





ORIGINAL RESEARCH ARTICLE

Development of Sterilization protocol for *In Vitro* Germination of *Prosopis Africana* on Cocopeat as a low-Cost Alternative

Rahanatu Adamu Kakudi¹, Tijjani Sabiu Imam², Hauwa Bah Ibrahim¹, Safiya Balarabe¹,
Abdulmutallib Isah Muhammad³ and Ismail Adamu Kakudi⁴

¹Department of Integrated Science, Yusuf Maitama Sule Federal University of Education, Kano, Nigeria

²Department of Life Sciences, Bayero University Kano, Nigeria

³Centre for Dryland Agriculture, Bayero University, Kano, Nigeria

⁴Department of Animal Health and Technology, Audu Bako College of Agriculture, Kano, Nigeria

ABSTRACT

Prosopis africana, a valuable tree species in West Africa, is the only species of *Prosopis* that is indigenous to tropical Africa. Due to extensive overexploitation, this species has become extinct in many parts of the southern Sahel and the adjacent Sudan savannahs. In the first experiment, scarified seeds were surface sterilized with 10% and 20% NaOCl solution for 10 and 20 minutes. In the second experiment, seeds were soaked in 1% antifungal solution for 30 and 60 minutes before final sterilization with 10% and 20% NaOCl. Treating seeds with 10% and 20% NaOCl alone for 10 and 20 minutes resulted in very low contamination levels of 5.0 ± 0.04 and 2.5 ± 0.03 , respectively, with no significant difference between the treatments at $p < 0.05$. Seed germination was influenced by treatments, with significant differences at $p < 0.05$, where the highest germination was obtained with 20% NaOCl for 10 minutes (70.0 ± 13.00). In the second experiment, the lowest contamination ($20\% \pm 2.41$) and the highest germination ($50\% \pm 14.1$) were recorded for seeds treated with 10% NaOCl + 1% antifungal for 30 min. The best sterilization treatment was 20% NaOCl for 10 minutes, which had the highest germination and one of the lowest contamination percentages. Cocopeat can be successfully used as a low-cost medium to generate sterile explants of *P. africana*, which can be used for micropropagation and genetic improvement of the species. This can be used for the conservation of the threatened tree in Nigeria.

ARTICLE HISTORY

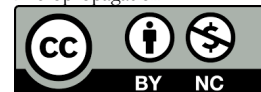
Received July 07, 2025

Accepted September 16, 2025

Published September 30, 2025

KEYWORDS

Prosopis africana, Sterilization, Sodium Hypochlorite, Germination, micropropagation



© The Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License [creativecommons.org](https://creativecommons.org/licenses/by-nc/4.0/)

INTRODUCTION

Prosopis africana, a valuable tree species in West Africa, is the only species of *Prosopis* that is indigenous to tropical Africa (Asoiro and Ohagwu 2017). It is highly valued for its wood, which is sought after for firewood, charcoal, and other essential products, as well as for its environmental services. *Prosopis africana* enriches the soil by fixing nitrogen; its leaves are rich in protein, and sugar pods are used as foodstuffs for feeding ruminants in Nigeria (Ancha et al., 2020; Annongu et al., 2004).

However, the species is facing degradation and regeneration problems due to over-exploitation (Bello et al., 2019). Ekpo et al. (2021) stated that *P. africana* is among the most endangered species due to commercial charcoal production. Despite its numerous benefits, the *P. africana* is listed as “Least Concern” on the IUCN Red List but locally it has been classified as critically threatened (Bello et al., 2019; Danjuma & Abubakar, 2017; Nodza et al., 2024). Due to extensive overexploitation, this species

has disappeared from extensive parts of the southern Sahel and the adjacent Sudan savannahs (Agboola, 2004).

