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Physiological ecology of Carob trees "*Ceratonia silique* L." at El-Jabal Elakhdar area

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El-Gabal El-Akhdar,
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A B S T R A C T:
According to its location, geography, habitats, and its vegetation cover, El-Gabal El-Akhdar was described as one of the important ecological regions in Libya. *Ceratonia silique* L. is one of the plant species in the area and forms with other plants in the maquis plant community. Because of the ecological situation of the vegetation cover due to anthropogenic and physical factors, this plant was suggested to be in any rehabilitation programs in the future. Thus, the study of its ecological and physiological characteristics is very important. In this study fo,ur different locations in El-Gabal El-Akhdar were evaluated for carob plant population differences. Generally, no significant morphological differences were found among these populations except in highly significant leaf size. the second part of the study evaluated the germination of seeds using four pretreatments, compared to control all pretreatments significantly enhanced the germination of carob seeds, a relatively higher germination percentage was noticed in the seeds soaked in boiling water (96%).

دراسة بيئية فسيولوجية لنبات الخروب (*Ceratonia silique* L.) بمنطقة الجبل الأخضر

سالم الشطشاط و فاطمة بورزيزة

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الكلمات المفتاحية:
الجبل الأخضر ، نبات الخروب، برامج إعادة التأهيل، الغطاء النباتي، ليبيا، *Ceratonia silique* L.

المستخلص:
وصف الجبل الأخضر بناء علي موقعه وجغرافيته وموائله وغطائه النباتي بأنه من المناطق البيئية المهمة في ليبيا. يعتبر نبات الخروب *Ceratonia silique* L. أحد الأنواع النباتية في المنطقة ويشكل مع نباتات أخرى مجتمع نباتات المكاي Maquis. وبسبب الوضع البيئي للغطاء النباتي السيء الناجم عن العوامل البشرية والفيزيائية، فقد تم اقتراح هذا النبات في أي برامج إعادة تأهيل للغطاء النباتي في المستقبل. وبالتالي فإن دراسة خصائصه البيئية والفسيولوجية مهمة للغاية. في هذه الدراسة تم تقييم أربعة مواقع مختلفة في الجبل الأخضر لدراسة الاختلافات المورفولوجية لعشائر نبات الخروب. بشكل عام، لم يتم العثور على اختلافات شكلية معنوية بين هذه التجمعات باستثناء حجم الورقة التي كانت ذات دلالة إحصائية عالية. في الجزء الثاني من الدراسة تم تقييم إنبات البذور باستخدام أربع معالجات مسبقة، ومقارنةً بالشاهد فان جميع المعالجات المسبقة ساهمت وعززت بشكل كبير إنبات بذور الخروب بدرجات متفاوتة، ولوحظت نسبة إنبات أعلى نسبيًا في البذور المنقوعة في الماء المغلي (96٪).

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INTRODUCTION

The carob tree's scientific name, *C. siliqua* L., is from the Latin words siliqua, which refers to the pod's hardness and form, and the Greek word keras, which means horn. On the other hand, according to Hammer et al. (1992), the popular name comes from the Hebrew kharuv, from whence the Arabic kharrub is derived.

Ramón-Laca and Mabberley (2004) described the carob tree (*C. siliqua* L.) as a native plant of the Mediterranean Basin that grows slowly and is an evergreen sclerophyll plant. The Ceratonia species is a member of the Leguminoseae (Syn. Fabaceae) family's Caesalpiniodeae subfamily. Under ideal climatic circumstances, this species, which is primarily dioecious and an evergreen sclerophyllous tree, may reach a height of 20 m (Catarino, 1993), but more often than not, it reaches heights of 8 to 15 m (Goor and Barney, 1968; Ortiz et al., 1995). During the last few decades, Carob has been a widely common plant and it has been produced and grown extensively in around fifteen Mediterranean countries like Cyprus, Greece, Italy, Spain, Lebanon, Syria, Turkey…. etc. and the coastal regions of North Africa Egypt, Tunisia, Morocco and of course, Libya. In more recent years, it has even spread to parts of North America (Manso et al., 2010).

Carob is considered a tropical plant that has adapted well to Mediterranean conditions like soil, rainfalls and temperature. It is able to avoid water stress because of its adaptations such as deep rooting habits and xerophilous leaves (Catarino, 1993). Since ancient times, it has been grown across the majority of the Mediterranean basin, often in arid, moderate climates and soils with low content of nutrients. The ancient Greeks introduced it from its native Middle East to Greece and Italy, and the Arabs, who spread it along the coast of North Africa and farther north into Spain and Portugal, both recognized its significance value. Recent emigrant from the Mediterranean region brought it to other Mediterranean-like locations including California, Arizona, Mexico, Chile, parts of Australia, and Argentina. It was also brought by the English to South Africa and India (Battle and Tous, 1997).

