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Effect of Mercuric Chloride ($HgCl_2$) on the Establishment of Contamination-Free Cultures from Different Explants of *Viola odorata* L.

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Abstract

Surface sterilization is a critical prerequisite for the successful establishment of contamination-free cultures in plant tissue culture. The present study aimed to standardize the concentration of mercuric chloride ($HgCl_2$) for effective surface sterilization of different explants of *Viola odorata* L., including leaves, petioles, and nodal segments. Healthy explants were collected from field-grown plants and pre-treated with running tap water, Bavistin, and Tween-20 before exposure to different concentrations of $HgCl_2$ (0.05%, 0.10%, 0.15%, 0.20%, and 0.25%) for 3 minutes. Sterilized explants were inoculated onto Murashige and Skoog (MS) medium under aseptic conditions, and observations on contamination, mortality, and survival were recorded after four weeks. Increasing $HgCl_2$ concentration progressively reduced microbial contamination but also increased explant mortality at higher concentrations. For leaf explants, treatment with 0.15% $HgCl_2$ resulted in the highest survival rate (88.80%) with low contamination (9.62%) and minimal mortality (1.57%). Petiole explants exhibited maximum survival (81.31%) at 0.10% $HgCl_2$, whereas nodal segments showed the best response at 0.20% $HgCl_2$ with 70.20% survival and only 11.60% contamination. The findings demonstrate that the optimal $HgCl_2$ concentration varies with explant type and that appropriate sterilization protocols are essential for achieving aseptic cultures while maintaining explant viability. The standardized protocol developed in this study provides a reliable foundation for subsequent *in vitro* propagation and tissue culture applications in *Viola odorata* L.

Keywords: *Viola odorata* L., mercuric chloride ($HgCl_2$), surface sterilization, aseptic culture, explant survival, contamination control, tissue culture, *in vitro* propagation, MS medium, medicinal plant.

Introduction

The establishment of aseptic cultures is a fundamental prerequisite for successful *in vitro* plant tissue culture, making explant sterilization a crucial step in the propagation process. Explants obtained from field-grown plants often harbor bacteria, fungi, yeasts, and other microorganisms on their surfaces or within their tissues, posing a significant risk of contamination. If these microbial contaminants are not adequately removed, they can rapidly proliferate in the culture medium, suppress explant growth, reduce regeneration efficiency, and eventually cause complete culture failure (George *et al.*, 2008; Cassells, 2012).

Surface sterilization efficiency varies depending on factors such as the explant type, source, physiological age, environmental conditions, and the associated microbial load. Young and actively growing tissues generally carry fewer contaminants than mature, woody, or field-grown materials, making the latter more challenging to establish under aseptic conditions. Consequently, sterilization protocols should be carefully optimized for each explant to achieve effective decontamination while maintaining tissue viability and regenerative potential (Bhojwani & Dantu, 2013). Commonly used sterilizing agents in plant tissue culture include ethanol, sodium hypochlorite, calcium hypochlorite, hydrogen peroxide, and mercuric chloride, often applied with surfactants and followed by sterile water rinses to reduce phytotoxic effects. Among these, sodium hypochlorite is widely favoured for its efficacy and comparatively lower toxicity, whereas mercuric chloride, despite its strong disinfectant properties, requires cautious use because of its hazardous nature and environmental concerns (George *et al.*, 2008; Leifert & Cassells, 2001).

Several studies have reported that the successful establishment of aseptic cultures in *Viola odorata* L. is highly dependent on the explant type and the surface sterilization protocol employed. Different explants, including seeds, pistils, leaves, petioles, axillary buds, and apical buds, have been effectively sterilized using combinations of disinfectants such as 70% ethanol, sodium hypochlorite (NaOCl), commercial bleach, and mercuric chloride ($HgCl_2$). The concentration of these sterilizing agents and the duration of treatment vary according to the explant to achieve maximum decontamination while minimizing tissue damage and maintaining regenerative potential. Previous reports have highlighted the successful use of NaOCl for seed sterilization, bleach for floral tissues, and $HgCl_2$ for vegetative explants, emphasizing the importance of optimizing sterilization conditions for different plant materials

(Wijowska & Kuta, 1999; Barekat *et al*., 2013; Kaloo *et al*., 2013; Chadha & Shrivastava, 2017).

