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Evaluation of different disinfection protocols for seed germination of *Ocimum basilicum* L.

Evaluación de diferentes protocolos de desinfección para la germinación de semillas de *Ocimum basilicum* L.

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Abstract

The aim of this study was to discriminate between different disinfection protocols applied to common basil seeds for in vitro establishment on Murashige & Skoog medium. The aim of this approach was not only to overcome seed dormancy, but also to decisively influence the germination process in order to achieve a significant increase in the proportion of seeds that germinate successfully. In the Colombian context, where some 156 species of medicinal plants are currently marketed, basil (*Ocimum basilicum* L.), originally from Asia Minor, stands out as one of the most widely used plants in the world. In the department of Valle del Cauca, medicinal plants have emerged as protagonists in contrast to the monoculture of sugar cane (*Saccharum Officinarum* L.) that dominates the region. However, basil faces significant challenges related to seed dormancy, a condition that negatively affects germination and therefore directly affects yield. In this scenario, 21 treatments were carried out in the Biological Sciences Laboratory of the Central Unit of Valle del Cauca, which showed a marked variability and influence in phenological development, especially in stem and leaf growth. Treatment 12 (0.7% NaClO) showed the highest percentage of basil seed germination (80%). It is imperative to explore new protocols that effectively contribute to the basil production process by overcoming seed dormancy.

Resumen

El objetivo de este estudio fue discernir entre diversos protocolos de desinfección aplicados a las semillas de albahaca común para su establecimiento in vitro en el medio de cultivo Murashige & Skoog. Este enfoque buscó no solo superar la latencia de las semillas, sino también influir de manera determinante en el proceso de germinación, con el fin de lograr un aumento sustancial en la proporción de semillas que germinan exitosamente. En el contexto colombiano, donde actualmente se comercializan alrededor de 156 especies de plantas medicinales, la albahaca común (*Ocimum basilicum* L.), originaria de Asia Menor, destaca como una de las más ampliamente utilizadas a nivel mundial. En el departamento del Valle del Cauca, las plantas medicinales han emergido como protagonistas en contraposición al predominante monocultivo de caña de azúcar (*Saccharum Officinarum* L.) en la región. Sin embargo, la albahaca enfrenta desafíos significativos relacionados con la latencia de sus semillas, una condición que repercute negativamente en la germinación y, por ende, afecta directamente su rendimiento. En este escenario, se llevaron a cabo 21 tratamientos en el laboratorio de Ciencias Biológicas de la Unidad Central del Valle del Cauca, revelando una marcada variabilidad y afectación en el desarrollo fenológico, específicamente en el crecimiento del tallo y las hojas. El tratamiento 12 (NaClO al 0.7%), exhibió el más alto porcentaje de germinación de semillas de albahaca (80%). Se hace imperativo explorar nuevos protocolos que contribuyan de manera efectiva al proceso productivo de la albahaca al superar la latencia de sus semillas.

Introduction

Around 156 species of medicinal, aromatic and culinary plants are marketed in Colombia [1]. 65% go to the United States, 10% to Canada, 8% to the United Kingdom and 5% to the European Union, mainly Germany, Holland and Belgium, and it is considered that the market for the European Union is very large and there is still much to be explored [1,2]. Basil (*Ocimum basilicum* L.), from Asia Minor, is a medicinal plant of great importance, it is used in many cases, from the gastronomic aspect as condiments and drinks to the medicinal aspect thanks to its antiseptic, anti-inflammatory and digestive properties due to the properties of its essential oils [3], its cultivation generally takes place in tropical climates [4].

Due to its adaptability, basil is one of the most important medicinal, aromatic and culinary plants grown in Colombia, especially in the Valle del Cauca region [5]. Basil, which belongs to the genus Ocimum of the Lamiaceae family, is characterised by a high morphological and photochemical variability [6,7], which allows the different varieties to adapt to the different thermal soils found in Colombia. The geographical origin of the basil species determines the traits of the seed and its chemical and nutritional composition, as well as its morphological characteristics [8].

