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"EVALUATION OF GERMINATION AND GERMINATION VELOCITY IN SOTOL (DASYLIRION SPP.) THROUGH PHYSICAL AND CHEMICAL TREATMENTS IN THE FACE OF NATURAL SCARCITY SCENARIOS"

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ABSTRACT

The evaluation of physical and chemical treatments as tools to break dormancy in seeds and improve their germination capacity is important for sustainability and economic and ecological pressure on the sotol plant (Dasylirion spp.). Sotol is a species of great ecological and economic importance in arid and semi-arid regions, used in the production of the homonymous drink, as fodder and in environmental restoration projects. The objective of this study was to evaluate the effect of different stratification and chemical treatments on the germination of sotol seeds, determining their effectiveness through germination rates. The study was carried out under controlled laboratory conditions, using treatments such as chemical scarification and cold stratification, in addition to a novel treatment with alcohol plus hydrogen peroxide, which has not been previously reported in the scientific literature. The results indicated that stratification at low temperatures for 4 days was the most effective treatment, reaching a germination percentage above 70%, with a mean of 73%, followed by treatment with 3% H2O2 (72%) and treatment with 10% alcohol + 3% H2O2 (70%). In contrast, chemical treatments with sulfuric acid and 15% phosphoric acid showed the lowest percentages of germination, with means of 23% and 27%, respectively. Statistical comparisons using ANOVA and Tukey's tests (a = 0.10) showed high significance (p < 0.0002), explaining 72.32% of the variability in the data. Stratification treatments and the use of alcohol in combination with hydrogen peroxide proved to be more effective than chemical treatments in promoting seed germination. These findings provide valuable information to optimize sotol germination in reforestation and commercial production programs, highlighting the importance of cold stratification and alcohol treatment as promising options for the propagation of this species. These findings provide key information for the establishment and efficient propagation of sotol, with applications in reforestation, conservation and sustainable use programs of this native species.

KEYWORDS: Sotol (*Dasylirion spp.*), Germination, Dormancy, Sustainability, Ecological Restoration, Stratification, Scarification.

1. INTRODUCTION

Sotol (*Dasylirion spp*.) is a perennial plant native to the arid regions of northern Mexico and the southwestern United States, belonging to the Asparagaceae family. Its resistance to extreme environmental conditions, such as arid soils and low rainfall, makes it an essential resource for the restoration of degraded ecosystems and soil conservation (Martínez-García et al., 2022). This adaptability allows sotol to contribute significantly to moisture retention and erosion prevention, thus favoring the regeneration of vegetation in areas affected by desertification.

From an ecological and sustainable development perspective, sotol plays a fundamental role in soil conservation and in the maintenance of biodiversity (Rojas-Aréchiga & Vázquez-Yanes, 2000). In addition, it has gained relevance in ecological restoration programs and in the sustainable production of distillates, which has increased its economic and environmental value (Medina *et al*., 2019). However, the dormancy in its seeds represents a challenge for its propagation and availability, highlighting the need to develop effective strategies to optimize its germination in conservation and sustainable use projects.

One of the most recognized applications of sotol is the production of the eponymous distilled beverage, a cultural tradition of more than 800 years in communities in northern Mexico (Juárez-López *et al.,* 2023), Its ability to thrive in arid environments makes it ideal for land reclamation and reforestation initiatives, contributing to the sustainability and resilience of arid ecosystems (Rodríguez *et al*., 2021), but the exponential growth in commercial demand for this beverage has led to an overexploitation of sotol under natural conditions.

In this context, the dormancy of sotol seeds has been the subject of several studies that seek to optimize germination under controlled conditions. According to Martínez-García *et al*. (2022). Despite this, there is little scientific information on efficient methods to determine the viability and vitality of sotol, particularly through the use of physical and chemical treatments that allow standardizing seedling emergence and reducing pressure on native populations. Given the ecological and economic value of sotol, improving the germination capacity of its seeds is crucial to increase its availability in restoration programmes and in commercial production. The present study aims to comparatively evaluate the effectiveness of different physical and chemical treatments to break seed dormancy, using for its evaluation a completely randomized block experimental design and mean comparison tests.

