AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Evaluation of different antioxidants during *in vitro* establishment of allspice (*Pimenta dioica* L. Merrill): a recalcitrant species

Vásquez-Hernández, Sugey[1]; Cruz-Cruz, Carlos A.[1]; Santiago-Santiago, Maricela[2]; Bello-Bello, Jericó Jabín[3]*

[1] Universidad Veracruzana. Facultad de Ciencias Químicas, Orizaba, Veracruz, México. C. P. 94340.
[2] La Joya del Totonacapan, El Progreso, Atzalan, Veracruz, México. C. P. 93680.
[3] CONACYT-Colegio de Postgraduados Campus Córdoba, Amatlán de los Reyes, Veracruz, México. C. P. 94953.
* Correspondence: jericobello@gmail.com

ABSTRACT

Objective: To evaluate the effect of different antioxidant agents during *in vitro* establishment of allspice (*Pimenta dioica* L. Merrill).

Design/methodology/approach: The effect of different antioxidant agents (Methylene blue, L-cysteine, and silver nanoparticles [AgNPs]) added to Murashige and Skoog culture medium at different concentrations were studied during axenic establishment of *P. dioica*. A completely randomized experimental design was used. All trials were performed in triplicate. The percentage of survival, oxidation, contamination was determined, the phenols content, antioxidant capacity and lipid peroxidation.

Results: The highest survival occurred with the addition of L-cysteine. The lowest percentage oxidation were observed in explants treated with L-cysteine. Treatments with 100 and 200 mg L^{-1} AgNPs had the lowest contamination values. L-cysteine and 50 and 100 mg L^{-1} AgNPs resulted in an increase in the content of soluble phenols.

The highest contents of cell wall-linked phenols were obtained in treatments with 200 mg L^{-1} methylene blue, L-cysteine, and 200 mg L^{-1} AgNPs. In this study, all treatments had a reaction of scavenging/reduction mechanisms free radicals. The highest content of malondialdehydes was observed in the control treatment and 200 mg L^{-1} methylene blue. The highest content of malondialdehydes was observed in the control treatment and 200 mg L^{-1} methylene blue.

Limitations on study/implications: The highest percentage of oxidation was observed in the control treatments, 100 and 200 mg L^{-1} methylene blue, causing cell death.

Findings/conclusions: The addition of L-cysteine to the culture medium is alternative to reduce oxidation during *in vitro* introduction of *P. dioica*.

Keywords: methylene blue, antioxidant capacity, L-cysteine, silver nanoparticles, lipid perioxidation, polyphenols.

Citation: Vásquez-Hernández, Sugey; Cruz-Cruz, Carlos A.; Santiago-Santiago, Maricela; Bello-Bello, Jericó Jabín. Evaluation of different antioxidants during *in vitro* establishment of allspice (*Pimenta dioica* L. Merrill): a recalcitrant species. *Agro Productividad*. https://doi.org/10.32854/agrop.v14i11.2167

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: April 21, 2021.
Accepted: October 13, 2021.
Published on-line: November 30, 2021.

INTRODUCTION

The "pimienta gorda" or allspice (*Pimenta dioica* L. Merrill), is native to Mexico and Central America (López *et al.*, 2021). The cultivation of this species plays an important role

Image by mkupiec7 at Pixabay

in the agro-food and pharmaceutical industry because its fruits are used as a spice and in therapies due to their high level of eugenol (Marques *et al.*, 2019). Allspice is propagated through seeds and cuttings. The propagation by seeds presents high genetic variability, while the propagation method by cuttings does not guarantee phytosanitary quality for planting. The Plant Tissue Culture is an alternative for *in vitro* production of pest and disease free clonal seedlings (Cardoso *et al.*, 2018). However, the *in vitro* establishment stage is the main constraint on initiating commercial micropropagation of plants, due to the *in vitro* manipulation causes tissue damage producing the oxidation of phenols, causing the darkening of the tissue affecting its growth (Martínez Rivero *et al.*, 2020). This oxidation and other stress conditions *in vitro* trigger the overproduction and accumulation of Reactive Oxygen Species (ROS) in plant cells (Zhou *et al.*, 2021), the generation of ROS promote changes in phenolic content, antioxidant capacity and lipid peroxidation (Wei *et al.*, 2019).

