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EFFECT OF SURFACE STERILANT TYPE AND EXPOSURE DURATION ON *IN VITRO* ESTABLISHMENT AND SHOOT REGENERATION OF NODAL EXPLANTS IN SPINE GOURD (*Momordica dioica* ROXB.)

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ABSTRACT

The present study was conducted to evaluate the effect of different surface sterilants and exposure durations on the *in vitro* establishment and shoot regeneration of nodal explants of spine gourd (*Momordica dioica* Roxb.) during August 2025 to July 2026. The experiment was laid out in a 2 × 3 factorial completely randomized design (FCRD) with two factors: surface sterilants and exposure duration. The surface sterilants included 0.1% mercuric chloride ($HgCl_2$) and 1% sodium hypochlorite (NaOCl), while the exposure durations were 4, 8 and 12 minutes. The treatment effects were evaluated based on percentage of contamination-free explants, percentage of contaminated explants, days to shoot initiation and percentage of shoot proliferation, with observations recorded up to 30 days after inoculation. Significant differences were observed among the surface sterilants, exposure durations and their interaction.

Among the surface sterilants, 0.1% $HgCl_2$ recorded the highest mean percentage of contamination-free explants (75.00%) and shoot proliferation (79.00%). Among exposure durations, 12 minutes resulted in the highest mean percentage of contamination-free explants (63.30%) but also delayed shoot initiation and reduced shoot proliferation. At the interaction level, 0.1% $HgCl_2$ for 12 minutes recorded the maximum contamination-free explants (86.00%) and minimum contamination (14.00%). However, 0.1% $HgCl_2$ for 4 and 8 minutes recorded the highest shoot proliferation (93.00% each), with shoot initiation occurring at 5.45 days. Thus, 0.1% HgCl, for 8 minutes was considered the most suitable treatment under the conditions of the present study because it provided 80.00% contamination-free explants, while maintaining 93.00% shoot proliferation and early shoot initiation. Although, 12 minutes improved decontamination further, it reduced shoot proliferation to 52.00% and delayed shoot initiation to 12.90 days.

Key words : Spine gourd, *Momordica dioica* Roxb., Surface sterilization, Mercuric chloride, Sodium hypochlorite, Exposure duration, Contamination, Shoot initiation, Shoot regeneration.

Introduction

Spine gourd (*Momordica dioica* Roxb.) is a valuable cucurbitaceous vegetable known for its nutritional, medicinal and economic importance. Its tender fruits contain carbohydrates, proteins, fibre, fats, vitamins and minerals, while different plant parts have been traditionally used for various medicinal purposes (Kushwaha *et al*., 2005; Shreedhar *et al*., 2001; Nabi *et al*., 2002; Deokule *et al*., 2006).

Despite its potential, spine gourd remains underexploited because of the limited availability of quality planting material. It is a perennial, dioecious and cross-pollinated climber and approximately 5–10% male plants are required for effective pollination. Conventional propagation through tuberous roots is slow, labour intensive and costly, while seed propagation is affected by poor germination, prolonged dormancy, unpredictable sex expression and genetic variability.

Micropropagation provides an efficient alternative for rapid production of genetically uniform, true-to-type and disease-free planting material throughout the year. However, successful *in vitro* culture establishment depends on several factors, particularly the type and duration of surface sterilization. An effective sterilization treatment must eliminate microbial contaminants without adversely affecting explant viability and regenerative capacity.

Previous studies have demonstrated the feasibility of *in vitro* propagation of spine gourd and related cucurbit species. However, the optimum balance between microbial decontamination and maintenance of explant regeneration capacity can vary with explant type, donor-plant condition, sterilant and exposure duration. Comparative evaluation of 0.1% $HgCl_2$ and 1% NaOCl at different exposure durations under the present culture conditions therefore provides a useful basis for optimizing nodal culture establishment.

Therefore, the present study was undertaken to evaluate the effects of different surface sterilants and exposure durations on contamination control, shoot initiation and shoot proliferation of spine gourd nodal explants and to identify the treatment providing the most favourable balance between aseptic culture establishment and regeneration.