Sotol is a plant of great ecological, cultural, and economic relevance in the arid regions of northern Mexico and the southwestern United States. In addition to its commercial value, sotol plays a crucial role in environmental conservation and sustainability as it contributes to the preservation of soils in arid areas, where it is essential to prevent erosion and restore degraded lands. According to Martínez-García *et al*. (2022), sotol acts as a pioneering species in soil recovery, helping to retain moisture and favouring the regeneration of other plant species in its environment.

The growing interest in the production of sotol for the production of distilled beverages has raised concerns about its sustainability. Juárez-López *et al*. (2023) highlight that overexploitation of wild sotol populations could lead to the degradation of their natural habitat if sustainable management practices are not implemented. To mitigate this impact, initiatives have been developed that promote the controlled cultivation of sotol, which would allow to meet commercial demand without compromising natural populations, so it was proposed as a general objective to evaluate the effect of physical and chemical treatments on the breakdown of dormancy, germination and germination velocity of sotol seeds (*Dasylirion spp.),* by characterizing the physiological quality of sotol seeds, through comparative analysis of the effect of different physical and chemical treatments to identify the most efficient treatment.

From an ecological point of view, sotol is not only important for its ability to adapt to arid environments, but also for its potential in carbon sequestration and its role in the restoration of ecosystems affected by climate change (Rodríguez *et al*., 2021). These characteristics underscore the importance of promoting sotol reforestation in areas affected by desertification, which in addition to generating economic benefits, contributes to long-term environmental sustainability.

To ensure the sustainable use of sotol, it is essential to balance its commercial use with conservation measures and responsible agricultural practices. The implementation of sustainable forest management programs, including controlled collection and population regeneration, is important to maintain the balance between the exploitation of sotol and the preservation of its ecosystem (Martínez-García *et al*., 2022).

2. MATERIALS AND METHODS

Location of the study area

The present research was carried out in the physiology and seed laboratories of the Faculty of

Agricultural and Forestry Sciences, of the Autonomous University of Chihuahua, in the city of Delicias, Chihuahua, Mexico. It is located in northern Mexico, south-central region of the state of Chihuahua at 28° 10' 50.4'' north latitude and 105° 30' 2.8'' west longitude, with an altitude of 1,170 meters above sea level. The climate of the study area according to Köppen and Geiger is classified as BSh, being semi-warm dry, with an average annual rainfall of 700 mm with a greater incidence in the months of July, August and September, the climate is extreme its average annual temperature is 18 °C and temperatures are normally present in winter from -5 °C to 40 °C in summer (CONAGUA, 2024). The vegetative material was obtained from a local producer in the city of Delicias, Chihuahua, however, there is no certainty about its history of regeneration or its use in previous cycles.

Type of Research

This research was descriptive and conclusive. Descriptive because it determines important properties and characteristics of the analyzed process. Conclusive because its objective is to help in decision-making by presenting conclusions of the processes through the analysis of information.

Materials

- Sotol seeds *Dasylirion spp*. • Petri dishes
- Beakers • Dissection forceps
- 250 ml Sauce • Brown paper
- Filter paper• Cotton
- 10% alcohol• Hydrogen peroxide (H2O2) 3%
- Distilled water (H2O)• Hydrochloric Acid (HCl) 1%
- Hydrochloric Acid (HCl) 10% • Sulfuric Acid (H2SO4) 15%
- Sulfuric Acid (H2SO4) 10% • Phosphoric Acid (H3PO4) 15%

Material Preparation

For the present work, sotol seeds provided by a seedling producer in the region were used, taking these as *ceteris paribus* due to ignorance of its origin. As preparation of the vegetative material, the seeds were cleaned, separating them from post-harvest residues, homogenizing them by stirring them manually prior to each count in their selection for the different treatments to be evaluated.

Experimental unit

The experimental unit was made up of each Petri dish, made of transparent Pyrex glass of 9 cm in diameter, in which the seeds were deposited, where a layer of cotton was previously placed and on top of it a layer of medium-pore filter paper as a substrate, a material that is internationally accepted in germination tests. 25 seeds were placed in each Petri dish for each repetition, using four repetitions to be evaluated per treatment, moistening what was necessary for the germination process.