Basil seeds tend to show erratic behaviour due to the non-domestication of the species [9]. Therefore, studies are needed to improve germination, break seed dormancy or stimulate germination, rooting of cuttings and plant development [10]. In the development of medicinal, aromatic and spice plants (MASP), research often "fails" due to the scarcity of propagation material, either by seed or cuttings. In addition, there is insufficient information on the conservation process [11]. The growing demand for products made from MASP, requires a rapid response in terms of new varieties to increase production, while maintaining the quality of seeds and plants to ensure a clear response to the growing demand [8,11].

Currently, medicinal and aromatic plants are part of many rural community development programmes and development projects of various governmental and private institutions and NGOs [12]. There are four departments that produce this medicinal plant, with Boyacá being the main producer, followed by Valle del Cauca, Cundinamarca and Antioquia. The aim of this research was to evaluate the propagation of basil (*O. basilicum*) in an in vitro culture medium with different disinfection protocols, in order to determine which one has a higher germination and effectiveness, and thus, increase the production of this plant species in the Agroecological Farm of the Unidad Central del Valle del Cauca.

Methods

Basil seed collection

The basil seeds for the research process were obtained from the agroecological farm of the Unidad Central del Valle del Cauca, strategically located in Tulua, close to the town centre. This farm has a special plot for medicinal plants and this is where the seeds used in this study came from. The basil plant in question is shown in figure 1, which provides a visual reference of the species used in the seed collection process. This systematic approach to the selection and collection of botanical material supports the validity and reproducibility of the results obtained in the research.

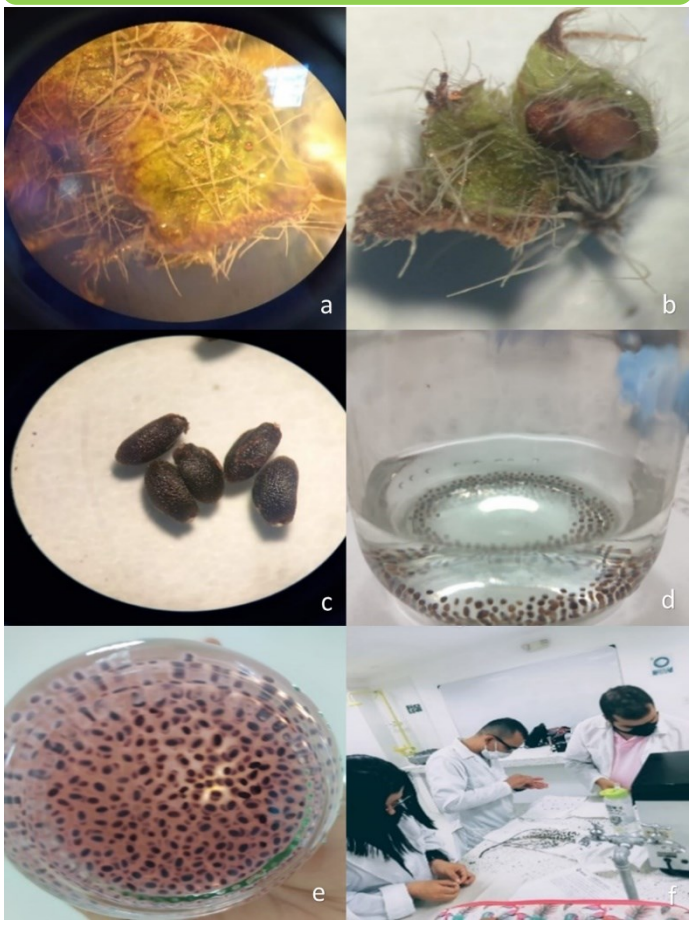
Figure 1. Basil plant in the field, for seed collection