This variability underscores the need to standardize explant-specific sterilization protocols for reliable *in vitro* culture establishment in *V. odorata*. Establishment of contamination-free cultures of *V. odorata* L. is especially important because successful callus induction, organogenesis, and micropropagation rely on healthy explants with minimal microbial interference. Developing an efficient sterilization protocol for different explant types not only improves culture initiation but also enhances the reproducibility of downstream applications, including large-scale propagation, germplasm conservation, genetic transformation, and secondary metabolite production. Therefore, the present study aims to evaluate and optimize sterilization procedures for different explants of *V. odorata* to establish reliable aseptic cultures for tissue culture applications.

Materials and Methods

Collection of Explant:

Present research work has been carried out on an important medicinal plant *Viola odorata* L. which belongs to the family Violaceae. The young and healthy plants of *Viola odorata* L. were collected from Sanjeevini Vatika, Department of Horticulture, University of Agriculture Science, GKVK Bangalore - 65, Karnataka (India) with authentication. The plant material was collected from young and healthy plants. Leaves, petiole and nodal segments are used as explant.

Surface sterilization of explants:

This step included sterilization of living plant material. The commonly used sterilization agents are mercuric chloride. Proper care was taken to see that the explant should not lose its biological activity and it should help to eliminate bacterial, fungal and other contaminants, during sterilization (Razdan 1993). The type of sterilizing agent, its concentration and duration time was dependent on the nature of the explant used.

Viola odorata L. leaves, petioles and nodal segments were collected from the field and were washed in running tap water for at least 20 minutes and later washed with distilled water. They cut into petiole and leaf separately; later it was treated with 0.1 percent Bavistin, containing one or two drops of Tween 20 for 15 min. Later they were rinsed with sterile water thrice each for 5 min. After that they were treated with different concentration of mercuric chloride solution (0.05% to 0.25%) for 3 min. After that they were again washed with sterile water twice to remove the traces of $HgCl_2$.

Inoculation of explant

During culturing UV light was switched off, and fluorescent tube and airflow were switched on, hands were thoroughly washed with soap and water before starting the aseptic operations. The hands were frequently wiped with 70% alcohol, thereafter the metallic tools such as scalpel and forceps were flame sterilized prior to culturing. The surface sterilized explants were aseptically inoculated on MS medium (Murashige and Skoog, 1962) containing different concentrations and combinations of growth regulators. The process of surface sterilization and inoculation was carried out by maintaining aseptic conditions. Observations on explant survival and contamination were recorded four weeks after inoculation.

Results

Effect of $HgCl_2$ on survival of explant of *Viola odorata* L. (Table no. 1, Graph no.1)

The plant material was collected from young and healthy plants in the form of leaves, petiole and nodal segment of explant. Explants were surface sterilized with mercuric chloride ($HgCl_2$). To control the contamination, surface sterilizing agents protocol was standardized. The leaves and petioles of *Viola odorata* L. were sterilized with different concentrations of mercuric chloride (0.05%-0.25%). After 4 weeks of inoculation, observations were recorded for survival of explants and contamination.

With an increase in concentration of mercuric chloride treatment, there was a progressive reduction in contamination percentage of leaf explant. The lowest percentage of contamination (3.75%) but mortality rate was high (82.25%) recorded when leaves were treated with 0.25% mercuric chloride for three minutes. The 0.15% $Hgcl_2$ for 3 minutes was best for leaf explant with survival (88.8%) rate, contamination (9.62%) and mortality (1.57%).

Table no. 1. Effect of mercuric chloride ($HgCl_2$) on the establishment of contamination free cultures from explants of *Viola odorata* L.