Some studies (Correia and Martins-Loucao, 1994; Lo Gullo and Salleo, 1988) showed that carob can tolerate drought and this reason might be behind its widespread distribution in the Mediterranean climatic and soil conditions, which are characterized by the area. Even though this plant has been domesticated since 4000 BC, and its widespread dates from at least 2000 BC culture, but it has a long lifespan (up to 200 years).

Because of its feed and food properties, the tree can therefore be used to provide food for both humans and animals (El Kahkahi et al., 2014).

Based on what is known, only a few research have dealt with C. siliqua seed germination (Tsakaldimi and Ganatsas, 2001) and in contrast, there is a lot of conflicting information available on the methods that should be used to break the dormancy of carob seeds. Carob seeds have an extremely hard seed coat, which results in a very low germination percentage and a lengthy germination period. Therefore, Carob seeds should be chemically treated or sacrificed to increase germination in order to get a high germination rate.

This study aims to evaluate the possibility of propagation using seeds of *C. siliqu* under local Libyan environmental conditions and provide the authorities with the data to use this plant in the El-Gabal El-Akhdar area in rehabilitation programs and replacement processes of some threatened plants. In addition, to use it in sustainable development, gardening and forestation programs.

MATERIALS AND METHODS

Study location:

El-Gabal El-Akhdar mountainous region which is located in northeastern Libya extends for about 300 km along the coast and climbs reaching 881m above sea level and is characterized by a Mediterranean climate with cool rainy winter and hot dry summer (Elshatshat and Mansour, 2014). Four selected locations of El-Gabal El-Akhdar were used in this study (Table 1). The study of morphological characters was carried out during the spring of 2020 while the study of germination was conducted in summer 2021, both growth characters and germination study were carried out in the faculty of sciences laboratory at Benghazi University.

Measurements of morphological characters:
The plant material was collected from four different locations. Ten pods were measured for their mean size using a ruler (multiplied by length and diameter). The mean of seeds in 10 pods was also estimated and the mean of the seeds number was calculated in each area, the weight of 100 seeds collected from the same tree was performed three times for each location and the mean of the three weights was calculated, seeds size were calculated by multiplying the length and width of the 10 seeds and the mean was also calculated for each location, finally the sizes of 10 leaves from the same trees were also estimated for each location, the tree diameter and height were directly measured in the study area for three times for each location, the diameter was measured by measuring tape.

Table (1). Sites data of the examined carob populations information.

Locations	Longitude	Latitude	Altitude m (a.s.l)
Al byadah	32°46'20.123"N	21°45'44"	316
Tokhra	32°32'19.252"N	21° 46′ 33″	26
Wadi El-Kouf	32° 43'16.513"N	21° 30' 10"	432
Al Himadah	32° 24' 37.149" N	20° 36' 38"	335

Carob seed Material:
Ripe fruits (pods) containing mature seeds of *C. siliqua* were collected from their natural habitat (study locations). Seeds were randomly selected and cleaned manually, placed and subsequently stored under laboratory conditions (22 – 25C°) using paper bags until they were used. To test seed viability, seeds were soaked in water and fluted seeds were excluded. After that, 95% ethanol was used to clean and disinfect the surfaces of the experiment place. To prevent microbial growth on seeds during the germination process, Seeds were immersed in 70% ethanol for 1 minute and then rinsed 4 to 5 times in sterilized distilled water.

Pre-treatments of seeds:
A. Soaking in tap Water: Seeds were soaked in distilled water by using 3:1 volumes of water to seeds, at laboratory temperature conditions (approximately 22C˚) for 72 h. The water was daily changed and new water was added.
B. Hot Water treatment (100 C˚): 3 volumes of boiling water for each volume of seeds were used. after immersed seeds in the water, they left for 24 h to cool at laboratory temperature (Pérez-García, 2009).
C. Acid scarification with Sulfuric acid (H_2SO_4): Concentrated sulfuric acid (96%) was used to soak seed individually for 5 minutes, 10 minutes and 15 minutes (3:1 volumes of acid to seeds, respectively). Before being examined for germination, the seeds were rinsed in running water for an hour to eliminate any remaining acid (Pérez-Garca, 2009).
D. Mechanical scarification: Chipping was achieved using a rough paper to carefully remove the seed coat, without damaging the radical, at the radical end of the seed (Pérez-García, 2009).
E. Control: untreated seeds were left and allowed to germinate without any treatment.
After these previous treatments, seeds were allowed to germinate according to available *in vitro* seed germination tests found in previous literature.

