ASSESSMENT OF ALTERNATIVE ASEPTIC CONDITIONS FOR *ALOE VERA* MICROPROPAGATION: EFFECTS ON CALLUS INDUCTION AND CULTURE CONTAMINATION

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Microbial contamination and inadequate sterilization infrastructure are critical constraints in *Aloe vera* tissue culture. This study optimized callus induction protocols for *Aloe barbadensis* by comparing the Seesap / CSUP method (Chemical Sterilization Using Sodium Hypochlorite), a low-cost alternative aseptic method, against conventional Laminar Airflow Cabinet (LAF)-based sterilization at the Department of Urban Bioresources, Faculty of Urban and Aquatic Bioresources, University of Sri Jayewardenepura. A factorial design was applied using full-strength and half-strength Murashige and Skoog (MS) medium, two 2,4-D concentrations (1.0 and 2.0 mg L^{-1}) with 6-Benzylaminopurine (BAP) fixed at 2.0 mg L^{-1}, and two vessel types (glass bottles and petri dishes). Leaf base explants were surface-sterilized using 1–2% NaOCl and cultured on MS agar medium. Contamination, survival, browning, and callus induction frequency were recorded at 5–7-day intervals over a 28-day observation period. Statistical analyses employed independent t-tests and two-way ANOVA. The CSUP method reduced contamination by 25–50 percentage points across all eight treatment combinations compared with the LAF method (binomial $p = 0.004$). Full MS + CSUP + glass bottle cultures achieved the lowest contamination rates. Full-strength MS bottle cultures recorded the highest survival rate (66.7%) and lowest overall contamination (26.7%), while half-strength MS petri dish cultures showed contamination up to 75%. Bottle cultures demonstrated a statistically significant survival advantage over petri dish cultures ($\chi^2 = 6.667$, $p = 0.010$). Browning was prevalent in NaOCl-treated cultures (71.4–76.9%), with earlier onset (mean 3.2 days after culture) and elevated PPO activity (0.85 vs 0.61 OD_{420}/min/g FW) compared to CSUP-treated material. Early tracheary element differentiation with spiral wall thickenings, observed exclusively in CSUP explants at 14 days after culture, confirmed callus initiation potential. These findings validate the CSUP method as a scientifically defensible and cost-effective alternative to conventional tissue culture infrastructure.

Keywords: *Aloe vera*, CSUP method, callus induction, contamination control, micropropagation, MS medium