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The Effect of Cold and Acid Scarification on Seed Germination of Three Green Space Tree Species

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Abstract

Seed germination of some woody species is done with difficulty and at a low rate due to various reasons, including physiological and mechanical dormancy and the presence of inhibitory substances in the seed tissues. Priming is one of the most important treatments to increase the germination capacity of seeds. The purpose of this study was to investigate the effect of different treatments of stratification and sulfuric acid on the growth and germination percentage of *Robinia pseudoacacia*, *Ailanthus altissima* and *Fraxinus excelsior* seeds. This research was done as factorial in the randomized completely block design with two factors; plant and stratification in four replications. The results showed that the highest percentage of germination (72%) was obtained in the 50% sulfuric acid treatment on *R. pseudoacacia*, seeds and the lowest one (18 and 12%) in the control and 95% sulfuric acid treatments on *F. excelsior* seeds, respectively. The overall results of this research showed that the best stratification treatment for most of the measured traits was 50% sulfuric acid and 3°C cold for 20 days.

Keywords: Forest trees, Plant stablishment, Priming, Scarification, Seed dormancy.

INTRODUCTION

Seed germination of some woody species are difficult and with a low rate due to embryo abortion, immaturity of the embryo, physiological dormancy, planned winter dormancy, chemical dormancy (presence of growth inhibitors) and mechanical dormancy (hard fruit endocarp) and the presence of inhibitory substances in the seed tissues (Aboutalebi *et al*., 2012; Mirzajani Fathkouhi *et al*., 2022). Different treatments including mechanical scarification, chemical scarification and stratification have been used to accelerate seed germination (Imani, 2011; Arji *et al*., 2020). Due to the remove the mechanical dormancy caused by the hard fruit endocarp and the facilitation of water and gas diffusion, concentrated sulfuric acid is mainly used, and to remove the physiological dormancy, the wet stratification is mainly utilized (Mirzajani Fathkouhi *et al*., 2022).

Black locust (*Robinia pseudoacacia*) is a medium-sized hardwood deciduous tree, belonging to the family Fabaceae. It is endemic to a few small areas of the United States, but it has been widely planted and naturalized elsewhere in temperate North America, Europe, Southern Africa and Asia and is considered an invasive species in some areas (Contu, 2012). Varnish tree (*Ailanthus altissima*) is a deciduous tree in the family Simaroubaceae. It is native to northeast and central China, and Taiwan. It is found in temperate climates rather than the tropics. It is considered a noxious weed and vigorous invasive species, and one of the worst invasive plant species in Europe and North America (Sladonja *et al*., 2015). Ash (*Fraxinus excelsior*), is a flowering plant species in the family Oleaceae. It is native throughout mainland Europe, east to the Caucasus and Alborz mountains, and Britain and Ireland, the latter determining its Western boundary. The Northernmost location is in the Trondheimsfjord region of Norway. The species is widely cultivated and reportedly naturalised in New Zealand and in scattered locales in the United States and Canada (Thomas, 2016).

Researches have shown that chemicals such as some hormones such as gibberellic acid (GA_3) and some cytokinins, potassium nitrate, ammonium nitrate, sodium nitrate and thiourea, as well as scarification and placing the seed in low and high temperature and some light spectrums are effective in stimulation of seed germination (Lee and Kim, 2000; Eda and Hori, 2007; Ebrahimi *et al*., 2008). The positive effect of priming and stratification on removing seed dormancy and increasing the speed and strength of its germination has been reported in some species including forest trees (Nejadsahebi *et al*., 2007; Sadrabadi Haghighi, 2014; Hosseini *et al.*, 2016; Kaviani and Sedaghathoor, 2020; Mirzajani Fathkouhi *et al*., 2022). The highest percentage of seed germination in some olive cultivars was obtained by removing the fruit endocarp, stratification and chemical scarification treatment with sulfuric acid (Rostami and Shahsavar, 2009; Sadeghi and Aboutalebi, 2011; Arji *et al*., 2020). Three weeks of stratification at a temperature of 4-5°C caused the germination of 78.80% of wild ziziphus (*Ziziphus spina-christi*) seeds (Aboutalebi *et al.*, 2012). The increase in seed germination rate and seedling growth of primed seeds compared to non-primed ones is related to the higher activity of amylase, invertase, sucrose synthetase, sucrose phosphate synthetase and increasing the level of biological energy charge in the form of increasing the amount of ATP, increasing DNA and RNA synthesis and increasing the number and function of mitochondria. The higher activity of these enzymes, especially amylase, leads to the rapid hydrolysis of starch to glucose (Sedghi *et al*., 2010; Kaviani and Sedaghathoor, 2020).

