*Plant Archives* Vol. 26, No. 2, 2026 pp. 3084-3088 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.2.426

# EFFECT OF DIFFERENT SURFACE STERILENTS AND STERILIZATION DURATION ON CULTURE ESTABLISHMENT OF RASPBERRY EXPLANTS (*Rubus niveus*) NODAL EXPLANTS

**Navaneetha Chakala[1]*, Veena Joshi[2], Rajasekhar Marri[3], M. Gopala Krishna[4] and G. Sathish[5]**

[1]Department of Fruit Science, PGIHS, SKLTGHU, Mulugu, Siddipet-502279, Telangana, India

[2]Department of Horticulture, PGIHS, SKLTGHU, Mulugu, Siddipet-502279, Telangana, India

[3]College of Horticulture, Mojerla, Sri Konda Laxman Telangana SKLTGHU, Telangana, India.

[4]College of Horticulture, Malyal, Sri Konda Laxman Telangana Horticultural University, Telangana, India.

*Corresponding author E-mail: navannethanavvi@gmail.com

(Date of Receiving-15-06-2026; Date of Revision-15-08-2026; Date of Acceptance-29-08-2026)

**ABSTRACT**

The study entitled the effect of different surface sterilents and surface sterilization duration on culture establishment of raspberry (*Rubus niveus*), nodal explants was conducted in Agri Biotech Foundation, PJTAU Campus Hydrabad, Telangana between September 2025 to March 2026.The experiment conducted was Factorial Completely Randomized Design (FCRD)with two surface sterilants (0.1% mercuric chloride ($HgCl_2$) and 1% sodium hypochlorite (NaOCl)) and three exposure durations (5, 10 and 15 minutes), with four replications of each treatment combination. The interaction treatment 0.1% $HgCl_2$ for 15 minutes ($T_1S_3$) had the highest percentage of uncontaminated explants (82.50%) while 0.1% $HgCl_2$ for 10 minutes ($T_1S_2$) had the highest shoot regeneration (87%) and the minimum time required for shoot bud proliferation (5.7 days).

***Key words*:** Raspberry, Micropropogation, $HgCl_2$, NaOCl

## Introduction

Raspberry (*Rubus* sps L., Rosaceae family, origin Europe, 2n=2x=14) is one of the most important berry cultures in the world, second only to strawberries, with an estimated production of 895,771 tons per year worldwide. It is highly valued for its taste, colour and high antioxidant, polyphenol, fibre and Vitamin A and C content, and has significant economic value as fresh fruit and processed products (Barney *et al*., 2007).

The genus Rubus is widespread throughout temperate regions, but some species such as Rubus niveus (Mysore raspberry) can bear fruit throughout the year in frost-free areas like subtropical areas. In Ukraine, Bulgaria and Kazakhstan, there is a growing demand for commercial planting and an increasing area of cultivation, but the production is significantly restricted by the lack of certified pathogen-free planting material (Archer *et al*., 2016). Traditional propagation methods such as cuttings, root suckers or layering are slow and often infected with vector-borne viruses transmitted by aphids, leafhoppers and nematodes, and seed propagation is hampered by seed coat dormancy (Debnath *et al*., 2006).

To overcome these traditional propagation problems, tissue culture micropropagation is used to produce large quantities of uniform, virus-free and genetically identical planting material at any time of the year ( Iliev *et al*., 2010). The establishment of optimal culture media protocols is important because different cultivars need different nutrient balances and are exposed to different challenges such as inadequate winter chilling (Wuen *et al*., 2009). In addition to commercial multiplication, micropropagation is also important for germplasm conservation, disease eradication and for production of elite cultivars to meet market demand (Rathore *et al*., 2004).

## Materials and Methods

### Origin of plant material (Explant)

Nodal segments of Raspberry (*Rubus niveus.*) were used as explants in this study. The nodal segments of *Rubus niveus* L. were taken from one year old healthy mother plants grown in the nursery of Agri Biotech Foundation, PJTSAU Campus, Rajendranagar, Hyderabad.