During *in vitro* establishment, in some species, the use of antioxidants is necessary to prevent oxidation of the explant (Taghizadeh *et al.*, 2020). Methylene blue, L-cysteine and AgNPs are an alternative of reducing oxidation of phenolic compounds and maintaining a survival rates of the explants in species recalcitrant to *in vitro* morphogenesis. The aim of this study was to evaluate different antioxidant agents to reduce oxidation, as well as the phenolic content, antioxidant capacity and lipid peroxidation of *P. dioica* explants during *in vitro* establishment.

MATERIALS AND METHODS

Plant material and *in vitro* establishment

Nodal cuttings 2 cm high of female plants of *P. dioica* ecotype Totonacapan from the Totonacapan region, Veracruz, Mexico was taken as explants. The explants were washed with running water and Tween® 20 soap (Sigma-Aldrich®, St. Louis, MO, USA) in continuous circulation for 30 min. Subsequently, they were rinsed with drinking water. The explants were transferred to a laminar flow hood where they were immersed in 70% (v/v) ethanol for 1 minute and rinsed three times with sterile distilled water; they were then immersed in sodium hypochlorite (NaClO, 6% active ingredient Clorox®; Monterrey, NL, Mexico) at 0.6 and 0.9% (v/v) for 15 and 10 min, respectively, adding 2 drops of Tween® 20 per 100 mL of solution. The explants were rinsed three times with sterile distilled water and were cultured in different treatments with different antioxidant agents.

Treatments with antioxidant agents

The antioxidant response of methylene blue (Merck; Darmstadt, Germany), L-cysteine (Sigma-Aldrich®) and Silver nanoparticles (Argovit™) at concentrations of 0, 50, 100 and 200 mg L^{-1} was evaluate to equalize conditions. The antioxidant agents were added to MS (Murashige and Skoog, 1962) culture medium, supplemented with 3% sucrose (w/v) (Fermont®; Monterrey, NL, Mexico) and 2.5 g L^{-1} phytagel (Sigma-Aldrich®) as gelling agent. The pH was adjusted to 5.8 and sterilized in an autoclave at 120 °C and 115 kPa for 20 min. Finally, 10 explants per treatment were seeded individually in test tubes (15×2 cm) containing 10 mL of culture medium. After 30 days of culture, the percentage of survival, oxidation, contamination, phenol content, antioxidant capacity and lipid peroxidation

were evaluated. Explants were incubated at 25±1 °C with an irradiance of 45 μmol m^{-2} s^{-1} provided by 60 W fluorescent lamps (Osram®, Munich, Germany) with a photoperiod of 16 h light.

Determination of phenols

Soluble phenols. Phenolic content was determined according to Payet *et al.* (2006). First, 18 mg dry weight of plant tissue was used in the different treatments with antioxidant agents. Extraction was performed with methanol: water (50:50) and 180 μL of the supernatant were taken by adding 100 μL of 10% Folin-Ciocalteua reagent (E. Merck), it was homogenized in a vortex (Corning® LSE™; Tewksbury, MA, U.S.A.), 30 μL of 20% calcium carbonate (Sigma-Aldrich®) were added and then it was incubated for 2 h at 26 °C. Finally, the absorbance was measured at 765 nm using distilled water as a blank. Phenolic content was calculated from a gallic acid calibration curve (0-10000 μg/mL) and expressed as milligrams of gallic acid equivalents (GAE) per g of dry weight (g DW) of *P. dioica* nodal explants.

Cell wall-linked phenols. The determination was carried out according to Payet *et al.* (2006). First, 250 μL of 1M NaOH (Fermont®) were added to the pellet obtained from the soluble phenols determination, homogenized in a vortex (Corning® LSE ™), and then incubated in a water bath at 70 °C for 16 h. Subsequently, 250 μL of 2 M HCl (Fermont®) were added and 100 μL of extract, 900 μL of distilled water and 100 μL of 10% Folin-Ciocalteu (Merck) reagent were taken; after 5 min, 600 μL of 1 M NaOH (Fermont®) were added, saturated with calcium carbonate (Sigma-Aldrich®) and incubated at 26 °C for 1 h. Absorbance and calibration conditions were the same as those used in the determination of soluble phenol.

