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Effects of Various Pre-Sowing Treatments on Seed Germination of Carob (*Ceratonia Siliqua* L.) From Al-Jabal Al-Akhdar Area (Balagrae, Al-Baida, Libya)

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Abstract: *This study was conducted on a wild carob genotype grown in Al-Jabal Al-Akhdar area (Balagrae, Al-Baida, Libya). Seeds belonging to three individual trees chosen at random were collected. Seven different pre-sowing treatments (soaking in distilled water, boiling water 100 C, soaking in sulphuric acid, mechanical scarification, mechanical scarification & soaking in distilled water, mechanical scarification & soaking in sulphuric acid and untreated seeds) were applied and examined for their effectiveness to stimulate carob seed germination. The results showed that mechanical scarification & soaking in distilled water treatment was the most effective method in increasing the germination percentage by (94.33%) compared to untreated seeds (5.99%). Mechanical scarification & soaking in distilled water treatment significantly decreased the mean germination time value (MGT) by (2.83 days). An MGT value was significantly higher in control treatment (23.22 days).*

Keywords: *carob, Ceratonia siliqua, pre-sowing treatment, seed germination.*

I. Introduction

Carob tree (*Ceratonia siliqua* L.), St. John's bread, or locust is a small to medium sized broadleaf. Ceratonia species belongs to the Caesalpiniodeae sub family of the family Leguminoseae (Syn. Fabaceae). This species is normally dioecious and it is sclerophyllous evergreen tree that may grow up to 20 m in height under best environmental circumstances [1] but typically attains heights of 8 to 15 m [2, 3].

Carob is widely cultivated in Mediterranean regions (Cyprus, Greece, Portugal, Italy, Spain, Lebanon, Southern Jordan, Syria, Turkey, Egypt, Libya, Tunisia, Morocco,) and in areas of North America [4]. Carob is considered a tropical plant that has modified well to Mediterranean climates. Its deep rooting habit and xerophilous leaves were used to evade water stress [1].

Carob considered as one of the most important indigenous species that constitute the plant cover of Al-Jabal Al-Akhdar area (Al-Wasita, Agfentta, WadiKouf, Al-Hania, Al-Hamama, Omar Al-Mukthar, Messa, Al-ghariqa and North of Labraq). Carob trees distribute naturally in all regions of Al-Jabal Al-Akhdar, it grows as pure carob populations or form mixed woodland together with *Juniperus phoenicea, Oleaeuropaea* L. var. oleaster, *Quercuscoccifera*, *Cupressussempervirens* and *Pinushalepensis*[5].

Carob is an economically significant tree and it can be used in many trees – planting activities: - charcoal, wood production, soil erosion management, land recovery, ornamental evergreen tree [6].

Carob seeds are difficult to germinate they are tremendously hard and not willingly absorb water [7]. Under natural conditions, only a reduced percentage of carob seeds are able to germinate. A number of factors (mechanical friction with soil particles, microbial action, passage through the digestive tract of mammals that feed on them, etc) can change seed coat [6].

Similar to other legume seeds, carob reveals seed coat impermeability to water therefore the seed will not germinate without seed coat scarification [8, 9 and 10]. The impermeable seed coat is the cause of the physical dormancy of carob seeds. Germination can be improved by treating seeds with tap water, boiling water, sulphuric acid (H_2SO_4) or gibberellic acid (GA_3) [11, 12 and 13]. Mechanical or acid scarification is commonly employed to break hard seed coat and enhance seeds germination [14]. The dormancy-breaking treatments used in these instances must raise moisture uptake and gas exchange and should not produce alterations in the embryo and endosperm [15], in the intention to prevent seed damage, taking into account that several physiological reactions are occurred during the germination process. Based on available knowledge, only few studies have dealt with seed germination of the *C. siliqua*[16]. In addition, information regarding the pre-treatment to be used to break the dormancy of carob seeds is often contradictory.