### The selection of plant material and preparation of explants for culture.

In vitro culture establishment was done with healthy nodal segments taken from plants that were sprayed with Bavistin solution (1 mg/L) in the morning or evening two days prior to collection. Leaves were carefully removed, one by one, without damaging the nodal buds. The nodal segments were then cut off with a sharp blade and transferred to a beaker with a little water.

### The collection of explants and surface sterilization.

The explants were collected as shown in Plate 1 and brought in a beaker for sterilization. They were first washed thoroughly under running tap water to remove soil particles and dust followed by a mild soap wash to remove surface impurities. They were then treated with 1% Bavistin and 0.1% bactericide for 1 hour, rinsed several times with distilled water, and agitated in Tween-20 solution for 5–10 minutes before being re-rinsed. The explants were further treated with 0.5% Bavistin and 0.1% bactericide and transferred to a laminar airflow chamber, where the sterilizing solution was decanted and the material was washed with sterile distilled water. Using sterile forceps, the explants were moved to a sterile beaker, immersed in 70% ethanol for 2 minutes, and rinsed again with sterile distilled water. Surface sterilization was completed using 0.1% mercuric chloride ($HgCl_2$) and 1% sodium hypochlorite (NaOCl) for 5–15 minutes. followed by repeated sterile water rinses to remove residual sterilants.



**Plate 1:** Collection of raspberry explants.

**Table 1:** Treatment details.

| Factor: 1 (Surface sterilents) | |
|---|---|
| $S_1$ | 0.1%$Hgcl_2$ |
| $S_2$ | 1% NaOCl |
| **Factor: 2 (Time interval)** | |
| $T_1$ | 5 min |
| $T_2$ | 10 min |
| $T_3$ | 15 min |

### Explant inoculation

The browned ends of the explants were cut off after the excess moisture was absorbed by the filter paper. The sterilized explants were then placed in the laminar air flow chamber on initiation medium.

## Results and Discussion

### Percent of explants without contamination

Among surface sterilants, 0.1% $HgCl_2$ ($T_1$) recorded the highest percent of uncontaminated raspberry explants (71.67%), whereas 1% NaOCl ($T_2$) showed the lowest (39.17%). Sterilization duration also influenced outcomes, with 15 minutes ($S_3$) recording the maximum uncontaminated explants (66.25%), followed by 10 minutes ($S_2$, 58.75%), while 5 minutes ($S_1$) resulted in the minimum (41.25%). Among treatment interactions, $T_1S_3$ (0.1% $HgCl_2$ for 15 min) achieved the highest percentage of uncontaminated explants (82.50%), significantly superior to all other combinations and statistically comparable to $T_1S_2$ and $T_2S_3$; the lowest values were observed in $T_2S_1$ (30.00%) and $T_2S_2$ (37.50%).

