Düzce University Faculty of Forestry
Journal of Forestry
(DUJOF)

JUDOF Volume 20, Special Issue, pp. 342-356
Category: Research Article

https://dergipark.org.tr/tr/pub/duzceod
ISSN 2148-7855 (online), ISSN 2148-7871
Düzce University Faculty of Forestry
DOI:10.58816/duzceod.1559242

# The Role of Alternative Methods of Nutrient Medium Sterilization in Micropropagation of *Gardenia jasminoides*

## Besin Ortamı Sterilizasyonunda Alternatif Yöntemlerin *Gardenia jasminoides*'in Mikroçoğaltımındaki Rolü

**Elif Nur KABADAYI[1], Emrah ZEYBEKOĞLU[1,*], John L GRIFFIS Jr.[2], Mustafa Ercan ÖZZAMBAK[1]**

**Abstract**

The aim of this study was to develop alternative sterilization methods that could replace the conventionally used autoclave device for sterilization of nutrient medium used in gardenia micropropagation. These alternative methods may, at the same time, eliminate some negative changes that can occur in nutrient medium because of autoclaving. Either microwave irradiation for differing times or chemical disinfectants in differing doses were utilized as alternative sterilization methods for the nutrient medium. These were compared with standard autoclave methods in the sterilization of the medium. In vitro Gardenia jasminoides shoots were used for all cultures. Microwaves were applied to the medium for 6, 8, and 10 minutes. Growth of gardenia shoots cultured on the autoclaved nutrient medium was significantly better compared to shoots grown on medium sterilized with the 10 minute microwave treatment in terms of shoot length, fresh and dry weight and shoot quality. In the second experiment, no pre cultured contamination occurred in the nutrient media in which either NaOCl was added at four concentrations of 0.002% -0.02% active chlorine or NaDCC was added at four dosages of 25-200 mg /l as sterilization agents. It has been determined that sterilization of nutrient media with either all concentrations of NaOCl or NaDCC at the low concentrations used in this study could be used as an alternative sterilization method to the autoclaving of nutrient media.

**Keywords:** Microwave sterilization, Autoclaving, Sodium hypochlorite, Sodium dichloroisocyanurate, Contamination

**Özet**

Bu çalışmanın amacı gardenyanın mikroçoğaltımında besin ortamlarının sterilizasyonunda geleneksel olarak kullanılan otoklavın yerini alabilecek alternatif sterilizasyon yöntemlerinin geliştirilmesidir. Bu alternatif yöntemlerin, aynı zamanda otoklavlama nedeni ile besin ortamında meydana gelebilecek bazı olumsuz değişiklikleri ortadan kaldırması da beklenmektedir. Çalışmada farklı sürelerde mikrodalga uygulaması ve farklı dozlarda kimyasal dezenfektanlar kullanılmış ve standart otoklavlama yöntemleri ile karşılaştırılmıştır. Tüm kültürler için *Gardenia jasminoides* sürgünleri kullanılmıştır. Ortalama 6, 8 ve 10 dakika mikrodalga uygulanmıştır. Otoklavlanmış besin ortamında kültüre alınmış sürgünlerin büyümesinin, sürgün uzunluğu, taze ve kuru ağırlık ve sürgün kalitesi açısından 10 dakikalık mikrodalga işlemiyle sterilize edilen ortamda yetiştirilen sürgünlere kıyasla daha iyi olduğu görülmüştür İkinci denemede besin ortamlarında dört farklı dozda NaOCl veya dört dozda NaDCC eklendiğinde kontaminasyon meydana gemediği belirlenmiş, bu çalışmada Kullanılan tüm Na=cl veya düşük konsantrasyonlarda NaDCC ile besin ortamının sterilizasyonunun besin ortamının otoklavlanmasına alternatif bir sterilizasyon yötemi olarak kullanılabileceği belirlenmiştir.

**Anahtar Kelimeler:** Mikrodalga sterilizasyon, Otoklavlama, sodyum hipoklorit, Sodyum dikloroizosiyanürat, Kontaminasyon

Received: 01.10.2024, Revised: 02.11.2024, Accepted: 13.11.2024
Address: [1]Ege University, Faculty of Agriculture, Department of Horticulture
E-mail: emrah.zeybekoglu@ege.edu.tr
[3]Florida Gulf Coast University, Department of Ecology and Environmental Studies

## 1. Introduction

Sterilization of the nutrient medium is one of the basic principles of plant tissue culture. The sterilization of the nutrient medium is usually carried out with an autoclave, which is set conventionally and universally at 121˚C temperature and at 1.05 kg/cm2 pressure conditions for 15-20 minutes for small volumes of medium (Kothari, et al., 2011). However, the sterilization time, although it seems to be rather short, is actually quite long when the preheating of the autoclave and the cooling times required for depressurizing before opening of the cover after sterilization are considered (Prijana, et al., 2016). In addition to these, the autoclave is not only an expensive apparatus, but also its high energy consumption is an important factor that increases operating costs in classical tissue culture technique (Tiwari, et al., 2012, George and Manuel, 2013). Sterilization by autoclaving can have negative effects on heat sensitive substances such as 2IP, gibberellic acid, zeatin, IAA, sugar (carbohydrate hydrolysis) and it also decreases the pH of the nutrient medium and may create precipitation and gelling problem due to agar depolymerization (Pierik, 1989, Schenk, et al., 1991, Narasimhan and Simran, 2021). Autoclaved medium may have toxic effects on in vitro plant growth (Buter, et al., 1993, Sawyer and Hsiao, 1992, Wang and Hsiao, 1995).

