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SULFURIC ACID AND HOT WATER TREATMENT EFFECTS ON THE SEED GERMINATION AND GROWTH TRAITS OF *SESBANIA PUNICEA* L.

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SUMMARY

Like many species within the Fabaceae family, *Sesbania punicea* L. seeds experience seed coat dormancy, affecting their germination from a hard shell coating the seed, preventing water absorption and gaseous exchange into the seeds. The presented research sought to overcome the outer dormancy phase in *Sesbania punicea* seeds by treating them with two concentrations of sulfuric acid (50% and 98% H_2SO_4) for periods of 0, 10, and 15 minutes and soaking in hot water (without, with) for 24 hours. The results revealed that seed pretreatment of immersion in sulfuric acid at a 98% concentration was significantly superior to the 50% and showed the highest mean values for the studied parameters, germination percentage, seedling height, stem diameter, leaves per seedling, and shoots dry weight at 78.33%, 61.61 cm, 5.79 mm, 31.27 leaves/seedling, and 7.32 g, respectively. In the same line, the immersion in sulfuric acid for 15 minutes was superior compared with 10 minutes, providing the highest values for the same traits at 87.04%, 64.08 cm, 6.16 mm, 32.08 leaves/seedling, and 8.12 g, respectively. The hot water treatment was notably dominant to the one without soaking and exhibited the maximum values for the above traits (78.55%, 59.83 cm, 5.94 mm, 31.05 leaves/seedlings, and 7.32 g, respectively). The interaction effects of three factors (immersion in 98% sulfuric acid for 15 minutes and soaking in hot water) excelled other treatments for the traits, i.e., germination rate (99.30%), seedling height (71.00 cm), main stem diameter (7.28 mm), leaves/seedling (36.66), and shoot's dry weight (11.42 g). The most remarkable achievement was that chemical scarification using sulfuric acid and hot water, either individually or in combination, proved effective in breaking the seed dormancy of *Sesbania punicea*.

Keywords: *Sesbania punicea* L., seed dormancy, sulfuric acid, hot water, germination, growth traits

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Key finding: For better germination and early growth of *Sesbania punicea* L. seedlings, the combination of seed immersion in 98% sulfuric acid for 15 minutes and soaking in hot water for 24 hours proved recommendable since it leads to an increase in the germination percentage from 36.9% to 99.3%, enhancing the growth parameters.

INTRODUCTION

Sesbania punicea L., commonly known as Spanish gold, purple sesban, and Scarlet sesban, belongs to the legume family Fabaceae and the subfamily Faboideae, which contains most species. Genus *Sesbania* comprises about 70 diverse species scattered across numerous geographical areas (Joshi-Saha and Gopalakrishna, 2007). *Sesbania* often exists in tropical and subtropical regions of the Philippines, Indonesia, Malaysia, and India (Bhat, 2003) and across coastal areas of the Southern United States, Brazil, Argentina, Paraguay, Uruguay, and other regions of South America and some parts of Africa. *Sesbania punicea* often proliferates close to rivers and streams because it prefers humid habitats with high soil moisture levels; however, it can also abound in dry areas, particularly in grasslands and undisturbed zones (Stumpf, 2016).

Sesbania is a nitrogen-fixing shrub that can restore disturbed soils by improving soil nitrogen (N2) and fertility via the nodulation process between bacterial nodules and sisban roots and can also serve as fodder and wood fuel (Grzeça *et al*., 2021). *Sesban* species propagation is often by the seeds and represents natural resources for reproduction and maintenance of genetic diversity in crop plants (Taiz and Zeiger, 2002; Gins *et al*., 2022; Narimonov *et al*., 2023).

Seed dormancy happens due to various factors, including the coating's non-absorption of oxygen and water because of the hardness of the seed's outer covering. It causes mechanical resistance, preventing the seed germination process connected to the seed itself. It may refer to the nature of the genetic composition of seeds and others, as the seed does not germinate even with appropriate conditions, and it defines internal physiological dormancy. In that apparent inactivity, the seed coat is hard to split and impermeable to water, hindering water and oxygen access to the embryo and inhibiting germination, as the case with the seeds of families Rosaceae (Stoian-Dod *et al.*, 2023) and Leguminosae (Long *et al*., 2012). The three subfamilies of the legume family, Caesalpinioideae, Mimosoideae, and Papilionoideae, also have the traits of integument dormancy (Funes and Venier, 2006). The hardness of the seed coat, causing impermeability to water and gases, is also responsible for the Sesbanum's dormancy (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014).