Antioxidant capacity

The determination of DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich®, St. Louis MO) was performed by the methodology proposed by Huang *et al.* (2002). An aliquot of 3900 μL of DPPH and 100 μL of methanolic extract obtained in the soluble phenols determination was carried out. A calibration curve with Trolox (Sigma-Aldrich®, St. Louis, Missouri Ohio) was used. The mixture was incubated at 30 °C for 30 min and the absorbance was measured at 515 nm. Data were expressed as Trolox equivalents (TE) per g of dry weight (g DW) of *P. dioica* nodal explants.

Determination of lipid peroxidation

The methodology by Heath and Packer (1968) was used. First, 50 mg of fresh plant material were used by adding 1 mL of 0.1% trichloroacetic acid (TCA) (Sigma-Aldrich®) and homogenizing it in a vortex (Corning® LSE™), after which it was centrifuged at 10,000 xg for 15 min. Next, 500 μL of the supernatant was taken and 1 mL of 20% TCA plus 1 mL of 0.5% thiobarbituric acid (TBA) (Sigma-Aldrich®). The supernatant was incubated in a water bath at 95 °C for 30 min followed by an ice bath. MDA content was calculated by the absorbance difference at 532 and 600 nm.

Experimental design and statistical analysis

A completely randomized experimental design was used. All trials were performed in triplicate. Data were processed with the Statistical Package for the Social Sciences (SPSS) version 22 software for Windows and statistical analysis was carried out using an analysis of variance (ANOVA) and a comparison of means using Tukey's test ($p \leq 0.05$). Values expressed in percentages were transformed by the arcsine function before performing the analysis.

RESULTS AND DISCUSSION

Treatments with antioxidant agents

The evaluation of different antioxidant agents showed significant differences for the variables survival, oxidation and contamination *in vitro* in *P. dioica* nodal explants (Table 1).

The highest survival was observed with the addition of L-cysteine, with percentages greater than 40%, while the lowest survival occurred in the control treatment, 100 and 200 mg L^{-1} methylene blue, with 0, 3.3 and 0% survival, respectively. The highest percentage of oxidation was observed in the control treatment, 100 and 200 mg L^{-1} methylene blue with 96.67% oxidation, while the lowest percentages of oxidation were observed in explants treated with L-cysteine, with 30% oxidation. Regarding the percentage of contamination, treatments with 100 and 200 mg L^{-1} AgNPs had the lowest contamination values, with only 20.00%, while the rest of the treatments had contamination values between 30.00 and 46.67%.

In this study, the use of antioxidant agents had an effect on the survival, oxidation, and contamination *in vitro* of *P. dioica* nodal explants. Adding methylene blue to the culture medium showed a negative effect on survival in the explants; the decrease in survival was probably caused by the high percentages of oxidation observed. Regarding the percentage of contamination, no effect was observed because methylene blue does not

Table 1. Effect of three antioxidant agents on the survival, oxidation and contamination *in vitro* of *Pimienta dioica* (L.) Merrill nodal explants.

Treatment	Concentration (mg L^{-1})	Survival (%)	Oxidation (%)	Contamination (%)
Control	0	0.00 ± 0.00^{c}	96.67 ± 3.33^{a}	46.67 ± 3.33^{a}
Methylene blue	50	13.33 ± 3.33^{b}	66.67 ± 3.33^{b}	43.33 ± 3.33^{a}
	100	3.33 ± 3.33^{c}	96.67 ± 3.33^{a}	46.67 ± 3.33^{a}
	200	0.00 ± 0.00^{c}	96.67 ± 3.33^{a}	43.33 ± 3.33^{a}
L-cysteine	50	43.33 ± 3.33^{a}	30.00 ± 0.00^{c}	43.33 ± 3.33^{a}
	100	43.33 ± 3.33^{a}	30.00 ± 5.77^{c}	46.67 ± 3.33^{a}
	200	40.00 ± 5.77^{a}	30.00 ± 5.77^{c}	43.33 ± 3.33^{a}
AgNPs°	50	13.33 ± 3.33^{b}	66.67 ± 3.33^{b}	30.00 ± 0.00^{ab}
	100	33.33 ± 3.33^{ab}	83.33 ± 3.33^{ab}	20.00 ± 5.77^{b}
	200	33.33 ± 3.33^{ab}	83.33 ± 3.33^{ab}	20.00 ± 5.77^{b}