Preparation of the seed for further propagation in vitro

Seed preparation for subsequent in vitro propagation, followed a meticulous protocol. First, the seeds were thoroughly cleaned, followed by immersion in tap water

at a controlled temperature of 26°C. This procedure was strategically designed to reduce seed dormancy and involved an immersion period of approximately 24 hours. Figure 2 illustrates in detail the cleaning process to which the seeds were subjected, highlighting the thoroughness applied at each stage to ensure the efficacy of the treatment and to optimally prepare the seeds for subsequent in vitro propagation. This meticulous approach underlines the importance of precision in the preparation techniques, laying the foundation for reliable and meaningful results in the subsequent conduct of the experiment.

Figure 2. Basil seeds soaked in water



a) Initial seed condition; **b)** Seed cleaning process; **c)** Cleaned seed; **d)** Seed initially immersed in water at 26°C; **e)** Seed after soaking for 24 hours; **f)** Team work.

Preparation of disinfectants and culture media for disinfection

Sodium hypochlorite (NaClO) and iodine were used to formulate disinfectants tailored to the treatments and protocols in this research project. The iodine solution was carefully prepared at a concentration of 0.1%, while sodium hypochlorite solutions were prepared at concentrations of 0.2%, 0.5%, 0.7% and 1%. Sodium hypochlorite in particular is a versatile germicide and oxidising agent, widely recognised for its effectiveness. However, its strong bleaching action, documented by Chung et al. [13], can induce lesions, prompting the establishment of different concentrations. Starting with the 0.5% concentration commonly found in commercial hypochlorite, various concentrations were systematically derived through precise calculations, as detailed in table 1.

Following seed disinfection, the various protocols underwent a thorough rinsing process lasting 30 seconds, effectively eliminating any residual traces of disinfectants. This meticulous approach, ensures the integrity of subsequent germination experiments and underlines the methodical consideration given to the selection and preparation of disinfectant concentrations in the context of enhancing seed viability and overall experimental reliability (see table 1).

Table 1. Protocols for disinfection

Exposure times (sec)	NaClO 0.2%	NaClO 0.5%	NaClO 0.7%	NaClO 1%	Yodo 0.1%	Control
20	T1	T5	T9	T13	T17	
40	T2	T6	T10	T14	T18	T21
60	T3	T7	T11	T15	T19	
80	T4	T8	T12	T16	T20	

Preparation of culture medium. MS culture medium was used for all disinfection treatments

For the preparation of the DM, 1.10 g was weighed and prepared on a magnetic stirrer with heating, with 750 ml of distilled water to dilute the DM. The DM was supplemented with 22.5 g of sucrose as a carbon source, the pH was adjusted to 5.5, Phytogel 1.87 g, BAP 0.375 mg, 5 mg of antibiotic diluted in distilled water and ten antifungal drops were added; sterilised in an autoclave for 20 minutes at 121°C and 15 lbs of pressure. The sowing process is carried out in the Petri boxes and they are transferred to the in vitro chamber where the seed germination and stem and leaf elongation monitoring process was carried out.

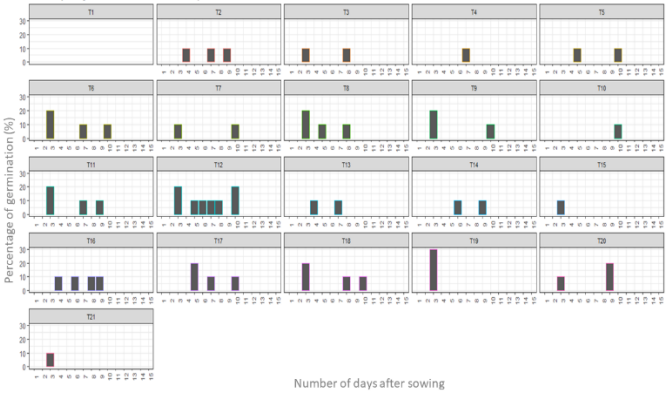
Results

In the present study, the most effective treatment for assessing germination was identified as treatment 12,

characterised by a concentration of 0.7% NaClO and an exposure time of 80 seconds, which showed an impressive 80% germination. This finding underlines the importance of careful selection of concentrations and exposure times in the disinfection process, and highlights how a higher concentration of disinfectant requires longer exposure to break down the seed slime efficiently, allowing for optimum nutrition and water uptake.

