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The Graded Concentration of Bleach Solution and Material Size Plays an Important Role in the In Vitro Sterilization of *Thyphonium flagelliforme* Plants

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ABSTRACT

The sterilization is a primary step in in-vitro culture. plant breeding through in-vitro culture hinges on the successful effectiveness of the sterilization method in producing sterile plants. This research aims to optimize sterilization methods using a completely randomized factorial design. The first factor is the size of the material (small and large), and the second factor is the graded concentration of the bleach solution. The study revealed that Material sizes do not have a significant effect on contamination level, all the same, large size materials increase the survival rate of sterilized rodent tuber plants. The application of graded bleach solution concentrations at 25%, 20%, and 15% is able to reduce contamination to 0% on small size materials and 5 % on large size materials. Material sizes do not have a significant effect on contamination level, all the same, large size materials increase the survival rate of sterilized rodent tuber plants.

Keywords:
Sterilization; bleach solution; material sizes; Thyphonium flagelliforme.

1. Introduction

The rodent tuber plant (*Typhonium flagelliforme*) is indigenous to the Southeast Asian region, including Indonesia, and is conventionally propagated vegetatively, resulting in relatively low genetic diversity (Mankaran et al., 2013; Sianipar et al., 2015). Plant breeding represents an endeavor aimed at augmenting plant diversity. In vitro culture stands out as one of the techniques that can be used in this discipline.

In vitro culture or tissue culture is a technique to isolate a part of the plant, such as protoplasts, cells, cell groups, tissues, and organs, and cultivate them under aseptic conditions, enabling their multiplication and regeneration into whole plants (Kaya and

Huyop, 2020; Norouzi et al., 2022). In-vitro culture has the advantage of producing a large number of plants in a limited space and a relatively short time (Bhatia and Sharma, 2015; Kavya et al., 2021; Suman, 2017). Tissue culture techniques are based on the principle of cell totipotency. Cell totipotency is the ability of a single cell, tissue, or organ to regenerate into a complete plant (Espinosa-Leal et al., 2018; Hussain et al., 2012).

In in-vitro culture, the sterilization is a primary step. Plant breeding through in-vitro culture cannot be successful if the sterilization method does not yield sterile plants. Chemicals such as sodium hypochlorite (NaOCl) (Kidasi et al., 2023; Peiris et al., 2020; Silva et al., 2015), calcium hypochlorite ($Ca(OCl)_2$) (Quynh et al., 2022), ethanol (Kidasi et al., 2023), mercuric chloride ($HgCl_2$) (Boruah, 2020), benzalkonium chloride (Badhane and Patil, 2016; Zhao et al., 2024), and hydrogen peroxide (H_2O_2) (Chen et al., 2017) are common disinfectants used for the surface sterilization of materials. However, in many laboratories, NaOCl, which are different commercial bleaches (Mihaljevic et al., 2013), is commonly employed along with fungicides and antibiotics (Parzymies, 2021) to eliminate contaminations (Elbasheer et al., 2019; Malwattage et al., 2021).

The determination of both the concentration and exposure time of bleach solution containing NaOCl in sterilization process must be carried out empirically to establish an effective method that yields explants with minimal mortality rates (Hashim et al., 2021; Pais et al., 2016; Si et al., 2022). Chika et al. (2022) reports that Multilevel sterilization (10%, 20%, and 30%) is the most effective method of sterilization to reduce contamination and browning. reports that immersion of explants in 10% NaOCl for 10 minutes demonstrated good effectiveness in sterilizing oil palm plants. Similar research was conducted by Rahayu et al. (2019) and Pratiwi et al. (2021) on *Elaeocarpus grandiflorus* L and oil palm plants in vitro on the 5%-25% concentration of bleach solution. These study report that the use of graded concentrations of bleach solution is effective for obtaining sterile and alive explants.

It is important to consider that the effectiveness of sterilization agents may vary depending on the size of the explant. For example, smaller explants may require a shorter contact time with the sterilization agent to achieve optimal sterilization, while larger explants may require a longer contact time. Therefore, the selection of explant size is one of the key considerations in planning sterilization procedures in tissue culture. However, no recent research has revealed this. Thus, this study was conducted to investigate the influence of commercial bleach solution concentration and the material size of rodent tuber plants on the sterilization process.