One of the topics that have been emphasized and studied in tissue culture and micropropagation in recent years has been the investigation of low-cost options (Purohit, et al., 2011, Datta, et al., 2017). In addition to reducing investment costs, lowering high energy costs are one of the issues focused on in these studies. Sterilization of the nutrient medium with alternative methods instead of autoclaving could reduce the costs of medium preparation and also eliminate the possible negative effects of autoclaving on the medium. Therefore, various alternative methods are required in the sterilization of the nutrient medium (Mvuria and Ombori, 2014, Biswas and Biswas, 2017). Sterilization of the nutrient medium with microwaves has been used in micropropagation of plants such as orchids and bananas (Vora and Jasrai, 2012, Venturieri, et al., 2013). Microwave energy is high frequency radio waves. Microwaves vibrate water molecules with high speed, temperature rises with friction, and bacteria, viruses and fungi become inactivated; sterilization takes place.

Microwave radiation is also an effective method commonly used in the sterilization of plastic culture containers (Sanborn, et al., 1982) and various laboratory supplies. The use of inexpensive household microwave ovens is another advantage of this method, but sterilization time is a critical parameter in practice (Hicks, 2009). Chemical substances such as chlorine dioxide, sodium hypochlorite, calcium hypochlorite, mercury chloride, hydrogen peroxide,

peracetic acid, sodium dichloroisocyanurate, biocides, and methylchloroisothiazolinone are used to prevent contamination in the nutrient medium (Özzambak and Zeybekoğlu, 2020, Thepsithar, et al., 2013).

Sterilization of the nutrient medium without autoclaving, using sodium hypochlorite for gerbera (Pais, et al., 2016), orchid (Yanagawa, et al., 2006, Thepsithar and Thongpukdee, 2013), eucalyptus (Porondani, et al., 2013), carnation, chrysanthemum, orchids (Yanagawa, et al., 2016), pineapple (Teixera, et al., 2006) with chlorine dioxide and peracetic acid for gerbera (Cardoso and Imthurn, 2018), sodium dichloroisocyanurate, calcium hypochlorite, chlorine bleaching agent with phalaenopsis, chrysanthemum (Yanagawa, et al., 2006) was carried out. In addition to these, there are substances that are used in the sterilization of the nutrient medium such as plant essential oils that could be included in the group of chemical substances. Bergamot oil, betel oil, cinnamon oil, lavender oil, tumeric oil alone and together with 10% povidone-iodine or 2% iodine + 2.4% potassium iodide were used in the sterilization of chrysanthemum in vitro culture media (Thepsithar, et al., 2013). The type and concentration of chemical substances included in the media are the most important factors that determine both sterilization efficiency and in vitro plant growth.

The principal aim of this study was to determine the efficacy of either microwave energy application time or the efficacy of chlorine-based chemicals and their dosages on the sterilization of nutrient media. Some possible effects of the alternatively-sterilized nutrient media on the growth of Gardenia jasminoides in vitro shoots were also determined. Gardenia shoots grown on the alternatively-sterilized media were compared for similarities and differences to shoots grown on similar nutrient media that was autoclave sterilized.

## 2. Material and Method

This study was carried out at Ege University Faculty of Agriculture, Department of Horticulture, Tissue Culture Laboratory. Gardenia jasminoides in vitro shoots grown on a nutrient medium containing MS macro- and micronutrients supplemented with 2.0 mg/l BAP, 0.1 mg/l NAA, 150 mg/l adenine sulfate, 30g/l sucrose and 7 g/l agar (Duchefa Biochemie, the Netherlands) were used as plant stock material for the experiments.

The study was conducted in two trials. In the first experiment, microwave energy application times (6, 8, 10 min) for the sterilization of the MS nutrient medium was compared with autoclave sterilization. The control nutrient medium was sterilized in the autoclave at 121 °C and 1.05 kg/cm2 pressure conditions for 20 minutes, then it was poured into sterile culture

containers in a laminar flow cabinet. For the application of microwave energy, a 1000W household microwave oven was used and the 250 ml of modified MS medium were sterilized for the different durations in 500 ml flasks, then poured into sterile culture tubes in a laminar flow cabinet. The MS nutrient medium for this first experiment was supplemented with 2.0 mg/l BAP, 0.1 mg/l NAA, 30g/l sucrose and 7g/l agar (Duchefa Biochemie, the Netherlands). In both the microwaved and autoclaved nutrient medium, the pH of the medium was adjusted to 5.8 with 1.0N NaOH and 0.1N HCl before addition of agar. Medium contamination rates were noted as were any medium treatment effects on in vitro growth of gardenia.