On the other hand, treatments T6, T8, T11 and T16, characterised by lower concentrations of NaClO and shorter exposure times, showed a germination percentage of 40%. This trend suggests that a higher concentration of disinfectant and a longer exposure time do indeed contribute to obtaining better results in the germination of basil seeds, confirming the correlation between these factors and the success of the germination process. Figure 3 provides a detailed visualisation of germination percentage in relation to time since sowing. Of the 21 treatments applied, 57.1% showed germination between days 2 and 3. Treatment T10 showed a germination time of 10 days, while T14 germinated 6 days after establishment in the laboratory. In contrast, treatment T1 showed no germination, suggesting that these treatments are not suitable for the disinfection process of basil seeds intended for in vitro culture.

Figure 3. Percentage of germination as a function of time



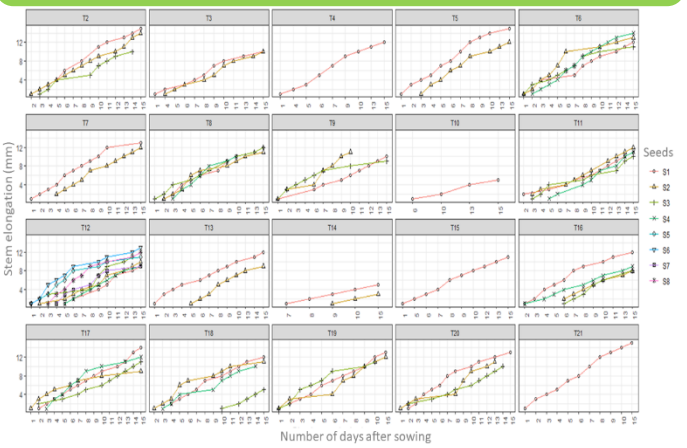
Note: Germination percentages are calculated based on 10 seeds per treatment.

In addition, a careful measurement of stem and leaf elongation was carried out during a period of 15 days after germination. This detailed analysis of growth and development provides valuable information on the effects of different treatments in the early stages of the plant's life cycle, thus contributing to a more complete understanding of the results obtained in the study (figures 4,5).

Stem elongation

Figure 4 shows the dynamics of stem elongation in germinated seedlings. For statistical analysis, only those treatments in which more than one seed germinated were considered. In this context, it was observed that in treatments T2 and T5, S1 stood out by showing greater stem elongation compared to other seeds and treatments, while T14 was characterised by showing the lowest growth rate of all treatments.

Figure 4. Stem elongation of basil plants as a function of time



It should be noted that the S3 seed of T18, showed a significant delay in starting its stem growth process. An analysis of figure 4, shows that 66.6% of the treatments started their stem elongation on the first day after germination.

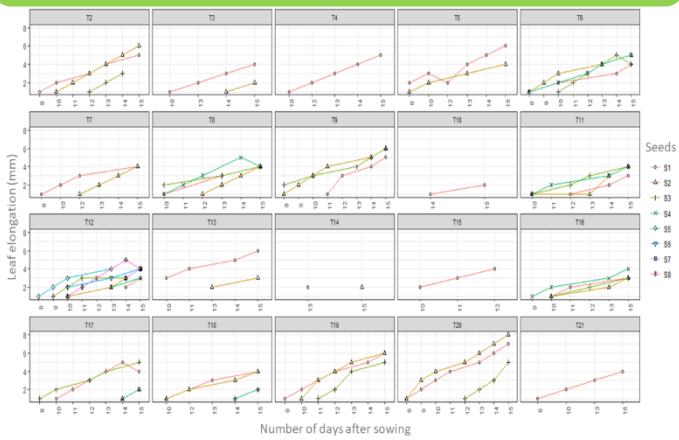
Regarding the statistical parameters, T9 is characterised by a mean behaviour of 46.66, a standard deviation of 5.50, which indicates a low dispersion of the data and, therefore, suggests that it is the ideal treatment for the stem elongation process. On the other hand, T12, despite having a high dispersion with a mean of 52.50, a standard deviation of 13.58 and a coefficient of variation of 25.87, is shown to be the most suitable for seed germination. The stem elongation data support the suitability of T12 not only for germination but also for subsequent stem development and growth, underlining its effectiveness in the early stages of the basil seedling life cycle.

