

## **IMPACT OF ACID SCARIFICATION AND COLD MIST STRATIFICATION ON ENHANCING SEED GERMINATION AND SEEDLING EARLY GROWTH OF *Albizia lebbek* (L.) Benth.**

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### **ABSTRACT**

The thick, hard and water-impermeable seed coat cause physical dormancy and low germination rate in *Albizia lebbek* (L.), to overcome this physical dormancy, seeds were pretreated with concentrated HCl and cold mist condition. Immersing seeds in concentrated HCl acid 37% for 30 and 45min effectively raised seed germination rate (35.56% and 46.67%) respectively as compared to control treatment (15.56%). Cold mist storage at 5°C for 4 and 8 weeks also significantly increased germination percentage (51.11% and 68.89%) respectively. Furthermore, the combination of cold mist stratification for 8 weeks and immersion in HCl for 15min boost the germination rate to 75.56% an increase of 59.90% comparing to control treatment. For seedling parameters, all treatments have positive effects on the studied characters highest seedling height (37cm) and number of leaves per plant (18 leaves) where found in, 8 weeks cold mist condition, however, the combination of 15min acid scarification and 8 week cold mist stratification resulted in higher leaves area (207.52). Acid scarification and cold mist stratification both increased germinations and improved seedling features, but germination rates were highest and fastest when these treatments were applied together. The results suggest that to enhance seedling qualities, seed germination rate and speed, a combination of scarification and stratification should be used.

**Keywords:** *Albizia lebbek*, seeds, acid scarification, cold mist stratification.

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### **INTRODUCTION**

*Albizia lebbek* L. is a deciduous fast growing tree, attain an average maximum height of 8 to 15m, with a thin spread crown, native to tropical Asia, belongs to Mimosaceae family, the leaves are bipinnate, 7.5–15cm long with 1 to 4 pairs of pinnae, each pinna with 6–18 leaflets. The flowers are very fragrant; it has a long flat and oblong seed pods, 3-12 seeds per pod, brown and flattened (Warrier, 2010). *A. lebbek* (L.) multi-purpose tree has the ability to fix atmospheric nitrogen that improves the chemical structure of the soil, drought resistant, and provides shade (Faisal *et al.*, 2012)

The tree has been suggested as an ornamental and plantation tree throughout the tropics (Parrotta, 2002), used in home gardens, parks and roadsides. It grows in a wide range of climates including an annual rainfall range of 600- 2500 mm. However, it also grows successfully in areas with annual rainfall as low as 400 mm. while the species is adapted to different soil types, from acid soils to alkaline and saline soils, the tree grows best on well-drained, moist soils (Prinsen, 1986).

The seeds of this family have impermeable seed coat (Al-Menaie *et al.*, 2010). The thick hard seed coat and impermeable to water caused to physical dormancy which due to irregular, slow germination and low germination rate (Nongrum and Kharlukhi, 2013; Jackson, 1994a; Baskin and Baskin, 2004). Physical dormancy could be overcome through many ways, such as stratification at cold temperature storage that effectively break seed dormancy and enhance germination and increase its rate to more than 80% (Pipinis *et al.* 2009; Roh *et al.* 2004). As well as acid scarification treatment of species with hard seed coats is known to be highly effective in improving induce germination (Youssef, 2008; Kobmoo and Hellum, 1984).

This study aims to determine the effectiveness of acid (HCl) scarification and cold mist stratification on breaking down the dormancy and increasing germination rate of *Albizia lebbek* (L.) tree seeds.

## MATERIALS AND METHODS

### Seed collection and processing

Mature pods were collected on November 2018 from low branches of *Albizia lebbek* (L.) trees by hand, in the private garden at Sitak district located at elevation 1130masl. (35°38'N and 45°30'E) about 25km northeast of Sulaimani city. Then, seeds were extracted manually from the pods, after removing all abnormal seeds, the cleaned seeds packed in plastic bags and stored in a dark place at room temperature till sowing time.

### Experiment site

The experiment was performed during the period (20<sup>th</sup>, February to the end of May) 2018 in the lath house of Horticulture Department, College of Agricultural Sciences, University of Sulaimani at Bakrajo district 10km Westside of Sulaimani city, which is located at elevation 743masl. (35°32'N and 45°21'E).

### Preparation of growing medium

The growing medium was prepared by mixing peat moss and river sand with the proportion (1:1) V/V, the growing medium was distributed to 540 plastic pots with (6×8) cm.

