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Effect of treating *Ceratonia siliqua L.* seeds with sulfuric acid and electrical shock in the some physiological characteristics of the resulting seedlings

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Abstract

This study was conducted in the Nursery of the Forestry Department of the College of Agriculture and Forestry, University of Mosul on the seeds of *Ceratonia siliqua* L. pre-treating their seeds with concentrated sulfuric acid 98% for different periods (0, 5, 10 and 15 minutes) and then exposing them to electric shock for different periods also (0, 3 and 6 minutes). Some physiological characteristics chlorophyll (a) and (b), total chlorophyll, proteins, and percentage of nitrogen, phosphorous and potassium were studied. The results showed the superiority of immersion treatment in sulfuric acid (15) minutes for the percentage of total proteins, nitrogen and potassium elements and treatment for 10 minutes gave the highest rate of total phosphorous compared with the control. The treatment with 5 minutes gave the highest rate of chlorophyll (a and b) compared with the control treatment. As for the effect of electric shock, treatment with 6 minutes recorded the highest rates for the percentage of total proteins, nitrogen, potassium and chlorophyll (a and b), while treatment with 3 minutes gave the highest rate of phosphorous compared with the control treatment. As for the interaction effect for both factors, treatment (5 minute of H_2So_4 x 6 minute of electrical shock) recorded the highest rates for the leaves content of chlorophyll (b) and total chlorophyll, and the interaction treatment (15x6) gave the highest rates of protein and for nitrogen and potassium %, except for phosphorous in the leves, the highest rates were recorded with (15x3) treatment.

Keywords: Carob, chlorophyll, Potassium, Nitrogen and phosphorous.

Introduction

Ceratonia siliqua L., an evergreen tree belonging to the family Fabaceae, is widely cultivated in the Mediterranean basin and is distinguished by its biological diversity [1]. Carob is used in pharmaceutical, food and cosmetic industries [2]. Several studies indicated that carob has many biological activities, such as antioxidants, antitumor, and antibacterial [3, 4].Carob pods and seeds are often used in traditional Tunisian medicine as an analgesic, anti-constipation, anti-glucose, and in gastrointestinal and anti-diarrheal

activities [5]. Propagation of this tree has been by many researchers by seed and cutting but the rooting of the cutting is weak [6]. Therefore, the best way for propagation is by seeds. The carob seeds suffer from dormancy as a result of the hard shell covering them, which prevents water intrusion and gas exchange inside the seed [7].

To enhance the germination of carob seeds, the seeds are treated before sowing with various treatments such as hot water and chemical scratching by acids such as sulfuric acid, gibberellic acid and electrocution. These treatments lead to increase moisture absorption and gas exchange process without affecting the embryo. Most of research focused on mechanical treatment or chemical scratching with acid in order to break the dormancy phase in carob seeds [8, 9] stated that the biological processes in the plant generate electric fields, and the plasma membranes transmit electrical signals.The phloem transmits electrical signals through its cells that act as a communication between plasma membranes. Therefore, the phloem is the electrical conductor of bio-electrochemical impulses [10]. Also, the electric shock has an effect on the phenotypic and physiological characteristics of plant. It was found [11] that the treatment of *Albizia libbcke* seeds with electric shock affected the physiology of the subsequent growth of seedlings in some of the studied chemical properties, as it gave the highest percentage of chlorophyll a and b and total (18.78%, 20.60%, 19.67%) respectively as well as the highest protein content of (60.52%). It was indicated [12] the effect of the duration and intensity of the electric shock on some physiological characteristics of *Pinus brotia* leaves, where the treatment (6 amps. for 6 mins.) significantly outperformed all studied characteristics compared to the comparison treatment, and the same current 6 amperes for 8 minutes outperformed the performance, giving the highest significant value of the content of total protein in the leaves with an increase (18.79%). Furthermore, it was mentioned [13] that when treating the seeds of *Pinus halepensis* with Electric shock with intensity (2, 4 and 6) amps for periods of time (0, 2, 4 and 6) mins., the results of chemical analysis showed that the content of the seedling leaves of proteins, total chlorophyll, total chlorophyll and nitrogen increased significantly with the increase of the electric shock intensity of 4 and 6 amps., and the total phenols and potassium increased at the intensity of 6 amperes, while the content of chlorophyll b leaves increased at the intensity of electric shocks at 2 amps. The intensity of the electric shock did not have a significant in the two characteristics of the leaf content of phosphorous and calcium, and all periods of electric shock caused a significant superiority in all the studied chemical characteristics compared to the comparison treatment except for the leaves content of potassium and phosphorus. The research aims to study the effect of exposing carob seeds *Ceratonia siliqua* to immersion in concentrated sulfuric acid and electric shock for different periods of time to know their effects on the course of some physiological processes in seedlings.

