Response of seeds and growth of *Cassia fistula* L. seedlings to immersion with different periods and concentrations of sulfuric and gibberellic acid

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Abstract
This study was conducted in the nursery of the Forestry Department, College of Agriculture and Forestry/University of Mosul. On *Cassia fistula* seeds during 4/10/2023 to 10/15/2023, they were treated with sulfuric acid at two concentrations 98% and 50% for different periods (0, 5, 10, 15, 20 minutes) and then immersed in gibberellic acid at concentrations (0, 500, 1000 mg. L^{-1}). The results showed that the 20-minute immersion treatment with sulfuric acid recorded the highest averages for the traits (germination percentage, germination speed, seedling height, dry weight of the vegetative group). The study also showed that sulfuric acid at a concentration of 98% was superior to 50% in the characteristics (germination percentage, germination speed, seedling height, number of leaves, and dry weight of the vegetative group), and gibberellic acid at a concentration of 500 mg. L^{-1} had a positive effect and recorded the highest averages in (germination percentage, germination speed, seedling height, number of leaves, dry weight of the vegetative group).
Key words: *C. Fistula*, Sulfuric acid, Gibberellic acid, Germination.

Introduction
The genus *C.* belongs to the subfamily Caesalpiniaceae of the legume family (Fabaceae) and includes about 400 different species [1]. It is one of the most economically important flowering genera [2]. *C. fistula* L. is one of its most important species. It is a medium-sized, fast-growing tree with an irregular, round crown covered with yellow flowers that bloom from spring to mid-summer. Its trunk is straight, its branches are spreading, and it reaches a height of 15 meters and reaches a height of 20 meters in its natural growth areas. It grows in a wide range of soils (dry, nutrient-depleted soils, calcareous, and sandy soils) [3,4]. It needs moderate irrigation [5], its original homeland is Southeast Asia, in India and Sri Lanka, and it has spread widely in various countries of the world [6]. *C. fistula* L. is widely used for ornamental purposes for its aesthetics in landscaping public gardens, parks, and roadsides, and has great uses in the pharmaceutical industries. Its plant parts, especially the leaves, contain many phenolic compounds [7]. Its wood is also used for fuel purposes [8]. Seed propagation is the most common method of propagation of the C. genus in general [9]. *C. fistula* seeds

have a hard covering that prevents water and oxygen from reaching the embryo and activating it. Therefore, *C. fistula* seeds suffer from physical dormancy, which is one of the important limiting factors in the cultivation of leguminous trees in general [10]. Species belonging to the subfamily Caesalpiniodeae have embryos with an embryonic primordium attached to the endosperm. This type of embryo primordium requires active assistance for germination, and without such assistance, it exhibits significant developmental variability. [11, 12]. To enhance seed germination and break the dormancy phase, seeds are treated before sowing with different treatments such as chemical scarification using acids such as sulfuric acid [13]. This treatment leads to increased moisture absorption and gas exchange without affecting the embryo [14]. The duration of soaking in sulfuric acid depends on the thickness and hardness of the seed coat [15]. Scarifying Senna macranthera seeds with sulfuric acid at a concentration of 98% for 45 minutes caused an increase in the germination percentage and germination speed index [16]. Scarifying Sesbania punicea seeds in sulfuric acid at a concentration of 98% significantly outperformed seeds scarified at a concentration of 50%. They showed the highest average values of the studied traits, which are the germination percentage, seedling height, stem diameter, number of leaves per seedling, and shoot dry weight [17]. In addition to scarifying with sulfuric acid, there is an important role for plant growth regulators in regulating most of the vital and physiological activities of plant growth. Gibberellic acid is one of the basic plant growth regulators that are widely used in agriculture due to its physiological effects within plant tissues [18], as it works to break the dormant phase of seeds and accelerates the germination processes and works to elongate and expand cells by contributing to regulating the distribution of cellulose fibers in the cell walls, thus reducing their hardness and increasing their flexibility. It also accelerates the metabolic processes of nutrients [19]. Soaking Aleppo pine seeds, *Pinus halepensis* Mill., in a solution of gibberellic acid at a concentration of 300 mg. $L^{-1}$for 72 hours had a significant effect on the rate of germination rate and speed, and germination energy compared to the comparison treatment[20]. *Acacia cyanophylla* seeds immersed in a concentration of 200 mg. L^{-1} of gibberellic acid for 24 hours significantly outperformed seeds immersed in a concentration of 100 mg/L and recorded the highest rates for the studied traits (seedling height, stem diameter, number of leaves, leaf area, root length, and root diameter compared to the comparison treatment [21].