Coit[7] stated that mechanical scarification is an effective treatment in increasing the rate of water absorption with carob seeds.In addition, Goor and Barney [3] showed that the best treatment to defeat seed coat impermeability are soaking in concentrated sulfuric acid (H_2SO_4), or alternatively soaking in warm water.

Martins – Loucao*et al.*[17] indicated that the superlative pre-sowing treatments for augmenting carob seeds germination were acid scarification, treatment with warm water and mechanical scarification. Though, that information is difficult to be applied in another origin without any investigation, mostly because of the immense genetic variability usually existed within a species [15]. Also, to generate a protocol for seed germination, information from a variety of countries is required to document the rule.Tsakaldimi and Ganatsas[16] examined treatments enhancing seeds germination of *C. siliqua* from Crete, such as acid scarification (concentrated sulphuric acid, H_2SO_4) and hot water. Acid scarification for 15 minutes was confirmed the most efficient pre-treatment for carob seeds, caused 86.7% germination percentage and 10.19 days MGT. In contrast, hot water soaking was less efficient although it enhanced the germination percentage (58.7%, 9.08 days MGT). The results are compatible with that found by Martins-Loucao*et al.*[17] in Portugal. Therefore, it can be concluded that the response of this species against acid scarification with sulphuric acid appears to be identical for the West and East Mediterranean areas.Piotto and Di Noi[10] stated that the germination percentage never exceeds 10% without any pre-treatment. Seeds have to be subjected to one of the following treatments: soaking in hot water, soaking in concentrated sulphuric acid, or mechanical scarification. All these pre-treatments considered as some of the most used dormancy-breaking treatments.Pérez-García [6] used various pre-treatments (mechanical scarification, soaking in distilled water, boiling water, dry heat, sulphuric acid and gibberellic acid) to enhance seed germination in carob. Seeds were collected from 17 different individual trees. The results clarified that the final germination percentages of control (untreated) seeds that were bulked from 17 trees, varied from 23 to 28%. Mechanical scarification, sulphuric acid and boiling water drastically improved final germination percentages (99, 88 and 80%, respectively). The germination percentage reached by untreated seeds and preheated seeds were very similar. Soaking seeds in distilled water (24, 48 and 72 h) and in gibberellic acid solution did not significantly increase the final germination percentages compared to control seeds. Exposure to dry heat at 100°C for different periods did not significantly augment the final germination percentages over the untreated seeds. This could designate that carob seeds are not dependent on fire for seed germination. The results are similar to those achieved by Ortiz *et al.* [2]The authors showed that investigations motivating fire effect caused a reduced germination. Furthermore, the results of Pérez-García [6] proved that there is a substantial variability in seed weight, seed water content and germination characteristics within the studied population. Final germination percentages of control seeds belonging to 17 different individual trees ranged from 7 to 50%. These findings demonstrated that there is a high degree of variability among seeds to the extent that the hardiness of the seed coats is concerned. The production of seeds with different germinability is one of the main significant survival approaches for species growing beneath changeable and unpredictable environmental circumstances [15]. The capacity to yield seeds with diverse level of dormancy is a mechanism by which *C. siliqua*, as other species [18, 19], adjusts to new environmental conditions and guarantees its endurance by supporting the germination of seeds over time. It is likely that this variation, as happen in numerous other species [18, 20], can be ascribed to genetic variations among individual parent plants, even within a small geographic region. Günes *et al.* [12] assessed the effect of several H_2SO_4 concentrations on seed germination of carob. Seeds used in the study were wild genotype of carob. The findings designated that the lowest germination percentage was 22.92% in untreated seeds followed by 23.61% by dipping seeds in 60% H_2SO_4. The treatment of 98% H_2SO_4 resulted in the highest germination percentage (93.06%). Günes *et al.*[13] investigated the influences of different treatments on seed germination and growth of carob collected from both wild and cultivated genotypes in Turkey, which were exposed to mechanical scarification, boiling water and soaking in sulphuric acid. All treatments accelerated seed germination and seedling growth compared to control. The superlative treatment was soaking in concentrated H_2SO_4 acid, followed by mechanical scarification and hot water treatment, in that order. The germination percentage of control seeds were alike for both wild and cultivated genotypes (13%) and by applying sulphuric acid treatment, the germination percentage was raised up to 95and 93% in wild and cultivated genotypes, respectively. Formerly, pre-treatment on seed germination of carob have only been performed with wild carob genotypes [17, 16, 10 and 6] with no similar work being carried out on cultivated genotypes. El Deen*et al.*[21] studied carob seed propagation. Seeds and cuttings were scarified and disinfected under aseptic circumstances with some treatments to increase the germination percentage. Six treatments were applied to the seeds: control (untreated seeds), soaking in water at 30°C for 72 h, soaking in hot water for 10 min, dipping in 60% H_2SO_4 for 30 min, dipping in GA_3 and dipping in 80% acetone. Their findings illustrated that the highest values of seed germination percentage, the fastest germination, the greatest plant length, number of leaves/plant, root length and dry weight were acquired by soaking seeds in 60% H_2SO_4. Bostan and Kilic[22] studied the influences of various treatments on seed germination on a wild carob genotype grown in Turkey. Seeds were stratified then the seeds were subjected to sulphuric acid and gibberellic acid. The results illustrated that there was no seed germination in untreated seeds, the highest germination percentage for sulphuric acid treatment was detected in 95% sulphuric acid as 88.90%, and the highest germination percentage for gibberellic acid (GA_3) treatments was noted in 1000 ppm dose as 28.90%.