There are several reports on the *in vitro* propagation of *Prosopis* species (Rivera et al., 2020; Haroon et al., 2018; Morales-Domínguez et al., 2019; Ortiz-Robles et al., 2022), but no work has been reported yet for *P. africana*. Excessive falling, in conjunction with a slow growth rate and difficulty in seed germination, prevents trees from multiplying in considerable numbers in the wild. Biotechnological techniques are widely used in conservation practices, with micropropagation emerging as a suitable tool for *in vitro* conservation purposes. However, plant tissue culture cannot be achieved without establishing a sterilization protocol, as the success of the process depends on it. Another factor to consider in tissue culture is cost; a cheap yet efficient alternative is crucial for tissue culture, especially in developing countries like Nigeria. Several media (Murashige and Skoog Media,

Correspondence: Rahanatu Adamu Kakudi. Department of Integrated Science, Yusuf Maitama Sule Federal University of Education, Kano, Nigeria. ✉ kakudirayhan@gmail.com.

How to cite: Kakudi, R. A., Imam, T. S., Bah, H. I., Balarabe, S., Isah, A. M. & Kakudi, I. A. (2025). Development of Sterilization protocol for *In Vitro* Germination of *Prosopis Africana* on Cocopeat as a low-Cost Alternative. *UMYU Scientifica*, 4(3), 166 – 171. <https://doi.org/10.56919/usci.2543.016>

Woody Plant Media, White's Media, etc.) are used in conjunction with gelling agents as substrates for *in vitro* plant growth, which is costly.

The use of cocopeat as an alternative medium to generate sterile explants will reduce cost. Having excellent air permeability, water-holding and nutrient-holding capabilities, low bulk density, high cation exchange capacity, and high expansion up to four or five times in volume when combined with water are some of the key attributes of coco peat, the last attribute contributing more to its economic value (Kim & Yoo, 2024). The first step in establishing plant tissue-cultured plants is to develop a sterilization protocol to produce aseptic explants. This research is the first to aim at developing a sterilization protocol and cost-effective media for the *in vitro* generation of sterile explants, which can be used in the micropropagation and conservation of *P. africana*.

MATERIALS AND METHODS

Seed collection and experimental site.

Samples were collected from Yobe state at Dazigaw village in Nangere Local Government Area of Yobe State, North-eastern Nigeria at latitudes of 11°42'44" N and Longitudes of 11°0' 33" E. The experiment was conducted at the Plant Tissue Culture Laboratory of the Centre for Dryland Agriculture, Bayero University, Kano, Nigeria.

Seed scarification

Seeds were scarified in 98% Sulfuric acid (H₂SO₄, Sigma Aldrich) for 20 minutes, then washed under running tap water until no traces of acid were left (Dera et al., 2019) with modification. Seeds were then soaked for 48 hours for water imbibition.

Cocopeat Preparation

Cocopeat was soaked in water and drained, then fifteen grams (15g) was weighed into test tubes (50ml) and sterilized by autoclaving at 121 °C and 103.4 kPa pressure for 20 minutes.

Seed Sterilization

In the first sterilization method, soaked seeds were surface sterilized in the laminar flow hood by stirring in 10 and 20% (v/v) NaOCl solution (3.5% Jik, Uniliver) with 2 drops of liquid soap (Morning fresh Uniliver) for 10 and 20 minutes, then rinsed several times with sterile distilled water to remove all traces of NaOCl.

In the second setup, seeds were pretreated with 1% w/v antifungal (SAAF Carbendazim 12% + Mancozeb 63%) for 30 and 60 mins then then soaked for 48 hours. After soaking, they were sterilized in a laminar flow hood by shaking in 20% NaOCl with one drop of liquid soap for 20 minutes, followed by several rinses with sterile distilled water. The sterilized seeds were inoculated on sterilized coconut coir (cocopeat) and kept in a dark room for

germination. Seeds that germinated were removed from the dark room and incubated at 28 ± 2 °C and in the light (16 h light /8 h dark) for seedling growth.\

Germination

Germination, defined as the emergence of the radicle from the seed coat, was recorded every week after the first radicle emerged. Final germination was evaluated after 60 days for all germination experiments.

Final germination percentage (FG) was calculated with the following equations (NASR et al., 2013):

$FG = n/N \times 100$ (Where n is the number of germinated seeds, N is the total number of seeds).