2. Materials and Methods

2.1 Place and Time

The experiment was carried out at the Laboratory for Biotechnology BRIN, building 630, Directorate of Laboratory Management, Research Facilities, and Science and Technology Park, located in Serpong, South Tangerang City, Indonesia. The experiment started on 1 March 2023 and ended on 15 August 2023.

2.2 Materials

The materials used for this study were rodent tuber plants derived from the Collection Park, the Laboratory for Biotechnology, National Research and Innovation Agency (BRIN), Indonesia. The materials used in this study were obtained from Balikpapan, Indonesia, which is the accession with the highest bioactive compounds compared to other accessions in the Collection Park, the Laboratory for Biotechnology, BRIN, Indonesia

2.3 Research Design

This study used a completely randomized factorial design. The first factor was the size of the materials, namely large (± 5 x 5 mm) and small (± 3 x 3 mm). The second factor was the graded bleach solution concentration. They were (1) method 1: 25%; 20%; 15%, (2) method 2: 20%; 15%; 10%, (3) method 3: 15%; 10%; 5%, and (4) method 4: 10%; 5%; 5%. There were 8 treatment combinations which will be repeated 3 times, therefore, there were 24 experimental units. Each experimental unit consisted of 20 plant samples, hence, 480 observation units were obtained.

2.4 Procedure Methodology

2.4.1 Materials preparation

The preparation process began by isolating the materials in the form of shoot buds from rodent tuber plants with sizes of ± 3 x 3 mm (small) and ± 5 x 5 mm (large). The materials were washed thoroughly with running water for 10 minutes.

Figure 1. Rodent tubber material a. small size b. large size

2.4.2 Sterilization Process

The Materials were soaked in a floor cleaner solution (containing 1.5% benzalkonium chloride) for 3 minutes, then soaked in 70% alcohol for 3 minutes. Afterwards, the materials were soaked in the floor cleaner solution again for 30 minutes, and then they were washed with running water for 1 hour. Subsequently, the materials were immersed in a fungicide and bactericide solution (2 g L^{-1}) for 30 minutes. Afterwards, the materials were transferred into a laminar air flow, and then they were soaked in the three-graded bleach solution (containing 5.25% NaOCl) for 5 minutes, 10 minutes and 15 minutes (Table 1). Finally, the materials were planted in MS medium without plant growth regulators (MS0).

Table 1. Comparison of soaking treatment at graded bleach solution concentration

Soaking	Method			
	1	2	3	4
First Soaking	25 % for 5 minutes	20% for 5 minutes	15% for 5 minutes	10% for 5 minutes
Second soaking	20% for 10 minutes	15% for 10 minutes	10% for 10 minutes	5% for 10 minutes
Third Soaking	15% for 15 minutes	10% for 15 minutes	5% for 15 minutes	5% for 15 minutes

2.5 Data analysis

Data were analyzed using the Analysis of Variance (ANOVA) test, and if there were significant differences, they would be tested using Turkey's Honestly Significant Difference (HSD) method with Minitab software.

3. Results and Discussion

3.1 Result

The interaction between material size and bleach solution concentration had the same impact on the contamination level of rodent tuber plants after sterilization. Similar results were also obtained from testing the single factor of material size on contamination levels. While a single concentration test factor exerts a different effect on the contamination level of rodent tuber plants after sterilization (Table 2) on the 95% confidence level.

Table 2. The result of ANOVA test

	Material sizes	Bleach Solution Concentration	Interaction
Contamination level (%)	1.39^{ns}	657.39**	1.69^{ns}
Survival rate (%)	6.56*	27.34**	11.23**

Remarks: **Highly significant at 1% level (P<0.01), *Significantly influential at 5% level (0.01<P<0.05), ns: Not significant (P>0.05).

The result of the post hoc test used Turkey's HSD test at a 95% confidence level for various graded bleach solution concentrations illustrated that method 4 had the highest contamination level. It was 90%. Meanwhile, method 1 had the lowest contaminant level. It was 2,5%. Consequently, it was the best method for suppressing contamination in the sterilization process of rodent tuber plants (Table 3).

Table 3. Contamination level analysis using Tukey's HSD test at 95% confidence

Methods	Size of material		Means (%)
	Small	Large	
1	0.00 %	5.00 %	2.50^{d}
2	8.33 %	15.00 %	11.67^{c}
3	80.00 %	80.00 %	80.00^{b}
4	91.67 %	88.33 %	90.00^{a}
Mean (%)	45.00^{a}	47.08^{a}	

Remarks: Means that do not share a letter are significantly different.