Variables

The variables evaluated (dependent) were the percentage of germination and the germination speed that is defined by the equation: , = germination rate, number of germinated seeds per day and = germination day. These were calculated for the 8 treatments: hydrogen peroxide (H$V_{ge} = \sum \frac{n_i}{t} V_{ge} n_i = t$ (Morales Nieto, 2016) $_{2O2}$) at 3%, hydrogen peroxide (H2O2) at 3% plus alcohol at 10%, hydrochloric acid (HCL) at 1%, hydrochloric acid (HCL) at 10%, phosphoric acid (H3PO4) at 15%, stratification with four days, sulfuric acid (H$_{2SO4}$) at 10% and sulfuric acid (H2SO4) at 15% and also for the control.

Germination

The incubation of the seed was carried out at a controlled temperature, monitoring the germinated seeds for 24 days considering the size of the radicle (at least half a centimeter) and recording in a log for subsequent analysis, the experiment began on December 22, 2024, taking this as day zero.

Treatments

Nine treatments were used, including a control (Table 1), with four replications each. The treatments used were the control (T1), hydrogen peroxide (H2O2) at 3% (T2), hydrogen peroxide (H2O2) at 3% plus alcohol at 10% (T3), hydrochloric acid (HCL) at 1% (T4), hydrochloric acid (HCL) at 10% (T5), phosphoric acid (H$_{3PO4}$) at 15% (T6), sulfuric acid (H2SO4) at 10% (T7), stratification with four days at 4° C (T8), sulfuric acid (H2SO4) 15% (T9).

Table 1: Treatments used in the test.

Treatment	Description	Time
S1	Deponent	0
S2	Hydrogen peroxide 3%	10 min
S3	H2O2 3% plus Alcohol 10%	10 min
S4	Hydrochloric acid 1%	10 min
S5	Hydrochloric acid 10%	10 min
S6	Phosphoric acid 15%	15 min
S7	Sulfuric acid 10%	15 min
S8	Stratification (4° C)	4 days
S9	Sulfuric acid 15%	15 min

Information processing

The information processing was carried out through counts, using the Microsoft Office Excel program (Office, 2013) creating a database, for its description and characterization.

Statistical Analysis

For the statistical analysis of the information, a simple linear regression model was used, with a completely randomized block design (DCA) with nine treatments and four replications, using the analysis of variance procedure, using PROC ANOVA of the SAS 9.4 statistical package (SAS, 2012), considering treatments as the predictive variables and the percentage of germination as the response variable.

Tests were carried out to check the normality of the data using the PROC UNIVARIATE of the SAS 9.4 statistical package (SAS, 2012). And for the mean comparison tests, the analyses were performed using LSD, Duncan and Tukey tests.

Statistical model

The statistical model used to represent a completely random block design with an equal number of repetitions is as follows:

$Y_{ij} = \mu + \tau_i + b_j + \varepsilon_{ij}$

Where:

Yij = Value of the response variable expressed by the j-th measurement of the i-th treats I am not going to know what I

μ = Overall mean of the experiment.

The = Effect of the i–th treatment.

Bj = Effect of the block j.

eij = Error effect associated with Yij.

In this case, the effect in blocks was cancelled, since it did not occur as such, so it can be concluded that the blocks did not occur under different conditions

Germination Test

The results obtained in this study show that the stratification treatment at low temperatures for 4 days was the most effective, reaching a germination percentage of more than 70%, as observed in Figure 1, confirming that stratification is an effective method to break dormancy in sotol seeds.

With respect to the chemical treatments of 15% sulfuric acid and 15% phosphoric acid, they showed the lowest germination percentages, with averages of 23% and 27%, respectively, as shown in Figure 1. These results differ from those obtained by Juárez-López *et al*. (2023) where immersion in sulfuric acid considerably improved the ability of seeds to absorb water, promoting germination by a higher percentage.

According to the results based on the cumulative counts of germinated seeds, the treatments were classified into two groups: those in which acids were used and those in which acids were not, in order to facilitate their representation (Figures 2 and 3).

In the group of treatments with acid application (Figure 2), significant differences were observed in accumulated germination. The best response was obtained with the application of 10% sulfuric acid, 15 min, reaching an accumulated germination of more than 60%, while the lowest germination was recorded with 15% sulfuric acid, (15 min) with only 23%. These results suggest that the optimal concentration of sulfuric acid for dormancy breakdown in sotol (*Dasylirion spp*.) seeds is around 10%.