In the second experiment, the efficacy of chlorine-based chemicals added to nutrient medium at various dosages for the purpose of sterilizing the medium was investigated. Driver and Kuniyuki Walnut (DKW) nutrient medium supplemented with the same plant growth regulators as used the first experiment (2.0 mg/l BAP + 0.1 mg/l NAA, 30g/l sucrose, 7g/l agar) was used in this experiment (Driver and Kuniyuki, 1984).

Laundry bleach Klorak (Klorak Kimya Ve Temizlik Ürünleri Sanayi Ticaret A.Ş, Izmir Turkey) that has 5% w/v active chlorine was used for the source of sodium hypochlorite (NaOCl) in these experiments. Aliquots of bleach solution at 0.4 ml, 1.0 ml, 2.0 ml, or 4.0 ml were added to the 1000 ml nutrient medium and the final active chlorine concentrations were of 0.002%, 0.005%, 0.01%, and 0.02% (w/v). Sodium dichloroisocyanurate (NaDCC) at aliquots of 25 mg/l, 50 mg/l, 100 mg/l, and 200 mg/l were added to the DKW nutrient medium, both chemicals were added to the nutrient medium before the agar solidified (medium was 45-50 °C). After mixing the chlorine-based chemicals with the hot liquid nutrient medium thoroughly, the pH of the medium was adjusted to 5.8 and the medium was poured into sterile culture containers in a laminar flow cabinet. The 250 ml control nutrient DKW medium was sterilized in the autoclave at 121°C and 1.05 kg/cm2 pressure conditions for 20 minutes and was poured into sterile culture containers (jars) in a laminar flow cabinet.

Fourteen gardenia small shoot tips (1-2 mm) were transferred in the first experiment (Fig.1), and 6-8 mm larger shoot tip explants were cultured in the second experiment for each replication. In vitro cultures were placed under light conditions of 24 ± 1°C, a 16-hour photoperiod with daylight fluorescent lamps and 30-35 micromole m-2s-1.

For both experiments, the sterility rate of the nutrient medium was evaluated after the first two weeks after the sterilization procedures, but before inoculation with gardenia explants. Proliferation rate, shoot length, number of leaves, total shoot fresh and dry weight, and shoot quality-appearance of gardenia were the parameters recorded six weeks after inoculation. In

microwave sterilization, evaporation loss during boiling was determined by measuring with a sterile graduated cylinder after the application period. Shoot quality in vitro was determined by scoring on a scale of 1 = poor to 5 = very good (Parkinson, et al., 1996, Özzambak, et al., 2018).

The trials were arranged in four replications in a randomized block experimental design. All computation and statistical analyses were done using IBMR SPSS Statistics 19, a statistical software (IBM, NY, USA). Significant differences between the means were determined employing Duncan's multiple range tests at $p< 0.05$ or $p< 0.01$.

## 3. Results

### 3.1. First experiment (Medium microwaved or autoclaved)

a) Effect of irradiation time of nutrient medium sterilized on evaporation losses

As the erlenmeyer flasks containing medium were not tightly covered with stretch film, it was determined that with prolonged microwave irradiation time, volumetric losses increased as a result of evaporation with boiling of the nutrient medium. There was 15.6% evaporation loss in the nutrient medium irradiated in the microwave oven for 6 minutes and 28.4% in the medium where the microwave time was 8 minutes. A high rate of 40.5% loss occurred in the nutrient medium kept at full power for 10 minutes. There was no volumetric change in the autoclave sterilized medium.

b) Effect of microwave sterilization time on pre-culture contamination rate

Before inoculation, contamination was observed in microwaved sterilized media for all microwave application times. Contamination rates of 46.1%, 26.9% and, 30.8% were determined at 6, 8 and, 10 minutes of microwave sterilization times, respectively. No contamination occurred in the autoclaved nutrient medium.

c) Effect of microwave sterilization time on the growth of in vitro gardenia shoots

The application time of microwave radiation did not make a significant difference in terms of the number of shoots per explant and the number of leaves per explant compared explants grown on media that was autoclaved, and all applications were statistically included in the same group with autoclaving and sterilization (Table 1).

The autoclaved nutrient mediums created a statistically significant difference in the development of gardenia shoots in terms of shoot length, fresh and dry weight, appearance-quality criteria compared to shoots grown on 10 min. microwave application medium (Table 1). It was an important disadvantage that the shoots fell behind autoclaved media in terms of appearance and quality in the microwaved nutrient medium (Fig. 1).