Esvand and Nasiri (2001) showed that the application of 50% sulfuric acid increased the percentage and speed of germination of *A. altissima* and *F. excelsior* seeds. With increasing concentration of sulfuric acid, the percentage and speed of seed germination decreased. In

R. pseudoacacia, the highest percentage of germination, germination speed and germination capacity were related to sulfuric acid and hot water treatment (Yari *et al*., 2011). In the seeds of *Leucaena leucocephala* (Lam.) and *Acacia farnesiana*, the pretreatments of scarification and soaking in hot water at 70°C and their combination showed that soaking in hot water had a better effect on germination (Tadoos *et al*., 2012). The highest germination rates for the seeds of *Acacia victoriae* and *Prspopis juliflora* were obtained by applying 98% concentrated sulfuric acid treatment and hot water treatment, respectively (Mahmoodi *et al*., 2013). The combined application of cold priming and sulfuric acid on three cultivars of olive (Arbequina, Balidi and Mari) after full fruit ripening reduced the number of days to seed germination but increased the germination percentage, germination rate, germination average and seedling vigor index in all three cultivars (Mirzajani Fathkouhi *et al*., 2022). Seeds of some woody plants, including *F. excelsior*, *A. altissima* and *R. pseudoacacia* face two problems (physiological and mechanical dormancy) for germination. Various treatments such as mechanical scarification, chemical scarification and cold stratification (wet cooling) are used to accelerate seed germination. Concentrated sulfuric acid is used to relieve mechanical dormancy and to eliminate physiological dormancy.

The aim of the present research was to evaluate the effect of stratification treatments, different concentrations of sulfuric acid and effective temperature on the strength and speed of germination of *F. excelsior*, *A. altissima* and *R. pseudoacacia* seeds.

MATERIALS AND METHODS

The place of research

This research was carried out on 2019 in the laboratory of the Seed Certification Control, Department of the East Azarbaijan Province Agricultural Research Center, Tabriz Branch. East Azarbaijan province is located in the northwest of Iran at an altitude of 1800 meters above sea level at 46° and 25´ East longitude and 38° and 2´ North latitude from the Greenwich meridian.

Experimental design

The factorial experiment was conducted based on a randomized completely block design with two factors; plant and stratification treatment in four replicates. The plant factor included the seeds of three types of plants (*Robinia pseudoacacia, Ailanthus altissima* and *Fraxinus excelsior*). Also, the second factor, stratification included seven treatments (control, concentrated sulfuric acid 95%, diluted sulfuric acid 50%, boiling water 95°C and cold for 10, 15 and 20 days). The control treatment was distilled water.

Preparation of plant specimens

The seeds of the three investigated species were purchased from the Azar Sabzineh company, Tabriz, East Azarbaijan Province, Iran. The required equipments were sterilized in an oven at 110°C. Whatman No. 2 filter paper was placed in the required Petri dishes. The 100 seeds were prepared from each species. The 25 healthy and uniform seeds was considered for each Petri dish. The seeds were disinfected with hydrogen peroxide (H_2O_2) for 40 sec. and then washed with distilled water and placed at the bottom of each Petri dish.

Treatments

The duration of placing the seeds in sulfuric acid and hot water was 10 and 15 min, respectively. After this time, the seeds were completely washed and cultivated in Petri dishes containing two sheets of Whatman paper soaked with distilled water in the amount of 7 ml and transferred to the germinator at a temperature of 20 °C. In order to apply the cold treatment, the seeds were kept in the refrigerator at 3 °C for 10, 15 and 20 days and then transferred to the germinator at 20 °C for 10 days.

Measured traits

The sprouting (germination) time of buds, bud length, leaf rupture rate, root length, root number, number of germinated seeds, number of non-germinated seeds and germination percentage were measured. Organs length was measured with a digital caliper. The percentage of seed germination was calculated from the following formula:

$$\text{Germination percentage} = \frac{\text{The number germinated seeds } \times \text{ Repeat}}{100}$$

Data analysis

Data were subjected to the analysis of variance (ANOVA) and means were compared by Duncan's test at $P < 0.05$ using the SAS ver. 9.1 software.

RESULTS AND DISCUSSION

The results of the variance analysis of the studied traits (Table 1) showed that there was a significant difference between different levels of studied treatments and all the studied traits including sprouting time of buds, bud length, bud rupture, root length, root number, number of germinated seeds, number of non-germinated seeds and germination percentage. Also, the interaction effect of all studied treatments was significant at the 1% probability level, which indicated that the treatments did not act equally in the traits. The coefficient of variation, as a standard criterion for experiment accuracy and reproducibility, showed that all traits had high accuracy and the coefficient of variation was lower than 30%. Maximum coefficient of variation (8.41%) was observed in the root length and the lowest value (3.22%) in the sprouting time.

