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Research Article
The Effect of Seeds Age and Chilling on Water Imbibition and Germination for *Ziziphus spina-christi* Seeds

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Abstract

Background and Objective: Sidr honey is considered one of the most valuable resources that could improve the economy of Yemen. Therefore increasing the number of *Ziziphus spina-christi* (Sidr) trees becomes a priority. In this study, the percentage and speed of germination, as well as water imbibition, were checked for *Ziziphus spina-christi* seeds of 2017 and 2018 production. **Materials and Methods:** The seeds were obtained from the Doan Honey Bee Center, Hadhramout, Yemen. Four treatments (immersing in water, chilling, immersing first then chilling and chilling first then immersing) along with water control were applied to the seeds. The immersing time was kept to 24 hrs. Also, the temperature and time of the chilling process were 4°C and 4 hrs, respectively. **Results:** The seeds of 2018 significantly outperformed the seeds of 2017 in terms of water imbibition ability. The largest amount of adsorbed water was noticed for seeds treated by chilling first and then immersing. On the other hand, the lowest amount of adsorbed water was observed when seeds were treated by immersing first and then chilling. **Conclusion:** The expected promising results in the water imbibition and seed germination will be obtained if old seeds are chilled first and then immersed in water.

Key words: Chilling, immersing, seeds, seeds age, *Ziziphus spina-christi*, deep-rooted, viability

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ziziphus spina-christi tree, also known as Sidr (or Seder) or Nabq, belongs to the Rhamnaceae family[1]. The tree is medium to large and growing rapidly. It is deep-rooted and long-lived[2]. The leaves of *Z. spina-christi* are evergreen, simple oval-cut and rarely opposite[3]. The flowers are small axillary and unnoticeable or unisexual (or polygamous) with 4-5 single sepals[4]. The fruits of *Z. spina-christi* are small to large and take many forms[5]. Moreover, *Z. spina-christi* seeds have a hard seed coat and have different shapes from circular to semi-oval to oval[6,7]. The stoned seed contains only 1-2 seeds and rarely three seeds[8-11]. When seeds fall into the field, their viability will last 12 months[6]. It was noticed that after 6 months, the germination rate of *Z. spina-christi* seed collected from the field dropped from 56 to 31 percent[12]. It was reported that storage conditions have a major impact on the percentage of seed germination[13]. Also, it was shown that storage at a low temperature of 0.5 to 4.5°C leads to longer seed viability[11]. Moreover, *Z. spina-christi* seeds can maintain their vitality when kept in a dry and cool climate for two and a half years[7]. All the available reports that studied *Z. spina-christi* tree are not enough to visualize the importance of this tree and identify the reason behind the use of tree parts in various fields. From the religious, economic, nutritional and medicinal aspects, *Z. spina-christi* plants are of great importance[14]. As far as medicinal and nutritional properties are concerned, the best honey bees in the world are made from the *Z. spina-christi* flower nectar[15]. The leaves and twigs are used as livestock fodder because of their high protein content[16]. Also, the wood of the tree is durable, insect-resistant and versatile[17]. The *Z. spina-christi* fruits are delicious and contain a good amount of complexes of vitamins C, A and B minerals, a high proportion of carbohydrates and proteins and a fair proportion of fats[18]. Also, the parts of plants are used to treat many diseases[19]. Therefore, *Z. spina-christi* trees could be considered as multiple sources to provide human beings with food and cures, provided their wide knowledge of this tree.

Ziziphus spina-christi trees can endure -10°C winter temperatures and 50°C summer temperatures. The tree could grow well at a temperature of 39-42°C[12]. When the temperature decreases, the dissolved oxygen in water increases. Therefore, a lower temperature provides the seeds with the oxygen necessary for breathing which enters with the water through the seed coat[12]. Also, it can be noted that the temperature affects directly or indirectly the vital functions of plants[20]. Also, the temperature affects the processes of diffusion, permeability, water imbibition and evaporation and all chemical processes related to food transformation. On the other side, increasing the chilling period improves the percentage and speed of germination of *Citrus sinensis* L.[21]. It was reported that there was a significant increase in germination rate when *Leucaena leucocephala* seeds were stored at 4°C before planting[20]. Because of the great benefits that the *Z. spina-christi* tree and its parts give to mankind, chilling and other treatments should be done on the seeds of *Z. spina-christi* to develop tree cultivation.