Materials and methods

Study site

The seeds were brought from Jordan and were treated in the silviculture Laboratory of the Forestry Department, College of Agriculture and Forestry, Mosul University.

The seeds were planted after the treatment in the greenhouse in September 2020 at the rate of one seed / bag (of bags Black polyethylene). and the service operations continued from watering, hoeing until the end of the experiment at the end of April 2021. The germination took place after ten days of planting and seedlings were obtained with a good vegetative growth and at the end of the experiment the final results of the studied traits were taken.

Studied factors

First: Immersion in sulfuric acid at a concentration of 98%, for four periods (0,5, 10 and 15 minutes).

Second: The electric shock at an intensity of 3 amps was used for three periods (0, 3 and 6 minutes).

The experiment becomes a combination of two factors (3 x 4) and the number of the coefficients becomes (12) treatments (Table 1). The random sector design was used with three sectors, and so the number of the experimental units of this experiment is (36) experimental units; each of which consists of (10) observations and results were statistically analyzed using Genstat package. Means were tested using Dunkin test DMRT at 5% significance and levels were given different letters. The follow up of the research continued in terms of irrigation and weeding until the end of the experiment in October 2018.

Seed treatment

The seeds were treated with concentrated sulfuric acid for the durations (5, 10 and 15 minutes) as well as the comparison treatment without immersion in sulfuric acid. Then the treated seeds were washed with running water several times to remove the traces of the acid. The seeds were divided according to the treatments and placed in perforated cellophane paper and then placed in a device of Electric shock that contains water and sodium chloride salt at the concentration of 1% to increase the electrical conductivity that applied to it by using a device that conforms to what is stated in Patent No. 3112 dated 10/21/2002 issued by the Central Organization for Standardization and Quality Control in the name of Dr. Medhat Al-Sahouki and Mr. Ahmed Ali [14]. A constant intensity of (3 amperes) was used for two periods (3 and 6 minutes), as well as the comparison treatment without shock. After completing the electric shock for each treatment, the seeds were placed in a container containing running water for three hours to wash the salt from the seeds to avoid damage to the seeds [15].

Studied traits

1. Determination of chlorophyll a, b and total content of fresh leaves (mg. g-1 wet weight): based on McKinney, (1941)
2. Estimation of the nutrients in the leaves:

a- Nitrogen (%): The percentage of nitrogen was estimated by the Kjeldahl method using the Micro-Kjeldahl device, from which the crude protein percentage was calculated based on [16] and according to the following equation:

percentage of crude protein = percentage of nitrogen x 6.25

b- Phosphorous (%): The percentage of phosphorous was estimated chromatically by Ascorbic acid method according to [17].

c-Potassium (%): The potassium content was estimated according to the method [18] using a Flame Photometer.

Results and Discussion

Effect of sulfuric acid and electrical shock in some physiological characteristics for *Ceratonia siliqua* L. seedling