3. RESULTS

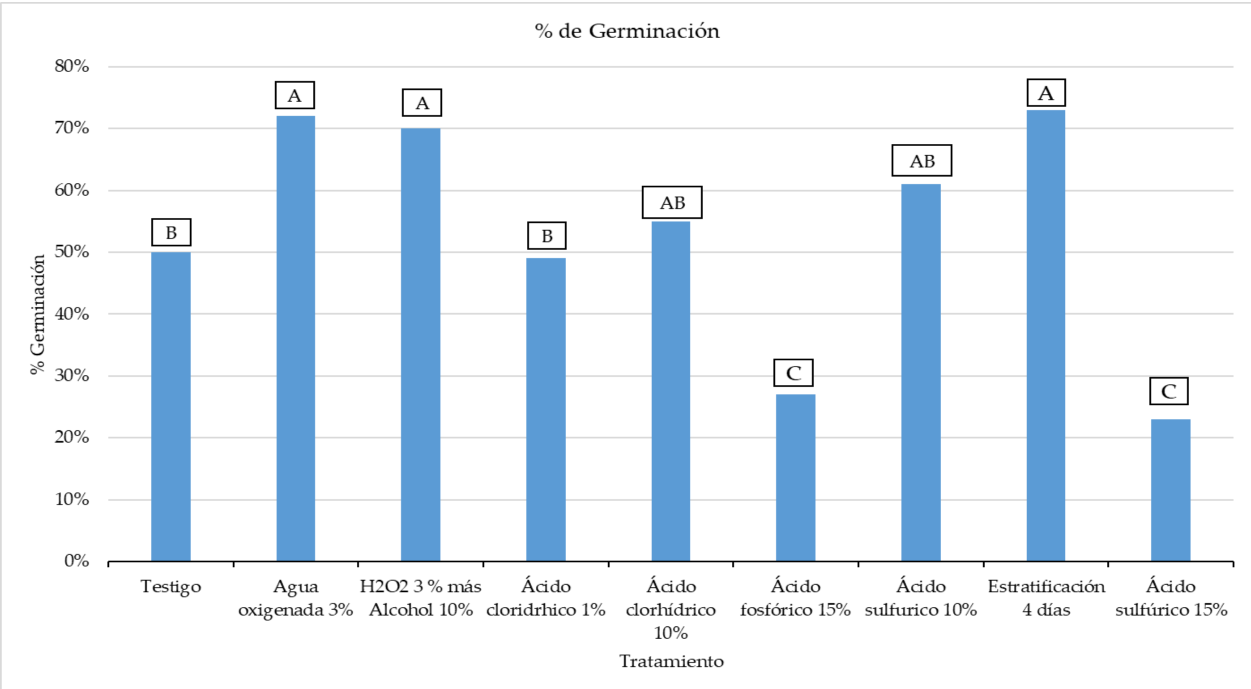

Figure 1: Comparison of means of the different treatments in germination.