Germination Procedure:
Under aseptic conditions, 5 seeds of karob were platted on sterile 9 cm Petri dishes lined with whatmann filter paper and moistened with 10 ml of water. Petri dishes were incubated in the dark at 22±0.5°C, this process was in 5 replicates for each pre-sowing treatment with a total number of 25 seeds. Plates were watered as needed with 10 ml of water and allowed to germinate. since carob seeds take a long time to germinate, seeds were allowed to germinate for 21 days. Germinated seeds were counted daily for the calculations of daily and final germination percentages (g%) and mean germination time (MGT). seeds considered germinated when the radical had protruded 5 mm.

Measurements of germination experiment:
The Germination percentage was calculated according to (ISTA, 1999) using the following formula:

$$(\textbf{Number of germinated seeds} / \textbf{Number of total seeds}) \times \mathbf{100}$$

The mean germination time (MGT) was calculated according to (Ranal and Santana, 2006) by the expression of:

MGT = Σniti/Σni

Where ni is the number of seeds that germinated per day, while it reveals the incubation period of seeds (days).

Statistical analysis:
Variables were displayed as means and standard deviation. The statistical analysis was performed using SPSS (Statistical Package for Social Sciences, version 26). The first part of the study was analyzed by one-way analysis of variance (ANOVA) to find out if there were statistical differences in the mean of individual morphological parameters of carob plant in different four locations, Pearson correlation was used to correlate pod size and other morphological parameters of carob. the second part of the study was evaluated by an independent sample test to find out the differences in the means of germination percentage for each pre-sowing treatment and control, one way ANOVA was used to compare these pretreatments with each other LSD was performed for further statistical analysis.

RESULTS

Evaluation of morphological characters of Libyan carob: Carob

Pod size (cm^2): As shown in Table (2), the pod size of the Libyan carob is ranged between (11.26 – 23.12 cm^2). The mean pod size in the second location was (16.9 cm^2), while the smallest size was noticed in the fourth study location (mean 14.25 cm^2), with no significant differences in the mean of the volume of carob pods in all the study locations (p-value > 0.05).

Weight of 100 carob seeds: Seeds weights were found to range between (12-18.47g), increased weight of carob seeds was found in the first location, lighter carob seeds were noticed in the fourth location (mean = 15.164g), as described in the table (2). one-way ANOVA test of variance means showed no significant differences in the mean of the four locations of study (p-value > 0.05).

Seeds number per pod: The range of seeds numbers ranged between (5-13 seeds per pod), higher mean number of seeds were noticed in the first and second location (mean = 9.6 and 9.8) respectively, while the third and fourth locations showed relatively lower number of seeds (mean = 7.5 and 7.8) respectively, table (2) representing the number and meaning of the seeds per pods. but generally, no significant differences were noticed in the means of the seed numbers according to the one-way ANOVA test (p-value > 0.05).

Leaves area: The range of leaves size measurements was (3-10.26 cm^2), Carob leaves in Wadi El-Kouf location showed increased size (mean = 8.87 cm^2) compared with other locations, followed by Al Himadah (mean = 8.24 cm^2) and Albyadah (mean=7.44 cm^2), but small leaves were found in Tukhra (mean = 4.56 cm^2). The differences in the mean of the leaf size in the different four locations was significant according to the one-way ANOVA test (p-value <0.001), LSD multiple comparison were performed, and the main differences were coming from the first location Tukrah as shown in the table (2).

Seeds size (cm^2): Ten Carob seeds were individually measured for their dimensions, and the mean of the size of these seeds was calculated, higher mean was noticed in the first location (mean = 0.547), the other three locations showed very comparable means (0.452, 0.489 and 0.406), as shown in the table (2), one-way ANOVA test appeared no significant differences in the mean of the size in the four locations (p-value < 0.05).

Carob trees width: The means of the trunks width were compared, apparently the trees in the fourth location showed wider trunks compared with the other locations (mean =87.8 cm), followed by the carob trees trunks in the first and second locations (means = 81.6 and 84.8 cm) respectively, but relatively narrower trunks of carob trees were noticed in the third location (mean = 67.6 cm). No significant differences in the mean of the trunks in these four locations were found according to the one-way ANOVA test (p- p-value>0.05 Table 2).