Chlorophyll (a) content of leaves mg.g^{-1} fresh weight

The results of Table (1) indicate a significant effect of the duration of immersion in concentrated sulfuric acid and electric shock in chlorophyll (a) content in leaves. The two treatments of immersion with acid concentrated sulfuric for (5 and 15) minutes gave the highest rate for the trait (1.0372 and 1.0159 mg.g-1 fresh weight) respectively and differed from the rest of the treatments. The electrocution treatment (6) minutes gave the highest rate of chlorophyll (a) and it amounted (1.019 mg.g-1 fresh weight), but it did not differ from the treatment (3) minute, which gave an average of (1.017 mg.g-1 fresh weight), but differed from the comparison treatment, which gave the lowest rate of the trait amounted (0.828 mg.g-1 fresh weight). The interaction effect had a significant impact where, the treatments (0x15, 0x5 and 0x10) gave the highest rates was (1.086, 1.076, ,1.075 mg.g-1 fresh weight) respectively in comparison with the control treatment, which gave the lowest rate for the trait, which was (0.911 mg.g-1 fresh weight)

Table (1): Effect of immersion in sulfuric acid and electrocution in chlorophyll content of leaves (a) mg/g^{-1} fresh weight

Duration of dipping in Sulfuric acid/minute Duration of exposure toelectrical shock	0	5	10	15	Period of exposure to electrical shock
0	0.911b	1.076 a	1.075 a	1.086a	0.828 b
3	1.01620ab *	1.019 ab*	1.029 ab*	0.954ab	1.017 a
6	0.945ab*	0.998 ab*	1.0119 ab*	1.037ab *	1.0194 a
Duration of dipping	0.8642b	1.0372a	0.876b	1.015 a	

2 - Chlorophyll (b) content of leaves mg.g^{-1} fresh weight:

Table (2) indicates the effect of the duration of immersion in concentrated sulfuric acid and electric shock in the content of chlorophyll leaves (b), as the two treatments, immersion in sulfuric acid (5 and 15) minutes, recorded the highest rate of chlorophyll content of leaves (b). It reached (0.858, 0.805) mg.g^{-1} respectively, compared to the comparison treatment, which recorded the lowest rate of the characteristic reached (0.517) mg.g^{-1}. As for the effect of electrocution treatment, the treatment (6) minutes

recorded the highest rate of chlorophyll (b) amounting to (0.831) mg.g^{-1} compared to the comparison treatment, which recorded the lowest rate of the characteristic reached (0,619) mg.g^{-1}, while the interaction of the treatments immersion in concentrated sulfuric acid for a period of (5) minutes and shock (3) amps. gave the highest rate of leaves content of chlorophyll (b) amounting to (0.912) mg.g^{-1} compared to the control treatment that gave the lowest rate for the character reached(0,665) mg.g^{-1} **.**

Table (2): Effect of immersion in sulfuric acid and electrocution in chlorophyll content of leaves (b) mg/g wet weight

Duration of dipping in sulfuric acid /minute Duration of exposure to electrical shock	**0**	**5**	**10**	**15**	**Period of exposure to electrical shock**
0	0.665 b	0.794 ab*	0.877a	0.829ab*	0.619b
3	0.834ab*	0.912 a	0.685b	0.673b	0.726ab*
6	0.780ab*	0. 936a	0.815ab*	0.821ab*	0.831a
Duration of dipping	0.517c	0.858 a	0.681b	0.805a	

3- Total chlorophyll content of leaves mg.g^{-1} fresh weight

Table (3) indicates the effect of the duration of immersion in concentrated sulfuric acid and electric shock in the (total) chlorophyll . The treatment of immersion with concentrated sulfuric acid (15) minutes gave the highest average of total chlorophyll amounted to(2.282) mg.g-1 fresh weight compared to the control treatment, which gave the lowest rate for the trait amounted to (1.422) mg.g-1 fresh weight. As for the effect of electric shock on the total chlorophyll content of the leaves, it is noted that the treatment of shock (3 minutes) gave the highest rate of the character amounted to (1.92) mg.g-1 compared with the treatment (6 minutes) and the comparison. As for the interaction the duration of acid immersion and exposure to shock, the factorial treatment (5x6) recorded the highest rate for the trait reached (2.319) mg.g-1 fresh weight in comparison with the control treatment which gave the lowest rate of the trait reached (0.842) mg.g-1 fresh weight.