Because Libya is one of the homeland regions of carob, it is important to conduct investigations into the ecophysiology of the germination performance in this Mediterranean species. The present study was conducted on a wild carob genotype grown in Al-Jabal Al-Akhdar area (Balagrae, Al-Bayda, Libya). The aim was to investigate the effects of several pre-sowing treatments on the germination of carob seeds.

II. Materials and Methods

2.1 Study Site

Balagrae (about 10 km south of Al-Baida, 32° 73′ – 32° 77′ N 21° 70′ – 21° 68′ E, 522.5 m above sea level) was chosen as the study site because it is the most diversified woodland close to Omar Al-Mukthar University campus. The study was carried out at the graduate studies laboratory (Botany Department, Science Faculty) at Omar Al-Mukthar University (Al-Beida, Libya) during the summer of 2014.

2.2 Seed Material

- Ripe fruits (pods) containing mature seeds of *Ceratonia siliqua* were collected in August 2014 from a wild growing population in Balagrae from Al-Jabal Al-Akhdar area.
- Seeds belonging to three individual trees chosen at random from the study site were collected. Seeds were cleaned manually, placed in paper bags and stored dry under laboratory conditions (20 ± 5°C) until the start of the experiment.

2.3 Seed Viability Test

To test seed viability (is a measure of the percentage of seeds that are alive after storage), seeds were placed in a beaker then were soaked in water. The seeds that float up were discarded.

2.4 Control of Pathogens

Aseptic technique is an important way to reduce pathogenic contamination by fungi, molds and bacteria by killing and minimizing their presence. All work surfaces were cleaned and disinfected with 95% ethanol. The easiest way to prevent contamination is by surface sterilization of seeds to be used in the research. Seeds were soaked in 70% ethanol for 1 minute then were thoroughly rinsed 4 to 5 times in sterilized distilled water, to minimize microorganism development at the early stages of germination.

The goal of the present study was the assessment of several dormancy-breaking treatments applied to enhance the germination of carob seeds from Al-Jabal Al-Akhdar area and to offer a contribution to the improved information of seed biology of carob, mainly for the intention of utilization in the nursery practice. Treatments enhancing seed germination presented in bibliography were investigated. Seven different pre-sowing treatments were applied and examined for their effectiveness to stimulate carob seed germination:

2.4.1. Soaking In Distilled Water

Seeds were soaked in distilled water (3 volumes of water for each volume of seeds), at room temperature (approximately 25°C) for 72 h. The water was renewed daily [6].