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Figure 2 illustrates the types of contamination in rodent tuber materials following sterilization. The predominant contaminant was bacteria in all treatments except for the treatment involving small-sized materials with method 1. Fungal contamination was observed in materials subjected to the treatment of large-sized materials with a graded bleach solution concentration of method 2, as well as in the treatments of both small and large-sized materials with graded concentrations of methods 3 and 4. Treatments with graded concentrations of methods 3 and 4 resulted in elevated contamination levels, both in small and large-sized materials.

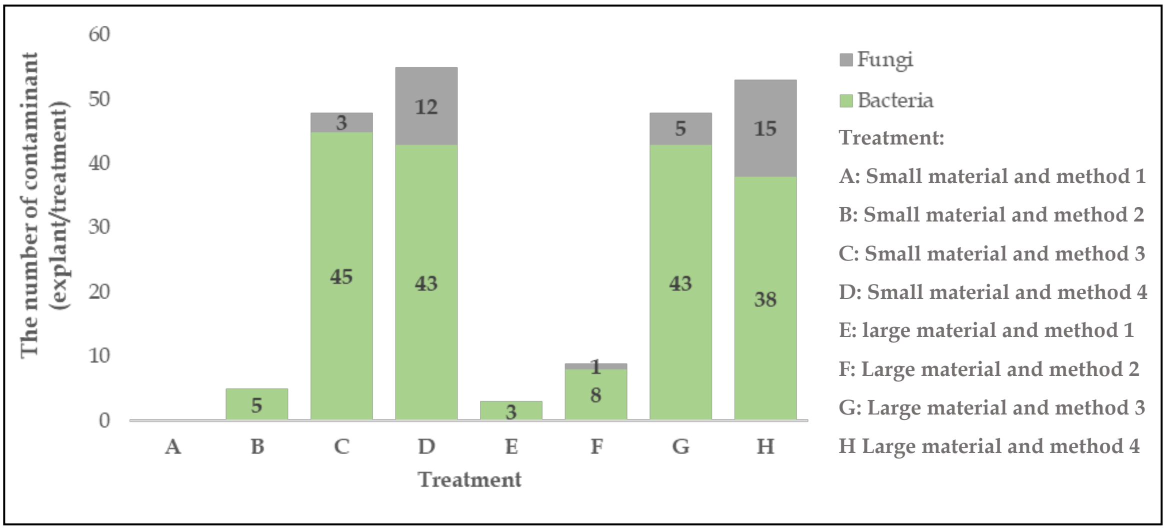

Figure 2. Type of contamination in different materials sizes

Interaction between material sizes and bleach solution concentration gives a different result of survival rate on rodent tuber plants after sterilization (Table 2). The survival rate of rodent tuber plants with method 1 was influenced by the material sizes used. Figure 3 illustrates the interaction between material size and bleach solution concentration on the survival rate of rodent tuber plants. Small materials have a low survival rate in the treatment with graded bleach solution concentrations in method 1. In contrast, small materials in the treatment with graded bleach solution concentrations in method 2 exhibit a high survival rate of 95%. The treatments with graded bleach solution concentrations in methods 3 and 4 gave the same result of survival rate, approaching 100%.