Experimental design and Data analysis

The experiments were laid out in a completely randomized design (CRD) with 20 explants per treatment, making a total of 160 seeds. Each treatment consisted of an average of 2 replicates, with 10 seeds in each replicate. The number of contaminated cultures after sterilization was recorded 2 days after culturing and continued weekly for 8 weeks.

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to test the statistical significance, and the significance differences among means were carried out using the Least Significant Difference (LSD) at a 5% probability. All results were analyzed using Microsoft Excel software version 2016.

RESULTS AND DISCUSSION

Generating aseptic explants is the most important step in tissue culture studies. The use of explants from mature plants growing in the field is often difficult or even impossible, mainly due to the high level of contamination (Bonga et al., 2010; Kirika et al., 2015). Therefore, sterile explants need to be produced *in vitro* using the most suitable sterilization protocol. Contamination control is mostly determined by the disinfectant used and the duration of exposure. Only microbial contamination should be removed during sterilisation; living material shouldn't lose its biological activity.

Consequently, the plant material needs to be surface sterilized using a disinfectant for a predetermined amount of time and at an appropriate concentration (Andelić et al., 2024). Contamination can be external, resulting from laboratories and used materials (media, glassware, culture vessels, tools, and explants), whereas it can also be internal, related to the endophytic microbes in mother plants (Abdalla et al., 2020).

It is easier to control external contamination, but very difficult when contamination is endophytic and can lead to an increase in the cost of producing explants. The high costs of equipment, water, chemicals (such as plant growth hormones or regulators, surface sterilants, disinfectants, etc.), and culture losses due to *in vitro* culture contamination, among other factors, can cripple

several *in vitro* plant culture techniques in many developing countries, especially in Africa. Possibly, this is the main reason we have limited or no interest in investing in tissue culture laboratories in Africa, except for academic institutions, which use them solely for learning the technique (Mng'omba et al., 2012). Several chemicals have been used to generate aseptic explants, including mercuric chloride, calcium hypochlorite, sodium hypochlorite, ethanol, antibiotics, and fungicides (Chornobrov & Tkachova, 2021; Payamnour et al., 2014), with high success rates.

NaOCl Sterilization

Even though the concentration of sterilant is very important when establishing aseptic cultures. Table 1 shows the contamination and germination percentages of *P. africana* seeds following different treatments with NaOCl. The lowest contamination (2.5% ± 0.03) was recorded when seeds were immersed in 10% and 20% NaOCl for 20 minutes. Similarly, the highest contamination (5.0% ± 0.04) was observed with 10 % and 20% NaOCl immersion for 10 minutes. The concentration of sterilant and exposure time of seeds were not significantly different at (p<0.05).

In a Procedure to establish aseptic seedlings of *Zehneria capillacea*, sterile seedlings were obtained using 20-100% NaOCl, with no difference in contamination percentages (Agogbua et al., 2022). Sometimes higher concentrations require optimized exposure time (Connell et al., 2016). If the treatment was too brief or too prolonged, microbial cells could survive, especially in micropores or crevices

that strong NaOCl cannot fully reach (Rozman & Doull, 2000). However, germination was influenced by concentration and exposure time, with significant differences even though not across all treatments. Treatment concentration of 20% NaOCl resulted in higher germination percentages (70.0% ± 0.07 and 67.5% ± 0.07) compared to 10%. This record indicated a high germination occurrence compared to 40.0 ± 2.46 and 52.5 ± 6.50 recorded for treatments with 10% NaOCl for 10 and 20 minutes, respectively (Table 1.0). The difference is statistically significant at (p<0.05). A high concentration of sterilant, with lower contamination and higher germination percentages, has been reported in other works. A similar result was reported by Maina et al. (2010), recording (40%) survival using the lower concentration of NaOCl (0.525%) and a higher survival percentage of 66% corresponding to the higher concentration of 1.050% NaOCl in ICGV-12991 groundnut variety.