Materials and Methods

Source of plant material (Explant)

Nodal segments of *Momordica dioica* Roxb. (spine gourd) were selected as explants for the present study. Nodes were collected from healthy, one-month-old mother plants maintained in the polyhouse of the Agri Biotech Foundation, PJTAU Campus, Rajendranagar, Hyderabad.

Selection of Planting material and preparation of Explants for Culturing

To establish *in vitro* cultures, healthy nodes were collected from mother plants that had been sprayed with 0.1% Bavistin solution two days before collection. The spraying was performed either in the morning or evening. The leaves were carefully detached one at a time without causing damage to the nodal buds. The nodal segments were subsequently excised with a sharp blade and transferred to a beaker containing a small quantity of water.

Collection and sterilization of explants

Nodal explants were collected and transported to the laboratory in a beaker. The explants were initially rinsed thoroughly under running tap water to eliminate adhering soil particles, dust and other surface impurities. They were then immersed in an aqueous solution containing 0.1% Tween-20 for 10 minutes. Following this treatment, the explants were washed three to four times with distilled water until all traces of detergent foam had been removed.

Subsequently, the explants were treated with a solution containing 0.2% Bavistin and 0.02% streptomycin for 8 minutes. All further sterilization procedures were conducted under aseptic conditions in a laminar airflow cabinet. The fungicide-bactericide solution was discarded and the explants were rinsed three to four times with sterile distilled water.

The washed explants were transferred to a sterile beaker and surface-disinfected with 70% ethanol for 1 minute. This was followed by treatment with either 0.1% mercuric chloride ($HgCl_2$) or 1% sodium hypochlorite (NaOCl) for 4, 8 or 12 minutes according to the treatment combination. After sterilization, the sterilant was removed and the explants were washed thoroughly three to four times with sterile distilled water to eliminate residual sterilant. Finally, the explants were taken out of the beaker and blotted dry on sterile filter paper before inoculation.

Inoculation of explants

Once the explants were blotted dry on sterile filter paper, the portions that had turned brown during sterilization were carefully removed. The sterilized explants were then inoculated onto the initiation medium under aseptic conditions inside a laminar airflow chamber. The explants were cultured on full-strength MS medium containing MS-A (25.0 mL L^{-1}), MS-B (12.5 mL L^{-1}), MS-C (10.0 mL L^{-1}), MS-D (10.0 mL L^{-1}), MS-E (1.0 mL L^{-1}), sucrose (30 g L^{-1}), myo-inositol (100 mg L^{-1}), agar-agar (7.5 g L^{-1}) and 6-Benzylaminopurine (BAP) at a constant concentration of (2 mg L^{-1}). The same concentration of BAP was used for all the treatments. The medium pH was adjusted to 5.8 ± 0.2 using 0.1N NaOH or 0.1N HCl, dispensed at 50 mL per culture bottle and autoclaved at 121° C and 15 psi for 15 min. The culture bottles containing the explants were maintained in a growth room at 27 ± 2°C under a light intensity of 1,800-2,000 lux.

Establishment of cultures

Culture initiation was carried out by placing the sterilized nodal explants on MS medium. The cultures were incubated under a 12-hour light/12-hour dark photoperiod for 15 days. Following the initial incubation period, explants that produced shoot buds were transferred to fresh medium having the same composition and kept under the same photoperiod for a further 15 days.

Treatment details

The experiment consisted of two factors in a 2 × 3 factorial completely randomized design.

Factor 1: Surface sterilants

S_1 – 0.1% $HgCl_2$

S_2 – 1% NaOCl

Factor 2: Exposure duration

T_1 – 4 minutes

T_2 – 8 minutes

T_3 – 12 minutes

The experiment comprised four replications, with three explants per replication, resulting in 12 explants per treatment and a total of 72 explants.

Observations recorded

Percentage of contamination-free explants was recorded as the proportion of inoculated explants showing no visible fungal or bacterial contamination during the observation period. Percentage of contaminated explants was recorded as the proportion of inoculated explants showing visible fungal and/or bacterial contamination.

Where possible, contamination-free explants should be further classified according to their physiological response, including viable explants, necrotic explants and shoot-regenerating explants. Contamination-free status alone should not be considered equivalent to successful culture establishment.

Days to shoot initiation was recorded as the number of days from inoculation until the first visible emergence of a shoot bud. Percentage of shoot proliferation was calculated as the proportion of inoculated explants that produced shoots within the observation period.