Germination time

The results of the mean comparison (Table 2) and eye observations (Figs. 1-4) of the effects of seven studied treatments in the seeds showed that the longest sprouting time (18.25 days) was observed in *F. excelsior* seeds in the 95% concentrated sulfuric acid treatment. The shortest sprouting time (5.5 days) was observed in *R. pseudoacacia* seeds treated with 50% sulfuric acid, which probably indicates the difference in the seed dormancy period. On the other hand, concentrated sulfuric acid increases the duration of germination, probably due to its destructive effect on the embryo.

The results of the study on three olive cultivars showed that the combined use of cold priming and sulfuric acid in the stage after the fully ripening of the fruit reduced the number of days to germination, but increased the germination percentage, germination speed, germination average and seedling vigoroty index in all cultivars. The shortest time for seed germination (one day) was obtained in Mari variety by applying the stratification treatment (6-7 °C) for 1000 h (Mirzajani Fathkouhi *et al*., 2022). Similar results were reported in some studies. In *Fraxinus* and *Ailanthus* seeds, the highest percentage of germination was observed by application of 50% sulfuric acid (Esvand and Nasiri, 2001). The results of this study showed that sulfuric acid has a different effect on breaking the seed dormancy of different species and in the case of some species (such as *Fraxinus*) it can be a suitable substitute for other scarified agents. In *R. pseudoacacia*, the highest amount of germination was related to sulfuric acid treatment and the lowest one was related to the control treatment (Yari *et al*., 2011). The highest percentage of seed germination in *Allium* kuhsorkhense occurred at 5 °C and high temperature prevented germination (Hosseini *et al*., 2016). Also, 90-day chilling not only increased the germination rate, but also increased the speed and number of germinated seeds. Sulfuric acid and GA_3 treatments had no positive effect on seed germination. Probably, the seed dormancy of this plant is of a physiological type, and long chilling had the most positive effect on germination.

Bud length

The highest bud length (7.6 and 7.15 cm) was obtained in the treatment of 95% sulfuric acid and cold for 20 days in R. pseudoacacia seeds, respectively. The lowest bud length (0.725 cm) was obtained in 50% sulfuric acid treatment (Table 2). The results of the mean comparison showed that with the increase in acid concentration, the length of the bud decreased, which is probably due to its effect on the epicotyl. The 20-day cold increased the length of the bud in all three types of seeds, which indicates the need of these seeds for cold to break the dormancy period.

Investigations on the germination characteristics in retama and twisted acacia by Everitt (1983) showed that seed germination and shoot length of these two species soaked in sulfuric acid for 45 minutes was increased. The percentage of seed germination and the length of bud and root were resistant to high pH and were the highest in acidic conditions. The effect of four pre-treatments; scarification, 50% dilute sulfuric acid, soaking in hot water at 70°C and the combination of scarification and hot water on *Acacia farnesiana* seeds revealed that dilute sulfuric acid had a greater effect on breaking seed dormancy than soaking seeds in hot water (Tadoos *et al*., 2012). Scarification alone had no effect on germination. The combination of scarification and hot water for 20 and 24 min. increased germination and bud length. The obtained results by these researchers were similar to the observations of this research. The study on Delonix regia showed that the combined treatment of seeds with 95% sulfuric acid for 3 h followed by immersion in boiling water at 90°C for 10 sec. compared to single treatment of these had a better effect on bud length, root length and germination acceleration (Nejadsahebi *et al*., 2007).

Table 1. Analysis of variance of the effect of stratification and sulfuric acid on seed germination and some other characteristics in *Robinia pseudoacacia*, *Ailanthus altissima* and *Fraxinus excelsior*.

S.o.V	df	Germination percentage	No. of germinated seeds	No non-germinated seeds	Time of buds appearance	Bud length	Bud tearing	Rootlet length
Block	3	30.159**	1.673**	1.885**	18.398**	1.357**	13.476**	10.426**
Seeds (S)	2	1796.762**	101.402**	112.298**	106.107**	101.894**	118.107**	106.995**
Treatments (T)	6	2759.937**	150.301**	172.496**	105.917**	17.652**	38.464**	9.806**
S × T	12	2.984**	0.205**	0.187**	0.024**	0.220**	0.107**	0.090**
Error	60	9.092	0.840	0.568	0.143	0.093	0.110	0.107
CV (%)		8.05	6.70	4.82	3.22	7.68	4.98	8.41

*: Significant atP < 0.01 based on the Duncan's test.

