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Evaluating the Efficacy of Mercuric Chloride as a Surface Disinfectant for *In Vitro* Establishment of *Stevia rebaudiana*

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Abstract: ***The present study aimed to standardize an effective surface sterilization protocol for the in vitro propagation of Stevia using nodal segments, shoot tips, and leaf explants. Different concentrations of mercuric chloride ($HgCl_2$; 0.05–0.5%) and exposure durations were evaluated to determine their effects on explant contamination and survival. Among the treatments tested, 0.2% $HgCl_2$ with a 2-minute exposure was found to be the most effective, resulting in maximum survival rates of 70% for leaf explants and 80% for both nodal segments and shoot tips. Higher concentrations of $HgCl_2$ resulted in increased tissue mortality and reduced explant viability, whereas lower concentrations were inadequate for effective contamination control. Therefore, treatment with 0.2% $HgCl_2$ for 2 minutes was established as an effective surface sterilization protocol for achieving aseptic culture establishment while maintaining high explant viability during the in vitro propagation of Stevia.***

Keywords: *Stevia rebaudiana*, Surface Disinfectant, $HgCl_2$, *in vitro,* tissue culture.

1. Introduction

Sugar is a ubiquitous component of the modern diet and contributes substantially to food palatability. However, excessive sugar consumption has been associated with several adverse health consequences, including dental caries and weight gain. Furthermore, emerging, although still controversial, evidence suggests that excessive sugar intake may play a role in the pathogenesis of certain degenerative diseases. Consequently, the demand for artificially sweetened food products and artificial sweeteners has continued to increase. Artificial sweeteners are synthetic food additives designed to mimic the taste profile of sucrose while providing reduced or negligible caloric content. Despite their potential benefits in reducing caloric intake, several studies have raised concerns regarding their possible adverse health effects [1].

A successful *in vitro* propagation protocol depends largely on effective surface sterilization to eliminate external microbial contaminants while preserving explant viability [2]. Explants collected from field-grown or greenhouse plants harbor diverse bacterial and fungal microflora that can rapidly proliferate on culture media, deplete essential nutrients, and cause explant necrosis. Among the various chemical sterilizing agents, mercuric chloride ($HgCl_2$) is widely recognized as a potent, broad-spectrum disinfectant capable of penetrating micro-crevices and effectively suppressing persistent epiphytic and endophytic pathogens [3]. Owing to its strong oxidizing and protein-precipitating properties, optimization of $HgCl_2$ concentration and exposure duration is critical; insufficient exposure may fail to eliminate microbial contamination, whereas excessive exposure can cause phytotoxicity, tissue browning, and reduced morphogenic potential [4]. Therefore, establishing a standardized $HgCl_2$ disinfection protocol is essential for achieving effective aseptic culture establishment and maintaining high explant survival rates during plant micropropagation [5].

Bleach, ethanol, sodium hypochlorite (NaOCl), and mercuric chloride ($HgCl_2$) are frequently served as sterilizing agents. The choice of sterilizing agent, its concentration, and the duration of exposure are dependent on the nature of the explant. Authors strictly adhered to all previously described procedures in this study.

2. Material and Methods

2.1 Explant Preparation and Sterilization

The standard protocol of MS medium for micropropagation of plants was followed, which is earlier used by Misal and Chavan (2025). The nodal segments used as an explant were collected from Khokarmoha, Beed (MS) where they maintained stock plants under observation [6].

Initially, the explants were thoroughly washed under running tap water for 20 minutes to remove adhering dust and surface debris. The explants were then treated with 70% (v/v) ethanol for 1–2 minutes, followed by rinsing under running tap water for an additional 10 minutes. After these pre-sterilization treatments, the explants were transferred to a laminar airflow cabinet for surface sterilization. Surface sterilization was carried out using a freshly prepared 0.05–0.5% (w/v) aqueous solution of mercuric chloride ($HgCl_2$) for 5 minutes. Subsequently, the explants were rinsed 3–4 times with autoclaved distilled water, with each rinse lasting 5 minutes, to ensure the complete removal of residual $HgCl_2$.

All of these explants were cut into tiny pieces and inoculated on the appropriate medium.