Leaf elongation

In the analysis of leaf elongation, it is noticeable that S2 of T20, showed the greatest leaf growth compared to the other seeds and treatments. It was also observed that S1,

S4, S2, S4 and S1 of T10, T18, T3, T17 and T12 respectively started their leaf growth from day 14. The detailed evaluation in figure 5, shows that 47.6% of the treatments showed leaf emergence between days 7 and 8 after germination, while 33.3% showed leaf emergence between days 9 and 10. These temporal findings provide a precise vision of the kinetics of leaf growth in basil seedlings, highlighting key moments in post-germination development.

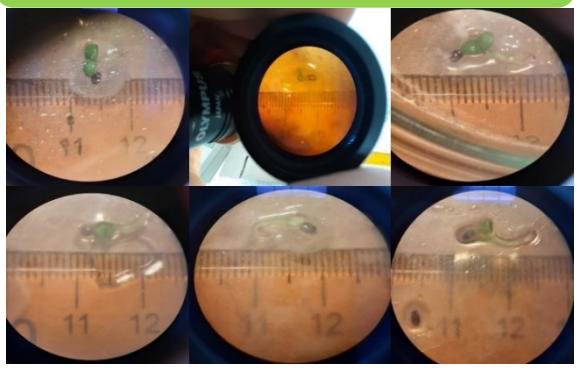
Figure 5. Elongation of the leaves of the basil plant as a function of time



Statistically, T9 is characterised by a mean behaviour of 18.00 and a standard deviation of 4.35, indicating a low dispersion of the data and suggesting that it is the most appropriate treatment for the leaf elongation process. On the contrary, T12, despite having a low dispersion with a mean of 9.25 and a standard deviation of 3.57, has a coefficient of variation of 38.65, indicating a greater variability with respect to its mean behaviour. Although the coefficients of variation of all the treatments are high, indicating a significant dispersion of the results, T12 was found to be the most suitable for the disinfection of basil seeds. This treatment is essential, both in traditional substrates and in Murashige & Skoog medium, due to its effectiveness in overcoming seed dormancy, which affects germination.

Finally, figure 6 summarises the monitoring activities carried out and provides a visual conclusion to the detailed evaluation of the leaf elongation results.

Figure 6. Monitoring of stem and leaf growth of basil plants



Discussion

Placing our results in the context of previous research on seed disinfection in the Lamiaceae family, it is clear that protocols using NaClO at concentrations of 0.2% to 0.4%, with exposure times of 1 to 15 seconds, tend to show a wide range of contamination in the culture medium. This phenomenon is consistent with the observation that increasing exposure times can inhibit seed germination, supporting previous findings [14]. On the contrary, other studies support the use of 20% NaClO with an exposure time of 3 s and a double wash, arguing that this approach favours the stable formation of plant tissues such as roots and stems [15]. This contrast highlights the importance of precision in the choice of concentrations and exposure times, and highlights the complexity and specificity of disinfection protocols.