This result is in conformity with other seeds of various plants which have been successfully sterilized with 10% or 20% NaOCl: *Parkia biglobosa* (Abbas et al., 2018), *Ficus religiosa* (Hesami et al., 2017), *Prosopis laevigata* (Morales et al., 2019), *Khaya senegalensis* (Hung and Trueman, 2011), and *Boscia senegalensis* (Khalafalla et al., 2011). Treating the seeds with 20% NaOCl for 10 minutes and 20 minutes did not show a statistically significant difference in the germination percentage of *P. africana* seeds. Therefore, a 20% NaOCl solution was used for 20 minutes throughout the research. Healthy seedlings were produced, which could be used as explants for micropropagation of *P. africana* (Plate 1).

Table 1.0: Effect of NaOHCL Treatment on Sterilization and Germination of *P. africana* Seeds

Treatment	Contamination%	Germination %
10% NaOHCL 10min	5.0 ± 0.04 ^a	40.0 ± 5.10 ^a
10% NaOHCL 20min	2.5 ± 0.03 ^a	52.5 ± 7.08 ^{ab}
20% NaOHCL 10min	5.0 ± 0.04 ^a	70.0 ± 13.00 ^b
20% NaOHCL 20min	2.5 ± 0.03 ^a	67.5 ± 11.13 ^b

Values represent means ±SD. Means followed by the same letter within columns are not significantly different (P < 0.05) using LSD.

Table 2.0: Effect of NaOHCL and Antifungal on Sterilization and Germination of *P. africana* Seeds

Treatment	Contamination (%)	Germination (%)
10% NaOCL + 1% FUNGICIDE 30MINS	20 ± 2.41 ^a	50 ± 14.1 ^a
10% NaOCL + 1% FUNGICIDE 60MINS	25 ± 4.20 ^a	30 ± 9.11 ^a
20% NaOCL + 1% FUNGICIDE 30 MIN	25 ± 4.20 ^a	35 ± 8.11 ^a
20% NaOCL + 1% FUNGICIDE 60 MIN	60 ± 12.33 ^b	25 ± 5.10 ^a

Values represent means ±SD. Means followed by the same letter within columns are not significantly different (P < 0.05) using LSD.

NaOCL+ Antifungal sterilization

The need to incorporate an antifungal often arises when dealing with contamination from various sources (Kakade and Nutan 2017). In Table 2.0, records show a contamination percentage of 20 ± 2.41% and 25 ± 4.20% for 10% NaOCl + Antifungal after 30 minutes. Using 10% NaOCl + Antifungal for 60 minutes and 20% NaOCl + Antifungal for 30 minutes had the same effect (25% ± 4.20), with no statistical difference between the three treatments. Similarly, sterilizing seeds of *Parkia biglobosa*

(Fabaceae) with 30% NaOCl and 60% NaOCl all resulted in 50% contamination percentages and a survival percentage of 25% and 37%, respectively (Abbas et al., 2018). Kirika et al. (2015) also found 10% NaOCl to be the most effective sterilizing agent, yielding 55% clean seeds compared to 50% clean seeds with a 30% NaOCl treatment. Contrary to the efficiency of the sterilant used at high concentration and longer exposure time, 20% NaOCl + 1% antifungal for 60 minutes resulted in the highest contamination and a significant difference at p < 0.05. This could be explained by extended exposure to

antifungal drugs, which may weaken seed defenses or reduce beneficial microbial populations, thereby facilitating the growth of opportunistic pathogens after treatment. High amounts of NaOCl may be phytotoxic. This might damage the outer seed coat or compromise the integrity of plant tissue. During sterilisation, damaged seeds may have been injected undetected, leaving them more susceptible to microbial invasion and subsequent contamination (Carmello & Cardoso, 2018). Both during culture initiation and from outside sources, contamination may be introduced. Germination, although higher at lower treatment levels, was not statistically different. A similar result was obtained by Silveira et al. (2022). Reports have shown that high levels of sterilization, which correspond to low contamination rates, usually result in low germination due to the inhibitory action of the chemicals in the sterilants (Akin et al., 2014; Hesami et al., 2017). Similar results obtained in this research also confirm that different concentrations and treatment durations of sodium hypochlorite did not affect *in vitro* germination of *Zehneria capillacea* seeds (Agogbua and Okoli 2022).