The aim of this was to know the effect of scratching the seeds and immersion in different concentrations of sulfuric acid and gibberellic acid on breaking the external dormant phase of the seed and achieving the best qualities for seedling growth**.**

Materials and Methods

This study was conducted in the nursery of the Forestry Department / College of Agriculture and Forestry / University of Mosul, on *C. fistula* tree seeds during the period from 10/4/2023 to 15/10/2023. To estimate the germination rate, Germination percentage, and Germination speed (days) were measured.. The final results were taken in

October 2023 for the rest of the studied phenotypic and chemical traits, including percentage and Germination speed (days) were measured. The seedlings are at the age of 6 months. Seed source.

The seeds were obtained from 15-year-old mother trees in Baghdad, located in the nurseries of the Upper Tigris Company for Farm Engineering and Landscape. The pods were collected from *C. fistula* trees in Baghdad, and the fruiting shell was broken with a hammer due to the hardness of the pod shell for the purpose of extracting the seeds. Then they were cleaned and treated later.

The experiment included three factors :

The first factor: **sulfuric acid**

Two concentrations were used: 50% and 98%.

The second factor: immersion periods in sulfuric acid

The factor included five levels.

Without immersion (comparison treatment)

- Immersion for 5 minutes
- Immersion for 10 minutes
- Immersion for 15 minutes
- Immersion for 20 minutes

Then the seeds were washed from the effect of the acid

- **The third factor:** Gibberellic acid
- The factor included three levels.
- Without immersion (comparison treatment)
- Immersion at a concentration of 500 mg. L^{-1} For 24 hours
- Immersion at a concentration of 1000 mg. L^{-1} For 24 hours

The experiment was implemented using a Randomized Complete Block Design (RCBD) [22]

The following indicators of the studied traits

1. **Germination percentage**: It was calculated according to the following equation: Germination percentage = number of germinated seeds/total number of seeds x 100
2. **Germination speed (day)** = calculated according to the Harrington equation [23]. This gives an idea of the strength of the seeds

   Germination speed = (N1T1+N2T2+N3T3…….)/(N1+N2+N3………)

   Where: N1 is the number of seeds germinating in time T
3. **Seedling height (cm/seedling):** Readings were taken using a measuring tape from the soil surface of the bag to the top of the plant for all seedlings.
4. **Number of leaves (leaf/seedling):** The number of leaves for all experimental units was taken for three replications.

The dry weight of the shoot (g/seedling): The shoot of five seedlings in each experimental unit was dried using an electric oven at a temperature of 70 °C ± 1 °C until the weight was confirmed, then weighed using a sensitive electric balance with a sensitivity of 0.01 mg, and the average was extracted.

Results and Discussion

The effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the germination percentage in *C. fistula* seeds %.