In relation to T17 and T18, where iodine was used as a disinfectant, it is interesting to note its use in previous research to establish species in in vitro culture [14]. Recognised for its antiseptic properties, iodine appears to be a more suitable alternative for working with biological organisms, as it minimises irritation and damage to living tissues during the cultivation process [16-18]. Our results support this perspective, indicating that the application of 0.1% iodine with exposure times between 20- and 40-seconds results in acceptable germination, highlighting the effectiveness of this disinfectant compared to other protocols evaluated [16].

In line with the observations of Bedoya et al. [14], it is confirmed that the use of iodine concentrations between 0.1% and 0.8%, with exposure times of 1 to 3 minutes, favours greater vegetative development in basil treatments. In our research, we found that an iodine concentration of 0.1% and exposure times of 20 to 40

seconds, produced acceptable germination results, suggesting an optimisation of the process that can contribute to vigorous seedling development. These results highlight the importance of considering the specificity of each disinfectant and adjusting the parameters according to the characteristics of the species studied.

Taken together, these results contribute to a more holistic understanding of basil seed disinfection protocols and their impact on germination, highlighting the need for a careful approach adapted to the characteristics of the species and the growing environment.

Conclusions

Extensive analysis of different laboratory disinfection protocols for basil (*O. basilicum*) seeds has led to the identification of the optimal approach to basil seed disinfection, which results in a gentle wash of the basil seeds with 0.7% hypochlorite, followed by a thorough rinse with distilled water for exactly 30 seconds. This nuanced protocol not only ensures effective disinfection, but also minimises the potential negative impact on seed viability.

A concentration of 0.7% hypochlorite has been found to be the most beneficial for basil seed germination. This concentration strikes a balance, demonstrating superior efficacy in seed disinfection without inducing adverse effects that could hinder subsequent germination. Careful consideration of disinfectant concentration is paramount in optimising the overall germination performance of basil seeds.

In addition, the optimum exposure time for basil seeds to the disinfectant is 80 seconds. This duration, established through rigorous experimentation, is consistent with the delicate balance required to effectively break seed dormancy while avoiding potential negative effects on seed vitality. In conclusion, it is safe to say that T12, stands out as a key intervention in the seed disinfection process. This particular treatment, using 0.7% hypochlorite with an exposure time of 80 seconds, not only outperforms in terms of germination percentage, but also demonstrates its unique ability to break seed dormancy. This breakthrough in dormancy paves the way for robust and healthy plant development in subsequent growth stages.

The present study identifies the most effective disinfection protocol for basil seeds, but also highlights the nuanced interplay between hypochlorite concentration, exposure time and subsequent germination success. These findings provide valuable insights for researchers and practitioners alike, informing future efforts to develop seed disinfection protocols tailored to the specific needs of basil cultivation.

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Consent for publication

The authors read and approved the final manuscript.

Competing interest

The authors declare no conflict of interest. This document only reflects their point of view and not that of the institution to which they belong.

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References

[1] Pájaro-González Y, Oliveros-Díaz AF, Cabrera-Barraza J, Cerra-Dominguez J, Díaz-Castillo F. A review of medicinal plants used as antimicrobials in Colombia. Medicinal Plants as Anti-Infectives: Current Knowledge and New Perspectives 2022:3–57. https://doi.org/10.1016/B978-0-323-90999-0.00005-7

[2] Marmolejo Liloy M, Ponce Mendoza JD, Hinestroza Córdoba LI, Moreno Holguín H. An ethnobotanical survey of spice, aromatic and medicinal plants used in La Molana, Atrato-Choco, Colombia: Basis for biodiversity conservation. Acta Agron 2018;67:9–16. https://doi.org/10.15446/acag.v67n1.59316

[3] Shahrajabian MH, Sun W, Cheng Q. Chemical components and pharmacological benefits of Basil (*Ocimum basilicum* L.): a review. Int J Food Prop 2020;23:1961–70. https://doi.org/10.1080/10942912.2020.1828456

[4] Filip S. Basil (*Ocimum basilicum* L.) a Source of Valuable Phytonutrients. International Journal of Clinical Nutrition & Dietetics 2017;3. https://doi.org/10.15344/2456-8171/2017/118