Values represent the mean±SE (standard error). Means with different letters in a column are statistically different (Tukey's test; $p \leq 0.05$), at 30 days of *in vitro* culture. AgNPs: Silver nanoparticles.

present any microbicidal action. Waranusantigui *et al.* (2003) state that methylene blue reduces light penetration and thus prevents oxidation; while Bruchey and Gonzalez-Lima (2008) point out that, at low doses, it decreases superoxide radicals produced in oxidative phosphorylation.

The L-cysteine, it increased survival and decreased the percentage of oxidation, without having an effect on contamination. The reduction of oxidation caused an increase in the survival rate. L-cysteine is an amino acid that has been used to reduce the oxidation of phenolic compounds, as reported in sandalwood (*Santalum album* L.) (Akhtar and Shahzad, 2019). AgNPs did not show a drastic effect on survival rate or oxidation; however, they caused a reduction in contamination rates. The use of AgNPs to reduce *in vitro* contamination has been reported by Spinoso-Castillo *et al.* (2017) in vanilla (*Vanilla planifolia* Jacks. ex Andrews). In fungi, AgNPs break the cell membrane of hyphae altering the mechanisms of infection (Bocate *et al.*, 2019). Lee *et al.* (2019) report that AgNPs that penetrate the cell increase Ag^{+} cations, which could affect the electrical potential of the membrane, denaturing proteins, leading to cell cycle arrest. Because *P. dioca* is a woody species, it has a high susceptibility to oxidation during *in vitro* establishment.

Content of soluble phenols and cell wall-linked phenols

P. dioica buds cultured in medium with methylene blue showed no differences in soluble phenol content; however, treatments with L-cysteine and 50 and 100 mg L^{-1} AgNPs had a significant increase in soluble phenol content, with values above 3.843 mg GAE g^{-1} DW, while the rest of the treatments showed the lowest values (Figure 1A). Regarding cell wall-linked phenolic compounds, the highest contents were obtained in the treatments with 200 mg L^{-1} methylene blue, L-cysteine, and 200 mg L^{-1} AgNPs, with concentrations higher than 7.185 mg GAE g^{-1} DW; while the control and the rest of the treatments did not show significant statistical differences (Figure 1B). The production of phenolic compounds is an indicator of defense against the mechanical damage involved in the healing process. The addition of methylene blue showed no effect on the content of soluble phenols; however, changes were observed in the content of cell wall-linked phenols at the highest concentration evaluated, probably because some dyes have a toxic effect on plant tissues, due to the formation of chelates that produce toxicity (Castellar *et al.*, 2018). On the other hand, when L-cysteine was added, an effect on the content of phenolic compounds was observed for due to its ability to remove quinones (Cruz-Gutiérrez *et al.*, 2020). The use of L-cysteine as an *in vitro* antioxidant agent has been reported by Ricco *et al.* (2018) in mistletoe (*Ligaria cuneifolia*). Regarding the use of AgNPs, they did not decrease the production of soluble phenols, probably due to the reaction of Ag^{+} with the thiol, carboxylate, phosphate, hydroxyl, amine, imidazole and indol groups of some enzymes, producing their inactivation and cell death (Ashraf *et al.*, 2013). However, the addition of AgNPs increased the content of cell wall-linked phenols at the highest evaluated concentration, due to possible toxic damage caused by an excess in the Ag^{+} ion in the culture medium. Toxicity at high concentrations of AgNPs has been reported by Hussain *et al.* (2018) in tangerine (*Citrus reticulata*).

