

Effect of Physical, Chemical and Physiochemical Treatments of Surface Sterilisation on Medicinal Plants *Salvadora persica* and *Solanum surattense* for *In-vitro* Propagation

Beena Naqvi^{a*}, Saleema Mehboob Ali^b, Kiran Makhani^b and Kamran Yousuf^b

^aPCSIR Laboratories Complex, Shahrah-e-Dr.Salimuzzaman Siddiqui, Karachi-75280, Pakistan

^bDepartment of Biotechnology, University of Karachi, Karachi-75270, Pakistan

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Abstract. Effect of various surface sterilisation treatments on medicinal plants *Salvadora persica* and *Solanum surattense* has been studied for *in-vitro* propagation. Surface sterilisation treatment was applied by physical, chemical and physiochemical means. Chemical sterilisation was done with sodium hypochlorite, physically with sonication and physiochemically with combination of both. Sodium hypochlorite alone was found to be effective for smooth textured plant i.e., *Salvadora persica*, whereas for rough textured plant *Solanum surattense* a combination of both physiochemical means yielded good results. However, sonication alone did not render the effective way of surface sterilisation. Moreover, for *Salvadora persica*, 10% sodium hypochlorite was effective in eliminating bacterial or fungal growth whereas in *Solanum surattense*, a concentration of 15% sodium hypochlorite proved to be effective. In general, best results were achieved in the combined treatment by physiochemical means.

Keywords: physiochemical treatment, surface sterilisation, *in-vitro* propagation

Introduction

The first and most important step to start *in-vitro* propagation is surface sterilisation either by physical, chemical or physiochemical means. Different protocols of surface sterilisation may vary with the type of chosen plant for culture and selected explants to use. Washing with sodium hypochlorite (NaOCl) is one of the most common methods used for surface sterilisation. Several studies have reported that NaOCl is better for controlling the infection without any adverse effect on plant growth (Badoni and Chauhan, 2010), although negative effects of NaOCl have also been reported by Hong and Gross (1998). This depicts the role of sodium hypochlorite in the destruction of not only microbes but also plants cells. In addition to NaOCl, sonication is also being used as a decontamination method. This procedure succeeds in detaching the adhering bacteria without apparently damaging the meristematic RNA (Aller *et al.*, 1978). In various studies considerable decrease in bacterial contamination was observed by using ultrasonic sonicator.

Medicinal plants are sources of important therapeutic aid for alleviating human ailments. With increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and

antibiotics, interest in the use of plants and plant-based drugs has revived throughout the world (Nalawade *et al.*, 2003). Numerous medicinal plants grow naturally in Karachi vicinity and *Salvadora persica* and *Solanum surattense* are two of them. *Salvadora persica*, a species of Salvadoraceae family is an evergreen small shrub or tree with a height of 3 m. It is widely distributed in the drier parts of Balochistan and Sindh (Khatak *et al.*, 2010; Korejo *et al.*, 2010). *S. persica* has wide range of medical benefits. It can be used to produce promising products like antiplaque, analgesics, antifungal, anti-convulsant, antifertility, de-obstruent, diuretic drugs. Its leaves are used in cough, asthma, scurvy, rheumatism, piles while its bark is highly recommended by world health organization (WHO) as chewing stick for cleaning teeth (Khatak *et al.*, 2010). *In-vitro* study on *Salvadora persica* for salinity-induced modulation of growth and antioxidant activity has been conducted by Sharma *et al.* (2013).

Solanum surattense, belonging to the family Solanaceae is a prostrate herb (Yousaf *et al.*, 2010). It grows in Pakistan up to 1300 m altitude including the regions from Himalayan plateau to Kirthar range (Yousaf *et al.*, 2010; Ahmad *et al.*, 2010; Perveen *et al.*, 2008). *Solanum surattense* is also a highly important medicinal plant. Its antifungal activity against *A. fumigates* and antibacterial activity against *Salmonella typhi*, *Escherichia*

*Author for correspondence; E-mail: nbeena25@hotmail.com

coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus flavus*, *Aspergillus niger* and *Candida albican* has already been reported by Dabur *et al.* (2007). The plant extract is reported to has significant anti hyperglycaemic action and is also widely used in treatment of gonorrhoea, rheumatism, fever, bronchitis and asthma (Muruhan *et al.*, 2013). *In-vitro* micropropagation of *Solanum surattense* has been done by Rama Swamy *et al.* (2004) for testing best explant for plantlet regeneration.

Owing to the medicinal importance of the test plant species, their active compounds need to be isolated in bulk quantities for commercial use. Harvesting from the wild (main source of raw material) is not only causing loss of genetic diversity and habitat destruction but it is also difficult to collect enough quantity throughout the year. Unfortunately, cultivation fields for commercial scale are not available in Pakistan for these plants. Moreover, plants propagated by vegetative means contain systemic bacteria, fungi and viruses (Sharma *et al.*, 2010). Hence, there is a need of *in-vitro* mass propagation of *Salvadora persica* and *Solanum surattense* to produce good quality disease or infection free plants. To start *in-vitro* culture of *Solanum surattense* and *Salvadora persica*, there is a need to achieve successful surface sterilisation. *Salvadora persica* has smooth surface texture and fleshy leaves (Khatak *et al.*, 2010), whereas *Solanum surattense* has hairy and thorny stem with rough textured leaves (Yousaf *et al.*, 2010). Taking the texture of the two plants into consideration, the research on surface-sterilisation has been conducted in the present study.