Table (3): Effect of immersion in sulfuric acid and electrocution in leaves content of total chlorophyll mg.g^{-1} wet weight

Duration of dipping in sulfuric acid/minute Duration of exposure toelectrical shock	0	5	10	15	Period of exposure to electri-cal shock
0	0.842 e	1.304 d	2.122ab*	1.894b	1.747b
3	1.966 b	1.683c	1.934b	2.106ab	1.924a
6	1.480 cd	2.319a	2.132ab*	2.125ab*	1.852b
Duration of dipping	1.422c	1.847 b	1. 840 b	2.282a	

4- percentage protein content of leaves (%)

It is noted from the results of Table (4) that the effect of the immersion period with concentrated sulfuric acid gave a significant effect on the protein content of leaves. The treatment of (15) minutes outperformed and gave the highest rate of protein rate of (15.77%) compared with the comparison treatment that gave the lowest rate of the trait (13.47%), and the treatment of shock (6 minutes) outperformed and gave the highest percentage of protein was 16.213% compared with the comparison treatment that gave the lowest rate of the trait (13.51%), and the interaction had a clear effect, as the treatment (15 x 6) minutes gave the highest rate (19.47%), and it differed significantly from the comparison treatment, which gave the lowest rate of protein amounted to (12.22)%

Table (4): Effect of immersion in sulfuric acid and electrocution in the protein content of leaves %

Duration of dipping in sulfuric acid/minute Duration of exposure toelectrical shock	0	5	10	15	Period of exposure to electri-cal shock
0	12.229l	13.167k	15.042d	14.479f	13.510c
3	15.792c	13.917h	14.042g	13.354i	14.213b
6	16.417b	13.292j	14.542e	19.479 a	16.213a
Duration of dipping	13.47d	14.72b	14.60 c	15.77 a	

Percentage of nitrogen content of leaves (%)

The results of table (5) indicate that the effect of the duration of immersion in concentrated sulfuric acid and electric shock gave a significant effect individually in the nitrogen content of leaves. 15 minute gave the highest rate of nitrogen at a rate of (2.523%)، and differed from the treatment of comparison, which gave the lowest rate for the recipe was (2.157%), as for the effect of treatment (6) minutes gave the highest rate of nitrogen amounted to(2.597%) compared with the comparison treatment that gave the lowest rate of the trait (2.162%), whill the interaction had a clear effect on the trait. The treatment (15 x 6) minutes gave the highest nitrogen rate of (3.117%), and it differed significantly with the comparison treatment, which gave the lowest rate of nitrogen percentage, which amounted to (1.957%) minutes.

Table (5): Effect of immersion in sulfuric acid and electric shock in the nitrogen content of leaves %

Duration of dipping in sulfuric acid/minute Duration of exposure toelectrical shock	0	5	10	15	Period of exposure to electrical shock
0	1.957 l	2.107 k	2.407d	2.317f	2.162 c
3	2.527c	2.227 h	2.247 g	2.137 i	2.274 b
6	2.627 b	2.127 j	2.327 e	3.117 a	2.594 a
Duration of dipping	2.157 d	2.337 c	2.357 b	2.523 a	

Percentage of phosphorous content of leaves (%)

Table (6) indicates the effect of the duration of immersion in concentrated sulfuric acid and electric shock in the of phosphorous element in the leaves. As the treatment of immersion with concentrated acid excelled (10) minutes gave the highest rate reached (0.320%) compared with the control treatment which gave the lowest rate reached to (0.118 %), As for the effect of electric shock (3) minutes treatment gave the highest rate of the trait, which reached(0.490%) which varied significantly with the comparison treatment that gave the lowest rate of the percentage of phosphorous element amounted to (0.132%). It is noted from the data of the binary interaction between the studied factors that the percentage of phosphorous element increased to its maximum rates (15 x 3) minutes, which recorded the highest rate of phosphorous element amounting to (0.310%) which varied Significantly with the comparison treatment that gave the lowest rate of the percentage of phosphorous element amounted to (0.082%).

