

Original Research Article

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**Effect of 0.1% HgCl₂ on Surface Sterilization of
Som (*Persea bombycina* King) Explant during Tissue Culture
- A Major Host Plant of Muga Silkworm**

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In the current study we analysed the effect of 0.1% mercuric chloride (HgCl₂) on surface sterilization of the explants at different durations of time (1-5mins). It was observed that, longer the duration of treatment, lower was the bacterial contamination (8% contamination at 5 min duration of HgCl₂ treatment) but the survival percentage of the culture decreased as exposure time increased (35% at 5 min). 3 min of exposure gave the best result for both bacterial contamination (18%) and survival percentage (68%) among other time durations. Fungal contamination doesn't showed any significant difference at different time intervals.

Introduction

Som, *Persea bombycina* king is evergreen, monoecious, medium sized tree with spreading branches is a primary food plant of muga silkworm. Due to evergreen nature of the plant, muga silkworm can be reared on it throughout the year (Siddiqui, 2012). Conservation through sexual and vegetative propagation is slow, time consuming and seasonal. Considering these constrains, problems faced by the plant breeders in propagating this plant by conventional method can be overcome by using the

applications of tissue culture technology. Axillary buds are widely used for micropropagation as they have entire rudimentary vegetative shoot and can be induced to develop into new plants easily, which are true-to-type to the parental type (Sajeevan *et al.*, 2011).

Phenolic compounds are present in leaves of som, a total of 1.946% phenol present in tender leaves (Neog *et al.*, 2011). Surface sterilization is the main aspect of tissue culture to prevent it from contamination, to obtain sterile plant material is difficult

because in the process of sterilization living materials should not lose their biological activity (Razdan, 2012). Mercuric chloride ($HgCl_2$) has been effective in decontaminating pre conditioned mature *U. kirkiana* stock plants where sodium hypochloride ($NaOCl$) and calcium hypochloride ($Ca(OCl_2)_2$) have not been effective in decontaminating the stock plant (Mng'omba *et al.*, 2007). Therefore here in this experiment we are using $HgCl_2$ as sterilizing agent and optimizing its perfect time of explant exposure to it.

Materials and Methods

The axillary buds and tender shoot tips were excised from the experimental field, Department of Sericulture, Assam Agricultural University, Jorhat. The material was excised with a scissor and kept in a flask containing ascorbic acid (0.5%) from 10-12 year old plant and brought to the tissue culture lab. The explants were generally collected in the morning or in evening time. Explants were washed in running tap water by adding a drop of Tween 20 (5% v/v) followed by washing in distilled water. The explants were treated with 0.5% ascorbic acid solution for 2 hours in an orbital shaker. After 2 hours the explants were washed several times with sterile double distilled water.

Surface sterilization of explants was done using 0.1% mercuric chloride ($HgCl_2$) followed by blot drying before inoculation. Different time durations (ranging from 1min-5min) were tried during the process of sterilization and observed rate of fungal and bacterial contamination along with survival rate.

Results and Discussion

However, there is no report on *in vitro* regeneration of *Persea bombycina* which is

very essential for propagation and genetic improvement of the species (Kumar *et al.*, 1998). A very preliminary study on tissue culture of *P. bombycina* by Bhagawati (1991), Yadav, G.S. and Goswami, B.C. (1993). Therefore there is no such standard protocol for surface sterilization of explants.

To remove the phenolic exudates from explants 0.5% of ascorbic acid (an antioxidant) was used during collection of explants and further treatment. Shirin and Sarkar (2003), studied various antioxidants and adsorbents on removal of phenolic exudates from plants of *Tectona grandis*. They treated the explants with 0.1% (w/v) solution of various inorganic compounds and adsorbents *viz.* ascorbic acid, citric acid, glutamine, polyvinylpyrrolidone, boric acid and activated charcoal for 18hours prior to their surface sterilization with 0.1% (w/v) mercuric chloride solution. Among these boric acid and ascorbic acid was proved to be the most effectiveresulting in 50-60% establishment of nodal segments on culture media.

Mercuric chloride ($HgCl_2$) is stronger than sodium hypochloride ($NaOCl$), which is the likely reason for its effectiveness in combating fungi, bacteria and endogenous species (Mng'omba *et al.*, 2012). The mortality of the cultures may be higher due to damage caused by stronger disinfectants, as was the case with *Calophyllum apetalum* (Nair and Seenii, 2003).

Here in this experiment, Surface sterilization of explants was done using 0.1% mercuric chloride. Different time durations (ranging from 1-5 min) were tried during the process of sterilization. In vitro established explants showed contamination with fungi after 7 days of culture, whereas the bacterial contamination observed after 14 days of culture.

Table.1 Effect of $HgCl_2$ treatment at different time durations on cultures (Bacterial contamination, fungal contamination and survival rate)

Time duration (min)	Bacterial contamination (%)	Fungal contamination (%)	Survival percentage (%)
1 min	30	31	47
2 min	26	32	52
3 min	18	28	68
4 min	10	30	56
5 min	8	31	35



Fig.1 A. Surface sterilization of explants with 0.1% $HgCl_2$, **B.** Blot drying of explants before inoculation