2.4.2. Boiling Water (100°C)

Seeds were immersed in boiling distilled water (3 volumes of water for each volume of seeds) and then left to cool at room temperature (approximately 25°C) for 24 h [6].

2.4.3. Acid Scarification With Sulphuric Acid (H_2SO_4)

Concentrated sulphuric acid (96% H_2SO_4) was used to soak seed for 1 to 30 minutes (3 volumes of acid for each volume of seeds). Fifteen minutes were applied. The seeds were then washed in running water for 1 hour to remove any trace of acid, before being tested for germination [6].

2.4.4. Mechanical Scarification

Chipping was achieved using a sharp blade to carefully remove the seed coat, without damaging the radicle, at the radicle end of the seed [6].

2.4.5. Mechanical Scarification & Soaking In Distilled Water

Seeds were scarified first and then soaked in distilled water for 24 h.

2.4.6. Mechanical Scarification & Soaking In Sulphuric Acid

Seeds were scarified first and then were soaked in concentrated sulphuric acid 96% for 15 minutes (3 volumes of acid for each volume of seeds). The seeds were then washed in running water for 1 hour to remove any trace of acid.

2.4.7. Control (Untreated Seeds)

Seeds were set to germinate without any treatment. After these pre-sowing treatments, seeds were set to germinate at room temperature (20 - 25°C) and were tested for germination by using three replicates (each contained 10 seeds). Germinated seeds were counted daily and removed.

2.5. Measurements

The germination percentage (G%) and mean germination time (MGT in days) were recorded for all treatments. The Germination percentage was calculated using the following formula [23]:

Germination % = Number of germinated seeds /Total number of seeds X 100

The mean germination time (MGT) was calculated by the expression of [24].

$MGT = \Sigma n_i t_i / \Sigma n_i$

Where *ti*: time from the start of the experiment to the i^{th} observation (day for the example; n_i: number of seeds germinated in the i^{th} time (not the accumulated number, but the number correspondent to the i^{th} observation)

2.6. Statistical Analysis

A One-way analysis of variance (ANOVA) was used to test the effect of the different pre-sowing treatments on seed germination. Percentage values were arcsine transformed prior to statistical analysis to improve normality and stabilize variances [25]. Variables were displayed as means ± standard errors of the means (SEM). The statistical analysis was performed using SPSS (Statistical Package for Social Sciences, version 20). Mean comparison was performed using Duncan's Multiple Range Test (DMRT) [26]. The alpha level was set at 5%.

III. Results

TABLE (1) illustrates the effects of seven pre-sowing treatments on seed germination of *C. siliqua* from Al-Jabal Al-Akhdar area. Data were analyzed using a one-way ANOVA. The highest germination (94.33%) was recorded when seeds were treated with mechanical scarification & soaking in distilled water treatment. The lowest germination (5.99%) was found in untreated seeds (control). This confirms the presence of a strong hard seed coat physical dormancy in the studied species. Likewise, a combination of (mechanical scarification & soaking in sulphuric acid treatment) and soaking in sulphuric acid treatment significantly improved the germination of carob seeds by (92.44 and 90.78%, respectively) compared to untreated seeds (5.99%). When seeds were boiled, the germination percentage was increased to 84.22%. In contrast, mechanical scarification treatment improved the germination percentage by only 70.45%. Our results contradict the findings of Pérez-García[6] who stated that mechanical scarification was the most effective treatment that enhanced the germination percentage by (99%) of carob seeds. Soaking seeds in distilled water treatment increased the germination percentage by only 12.78%. However, differences with control seeds were significant. Figs(1a, 1b, 2, 3, 4, 5, 6, 7, 8) display the effect of different pre-sowing treatment on germination percentages and mean germination time for *C. siliqua* from the studied site.