Carob trees height (m): Generally, the Carob height ranges are between (12.2-16.8 m), the mean of these measurements was calculated and compared and the higher carob trees were found in the first location (mean = 15.07 m) followed by the third location (mean = 14.9 m) the means of height were very comparable in the other two locations (means = 14.3 and 14.03 m). The differences in the means of these measurements were not significant (p-value> 0.05).

Correlating morphological characters of Carob in the four locations:

In this part of the calculations, the interrelations of each morphological parameter were evaluated according to a multi-correlation test. No significant correlations were found between all growth parameters, as represented in Table (2).

Table (2). Evaluating morphological parameters relations of Libyan Carob in El-Jabal El-Akhdar.

Parameters		**Pods size**	**No. of seeds**	**Leaves areas**	**Seeds sizes**
Pods size	Correlation	-	0.088	0.192	-0.169
	Sig.	-	0.302	0.128	0.159
No. of seeds	Correlation	0.088	-	0.01	-0.005
	Sig.	0.302	-	0.477	0.489
Leaves area	Correlation	0.192	0.01	-	0.026
	Sig.	0.128	0.477	-	0.439
Seeds size	Correlation	-0.169	-0.005	0.026	-
	Sig.	0.159	0.489	0.439	-

Germination experiment results:

The germination treatment with boiling water was evaluated, and 24 seeds (96%) were germinated starting from the fifth day. carob seeds treated with mechanical scarification revealed only 10 seeds of 25 (40%) were germinated starting from the fifth day, the results were higher than the control. In Sulphuric acid treatment**,** the germination percentages were (32%), (40%) and (60%) for 5, 10, and 15 minutes, respectively. Treatment with regular tap water showed no germination during the period of the experiment and 0% germination was recorded (Figure1).

Comparing the results of carob seed germination in all treatments showed that a relatively higher germination percentage was noticed in the seeds treated with boiling water (96%), followed by carob seeds treated with the acid for 15 minute (60%) germination, the mechanical scarification and sulphuric acid for 10 minutes revealed similar germination percentages(40%), and the seeds treated with sulphuric acid for 5 minutes gave relatively the smaller germination percentage (32%), while the treatment with regular tap water appeared zero germination as shown in the figure (1).

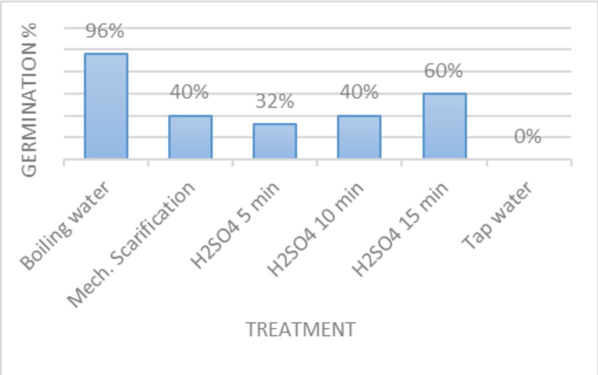

Figure (1). seed germination percentage (%) of *C. silique* seeds under different treatments.

The rate of germination was plotted vs time, and faster germination was noticed in sulfuric acid 15 minutes' pretreatment (4 days) which reached maximum germination by the seventh day of incubation, followed by seeds pretreated with boiling water (5 days) that reached the maximum germination by the seventh day of incubation. sulphuric acid 10 minutes' pretreatment also started to germinate in (5 days) but delayed final germination to the tenth day of incubation was noticed, seeds pretreated with sulphuric acid 5 minutes started to germinated during (6 days) also delayed final germination to the ninth was noticed, mechanical scarification showed slower rate (10 days) and the final germination was delayed to 14 days (Figure 2).

DISCUSSION

Carob (*C. siliqua* L.) has recently become an important tree in environmental and economic fields. The carob tree can reach more than 100 years of its life period and mostly starts fruiting from 6 to 7 years after planting as seed, and for this, it can be classified as a slow-growing species. According to increasing the importance of carob plants in industries and using the plant in horticultural and ecological fields, Consequently, there is a greater need for carob trees to be produced. The Carob tree serves as an ornamental tree and roadside tree as the tree helps protect soil from erosion and sand storms. Carob tree seeds with a hard coat and it is impermeable to water. In addition to that, carob tree seeds pass through some physiological dormancy based on the environmental conditions where the tree lives. APAT (2003) reported that average germination of carob seeds was 60-95% after different treatments. Without any treatment, the germination percentage rarely exceeds 10%. While the natural germination of the seeds is lower than 10% (Piotto and Di Noi, 2003).