### 3.2. Second experiment: (Chlorine-based chemical sterilization)

a) The effect of chemical materials and dosses on contamination and solidification of the nutrient medium

It was determined that NaDCC (Sodium dichloroisocyanurate) and NaOCl (Sodium hypochlorite) added to the nutrient medium at different concentrations provide 100% sterilization compared with the autoclaved nutrient medium, no contamination occurs and they have no negative effects on the gelling of the nutrient medium.

b) The effect of the chemical substances and dosages on in vitro growth of gardenia shoots

The effects of the NaOCl sterilized media on the in vitro growth of gardenia shoots and the leaf number of the shoots were found to be insignificant compared to the autoclaved medium (Table 2). In the shoot length criterion, the longest shoots (40.1 mm) were obtained with NaOCl 0.02% active chlorine concentration. Other NaOCl treatments also made a difference in shoot length at the level of $p \leq 0.05$ compared to the autoclaved nutrient medium. The fact that increasing concentrations of NaOCI had no negative effects on development and fresh-dry weight of the explants creates an important advantage in practice.

Shoots grown in nutrient medium sterilized with different concentrations of NaDCC material were generally inferior in terms of height, shoot quality, fresh and dry weight values compared to the explants grown on NaOCI-added nutrient medium (Table 2, Fig 3). Shoot length and shot quality were negetively by increasing doses of NaDCC (50-100 mg/l) compared to autoclaved medium. There were no any important differences between in all treatments in multiplication rate and leaf number.

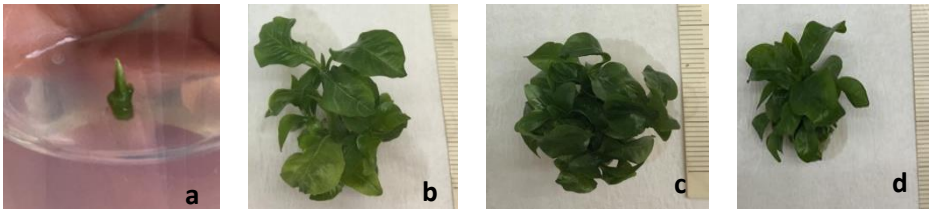


**Figure 1.** Cultured shoot tip explant and Gardenia shoots on autoclaved(A) and microwaved medium(B,6min; C,10 min) in the first experiment

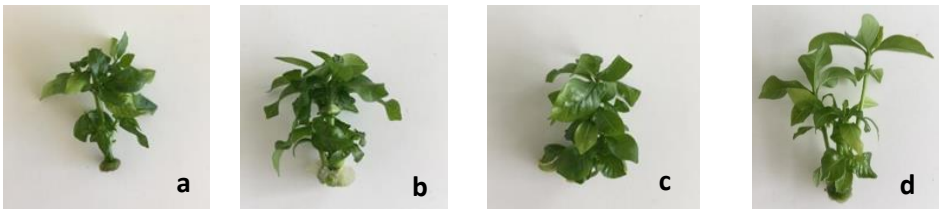


**Figure 2.** Growth of gardenia shoots on chlorine sterilized media with NaOCl contains different doses of (a,0.002; b,0.005; c,0.01; d,0.02 m/v )active Chlorine and NaDCC (e,25; f, 50; g, 100; h, 200 mg/l) and autoclaved sterized media (i)

**Table 1.** Effect of microwave sterilization time on the growth of in vitro gardenia shoots

| | **Shoot length (mm)** | **Multiplication rate (shoot/expl)** | **Leaf number** | **Shoot quality** | **Shoot fresh weight (mg/expl.)** | **Shoot dry weight (mg/exp.)** |
|---|---|---|---|---|---|---|
| Autoclaved | 34,3 ±5,21 a | 10,3± 2,1 | 54,9±10,2 | 3,8±0,7a | 967,2± 205,7 a | 106,4 ± 19,5 a |
| Microwaved 6 min. | 22,9±2,0 ab | 9,7 ± 2,6 | 50,6±13,1 | 2,3±0,3 b | 674,4±145,5 ab | 77,7± 13,1 ab |
| Microwaved 8 min. | 24,2±2,1 ab | 8,5 ± 2,1 | 53,0±5,3 | 2,5± 0,2 b | 489,6±100,2 ab | 61,2 ± 8,1 ab |
| microwaved 10 min. | 22,2±1,4 b | 9,8 ± 4,1 | 56,8±15,9 | 2,3±0,3b | 456,3 ± 51,6 b | 57,7± 6,1 b |

Mean values followed by the same letter in the column do not differ statistically in Duncan's MRT with 5% significance.