Materials and Methods

Effect of various surface sterilisation treatments for *in-vitro* propagation have been studied on medicinal plants *Salvadora persica* and *Solanum surattense*. Physical treatment was done with sonication, chemical treatment with sodium hypochlorite and physiochemical

treatments of surface sterilisation was a combination of both. Shoot tip and nodal explants of *Salvadora persica* and *Solanum surattense* were used as an explants, collected from the experimental field of Plant Tissue Culture Laboratory, PCSIR Labs Complex Karachi. Explants were first placed under running tap water for few minutes followed by rinsing with distilled water for 5-10 min. Few explants were inoculated in MS basal media (Murashige and Skoog, 1962) just after washing with distilled water as a control. Remaining explants were treated chemically with 10 and 15% sodium hypochlorite solutions for 15 min on orbital shaker followed by washing with distilled water for 10 min under laminar flow hood. The physical treatment was sonication and explants were subjected to sonication for 20 min followed by washing with distilled water for 10 min under laminar flow hood. In physiochemical treatment explants were treated with 10 and 15% sodium hypochlorite under sonication for 20 min followed by washing with distilled water for 10 min under laminar flow hood. Explants were then finally trimmed into 5 mm small pieces and inoculated on MS basal media and incubated under controlled condition at 25 ± 2 °C and 16/8 h photo period. Observations were taken on every alternate day for 15 days.

Results and Discussion

Commonly surface sterilisation may be done by physical, chemical and physiochemical means to disinfect explants for tissue culture. Simple washing with tap water and distilled water is not enough to control contamination in culture. When explants of both *Solanum surattense* and *Salvadora persica* were simply washed with running tap water and distilled water even for longer time duration contamination appeared under *in-vitro* conditions (Table 1).

It was observed that effect of chemical treatment with 10 and 15% NaOCl solutions for 15 min varied with the plant texture. It was reported that sodium hypochlorite

Table 1. Mean number of explants survived after physical (sonication), chemical (sodium hypochlorite) and physiochemical treatments (both) for surface sterilisation on *Solanum surattense* and *Salvadora persica* for *in-vitro* studies

Plant	Chemical treatment		Physical treatment 20 min	Physiochemical treatment	
	10%	15%		10% +20 min	15% +20 min
<i>Solanum surattense</i>	0.20 ± 0.13	1.00 ± 0.37	0.70 ± 0.21	1.20 ± 0.20	2.40 ± 0.27
<i>Salvadora persica</i>	2.60 ± 0.16	2.80 ± 0.13	1.30 ± 0.15	2.70 ± 0.15	2.80 ± 0.13

reacted with microbes and stimulated their role which ultimately cause bacteria to form clumps that would eventually die off (Jakob *et al.*, 2008). For rough texture plant i.e., *Solanum surattense* washing with 15% NaOCl was more effective (1.00 ± 0.37). Yousaf *et al.* (2010) also found that rough and spiky textured plant requires 15% of bleaching to kill the bacteria residing amidst its spikes. In the case of smooth texture plant, *Salvadora persica* washing with 10% NaOCl appeared successful in controlling the contamination. There was insignificant difference in the results of *Salvadora persica* by increasing the concentration of sodium hypochlorite (Table 1). These results are in agreement with Ahmed *et al.* (2008) in which NaOCl was used for surface sterilisation of *Salvadora persica* explants. One reason of contamination in culture even after treating with bleach may be the presence of endogenous microbes. In several studies, it is reported that despite of surface sterilisation treatment of explants in either ethanol or NaOCl, the problem of frequent endogenous bacterial and fungal contamination persists (Valizadah and Valizadah, 2011).

Effect of physical treatment with sonication for 20 min was not very effective in controlling the contamination on both types of plants. It was observed that there was only fungal contamination in the culture and there was no bacterial growth appearance till the end of the experiment. It might be due to the sound waves of frequency $>20,000$ cycle/sec that kills bacteria and some viruses on exposing for some time. However, their growth was adversely affected due to fungal contamination. Our results are in agreement with the results of experiment performed by Aller *et al.* (1978) in which sonication has been shown effective in controlling bacterial contamination in particular. These results suggest that sonication is effective against bacteria but it was not able to prevent fungal contamination. In *Solanum surattense* only few plants survived (0.70 ± 0.21) whereas on *Salvadora persica* it showed comparatively better results (1.30 ± 0.15). Sonication is more effective because the ultrasonic waves are able to inactivate bacteria and deagglomerate bacterial clusters or flocs through a number of physical, mechanical and chemical effects arising from acoustic cavitation (Joyce *et al.*, 2003).

Effect of physiochemical treatment with 10 and 15% sodium hypochlorite under sonication for 20 min found the most suitable mean of controlling contamination on both rough and smooth texture plants. In both *Solanum*

surattense and *Salvadora persica* explants treated with 15% sodium hypochlorite under sonication for 20 min highest number of plant survival recorded i.e., *Solanum surattense* (2.40 ± 0.27) and *Salvadora persica* (2.80 ± 0.13). These results are also supported by Yousaf *et al.* (2010) and Khatak *et al.* (2010) that the combination of surface sterilization methods are more effective for both rough and smooth textured plants.

For obtaining contamination free cultures the most important step is the sterilisation of explants. In the present study the sterilisation procedure was standardised for *Solanum surattense* and *Salvadora persica*. Amongst the three treatments applied physiochemical treatment with sodium hypochlorite under sonication was found the most suitable for controlling contamination on both rough and smooth texture plants.

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