According to its habitat, morphological and physiological characteristics and adaptations, this tree is one of the hardest plant species to reproduce using seeds or another method of propagation, this study looked at and investigated treatments that improve seed germination of carob trees.

Evaluation of morphological characters of Libyan carob in four locations:

The first part of this study was done to assess the morphological differences of carob population diversity in 4 different locations in the El-Gabal El-Akhdar region in Libya, we use 5 characters of carob (pods, seeds number, weight, leaves sizes and seed sizes) to evaluate the morphological differences. Several researchers (Marakis et al., 1988; Tous et al., 1996; Battle and Tous, 1997; Gharnit et al., 2001) used pod features to identify, label, or define various carob populations, collections, or germplasms. Additionally, morphological features can be utilized as quantitative markers to separate carob populations based on productivity, disease resistance, and susceptibility to environmental stress (Konaté et al., 2007).

The results of this study revealed that the Libyan carob's pod size ranges from 14 to 26.5 cm, which is regarded to be medium. This finding is agreed with Battle and Tous (1997), who categorized pod sizes into three lengths: long, medium, and short pods, with an average pod size that may range from 10 to 30 cm. The pod sizes were compared in the four locations and no significant differences in the mean of the volume of carob pods in all the study locations. Highly significant differences (p-value = 0.00) were found in Carob leaves in the four locations Carob leaves, the Wadi El-Kouf location showed increased size (mean = 19.3) compared with other locations, followed by Alhmeda (mean = 18.3), then Albyadah (mean=16.27), but small leaves were found in Tukrah (mean = 10.13). Other morphological parameters like seed weight, pod yield (number of seeds per pod), seed size, tree height and diameter showed no significant differences among the four study locations.

Libyan carob showed no significant differences between the studied locations for any of the evaluated characters except leaf sizes. Contrary to our findings, numerous authors have established that carob exhibits high diversity in morphological parameters in populations from Libya (Ali et al., 2019), Spain (Albanell et al., 1991), Italy, Portugal (Barracosa et al., 2007), Morocco (Konaté et al., 2007), and Tunisia (Naghmouchi et al., 2009).

Correlation of morphological characters of Libyan carob:

The morphological features of the Libyan carob exhibited a positive or negative association with one another, according to the Pearson coefficient correlation analysis. On the other hand, outside of seed size at the Albyadah site, there is no link between variables defining the pods and those describing the seeds. Numerous writers have highlighted the substantial relationships that were discovered among the parameters describing the pod, which are in agreement with our findings (El Kahkahi et al., 2014; Elfazazi et al., 2017; Sreec et al., 2016; Ali et al., 2019).

Germination experiment results:

Piotto and Di Noi, (2003) reported that the natural germination of the seeds of carob is lower than 10%. Since the tree is growing naturally in many areas of the Mediterranean, sometimes farm animals eat seeds and pods of this plant, this leads to exposure to acids and digestion enzymes in the stomach of those animals, and subsequently, they grow naturally better. This fact led to apply some artificial methods that can improve seed development. Those treatments include mechanical scarification, soaking in hot/cold water, and soaking in mineral acids such as hydrochloric, sulfuric, nitric, and phosphoric acid.

The results have shown that the germination of control seeds was significantly lower than that of mechanically treated ones (Figure 1). Piotto and Di Noi (2003) got the same results. Many studies (Baskin and Baskin, 1998, 2004; Pérez-García *et al.*, 2008), reported that the hard coat which covers the seeds in many *Fabaceae* plants is behind the physical dormancy of seed germination. Lowering water uptake due to the impermeable coat in carob seed plays a major role in its germination, thus, the coat must be partially removed or damaged and this leads to germination enhancement and subsequently, increasing (Piotto and Piccini, 1996).

In this study, the treatments increased the seed coat permeability to different degrees which caused higher imbibition in carob compared with control treatments. results of carob seed germination in the pre-sowing treatments (Figure 1), showed that a higher germination percentage was noticed in the seeds soaked in boiling water 96%, followed by carob seeds treated with sulphuric acid for 15 minutes which showed 60% germination, the mechanical scarification and sulphuric acid for 10 minutes showed similar germination percentages 40%, the seeds treated with sulphuric acid for 5 minutes showed relatively the smaller germination percentage 32%, while the treatment with regular tap water showed zero germination.