### Treatment and experimental design

The experiment included study the effect of two factors on seed germination and seedling early growth.

The combination treatment comprised of two factors:

**Factor 1: Acid scarification:** Seeds were immersed in HCl concentrations %37 for 15, 30 and 45 minutes in addition to control (without immersion) in beakers, then the acid was discharged, and seeds were repeatedly rinsed in running tap water followed by distilled water until considered safe to handle. Then seeds were sown in plastic pots.

**Factor 2: Cold mist stratification:** Seeds were stored in plastic bags mixed with wet construction sand, closed tightly and stored for 4 and 8 weeks in a refrigerator at 5°C, and monitored weekly to maintain the sand moist.

The treated seeds of each treatment were seeded in plastic pots (6×8) cm filled with growing medium singly and in combination on 20<sup>th</sup> February 2018. The

experiment was applied as a factorial experiment according to randomized complete block design (RCBD) under lath house condition with total 12 combination treatments, each treatment was replicated three times, using 15 pots of each replication seeded by one seed.

The combination treatments were denoted as follows:

**T1:** Control (without treatments).

**T2:** Seeds immersed in 37% concentration of HCl acid for 15min.

**T3:** Seeds immersed in 37% concentration of HCl acid for 30min.

**T4:** Seeds immersed in 37% concentration of HCl acid for 45min.

**T5:** Cold mist storage at 5°C for 4 weeks.

**T6:** Cold mist storage at 5°C for 8 weeks.

**T7:** Cold mist storage at 5°C for 4 weeks with immersion in 37% concentration of HCl acid for 15min.

**T8:** Cold mist storage at 5°C for 4 weeks with immersion in 37% concentration of HCl acid for 30min.

**T9:** Cold mist storage at 5°C for 4 weeks with immersion in 37% concentration of HCl acid for 45min.

**T10:** Cold mist storage at 5°C for 8 weeks with immersion in 37% concentration of HCl acid for 15min.

**T11:** Cold mist storage at 5°C for 8 weeks with immersion in 37% concentration of HCl acid for 30min.

**T12:** Cold mist storage at 5°C for 8 weeks with immersion in 37% concentration of HCl acid for 45min.

During the experiment period, watering and weeding processing were performed regularly as needed. The germination process was monitored every day, and germinated seeds were counted for 12 weeks from appearing first germinated seed on 6<sup>th</sup>, March 2018 until the end of May 2018.

#### **Parameters measurement:**

At the end of experiment the following parameters were measured.

##### **1- Germination parameters:**

- Germination %, calculated by using the following formula:

Germination % = Number of germinated seeds / Total number of seeds × 100  
ISTA (1999).

- Speed of Emergence Index, and calculated by the following formula:

Speed of germination =  $n_1/d_1 + n_2/d_2 + n_3/d_3 + \dots$  Czabator, F. J.  
(1962).

Where, n = number of germinated seeds, d= number of days.

- Time request for 50% of germinated seeds (days).

##### **2- Seedling Growth Parameters:**

Growth parameters such as shoot and taproot length, number of leaves and leave areas were measured at the end of May 2018. Shoot height (cm) was measured by the ruler. Taproot length (cm) also measured by the ruler. Leaves area (cm<sup>2</sup>) measured by using (Digimizer image analysis) software program application ([https:// www. digimizer.com/](https://www.digimizer.com/)); downloaded on the personal computer, and based on image analysis by determining the dark spots at the images of the leaves.

#### **Statistical analysis:**

Data obtained were subjected to analysis of variance (ANOVA) by using XLSTAT 2016 data analysis software program (<https://www.xlstat.com>) with Duncan's Multiple Range Test (DMRT)  $P \leq 0.05$ , to compare the differences between treatment means.

## RESULTS

### 1- Germination parameters (Table 1):

Scarification by HCl acid and increasing immersion time from 0.0 minute (control) to 15, 30 and 45 minutes significantly increased germination percentage of *Albizia lebbeck* (L.) seeds from (15.56%) to (26.67%), (35.56%) and (46.67%) respectively.