The statistical analysis table (1) indicates that the germination percentage differed significantly according to the three studied factors (sulfuric acid, immersion periods in sulfuric acid, and gibberellic acid). The immersion treatment for 20 minutes in sulfuric acid significantly outperformed the rest of the treatments by a rate of 42.33% compared to the non-immersion treatment, which gave the lowest rate of 14.33%. The same table indicates that the seeds immersed in 98% sulfuric acid gave the highest germination rate of 48.98% compared to the seeds immersed in 50% sulfuric acid, which gave the lowest rate of the trait of 13.01%. It also showed that the seeds immersed before planting in different concentrations of gibberellic acid had a significant effect on the germination rate trait, as the concentration of 500 mg. L^{-1} gave the highest significant increase in the germination trait of 34.382% compared to the non-immersion treatment, which gave the lowest rate of germination of 28.23%. It is noted from the interaction table of the study factors that the germination rate reached its highest rate when the seeds were treated with 98% sulfuric acid for 20 minutes and 500 mg. L^{-1} gibberellic acid. 1 liter at a rate of 91.33% compared to the comparison treatment, which gave the lowest germination rate of 10.33%.

Table (1): Effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the percentage of *C. fistula* seed germination

<table>
<tr><th rowspan="2">Sulfuric acid concentration%</th><th rowspan="2">Gibberellic acid concentration mg.L⁻¹</th><th colspan="5">Sulphuric acid immersion time/min</th><th rowspan="2">Interaction between sulfuric and gibberellic acids</th><th rowspan="2">Average sulfuric acid</th></tr>
<tr><th>0</th><th>5</th><th>10</th><th>15</th><th>20</th></tr>
<tr><td rowspan="3">50%</td><td>0</td><td>10.33j</td><td>11.33i</td><td>16.33h</td><td>12.33i</td><td>11.33i</td><td rowspan="2">28.2327bc</td><td rowspan="3">13.01b</td></tr>
<tr><td>500</td><td>11.33i</td><td>16.33h</td><td>16.33h</td><td>11.33i</td><td>11.33i</td></tr>
<tr><td>1000</td><td>11.33i</td><td>16.33h</td><td>16.33h</td><td>11.33i</td><td>11.33i</td><td rowspan="2">34.8327 a</td></tr>
<tr><td rowspan="3">98%</td><td>0</td><td>15.33h</td><td>46.33d</td><td>41.33e</td><td>46.33d</td><td>71.33b</td><td rowspan="3">48.98a</td></tr>
<tr><td>500</td><td>16.33h</td><td>31.33f</td><td>71.33b</td><td>71.33b</td><td>91.33a</td><td rowspan="2">29.933 b</td></tr>
<tr><td>1000</td><td>21.33g</td><td>41.33e</td><td>56.33c</td><td>56.33c</td><td>76.33c</td></tr>
<tr><td colspan="2">Average immersion</td><td>14.33e</td><td>27.16d</td><td>36.33b</td><td>34.83c</td><td>42.33a</td><td></td><td></td></tr>
</table>

*Means that share the same letters for individual factors and their interactions do not differ significantly from each other according to Duncan's multiple range test at the 0.05 probability level.

The effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the germination rate of *C. fistula* seeds.

It is clear from the statistical analysis table (2) that the single effect of immersion periods in sulfuric acid led to significant differences in the germination speed trait, as the immersion treatment in sulfuric acid for 20 minutes recorded the lowest average germination period of 16.22 days compared to the comparison treatment, which gave the most extended germination period of (23.51 days). The table also shows that treating the seeds with a concentration of 98% sulfuric acid had a significant effect on the studied trait and achieved the lowest germination period of 13.02 days. Compared to the immersion treatment with a concentration of 50% sulfuric acid, which gave the longest average number of germination days (24.07 days). The table indicates that treating seeds before planting with different concentrations of gibberellic acid produced significant differences in the studied trait, as the concentration of 1000 mg. L^{-1} gave the shortest germination period at an average of 17.2 days compared to the comparison treatment that recorded the most extended germination period of 19.837 days. The interaction table between the studied factors also shows that treating seeds with sulfuric acid at a concentration of 98% for 20 minutes and gibberellic acid at a concentration of 1000 mg. L^{-1} gave the lowest average number of germination days for *C. fistula* seeds at 3.35 days) compared to the comparison treatment that gave the highest average number of germination days at 29.2 days.