Plate 1: Sterile *P. africana* seedlings generated on cocopeat

CONCLUSION

For the first time, an effective sterilization protocol was developed for *P. africana*. A 10% NaOCl treatment for 20 minutes resulted in a low contamination percentage of 5% and a high germination percentage of 70%, making it the most suitable treatment. Exposure time and concentration of NaOCl did not affect the contamination percentage but had a significant effect on germination percentage. Adding an antifungal to NaOCl resulted in a higher contamination percentage compared to NaOCl alone in this experiment. Sterile explants of *P. africana* can be successfully generated on low-cost cocopeat as a new medium to produce sterile explants. This research introduces a technique that can be utilized for the conservation of *P. Africana*. Future research should focus on multiplication using cocopeat-grown explants and rooting to establish a micropropagation protocol for the species, including the assessment of genetic stability *in vitro* seedlings and genetic improvement of the plant.

Additionally, the isolation of fungi can help identify the best antifungal treatment.

ACKNOWLEDGEMENT

We appreciate the financial support given by the Nigerian Tertiary Education Trust Fund (TETFUND). We also thank the Centre for Dryland Agriculture at Bayero University, Kano, Nigeria, for providing the necessary support to accomplish this research.

REFERENCES

- Abbas, M. S., El-Shabrawi, H. M., Soliman, A. S., & Selim, M. A. (2018). Optimization of germination, callus induction, and cell suspension culture of African locust beans *Parkia biglobosa* (Jacq.) Benth. *Journal of Genetic Engineering and Biotechnology*, 16(1), 191–201. [Crossref]
- Abdalla, N., El-Ramady, H., Seliem, M. K., El-Mahrouk, M. E., Taha, N., Bayoumi, Y., & Dobránszki, J. (2022). An academic and technical overview on plant micropropagation challenges. *Horticulturae*, 8(8), 677. [Crossref]
- Agboola, D. A. (2004). *Prosopis africana* (Mimosaceae): stem, roots, and seeds in the economy of the savanna areas of Nigeria. *Economic Botany*, 58(1), S34–S42. [Crossref]
- Agogbua, J. U., Okoli, B. E., & Osuji, J. O. (2022). Procedure for *in vitro* seed sterilization, germination and aseptic seedling establishment of *Zehneria capillacea* (Schumacher) C. Jeffrey. *GSC Biological and Pharmaceutical Sciences*, 19(1), 143–148. [Crossref]
- Akin, B., Kocaçalışkan, İ., & Güleriyüz, G. (2014). Micropropagation of *Erodium sibthorpiatum* subsp. *sibthorpiatum*, an endemic threatened species of Uludağ Mountain (Bursa-Turkey). *Turkish Journal of Botany*, 38(1), 148–155. [Crossref]
- Ancha, P. U., Chukwu, O., Ezeano, C. I., Udekwe, M. A., & Iheme, F. C. (2020). Effect of growth media on the early performance of *Prosopis africana* (Guill. and Perr.) Taub. seedlings. *European Journal of Biological Research*, 10(3), 257–262.
- Anđelić, T., Vujović, T., Jevremović, D., Tomić, J., & Radivojević, D. (2024). Comparative study of different surface sterilization treatments and optimal month for establishment of aseptic cultures of raspberry cultivars. *Journal of Central European Agriculture*, 25(2), 470–480. [Crossref]
- Annongu, A. A., Joseph, J. K., & Liebert, F. (2004). Effect of anaerobic fermentation and Lyle treated *Prosopis africana* seed meal on the nutritional and haematological responses of Harco chicks. *Journal of Raw Materials Research*, 1, 33–41.
- Asoiro, F. U., & Ohagwu, C. J. (2017). Some Moisture Dependent Thermal Properties and Bulk Density of *Prosopis africana* Seeds (Okpeye). *Nigerian Journal of Technology*, 36(3), 936–943. [Crossref]