**Table 2.** Effect of NaOCl doses contain different active chlorine concentration and NaDCC (mg/l) on growth parameters of in vitro G. jasminoides shoots.

| **Chlorine agents** | **Shoot length (mm)** | **Multiplication rate (shoot/exp)** | **Total Leaf number** | **Shoot quality** | **Fresh weigth (mg/exp)** | **Dry weigth (mg/expl)** |
|---|---|---|---|---|---|---|
| NaOCl (0,002% ac. Chl.) | 30,8±0,8 ab | 6,8 ± 0,9 | 40,2 ± 3,8 | 3,6±0,1 ab | 368,5±15,7 ab | 61,0 ± 5,1 ab |
| NaOCl (0,005% ac. Chl.) | 28,2±1,6 b | 7,3 ± 0,2 | 43,2 ± 2,9 | 3,5 ± 0,1 ab | 330,5±13,3 ab | 52,6±2,9 abc |
| NaOCl ( 0,01% ac. Chl.) | 29,7±1,1 b | 9,8 ± 3,7 | 46,1 ± 1,5 | 3,6 ± 0,3 a | 339,2±55,1 ab | 56,2±11,2 abc |
| NaOCl (0,02% act. Chl) | 40,2±3,4 a | 7,6 ± 2,2 | 44,2 ± 9,0 | 4,4 ±0,3 a | 464,5±52,3 a | 71,8±6,13 a |
| NaDCC (25 mg/l) | 15,0±2,2 cd | 10,9 ± 1,3 | 53,9 ± 6,0 | 2,5 ± 0,2 cd | 363,0±39,0 ab | 43,6±4,6 bcd |
| NaDCC (50 mg/l) | 14,1±2,2 cd | 10,7 ± 1,9 | 50,3 ± 4,5 | 2,6±0,2 bcd | 326,7±51,5 ab | 38,2±4,4 cd |
| NaDCC5 (100 mg/l) | 10,1±0,5 de | 9,1 ± 2,7 | 41,1 ± 3,3 | 2,1 ± 0,5 d | 263,3±32,8 b | 29,6±4,5 d |

| | | | | | | |
|---|---|---|---|---|---|---|
| NaDCC (200 mg/l) | 6,7 ±1,0 e | 9,2 ± 1,1 | 49,4 ± 0,4 | 1,7 ± 0,2 d | 322,1±55,3 ab | 32,7±5,01 d |
| Autoclaved | 19,0±1,3 c | 8,6 ± 2,1 | 50,0 ± 8,3 | 3,4±0,2 abc | 285,4 ± 32,9 b | 38,0±3,3cd |
| Duncan | ** | ns | ns | ** | ** | ** |

Mean values followed by the same letter in the column do not differ statistically in Duncan's MRT with 5% significance.

## 4. Discussion

In our study, 25-45% evaporation losses and a very high contamination rate were determined as one of the most important disadvantages of the microwave sterilization method. Similarly, Venturieri, et al. (2013) stated that there was a contamination problem in microwave and sterilization under 15 minutes of sterilization time, and to overcome this, 2ml/l 30% H2O2 should be added to the medium or the nutrient medium could be sterilized without contamination with a household microwave oven for more than 15 minutes. However, Vora and Jasrai (2012) successfully achieved sterilization of 200 ml of medium in four minutes with 900W power microwave oven. From these results, the most important criteria in microwave sterilization are the volume of the medium to be sterilized, the application time and the electrical power of the device (Youssef and Amin, 2001, Hicks, 2009). The difference between the reports and our results may have been caused by these factors. In these articles, there were no conclusions about the evaporation losses caused by microwave sterilization, but this drawback should be taken into account and final medium volume changes should be prevented and/or volume decreases of the nutrient medium should be regulated before sterilization. Addition of extra quantities of water may be required for smaller medium volumes before microwave sterilization for these medium volumes to arrive at the final correct volume after microwave treatment. The amount of additional water needed can only be determined by trial and error with selected containers, medium volumes and specific microwave units, there are no generally correct supplemental amounts. It has also been reported that there may be problems such as boiling and overflow in the microwave sterilization procedure (Venturieri, et al., 2013). It is thought that the contamination problem seen in microwaved medium may arise from the fact that some areas within the liquid medium are not fully affected by irradiation (Vadivambal and Jayas, 2010).

Our results, that there were no significant differences in shoot proliferation between explants grown on either autoclaved or microwaved media, are not in agreement with the findings of either Youssef, et al. (2001) or Vora and Jasrai (2012) who reported a higher multiplication rate

for explants grown on microwave media for populus and banana respectively. However, for explants grown on the autoclaved medium, shoot size, fresh weight, dry weight, and shoot quality criteria were superior compared to explants grown on medium with prolonged microwave radiation. These results are partially similar to Tisserat, et al. (1992), Arditti (2008), Venturieri, et al. (2013).

Sterilization of the medium with chemical agents was also one of the alternative sterilization methods. Yanagawa, et al. (2006) successfully sterilized nutrient medium with chlorine-based disinfectants. It was reported that 0.005%-0.001% active chlorine could be used in chrysanthemum, carnation, cymbidium, and phalaenopsis micropropagation cultures without contamination and without damaging the tissues. (Yanagawa, et al. 2006)

Similarly, Brondani, et al. (2013) reported that there were no statistical differences between autoclaved medium and medium sterilized with different dosages of NaOCI in terms of bacterial and fungal contamination and the number of shoots per explant and explant shoot length criteria were also not different for explants where 0.001% to 0.003% active chlorine concentrations were sufficient for medium sterilization. Teixeira, et al. (2006) reported that contamination was prevented and the number of shoots and fresh weight in pineapple explants doubled compared to pineapple explants grown on autoclaved medium.