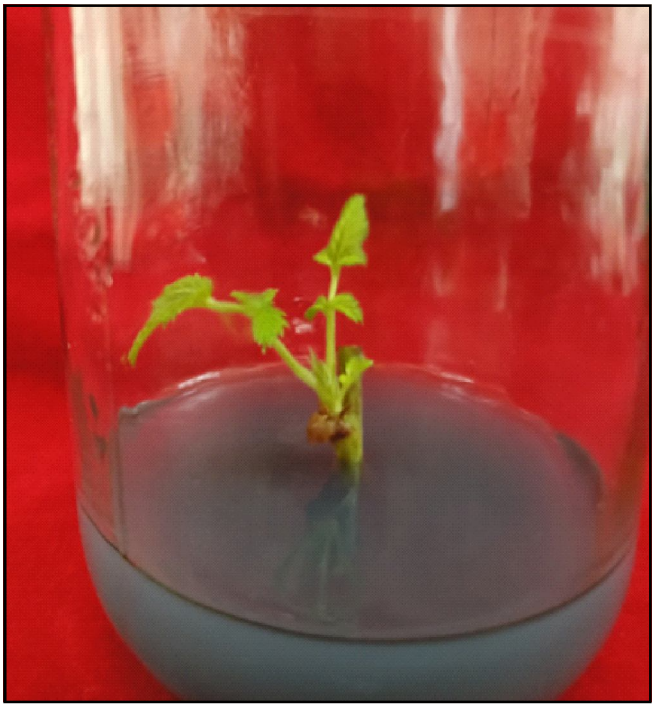


**Plate 2:** Shoot proliferation.

**Table 2:** Effect of different surface sterilents and time interval on percent of explants without contamination.

| Factors | $S_1$ | $S_2$ | $S_3$ | Mean A |
|---|---|---|---|---|
| $T_1$ | 52.50 (7.27) [a] | 80.00 (8.97) [a] | 82.50 (9.11) a | 71.67 (8.45) [a] |
| $T_2$ | 30.00 (5.52) [d] | 37.50 (6.15) [c] | 50.00 (7.11) [a] | 39.17 (6.26) [b] |
| Mean B | 41.25 (6.40) [c] | 58.75 (7.56) [b] | 66.25 (8.11) [a] | |
| Factor | | SE(m) | C.D. 1% | CV |
| Factor(A) | | 3.00 | 0.10 | 3.34 |
| Factor(B) | | 0.04 | 0.13 | |
| Factors (A×B) | | 0.06 | 0.18 | |

• Four replications were performed
• Figures mentioned in the parentheses are ARC Sin values
**Factor 1**: $S_1$: 0.1% $HgCl_2$, $S_2$: 1% NaOCl
**Factor 2**: $T_1$: 5 min, $T_2$: 10 min, $T_3$: 15 min

These results indicate that 0.1% $HgCl_2$ for 15 minutes is the most effective protocol for maximizing uncontaminated raspberry explants in tissue culture, attributable to the superior antimicrobial efficacy of mercuric chloride combined with prolonged exposure. The findings align with earlier reports in strawberry Sharma *et al*., 2009, Jan *et al*., 2016, Munir *et al*., 2020, Panday *et al*., 2023, Pooja *et al*., 2024 and Guranna and Hoolageri, 2017.

**Percent of explants with contamination**

Among the surface sterilents $T_1$ (0.1% $HgCl_2$) recorded lowest contamination with (28.33%) and the highest with (60.83%) in $T_2$ (1% NaOCl) . The shortest sterilization time interval ($S_3$, 15 min) gave the lowest contamination (33.75%), the longest ($S_2$, 10 min) gave the highest (41.25%) and the intermediate ($S_1$, 5 min) gave the highest (58.75%). The contamination of the different treatment combinations was compared, with $T_1S_3$ (0.1% $HgCl_2$ for 15 min) showing the lowest contamination (17.50%), significantly better than all other treatment combinations and statistically similar to $T_1S_2$ (20.00%); the NaOCl treatments showed significantly

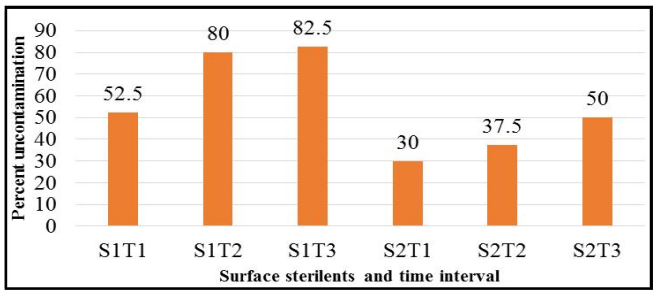



**Fig. 1:** Effect of different surface sterilents and time interval on percent of uncontaminated explants on raspberry explants. **Surface sterilents**: $S_1$: 0.1% $HgCl_2$, $S_2$: 1% NaOCl; **Time interval**: $T_1$: 5 min, $T_2$: 10 min, $T_3$: 15 min.