- Bello, A., Jamaladdeen, S., Elder, M. T., Yaradua, S. S., Kankara, S. S., Wagini, N. H., & Muasya, M. (2019). Threatened medicinal and economic plants of the Sudan Savanna in Katsina State, northwestern Nigeria. *Bothalia-African Biodiversity & Conservation*, 49(1), 1–17. [\[Crossref\]](#)
- Bonga, J. M., Klimaszewska, K. K., & Von Aderkas, P. (2010). Recalcitrance in clonal propagation, in particular of conifers. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 100, 241–254. [\[Crossref\]](#)
- Carmello, C. R., & Cardoso, J. C. (2018). Effects of plant extracts and sodium hypochlorite on lettuce germination and inhibition of *Cercospora longissima* in vitro. *Scientia Horticulturae*, 234, 245–249. [\[Crossref\]](#)
- Chornobrov, O., & Tkachova, O. (2021). Optimisation of the protocol for sterilising explants of some deciduous woody plants in culture in vitro. *Ukrainian Journal of Forest and Wood Science*, 12(3), 80. [\[Crossref\]](#)
- Danjuma, M. N., & Abubakar, S. M. (2017). Conservation status of Vegetation in the Dryland of Northwestern Nigeria. *International Journal of Agriculture, Forestry and Fisheries*, 5(2), 18–24.
- Dera, B. A., Agera, S. I. N., & Ezugwu, E. U. (2019). Effect of Seed Size and Acid Scarification on Germination and Early Growth of *Prosopis africana*. *Journal of Global Biosciences*, 8(1), 5774–5788.
- Ekpo Abraham Salamatu, S., Ayuba, H. K., Marcus, D. N., & Ogah, A. T. (2021). Analysis of Tree Species Preference and Reasons among Commercial Charcoal Producers in Nasarawa State, Nigeria. *European Journal of Environment and Earth Sciences*, 2(2), 24–29. [\[Crossref\]](#)
- Haroon, A., Manzoor, N., & Iqbal, M. (2018). Micropropagation of *Prosopis cineraria* (L.) Druce, an endangered tree of Pakistan. *Pakistan Journal of Botany*, 50(6), 443–449.
- Hesami, M., Daneshvar, M. H., & Lotfi, A. (2017). In vitro shoot proliferation through cotyledonary node and shoot tip explants of *Ficus religiosa* L. *Plant Tissue Culture and Biotechnology*, 27(1), 85–88. [\[Crossref\]](#)
- Hung, C. D., & Trueman, S. J. (2011). In vitro propagation of the African mahogany *Khaya senegalensis*. *New Forests*, 42, 117–130. [\[Crossref\]](#)
- Ilahi, W. F., & Ahmad, D. (2017). A study on the physical and hydraulic characteristics of cocopeat perlite mixture as a growing media in containerized plant production. *Sains Malaysiana*, 46(6), 975–980. [\[Crossref\]](#)
- Kakade, P. S., & Malpathak, N. P. (2017). Micropropagation of *Plumbago zeylanica* L. using in vitro germinated seedling explants. *International Journal of Life Sciences*, 5(2), 211–218. [\[Link\]](#)
- Khalafalla, M. M., Daffalla, H. M., Abdellatef, E., Agabna, E., & El-Shemy, H. A. (2011). Establishment of an in vitro micropropagation protocol for *Boscia senegalensis* (Pers.) Lam. ex Poir. *Journal of Zhejiang University Science B*, 12(4), 303–312. [\[Crossref\]](#)
- Khatak, S., Sharma, A., & Saini, R. (2018). Comparative analysis of plant growth regulators on bud break in *Prosopis* and *Tecomella* for sustainable agriculture in arid and semi-arid regions of India. *Bioscience Biotechnology Research Communications*, 11(2), 208–215. [\[Crossref\]](#)
- Kim, S. K., & Yoo, Y. K. (2024). Mixing ratio of peat moss, coco peat, and perlite affects the physicochemical properties of media and growth of *Tetragonia tetragonoides*. *Horticultural Science and Technology*, 42(1), 15–27. [\[Crossref\]](#)