In finding the most effective methods for breaking seed dormancy, numerous studies have proceeded in overcoming dormant seeds of trees and shrubs. Various chemical and mechanical treatments in forestry practice helped boost the germination percentage. Ali *et al.* (2021) revealed that immersing Carob seeds (*Ceratonia siliqua*) in concentrated sulfuric acid for 15 minutes gave the highest values for the growth traits. Shreelalitha *et al*. (2015) evaluated the germination capacity of two species, *S. bispinosa,* and *S. speciosa,* by treating the seeds with physical and chemical solutions, resulting in the utmost seed germination and the vigor index in both plant species.

Masaka and Yamada (2009) revealed that the seeds exhibited the highest germination rates (45.3%) when soaked for three hours in hot water at 60 °C. Furthermore, with the earlier mentioned scalding approach, it also seemed adequate to employ the chemical method. This method entails immersing the seeds in concentrated sulfuric acid for 60 minutes, rinsing them with cold water, and subjecting them to a 24-hour drying period in a temperature-controlled chamber. *Dalbergia cochinchinensis* seed treatment with hot water at a temperature of 70 °C significantly increased the germination rate compared with the cold-water and control treatments (Asl *et al.*, 2011; Kheloufi, 2017; Seng and Cheong, 2020). The presented study investigated methods to overcome seed dormancy in *Sesbania punicea* to identify the most effective seed pre-sowing solutions for breaking the dormancy and increasing the germination percentage. Additionally, the study aims to enhance seedling growth and achieve desirable improvement in growth traits, i.e., seedling height, stem diameter, leaves per seedling, and dry weight.

MATERIALS AND METHODS

The experiment on *Sesbania punicea* L. began in the nursery at the Department of Forestry, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq. The sowing of *Sesbania punicea* seeds started in the first week of May 2022. Their vitality evaluation employed immersing them in distilled water, removing the floated seeds, and retaining the seeds that fell at the bottom, eventually dried and divided into two groups. The experiment ran following a completely randomized design (CRD) with three replications and three factors. The first factor consisted of two concentrations of sulfuric acid (50% and 98%), the second comprised three different soaking durations in sulfuric acid (0, 10, and 15 minutes), with the third factor including two hot water drenching treatments (without and with hot water from 40 °C to 45 °C).

Studied traits

Germination percentage calculation was according to the following equation:

$$Germination\ percentage\ (\%) = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds} \times 100$$

For seedling height (cm), the measurements used a measuring tape from the top of each seedling to the soil surface. The main stem diameter (mm) measured for each seedling used a digital foot (Vernier) at a height of one centimeter from the soil's surface. The leaves per seedling employed counting and averaging in each experimental unit. Shoots dry weight (g) computation transpired after drying them in an electric oven at 70 °C ± 1 °C and weighing them in each experimental unit using an electric balance with a sensitivity of 0.01 mg.

Statistical analysis

The recorded data analysis for all the traits engaged the SAS program, with the averages tested using the Duncan Multiple Range Test (DMRT) at a 5% probability level (Al-Rawi and Khalaf-Allah, 2000).

RESULTS AND DISCUSSION

Germination percentage

The results revealed that exposing *Sesbania punicea* seeds to various sulfuric acid concentrations at varying times and hot water treatment substantially impacted the germination percentage (Table 1). The *S. punicea* seeds immersion in sulfuric acid concentrations (50% and 98%) had a significant effect, with the H_2SO_4 98% concentration giving the highest germination percentage (78.33%), followed by the H_2SO_4 50% concentration (68.18%). The results further indicated a significant impact of immersion periods (0, 10, and 15 min) with different H_2SO_4 concentrations. The duration of 15 min showed the maximum germination percentage (87.04%) compared with the control treatment with the lowest (51.22%). Soaking seeds in hot water or not also had a sizable influence on the germination percentage of *S. punicea* seeds. The treated seeds with hot water showed the utmost germination percentage (78.55%), followed by no hot water soaking, which revealed the lowest (57.81%). The interaction of three factors (H_2SO_4 concentration of 98%, with immersion duration of 15 min, and hot water soaking) significantly impacted the germination percentage (99.30%), followed by the interaction of H_2SO_4 concentration of 98%, immersion time of 10 min, and hot water soaking, recording a germination percentage of 97.50%.

