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## **A comparative analysis of the in-vitro effects of various sterilization techniques and different disinfectants on the micropropagation of explants of *Asparagus Racemosus***

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**Abstract**---*Asparagus racemosus* belongs to the Asparagaceae family and the *Asparagus* genus is an endangered plant species that have been listed in the red data book. For the conventional breeding of the *Asparagus racemosus*, its tubers are used which possess a wide range of antioxidants, leading to the wastage of highly oxidative tuber roots. Using the in-vitro propagation method we can propagate *Asparagus racemosus* using its nodal explants successfully only in a contamination-free environment. The sources of contamination can be present in the media or associated with the explants which need to be eradicated using various sterilization techniques and disinfectants. This paper presents two different sterilization techniques and treatment of disinfectant Sodium hypochlorite used to sterilize the media and the explants. The media was sterilized using an autoclave and microwave oven with the treatment of explant with sterilizing agent sodium hypochlorite in three concentrations of 5%, 10%, and 15% for 5 min. The nodal explants were cultured in MS basal medium supplemented with 30g sucrose, as a carbon source and 7g agar, as a gelling agent. The temperature inside the growth chamber was maintained at 24±2°C with a photoperiod of 16 h daylight and 8 h dark. The results were assessed based on survival rate, shoot growth, and the percentage of contamination during the initiation phase.

Among all the different sterilization variations, 10 % sodium hypochlorite disinfectant treatment with 30 min autoclaved media was found to be the most effective treatment with an 85% initiation rate and 70% survival rate. After sterilization, shoots continued to grow vigorously and the multiplication phase was initiated.

**Keywords**---sterilization, autoclave, microwave oven, sodium hypochlorite, *asparagus racemosus*.

## Introduction

*Asparagus racemosus* is an important medicinal plant that grows throughout India's tropical and subtropical parts and is known for its phytoestrogen properties. The plant is used to increase lactation in women and treat problems related to their menstrual cycle (1,2). Steroidal saponins are the main active constituent of the *Asparagus racemosus* which is majorly deposited in the tubers of the explant (3,4). The rise of demand for the plant owing to its high medicinal value leads to destructive harvesting of the plant has shrunk the natural population of *Asparagus racemosus*. Micropropagation can be used as a powerful tool to meet the increasing demand of the plant by culturing its explant in artificially favourable conditions (5,6). The successful micropropagation of *Asparagus racemosus* has been done using its nodal explants (7,8,9), but these explants may be associated with the source of contamination. The contamination in the culture can also invade if the media is not sterilized in the proper conditions. Therefore, proper sterilization of the media and the explants plays a key role in the successful micropropagation of the explants (10).

Autoclaving of the media is used as a traditional method of sterilization of media, but consumption of less time, labor, and energy has popularised the usage of microwave ovens among tissue culturists (11,12). Research has been done to find out the efficiency of the microwave oven in the sterilization of the plant tissue culture media and found the usage of microwave efficient to avoid the contamination however case of overboiling of the media can lead to dehydration of the media. (13,14,15,16). Surface sterilization of explants is done using various disinfectants such as sodium hypochlorite, mercuric chloride, boric acid, ethanol, hydrogen peroxide, etc. Sodium hypochlorite is the most commonly used disinfectant that has been used in the sterilization of explants of various plant species (17,18,19). In the present study, a comparison of the efficiency of sterilizing technique using autoclave and microwave oven in combination with various concentrations of disinfectant sodium hypochlorite is done for *Asparagus racemosus* micropropagation to find out the best, most efficient, and cost-effective sterilization procedure that may result in fewer chances of contamination or no contamination in tissue culture of *Asparagus racemosus* (20,21,22,23).

