookruksawong & Pilahome, 2025; Wang et al., 2025). Excessive sterilization may damage cell membranes, triggering the release of phenolic compounds and accelerating this process (Xu & Wang, 2025).

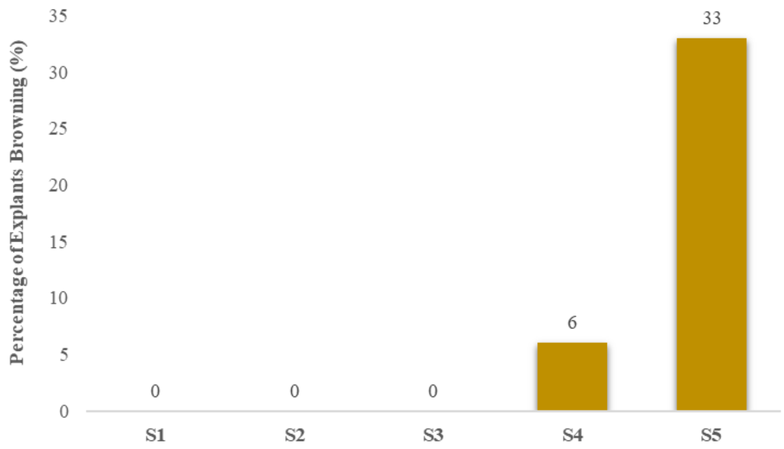

Figure 3. Percentage of explants browning at 28 days after inoculation. S1 = NaOCl 1% for 25 min; S2 = NaOCl 1% for 20 min; S3 = NaOCl 1% for 15 min; S4 = NaOCl 1.5% for 10 min; and S5 = NaOCl 1.5% for 5 min.

These findings are in agreement with Xu et al. (2023), who reported that chemical stress during sterilization can enhance phenolic oxidation and reduce explant viability. The absence of browning in S3 further confirms that moderate sterilization conditions are optimal for maintaining cellular integrity while suppressing contamination, reinforcing its effectiveness as the best treatment.

This study provides a specific and optimized NaOCl-based sterilization protocol for leaf lamina explants of *Lansium domesticum*, which has not been previously well-documented. The novelty lies in identifying the optimal combination of concentration and exposure time that simultaneously minimizes contamination and prevents browning while maintaining high explant survival. Practically, this protocol can be directly applied in tissue culture laboratories to improve the efficiency of large-scale propagation of *Lansium domesticum*, particularly for conservation and commercial production purposes.

These findings suggest that optimizing sterilization parameters is critical for improving in vitro culture success, particularly in woody plant species with high susceptibility to contamination. This study was limited to surface sterilization treatments and did not evaluate internal contamination control or the effect of different plant growth regulator combinations on morphogenesis. Future studies should therefore explore the integration of sterilization protocols with hormonal optimization to enhance shoot or callus induction, as well as investigate methods to eliminate endophytic microorganisms.

CONCLUSION

This study demonstrated that the effectiveness of surface sterilization in *Lansium domesticum* is determined by the balance between antimicrobial activity and tissue tolerance. Among the tested treatments, 1% NaOCl for 15 minutes (S3) was identified as the optimal protocol, achieving the highest explant survival (63%), zero browning, and relatively low contamination levels. These findings contribute to the development of an efficient in vitro propagation protocol for *Lansium domesticum*, particularly in improving culture establishment success. Practically, the optimized sterilization method can be applied to support large-scale propagation and conservation of this economically important species. This study also contributes to the limited knowledge on sterilization optimization in tropical woody plant tissue culture systems. Future research should focus on integrating sterilization protocols with growth regulator optimization and strategies to control endophytic contamination to further enhance in vitro propagation outcomes.

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AUTHOR CONTRIBUTIONS

Conceptualization, I.W.; Methodology, I.W., S.W.; Software, I.W., W.H.; Validation, I.W., S.W.; Formal Analysis, I.W.; Investigation, I.W., H.L., R.I.; Resources, I.W.; Data Curation, I.W., H.L., R.I.; Writing – Original Draft Preparation, H.L., R.I.; Writing – Review & Editing, I.W., S.W.; Visualization, I.W., W.H.; Supervision, S.W.; Project Administration, I.W.; Funding Acquisition, I.W. All authors have read and agreed to the published version of the man-uscript.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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