Table 1. Effect of concentration and duration of soaking in sulfuric acid and hot water on germination percentage of the *Sesbania punicea* seeds.

Sulfuric acid concentration (%)	Hot water	Soaking times sulfuric acid (minutes)		
		0	10	15
50	Without hot water	36.93L	65.60h	70.92g
	With hot water	55.5j	87.60d	92.55c
98	Without hot water	50.75k	75.35f	85.40e
	With hot water	61.7i	97.5b	99.3a
Soaking time means (%)		51.22c	81.51b	87.04a
H_2SO_4 concentration means (%)	50	68.18b		
	98	78.33a		
Hot water treatment means (%)	Without hot water	57.81b		
	With hot water	78.55a		

Table 2. Effect of concentration and duration of soaking in sulfuric acid and hot water on the height of *Sesbania punicea* seedlings.

Sulfuric acid concentration (%)	Hot water	Soaking times sulfuric acid (minute)		
		0	10	15
50	Without hot water	42.00L	55.66h	58.33g
	With hot water	44.66k	63.66d	68.00c
98	Without hot water	47.00j	59.66f	59.00e
	With hot water	54.00i	68.33b	71.00a
Soaking time means (cm)		46.91c	61.83b	64.08a
H_2SO_4 concentration means (cm)	50	53.61b		
	98	61.61a		
Hot water treatment means (cm)	Without hot water	55.38 b		
	With hot water	59.83 a		

In ornamental shrubs, seed dormancy commonly occurs due to the rigidity of the seed coat, consisting of a few layers of cells not allowing water to penetrate through (Tiwari *et al.*, 2016). Seeds treated with hot water usually provide an appropriate range of temperatures for the enzymes that activate the reactions and break down the seed coat. Sharma *et al*. (2008) confirmed that scratching with hot water has a positive effect on breaking the dormancy and effectively removes the oily layer found on the seed coat, which can otherwise hinder water absorption and gas exchange between the interior and exterior of the seed. This situation was commonly evident in *Leucaena* and leguminous plants as a general characteristic of various factors, such as, the permeability of the seed coat to water and gas exchanges in *Tamarindus indica* L. (Muhammad and Amusa, 2003). Likewise, scratching with acid cracks the seed coat layer and, thus, allows water and gas absorptions for germination to occur in *Sesbania sesban* (Langat and Maina, 2018). Wan and Hor (1983) tested the use of scarification with sulfuric acid to enhance germination in rose seeds, and a significant increase was apparent in the germination.

Seedling height

The outcomes verified that various concentrations of sulfuric acid with varied immersion times and hot water soaking markedly impacted the Sesban seedlings' height (Table 2). The data demonstrated a substantial influence of immersion in sulfuric acid concentrations (50% and 98%), and the H_2SO_4 98% concentration gave the highest seedling height (59.83 cm), followed by a decreased value (55.38 cm) obtained with 50% concentration H_2SO_4. The results further indicated a significant effect of the immersion periods (0, 10, and 15 min) with different concentrations of sulfuric acid, and the length of 15 min showed the maximum seedling height (64.08 cm), while the shortest seedling obtained with the control treatment (46.91 cm). Soaking and no hot water drenching notably affected the seedling height. Treating the *S. punicea* seeds with hot water gave the tallest seedling point (61.61 cm), followed by no hot water with the lowest value (53.61 cm). The interaction of three factors also provided a significant effect on seedling height, and the triple interaction (H_2SO_4 concentration of 98%, with immersion duration of 15 min, and hot water soaking) displayed the utmost seedling peak (70.00 cm) compared with the rest treatments and the control treatment, which showed the least seedling height (42.00 cm).