Cold mist stratification of *Albizia lebbeck* (L.) seeds significantly increased germination percentage; from only (15.56%), to (51.11%) for cold mist stratification at 5°C for 4 weeks and most significantly in 8 weeks stratification (68.89%),

The combination interaction effects of acid scarification and cold mist stratification revealed that treating seeds with cold mist stratification at 5°C for 8 weeks followed by acid scarification for 15minutes delivered highest germination percentage (75.56%) amongst all the treatments, also stratification at 5°C for 4 weeks followed by scarification for 15 and 30minutes resulted in (53.33%) and 60% germination respectively. Moreover, increasing emersion time in HCl acid to 30 and 45 minutes in stratification for 8 weeks, and immersing in concentrated HCl acid for 45minuts and 4 weeks stratification; the germination percentage reduced to (51.11%), (44.44%) and (55.56%).

The results also revealed that HCl scarification accelerates the speed of germination significantly and decrease the time (day) to 50% of seed germination of *Albizia lebbeck* (L.) from 84 days for the control to 70 days for immersed in HCl acid for 45 minutes. While the average of 50% of seed germination time varied to 55 and 50 days when stratified at 5°C for 4 and 8 weeks respectively. The shortest time was 49 days in which seeds stored 8 weeks at 5°C and followed by immersion in concentrated HCl acid for 15 minutes.

Table (1): Mean comparison of different treatments of HCl acid scarification, cold mist stratification and interaction between them on germination percentage, germination speed index and time to 50% germination (day) of *Albizia lebbeck* (L.).

| Treatments | Germination % | Germination speed index | Time to 50% germination (day) |
|------------|---------------|-------------------------|-------------------------------|
| T1         | 15.56 g       | 0.08                    | 84                            |
| T2         | 26.67 fg      | 0.14                    | 77                            |
| T3         | 35.56 ef      | 0.15                    | 75                            |
| T4         | 46.67 de      | 0.19                    | 70                            |
| T5         | 51.11 cd      | 0.27                    | 55                            |
| T6         | 68.89 ab      | 0.40                    | 50                            |

|       |       |    |      |    |
|-------|-------|----|------|----|
| T7    | 53.33 | cd | 0.29 | 63 |
| T8    | 60.00 | bc | 0.30 | 54 |
| T9    | 55.56 | cd | 0.35 | 56 |
| T10   | 75.56 | a  | 0.44 | 49 |
| T11   | 51.11 | cd | 0.23 | 65 |
| T12   | 44.44 | de | 0.20 | 50 |
| Std** | 3.259 |    |      |    |

\*Values followed by the same letter did not differ significantly ( $P \leq 0.05$ ) according to Duncan's multiple range tests.

\*\* Standard error of the mean.

## 2- Seedling Growth Parameters:

The effect of different exposure of scarification period and cold mist stratification significantly ( $p \leq 0.05$ ) influenced the mean shoot length values of *Albizia lebbek* (L.) Figure 1(a) the highest value was 22.00 cm from immersing seeds for 45minutes in 37% HCl which did not differ significantly with those treated for 30min. The lowest value 13.5cm was for non-treated seeds (T1 control), and also there were no significant differences between the control and each of scarifying (T2) for 15minutes and acid scarification for 30min (T3).

Furthermore, Figure 1(b) shows that the significant influence of cold mist stratification on shoot height (cm) and the results were 24.67 and 37.00cm seedling height stratifying at 5°C for 4 weeks and 8 weeks. It can be concluded that immersion seeds in 37% HCl for 15, 30 and 45minutes and also storage of seeds at 5°C for 4 or 8 weeks can improve and invigorate the germination of *Albizia lebbek* (L.) tree seeds.