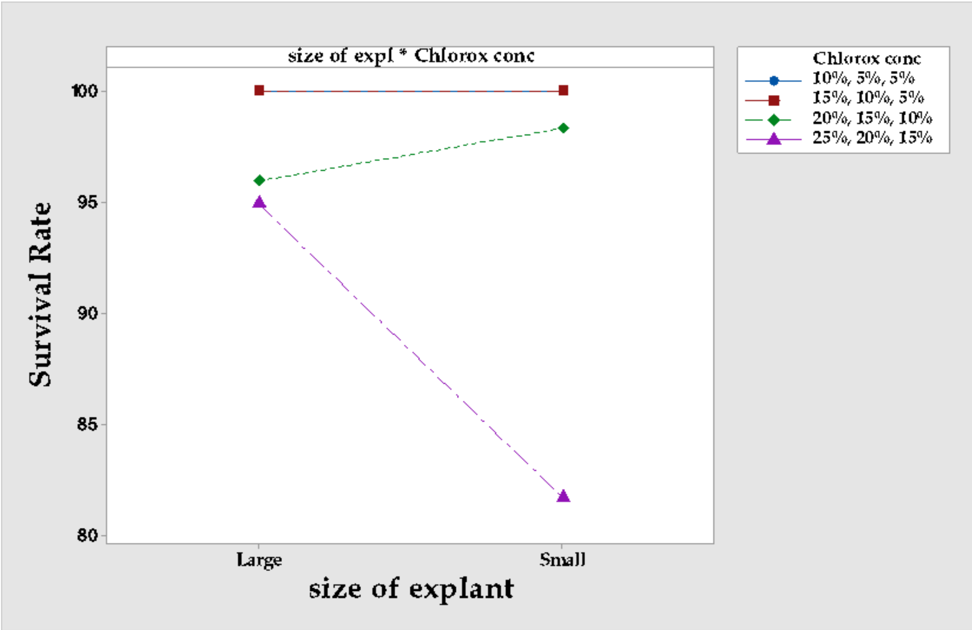

Figure 3. Interaction plot for survival rate

The result of the post hoc test using Tukey's HSD test illustrates that the large-sized materials have a better survival rate compared to small-sized materials, namely at 97.72% (Table 4). Meanwhile, the application of various concentrations of bleach solution significantly influenced the survival rate of rodent tuber plants after sterilization. The graded concentration of bleach solution in methods 2, 3 and 4 had the same impact on survival rate of rodent tuber plants. The different result was shown in method 1. It had the lowest survival rate, it is 88.29%.

Table 4. Survival rate analysis using Tukey's method and 95% confidence

Methods	Size of material		Mean (%)
	Small	Large	
1	81.67 %	94.91 %	88.29[b]
2	98.33 %	95.96 %	97.15[a]
3	100.00 %	100.00 %	100.00[a]
4	100.00 %	100.00 %	100.00[a]
Mean (%)	95.00[a]	97.72[a]	

Remarks: Means that do not share a letter are significantly different.

3.2 Discussion

Contamination of microorganisms, such as bacteria and fungi, is a challenge in in-vitro plant culture. This hinders cultivation in in-vitro culture, reducing the efficiency of propagation and rooting of shoots for commercial purposes. In research laboratories, contamination can cause inaccurate physiological experiment results (Kidus and Teka, 2020). The diversity and abundance of genera and species of contaminating microorganisms accompanying plants is a major obstacle in the initial material sterilization process. In many cases, contaminating microorganisms enter the culture along with the starting material. Sterilization aims to suppress the growth of contaminants under in-vitro culture and to obtain sterile plants free from contaminants and capable of growth (Figure 4).

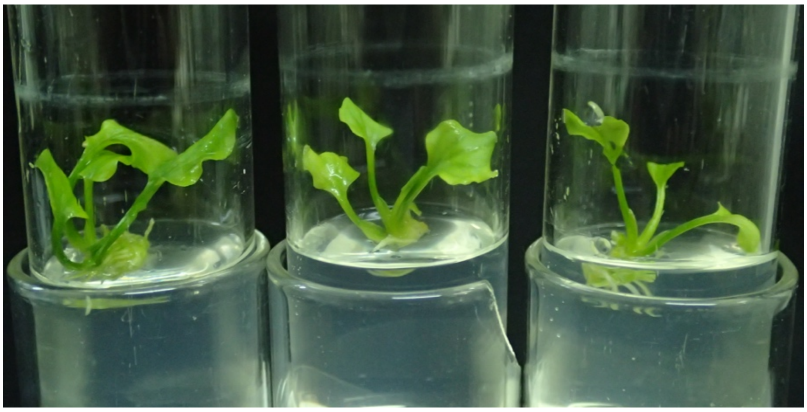

Figure 4. Rodent tuber plants are sterile, and growth

The commercial bleach solution (containing 5,25% NaOCl) has been proven effective as a sterilization agent because of its properties, which can kill various microorganisms, including bacteria, viruses, and fungi (Ersoy et al., 2019). Generally, in research laboratories, bleach solutions containing NaOCl are used as additional compounds in the process of sterilizing microorganisms. This is because it is cheap and easy to find.