Scratching with acid or hot water breaks the physiological dormancy of seeds with hard covers that prevent water absorption and gas exchange in *Acacia polyacantha* seeds (Missanjo *et al*., 2014). Premature germination of scratched seeds is the result of cracks that occur in the seed coat, which facilitates the entry of water, gas exchange, and the transformation of the embryo into a seedling of *Acacia auriculiformis* (Olatunji *et al*., 2013). Past findings revealed that the tallest plants (28.82 and 37.76 cm) of *Cassia fistula* L. came from seeds treated with H_2SO_4 for two minutes and then soaked in hot water (100 °C) for three and six minutes, respectively (Soliman and Abbas, 2013). Nourmohammadi *et al*. (2019) reported that treating the *Gleditsia caspica* seeds with sulfuric acid has the best potential to break seed dormancy and produce high-quality seedlings.

Main stem diameter

The results showed a significant effect of both seed immersion in different concentrations of sulfuric acid for different periods and treatment with hot water soaking on the main stem diameter of *Sesbania punicea* (Table 3). The seeds' immersion in sulfuric acid concentrations (50% and 98%) considerably enhanced, with the 98% concentration giving the highest stem diameter (5.79 mm), followed by H_2SO_4 50% concentration (5.27 mm). The results of Table 3 detailed a significant effect of immersion times (0, 10, and 15 min) with sulfuric acid. The duration of 15 min gave the highest stem diameter (6.16 mm) compared with the control treatment (4.58 mm). Soaking in hot water substantially influenced the stem diameter of the *Sesbania punicea* seedlings, and the treatment with hot water gave a stem diameter of 5.94 mm.

The control treatment provided the least stem diameter (5.12 mm). The interaction of three studied factors markedly impacted the stem diameter. The interchange, H_2So_4 concentration of 98%, with an immersion duration of 15 min and hot water soaking, delivered the highest stem diameter (7.28 mm), followed by the same two factors with an immersion time of 10 min (6.56 mm), and the shortest stem diameter appeared in the control treatment (4.20 mm).

The stimulatory and positive effects of scarification on germination and growth traits have released reports for some tree species of the family Fabaceae as *Tamarindus indica* (Muhammad and Amusa, 2003), *Prosopis juliflora* (Zare *et al*., 2011), *Centrosema pubescens* (Rusdy, 2015) and *Gleditsia caspica* (Nourmohammadi *et al*., 2019). The pre-germination methods of chemical scarification positively influenced the growth and quality of the *Tamarindus indica* L. seedlings (Gomes *et al*., 2019).

Leaves per seedling

The investigated treatments and their interactions significantly influenced the number of leaves in *S. punicea* seedlings (Table 4). The *S. punicea* seeds' exposure to sulfuric acid at 50% and 98% concentrations had a discernible impact on the growth traits. The H_2SO_4 98% concentration produced the maximum number of leaves per seedling (31.05), with the 50% concentration producing 29.31 leaves per seedling. A significant relationship was evident between the immersion times (0, 10, and 15 min) and sulfuric acid concentrations. The 15-minute period caused the maximum number of leaves per seedling (32.08) compared with the control treatment (27.72 leaves per seedling). The *S. punicea* seedling leaves sustained notable effects by hot water treatment, with

Table 3. Effect of concentration and duration of soaking in sulfuric acid and hot water on the main stem diameter of the *Sesbania punicea* seedlings.

Sulfuric acid concentration (%)	Hot water	Soaking times sulfuric acid (minute)		
		0	10	15
50	Without hot water	4.20L	5.25h	5.42g
	With hot water	4.45k	6.14d	6.18c
98	without hot water	4.64j	5.44f	5.77e
	With hot water	5.03i	6.56b	7.28a
Soaking time means (mm)		4.58c	5.85b	6.16a
H_2SO_4 concentration means (mm)	50	5.12 b		
	98	5.94 a		
Hot water treatment means (mm)	Without hot water	5.27 b		
	With hot water	5.79 a		

Table 4. Effect of concentration and duration of soaking in sulfuric acid and hot water on the number of leaves of *Sesbania punicea* seedlings.

Sulfuric acid concentration (%)	Hot water	Soaking times sulfuric acid (minute)		
		0	10	15
50	Without hot water	29.58f	28.33g	28.66g
	With hot water	26.33i	31.00d	32.00c
98	Without hot water	26.66h	30.33e	31.00d
	With hot water	28.33g	33.33b	36.66a
Soaking time means (leaves seedling^{-1})		27.72c	30.75b	32.08a
H_2SO_4 concentration means (leaves seedling^{-1})	50	29.09 b		
	98	31.27 a		
Hot water treatment means (leaves seedling^{-1})	Without hot water	29.31 b		
	With hot water	31.05 a		

seeds soaked in hot water producing the maximum number of leaves per seedling (31.27). The triple interaction (H_2SO_4 concentration of 98%, with immersion duration of 15 min, and hot water soaking) resulted in the highest number of leaves per seedling (36.66), which significantly differed from the other treatments, and the control treatment showed the minimum number of leaves per seedling (29.58).