These results are in agreement with another study that found carob seed germination improved to 95.69% by mechanical scarification and making some cracks on the seed coat after concentrated sulfuric acid treatment. Perez-Garcia (2009) examined a variety of treatments to improve seed germination in carob and discovered that scarified seeds had the greatest germination rates (99%), while control seeds had the lowest rates (25%) of germination. Similar to this, Tsakaldimi & and Ganatsas (2001) reported that seeds soaked in sulfuric acid for 15 minutes had the highest germination rate (87%). Additionally, Gunes et al. (2013) found that sulfuric acid treatment combined with mechanical scarification gave the best results in the percentage of germination.

In germination, water passes through the seed coat and starts swelling, the metabolites in the seed change into soluble materials and hydrolytic reactions occur in addition to gas exchange. When the seed coat is not permeable to water due to physiological or natural dormancy, water cannot go into the seed and seed germination will not occur. This phenomenon is normal in the plant life cycle to preserve the species in the life cycle or overcome some unfavourable environmental conditions. Many chemical, physical, and mechanical methods are applied to do so on dormant seeds. In our experiment, the results are in agreement with what was reported on the hard seed coat of Carob (Martins-Loução,1996) and some other plant species (NAS, 1980, Abdullah *et al*., 2019).

Mean germination time:

faster germination was recorded by seeds pre-treated with sulphuric acid for 15 minutes, seeds started to germinate by the fourth day of incubation with mean germination time (MGT 5.125) started with 32% germination in the fourth day and the final germination was recorded in the seventh day (60%), germination recorded by carob seeds pre-treated with boiling water started to germinate in the fifth day of incubation (28%) with mean germination time

(MGT 5.79), the final germination was achieved in seventh day of incubation (96%), seeds pre-treated with sulphuric acid 10 minutes started to germinated in the fifth day of incubation by (16%) with mean germination time (MGT6.9) the final germination was recorded in the tenth day (40%), seeds pre-treated with sulphuric acid 5 minutes and mechanical scarification showed somewhat delay in germination (6 days , 10 days) respectively, mean germination times (MGTs 7.8 and 12.5) respectively with higher final germination for the mechanical scarification treatment (Figure 2).

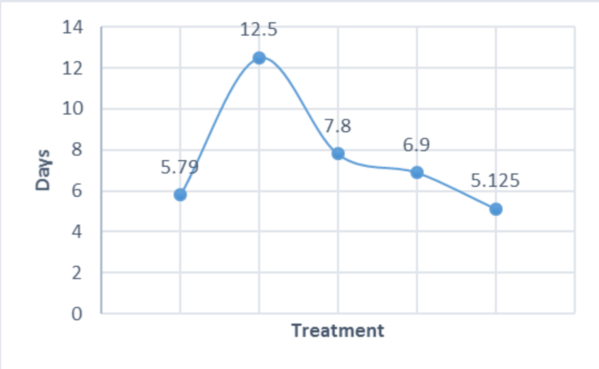

Figure (2) The mean germination time (MGT) of different treatments. From left to right, boiling water, mechanical scarification, and H2So4 for 5, 10 and 15 minutes respectively.

Few authors evaluated the mean germination time in carob seeds, Gunes et al., (2013) found that the greatest rate of germination percentage was achieved using a company mechanical method (scarification and sulfuric acid treatment), while using scarification only presented the shortest mean germination time. Additionally, from the same study, Gunes et al. (2013) found that the germination rate of seeds treated with boiling water increased to 90% after five days of incubation, compared to 42.5% for seeds treated with concentrated sulfuric acid and 12.5% for the control. For seeds soaked in sulfuric acid, the germination rate gradually increased over the course of twelve days of incubation, reaching a maximum value of 100%, compared to 80% for seeds soaked in boiling water and 22.5% for seeds soaked in sterile distilled water (El Kahkahi et al., 2014).

CONCLUSION

Generally, no significant morphological differences were found among carob populations except in highly significant leaf size. Compared to the control, all pre-treatments significantly increased the seed germination, a relatively higher germination percentage was noticed in the seeds soaked in boiling water 96%, followed by other carob seeds treated with different treatments. Using boiling water in seed germination is more useful because of its simplicity, safety, cheapness and faster than the other methods. Thus, we highly recommend using this method in propagation by seeds. More studies about seedling development, especially in its natural habitat must be taken into account.

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