Antioxidant capacity

The evaluation of antioxidant capacity showed that all antioxidant treatments had a reaction of DPPH free radical capture, with values greater than 990 TE g^{-1} DW, differing significantly from the control treatment (Figure 1C). Antioxidant capacity is a defense mechanism against oxidative stress. All treatments with antioxidant agents showed antioxidant capacity. Callaway *et al.* (2004) report that methylene blue at low concentrations can function as a free radical scavenger. However, at high concentrations it can lead to oxidative stress at the cellular level. L-cysteine acts as an intracellular precursor of glutathione biosynthesis, whose function is to remove excess ROS molecules (Koramutla *et al.*, 2021). Haase *et al.* (2012) point out that exposure to AgNPs induces the synthesis of antioxidant enzymes, such as catalase and superoxide dismutase. Pace *et al.* (2015) report that the addition of L-cysteine in lettuce (*Lactuca sativa*) plants increases antioxidant capacity. Regarding the effect of AgNPs, Chung *et al.* (2018) report their antioxidant capacity in cucumber (*Cucumis anguria*) roots.

Lipid peroxidation

Lipid peroxidation showed significant differences in malondialdehyde (MDA) content among treatments with antioxidant agents. The highest MDA content was observed in the

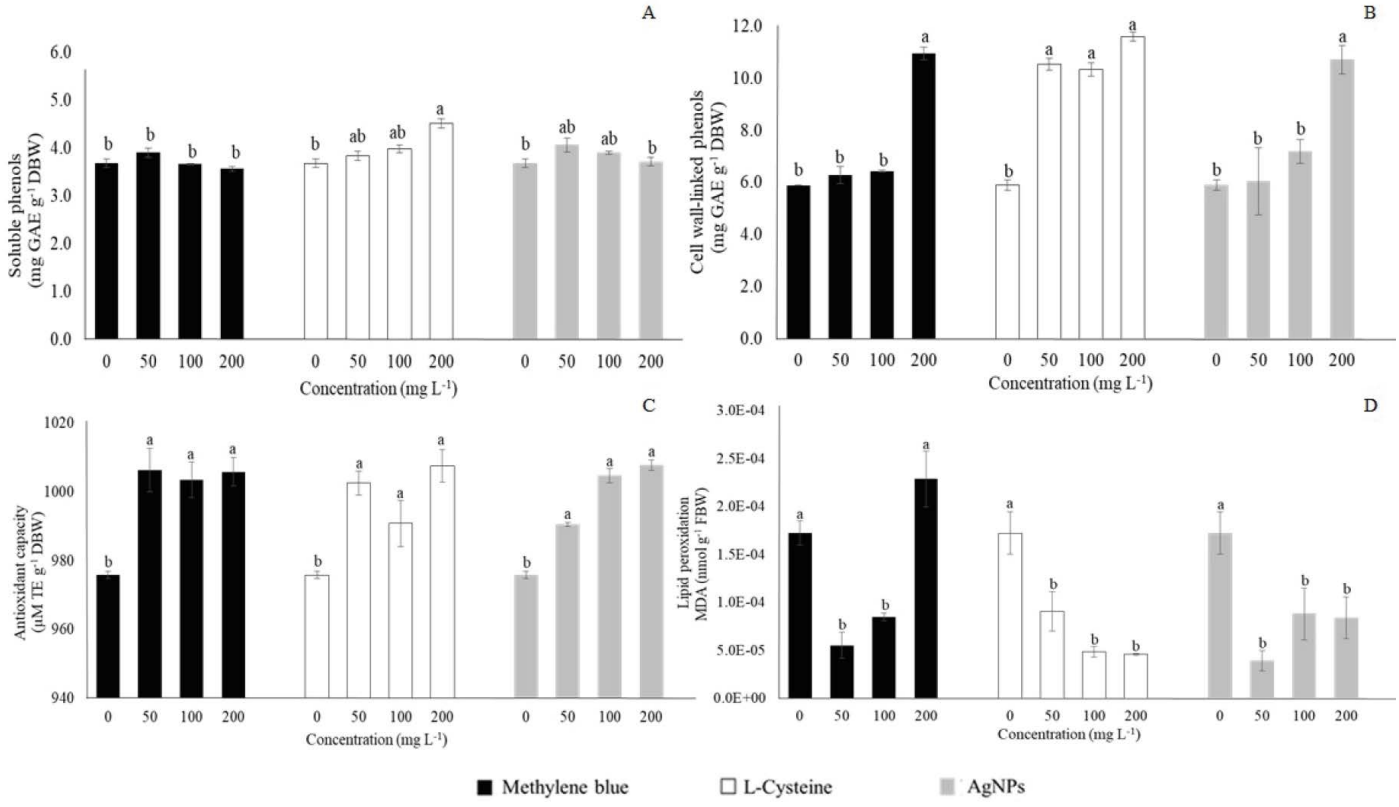

Figure 1. Effect of three antioxidant agents on A) soluble phenols, B) cell wall- linked phenols, C) antioxidant capacity and D) lipid peroxidation in *P. dioica* after 30 days of *in vitro* culture. Different letters denote statistically significant differences according to Tukey's test ($p \leq 0.05$). DBW: Dry biomass weight. FBW: Fresh biomass weight.

control treatment and 200 mg L^{-1} methylene blue, with values higher than 1.72×10^{-4} nmol MDA/g fresh weight, while the lowest content was obtained in the treatments with the addition of L-cysteine and AgNPs, with values lower than 9.07×10^{-5} nmol MDA g^{-1} fresh weight (Figure 1D). An increase in the oxidative degradation of lipids is the result of the capture of electrons that make up the fatty acids present in the cell membrane. The increase in MDA content in the control treatment was due to oxidative stress caused by the lack of antioxidants as reducing agents to prevent and protect oxidative damage, in this regard, Mostofa *et al.* (2015) point out that malondialdehydes produced because of some type of stress can act as molecules that inhibit development. The addition of methylene blue at low concentrations resulted in a decrease in MDA production, because it interacts directly with the mitochondrial electron transport chain (Yang *et al.