## **Material and Methods**

### **Preparation and Sterilization of Media**

For the micropropagation of *Asparagus racemosus* MS basal media is chosen which is a combination of macronutrients, micronutrients, and vitamins supplemented with 30 g sucrose and 8g agar for the preparation of 1000 ml media. The bottles were packed in the autoclave bags and were placed in the autoclave. The media is sterilized at 121°C for 20 min and 30 min at 15psi. The bottles were taken out from the autoclave and were kept inside the laminar airflow to get the media cool and solidified (24). Another batch of 1000 ml media was sterilized using a microwave for 10 min and 15 min. The microwave surface was cleaned with 70% ethanol to avoid any contamination. The media was poured inside the small bottles and was heated under continuous supervision by stirring the media every 2 min to avoid the overboiling of the media and for the uniform mixing of the MS media. The bottle caps which were kept loose to avoid the bursting of bottles were tightened before taking out the bottles from the oven. The bottles were transferred to the laminar airflow in a sterilized box wiped with 70% ethanol. All the media were left in the bottles in the Tissue culture room for two days to observe the presence of contamination in the media.

### **Explants surface sterilization**

Nodal segments of *Asparagus racemosus* were collected from the plants in the nursery at Maharishi Dayanand University. Explants were washed for 30 min with detergent in tap water to remove any dust and soil particles associated, the explant is washed with water until all the detergent gets removed. After that, the explant is transferred to the laminar airflow and treated with 70% ethanol for 2-3 min and washed with distilled water before the treatment with the disinfectant of various concentrations. For *Asparagus racemosus*, treatment of sodium hypochlorite is given in three concentrations 5%, 10%, and 15% for 5 min.

### **Media Culture and Surface Sterilization**

All the sterilized explants were planted in two sets. The first one is sterilized MS media using an autoclave. The other explants were planted on the MS media and sterilized using a microwave oven. The plants were allowed to grow *in vitro* in the growth chamber having 24±2°C temperature and a photoperiod of 16 h daylight and 8h dark.

### **Data Collection**

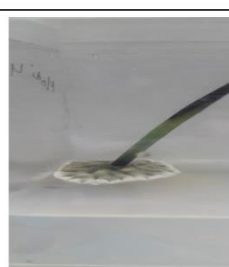
The experiment was carried out in triplicate consisting of ten explants in each set of treatments. A total of 12 variations were used in culturing the explants. The data was collected after 15 and 30 days in terms of Initiation percentage, percentage of contamination, and percentage of explant survived.

## Results and Discussions

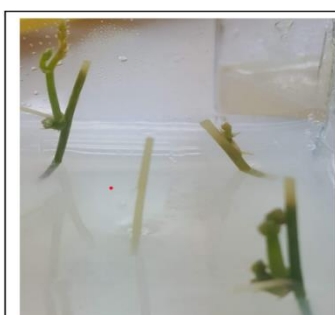
In the study, we found that out of both sterilization methods, with variable sterilization time Microwave oven-assisted sterilization for 10 minutes was found to be least effective when observed after two days with the presence of contamination in 90% of bottles, and some bottles were having semi-solid media. The reason for semi-solid media can be the uneven mixing of agar in the media. All the contamination-free media were used for the culture of explants treated with various disinfectants. Out of all the sets of experiments, media sterilization using Autoclave for 30 min with 10% sodium hypochlorite is found to be the most effective mode of sterilization with a maximum initiation rate and explant survival rate of 85% and 70% respectively with minimal contamination. The maximum percentage of contamination was observed in microwave-assisted sterilization of 10 min with 5% sodium hypochlorite. 15-minute microwave-assisted sterilization is effective in minimizing contamination in Tissue culture. 15% Sodium hypochlorite is most effective in combating contamination with maximum initiation and explant survival rate, 20% sodium hypochlorite can be used to minimize the contamination but high concentrated form affects the growth of explants.