Table 2. Mean comparison of the effect of stratification and sulfuric acid on seed germination and some other characteristics in *Robinia pseudoacacia*, *Ailanthus altissima* and *Fraxinus excelsior*.

Treatments	Germination percentage	No. of germinated seeds	No. of non-germinated seeds	Time of buds appearance	Bud length	Bud tearing	Rootlet length
A_1B_1	34^{e}	8.5^{h}	16.5^{e}	12.00^{f}	2.250^{f}	6.00^{g}	5.100^{cd}
A_1B_2	28^{f}	7^{i}	18.0^{c}	14.250^{h}	3.650^{e}	7.25^{f}	4.600^{d}
A_1B_3	72^{a}	18^{a}	7.00^{l}	5.50^{a}	7.60^{a}	2.00^{k}	7.225^{a}
A_1B_4	48^{c}	12^{e}	13.0^{h}	9.00^{c}	6.425^{b}	4.00^{i}	6.250^{b}
A_1B_5	60^{b}	15^{b}	10.0^{k}	7.00^{b}	7.150^{a}	3.00^{j}	6.800^{a}
A_1B_6	40^{d}	10^{g}	15.0^{f}	10.0^{d}	5.825^{c}	5.00^{h}	5.625^{c}
A_1B_7	38^{e}	9.5^{gh}	15.0^{f}	11.0^{e}	5.375^{c}	5.00^{h}	5.475^{c}
A_2B_1	26^{g}	6.5^{gh}	18.5^{c}	14.0^{h}	3.250^{e}	8.00^{e}	3.000^{f}
A_2B_2	20^{h}	5^{k}	20.0^{ab}	16.25^{i}	1.650^{g}	9.25^{cd}	2.60^{g}
A_2B_3	60^{b}	15^{b}	10.0^{k}	7.50^{bc}	5.6^{c}	4.00^{i}	5.225^{c}
A_2B_4	40^{d}	10^{g}	15.0^{f}	10.75^{de}	4.425^{d}	6.00^{g}	4.250^{d}
A_2B_5	52^{bc}	13^{d}	12.0^{i}	9.00^{c}	5.150^{c}	5.00^{h}	4.928^{cd}
A_2B_6	32^{ef}	8^{h}	17.0^{d}	11.75^{e}	3.825^{e}	7.00^{fg}	3.625^{e}
A_2B_7	29^{f}	7.25^{i}	17.75^{d}	12.75^{f}	3.50^{e}	7.00^{fg}	3.325^{ef}
A_3B_1	18^{i}	4.5^{l}	20.5^{a}	16.00^{i}	1.300^{g}	10.0^{b}	1.400^{gh}
A_3B_2	12^{i}	3^{l}	22.0^{a}	18.25^{j}	0.725^{h}	11.25^{a}	0.925^{i}
A_3B_3	56^{b}	14^{c}	11.0^{j}	9.50^{cd}	3.55^{e}	6.00^{g}	3.225^{ef}
A_3B_4	32^{ef}	8^{h}	17.0^{d}	12.75^{f}	2.5^{f}	8.00^{e}	1.900^{gh}
A_3B_5	44^{c}	11^{f}	14.0^{g}	11.0^{e}	3.15^{e}	7.00^{fg}	2.825^{g}
A_3B_6	24^{g}	6^{j}	19.0^{b}	13.75^{g}	1.75^{g}	9.00^{d}	1.850^{h}
A_3B_7	22^{g}	5^{k}	19.0^{b}	14.75^{h}	1.60^{g}	9.75^{c}	1.587^{h}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test. A_1: *R. pseudoacacia* seed, A_2: *A. altissima* seed, A_3: *F. excelsior* seed, B_1: control, B_2: Sulfuric acid (95%), B_3: Sulfuric acid (50%), B_4: Hot water (95°C), B_5: Cold (3°C) for 20 days, B_6: Cold (3°C) for 15 days, and b_7: Cold (3°C) for 10 days.