*, 2020) and having a redox reaction avoiding the oxidative stress of tissues; however, at the highest concentration evaluated it produced greater stress in the *P. dioica* explants, resulting in higher MDA production, the effect on lipid peroxidation using methylene blue has not yet been reported; however, this fact was probably due to toxic damage caused by the high concentration of methylene blue. The addition of L-cysteine resulted in a decrease in MDA content. Ali *et al.* (2016) report decreased MDA production in lychee (*Litchi chinensis*) fruits with the addition of L-cysteine. Regarding the addition of AgNPs, like L-cysteine, they reduced lipid peroxidation. In our study, the decrease in MDA content was probably due to the low effect of silver ions (Nuñez-Anita *et al.*, 2014). Spinoso-Castillo *et al.* (2017) found that 50 mg L^{-1} of AgNPs in *V. planifolia* has an effect on the reduction of MDA.

CONCLUSION

The present study, L-cysteine is an alternative to reduce oxidation of *P. dioica* and could be evaluated for other species with *in vitro* recalcitrance caused by oxidation during establishment.

ACKNOWLEDGMENTS

To La Joya del Totonacapan, S. de P.R. de R.L., for financial support with the support of the Stimulus Program for Research, Technological Development and Innovation (PEI-2018, Project No. 251642).

REFERENCES

Akhtar, R., & Shahzad, A. (2019). Morphology and ontogeny of directly differentiating shoot buds and somatic embryos in *Santalum album* L. *Journal of Forestry Research, 30* (4), 1179-1189. DOI: 10.1007/s11676-018-0679-5

Ali, S., Khan, A. S., & Malik, A. U. (2016). Postharvest L-cysteine application delayed pericarp browning, suppressed lipid peroxidation and maintained antioxidative activities of litchi fruit. *Postharvest Biology and Technology, 121*, 135-142. DOI: 10.1016/j.postharvbio.2016.07.015

Ashraf, S., Abbasi, A. Z., Pfeiffer, C., Hussain, S. Z., Khalid, Z. M., Gil, P. R., & Hussain, I. (2013). Protein-mediated synthesis, pH-induced reversible agglomeration, toxicity and cellular interaction of silver nanoparticles. *Colloids and Surfaces B: Biointerfaces, 102*, 511-518. DOI: 10.1016/j.colsurfb.2012.09.032

Bocate, K. P., Reis, G. F., de Souza, P. C., Junior, A. G. O., Durán, N., Nakazato, G., & Panagio, L. A. (2019). Antifungal activity of silver nanoparticles and simvastatin against toxigenic species of *Aspergillus*. *International Journal of Food Microbiology, 291*, 79-86. DOI: 10.1016/j.ijfoodmicro.2018.11.012

Bruchey, A. K., & Gonzalez-Lima, F. (2008). Behavioral, physiological and biochemical hormetic responses to the autoxidizable dye methylene blue. *American Journal of Pharmacology and Toxicology, 3*(1), 72-79.