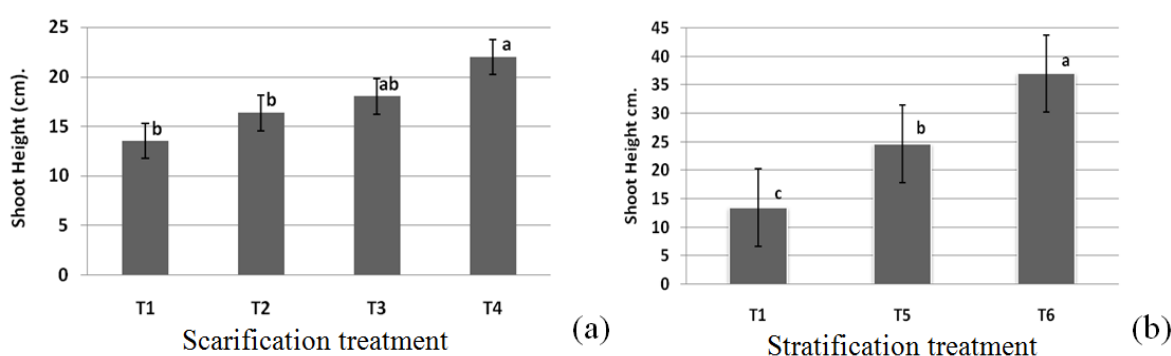


Figure (1): Effect of (a ) acid scarification and (b) cold mist stratification on shoot height (cm) of *Albizia lebbek* (L.) seedling. Means not followed by the same letter differ significantly at ( $P \leq 0.05$ ).

There were significant differences between acid scarification of *A. lebbek* seeds, which immersion in HCl acid (37%) for 30minutes enhance seedlings to tallest taproot 42.83cm, followed by 45min (33.50cm.) and 20.50 for 15min

immersion as compared to 12.33cm for control Figure 2(a). Cold mist storage at 5°C for 4 weeks and 8 weeks showed significant effects on taproot length cm, which were 27.0, 23.00 and 12.33cm; for 4 weeks, 8 weeks and control respectively Figure 2(b).

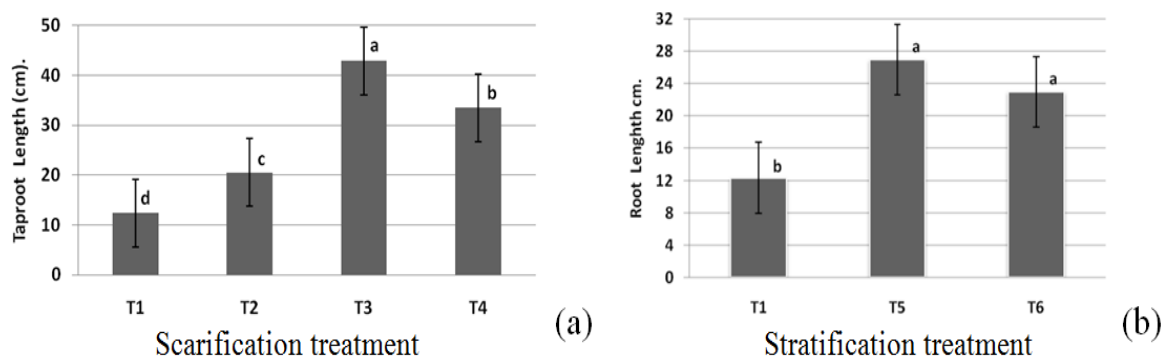


Figure (2): Effect of (a) acid scarification and (b) cold mist stratification on taproot length (cm) of *Albizia lebbek* (L.) seedling. Means not followed by the same letter differ significantly at ( $P \leq 0.05$ ).

Results presented in Figure 3(a;b); suggest that there are no statistical differences between acid scarification durations and cold mist stratification duration, while all treatments significantly higher than non-treated seeds.

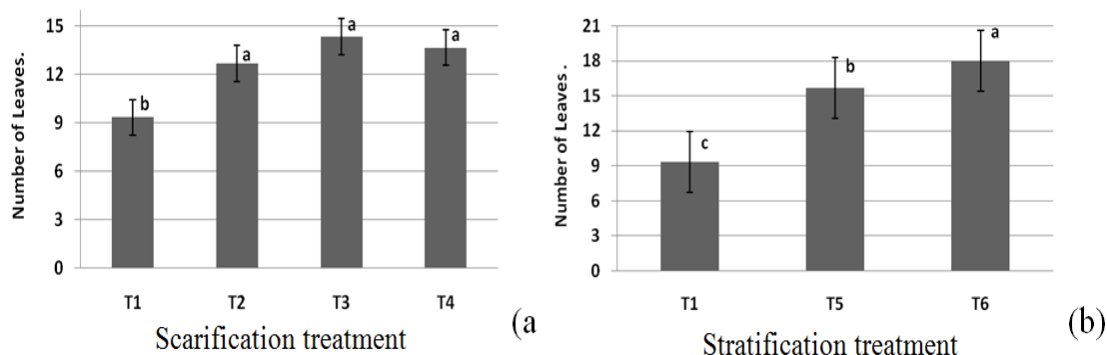


Figure (3): Effect of (a) acid scarification and (b) cold mist stratification on number of leaves of *Albizia lebbek* (L.) seedling. Means not followed by the same letter differ significantly at ( $P \leq 0.05$ ).