The application of graded concentrations of bleach solution method 1 (25%, 20%, 15%) has been demonstrated to effectively eliminate contamination in small-sized rodent tuber materials, reducing it to 0%. NaOCl can effectively eliminate contamination due to its potent oxidizing properties. Within the sterilization solution, the hypochlorite ion (OCl^-) it generates reacts with the cellular structures of microorganisms, including proteins, nucleic acids, and lipids, leading to their disruption and damage. Moreover, NaOCl can alter the structure of microbial proteins through denaturation, thus impeding the functionality of proteins crucial for microbial survival. This denaturation results in structural and functional impairment, ultimately hindering microbial viability and reproduction. In contrast, the application of bleach solution at graded concentrations in low concentration (methods 3 and 4) has proven insufficient in effectively reducing contamination levels. These concentrations of bleach solution lack the potency to thoroughly eradicate the cell membranes of contaminants and disrupt their DNA, proteins, and other components.

However, despite employing commercial cleaning solutions in method 1, contamination by bacteria persisted, with an average rate of 2.5%. This occurrence can be attributed to the presence of endophytic bacteria within the rodent tuber plant, which live symbiotically within its tissues. Lekatompessy (2023) documented the presence of 25 endophytic bacteria in rodent tuber plants. These bacteria have the potential to act as contaminants in in-vitro cultures. Efforts to mitigate endophytic bacterial contaminants in the sterilization process of rodent tuber plants can be addressed through the addition of antibiotics, which interfere with bacterial cell wall synthesis, and nucleic acid synthesis, and disrupt bacterial cellular metabolism.

The application of graded concentrations of bleach solution reduces the survival rate of rodent tuber plants. The higher the concentration of bleach solution, the lower the survival rate becomes. This occurs because elevated levels of NaOCl can induce greater damage to the cell structure and essential components of the plant. NaOCl, functioning as a potent oxidative agent, can inflict damage upon proteins, nucleic acids, and cell membranes of microorganisms, including rodent tuber plants. Consequently, the plant cells undergo significant damage or even perish, diminishing the plant's capacity to survive and thrive. Plants subjected to method 1 exhibited the lowest survival rate, recorded at 88.29%. Nevertheless, the survival rate remains relatively high (>80%).

4. Conclusion

Overall, this study demonstrated that choosing the right graded concentration of commercial bleach solution was able to suppress contamination. The graded concentrations of 25%, 20%, 15% yielded the best results with the low contamination rate (average 2.5%) and a high survival rate (average 88.29%). In contrast to size materials, there were no differences in results between small and large material sizes. Material sizes did not have a significant effect on contamination level, all the same, large size materials increased the survival rate of sterilized rodent tuber plants. Contaminants in rodent tuber plants were generally in the form of bacteria. The addition of antibiotics on media or during the sterilization process had the potential to suppress this type of contamination. However further studies were needed to confirm this.

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References

Badhane, B.S. and R.H. Patil. (2016). Data on the cost effective surface sterilization method for *C.carandas* (L.) seeds and callus induction from aseptic seedling. *Data Brief*, 7: 1551–1555. https://doi.org/ 10.1016/j.dib.2016.04.047.

Bhatia, S., and K. Sharma. (2015). Chapter 11 - Micropropagation, Editor(s): Saurabh Bhatia, Kiran Sharma, Randhir Dahiya, Tanmoy Bera, Modern Applications of Plant Biotechnology in Pharmaceutical Sciences. Academic Press: 361-368, ISBN 9780128022214. https://doi.org/10.1016/B978-0-12-802221-4.00011-X.

Boruah, J. (2020). Effect of 0.1% HgCl on surface sterilization of *Som* (*Persea bombycine* King) explant during tissue culture - a major host plant of muga silkworm. *Int. J. Curr. Microbiol. App. Sci.,* 9(7): 954-958. https://doi.org/10.20546/ijcmas.2020.907.111.

Chen, L.R., T.C. Hsiung, K.H. Lin, T.B. Huang, M.Y. Huang, and A. Wakana. (2017). Supplementary effect of hydrogen peroxide as a pre-disinfectant for sterilizing rhizome bud explant of *Zantedeschia aethiopica* L. with chlorine dioxide. *J. Fac. Agr., Kyushu Univ*, 62(1): 81-86. https://doi.org/10.5109/1799306.