Four treatments (mechanical scarification & soaking in distilled water, mechanical scarification & soaking in sulphuric acid, soaking in sulphuric acid and boiling water) significantly decreased ($P = 0.000$) the MGT values (TABLE 1). There were no significant differences among MGT values reached by these treatments.

Mechanical scarification treatment also decreased MGT value but was significantly different than the other four mentioned treatments. An MGT value was significantly higher in control treatment (23.22 days). However, no significant differences were found between the values reached by control seeds (23.22 days MGT) and those values reached by seeds soaked in distilled water (21.42 days MGT). Briefly, treatment that resulted in the highest germination percentage (in this instance, mechanical scarification & soaking in distilled water) was the shortest in terms of MGT values reached by it and vice versa.

TABLE 1. Final germination percentages (mean values ± standard error) and mean germination time (mean values in days ± standard error) of *Ceratonia siliqua* seeds after different pre-sowing treatments. Seeds belonging to pods from three trees were bulked.

Treatments	Germination percentage (%)	Mean germination time (MGT)
Untreated seeds (control)	**5.99 ± 0.67^a**	**23.22 ± 0.22^c**
Mechanical scarification	**70.45 ± 1.18^c**	**9.87 ± 0.35^b**
Boiling water (100 □C)	**84.22 ± 1.73^d**	**5.73 ± 0.52^a**
Soaking in sulphuric acid (H_2SO_4)	**90.78 ± 0.29^e**	**3.77 ± 0.09^a**
Mechanical scarification & soaking in distilled water	**94.33 ± 0.84^f**	**2.83 ± 0.35^a**
Mechanical scarification & soaking in sulphuric acid	**92.44 ± 0.56^{ef}**	**3.27 ± 0.17^a**
Soaking in distilled water	**12.78 ± 2.29^b**	**21.42 ± 1.72^c**
***P*-value**	**<0.000**	**<0.000**
R^2	**0.9975**	**0.9847**

Mean values within a column followed by the same letter are not significantly different ($\alpha = 0.05$, One-way ANOVA).