- Kirika, M. W., Kahia, J. W., Diby, L. N., Njagi, E. M., Dadjo, C., & Kouame, C. (2015). Micropropagation of an Endangered Medicinal and Indigenous Multipurpose Tree Species: *Erythrina abyssinica*. *HortScience*, 50(5), 738–743. [\[Crossref\]](#)
- Lara, E. Y. C., Imakawa, A. M., Da Silva, D., & Sampaio, P. D. T. B. (2022). In vitro regeneration of *Senna alata* (L.) Robx. (Fabaceae): An ancient medicinal plant. *Advances in Forestry Science*, 9(3), 1831–1838. [\[Crossref\]](#)
- Maina, S. M., Emongor, Q., Sharma, K. K., Gichuki, S. T., Gathaara, M., & de Villiers, S. M. (2010). Surface sterilant effect on the regeneration efficiency from cotyledon explants of groundnut (*Arachis hypogea* L.) varieties adapted to eastern and Southern Africa. *African Journal of Biotechnology*, 9(20), 2866–2872.
- Mng'omba, S. A., du Toit, E. S., Akinnifesi, F. K., & Sileshi, G. (2012). Efficacy and utilization of fungicides and other antibiotics for aseptic plant cultures. InTech Open Access Publisher.
- Morales-Domínguez, J. F., de León, D. S. D., Garcidueñas-Piña, C., & Pérez-Molphe-Balch, E. (2019). Germination, in vitro propagation and soil acclimatization of *Acacia farnesiana* and *Prosopis laevigata*. *South African Journal of Botany*, 124, 345–349. [\[Crossref\]](#)
- Morales-Domínguez, J. F., de León, D. S. D., Garcidueñas-Piña, C., & Pérez-Molphe-Balch, E. (2019). Germination, in vitro propagation and soil acclimatization of *Acacia farnesiana* and *Prosopis laevigata*. *South African Journal of Botany*, 124, 345–349. [\[Crossref\]](#)
- Nasr, S. M. H., Savadkoobi, S. K., & Ahmadi, E. (2013). Effect of different seed treatments on dormancy breaking and germination in three species in arid and semi-arid lands. *Forest Science and Practice*, 15(2), 130–136. [\[Crossref\]](#)
- Nodza, G. I., Tochukwu, E., Igbari, A. D., Onuminya, T. O., & Ogundipe, O. T. (2024). Application of IUCN Red List Criteria for Regional Assessment of Some Northern Savanna Trees of Nigeria, West Africa. *Preprints*. [\[Crossref\]](#)
- Ortiz-Robles, M. D., Gómez-González, A., Flores-Benítez, S., Montero, L. L. V., Amador, L. D. R., Segovia, C. P., & Silos-Espino, H. (2022). Characterization and in vitro spread of mesquite (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) MC Johnst.). *Agro Productividad*, 15(8), 137–145. [\[Crossref\]](#)

- Payamnour, V., Bezdi, K. G., Mehrdad, M., & Ahmadi, A. (2014). Optimization of in vitro sterilization protocol for obtaining contamination-free cultures of *Tilia platyphyllos*. *Nusantara Bioscience*, 6(1), 31–35. [[Crossref](#)]
- Rivera Curi, J. C., Cabrera Pintado, R. M., & Bulnes Soriano, F. (2020). Micropropagación de *Prosopis pallida* (Humb & Bonpl. Ex Willd.) Kunth a partir de yemas apicales. *Revista Colombiana de Biotecnología*, 22(1), 18–26. [[Crossref](#)]
- Rozman, K. K., & Doull, J. (2000). Dose and time as variables of toxicity. *Toxicology*, 144(1-3), 169–178. [[Crossref](#)]
- Silveira, A. A. D. C., Silva, L. C. D., & Sibov, S. T. (2022). In vitro germination and shoot proliferation of *Dipteryx alata* Vogel (Fabaceae) under conventional and natural ventilation. *Ciência Florestal*, 32(2), 996–1010. [[Crossref](#)]