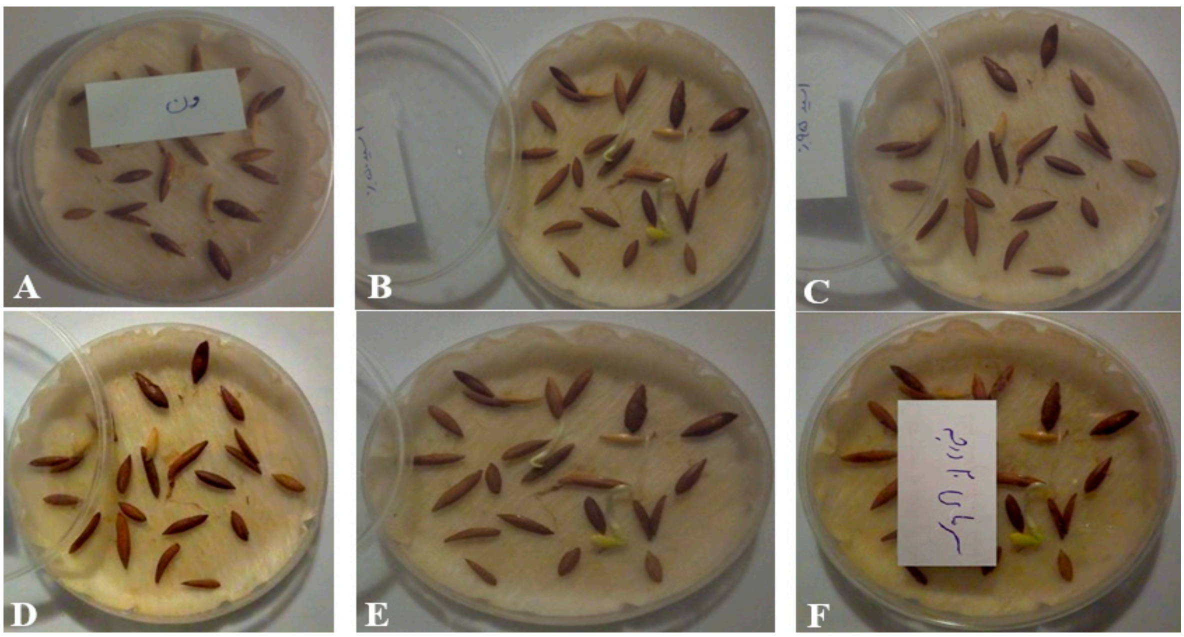

Fig. 1. The effect of stratification and sulfuric acid on seed germination and some other characteristics in *Fraxinus excelsior*. A: Control, B: Sulfuric acid (50%), C: Sulfuric acid (95%), D: Cold (3°C) for 10 days, E: Cold (3°C) for 15 days, and F: Cold (3°C) for 20 days.