In this study, chilling/immersing treatments were applied to *Z. spina-christi* seeds. The seeds were collected from Doan District to discover new methods by which the economy of the Republic of Yemen increases through the production of the Sidr honey gate.

MATERIALS AND METHODS

Study area: All the experiments were carried out at Hadhramout University's postgraduate laboratory College of Science.

Research protocol: The experiments were performed to study the effect of chilling/immersed treatment of seeds *Z. spina-christi*. The effects on seed water imbibition, the percentage of seed germination and germination speed were investigated. Two types of seeds were produced in 2017 and 2018 and the seeds were obtained from the Honey Bee Center-Doan, Hadhramout.

The used treatments (T) were listed below:

T_1 = Immersion in water for 24 hrs
T_2 = Chilling for 4 hrs at a temperature of 4°C
T_3 = Immersing in water for 24 hrs, then chilling for 4 hrs at a temperature of 4°C (immersing/chilling)
T_4 = Chilling for 4 hrs at a temperature of 4°C, then immersing in water for 24 hrs (chilling/immersing)

After all treatments, seeds were removed from dishes, blotted dry on paper towels and re-weighed to calculate water uptake. Seeds were then returned to Petri dishes and incubated in the dark. The experiment was carried out in a fully random design with three replications and 24 seeds per replication. After treatment, the seeds were planted in Petri dishes with filter paper. The pots were watered regularly and shaded in the laboratory with an average temperature of 25±5°C and relative humidity of 40%. Seed germination was monitored twice a day and a seed was considered to be germinated when the radicle was 2 mm long. The seed germination percentage (SG%) was calculated according to the formula reported by Pyar *et al.*[22], as follows:

$$\text{SG (\%)} = \frac{N_g}{N_s} \times 100$$

Where:
N_g = Number of t germinated seed days
N_s = Presents the number of total seeds

The germination speed (GSP) per day is defined according to the formula reported by Pyar *et al.*[22]:

$$\text{GSP (day)} = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3}$$

Where:
n = Number of germinating seeds
d = Number of days

Statistical analysis: The standard deviation was taken to calculate the experimental error. A completely random design was used and data were analyzed statistically using a system (Genstat 5) 3.2. The results were analyzed with the least significant difference (LSD) $p>0.05$ to assess the significant difference between the means of samples.

RESULTS

Figure 1 shows *Z. spina-christi* seeds out of their solid woody shell (stone seed). Two types of seeds were used in the experiments. Figure 1a shows the new seeds (2018) and Fig. 1b show the old seeds (2017). It can be noted from Fig. 1 that the new seeds were fuller and have a light brown colour, while the old were much wrinkled (depressions) and were dark brown with a tendency to be black. Moreover, the new seeds were found to be heavier than the old ones.

Immersed in water (T_1): The weight of the seeds grew considerably after being immersed in water for 24 hrs. The rise in seed weight is most likely due to impregnation in Fig. 2a. As a result, the fresh seeds absorb far more water than the old seeds. The fresh seeds gained in weight as compared to the old dry seed. Furthermore, when compared to the dry seeds, the weight gains for the older ones increased, representing weight growth. As a result, the new seeds' weight increased roughly when compared to the old.

Chilling (T_2): When fresh and old dry seeds were subjected to a chilling treatment, it was observed that there was a little increase in weight for both types of seeds, as shown in Fig. 2.

Immersing/chilling (T_3): Figure 2c shows how the weight of seeds behaves when T_3 was applied to the dry seeds. When the first stage of T_3 (immersing in water) is done. This means that the new seed consumed more water than the old one absorbed. Therefore, it was found that the weight of the new seeds increased. When the second step of T_3 (chilling) was applied, the seed's weight decreased.

Chilling/immersing (T_4): As the first step in this treatment was the chilling process, a slight increase in seed weight in Fig. 2d is noticed. The gain in weight was 0.0359 and 0.0047 g for old and new seeds, respectively. The increase was in favour of the old seeds by 0.0312 g. Directly, when the second step of T_4 was performed (immersing process), an increase in the weight of the new and the old seeds was observed. Therefore, it was noticed that the new seeds increased in weight over the old ones when T_4 was applied.