**Table 3:** Effect of different surface sterilents and time interval on percent of explants with contamination.

| Factors | $S_1$ | $S_2$ | $S_3$ | Mean A |
|---|---|---|---|---|
| $T_1$ | 47.40 (6.923)[b] | 20.00 (4.52) [c] | 17.50 (4.20)[c] | 28.33 (5.22)[b] |
| $T_2$ | 70.00 (8.39) [a] | 62.50 (7.93) [a] | 50.00 (7.10)[b] | 60.83 (7.81) [a] |
| Mean B | 458.75 (7.66) [a] | 41.25 (6.23)[b] | 33.75 (5.65) [c] | |
| Factor | | SE(m) | C.D. 1% | CV |
| Factor (A) | | 0.09 | 0.28 | 5.04 |
| Factor (B) | | 0.11 | 0.34 | |
| Factors (A×B) | | 0.16 | 0.49 | |

• Four replications were performed
• Figures mentioned in the parentheses are ARC Sin values
**Factor 1**: $S_1$: 0.1% $HgCl_2$, $S_2$: 1% NaOCl
**Factor 2**: $T_1$: 5 min, $T_2$: 10 min, $T_3$: 15 min

higher contamination, with $T_2S_1$ (1% NaOCl for 5 min) having the highest (70.00%), followed by $T_2S_2$ (62.50%) and $T_2S_3$ (50.00%).

The results of the present investigation indicated that $HgCl_2$ (0.1%) was more effective than NaOCl (1%) in reducing microbial contamination of raspberry explants. Surface sterilization with 0.1% $HgCl_2$ for 15 minutes resulted in the lowest contamination (17.50%), which may be attributed to the greater antimicrobial efficacy of $HgCl_2$ and its prolonged contact time, leading to more efficient elimination of surface contaminants. The results are consistent with previous studies Sharma *et al*., 2009; Jan *et al*., 2016; Munir *et al*., 2020), (Bhatt and Dhar, 2000) (Georgieva *et al*., 2016; Sajjan *et al*., 2024).

**Number of days taken for shoot proliferation**

Among surface sterilants, $T_1$ (0.1% $HgCl_2$) recorded the minimum days for shoot proliferation, whereas $T_2$ (1% NaOCl) required the maximum (10.06 days). Regarding sterilization duration, $S_1$ (5 min) and $S_2$ (10 min) showed the earliest proliferation (7.12 days), while $S_3$ (15 min) recorded the maximum (12.42 days). Among

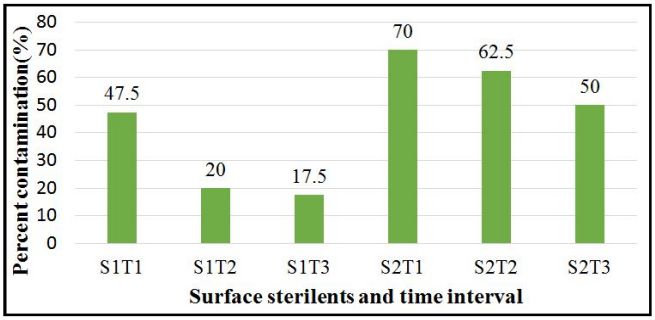



**Fig. 2:** Effect of different surface sterilents and time interval on percent of contaminated explants on raspberry explants. **Surface sterilents:** $S_1$: 0.1% $HgCl_2$, $S_2$: 1% NaOCl; **Time interval:** $T_1$: 5 min, $T_2$: 10 min, $T_3$: 15 min.

**Table 4:** Effect of different surface sterilents and time interval on number of days taken for shoot proliferation.

| Factors | $S_1$ | $S_2$ | $S_3$ | Mean A |
|---|---|---|---|---|
| $T_1$ | 5.75[d] | 5.75[d] | 11.65[b] | 7.71[b] |
| $T_2$ | 8.50[c] | 8.50[c] | 13.2[a] | 10.06[a] |
| Mean B | 7.12[b] | 7.12[b] | 12.42[a] | |
| Factor | | SE(m) | C.D. 1% | CV |
| Factor (A) | | 0.09 | 0.28 | 0.62 |
| Factor (B) | | 0.11 | 0.35 | |
| Factors (A×B) | | 0.16 | 0.50 | |