However, Ribeiro, et al. (2011) explained that satisfactory sterilization of the nutrient medium was achieved only at a higher NaOCl concentration (0.003%). Concerning shoot length for cultured Sequoia sempervirens, they obtained longer branches from explants grown on the nutrient medium sterilized in the autoclave, but all of the explants grown on medium sterilized with different dosages of NaClO produced a significantly greater number of branches. Combining these figures, there would be 13.75cm of total branch for the NaClO sterilized medium that presented the best results and 11.9cm of total branch for the treatment in which the nutrient medium was autoclaved - values favorable to the NaClO sterilized medium.

In addition, in the study conducted with Gerbera hybrida ‘Essandre’, it was found that contamination was completely controlled with a 0.003% active chlorine concentration. There were no significant differences in the number of leaves, number of roots, or shoot length between the plantlets growing on the autoclaved nutrient medium and those growing on the medium sterilized with NaOCl, but plants grown on autoclaved medium were superior in terms of dry matter. (Pais, et al. 2016).

The results obtained from this experiment were consistent with the reports described above. In the present study, the active chlorine concentration provided by NaOCl added to the nutrient medium was between (0.005% -0.015%). Since this dosage range was above the previously

recommended dosage of 0.003% active chlorine, there were no problems with the sterility or contamination of the medium.

In vitro gardenia plantlets growing in NaOCl-supplemented medium were not adversely affected by increasing active chlorine concentrations. Shoot length, growth quality, and fresh and dry weights observed in gardenia explants grown on medium supplemented with 0.015% active chlorine concentration indicated that the gardenia micro shoots were not affected by increasing chlorine concentration (at least within all concentrations in this trial). The superiority of NaOCl-supplemented media over autoclaved media is in agreement with the reports of Yanagawa et al. (2006), Teixeira et al. (2006), Ribeiro, et al. (2011).

However, the reports of Pais, et al. (2016) and Broydani, et al. (2013) with gerbera daisy and with eucalyptus, respectively and our results with gardenia in vitro do not exactly match. The reported results with gerbera daisy and eucalyptus showed that depending on the plant species, NaOCl applications and concentrations may have differing effects on in vitro growth. Gardenia plantlets were not affected by increasing active chlorine concentration in our experiments.

In this research project, another chemical added to the nutrient medium for sterilization was NaDCC, which is used for the disinfection of both drinking water and the water in swimming pools, as well as food industry surface sterilization (WHO, 2008). This substance was found to be more effective than NaOCl in explant surface disinfestation in tissue culture, and when 0.01% (w/v) NaDCC was added to the medium to sterilize it, both the growth rate and the quality of main and side shoots of Spathiphyllum plantlets were found to be better than for plantlets grown on medium sterilized with NaOCl (Parkinson, et al. 1996).

Yanagawa, et al., (2006) used NaDCC and other chlorine-containing compounds in chrysanthemum, carnation, phalaenopsis, cymbidium culture, and they reported that all Cl-containing substances provide sterilization of the nutrient medium and did not cause damage to plant tissues when compared to plants grown on autoclaved media. In our study, NaDCC medium sterilization was as effective as either autoclaving or NaOCl medium sterilization; complete sterilization was achieved. This efficacy of NaDCC in sterilization is consistent with the previously-mentioned reports. Narasimhan and Shetty, (2021) explained that NaDCC provides superficial sterilization of the nutrient medium and explants due to its effect on bacteria and fungi.

Since the in vitro shoot quality and growth (length-dry weight) of gardenia explants grown in the NaDCC medium lagged behind explants grown on the NaOCl medium, our results do not exactly match the results of Parkinson, et al. (1996). On the other hand, another important

advantage of NaDCC is that it decomposes into ammonia and CO2 easily and without harming the environment and it is very affordable and readily available (Narasimhan and Shetty, 2021).

## 5. Conclusions

These experiments, conducted with Gardenia jasminoides, attempted to determine acceptable alternative methods of nutrient media sterilization rather than using an autoclave for sterilization. Microwave sterilization of medium had a number of problems. Medium sterilization was incomplete and inconsistent at all microwave intervals in the trials and loss of medium to both evaporation and boiling over in the microwave oven resulted in incorrect medium component concentrations. The explants also had poorer shoot quality and reduced growth compared to explants grown on autoclaved medium. Other methods, such as the use of a water container in the middle of microwave oven plate that will provide energy reserve (Venturieri, et al., 2013) and intermittent application of the irridiation time should be tried for more consistent sterilization of nutrient medium and to prevent boiling over or evaporation. Different volumes of nutrient medium and different containers also need to be evaluated. Sterilization with microwaves emerges as an alternative method that could be preferred due to several advantages: it is economical in small-scale micropropagation, it consumes less energy and completes the sterilization process in a short time, and microwave ovens are easily obtainable and much less expensive than autoclaves (Scheppler, 2014).