As presented in figure 4(a); scarified *A. lebbek* seeds in HCl acid for 30 and 45min significantly had more leaf area than non-scarified seeds, the highest mean value were recorded ( $113.44\text{cm}^2$ ) from seeds pretreated with HCl (37%) acid for 30min followed by seedling originated from seeds immersed in HCl for 45min ( $61.62\text{cm}^2$ ), while the lowest mean leaf area ( $35.75\text{cm}^2$ ) obtained from untreated seeds but was not statistically different with 15min acid immersion. Cold mist stratification gave significantly higher leaves areas over the control (T1), the results were  $164.39\text{cm}^2$  for cold mist storage at 5°C for 8 weeks and  $51.61\text{cm}^2$  for storage at same condition for 4 weeks Figure 4(b).

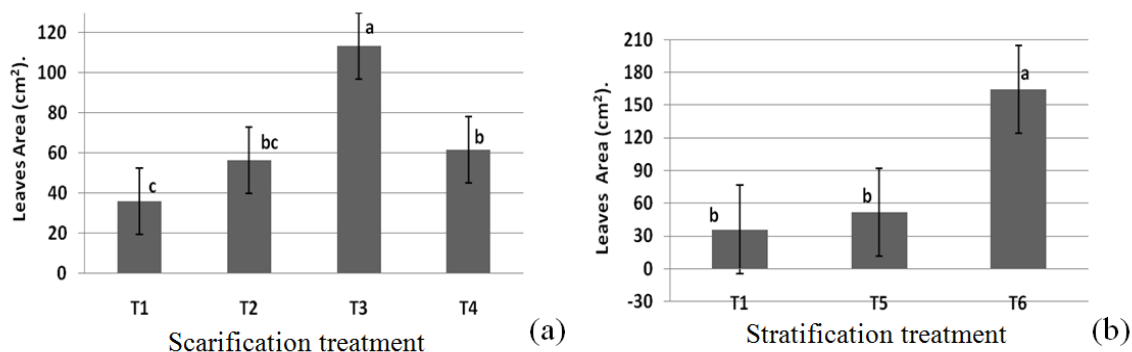


Figure (4): Effect of (a) acid scarification and (b) cold mist stratification on leaves area (cm<sup>2</sup>) of *Albizia lebbbeck* (L.) seedling. Means not followed by the same letter differ significantly at ( $P \leq 0.05$ ).

Finally, Table 2 shows how interactions of HCl acid scarification and cold mist stratification effectively act on growth parameters of *Albizia lebbbeck* (L.) seedlings. Results indicate that interaction of immersion in 37% concentration of HCl acid for 30min and cold mist storage at 5°C for 4 weeks (T8) superior in seedling height (33.17cm), root lengths (30.50cm), and number of leaves 19.00, while (T10) interaction of immersion in 37% concentration of HCl acid for 15min and cold mist storage at 5°C for 8 weeks, upper in seedling height (34.33cm) and leaves area (207.52cm<sup>2</sup>).

However, another interaction had a significant harmful impact on studied seedling characters in comparison to the other interactions the recorded values for (T12) were 20cm, 15.5cm, 9.67 and 43.54cm<sup>2</sup> for each of shoot height, root lengths, number of leaves and leaves area respectively.

Table (2): Interaction effect of HCl acid scarification and cold mist stratification on shoot height (cm), taproot lengths (cm), number of leaves and leaves area (cm<sup>2</sup>) of *Albizia lebbbeck* (L.).

| Treatments | Shoot Height cm. | Root Length cm. | No. of Leaves | Leaves Area cm <sup>2</sup> |
|------------|------------------|-----------------|---------------|-----------------------------|
| T1         | 13.50 d          | 12.33 f         | 9.33 e        | 35.75 d                     |
| T7         | 24.17 bc         | 23.50 b         | 16.33 bc      | 118.73 c                    |
| T8         | 33.17 a          | 30.50 a         | 19.00 a       | 162.56 b                    |
| T9         | 29.00 ab         | 19.00 d         | 15.33 c       | 53.61 d                     |
| T10        | 34.33 a          | 21.50 c         | 16.67 b       | 207.52 a                    |
| T11        | 25.50 bc         | 19.50 d         | 13.00 d       | 47.7 d                      |
| T12        | 20.00 c          | 15.50 e         | 9.67 e        | 43.54 d                     |
| Std**      | 2.060            | 0.500           | 0.380         | 8.721                       |

\* Mean values in a column followed by the same letter did not differ significantly ( $P \leq 0.05$ ) according to Duncan's multiple range tests.