These results are inconsistent with past findings that immersing seeds in hot water produced the highest performance for all the vegetative characteristics studied in the *Acacia polyacantha* (Missanjo *et al*., 2014). Mabundza *et al*. (2010) indicated that scarification of seeds with 95% H_2SO_4 for five minutes enhanced the germination of the pips, number of leaves, and seedling height in *Tamarindus indica* L.

Shoot dry weight

The results revealed that the vegetative dry weight of *S. punicea* incurred effects from each immersion period in various sulfuric acid concentrations and hot water treatment (Table 5). Significant differences occurred between soaking in sulfuric acid concentrations at 50% and 98% for shoot dry weights. The H_2SO_4 at 98% concentration produced the maximum dry weight (7.32 g), followed by the 50% (5.89 g). The seeds' immersion durations (0, 10, and 15 min) in sulfuric acid also significantly influenced the trait. The period of 15 min gave the highest vegetative dry weight (8.12 g), in contrast with the control treatment, showing a decreased dry weight (4.25 g). Considering the hot water treatments, the vegetative dry weight sustained relevant influences by hot water treatment, producing the maximum

Table 5. Effect of concentration and duration of soaking in sulfuric acid and hot water on the shoot's dry weight of the *Sesbania punicea* seedlings.

Sulfuric acid concentration (%)	Hot water	Soaking times sulfuric acid (minute)		
		0	10	15
50	Without hot water	2.47L	6.05h	6.26e
	With hot water	3.42k	8.48d	8.65c
98	Without hot water	5.51j	6.09g	6.14f
	With hot water	5.62i	9.15b	11.42a
Soaking time means (g)		4.25c	7.44b	8.12a
H_2SO_4 concentration means (g)	50	5.42 b		
	98	7.79 a		
Hot water treatment means (g)	Without hot water	5.89 b		
	With hot water	7.32 a		

vegetative dry weight (7.79 g), and non-soaking displayed the lowest vegetative dry weight (5.42 g). The triple interaction of H_2SO_4 at a concentration of 98%, an immersion period of 15 min, and soaking in hot water significantly increased the shoot dry weight at 11.42 g compared with the control treatment (2.47 g).

Soaking the seeds in hot water increases the germination rate and fresh weight due to destroying the cuticle layer and dissolving secondary metabolites deposited in them, encouraging cracks in the outer seed coat layer leading to increased permeability of the seed coat to water and oxygen. Water is the basic condition for germination, necessary for enzyme activation and for the breakdown and transport of stored food. Early induction of optimal germination will affect seeds' physiological processes and will lead to optimal seedling growth (Varier *et al*., 2010; Solichatun *et al*., 2016). For example, treating the seeds of *Rauvolfia serpentina* with hot water (80 °C for five minutes) and then cooling down to room temperature produced the highest germination percentage and better seedling vigor index in Sarpagandha (*Rauvolfia serpentina* Benth) (Bhuyar *et al*., 2000). Joshi and Pant (2010) findings indicated that H_2SO_4 scarification for two hours increased the growth characteristics of *Canna indica*. In the same line, Mehta and Sen (1990) reported that seeds of *Cassia italica* exhibited a seed dormancy and pretreatment with concentrated H_2SO_4 and mechanical scarification improved germination.

CONCLUSIONS

The results of this study conclude in germinating *Sesbania punicea,* its dormant seeds need treatment before sowing to increase the germination rate. The sulfuric acid at 98% concentration for 15 minutes and then soaking in hot water can be helpful for seed pretreatment to break the impermeable seed layer and allow germination. The results will help nursery owners solve this species' germination problems and produce good-quality seedlings suitable for planting. Further studies are necessary, especially when considering extending the immersion duration of seeds beyond 15 minutes since the results of the practical investigation suggest the potential for using longer than 15-minute durations.

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