• Four replications were performed
**Factor 1**: $S_1$: 0.1% $HgCl_2$, $S_2$: 1% NaOCl
**Factor 2**: $T_1$: 5 min, $T_2$: 10 min, $T_3$: 15 min

treatment interactions, $T_1S_1$ and $T_1S_2$ achieved the earliest shoot proliferation (5.70 days) and were statistically comparable, followed by $T_2S_1$ and $T_2S_2$ (8.50 days); prolonged sterilization delayed initiation, with $T_1S_3$ requiring 11.65 days and $T_2S_3$ the maximum (13.20 days)

The results show that the 0.1% $HgCl_2$ treatment for 5 or 10 minutes is more effective than the 1% NaOCl treatment in inducing shoot proliferation earlier in raspberry explants, whereas longer treatment times, particularly with NaOCl, delay shoot initiation, possibly due to phytotoxic effects. The results are consistent with previous reports in raspberry (Debnath, 2005) and strawberry (Sharma *et al*., 2009) where 0.1% $HgCl_2$ also caused earlier shoot proliferation and delayed shoot initiation with prolonged exposure to the sterilant.

**Percent of shoot proliferation (%)**

$T_1$ (0.1% $HgCl_2$) had the highest shoot proliferation (60.00%) while $T_2$ (1% NaOCl) had the lowest (30.00%). As for the duration of sterilization, $S_2$ (10 min) had the highest (60.00%) followed by $S_1$ (5 min) which was not statistically different, and $S_3$ (15 min) had the lowest (45.00%). The highest shoot proliferation (87.50%) was obtained from $T_1S_2$ and $T_1S_1$ which were statistically similar, followed by $T_1S_3$ (60.00%) while the lowest was obtained from $T_2S_3$ (30.00%) and $T_2S_1$ (40.00%) were the same.

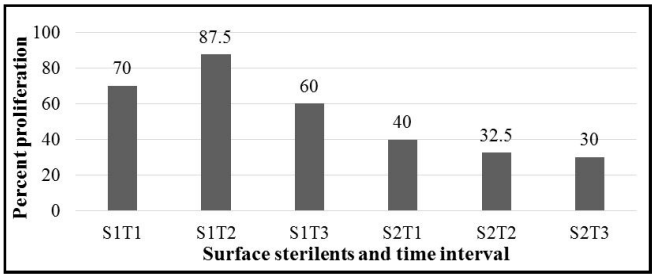



**Fig. 3:** Effect of different surface sterilents and time interval on percent of shoot proliferation raspberry explants. **Surface sterilents :** $S_1$: 0.1% $HgCl_2$, $S_2$: 1% NaOCl; **Time interval :** $T_1$: 5 min, $T_2$: 10 min, $T_3$: 15 min.

**Table 5:** Effect of different surface sterilents and time interval on percent of shoot proliferation.

| Factors | $S_1$ | $S_2$ | $S_3$ | Mean A |
|---|---|---|---|---|
| $T_1$ | 87.50 (9.4)[a] | 87.50 (9.4)[a] | 60.00 (7.8)[c] | 8.30 (8.52)[a] |
| $T_2$ | 40.00 (6.4)[d] | 40.00 (6.4)[d] | 30.00 (5.5)[e] | 36.00 (5.85)[b] |
| Mean B | 63.70 (7.90)[a] | 63.75 (7.90)[a] | 45.00 (6.65)[b] | |
| Factor | | SE(m) | C.D. 1% | CV |
| Factor (A) | | 0.04 | 0.12 | 4.11 |
| Factor (B) | | 0.05 | 0.15 | |
| Factors (A×B) | | 0.07 | 0.21 | |

• Four replications were performed
• Figures mentioned in the parentheses are ARC Sin values
**Factor 1**: $S_1$: 0.1% $HgCl_2$, $S_2$: 1% NaOCl
**Factor 2**: $T_1$: 5 min, $T_2$: 10 min, $T_3$: 15 min

The results show that 0.1% $HgCl_2$ for 5-10 minutes is more effective than 1% NaOCl in shoot proliferation because optimal exposure does not cause injury to the explants while reducing contamination. Prolonged sterilization (particularly with NaOCl) decreases proliferation, probably because of phytotoxicity. The results are consistent with the previous reports of strawberry (Sharma *et al*., 2009; Munir *et al*., 2020; Jan *et al*., 2016), pomegranate (Guranna and Hoolageri, 2017), and blueberry (Sharma *et al*., 2018; Pooja *et al*., 2024).