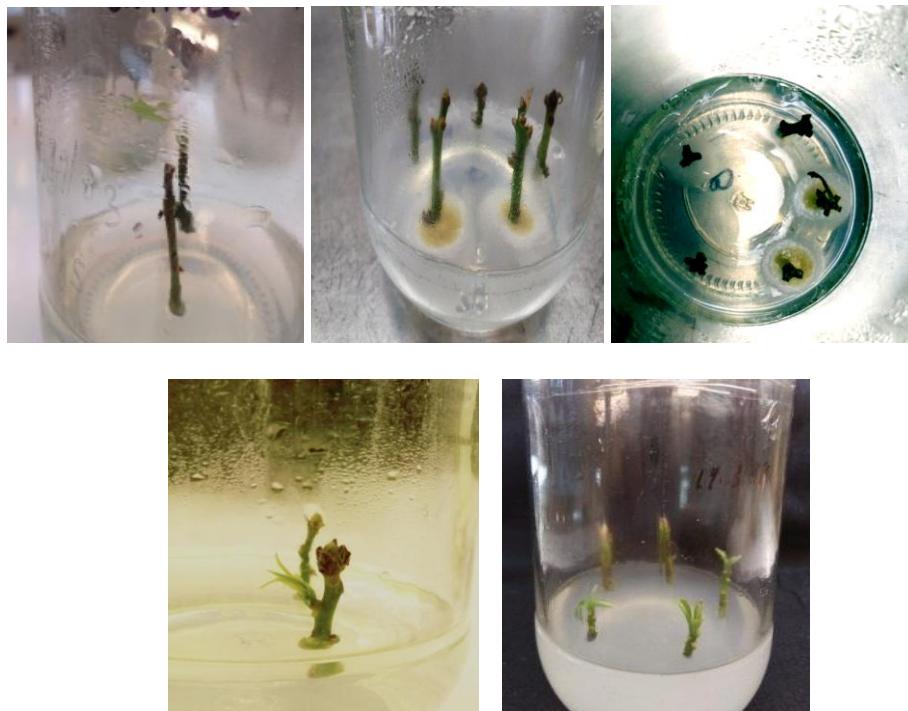


Fig.2 A. Browning and dying of explants due to longer exposure. **B&C.** Fungal contamination observed after 2 weeks of culture. **D&E.** Well established explants

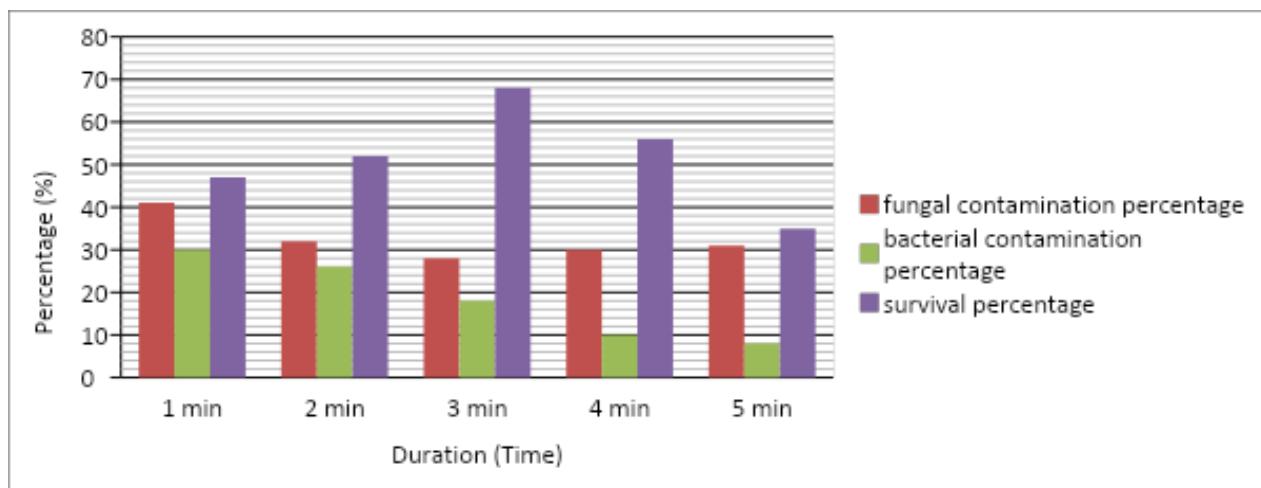


Fig.3 Percent analysis of survival rate, bacterial and fungal contamination when 0.1% HgCl₂ used for 2min, 3min and 4 min duration during surface sterilization of explants

Percentage of fungal contamination was observed more compared to bacterial contamination. The longer the exposure to HgCl₂, the lower the rate of bacterial contamination (8%; 5min). With increase in exposure to HgCl₂ led the explants turned brown and eventually died.

From the data furnished in table we observed that 3 min exposure of explant with 0.1% mercuric chloride showed less contamination by both fungal and bacterial agent with maximum percentage of survival (68%) compared to other time intervals. As the exposure time of 0.1% HgCl₂ increased the survival rate decreased (35%; at 5 min).

There is not much significance difference observed in the fungal contamination at different time intervals. Silveria *et al.*, (2016) reported that according to tukey's test, using two concentrations of mercuric chloride (HgCl₂) resulted in no significant differences between the means, indicating that this factor has no influence on infection rates or necrosis, but influenced survival of explants.

When considering length of exposure to the disinfectant agent, the differences between the results of bacterial contamination were

significant, indicating that the longer the exposure to HgCl₂, the lower the rate of bacterial contamination (Silveria *et al.*, 2016).

There is scanty of literature available in micropropagation of som plant and therefore there is no standard method of sterilization of som plant. The ultimate aim of the investigation was to optimize the effect of 0.1% HgCl₂ surface sterilization on *in-vitro* propagation of *Persea bombycina* king to overcome the problem of fungal and bacterial contamination.

In the present study we have succeed in controlling bacterial contamination whereas for fungal, use of fungicide in the culture media will be better for controlling the contamination.

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