Percentage of contamination-free explants = (Number of contamination-free explants / Total number of explants inoculated) × 100

Percentage of contaminated explants = (Number of contaminated explants / Total number of explants inoculated) × 100

Percentage of shoot proliferation = (Number of explants producing shoots / Total number of explants inoculated) × 100

Statistical analysis

All recorded observations were analysed statistically using procedures appropriate for a factorial completely randomized design. Analysis of variance (ANOVA) was performed to assess the main effects of surface sterilants, exposure duration and their interaction (surface sterilant x exposure duration). Percentage data were subjected to appropriate transformation before analysis, where required. The significance of treatment effects was tested using the F-test at the 5% level of significance ($p \leq 0.05$). Whenever the treatment effects were found to be significant, treatment means were compared using the Critical Difference (CD) values were calculated wherever applicable. P-values were obtained from the ANOVA and reported for the main surface sterilants, exposure duration and their interaction.

Results and Discussion

Percentage of explants without contamination

Data pertaining to the effect of different surface sterilants and exposure durations on the percentage of contamination-free explants are presented in Table 1 and graphically illustrated in Fig. 1 and Plate 1. Significant differences were observed among surface sterilants, exposure durations and their interaction.

The percentage of contamination-free explants varied with sterilant and exposure duration. The highest percentage of contamination-free explants (86.00%) was obtained with 0.1% $HgCl_2$ for 12 minutes, whereas the lowest percentage (22.90%) was recorded with 1% NaOCl for 4 minutes. Among the surface sterilants, 0.1% HgCl, recorded a higher mean percentage of contamination-free explants (75.00%) than 1% NaOCl (30.80%). Across exposure durations, 12 minutes recorded the highest mean percentage of contamination-free explants (63.30%).

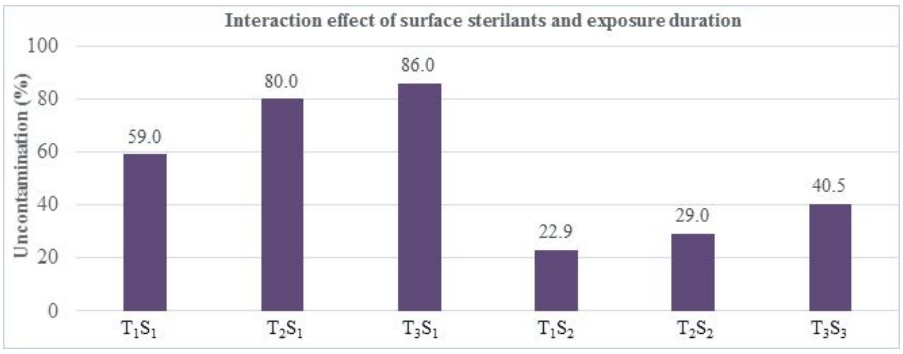
The greater decontamination response at the longer exposure duration may be associated with greater contact between the sterilant and microorganisms on the explant surface; however, this interpretation should be considered cautiously because tissue injury was not directly quantified.

These findings are broadly consistent with previous reports of effective $HgCl_2$ -mediated decontamination in spine gourd and related cucurbit species (Deokar *et al.*,

Table 1 : Effect of different surface sterilants and exposure durations on percentage of contamination-free nodal explants of spine gourd.

Surface sterilant	4 min	8 min	12 min	Mean
S_1 : 0.1% $HgCl_2$	59.00	80.00	86.00	75.00
S_2 : 1% NaOCl	22.90	29.00	40.50	30.80
Mean	40.95	54.50	63.30	

SE(m): Factor A = 0.05; Factor B = 0.06; A × B = 0.09. CD: Factor A = 0.17; Factor B = 0.21; A × B = 0.29. CV = 2.74 (Factor A). Four replications were performed. Transformed values were used for statistical analysis where applicable.



Surface sterilants: S_1- 0.1% $HgCl_2$; S_2 - 1% NaOCl Exposure duration- T_1 - 4 minutes; T_2 - 8 minutes; T_3- 12 minutes

Fig. 1 : Effect of different surface sterilants and exposure duration on percent of explants without contamination from culture establishment of spine gourd explants (%).



Plate 1 : Explants without contamination.