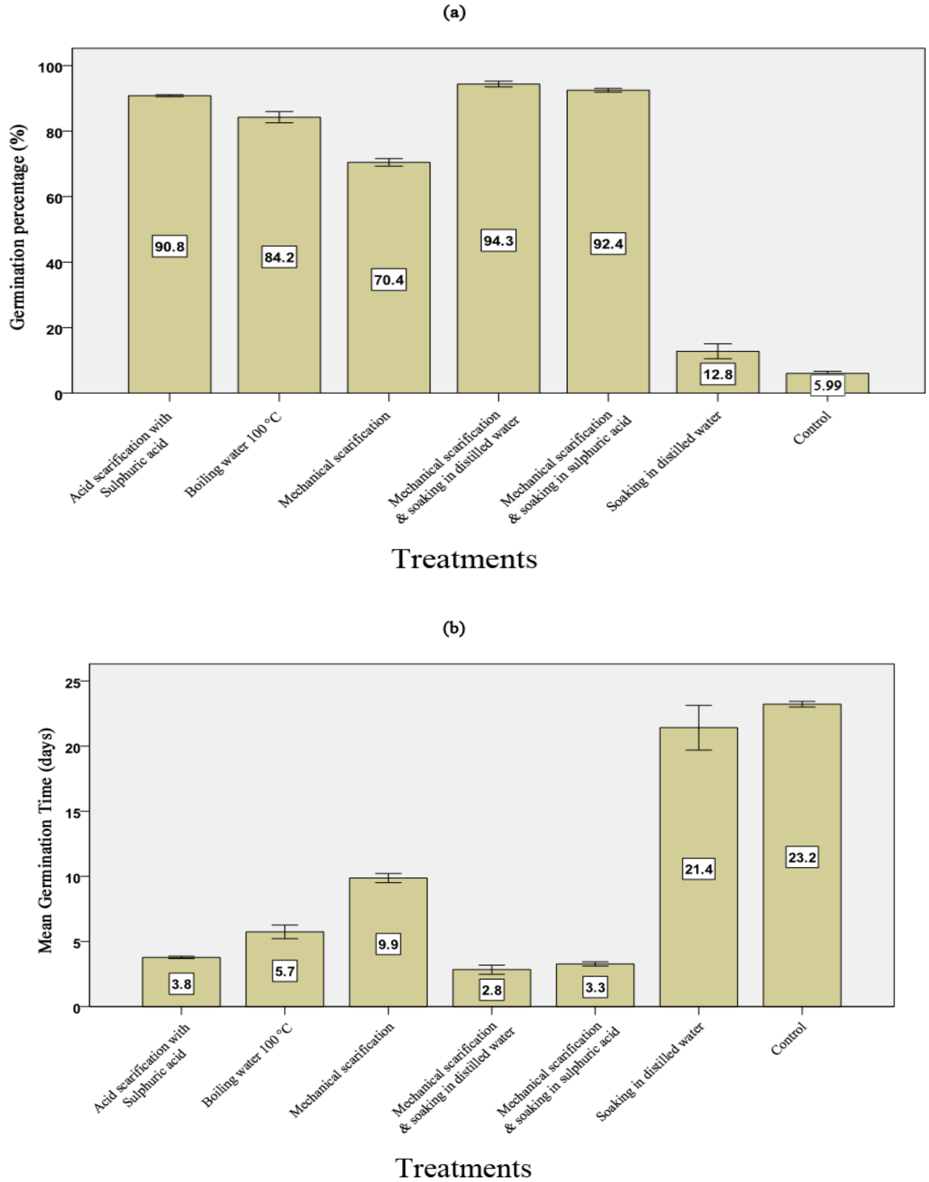

Figure 1. Cumulative germination percentages (a) and Mean germination time (b) of C. *siliqua* seeds in seven pre-sowing treatments. Each point represents the mean ± SE of three replicates of 10 seeds each.

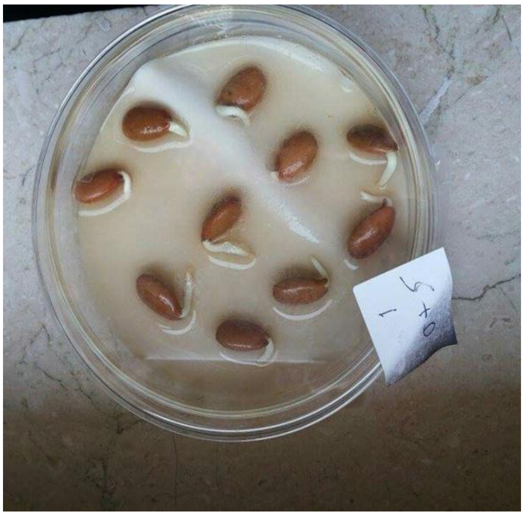

Figure 2. Mechanical scarification & soaking in distilled water treatment.

Figure 3. Mechanical scarification & soaking in sulphuric acid treatment.

Figure 4. Boiling water (100°C) treatment.

Figure 5. Acid scarification with sulphuric acid (H_2SO_4) treatment.

Figure 6. Mechanical scarification treatment.

Figure 7. Soaking in distilled water treatment.

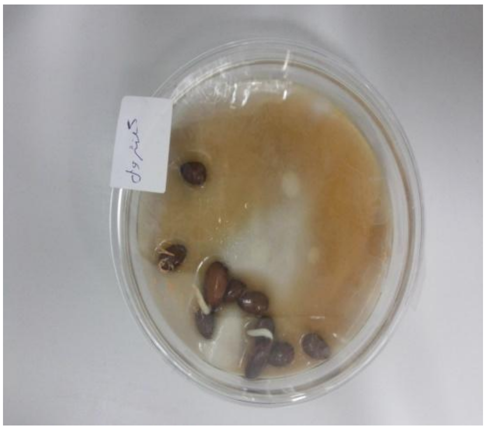

Figure 8. Control (untreated seeds).