Table (2): Effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on germination speed (days) for *C. fistula* seeds.

Sulfuric acid concentration%	Gibberellic acid concentration/ mg.L^{-1}	Sulphuric acid immersion time/min					Interaction between sulfuric& gibberellic acids	Average sulfuric acid
		0	5	10	15	20		
50%	0	29.2a	25.6 a-c-d	20.76 c-g	24.6a-e	23.2a-f	19.837a	24.07a
	500	28.26a-b	23.36a-f	20.5d-g	24.86 a-e	23.2a-f		
	1000	26.93a-c	22.03c-f	22.20c-f	24.86 a-e	21.56d-f	18.61a-b	
98%	0	20.1d-g	15.26g-j	14.04h-j	10.07j	15.52h-j		13.02b
	500	19e-h	13.15 h-j	13.82h-j	9.465j	10.48j	17.2b	
	1000	17.6f-h	11.04j	12.69i-j	9.70j	3.35k		
Average immersion		23.51a	18.41b	17.33b	17.26b	16.22b		

*Means that share the same letters for individual factors and their interactions do not differ significantly from each other according to Duncan's multiple range test at the 0.05 probability level

The effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the height of *C. fistula* seedlings (cm)

Table (3) shows significant differences between the effects of all individual treatments of the three studied factors on the seedling height trait. The immersion treatment in sulfuric acid for 20 minutes gave the highest rates in seedling height, reaching 21.96 cm, compared to the comparison treatment, which gave the lowest seedling height, reaching 10.86 cm. The same table indicates that seeds immersed in sulfuric acid at a concentration of 98% gave the highest rate in seedling length, reaching 23.817 cm, compared to seeds immersed in sulfuric acid at a concentration of 50%, which gave a lower rate for the trait, reaching 13.195cm. It was also shown that the seeds immersed before planting with different concentrations of gibberellic acid were significantly superior in the average length of seedlings at a concentration of 500 mg. L^{-1} and recorded the highest average length of seedlings of 19.79cm compared to the control treatment, which recorded the lowest average for the trait of 17.86 cm. It is noted from the interaction table of the study factors that the height of seedlings reached its maximum rate when the seeds were treated with sulfuric acid at a concentration of 98% for 20 minutes and gibberellic acid at a concentration of 500 mg. L^{-1} at a rate of 33.22 cm compared to the control treatment, which gave the lowest average height of seedlings of 9.408 cm.

Table (3): Effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the height of *C. fistula* seedlings. (cm)

Sulfuric acid concentration%	Gibberellic acid concentration/ mg.L^{-1}	Sulphuric acid immersion time/min					Interaction between sulfuric& gibberellic acids	Average sulfuric acid
		0	5	10	15	20		
50%	0	9.408 h-i	8.66i	12g-h-i	18.167f	13.5h-g	17.86c	13.195b
	500	11.44g-i	11.77 g-i	12.88 g-i	12.94 g-i	11.47g-h		
	1000	12.94g-i	12.50 g-i	20.33 e-f	13.66 g-h	16.22f-g	19.79a	
98%	0	9.778 h-i	24.88d	24.33 d-e	27.05 b-d	30.88a-b		23.817a
	500	11.66g-i	26c-d	26.33 b-d	30.22 a-c	33.22a	18.85b	
	1000	9.94h-i	24d-e	26c-d	26.44 b-d	26.5b-d		
Average immersion		10.86c	17.97b	20.31 a-b	21.41 a-b	21.96a		

*Means that share the same letters for individual factors and their interactions do not differ significantly from each other according to Duncan's multiple range test at the 0.05 probability level.

The effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the number of leaves of *C. fistula* seedlings

Table (4) shows the difference in the individual effect of the three studied factors on the number of leaves of *C. fistula* seedlings. The single effect of immersion periods in sulfuric acid led to significant differences in the number of leaves, as seeds immersed in sulfuric acid for 15 minutes gave the highest rate of the number of leaves trait, amounting to 5.36 leaves. seedling^{-1}, compared to the control treatment, which gave the lowest rate of the trait, amounting to 4,075 leaves. seedling^{-1}. The same table indicates that seeds immersed in sulfuric acid at a concentration of 98% gave the highest rate of the trait, amounting to 5,102 leaves. seedling^{-1}, compared to seeds immersed in sulfuric acid at a concentration of 50%, which gave a lower rate of the trait, amounting to 4,187 leaves. seedling^{-1}. It was also shown that the seeds immersed before planting with different concentrations of gibberellic acid were significantly superior at a concentration of 500 mg.L^{-1} and recorded the highest average number of leaves, amounting to 5 leaves.seedling^{-1} compared to the comparison treatment, which gave the lowest average for the trait, amounting to 4,098 leaves.seedling^{-1}. It is noted from the interaction table of the study factors that the average number of leaves reached its highest rates when the seeds were treated with sulfuric acid at a concentration of 98% for 15 minutes and gibberellic acid at a concentration of 500 mg.L^{-1} reached (8.66 leaves.seedling^{-1}) respectively compared to the comparison treatment which gave the lowest average for the trait amounting to 2.22 leaves.seedling^{-1}.

Table (4): Effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the number of leaves for *C. fistula* seedlings

Sulfuric acid concentration%	Gibberellic acid concentration/ g.L^{-1}	Sulphuric acid immersion time/min					Interaction between sulfuric& gibberellic acids	Average sulfuric acid
		0	5	10	15	20		
50%	0	2.22e	3.31 c-d-e	4.44c-e	3c-e	4.33c-e	4.098b	4.187b
	500	4.12c-e	4.55c-e	3.55d-e	6.66c-e	3.889c-e		
	1000	4.72c-e	4.11c-e	4.22c-e	4.55c-e	4.11 c-d-e	5. 000a	
98%	0	4.75c-e	4.77c-e	4.72c-e	4.33c-e	5.11a-b		5.102a
	500	3.81c	4.44c-e	5.33b-c	8.66a	5c-e	4.834a-b	
	1000	4.83c	6.33c-e	5.22c-e	5c-e	5.27c-e		
Average immersion		4.075b	4. 58 a-b	4.58 a-b	5. 36a	4.61a-b		

*Means that share the same letters for individual factors and their interactions do not differ significantly from each other according to Duncan's multiple range test at the 0.05 probability level.

Effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the dry weight of the vegetative mass (g) of *C. fistula* seedlings.

The statistical analysis table (5) shows that the dry weight of the vegetative group differed significantly according to the three studied factors. The seeds immersed in sulfuric acid for 20 minutes outperformed significantly and recorded the highest rates in the dry weight of the vegetative group, reaching 1.75 g, respectively, while the seeds immersed in sulfuric acid for 10 minutes gave the lowest rate of the trait, reaching 1.055 g. The same table indicates that the seeds immersed in sulfuric acid at a concentration of 98% gave the highest rate of dry weight of the vegetative group, reaching 2.53g, compared to the seeds immersed in sulfuric acid at a concentration of 50%, which gave the lowest rate of the trait, reaching 1.58g. It was also shown that the seeds immersed before planting in different concentrations of gibberellic acid had a significant effect on the dry weight of the vegetative group, as the concentration gave 500 g. L-1 The highest significant increase in the trait was 1.65g compared to the non-flooding treatment, which gave the lowest average dry weight of the vegetative group, which was 1.15 g. It is noted from the same table that the interaction of the study factors is that the dry weight of the vegetative group reached its highest rate when the seeds were treated with sulfuric acid at a concentration of 98% for 20 minutes and gibberellic acid at a concentration of 500 mg. L^{-1} at a rate of 3.05 g compared to the comparison treatment that gave the lowest rate of the trait at 0.18g.

