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Evaluation of the Effect of Chemical Acids on Breaking Seed Dormancy of Retama raetam

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A B S T R A C T

This study investigated the effectiveness of concentrated mineral acids—sulphuric (H_2SO_4), hydrochloric (HCl), and nitric (HNO_3)—in breaking the physical seed dormancy of *Retama raetam* (Forssk.) Webb, a key leguminous shrub in arid and semi-arid ecosystems of North Africa. Mature seeds were treated with each acid for 6, 12, 24, and 48 hours under controlled laboratory conditions using a completely randomised design. Germination percentage, mean germination rate, and germination speed index were measured to assess treatment efficiency. Results revealed significant differences among treatments ($p \leq 0.001$). Sulphuric acid proved most effective, achieving up to 95% germination when exposure lasted 12–24 hours, owing to its capacity to erode lignified and suberised seed coat layers without harming the embryo. Hydrochloric acid showed moderate efficacy at shorter durations (6–12 h), primarily softening the outer testa. In contrast, nitric acid caused oxidative injury and poor germination (<20%) due to its strong oxidising effect on embryonic tissues. The findings confirm that seed coat impermeability is the primary cause of dormancy in *R. raetam* and can be overcome effectively by controlled sulphuric acid scarification. The study provides a reproducible and low-cost germination protocol applicable to native desert legumes. Ecologically, enhancing R. raetam propagation supports sand dune stabilisation, rangeland rehabilitation, and desertification control in Libya and similar arid environments. This research contributes to the optimisation of propagation techniques for drought-adapted flora and underscores the ecological value of chemical scarification in restoration programmes.

1 Introduction

Seed dormancy represents one of the most critical adaptive strategies that allow plant species to synchronise germination with favourable environmental conditions. Dormant seeds fail to germinate even when moisture, temperature and oxygen appear adequate because intrinsic structural or physiological constraints temporarily restrict embryo growth. Modern seed biology recognises five principal classes of dormancy: physiological, morphological, morphophysiological, physical (coat-imposed), and combinational dormancy. Each class reflects distinct developmental, structural or metabolic mechanisms, and each contributes differently to ecological persistence, particularly in arid or semi-arid ecosystems where regeneration windows are narrow and unpredictable (Baskin & Baskin, 2014). Among these types, physical dormancy (PY) is especially widespread in Fabaceae, where hard seeds possess water-impermeable coats formed by thick, lignified palisade layers and hydrophobic cuticles. These structures prevent water uptake until specific “water gaps” open in response to external cues such as temperature oscillations, abrasion or fire, a trait that supports staggered germination and long-term seed bank stability (Mira et al., 2017; Wen et al., 2024). Hardseededness, while ecologically advantageous, poses a major challenge in restoration programmes and cultivation efforts because untreated seeds often germinate poorly or not at all under laboratory or field conditions.

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Breaking physical dormancy typically requires controlled disruption of the seed coat. Mechanical scarification—such as abrasion with sandpaper—can enhance imbibition but often offers variable results depending on seed size and coat thickness. In contrast, chemical scarification using strong acids, especially concentrated sulphuric acid (H_2SO_4), has demonstrated greater reliability in many species when exposure time is precisely controlled. Acid treatment erodes the palisade layer and opens the water gap while maintaining embryo viability, leading to marked improvements in germination percentage and reduced mean germination time (Argel & Paton, 1999; Miya et al., 2015). Nevertheless, excessive exposure may damage embryos, highlighting the need to optimise type and duration of acid exposure for each species.

The genus *Retama* (Fabaceae) comprises drought-tolerant shrubs widely distributed across North Africa and the Mediterranean basin. *Retama raetam* (Forssk.) Webb is particularly characteristic of arid and semi-arid landscapes, thriving on sandy and stony substrates where it contributes to dune stabilization, microhabitat formation, and soil improvement (Mahdhi et al., 2023).

Despite its ecological significance, natural regeneration in *R. raetam* is often poor, largely due to severe physical dormancy. Multiple studies across North Africa and the Middle East indicate that unscarified seeds of *R. raetam* exhibit extremely low germination, whereas chemical scarification—most commonly with concentrated H_2SO_4—can raise germination to near 100% when applied for optimised durations (Mehdadi et al., 2017). However, variation among populations suggests that standardised protocols are still lacking, particularly for local genotypes in Libya and adjacent regions.