## Conclusion

The present investigation Surface sterilization of raspberry (*Rubus niveus*) nodal explants with 0.1% mercuric chloride ($HgCl_2$) for 10 minutes was found to be the most effective treatment for successful culture establishment, as it produced the highest percentage of healthy regenerating explants with minimum contamination and early shoot bud proliferation.

## References

Archer, L., Carroll J., Heidenreich C. and Pritts M. (2016). Production and IPM guide for organic raspberries and blackberries. *New York State Integrated Pest Management Program, Ithaca*. **(59)**.

Barney, D.L. and Miles C. (2007). *Commercial red raspberry production in the Pacific Northwest*. Oregon State University, University of Idaho, and Washington State University.

Bhatt, I.D. and Dhar U. (2000). Micropropagation of Indian wild strawberry. *Plant Cell, Tissue and Organ Culture*. **60(2)**, 83–88.

Debnath, M., Malik C.P. and Bisen P.S. (2006). Micropropagation: A tool for the production of high quality plant-based medicines. *Current Pharmaceutical Biotechnology*. **7(1)**, 33-49.

Georgieva, L., Tsvetkov I., Georgieva M. and Kondakova V. (2016). New protocol for in vitro propagation of berry plants by TIS bioreactor. *Bulgarian Journal of Agriculture Science*. **22(5)**, 745-751.

Guranna and Hoolageri J.B. (2017). Influence of various surface sterilants and duration on surface sterilization of various explants of pomegranate (*Punica granatum* L.) cv. Bhagwa. *Environment & Ecology*. **35**, 1435–1440.

Iliev, I., Ivanova M. and Gajdosova A. (2010). Plant regeneration from *Rubus* and *Ribes* species. *Protocols for In Vitro Propagation of Ornamental Plants*. 315–334.

Jan, A., Bhat K.M., Bhat S.J.A., Mir M.A., Bhat M.A., Imtiyaz A. and Rather J.A. (2016). Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for *in vitro* culture. *African Journal of Biotechnology*. **12(39)**.

Munir, M., Iqbal S., Baloch J.U.D and Khakwani A.A. (2020). *In vitro* explant sterilization and bud initiation studies of four strawberry cultivars. *Journal of Applied Horticulture*. **17(3)**, 192-198.

Pandey, S., Tiwari A. and Singh S. (2023). Standardization of surface sterilization protocol for *in vitro* propagation of wild raspberry (*Rubus ellipticals* Smith). *The Pharma Innovation Journal*. **12(5)**, 2344–2349.

Rathore, M.S., Mastan S.G. and Shekhawat N.S. (2004). *In vitro* propagation of *Rubus ellipticus* Smith.A wild edible raspberry of sub-Himalayan region. *Journal of Plant Biochemistry and Biotechnology*. **13(2)**, 155–158.

Sajjan, P., Venkatalaxmi K. and Patil S. (2024). Effect of surface sterilization and basal media with plant growth regulators on micropropagation of blueberry (*Vaccinium corymbosum* L.). *Journal of Applied Horticulture*. **26(1)**, 84–89.

Sharma, S., Wall V.K., Klier R. and Sharma M. (2009). *In vitro* propagation of strawberry (*Fragaria x ananassa* duch.) cv. chandler. *Journal of Research, SKUAST–Journal*. **8(2)**, 157-167.

Wang, Q.M., Luan H.R. and Gao G.L. (2010). Optimization of sterilization protocol and *in vitro* propagation of strawberry (*Fragaria* × *ananassa* Duch.) cultivar 'Camarosa'. *Agricultural Sciences in China*. **9(8)**, 1121–1127.