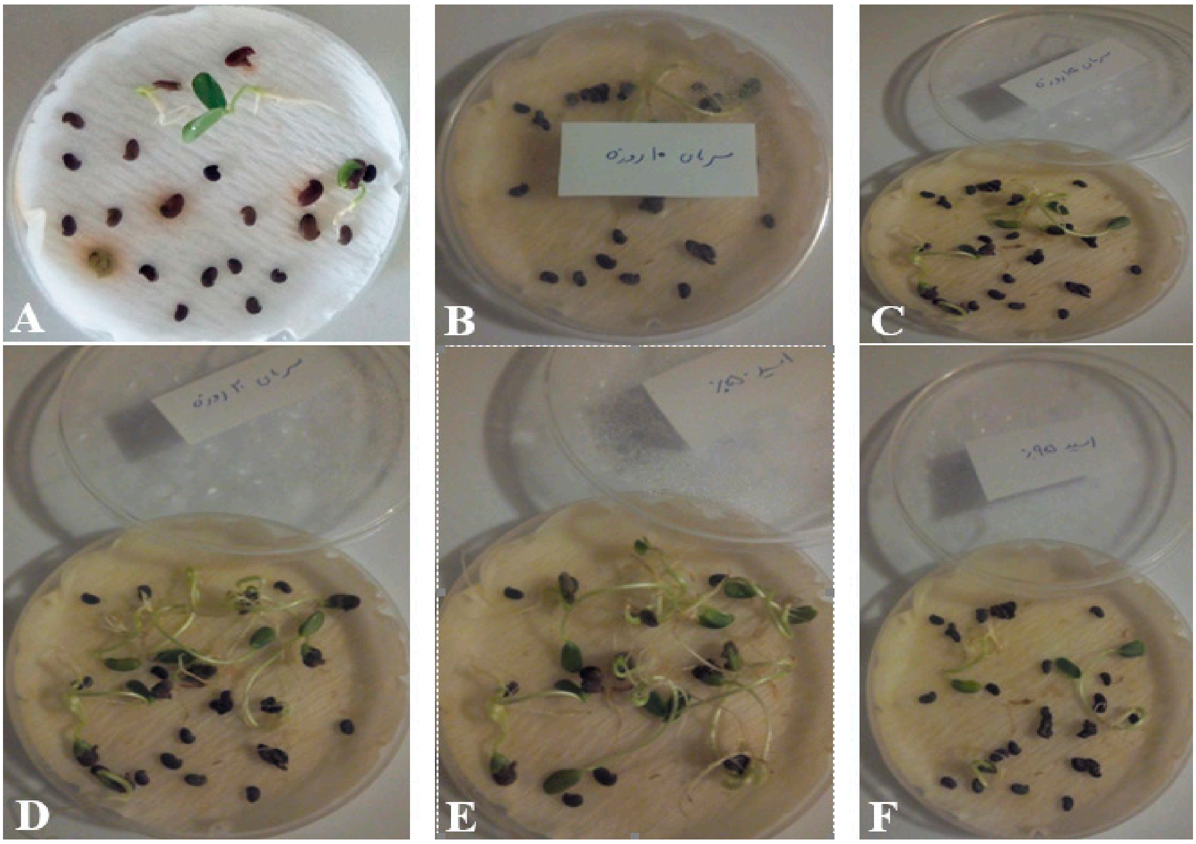

Fig. 2. The effect of stratification and sulfuric acid on seed germination and some other characteristics in *Robinia pseudoacacia*. A: Control, B: Cold (3°C) for 10 days, C: Cold (3°C) for 15 days, D: Cold (3°C) for 20 days, E: Sulfuric acid (50%), and F: Sulfuric acid (95%).

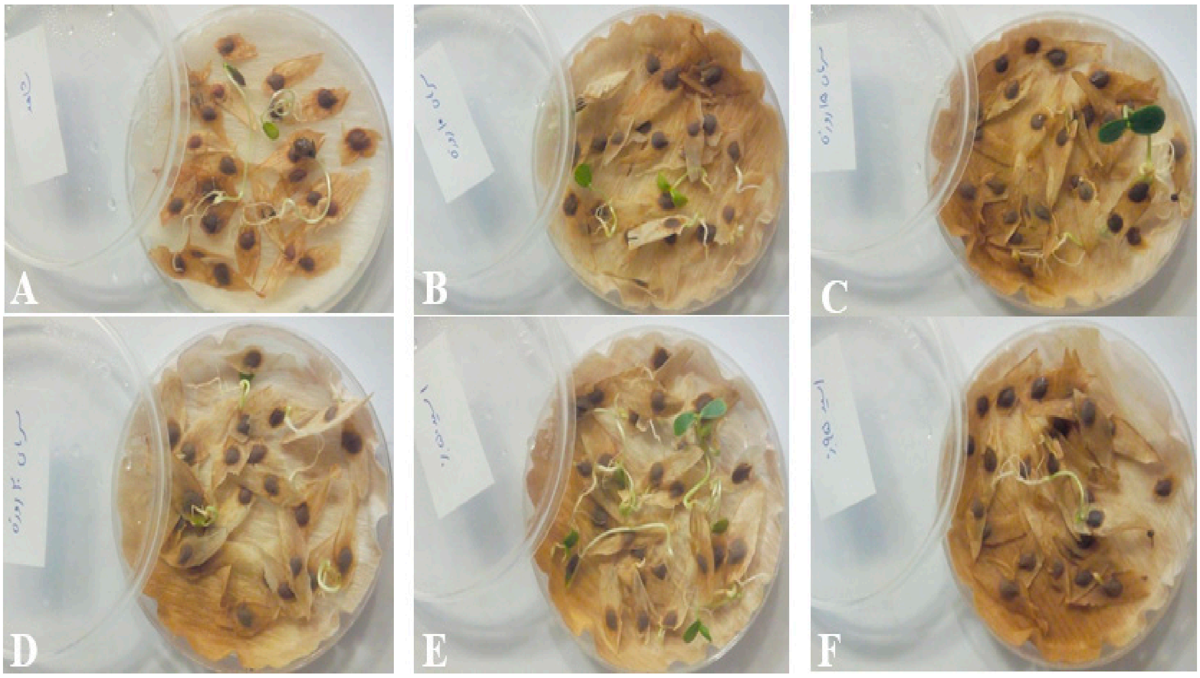

Fig. 3. The effect of stratification and sulfuric acid on seed germination and some other characteristics in *Ailanthus altissima*. A: Control, B: Cold (3°C) for 10 days, C: Cold (3 °C) for 15 days, D: Cold (3 °C) for 20 days, E: Sulfuric acid (50%), and F: Sulfuric acid (95%).