Unlike the sterilization with microwaves, the sterilization of the nutrient medium was fully assured when either NaDCC or NaOCl were added as supplements to the medium at the dosages trialed. In the nutrient media where these two substances were added, both in vitro shoot quality and shoot development were equal to or superior to shoots grown on autoclaved medium. Therefore, both chemicals have a high potential use as an alternative to autoclaving media. Nevertheless, the importance of the plant species should not be forgotten in such studies, the applicability of the methods should be decided by trying them on different plant species. The authors declare no conflict of interest.

## Acknowledgements

This study was presented as a poster presentation at the VIII. National Ornamental Plants Congress

## References

Arditti, J. (2008). Micropropagation of Orchids. Blackwell Publishing, 1: 106-109.

Biswas, K. and Biswas, R. (2017). Micropropagation of Lilium asiatic in an efficient low cost novel medium “KFA and KFA plus”. International Journal of Applied Agricultural Research 12(1), 33-41 http://ripublication.com/ijaar17/ijaarv12n1_04.pdf

Brondani, G. E., de Oliveira, L.S., Bergonci, T., Brondani, A.E., França, F.A.M., da Silva, A.L.L. and Gonçalves, A.N. (2013). Chemical sterilization of culture medium: a low cost alternative to in vitro establishment of plants. Scientia Forestalis 41(98), 257-264 https://www.ipef.br/publicacoes/scientia/nr98/cap11.pdf

Cardoso, J. C. and Imthurn, A. C. P. (2018). Easy and efficient chemical sterilization of the culture medium for in vitro growth of gerbera using chlorine dioxide (ClO2). Ornamental Horticulture 24(3), 218-224 https://doi.org/10.14295/oh.v24i3.1222

Datta, S. K., Chakraborty, D. and Janakiram, T. (2017). Low cost tissue culture: An overview. The Journal of Plant Science Research, 33(2), 181-199 https://search.proquest.com/scholarly-journals/low-cost-tissue-culture-overview/docview/1973308547/se-2?accountid=10919

Driver, J. A. and Kuniyuki, A. H. (1984). In vitro propagation of Paradox walnut rootstock. HortScience 19(4), 507-509.

Hicks, A. J. (2009). Asymbiotic Technique of Orchid Seed Germination (2ed.) Orchid Seedbank Project.

Kothari, V., Patadia, M. and Trivedi, N. (2011.) Microwave sterilized media supports better microbial growth than autoclaved media. Research in Biotechnology 2(5), 63-72.

Mvuria, J. M. and Ombori, O. (2014). Low cost macronutrients in the micropropagation of selected sweet potato [Ipomoea Batatas (L.) Lam.] varieties. Journal of Agriculture and Environmental Sciences 3(1), 89-101 https://doi.org/10.15640/jaes

Özzambak, M. E., Zeybekoğlu, E., Gün, İ. and Kılıç, T. (2018). The effects of sucrose concentrations on in vitro micropropagation of Spathiphyllum. Sakarya University Journal of Natural and Applied Sciences 22(3), 1015- 1023 https://doi.org/10.16984/saufenbilder.348480

Özzambak, M. E. and Zeybekoğlu, E. (2020). Different applications in sterilizing of the nutrient medium in the micropropagation of ornamental plants. In A. Çığ (ed.), Ornamental Plants in Different Approaches, (pp. 361-395). IKSAD Publishing House.

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

Pan M. J. and Van Staden J. (1999). Effect of activated charcoal autoclaving and culture media on sucrose hydrolysis. Plant Growth Regulation 29(3), 135-141 https://doi.org/10.1023/A:1006238300890

Parkinson, M., Prendergast, M. and Sayegh, A. J. (1996.) Sterilisation of explants and cultures with sodium dichloroisocyanurate. Plant Growth Regulation 20(1), 61-66 https://doi.org/10.1007/BF00024060

Pierik R.L.M (1989). In vitro Culture of Higher Plants. Martinus Nijhoff Publishers.

Prijana, C., Mulyana, Y. and Hidayat, B. (2016.) Roles of microwave oven in preparing microbiological growth media. Althea Medical Journal 3(1), 1-5 https://doi.org/10.15850/amj.v3n1.469

Purohit S. D., Teixeira da Silva, J.A. and Habibi, N. (2011). Current approaches for cheaper and better micropropagation techniques. Int J Plant Dev Biol 5(1), 1-36.

Ribeiro, J. M., Teixeira, S. L. and Bastos, D. C. (2011). In vitro culture of Sequoia sempervirens L. on nutritive media sterilized with sodium hypochlorite. Ciência Florestal 21(1), 77-82.