Callaway, N. L., Riha, P. D., Bruchey, A. K., Munshi, Z., & Gonzalez-Lima, F. (2004). Methylene blue improves brain oxidative metabolism and memory retention in rats. *Pharmacology Biochemistry and Behavior, 77*(1), 175-181. DOI: 10.1016/j.pbb.2003.10.007

Cardoso, J. C., Gerald, L. T. S., & da Silva, J. A. T. (2018). Micropropagation in the twenty-first century. *Plant Cell Culture Protocols*, 17-46. DOI: 10.1007/978-1-4939-8594-4-2

Castellar, G., Angulo, E., Zambrano, A., & Charris, D. (2018). Equilibrio de adsorción del colorante azul de metileno sobre carbón activado. *Revista UDCA Actualidad & Divulgación Científica, 16* (1), 263-271. DOI: 10.31910/rudca.v16.n1.2013.882

Chung, I. M., Rajakumar, G., & Thiruvengadam, M. (2018). Effect of silver nanoparticles on phenolic compounds production and biological activities in hairy root cultures of *Cucumis anguria*. *Acta Biologica Hungarica, 69* (1), 97-109. DOI: 10.1556/018.68.2018.1.8

Cruz-Gutiérrez, E. J., Hernández-Fuentes, L. M., & Gómez-Reyes, L. A. (2020). Uso de L-cisteina para el control de oxidación *in vitro* de *Annona muricata* L. *Revista Multidisciplinaria de Avances de Investigación, 6* (3), 1-6

Haase, A., Rott, S., Mantion, A., Graf, P., Plendl, J., Thünemann, A. F., & Reiser, G. (2012). Effects of silver nanoparticles on primary mixed neural cell cultures: uptake, oxidative stress and acute calcium responses. *Toxicological Sciences, 126*(2), 457-468. DOI: 10.1093/toxsci/kfs003

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics, 125* (1), 189-198. DOI: 10.1016/0003-9861(68)90654-1

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry, 50*(16), 4437-4444. DOI: 10.1021/jf0201529

Hussain, M., Raja, N. I., Naz, F., Iqbal, M., & Aslam, S. (2018). Green synthesis and characterisation of silver nanoparticles and their effects on antimicrobial efficacy and biochemical profiling in *Citrus reticulata*. *IET Nanobiotechnology, 12* (4), 514-519

Koramutla, M. K., Negi, M., & Ayele, B. T. (2021). Roles of glutathione in mediating abscisic acid signaling and its regulation of seed dormancy and drought tolerance. *Genes, 12*(10), 1620. DOI: 10.3390/genes12101620

Lee, B., Lee, M. J., Yun, S. J., Kim, K., Choi, I. H., & Park, S. (2019). Silver nanoparticles induce reactive oxygen species-mediated cell cycle delay and synergistic cytotoxicity with 3-bromopyruvate in *Candida albicans*, but not in *Saccharomyces cerevisiae*. *International Journal of Nanomedicine, 14*, 4801. DOI: 10.2147/IJN.S205736

López, I. M., Torres, J. M., & Hernández, D. M. (2021). Diversity of Volatile Organic Compounds in leaves of *Pimenta dioica* L. Merrill at different developmental stages from fruiting and no-fruiting trees. *Journal of the Mexican Chemical Society, 65* (3). DOI: 10.29356/jmcs.v65i3.1498

Marques, C. S., Carvalho, S. G., Bertoli, L. D., Villanova, J. C. O., Pinheiro, P. F., Dos Santos, D. C. M., & Bernardes, P. C. (2019).β-Cyclodextrin inclusion complexes with essential oils: Obtention, characterization, antimicrobial activity and potential application for food preservative sachets. *Food Research International, 119*, 499-509. DOI: 10.1016/j.foodres.2019.01.016