Chika, S., L. Ismaini, and D.T. Armanda. (2022). Explant sterilization technique Castanopsis argentea (Blume) A. DC. with the addition of ascorbic acid and sodium hypochlorite (NaOCl) In Vitro. *Berkala Ilmiah Biologi*, 13(2): 32-41. https://doi.org/10.22146/bib.v13i2.4692.

Elbasheer, M.M.A., A.G.A. Alkhidir, S.M.A. Mohammed, A.A.H. Abbas, A.O. Mohamed, I.M. Bereir, A.R. Abdalazeez, and M. Noma. (2019). Spatial distribution of breast cancer in Sudan 2010-2016. *PLoS ONE* 14(9): e0211085. https://doi.org/10.1371/journal. pone.0211085.

Ersoy, Z.G., O. Dinc, B. Cinar, Gedik, and A. Dimoglo. (2019). Comparative evaluation of disinfection mechanism of sodium hypochlorite, chlorin dioxide and electroactivated water on *Enterococcus faecalis*. LWT, 102: 2015-2013. https://doi.org/10.1016/j.lwt.2018.12.041.

Espinosa-Leal, C.A., C.A. Puente-Garza, and S. García-Lara. (2018). In Vitro plant tissue culture: means for production of biological active compounds. *Planta*, 248(1): 1-18. doi: 10.1007/s00425-018-2910-1.

Hashim, S.N., S.Z. Ghazali, N.J. Sidik, T.C. Chay, and A. Saleh. (2021). Surface sterilization method for reducing contamination of *Clinacanthus nutans* nodal explants intended for *in-vitro* culture in the proceeding of the First International Conference on Assessment and Development of Agricultural Innovation (1st ICADAI 2021): *E3S Web of Conferences 306: 01004.* https://doi.org/10.1051/e3sconf/202130601004.

Hussain, A., I. Ahmed, H. Nazir, and I. Ullah. (2012). Plant tissue culture: current status and opportunities. *InTech*. https://doi.org/10.5772/50568.

Kavya, V., R. Darla, N.P. Kalyan, H.N. Bai, and N. Kavitha. (2021). Recent advances of plant tissue culture in pharmaceuticals and opportunities. *Int. J. of Allied Med. Sci. and Clin. Research*, 9(2): 320-335. https://doi.org/10.61096/ijamscr.v9.iss2.2021.320-335.

Kaya, Y., and F. Z. Huyop. (2020). An easy and reliable method for establishment and maintenance of tissue cultures of nicotiana tabacum cv TAPM 26. *Int. J. Sci. Lett*, 2(2): 62–71. doi: 10.38058/ijsl.764947.

Kidasi, P.C., D.C. Kilalo, and A.W. Mwang'ombe. (2023). Effect of sterilant and plant growth regulators in regenerating commonly used cassava cultivars at the Kenyan coast. *Heliyon*, 9(6): e17263, ISSN 2405-8440, https://doi.org/10.1016/j.heliyon.2023.e17263.

Kidus, T., and Z. Teka. (2020). Isolation, characterization and identification of contaminant bacteria from surgacane (*Saccharum officinarum* L.) in vitro culture in tigray biotechnology center, Mekelle, Ethiopia. *J Bacteriol Parasitol*, 11: 372. https://doi.org/10.35248/2155-9597.20.11.372.

Lekatompessy, S.J.R., S.A. Puteri, R. Simarmata, T. Widowati, E. Erdayani, V.E. Windiastri, A.N. Nurhasanah, D. Widyajayantie, L. Nurjanah, Nuryanah, B.S. Maulana, I. Erlinawati, and S. Wahyuni. (2023). Isolation and characterization of extracellular enzymes from endophytic bacteria isolated from *Typhonium flagelliforme* and *Typhonium blumei* in the proceedings of the 8th International Symposium of Innovative Bioproduction Indonesia on Biotechnology and

Bioengineering 2021: Global Platform on Biodiversity and Biotechnology. *AIP Conf. Proc.* 2606, 020012. https://doi.org/10.1063/5.0118845.

Malwattage, G., R. Ratnayake, Peiris, Colin, Peiris, Sriyani, Seneviratne, dan Kasun. (2021). A novel surface sterilization technique for in vitro establishment of dianella tasmanica variegata nodal explants. Proceedings of National Symposium on Floriculture Research (NaSFloR), pp. 08-13.

Mankaran, S., K. Dinesh, S. Deepak, and S. Gurmeet. (2013). *Typhonium flagelliforme*: A multipurpose plant. *Int. Res. J. Pharm,* 4(3): 45-48. https://doi.org/10.7897/2230-8407.04308.