\*\* Standard error of the means.

## DISCUSSION

### 1- Germination parameters:

Scarified seeds of *Albizia lebbek* (L.) by HCl acid, and increasing immersion time to 45min significantly increased germination percentage to 46.67%. This increasing of germination rate also has been reported by other researchers (Abubakar and Maimuna, 2013; Babashpour and Sharifivash, 2010; Noorafkan and Khoshkhui, 2005; Gizachew and Scarisbrick, 1999). The effect of concentrated HCl on stimulation of seed germination might be due to that the acid may soften hard seed coats and allowing easier water uptake and oxygen diffusion resulting in enzymatic hydrolysis and hence transforming the embryo into a seedling (Olatunji *et al.*, 2012; Alamgir and Hossain, 2005).

Cold mist stratification of *Albizia lebbek* (L.) seeds also significantly increased germination percentage 68.89%; the results reported in this study agreed with those in literature (Gebre and Karam, 2004; Agrawal, 1995; Hartmann *et al.*, 1997), in which that stratification is necessary after embryo development when seed dormancy due to embryo factor.

The combination interaction effects of acid scarification and cold mist stratification revealed that treating seeds with cold mist stratification at 5°C followed by acid scarification delivered high germination percentage 75.56%; this might be due to that scarification and stratification together soften the hard seed coat which leads to allowing greater water absorption, gaseous exchange and finally enhances germination significantly. The previous data is in agreement with reports of Liu *et al.* (1981); Jones and Geneva (1995); Hamilton and Carpenter (1975), who worked on seeds of *Cercis* spp. Also, Harrie and Erick (1998) have reported that scarification or stratification of *Acacia senegal* (L.) seeds before sowing would produce the best germination result. Seeds could not sustain the shock of longtime immersion in concentrated HCl acid that may cause damage to the embryo and as at the results significantly decreased germination percentage to 44.44%.

Accelerating germination from 84 days to 70 days and decreasing the time (day) to 50% of seed germination of *Albizia lebbek* (L.) seeds treated with HCl acid; while the average of 50% of seed germination time varied when stratified period at 5°C differed, this indicates that impermeability and hardness of seed coat is the primary inhibitor of germination and the accelerate of germination and permits radical emergence might be due to absorbing more water after weakening seed coat structure. Where seed coat is softened, the process of hydrolysis could commence releasing simple sugars that could be readily utilized in protein synthesis, also releasing hormones such as auxins and ethylene could increase nucleic acid metabolism and protein synthesis (Irwin, 1982; Jackson, 1994b). HCL modified the seed coat that enhanced the germination process. It is evident from the present study that for optimum seed germination of *A. senegal*, the concentration of the acid should not exceed 50% (Harrie and Erick, 1998). Completion of pre-germination metabolic activities making the seed ready for soon germination compared with untreated seeds. Therefore, the rapid germination rate that was observed due to faster water uptake and earlier initiation of metabolism processes (Sedghi *et al.*, 2010; Eskandari and Kazemi, 2011).



## 2- Seedling Growth Parameters:

The effect of different exposure of scarification period and cold mist stratification significantly ( $p \leq 0.05$ ) influenced the means of all seedling growth parameters values; shoot length, root length, number of leaves and leaves area of *Albizia lebbek* (L.).

Increased seedling height also was reported by Thanuja *et al.*, (2018) in *Pterocarpus marsupium* Roxb. Adequate growth of seedlings is dependent on the ease of seed germination and thus increasing the growth potential of seedlings and improving seedling competitiveness in a shorter time compared with non-treated seeds. On the other hand, untreated seeds (control) also germinated but it took a very long period after sowing which gave a germination percentage lower and shorter height of the seedling as compared to those scarified with acid and stratified at 5°C.

Scarifying the seeds in concentrated hydrochloric acid increased imbibitions, and improved germination, seedling establishment and growth characteristics such as shoot and root length.

It can be concluded that the seedling growth rate and biomass accumulation such as leaves area cm<sup>2</sup> is dependent on the ease of seed germination.