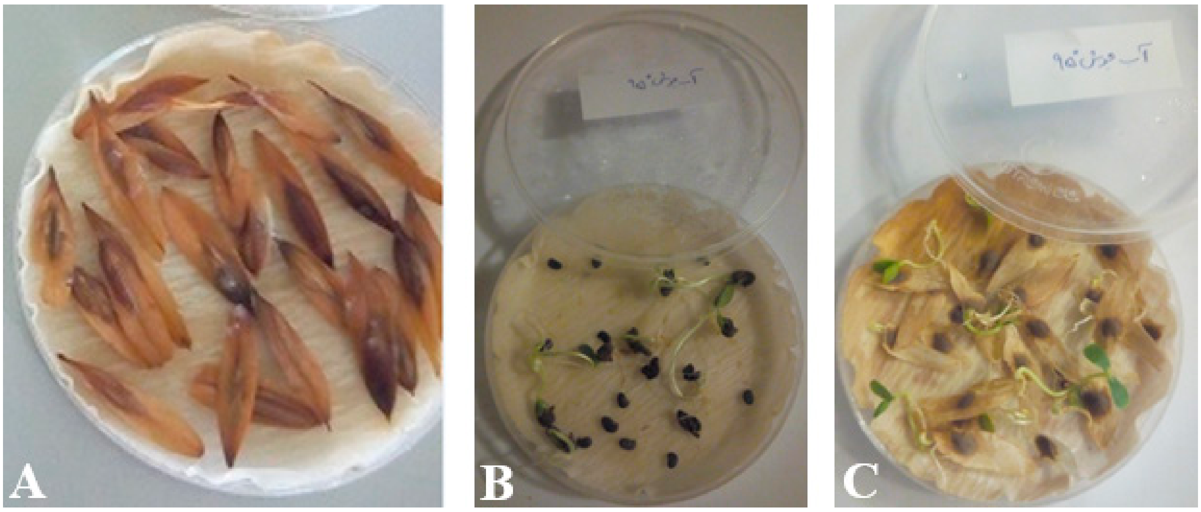

Fig. 4. The effect of hot water (95°C) on seed germination and some other characteristics in *Fraxinus excelsior* (A), *Robinia pseudoacacia* (B), and *Ailanthus altissima* (C).

Bud rupture

The highest number of bud rupture (11.25) was observed in 95% sulfuric acid treatment in *F. excelsior*, while the lowest number of bud rupture (2) was found in 50% sulfuric acid treatment in *R. pseudoacacia* (Table 2). The obtained results demonstrated that strong sulfuric acid caused an increase in leaf rupture by affecting the cotyledon axis, while dilute acid only caused less rupture by affecting seed dormancy and the growth of healthy buds. 20-day cold treatment is also introduced as a good treatment after dilute sulfuric acid treatment with a low amount of bud rupture.

Sulfuric acid increases the damage to the cell membrane. This event is related to the reduction of the total phospholipids of the membrane, which leads to a decrease in the total volume of the membrane, resulting in the weakening of the membrane and damage to the bud (Kouchekey and Sadrabadi Haghighi, 2000). Another evidences for the weakening of the membrane during the use of sulfuric acid has been reported. For example, the turgor pressure of soybean bud cells that were affected by sulfuric acid decreased dramatically as a result of dehydration and increased the negative effect of sulfuric acid treatments.

Root length

The root length (7.225 and 6.8 cm) was the highest in *R. pseudoacacia* seeds treated with 50% dilute sulfuric acid and 20 days cold, respectively (Table 2). The lowest root length (0.925 cm) was observed in 95% sulfuric acid treatment in *F. excelsior* seed. The obtained results showed that 20 days cold and diluted sulfuric acid had an effect on the root length due to the removal of the dormant period in the seed and its rapid germination. Also, 95% concentrated sulfuric acid reduced the length of the root due to damage to the cotyledon axis.

A study on *Prspopis juliflora* and *Acacia victoriae* showed that the maximum root length was obtained by applying 50% sulfuric acid treatment and hot water treatment, respectively (Mahmoodi *et al*., 2013). Nejadsahebi *et al*. (2007) in the study on the effect of different treatments of scarification with hot water and sulfuric acid on the germination parameters of the seeds of *Fraxinus* and *Delonix regia* reported that the seeds of *Fraxinus* treated with concentrated sulfuric acid for 3 h and then immersion in 90°C hot water for 10 sec. had the highest percentage and rate of regeneration and root length, while in *Delonix regia*, concentrated sulfuric acid for 30 min. compared to 95 °C hot water for 6 min. had the most effect on percentage and acceleration of germination and root length.

The number of non-germinated seeds

Mean comparison of the effect of treatments on the number of non-germinated seeds showed that *F. excelsior* treated with 95% sulfuric acid and the control with values of 22 and 20.5, respectively, had the highest number of non-germinated seeds (Fig. 1). The 50% sulfuric acid treatment showed the lowest number of non-germinated seeds (7) in *R. pseudoacacia* (Table 2, Fig. 2). Therefore, increasing the concentration of sulfuric acid had a negative effect on seed germination, and the control treatment also caused a decrease in seed germination due to its lack of effect on seed dormancy. Concentration of 50% sulfuric acid increased seed germination rate due to the breaking thef seed dormancy.