Mihaljevic, I.K., V. Dugalic, M. Tomas, A. Viljevac, Z. Pranjic, B. Cmelik, Puskar, and Z. Jurkovic. (2013). In Vitro sterilization procedures for micropropagation of 'Oblacinska' Sour Cherry. *J. Agric. Sci. Belgrade,* 58: 117–126. https://doi.org/10.2298/JAS1302117M.

Norouzi, O., M. Hesami, M. Pepe, A. Dutta, and A.M.P. Jones. (2022). In Vitro plant tissue culture as the fifth generation of bioenergy. *Scientific Reports*, 12: 5038. https://doi.org/10.1028/s41598-022-09066-3.

Pais, A.K., A.P. da Silva, J.C. de Souza, S.L. Teixeira, J.M. Ribeiro, A.R. Peixoto, and C.D. da Paz. (2016). Sodium hypochlorite sterilization of culture medium in micropropagation of *Gerbera hybrida* cv. Essandre. *African J. of Biotech*, 15(36): 1995-1998. https://doi.org/ 10.5897/AJB2016.15405 .

Parzymies, M. (2021). Nano-silver particles reduce contaminations in tissue culture but decrease regeneration rate and slows down growth and development of Aldrovanda vesiculosa explants. *Appl. Sci*, 11: 3653. https://doi.org/10.3390/app11083653.

Peiris, S.E., H. Hemathilaka, C.N. Peiris, and A.W. Wijeratne. (2020). Effects of silver nitrate in in vitro establishment of *Cordyline fruticosa* (L.) cv 'Purple compacta Tri Colour' with CSUP Media sterilization in proceedings of international conference on advancement in science and humanities. Presented at the International Conference on Advancement in Science and Humanities, Sri Lanka Institute of Information Technology (SLIIT), pp. 83–88.

Pratiwi, D.R., S. Wening, E. Nazri, and Y. Yenny. (2021). The use of alcohol and sodium hypochlorite as single sterilizing agents for oil palm explant sterilization. *J. Pen. Kelapa Sawit*, 29(1): 1-10. https://doi.org/10.22302/iopri.jur.jpks.v29i1.120.

Quynh, T.P., X.L. Thi Hoang, T.A. My, and N.T. Quang. (2022). Effect of calcium hypochlorite on surface sterilization and seedling growth of Vietnamese coconut varieties. *Vietnam Journal of Biotechnology*, 20(4): 663-673.

Rahayu, E.S., T. Widiatningrum, L. Herlina, N. Hermayani, and A.R. Amalia. (2019). The optimal sterilizing compound and culture medium in *Elaeocarpus grandiflorus* L. In Vitro Shoot Induction. *J. Phys.: Conf. Ser*. 1321 032040. https://doi.org/10.1088/1742-6596/1321/3/032040.

Sianipar, N.F., Ariadana, and Maarisit. (2015). Detection of gamma-irradiated mutant of rodent tuber (*Typhonium flagelliforme* Lodd.) In Vitro culture by RAPD molecular marker. *Procedia Chemistry*. 14: 285–294. http://doi.org/10.1016/j.proche.2015.03.040.

Si, Y., Y. Haxim, and L. Wang. (2022). Optimum sterilization method for In Vitro cultivation of dimorphic seeds of the succulent halophyte *Suaeda aralocaspica*. *Horticulturae,* 8(4):289. https://doi.org/10.3390/horticulturae8040289.

Silva, J.A.T., J. Winarto, Dobránszki, and S. Zeng. (2015). Disinfection procedures for In Vitro propagation of Anthurium. *Folia Hortic*. 27: 3–14. https://doi.org/10.1515/fhort-2015-0009.

Suman, S. (2017). Plant tissue culture: a promising tool of quality material production with special reference to micropropagation of banana. *Biochem. Cell. Arch.,* 17(1): 1-26.

Zhao, D., Y. Zhang, Z. Jin, R. Bai, J. Wang, L. Wu, and Y. He. (2024). Benzalkonium chloride and benzethonium chloride effectively reduce spore germination of ginger soft rot pathogens: *Fusarium solani* and *Fusarium oxysporum*. *Journal of Fungi*, 10(1): 8. https://doi.org/10.3390/jof10010008.