[5] Lal RK. Breeding for new chemotypes with stable high essential oil yield in Ocimum. Ind Crops Prod 2014; 59:41–9. https://doi.org/10.1016/J.INDCROP.2014.04.047

[6] Marotti M, Piccaglia R, Giovanelli E. Differences in Essential Oil Composition of Basil (*Ocimum basilicum* L.) Italian Cultivars Related to Morphological Characteristics. J Agric Food Chem 1996; 44:3926–9. https://doi.org/10.1021/jf9601067

[7] Beltrán Cifuentes M, Cantillo Maldonado M, Vivas Castaño A. Actividad antibacteriana de los aceites obtenidos de *Ocimum basilicum* L. var. cinammom, *O. album*, *O. thyrsiflorum*, para uso potencial en fitocosmética. Investigaciones Andina 2013; 15:798–810. http://scielo.org.co/pdf/inan/v15n27/v15n27a07.pdf.

[8] Nazir S, Wani IA. Functional characterization of basil (*Ocimum basilicum* L.) seed mucilage. Bioactive carbohydrates and dietary fibre 2021; 25:100261. https://doi.org/10.1016/j.bcdf.2021.100261

[9] Jirovetz L, Buchbauer G, Shafi MP, Kaniampady MM. Chemotaxonomical analysis of the essential oil aroma compounds of four different Ocimum species from southern India. European Food Research and Technology 2003; 217:120–4. https://doi.org/10.1007/s00217-003-0708-1

[10] Klimánková E, Holadová K, Hajšlová J, Čajka T, Poustka J, Koudela M. Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. Food Chem 2008; 107:464–72. https://doi.org/10.1016/j.foodchem.2007.07.062

[11] Sánchez Orozco MS, Durán Gaviria LA, Castro Vargas DF, Bonilla Correa CR. Calidad fisiológica de semillas de cinco variedades de Albahaca Ocimum spp. en condiciones del Valle del Cauca. Acta Agron 2015;65. https://doi.org/10.15446/acag.v65n1.45377

[12] Díaz Merchán J. Caracterización del mercado colombiano de plantas medicinales y aromáticas. Informe técnico. Bogotá, DC: 2003. http://repository.humboldt.org.co/handle/20.500.11761/32544?show=full

[13] Chung I, Ryu H, Yoon S-Y, Ha JC. Health effects of sodium hypochlorite: review of published case reports. Environ Anal Health Toxicol 2022;37:e2022006. https://doi.org/10.5620/eaht.2022006

[14] Bedoya Pérez JC, Sánchez Jaramillo CY, Bermúdez Gómez SM, Restrepo SR. Estandarización de un protocolo de desinfección y establecimiento de cultivo in vitro de *Aloysia tryphilla*. Biotecnología En El Sector Agropecuario y Agroindustrial 2016;14:38. https://doi.org/10.18684/BSAA(14)38-46

[15] Espinosa-Leal CA, Puente-Garza CA, García-Lara S. In vitro plant tissue culture: means for production of biological active compounds. Planta 2018; 248:1–18. https://doi.org/10.1007/s00425-018-2910-1

[16] Barreto R, Barrois B, Lambert J, Malhotra-Kumar S, Santos-Fernandes V, Monstrey S. Addressing the challenges in antisepsis: focus on povidone iodine. Int J Antimicrob Agents 2020; 56:106064. https://doi.org/10.1016/J.IJANTIMICAG.2020.106064

[17] Durani P, Leaper D. Povidone–iodine: use in hand disinfection, skin preparation and antiseptic irrigation. Int Wound J 2008; 5:376–87. https://doi.org/10.1111/j.1742-481X.2007.00405.x

[18] Eggers M. Infectious Disease Management and Control with Povidone Iodine. Infect Dis Ther 2019; 8:581–93. https://doi.org/10.1007/s40121-019-00260-x