Beyond its ecological roles, *R. raetam* has growing pharmacological interest. Extracts from aerial parts and seeds possess antioxidant, antimicrobial, antidiabetic and hepatoprotective activities attributed to diverse phenolic and alkaloid constituents (Algandaby et al., 2010; Saada et al., 2021; Alwasia et al., 2023). These bioactivities highlight the need for reliable propagation methods to support sustainable utilisation, conservation and potential domestication of the species.

In Libya, *R. raetam* is a native component of desert and semi-desert vegetation, yet its regeneration challenges limit its contribution to rangeland restoration and environmental management. Given the species' ecological and medicinal importance, developing a practical and reproducible method to break physical dormancy is essential for local nurseries, conservation projects and research programmes.

Therefore, this study focuses on evaluating the effectiveness of three concentrated acids—sulphuric (H_2SO_4), hydrochloric (HCl) and nitric acid (HNO_3)—in breaking the physical dormancy of *R. raetam* seeds under controlled laboratory conditions. By comparing different exposure times, the study aims to identify the most efficient treatment capable of enhancing germination while preserving seedling viability. The outcomes are expected to support propagation efforts, facilitate ecological restoration in arid Libyan environments, and contribute to broader understanding of dormancy-breaking protocols in hard-seeded desert legumes.

2 Materials and Methods

Study Location and Design

The experimental work was conducted at the Botany Department, Faculty of Science, University of Al-Zawia, Libya, during the 2023–2024 academic year. All laboratory procedures were carried out under controlled conditions to ensure accuracy and minimise environmental variability, following general seed testing guidelines described by ISTA (2023). The study aimed to evaluate the effect of three concentrated mineral acids—hydrochloric acid (HCl), sulphuric acid (H_2SO_4), and nitric acid (HNO_3)—on breaking the physical dormancy of *Retama raetam* seeds at four soaking durations (6, 12, 24, and 48 hours), a method widely used in legumes with hard seed coats (Flematti et al., 2011; Pérez-García, 1997).

Each treatment consisted of five replicates, with 10 seeds per replicate, and untreated seeds served as the control. The comparison among treatments was based on the mean germination response obtained for each acid and each exposure duration. Such replication and comparison structure align with standard experimental procedures recommended by Gomez and Gomez (1984).

Seed Collection and Preparation

Mature pods of *R. raetam* were collected in March 2023 from naturally growing shrubs on the University of Al-Zawia campus. Pods were air-dried for one week and manually opened to extract the seeds. Debris, broken seeds, and impurities were removed. The collection, drying, and handling procedures follow the protocols outlined by Baskin and Baskin (2014).
The seeds were washed under running tap water, spread on paper towels, and air-dried at room temperature (25 ± 2 °C) for one week. Only clean and intact seeds were selected. Seeds were stored in airtight containers until treatment. Seed storage and pre-treatment handling

were conducted as recommended by Bewley et al. (2013).

Surface sterilisation was not performed because concentrated acids provide sufficient disinfection during scarification, a practice also reported in previous studies on leguminous species (Al-Hadedy et al., 2024).

Chemical Treatments (Acid Scarification)
Three concentrated acids were used: HCl (37%), H_2SO_4 (98%), and HNO_3 (70%). For each treatment, 50 seeds (10 per replicate) were immersed in 50 mL of acid in heat-resistant glass beakers. Similar concentrations and soaking approaches have been used in classical and modern scarification studies (Goor & Barney, 1976; Al-Hadedy et al., 2024).
Soaking durations were 6, 12, 24, and 48 hours for each acid type. These soaking periods align with established experimental designs for breaking physical dormancy in species with extremely hard seed coats, including *Retama* (Pérez-García, 1997).

The beakers were maintained at room temperature (25 ± 2 °C) and gently agitated every two hours. After treatment, seeds were removed, washed thoroughly under running tap water for 10 minutes, rinsed with distilled water, and neutralised using a weak sodium bicarbonate ($NaHCO_3$) solution, following general seed-handling procedures described by Bewley et al. (2013). The seeds were then dried on sterile filter paper at room temperature for 24 hours.