Percentage and speed of germination: Table 1 shows that the new production seeds (2018) were significantly higher in germination percentage than the older seeds (2017), with a major variation. The treatment (T_4) was considered the best treatment for both seeds with a germination percentage of 48.3% (Table 1). This percentage suggested that the outcomes of applying T_4 were greater than the outcomes of T_3 with significant differences. The outcomes of T_4 also exceed the other treatments in this study by numerical differences. However, when the interaction between seeds and the treatment was studied, it can be obtained from Table 1. The interaction between the new seeds and T_4 gives the best germination rate of 66.66% with significant differences from what other interactions give. The new seeds recorded the lowest germination percentage (10%) when T_3 was applied.

Table 1, also showed that the old seeds (2017) significantly outperformed in germination speed of the new seeds (2018) with significant differences. The results showed that the germination speed reached 2.45 days and it was recorded when *Z. spina-christi* seeds were treated by chilling/immersing treatment (T_4). We also noted that the interaction between the old seeds and T_4 was found to be significantly surpassed the rest of the interactions except for the interaction between the new seeds and the chilling treatment (T_2).

Fig. 1(a-b): *Ziziphus spina-christi* seeds for the two seasons, (a) 2017 and (b) 2018

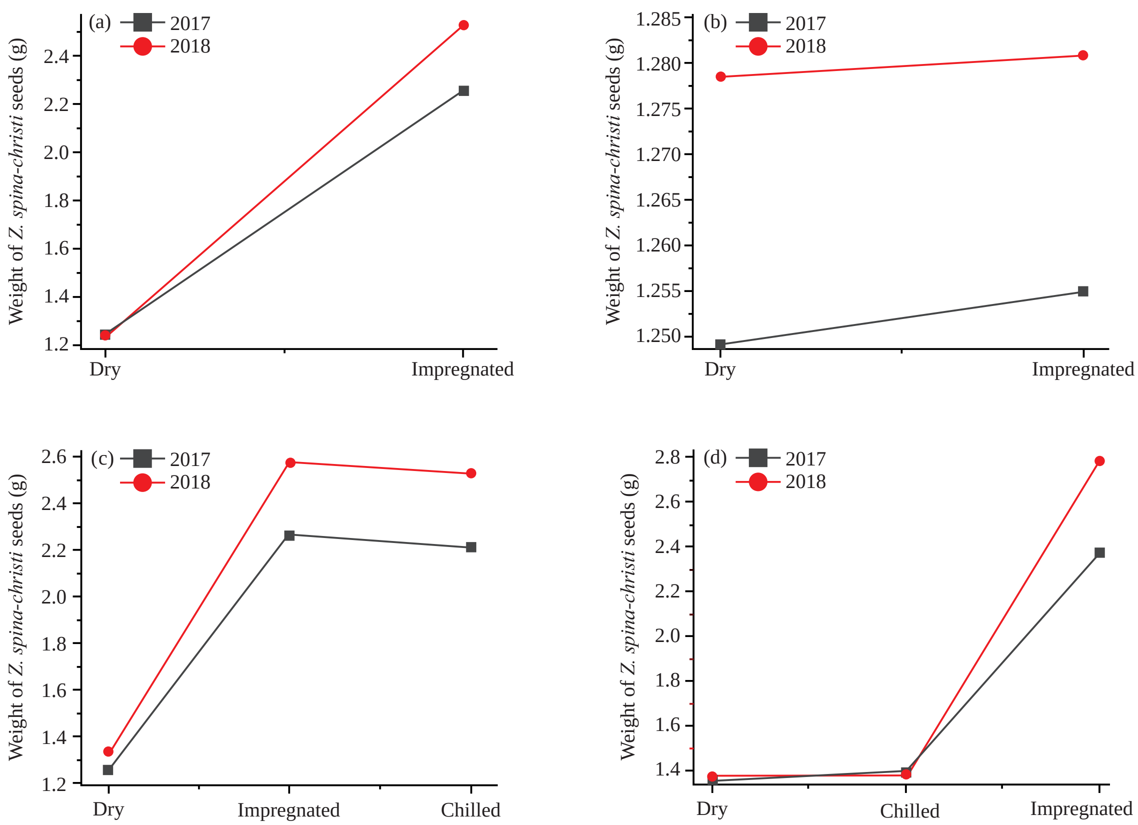

Fig. 2(a-d): Effect of chilling and immersing treatments on the weight of *Ziziphus spina-christi* seeds of different ages, (a) Effect of immersing, (b) Effect of chilling, (c) Effect of immersing, then chilling and (d) Effect of chilling, then immersion