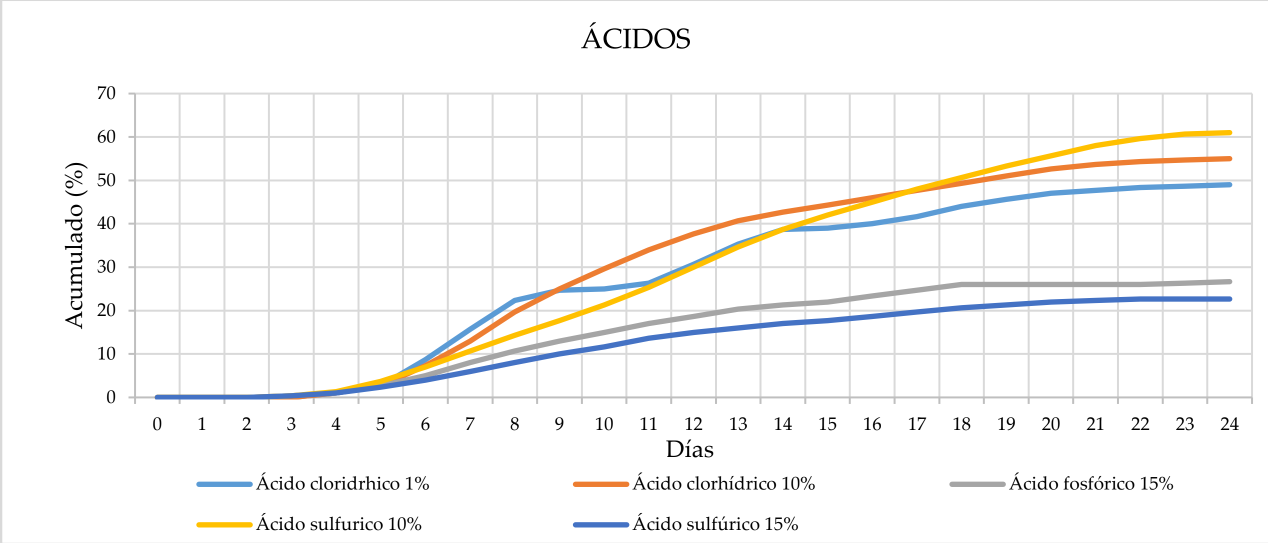

Figure 2: Graph of accumulation of germinated seeds from treatments with application of acids.

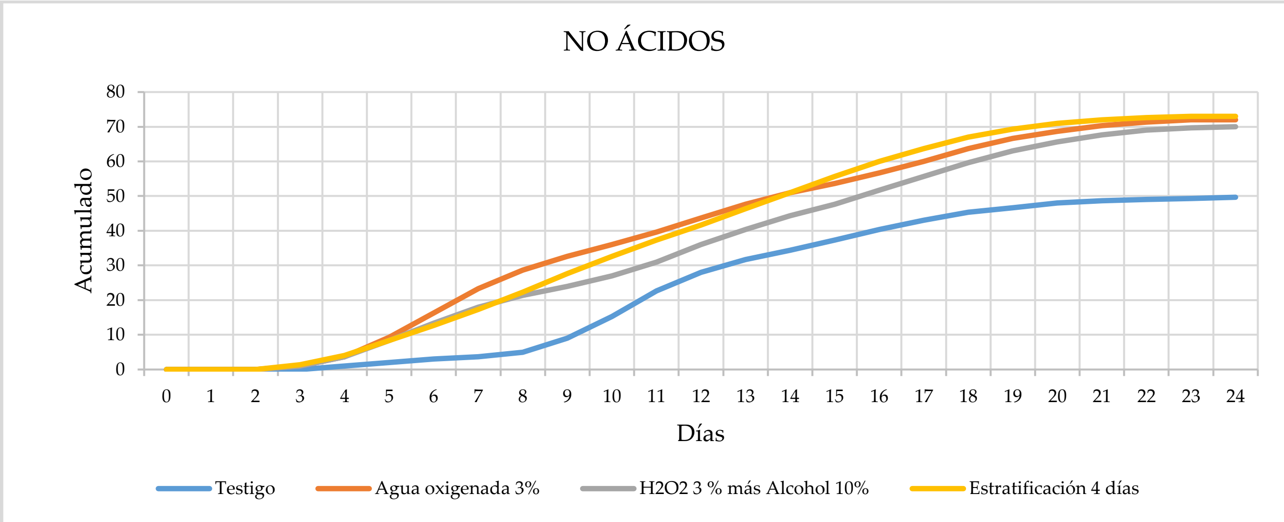

Figure 3: Graph of germinated seed accumulation from treatments without application of acids.

On the other hand, in the group of treatments without the application of acids, a greater homogeneity was observed in the percentages of accumulated germination. In particular, three treatments presented values close to 70%: 3% hydrogen peroxide, 3% hydrogen peroxide combined with 10% alcohol, and stratification for four days. (Figure 3). In contrast, the control showed lower germination, indicating that these treatments were effective in breaking dormancy and promoted a higher percentage of cumulative germination.

When comparing both groups, it was found that the most effective treatment in the acid group reached 61% of accumulated germination, while in the non-acid group the maximum value was 73%, that is, 12% higher. However, the control treatment recorded 50% germination, still exceeding the application of 10% sulfuric acid by 11%. It should be noted that, when comparing the control, it presented a higher percentage of germination compared to the treatments of the group with acids, surpassing 15% phosphoric acid, 15% sulfuric acid and 1% hydrochloric acid by 23%, 27% and 1%, respectively.