Germination Test
Germination was conducted in sterilised Petri dishes (9 cm) lined with two layers of Whatman No. 1 filter paper moistened with 5 mL distilled water. These procedures are consistent with international seed testing protocols (ISTA, 2023; AOSA, 2010). Each dish contained 10 seeds, with five replicates per treatment. All dishes were incubated at 25 ± 2 °C for 20 days under natural light–dark conditions. Moisture levels were maintained by adding distilled water daily. Dishes were randomly rearranged each day to minimise positional bias, as recommended by ISTA (2023).

Germination was recorded daily, using radicle protrusion of at least 2 mm as the criterion for germination, in accordance with AOSA (2010). Seeds that failed to germinate by the end of the test were dissected to assess embryo viability, following procedures suggested by Bewley et al. (2013).

Recorded Observations
Alongside germination counts, qualitative observations were documented to describe visible effects of acid treatments, including seed swelling, coat cracking, colour changes, and signs of tissue damage. Such morphological assessments are commonly used to evaluate the effects of scarification treatments (Nikolaeva et al., 2020).

Statistical Treatment of Data
Germination results for each exposure duration and each acid type were summarised using the mean and standard deviation (SD). Significance levels (P-values) were calculated to determine differences among exposure durations within each acid treatment.
The statistical analysis focused on comparing the mean response within each acid type independently rather than evaluating factorial interactions, aligning with the statistical approaches described by Gomez and Gomez (1984).

Safety and Precautionary Measures
All acid treatments were carried out under a chemical fume hood. Laboratory personnel used acid-resistant gloves, face shields, and protective lab coats. Acid residues were neutralised with sodium bicarbonate before disposal. Safety procedures followed OSHA Laboratory Safety Guidelines (2020).

Quality Control and Reproducibility
All glassware and Petri dishes were sterilised before use, and distilled water was used throughout. Temperature and humidity in the germination area were monitored daily. Daily logs were maintained to ensure consistency and reproducibility, as advised by ISTA (2023).

3 Results and Discussion

1. Effect of Hydrochloric Acid (HCl) on the Germination of *Retama raetam* Seeds
Seeds treated with hydrochloric acid for six hours exhibited noticeable swelling by the fifth day after sowing, followed by progressive cracking of the seed coat on the sixth day and radicle elongation on the seventh day. Soaking for twelve hours accelerated visible germination signs from the fourth to the sixth day, while germination percentages declined markedly after 24 hours and severe damage symptoms appeared after 48 hours.
Table (1) shows that the overall mean germination value was 1.42 with significant differences at $P \leq 0.001$.

Table. 1. Mean, Frequency, and Standard Deviation of *Retama raetam* Seed Germination at Different HCl Exposure Durations ($P \leq 0.001$)

Exposure Duration	Mean	Frequency	Standard Deviation
6 h	1.82	20	1.11
12 h	1.64	20	0.92
24 h	0.84	20	0.32
48 h	0.39	20	0.55
Total Exposure Duration	**1.42**	**80**	**0.86**

Level of Significance (P≤ value): 0.001

These findings indicate that short soaking periods (6–12 h) in HCl were sufficient to weaken the hard seed coat without injuring the embryo. This response agrees with **Hartmann and Kester (1975)**, who stated that moderate acid treatments dissolve the outer cuticular layer and improve water permeability. Similarly, **(Zhang et al. 2022)** reported that hydrochloric acid could moderately enhance germination in hard-coated seeds at controlled exposure times, though its efficiency is lower than that of sulphuric acid due to shallower penetration into lignified tissues.
The sharp decline after 24–48 hours indicates that prolonged exposure increases acid diffusion into embryonic tissues, leading to local necrosis and reduced viability.

2. Effect of Sulphuric Acid (H_2SO_4) on the Germination of *Retama raetam* Seeds

Seeds soaked in concentrated sulphuric acid for six hours began swelling within the first two days, their coats cracked by day three, and radicle emergence occurred by day four. The highest germination means were recorded at 12 and 24 hours (4.37 and 4.34, respectively). However, germination declined to 0.77 at 48 hours due to embryo damage. Table (2) shows an overall mean germination value of 3.34 with highly significant differences ($p \leq 0.000$).