Table 1: Effect of the treatments on germination rate and speed of *Ziziphus spina-christi* seeds

Category	Germination percentage (%)			Germination speed (day)		
Seed age (T_x)	2017	2018	Mean	2017	2018	Mean
Control treatment	20.0	40.0	30.0	6.2	6.5	6.4
T_1	16.7	40.0	28.3	6.8	6.7	6.8
T_2	20.0	30.0	25.0	4.7	6.8	5.8
T_3	26.7	10.0	18.3	5.3	6.3	5.8
T_4	30.0	66.7	48.3	2.4	2.5	2.5
Mean	22.7	37.3	30.0	5.1	5.8	5.4
LSD = 0.05	11.54	20.43	20.87	0.40	0.57	1.00

DISCUSSION

The increase in water imbibition by the new seeds (2018) was probably due to the higher vitality of the new seeds compared to the old ones (2017), these result was in the agreement with other researchers[7,22]. When both seeds (new and old ones) were subjected to the chilling process, the new seeds recorded a lower weight in comparison to the old seeds.

These findings were consistent with the findings of other researchers, who found that seeds aged one year outperformed seeds aged two and three years in germination test values[23]. The loss in weight of the new seeds was probably due to the higher amount of water content contained and as a matter of fact, water loses weight at lower temperatures. Increasing the water content of the seeds thus reduces their weight at the freezing temperature. Another study reported that chilling for 48 hrs was superior in germination energy, germination percentage, survival percentage, seedling length and seedling diameter[24]. The low temperature can significantly change the plants' internal environment by inhibiting the structural change and transition of the saved substances and increasing the water's oxygen. Temperature also affects the respiration process in a complex way, as does its effect on various vital processes. This outcome was consistent with Rezaee *et al.*[25] which reported that moist chilling for 40 days at 4°C is strongly suggested. Zait and Schwartz[12] found that 3 weeks of stratification (4-5°C) was the most effective treatment for germination, with a 78.8% success rate, on the other hand, the best treatment was moist-chilling at 5°C for 4-6 weeks which was similar to the current study[26]. On the other hand, other researchers discovered that cold stratifying canary pine seeds for 15 days at (4-5°C) resulted in a greater germination percentage (91.11%) when it came to soaking seeds, the superiority therapy had no effect[25]. Moreover, there were morphological and physiological adaptations that occurred in plants to resist the cold that they may be exposed to, such as a decrease in the water content of the protoplasm, excessive soluble content, high osmotic pressure, accumulation of hydrophilic colloids, increased permeability of protoplasmic membranes. All these and other processes lead to an increase in seed viability and hence an increase in its water imbibition, as well as an increase in the percentage and speed of germination. The consequences of chilling leading to an increase in cell wall extensibility, an increase in the rate of cell expansion and ultimately, an increase in the rate of radicle emergence[27].

CONCLUSION

The technique of freezing the *Z. spina-christi* seeds before submerging (T_4) had a much larger impact on germination than any of the other treatments (T_1, T_2 and T_3). Chilling the seeds before immersing increases the water imbibition of *Z. spina-christi* seeds. Chilling the seeds after the immersing process reduces their weight. It is possible to deduce that chilling seeds prior to immersion improves the weight of the *Z. spina-christi* seed as well as the percentage and speed of germination. In addition, for improved results, the old seeds might be chilled before being immersed in water. Even though the research was conducted on *Z. spina-christi* seed, it may be applied to other varieties of seed. Multiple investigations with a broad variety of chilling temperatures, chilling durations, plant types and cultivars are suggested as a future study objective.

SIGNIFICANCE STATEMENT

This study discovers the effect of seeds age and chilling on water imbibition and germination for *Ziziphus spina-christi* seeds. This study helps the researchers to study the two new varieties in Yemen and their activities which is not studied before.

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