**Figure 1: Fungal Contamination in the media was sterilized using a microwave oven for 10 min**



**Figure 2: Contamination in explant disinfected by 5% Sodium hypochlorite in media autoclaved for 20 min**



**Figure 3: Negative effect on explant viability in explants treated with 15% sodium hypochlorite**

Table 1

Evaluation of the presence of contamination in media sterilized after 2 days of preparation

S.No.	Mode of Sterilization	Sterilization Time	Contamination
1	Autoclave	20 min	NO
2	Autoclave	30 min	NO
3	Microwave	10 min	Yes
4	Microwave	15 min	NO

Table 2

Assessment of the presence of contamination, initiation rate, and explant survival rate for the explants disinfected using various concentrations of disinfectant in media sterilized using different modes

S.no	Media Sterilization method	Sterilization Time	Explant Sterilizer (Varied Concentration)	Initiation Percentage (After 15 days)	Contamination Percentage		Explant Survival Percentage (After 30days)
					After 15 days	After 30 days	
1	Autoclave	20 min	Sodium hypochlorite (5%)	30	40	70	10
2	Autoclave	20 min	Sodium hypochlorite (15%)	70	30	45	50
3	Autoclave	20 min	Sodium hypochlorite (20%)	55	15	25	40
4	Autoclave	30 min	Sodium hypochlorite (5%)	38	32	40	25
5	Autoclave	30 min	Sodium hypochlorite (10%)	85	5	18	70
6	Autoclave	30 min	Sodium hypochlorite (20%)	75	6	10	60
7	Microwave	15 min	Sodium hypochlorite (5%)	28	83	95	12
8	Microwave	15 min	Sodium hypochlorite (10%)	78	35	43	69
9	Microwave	15 min	Sodium hypochlorite (20%)	72	12	35	58

### Conclusion

Exactly 15-minute microwave-assisted sterilization is effective in minimizing contamination in Tissue culture media and gives the best result when the explant is treated with 10% sodium hypochlorite. Microwave oven sterilization has the potential to be used as a substitute for autoclaves in the Tissue Culture lab for a lesser number of media. Sterilization can be achieved in less time saving energy but the problem associated with the microwave oven is that it requires continuous supervision. 10% Sodium hypochlorite for 10 min is efficient in removing contamination and also does not affect the explant viability. A higher

concentration of Sodium hypochlorite is efficient in minimizing the contamination but at the same time, it negatively affects the initiation rate of the explant.

## References

1. Bopana , N . and Saxena , S . (2007) . Asparagus racemosus – Ethnopharmacological evaluation and conservation needs. *Journal of Ethnopharmacology* 110, 1 – 15.
2. Mitra , S.K ., Gopumadhavan , S ., Venkataranganna , M.V ., Sarma , D.N.K . and Anturlikar , S.D . (1999). Uterine tonic activity of U-3107 a herbal preparation in rats. *Indian Journal of Pharmacology* 31, 200 – 203.
3. Mayo, J.L . (1998). Black cohosh and Chasteberry: Herbs valued by women for centuries. *Clinical Nutrition Insights* 6 (15) , 1 – 4 .
4. Sabnis , P.B ., Gaitonde , B.H . and Jethmalani , M .(1968). Effect of alcoholic extract of Asparagus racemosus on mammary glands of rats. *Indian Journal of Experimental Biology* 6,55 – 57.
5. Kamat , J.P ., Bloor , K.K ., Devasagayam ,T.P.A . and Venkatachalam , S.R . (2000).Antioxidant properties of Asparagus racemosus against damage induced by g-radiation in rat liver mitochondria. *Journal of Ethnopharmacology* 71 (3), 425 – 435.
6. Rege , N.N ., Nazareth , H.M ., Issac , A.A .,Karandikar , S.M . and Dahanukar , S . A .(1989) . Immunotherapeutic modulation of intraperitoneal adhesions by Asparagusracemosus. *Journal of Postgraduate Medicine* 35, 199 – 203.
7. Kar, D.K . and Sen, S . (1985). Micropropagation of Asparagus racemosus. *Plant Cell, Tissue and Organ Culture* 5, 89 – 95
8. Saxena, S., &Bopana, N. (2009). In vitro clonal propagation of Asparagus racemosus, a high-value medicinal plant. In *Protocols for In Vitro Cultures and Secondary Metabolite Analysis of Aromatic and Medicinal Plants* (pp. 179-189). Humana Press, Totowa, NJ.
9. Patel, L. S., & Patel, R. S. (2015). Rapid in vitro micropropagation of Asparagus racemosusWilld. from nodal explants. *International Journal of Current Microbiology and Applied Sciences*, 4(5), 607-617.
10. Das, S. (2021). Bijendra Kumar Singh, Jitendra Prasad, Shikha Tiwari, and Nawal Kishore Dubey. *Evidence-Based Validation of Traditional Medicines: A comprehensive Approach*, 261.
11. Youssef, E. M. A., & Amin, G. A. (2000, July). Microwave sterilization of tissue culture media. In *IV International Symposium on In Vitro Culture and Horticultural Breeding* 560 (pp. 513-516).
12. Venturieri, G. A., Venturieri, A. R., & Leopoldo, G. (2013). Sterilization of culture media for orchids using a microwave oven. *In Vitro Cellular & Developmental Biology-Plant*, 49(2), 137-144.
13. Teixeira SL, Campanati M, Teixeira MT, Almeida RF (2005a) Esterilização de meiosnutritivos para cultura de tecidosvegetais, pela combinação de esterilizantesquímicos e forno de microondas.
14. Revista Ceres 52:343–349 Teixeira SL, Sousa RTS, Teixeira MT (2005b) Esterilização de meiosnutritivos para cultura de tecidosvegetaisemforno de microondas. Revista Ceres 52:499–507