2003; Nabi *et al*., 2002; Huq *et al*., 2012; Saurabh *et al*., 2017; Devi *et al*., 2017; Patel and Ishnava, 2015).

Percentage of explants with contamination

Data corresponding to the effect of different surface sterilants and exposure durations on the percentage of contaminated explants are presented in Table 2 and graphically illustrated in Fig. 2 and Plate 2. The lowest contamination (14.00%) was recorded with 0.1% $HgCl_2$ for 12 minutes, whereas the highest contamination (77.10%) was observed with 1% NaOCl for 4 minutes.

The response was the inverse of the contamination-free response. The lower contamination recorded with $HgCl_2$, particularly at 12 minutes, indicates greater decontamination efficiency under the conditions tested. However, maximum decontamination did not correspond to maximum shoot regeneration, as shown by the subsequent regeneration response.

The findings are in agreement with previous reports of effective $HgCl_2$ treatment for reducing microbial contamination during *in vitro* establishment in spine gourd and related species. Literature from other cucurbits presented as supporting rather than direct evidence for *Momordica dioica*.

Days to shoot initiation

Data associated with the effect of different surface sterilants and exposure durations on the number of days required for shoot initiation are presented in Table 3 and graphically illustrated in Fig. 3. The interaction between sterilant type and exposure duration significantly influenced the time required for shoot initiation.

The earliest shoot initiation was recorded with 0.1% $HgCl_2$ for 4 and 8 minutes, with both treatments requiring 5.45 days. In contrast, 1% NaOCl for 12 minutes recorded the longest duration of 13.45 days. The mean response across exposure durations was 7.93 days for $HgCl_2$ and 9.67 days for NaOCl.

The earlier response observed with 0.1% $HgCl_2$ at 4 and 8 minutes may reflect adequate decontamination, while retaining sufficient explant viability for rapid regeneration. Prolonged exposure, particularly for 12 minutes was associated with delayed shoot initiation. This may indicate adverse effects of extended sterilant exposure on explant physiological condition; however, direct tissue-injury measurements were not included in the present study.

The present findings are broadly comparable with reports of early shoot emergence following $HgCl_2$ treatment in spine gourd and related cucurbit species (Jamatia *et al*., 2016; Patel and Ishnava, 2015; Thasni *et al*., 2019). Species and experimental-condition differences should be considered when comparing absolute values.

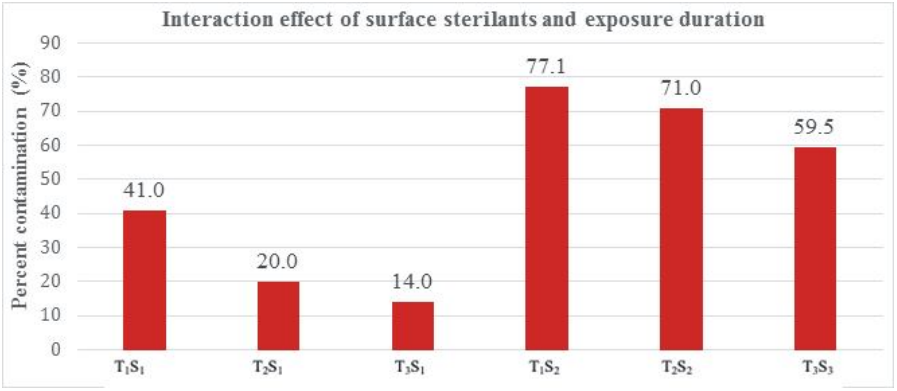
Percentage of shoot proliferation

Data associated with the effect of different surface sterilants and exposure durations on percentage of shoot proliferation are presented in Table 4 and graphically illustrated in Fig. 4. The interaction between surface

Table 2 : Effect of different surface sterilants and exposure durations on percentage of contaminated nodal explants of spine gourd.

Surface sterilant	4 min	8 min	12 min	Mean
S_1 : 0.1% $HgCl_2$	41.00	20.00	14.00	25.00
S_2 : 1% NaOCl	77.10	71.00	59.50	69.20
Mean	59.05	45.50	36.75	

SE(m): Factor A = 0.06; Factor B = 0.08; A × B = 0.11. CD: Factor A = 0.20; Factor B = 0.24; A × B = 0.34. CV = 3.48 (Factor A). Four replications were performed.