Table (6): Effect of immersion in sulfuric acid and electric shock in the content of phosphorous element % in leaves

Duration of dipping in sulfuric acid/minute Duration of exposure toelectrical shock	0	5	10	15	Period of exposure to electrical shock
0	0.082 l	0.160 e	0.106 k	0.119 i	0.132 c
3	0.116 j	0.126 h	0.152 g	0.310 a	0.490a
6	0.158f	0.175 d	0.176 c	0.192 b	0.146 b
Duration of dipping	0.118 d	0.121 c	0.320 a	0.181 b	

Percentage of potassium content of leaves (%)

Table (7) indicates the effect of the duration of immersion in concentrated sulfuric acid and electric shock in the of potassium element in the leaves. The treatment of immersion with concentrated acid (15) minutes outperformed and gave the highest rate of phosphorous element by (1.23%) compared to the comparison that gave the lowest rate of the trait amounted to (0.72%). As for the effect of electric shock treatment (6) minutes gave the highest rate of the trait amounted to (0.93%) which differed from the treatment of shocking (3) minutes, which gave the lowest rate for the trait amounted to (0.817%). It is noted from the results of the binary interaction between the studied factors that treatment (5 x6) minutes gave the highest rate of potassium element amounted to (1.296 %), which differed significantly with the comparison treatment that It gave the lowest percentage of potassium which was (0.426%).

Table (7): Effect of immersion in sulfuric acid and electrocution on potassium content of leaves%

Duration of dipping insul-furic acid/minute Duration of exposure to electrical shock	0	5	10	15	Period of expo-sure to electrical shock
0	0.426l	0.836g	0.896e	0.886f	0.830 b
3	0.616j	0.676i	0.726 h	0.586k	0.817c
6	0.983d	1.296a*	1.206b	1.186c	0.930a
Duration of dipping	0.720d	0.726c	0.765 b	1.230a	

From the results mentioned above, Chemical scratching of seeds such as soaking in gibberellic acid, concentrated sulfuric and acetic acid and other substances that encourage breaking the dormancy phase in seeds accelerate the germination process and work to stimulate the growth of the embryo and the subsequent growth of seedlings [19]. Because the process of scratching by acids, including concentrated sulfuric acid, affects the softness of the seed coat and thus leads to the permeability of moisture and oxygen to the embryo and stimulates it to grow, which in turn leads to acceleration of germination [8]. Thus, it affects the metabolism of physiological processes in seedlings for some traits, and this result is consistent with what was reached by [20] where he mentioned that treatment of carob seeds with acetic acid for 60 minutes gave the best results in terms of characteristics (length of seedlings, dry weight of the vegetative and root system, total chlorophyll content of leaves. The percentage of nitrogen in the leaves and the percentage of potassium in the leaves.

Exposing the seeds to electric shock also led to an increase in the studied traits. The results of tables (1, 2 and3) increased the content of chlorophyll (a, b and total) in the leaves. The reason may be through treating the seeds with electric shock, which increased the expansion of the xylem in the petioles of the leaves, which led to an increase in the rate of absorption of the necessary nutrients that contribute to the formation of the chlorophyll molecule, or the reason for the increase may be due to the effect of electric shock in changing the size and number of chloroplasts because the good growth of seedlings resulting from treated with electric shock is related to the contents of the leaves of these seedlings From the amount and activity of chlorophyll and this result matches what was mentioned [15]. From the results of Table (5, 6 and 7) it was found that there was an increase in the studied chemical properties (total proteins, nitrogen, phosphorous and potassium). The reason for the increase could be attributed to the role of electrocution in causing changes in vital and enzymatic activities inside the cell, and electrical treatments of seeds work to stimulate the necessary enzymes in the protein building process, such as enzymes that reduce nitrates and convert them to nitrites and then to ammonia and amino acids and then to the protein of [21] and this is consistent with what was reached [13] Which confirmed that when exposing Aleppo pine seeds to electric shock led to an increase in the content of leaves from chlorophyll, protein, potassium and nitrogen

Conclusions

Scratching by concentrated sulfuric acid and electric shock for different periods is one of the most important means that must be used to obtain good vegetative growth and a good content of nutrients in the leaves of carob trees *Ceratonia siliqua*. The seedlings produced from seeds treated with sulfuric acid and electrocution significantly outperformed the traits (the content of leaves from chlorophyll, protein, nitrogen, phosphorous and potassium).

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