Treatments might have softened the seed coat of very accelerating infiltration of possible enzyme activity in the endosperm. A general assessment of the results of different scarification and stratification treatments demonstrated that they affect on the early growth stages of *A. lebbek* seedlings wholly.

The exposure to acid was observed to cause the complete elimination of the integument, which possibly damaged the embryo and, as a consequence, the growth rate of different parts of the seedlings decreased, which agreed with a study by Sanabria *et al.*, (2004) on seeds of *Cratylia argentea*; that reported the seed immersion in acid did not improve germination rate with regards to the control, caused increasing a high percentage of the death. In the present study, the effect of HCl scarification and stratification at 5°C was minimal in breaking the seed coat as is reflected in poor seedling emergence and other growth parameters. Longer exposure of the seeds to HCl might have damaged the embryo. Similar views have been expressed by Aduradola & Adejomo (2005) for *Erythrophleum suaveolens* seeds. However, other factors such as the relative position of the seed on the parent plant, micro-environment, quantity of reserve food content and provenance seen in many species could be affecting on the variation in the timing, germination percentage of the seeds and also on initial growth parameters. (Gutterman, 1982; Gray & Thomas, 1982; Owoh *et al.*, 2011).

## CONCLUSIONS

To overcome the physical dormancy with obtaining best seedling growth parameters, seeds pretreated with 37% concentrated of HCl for different immersion time and cold mist storage for 4 and 8 weeks at 5°C the study found that the best pretreatment was obtained by immersion seeds in 37% concentration of HCl acid for 30min. for all growth parameters; cold mist storage at 5°C for 8 weeks for best germination percentage, shoot height (cm), number of leaves and leaves area (cm<sup>2</sup>)

and interaction effect of cold mist storage at 5°C for 8 weeks with immersion in 37% concentration of HCl acid for 15min for all germination parameters, shoot height (cm) and leaves area (cm<sup>2</sup>).

تأثير التخديش بالحامض والتنضيد البارد الرطب على تحسين إنبات البذور والنمو المبكر لشتلات الالبيزيا

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#### الخلاصة

الغلاف البذري السميك الصلب غير النافذ للماء يتسبب في السكون والانخفاض في نسبة إنبات بذور *Albizia lebeck* (L.)، و للتغلب على هذا السكون تم معاملة البذور بكل من حامض الهيدروكلوريك HCl والخزن البارد الرطب. حيث غمر البذور بحامض الهيدروكلوريك HCl بتركيز 37% لمدة 30 و 45 دقيقة رفعت نسبة إنبات البذور إلى (35.56% و 46.67%) على التوالي، حيث البذور غير المعاملة كانت (15.56%). كما وان الخزن البارد الرطب للبذور عند 5° مئوية لمدة 4 و 8 أسابيع أدى إلى زيادة معنوية لنسبة الإنبات لـ (51.11% و 68.89%) على التوالي. كذلك فإن التداخل او الجمع بين الخزن البارد الرطب لمدة 8 أسابيع والغمر في حامض الهيدروكلوريك لمدة 15 دقيقة عزز نسبة الإنبات إلى 75.56% بزيادة قدرها 60% مقارنة بالبذور غير المعاملة. بالنسبة للصفات المدروسة للشتلات، فإن جميع المعاملات كانت لها تأثيرات إيجابية على الصفات المدروسة؛ فوجد أعلى ارتفاع للشتلات (37 سم) وعدد الأوراق / نبات (18 ورقة) في الخزن البارد الرطب البذور لمدة 8 اسابيع، اضافة الى ذلك فإن الجمع بين غمر البذور لمدة 15 دقيقة والخزن البارد الرطب لمدة 8 أسابيع أدى الى زيادة في المساحة الورقية لـ (207.52 سم<sup>2</sup>). وقد أدى كل من التخديش بالحامض والتنضيد البارد الرطب إلى زيادة نسبة الإنبات وتحسين الصفات الظاهرية الشتلات، كذلك معدلات الإنبات كانت أعلى وأسرع عندما تم استخدام هذه العلامات معاً. تشير النتائج إلى أنه من أجل تحسين صفات الشتلات وزيادة معدل إنبات البذور وسرعتها، عليه تطبيق مزيج من التخديش والتنضيد معاً.

كلمات دالة: *Albizia lebeck*، بذور، التخديش بالحامض، التنضيد البارد الرطب.

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