15. Venturieri, G. A., Venturieri, A. R., & Leopoldo, G. (2013). Sterilization of culture media for orchids using a microwave oven. *In Vitro Cellular & Developmental Biology-Plant*, 49(2), 137-144.
16. Youssef EMA, Amin GA (2001) Microwave sterilization of tissue culture media. *Acta Hort* 560:513–516.
17. Tyagi, V. S. A., Chauhan, P., Kumari, P. O. O. N. A. M., & Kaushal, S. E. E. M. A. (2011). Identification and prevention of bacterial contamination on explant used in plant tissue culture labs. *Alcohol*, 3, 0-722.
18. Yildiz, M., FatihOzcan, S., T Kahramanogullari, C., & Tuna, E. (2012). The effect of sodium hypochlorite solutions on the viability and in vitro regeneration capacity of the Tissue. *The Natural Products Journal*, 2(4), 328-331.
19. Hussain A., Qarshi I.A., Nazir H., Ullah I., 2012. Plant Tissue Culture: Current Status and Opportunities. Qarshi University, Lahore, Pakistan
20. Sarasan V., Kite G.C., Sileshi G.W., Stevenson P.C., 2011. Applications of phytochemical and in vitro techniques for reducing over-harvesting of medicinal and pesticidal plants and generating income for the rural p
21. Singh, L., Antil, R., & Dahiya, P. (2021). Antimicrobial and Antioxidant Potential of Vernonia Cinerea Extract Coated AuNPs. *Indian Journal of Microbiology*, 1-13.
22. Singh, L., Antil, R., Ashmita, P., & Dahiya, P. (2020). In-vitro biological activities of Vernonia cinerea (L.). *Plant Arch*, 20(2), 4889-4900.
23. Singh, L., Antil, R., & Dahiya, P. (2020). Phytochemical screening and GC-MS analysis of a methanolic extract of Launaea procumbens (Roxb.) Ramayya and Rajagopal. *Annals of Biology*, 36(3), 436-441.
24. 24.Sulava, S., Andia, S., Bhol, R., & Jena, S. (2020). In Vitro Micropropagation of Asparagus Racemosus by Using of Nodal Explants. *Journal of Cell and Tissue Research*, 20(1), 6883-6888.
25. Nyandra, M., Kartiko, B.H., Susanto, P.C., Supriyati, A., Suryasa, W. (2018). Education and training improve quality of life and decrease depression score in elderly population. *Eurasian Journal of Analytical Chemistry*, 13(2), 371-377.
26. Nyandra, M., Suryasa, W. (2018). Holistic approach to help sexual dysfunction. *Eurasian Journal of Analytical Chemistry*, 13(3), pp. 207–212.