2.2 Preparation of culture medium

All experiments of present study were tried on MS media (Murashige and Skoog, 1962) supplemented with varying concentrations of auxin and cytokinin. Culture medium was enriched with sucrose and 2.5 to 3 gm clerigar for solidification, and the pH was set to 5.6-5.8. The media were steam sterilized in an autoclave at 15 psi and 121 °C. To check the impact of different sucrose concentrations on the growth of nodal explants 20, 30, 40, and 50 g/L sucrose were tried.

2.3 Culture conditions

After the inoculation, culture bottles were shifted to a culture room with a temperature of 25±2°C and a 16-hour photoperiod provided by cool white fluorescent cool tubes.

3. Results and discussion

Plant material was collected in the form of small branches, shoot tips, and leaves, which were used as explant sources. The collected explants were subjected to surface decontamination using mercuric chloride ($HgCl_2$). A standard surface sterilization protocol for Stevia explants was established through a trial-and-error approach by evaluating different concentrations of $HgCl_2$ ranging from 0.05 to 0.5%. Explant survival and contamination rates were recorded 21 days after inoculation, as presented in the table 1.

Healthy plant materials were initially washed thoroughly under running tap water, followed by rinsing with autoclaved distilled water. Surface sterilization of Stevia explants using different concentrations of $HgCl_2$ (0.05–0.5%) resulted in considerable differences in contamination and survival rates. The exposure duration to the sterilizing agent was evaluated from 1 to 5 minutes for different explant types. Among the tested durations, a 2-minute exposure was found to be the most effective and was subsequently used for all further experiments.

Leaf explants showed complete contamination at 0.05% $HgCl_2$, whereas the maximum survival rate of 70% was recorded at 0.2% $HgCl_2$ with a 2-minute exposure. At concentrations higher than 0.2%, both contamination and mortality increased markedly. Nodal segments exhibited better performance, with an 80% survival rate observed at 0.2% $HgCl_2$, while survival declined at higher concentrations. Similarly, shoot tips showed the highest survival rate (80%) at 0.2% $HgCl_2$; however, complete contamination was observed at 0.05%, and a drastic reduction in viability occurred at concentrations ≥0.3% (Table 1 and Graph 1).

Overall, treatment with 0.2% $HgCl_2$ for 2 minutes was found to be the most effective surface sterilization protocol for all explant types, as it minimized contamination while maximizing explant survival.

Table 1: Impact of mercuric chloride (HgCl2) on survival rate and control of contamination on different explant.

Source of explant	Concentration of $HgCl_2$ (%)	Treatment Time (minutes)	No. of cultures vessels	Contami-nation (%)	Survival (%)
Leaf	0.05	2	10	100	00
	0.1	2	10	80	20
	0.2	2	10	30	70
	0.3	2	10	30	30
	0.4	2	10	10	10
	0.5	2	10	00	00
Nodal segment	0.05	2	10	90	10
	0.1	2	10	70	30
	0.2	2	10	20	80
	0.3	2	10	20	40
	0.4	2	10	10	10
	0.5	2	10	00	00
Shoot Tip	0.05	2	10	100	00
	0.1	2	10	70	30
	0.2	2	10	20	80
	0.3	2	10	20	40
	0.4	2	10	10	10
	0.5	2	10	-	-

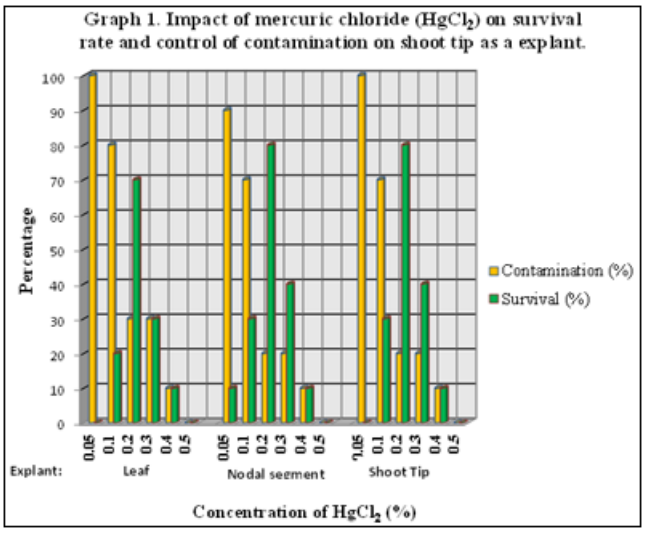

Figure 1: Impact of mercuric chloride ($HgCl_2$) on survival rate and control of contamination on shoot tip as a explant

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