Surface sterilants: S_1- 0.1% $HgCl_2$; S_2 - 1% NaOCl Exposure duration - T_1 - 4 minutes; T_2 - 8 minutes; T_3- 12 minutes

Fig. 2 : Effect of different surface sterilants and exposure duration on percent of explants with contamination includes explants with response and without response percent from culture establishment of spine gourd explants (%).

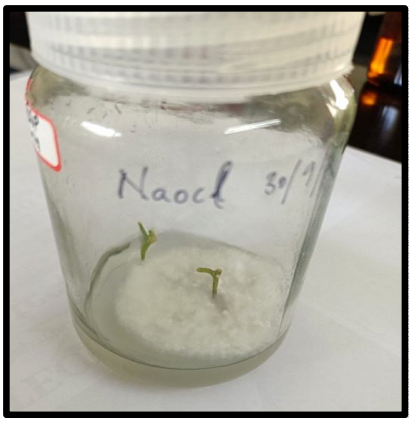
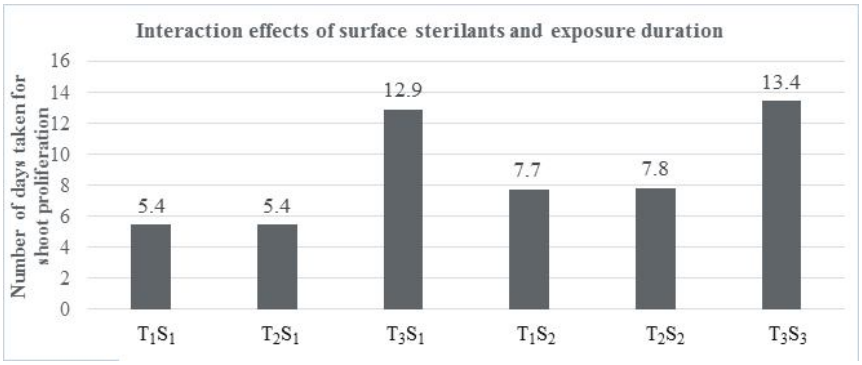


Plate 2 : Explants with contamination.



Surface sterilants: S_1- 0.1% $HgCl_2$; S_2 - 1% NaOCl Exposure duration - T_1 - 4 minutes; T_2 - 8 minutes; T_3- 12 minutes

Fig. 3 : Effect of different surface sterilants and exposure duration on number of days taken for shoot proliferation from culture establishment of spine gourd explants.

sterilant and exposure duration significantly influenced shoot proliferation.

The highest shoot proliferation (93.00%) was recorded with 0.1% $HgCl_2$ for 4 and 8 minutes, and these treatments were statistically on par with each other. The lowest response (39.00%) was recorded with 1% NaOCl for 12 minutes. With 0.1% $HgCl_2$, increasing exposure from 8 to 12 minutes reduced shoot proliferation from 93.00% to 52.00%, despite increasing contamination-free explants from 80.00% to 86.00%.

These findings demonstrate an important trade-off between decontamination efficiency and regeneration capacity. Although, 12 minutes of $HgCl_2$ exposure provided the highest contamination-free response, the associated reduction in shoot proliferation and delay in shoot initiation indicate that maximum decontamination is not necessarily the optimum treatment for successful culture establishment. The 8-minute exposure provided a better balance between microbial control and regeneration.

Table 3 : Effect of different surface sterilants and exposure durations on days to shoot initiation from nodal explants of spine gourd.

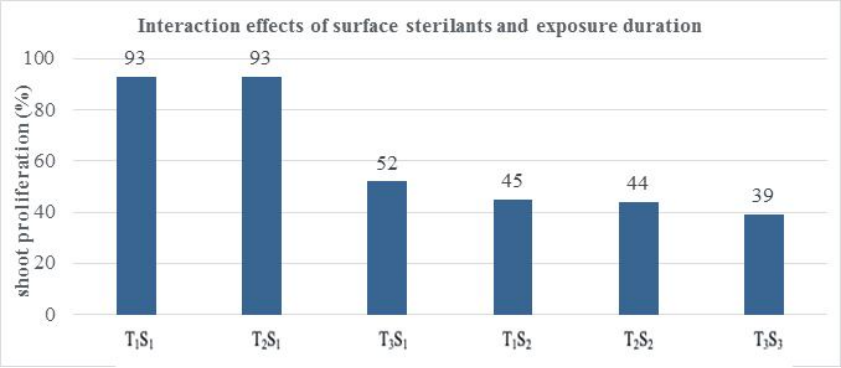
Surface sterilant	4 min	8 min	12 min	Mean
S_1 : 0.1% $HgCl_2$	5.45	5.45	12.90	7.93
S_2 : 1% NaOCl	7.72	7.85	13.45	9.67
Mean	6.58	6.65	13.17	