These results suggest that high concentrations of sulfuric acid and low concentrations of hydrochloric acid can generate adverse effects on germination. In addition, the presence of fungi was observed in treatments with higher concentrations of acids, which could indicate an additional negative impact on the viability of the seeds.

Germination Speed Test

The results show the importance of evaluating different treatments to optimize seed germination, considering not only the final germination percentage, but also the speed and uniformity of the process to determine their vigor. In the group of acid-free treatments (Figure 4), a more uniform trend is observed and, in general, with higher values compared to the control and acid treatments. In particular, treatments with four-day stratification and 3% hydrogen peroxide showed the highest values of , reaching approximately 3.8 in the last days of evaluation. This indicates that these treatments not only favor greater germination (Figures 2 and 3), but also have a better germination speed evidenced by greater vigor of the plant to respond to field conditions.$V_{ge}V_{ge}$

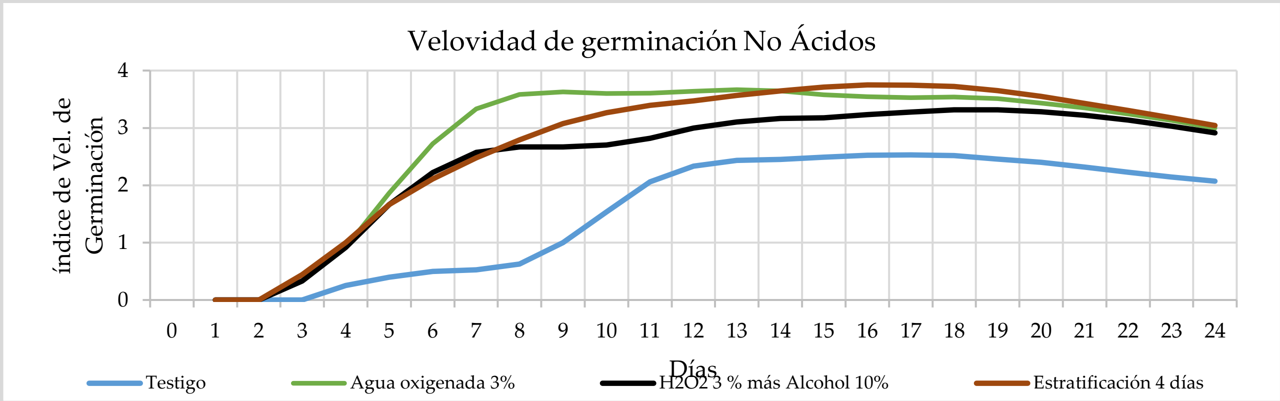

Figure 4: Germination rate of the group without application of acids.

In the group of acid-free treatments (Figure 4), a more uniform trend is observed and, in general, higher values compared to less effective acid treatments. In particular, treatments with four-day stratification and 3% hydrogen peroxide showed the highest values of , reaching approximately 3.8 in the last days of evaluation. This indicates that these treatments not only favor greater cumulative germination (as shown in Figures 2 and 3), but also promote faster germination.$V_{ge}V_{ge}$

On the other hand, in the acid treatment group (Figure 5), considerable variability in germination speed is observed. Treatments with 10% hydrochloric acid and 10% sulfuric acid had the highest values of , reaching a maximum close to 3.5 between days 12 and 15. In contrast, treatments with V_{ge}

15% sulfuric acid and 15% phosphoric acid exhibited the lowest values of Vge, with a slower progression throughout the evaluated period and a stabilization below 2.0. This behavior suggests that higher concentrations of acid may have adverse effects on germination, probably due to damage to seed integrity.