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Table (5): Effect of immersion periods and different concentrations of sulfuric acid and gibberellic acid on the dry weight of the vegetative group (g) of *C. fistula* seedlings

Sulfuric acid concentration%	Gibberellic acid concentration/ mg.L^{-1}	Sulphuric acid immersion time/min					Interaction between sulfuric& gibberellic acids	Average sulfuric acid
		0	5	10	15	20		
50%	0	1.31c-f	1.13c-f	0.94c-f	1.16c-f	1.32c-f	1.15b	1.58b
	500	1.38c-f	1.23c-f	0.95c-f	1.72b-c	0.87d-f		
	1000	1.3c-f	1.64b-c	0.89d-f	1.35c-f	1.12c-f	1.65a	
98%	0	0.18f	2.15b-c	0.94c-f	1.46b-c	1.75b-d		2.53a
	500	1.87b-c	1.63b-c	1.53b-c	1.87b-c	3.05a	1.34a-b	
	1000	0.32e-f	1.46b-c	1.05c-f	1.92b-c	2.39a-b		
Average immersion		1.06b	1.54a	1.055b	1.58a	1.75a		

*Means that share the same letters for individual factors and their interactions do not differ significantly from each other according to Duncan's multiple range test at the 0.05 probability level.

From Tables (1,2), it is noted that the positive effects of the duration of immersion in sulfuric acid on the values of the studied traits (germination percentage and germination speed). The immersion duration of 20 minutes had the most significant positive effect in recording the highest rates of germination traits. The reason may be attributed to the efficiency of sulfuric acid in scratching the hard seed coat and removing the inhibition of the outer seed coat and improving its permeability, thus encouraging germination. This result is consistent with what was mentioned by [15, 21, 24, 25, 26]. It is also noted that there is a powerful correlation between the germination characteristics as a whole with each other, which in turn was reflected in the morphological characteristics of the seedlings, as shown in Tables (3-5), which showed the positive effect of sulfuric acid on all the morphological characteristics of the seedlings, represented by (seedling height, number of leaves, dry weight of the vegetative group).

The same tables (1-2) show the role of gibberellic acid and its positive effects for the two concentrations (500 and 1000 mg.L^{-1}) on the germination traits (germination percentage and germination speed) and the occurrence of significant differences in the traits compared to the treatment of not immersing in gibberellic acid. The reason may be attributed to the fact that gibberellic acid led to increased germination by increasing cell division through stimulating hydrolysis enzymes such as amylase and alpha-amylase, which work to decompose starch into simple sugars, thus increasing the osmotic pressure of the cells and consequently leading to the rupture of the seed coat, which allows the germination process [27].

Hence, the importance of gibberellins in activating enzymes in structural processes emerges. Thus, this is reflected in activating vital activities within the seed with a decrease in the level of inhibitors, including abscisic acid (ABA), by stimulating the synthesis of alpha-amylase in the aleurone layer. Thus, the inhibitory role of abscisic acid decreases [28], meaning that seed dormancy and germination depend on the balance between endogenous growth inhibitors and growth hormone factors [20, 29]. A positive correlation was observed between germination traits and morphological traits of seedlings. The positive effect of increasing germination was reflected in the traits of the vegetative group represented by (seedling height, number of leaves, dry weight of the vegetative group) as shown in Tables (3-5). The reason may be attributed to the role of gibberellic acid in controlling the physiological processes related to plant growth, as it stimulates growth and development by enhancing water absorption in plant tissues. It also leads to activating the division and elongation of cells below the apical, which increases vegetative growth, especially longitudinal growth [30, 31].

The 98% sulfuric acid concentration was superior to the 50% concentration in all studied traits. The 20-minute immersion period in 98% sulfuric acid had an apparent positive effect on all studied traits of *C. fistula* seedlings, more than the other periods (15, 10, and 5 minutes). Soaking the seeds in 500 mg. L^{-1} gibberellic acid significantly improved the percentage and speed of seed germination, and had an apparent positive effect in improving most of the studied traits..

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