Table. 2. Effect of Sulphuric Acid (H_2SO_4) Exposure Duration on the Germination Index of *Retama raetam* Seeds

Exposure Duration	Mean	Frequency	Standard Deviation
6 h	3.90	20	1.80
12 h	4.37	20	1.90
24 h	4.34	20	1.57
48 h	0.77	20	0.50
Total Exposure Duration	**3.34**	**80**	**2.14**

Level of Significance (P-value)≤ 0.000

Sulphuric acid proved to be the most effective treatment in breaking the mechanical dormancy of *Retama raetam* seeds. Its efficiency is attributed to its strong capacity to remove lignin and suberin layers from the seed coat, allowing better water and oxygen penetration.
These results agree with **El-Dessougi (1994)**, who reported that controlled exposure to sulphuric acid achieved maximum efficiency in breaking hardseededness without harming the internal tissues. Furthermore, previous research has shown that treatment with concentrated H_2SO_4 for approximately 20–30 minutes can markedly enhance germination in hard-seeded leguminous species, outperforming other chemical scarification methods. For example, Zhang et al. (2022) reported that sulfuric acid immersion was substantially more effective in breaking physical dormancy and stimulating germination than alternative acid treatments.
Likewise, **(Al-Hadedy et al. 2024)** found that exceeding the optimum exposure period caused severe embryo deterioration due to the removal of protective layers.
Hence, the current results confirm the existence of an **optimal exposure window (12–24 h)** during which maximum germination can be achieved without embryo injury.

3. Effect of Nitric Acid (HNO_3) on the Germination of *Retama raetam* Seeds

Seeds soaked in HNO_3 for six hours started swelling on the third day, showing limited germination. A slight increase occurred at 12 hours, but germination dropped sharply after 24 and 48 hours. The overall mean germination value was 0.74 with significant differences (**$p \geq 0.0014$**).

T able. 3. Effect of Nitric Acid (HNO_3) Exposure Duration on the Germination Index of *Retama raetam* Seeds"

Exposure Duration	Mean	Frequency	Standard Deviation
6 h	1.33	20	0.74

Exposure Duration	Mean	Frequency	Standard Deviation
12 h	0.94	20	0.58
24 h	0.51	20	0.29
48 h	0.19	20	0.19
Total Exposure Duration	**0.74**	**80**	**0.65**

Level of Significance (P-value)≤ 0.0014

Nitric acid was the least effective among the tested treatments, likely due to its strong oxidative nature that causes partial degradation of cellular membranes and proteins in the embryo. Similar oxidative injury resulting from harsh chemical scarification has been reported in legume seeds exposed to strong acids (Wen et al., 2024).

The previous studies found that nitric acid treatments reduced germination in leguminous seeds when soaking durations exceeded the safe limit (wen et al.,2024).

4. General Analysis and Ecological Implications

GeneralInterpretation:
The overall analysis confirms that concentrated sulphuric acid applied for a moderate period (12–24 hours) is the most efficient treatment for removing physical dormancy in *Retama raetam* seeds. Hydrochloric acid offers a moderately effective but safer alternative, whereas nitric acid is not recommended due to its strong oxidative damage to embryos.
These findings hold not only physiological significance but also practical ecological value, as they can be applied to enhance seedling establishment of *Retama raetam* in reforestation and sand dune stabilisation programmes across arid Libyan regions. Similarly,(Mahdhi et al. 2023)highlighted the vital role of *Retama raetam* in improving soil fertility and combating desertification, underscoring the ecological importance of optimising its germination through controlled acid scarification.

4 Conclusions

This study confirmed that the hard, impermeable seed coat of *Retama raetam* imposes a strong physical dormancy that can be effectively broken using chemical scarification. Among the three acids evaluated, concentrated sulphuric acid (H_2SO_4) was the most efficient, with 12–24 hours of exposure yielding germination rates approaching 95% while preserving embryo viability. In comparison, hydrochloric (HCl) and nitric acid (HNO_3) produced limited improvement and caused partial embryo injury at longer exposure times. These findings provide a practical, low-cost, and reproducible protocol to enhance the propagation of *R. raetam*, supporting its use in ecological restoration, sand-dune stabilisation, and rangeland rehabilitation in arid regions. The results also offer a framework applicable to other hard-seeded legumes, highlighting the value of further research to validate performance under greenhouse and field conditions.

Conflict of interest: The authors declare that there are no conflicts of interest

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