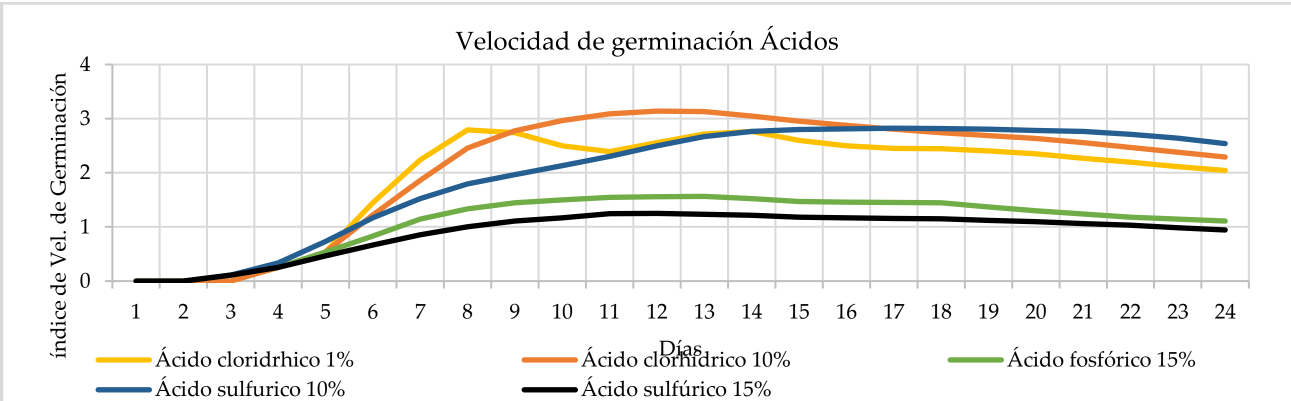

Figure 5: Germination rate of the group with application of acids.

Compared to the control treatment, which presented a progressive increase, but with values below 2.5, the alternative acid-free treatments were more effective in accelerating the germination process. The combination of 3% hydrogen peroxide with 10% alcohol showed an intermediate response, with a higher than the control, but without reaching the maximum values observed in the stratification and 3% hydrogen peroxide.$V_{ge}V_{ge}$

These results suggest that, to optimize both the rate and rate of germination in sotol (*Dasylirion spp.*) seeds, the use of 3% hydrogen peroxide and four-day stratification represent more effective strategies than the application of acids, especially at high concentrations.

Although most of the germination was expected to occur in the first days of the experiment, it lasted for a longer period, which led to prolonging the measurements up to 24 days, this coincided, in general, with the values found in other studies carried out by Villavicencio *et al.* (2007), Ávila *et al.* (2024), where they determined that the emergence of seeds in vitro is recorded from seven days and an average of 20 days is required to complete the germination process, but with the difference that with the treatments analyzed in this study they presented emergence from the third day and with the highest germination speed, but it coincides with what Ortiz-López (2024) found regarding the average germination rate values.

The analysis of variance (ANOVA), the results of which are shown in Table 2, indicated a high statistical significance ($p < 0.0002$) due to the treatments applied. The model explained 72.32% of the variability in the data, i.e. the response variable is explained in that percentage by the predictive variable (treatments) as shown in Table 3, although the coefficient of variation of 28.11% suggests that there is still considerable variability in the means of the treatments applied, the effect in the blocks was not taken into account as it was almost nil.

Table 2: Analysis of germination variance (ANOVA) with application of nine treatments with four blocks of 25 replications each in sotol seed.

Source	Mexico City	Sum of squares	Mean Square	F-Value	Pr > F
Model	11	12228	1111.6364	5.7	0.0002
Error	24	4680	195		
Total corrected	35	16908			

Table 3: Determination R2, coefficient of variation and mean.

R-square	Coef Var	MSE Root	GERM Media
0.723208	28.11592	13.96424	49.66667

When performing the mean comparison tests (Tukey, LSD and Duncan, $\alpha = 0.10$) as shown in Table 4, it was found that the treatments are grouped into different categories according to their effect on the percentage of seed germination. In the three trials, the treatment with the highest mean germination was stratification for 4 days, with a mean of 73%, followed by H_{2O2} at 3% with 72% and alcohol 10% + H_{2O2} at 3% with 70%. These three treatments were consistently classified in group A, indicating their superiority over the others.

At an intermediate level, the treatments were 10% sulfuric acid, 10% hydrochloric acid, control and hydrochloric acid with averages of 61%, 55%, 50% and 49% respectively. These treatments were grouped into groups B or A/B, depending on the statistical test used, indicating that, although they are less effective than those in group A, they do not present significant differences between them.