Narasimhan, S. and Shetty, S. C. (2021). Sodium dichloroisocyanurate: an eco-friendly chemical alternative for media autoclaving and explant sterilisation in plant tissue culture. International Journal of Research in Pharmaceutical Sciences 12(1), 107-12. https://pharmascope.org/index.php/ijrps/article/view/3943/9910

Sanborn, M. R., Wan, S.K. and Bulard, R. (1982). Microwave sterilization of plastic tissue culture vessels for reuse. Applied and environmental microbiology 44(4), 960-964 https://aem.asm.org/content/44/4/960

Savangikar, V.A. (2004). Role of low cost options in tissue culture. In Low cost options for tissue culture technology in developing countries. Proceedings of a Technical Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (pp. 11-15.) https://www.iaea.org/publications/6806/low-cost-options-for-tissue-culture-technology-in-developing-countries

Sawyer, H. and Hsiao, K. (1992). Effects of autoclave-induced carbohydrate hydrolysis on the growth of Beta vulgaris cells in suspension. Plant Cell, Tissue and Organ Culture 31(1), 81-86 https://doi.org/10.1007/BF00043479

Shareef, S. A., Hamasaeed, P. A. and Ismaeil, A. S. (2019). Sterilization of culture media for microorganisms using a microwave oven instead of autoclave. Rafidain Journal of Science 28(1): 1-6. https://doi.org/10.33899/rjs.2019.159390

Schenk N., Hsiao, K. C. and Bornman, C. H. (1991). Avoidance of precipitation and carbohydrate breakdown in autoclaved plant tissue culture media. Plant Cell Reports 10(3), 115-119 https://doi.org/10.1007/BF00232040

Scheppler, J. A. (2014). Watts Cooking: Using a microwave to prepare bacterial media for inquiry-based experiments. Staff Publications & Research. 2. http://digitalcommons.imsa.edu/sir_staffpr/2

Teixeira, S.L., Ribeiro, J.M. and Teixeira, M. T. (2006). Influence of NaClO on nutrient medium sterilization and on pineapple (Ananas comosus cv Smooth cayenne) behavior. Plant Cell, Tissue and Organ Culture 86(3), 375-378 https://doi.org/10.1007/s11240-006-9121-3

Thepsithar, C. and Thongpukdee, A. (2013). Sterilization of hyponex medium by chemicals without autoclaving and growth of Phalaenopsis protocorms. World Academy of Science, Engineering and Technology. International Journal of Bioengineering and Life Sciences 7(6), 403-406 http://doi.org/10.5281/zenodo.1058635

Thepsithar, C., Thongpukdee, A. and Daorat, A. (2013). Sterilization of in vitro culture medium of Chrysanthemum by plant essential oils without autoclaving. World Academy of Science, Engineering and Technology. International Journal of Bioengineering and Life Sciences 7(8), 802-805 https://doi.org/10.5281/zenodo.1086765

Tisserat, B., Jones, D. and Galletta P. D. (1992). Microwave sterilization of plant tissue culture media. Hortscience 27(4), 358-361 https://doi.org/10.21273/HORTSCI.27.4.358

Tiwari, A.K., Tripathi, S., Lal, M. and Mishra, S. (2012). Screening of some chemical disinfectants for media sterilization during in vitro micropropagation of sugarcane. Sugar Tech 14(4), 364–369 https://doi.org/10.1007/s12355-012-0178-5

Trivedi, N., Patadia, M. and Kothari, V. (2011). Biological applications of microwawes. International Journal of Life Sciences and Technology 4(6), 37-46

Vadivambal, R. and Jayas, D. S. (2010). Non-uniform temperature distribution during microwave heating of food materials—A review. Food and bioprocess technology, 3(2), 161-171. https://doi.org/10.1007/s11947-008-0136-0

Venturieri, G. A., Venturieri, A. R. and Leopoldo, G. (2013). Sterilization of culture media for orchids using a microwave oven. In Vitro Cellular & Developmental Biology-Plant 49(2), 137-144 https://doi.org/10.1007/s11627-012-9470-z

Vora, N. C. and Jasrai, Y. T. (2012). Microwave oven based steriIization of media for micropropagation of banana. CIBTech Journal of Biotechnology 1(2-3), 18-21 http://www.cibtech.org/J-Biotechnology/PUBLICATIONS/2012/Vol-1-No-2-3/04-003...Vora...Microwave...Banana...18-21.pdf

Wang X. J. and Hsiao, K. C. (1995). Sugar degradation during autoclaving: Effects of duration and solution volume on breakdown of glucose. Physiologia Plantarum 94(3), 415-418 https://doi.org/10.1111/j.1399-3054.1995.tb00947.x

World Health Organization (WHO) (2008). Sodium dichloroisocyanurate in drinking-water, Background document for preparation of WHO Guidelines for drinking-water quality. World Health Organization (WHO/HSE/AMR/08.03/3) https://www.who.int/water_sanitation_health/dwq/chemicals/sodiumdichloroisocyanuratesum_2nadd.pdf

Yanagawa, T., Tanaka, R. and Funai, R. (2006). Simple micropropagation of ornamentals by direct application of chlorine disinfectants without equipment. Acta Hortic 764, 289-298 https://doi.org/10.17660/ActaHortic.2007.764.40

Youssef, E. M. A. and Amin, G. A. (2001.) Microwave sterilization of tissue culture media. Acta Hortic 560, 513-516 https://doi.org/10.17660/ActaHortic.2001.560.104