SE(m): Factor A = 0.14; Factor B = 0.17; A × B = 0.24. CD: Factor A = 0.43; Factor B = 0.52; A × B = 0.74. CV = 5.60 (Factor A). Four replications were performed.

The present findings are consistent with earlier reports of high regeneration responses in spine gourd and related cucurbits under optimized *in vitro* conditions (Rai *et al.*, 2012; Jamatia *et al.*, 2016; Sundari *et al.*, 2011; Thasni *et al.*, 2019). However, the present study specifically demonstrates that extending $HgCl_2$ exposure beyond the optimum duration can reduce regeneration even when contamination control improves.

Overall interpretation and practical significance

Considering all response variables together, 0.1% $HgCl_2$ for 12 minutes was the most effective treatment for reducing visible contamination, with 86.00% contamination-free explants and only 14.00% contamination. However, this treatment required 12.90 days for shoot initiation and resulted in only 52.00% shoot proliferation. In contrast, 0.1% $HgCl_2$ for 8 minutes



Surface sterilants: S_1- 0.1% $HgCl_2$; S_2 - 1% NaOCl Exposure duration - T_1 - 4 minutes; T_2 - 8 minutes; T_3- 12 minutes

Fig. 4 : Effect of different surface sterilants and exposure duration on percent of shoot proliferation from culture establishment of spine gourd explants (%).

Table 4 : Effect of different surface sterilants and exposure durations on percentage of shoot proliferation from nodal explants of spine gourd.

Surface sterilant	4 min	8 min	12 min	Mean
S_1 : 0.1% $HgCl_2$	93.00	93.00	52.00	79.00
S_2 : 1% NaOCl	45.00	44.00	39.00	43.00
Mean	69.00	68.50	45.50	

SE(m): Factor A = 0.05; Factor B = 0.06; A × B = 0.09. CD: Factor A = 0.15; Factor B = 0.19; A × B = 0.26. CV = 1.35 (Factor A). Four replications were performed.

provided 80.00% contamination-free explants, only 20.00% contamination, 5.45 days to shoot initiation and 93.00% shoot proliferation.

Therefore, 0.1% $HgCl_2$ for 8 minutes was considered the optimum treatment under the conditions of the present study because it provided a more favourable balance between aseptic culture establishment and regeneration. This conclusion is based on the combined response of the measured variables rather than on contamination control alone.

Although, $HgCl_2$ showed superior performance in the present experiment, its toxicity and hazardous waste-generation potential are important limitations for routine laboratory and commercial use. Future studies should therefore evaluate effective non-mercurial surface sterilants and combinations that can provide comparable contamination control without mercury-associated environmental and safety concerns.

Conclusion

The present study demonstrated that surface sterilant type and exposure duration significantly influenced contamination control, shoot initiation and shoot proliferation of spine gourd nodal explants. The highest contamination-free response (86.00%) was obtained with 0.1% $HgCl_2$ for 12 minutes; however, prolonged exposure reduced shoot proliferation to 52.00% and delayed shoot initiation to 12.90 days. In comparison, 0.1% $HgCl_2$ for 8 minutes resulted in 80.00% contamination-free explants, 93.00% shoot proliferation and shoot initiation within 5.45 days. Thus, 0.1% $HgCl_2$ for 8 minutes was identified as the most suitable treatment under the present experimental conditions because it provided the best balance between contamination control and regeneration.

The recommendation should be interpreted within the limitations of the present experiment. In view of the toxicity and hazardous waste associated with $HgCl_2$, further work should focus on identifying safer, non-mercurial sterilization treatments for commercial micropropagation of spine gourd.

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