IV. Discussion

C. siliqua is known with very hard and impermeable seed coats that obstruct water absorption, therefore delaying germination[27, 28]. Without any pre-sowing treatment, the germination percentage in carob seeds rarely exceeds 10% [10]. Based on our results, it appears that the application of numerous dormancy-breaking treatments enhanced the seed germination of carob seeds. Germination percentage was only 5.99% in untreated seeds (control). This is in agreement with data obtained by [29, 16].

In this study, along with the traditional pre-sowing treatments, we implemented two new treatments (mechanical scarification & soaking in distilled water treatment and mechanical scarification & soaking in sulphuric acid treatment) that are a combination of two widely used treatments. Mechanical scarification treatment by itself improved germination by (70.45%) and soaking in distilled water treatment enhanced germination by only (12.78%), but when the two methods were applied together (mechanical scarification & soaking in distilled water treatment), the germination percentage was increased by (94.33%). Once more, mechanical scarification treatment by itself improved germination by (70.45%) and sulphuric acid treatment resulted in germination percentage of 90.78%, but when the two methods were used as one treatment (mechanical scarification & soaking in sulphuric acid), the germination percentage was improved by 92.44 %. Based on our findings, we decided to use mechanical scarification & soaking in distilled water treatment as the preferred method to enhance germination of our studied carob seed population. Although, there were no significant differences between this treatment and mechanical scarification & soaking in sulphuric acid treatment, we tried to avoid risky substances such as sulphuric acid in this case.

The positive reaction of soaking seeds in hot water (resulted in germination percentage of 84.22%) observed in our work, as well as in other studies, for example, Taskaldimi and Ganatsas [16], is most likely owing to the species adaptation to high temperatures offered in the superficial soils of the Mediterranean area. In addition, fire is a natural fraction of the sclerophyll woodlands where this species emerges, seeds germinate easily after fire. It can be concluded that heat from fire made seeds permeable and therefore it enhanced the germination through breaking the physical dormancy of seeds [15].

Acid scarification or heat treatments are frequently applied when it is required to break physical dormancy in large amounts of seeds [8]. Though, seed coat scarification with boiling water (resulted in germination percentage of 84.22%) and sulphuric acid (resulted in germination percentage of 90.78%) is not only dangerous, but the embryos can get hurt as well, in addition, both treatments can destroy seeds with thin coats. In contrast, mechanical scarification (resulted in germination percentage of 70.45%) merely produces fine cracks on the seed coat and can be achieved by numerous hand machineries as scalpels, knives, files and sandpapers. Pérez-García [6] stated that mechanical scarification of carob seeds is an appropriate method to enhance germination. It could substitute other hazardous pre-sowing treatments (e.g. boiling water or sulphuric acid treatments) that have a tendency to damage embryos in seeds with thin coats. However, in our study mechanical scarification treatment did not produce the highest germination percentage compared with other treatments such as mechanical scarification and soaking in distilled water treatment.

Since the carob seeds distinguished by physical dormancy, the variations in germination ability of the seeds exposed to different pre-sowing treatments were ascribed to differences in raising the permeability of the seeds coat to water.

V. Conclusion

It is obvious that seeds of carob (*C. siliqua*) reveal a high hard seed coat physical dormancy, which can be overcome by a number of dormancy-breaking treatments. Mechanical scarification & soaking in distilled water treatment, mechanical scarification & soaking in sulphuric acid treatment appear to be the most efficient treatments for the seeds of *C. siliqua* originated from Al-Jabal Al-Akhdar area. However, it is advisable to avoid working with hazardous substances such as sulphuric acid.

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