Finally, the treatments with the lowest germination values were 15 % phosphoric acid and 15 % sulfuric acid, with means of 26.67 % and 22.67 %, respectively. These were placed in groups C, D or E according to the test, which reflects their low effect compared to the other germination treatments coinciding with the LSD and DUNCAN tests, with the difference that Tukey makes groups with a greater number of treatments that do not present significant differences between them, this due to the rigor of the test, that is, statistical homogeneity, which indicates that they present less heterogeneity between groups, with respect to the other mean comparison tests carried out, which is why it is the one that was taken into account for the results.

Although in general, the three tests coincide in the formation of homogeneous groups, showing that there are significant differences between the most effective and the least effective treatments, but not within each group. This reaffirms the hierarchy of treatments at three levels: highly effective, intermediate and less effective, highlighting treatments with 3% H2O2 and stratification for 4 days as the best options to promote seed germination.

Table 4: Comparison of means using Tukey.

Tukey			
Tukey Group		Media	Treatment
	A	73.00	Estrat. 4 days
	A	72.00	H2O2 3%
	A	70.00	Alcohol 10% H2O2 3%
B	A	61.00	South Acid 10%
B	A	55.00	Cl Acid 10%
B		50.00	Witness
B		49.00	Cl Acid 1%
	C	27.00	PO Acid 15%
	C	23.00	SOUTH Acid 15%

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the evidence obtained and the vegetative material used, no significant differences were found between the chemical treatments and the control. However, stratification and immersion treatments in hydrogen peroxide and alcohol demonstrated better effects in breaking latency compared to chemical treatments. In particular, alcohol treatment, which has not been previously reported in the reviewed literature, highlights the importance of this finding, since it showed a positive effect in overcoming latency and allowed to achieve a high percentage of germination.

Within the chemical treatments evaluated, 15% sulfuric acid and 15% phosphoric acid were the ones that presented the least favorable results, obtaining the lowest germination percentages and the lowest germination speed indices. In contrast, cold stratification evidenced improvements in germination rate and speed, indicating its potential for mass propagation of sotol in ecological restoration and commercial production programs. These results coincide with those obtained by Rodríguez *et al.* (2021), who reported that cold stratification at 4 °C for 14 days significantly improved germination in *Dasylirion spp*. In addition, Martínez-García *et al.* (2022) pointed out that integument hardness is a key barrier to germination in sotol and that chemical scarification with sulfuric acid favors water absorption, promoting greater seedling emergence. However, this study found that high concentrations of sulfuric acid not only reduced germination rate, but also favored fungal proliferation, which may have adversely affected seed viability and seedling establishment.

On the other hand, Juárez-López *et al*. (2023) showed that the use of gibberellic acid and hydrogen peroxide improves the germination of sotol seeds, although with variability in their effects depending on the species and environmental conditions. In the present study, the combination of hydrogen peroxide and alcohol showed a similar behavior in terms of promoting germination, but without precedent in the scientific literature, suggesting the need for additional studies to understand its mechanism of action and its viability under different conditions. In addition, this treatment not only favored a higher percentage of germination, but also showed a higher IVG compared to less effective chemical treatments, suggesting that its action could accelerate the germination process.

These findings suggest the need for further research, extending the study to different sotol samples from various regions, as well as exploring variations in treatments, especially in the combination of 10% alcohol with 3% hydrogen peroxide. It is also recommended to exercise greater control over environmental conditions and seed origin to minimize variability and reduce experimental error. It is particularly important to evaluate the impact of fungal proliferation observed in some chemical treatments with high acid concentrations, as this could affect the sanitary quality of the seedlings and their subsequent establishment in the field.

In general, this study provides valuable information to optimize the germination of sotol in nurseries and crop fields, facilitating its sustainable use and its integration into different productive sectors. It is suggested to replicate the study in field conditions to evaluate the response of treatments in natural environments and determine its feasibility for reforestation programs. It is advisable to analyze the effect of different concentrations and exposure times on chemical scarification to optimize treatment. In addition, future research should focus on the detailed evaluation of alcohol treatment, exploring its effects at different concentrations. It is also recommended to investigate the impact of terrestrial and climatic conditions on the germination and establishment of sotol, in order to generate specific strategies for its propagation in different regions, ensuring a sustainable impact on the community.

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