Martínez Rivero, A., Ramírez-Mosqueda, M. A., Mosqueda, F. O., Rivas, P. M., & Bello-Bello, J. J. (2020). Influence of Vitrofural® on sugarcane micropropagation using temporary immersion system. *Plant Cell, Tissue, and Organ Culture, 141*(2), 447-453. DOI: 10.1007/s11240-020-01800-x

Mostofa, M. G., Hossain, M. A., & Fujita, M. (2015). Trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems. *Protoplasma, 252* (2), 461-475. DOI: 10.1007/s00709-014-0691-3

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15* (3), 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x

Nuñez-Anita, R. E., Acosta-Torres, L. S., Vilar-Pineda, J., Martínez-Espinosa, J. C., de la Fuente-Hernández, J., & Castaño, V. M. (2014). Toxicology of antimicrobial nanoparticles for prosthetic devices. *International Journal of Nanomedicine, 9*, 3999. DOI: 10.2147/IJN.S63064

Pace, B., Capotorto, I., Ventura, M., & Cefola, M. (2015). Evaluation of L-cysteine as anti-browning agent in fresh-cut lettuce processing. *Journal of Food Processing and Preservation, 39*(6), 985-993. DOI: 10.1111/jfpp.12312

Payet, B., Shum Cheong Sing, A., & Smadja, J. (2006). Comparison of the concentrations of phenolic constituents in cane sugar manufacturing products with their antioxidant activities. *Journal of Agricultural and Food Chemistry, 54*(19), 7270-7276. DOI: 10.1021/jf060808o

Ricco, M. V., Bari, M. L., Ricco, R. A., Wagner, M. L., & Álvarez, M. A. (2018). Análisis de polifenoles e iniciación de cultivos *in vitro* de *Ligaria cuneifolia* (R. et P.) Tiegh (Loranthaceae) de ejemplares provenientes de la localidad La Población, provincia de Córdoba, Argentina. *Dominguezia, 34* (1), 53-64

Spinoso-Castillo, J. L., Chavez-Santoscoy, R. A., Bogdanchikova, N., Pérez-Sato, J. A., Morales-Ramos, V., & Bello-Bello, J. J. (2017). Antimicrobial and hormetic effects of silver nanoparticles on *in vitro* regeneration of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. *Plant Cell, Tissue and Organ Culture, 129* (2), 195-207. DOI: 10.1007/s11240-017-1169-8

Taghizadeh, M., & Dastjerdi, M. G. (2020). Inhibition of browning problem during the callogenesis of *Spartium junceum* L. *Ornamental Horticulture, 27*, 68-77. DOI: 10.1590/2447-536X.v27i1.2230

Waranusantigui, P., Pokethitiyook, P., Kruatrachue, M., & Upatham, E. S. (2003). Kinetics of basic dye (methylene blue) biosorption by giant duckweed (*Spirodela polyrrhiza*). *Environmental Pollution, 125* (3), 385-392. DOI: 10.1016/S0269-7491(03)00107-6

Wei, Z., Shi, X., Wei, F., Fan, Z., Mei, L., Tian, B., ... & Shi, G. (2019). The cotton endocycle-involved protein SPO11-3 functions in salt stress via integrating leaf stomatal response, ROS scavenging and root growth. *Physiologia Plantarum, 167* (1), 127-141. DOI: 10.1111/ppl.12875

Yang, L., Youngblood, H., Wu, C., & Zhang, Q. (2020). Mitochondria as a target for neuroprotection: role of methylene blue and photobiomodulation. *Translational Neurodegeneration, 9*, 1-22. DOI: 10.1186/s40035-020-00197-z

Zhou, X., Joshi, S., Patil, S., Khare, T., & Kumar, V. (2021). Reactive oxygen, nitrogen, carbonyl and sulfur species and their roles in plant abiotic stress responses and tolerance. *Journal of Plant Growth Regulation*, 1-24. DOI: 10.1007/s00344-020-10294-y