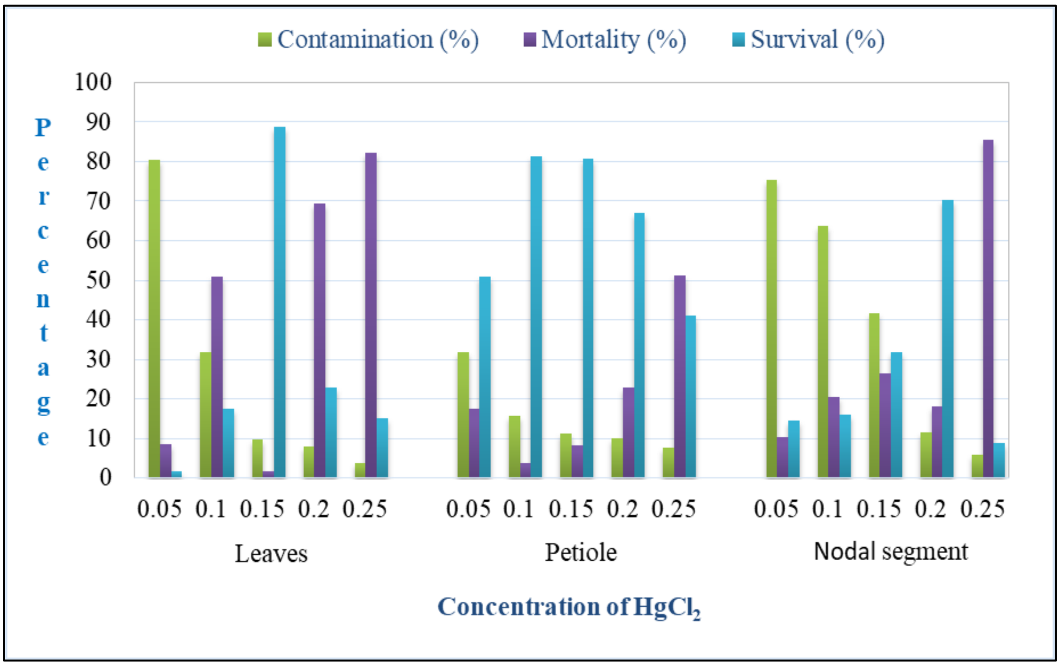
Source of explant	Concentration HgCl2 (%)	Time duration (minutes)	Contamination (%)	Mortality (%)	Survival (%)
Leaves	0.05	3	80.5	8.58	1.79

	0.10	3	31.66	50.83	17.51
	0.15	**3**	**9.62**	**1.57**	**88.8**
	0.20	3	7.87	69.37	22.75
	0.25	3	3.75	82.25	15
Petioles	0.05	3	31.66	17.51	50.83
	0.10	**3**	**15.6**	**3.63**	**81.31**
	0.15	3	11.25	8.2	80.62
	0.20	3	9.9	22.98	67.11
	0.25	3	7.68	51.2	41.11
Nodal segment	0.05	3	75.3	10.3	14.4
	0.10	3	63.6	20.5	15.9
	0.15	3	41.6	26.5	31.9
	0.20	**3**	**11.6**	**18.2**	**70.2**
	0.25	3	5.8	85.45	8.75

In case of petiole explant less contamination rate (7.68%) was recorded at 0.25% $Hgcl_2$ but survival rate (41.11%) was less and mortality rate (51.20%) was higher than survival rate. For petiole explant 0.10% $Hgcl_2$ was effective with high survival rate (81.31%) and less mortality rate contamination rate (3.63%) than contamination rate (15.6%).

When nodal segments treated with 0.25% mercuric chloride for three minutes and lowest contamination rate (5.8%), was recorded but mortality rate was high (85.45%).2.20% $Hgcl_2$ for 3 minutes was best for nodal segments with (11.6%), mortality (18.2%) and survival (70.20%)

Graph 1: Effect of mercuric chloride ($HgCl_2$) on explant survival and control of contamination.



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