The highest percentage of seed germination in *Acacia* was obtained in seeds treated with 50% sulfuric acid for 30 min after 15 weeks (Muhmmad and Amusa, 2003). Application of 50% sulfuric acid increased germination in *Acacia salicina* seeds which have a hard coat (Rehman *et al*., 1999). Therefore, increasing the contact time of seeds with this acid caused an increase in the number of non-germinated seeds.

The number of germinated seeds

The highest number of germinated seeds (18) was obtained in 50% sulfuric acid treatment in *R. pseudoacacia* seeds, while the lowest number (3) was obtained in *F. excelsior* seeds treated with the concentrated sulfuric acid (Table 2, Fig. 1). According to the obtained results, in the control seeds, we saw an increase in the number of germinated seeds, which was obtained by reducing the concentration of sulfuric acid, while concentrated acid caused a decrease in the number of germinated seeds due to its destructive effect on the embryo.

The highest number of germinated seeds in olive was obtained in the treatment of 97% sulfuric acid for 9 h together with 1000 h stratification. The lowest number was observed in the non-priming treatment with cold and acid (Mirzajani Fathkouhi *et al*., 2022). Germination in seeds of different species showed different responses to scarification treatments with sulfuric acid and cold (Heidari *et al*., 2008; Shahi Gharahlar *et al*., 2009; Aboutalebi *et al*., 2012). Chemical scarification with sulfuric acid and stratification for 15, 30, 45 and 60 days did not affect the germination of wild pear seeds (Akbari Mousavi and Saadat, 2006), which is contrary to the findings of this research. The combination of two methods, stratification and scarification with sulfuric acid, was effective on the germination of the seeds of two types of plum (Heidari *et al*., 2008). Mechanical scarification along with chemical scarification was effective on mustard seed germination (Rezvani *et al*., 2012).

In the comparison of the effect of priming and hydropriming on the germination of *Robinia* seeds, it was revealed that the highest percentage of germination was calculated in 48-h hydropriming treatments, 100 mM sulfuric acid prime for 24 and 48 h, and the control treatment. In general, the use of cheap and easy hydropriming treatment can be considered as a suitable technique to improve some germination parameters such as seed germination acceleration and increasing the fresh and dry weights of roots in *Robinia* seedlings (Norouzi Haroni and Tabari Kouchaksaraei, 2014). The strength of hydropriming can also be effective on improving germination traits. Ghassemi-Golezani *et al*. (2010) in their study on the effect of hydropriming period on three varieties of *Phaseolus vulgaris* in the laboratory and field observed that the best greenhouse results were related to 7-h hydropriming treatment.

Germination percentage

Treatment of 50% sulfuric acid induced the highest germination percentage (72%) in *R. pseudoacacia* seeds, while the control treatment and 95% sulfuric acid showed the lowest germination percentage (18 and 12%, respectively) in *F. excelsior* seeds (Table 2, Figs. 1 and 2). According to the obtained results, reduction of concentration of sulfuric acid due to the effect on seed dormancy, caused more buds to grow as a result of increasing the percentage of germination. On the other hand, it seems that breaking seed dormancy is easier in *R. pseudoacacia* seeds than in other seeds. Also, the control treatment induced the minimum percentage of germination, due to its lack of effect on seed dormancy, and concentrated sulfuric acid, due to its destructive effect on the embryo.

Treatment of olive seeds with sulfuric acid and stratification increased the germination percentage (Mirzajani Fathkouhi *et al*., 2022). The germination of *Acacia* and *Fraxinus* seeds was significantly stimulated under the influence of 35 °C treatment (Shahmoradi *et al*., 2014). Sulfuric acid 50% and cold for 30 days were more efficient than other used treatments. The highest percentage of germination was observed in 50% sulfuric acid treatment and this treatment increased germination compared to the control. The results of this research showed that seed dormancy can be removed by thermal stratification (35 °C) in *Fraxinus* plant and therefore it is a type of superficial physiological dormancy.

The highest percentage of seed germination in *A. kuhsorkhense* occurred at a temperature of 5°C and high temperature prevented germination. Chilling for 90 days not only increased the germination rate, but also increased the acceleration and number of germinated seeds. The effect of sulfuric acid and GA_3 on seed germination was completely negative and no seeds germinated. The use of GA_3 along with chilling for 60 days and also 45% sulfuric acid increased germination (Hosseini *et al*., 2016). Based on the results of the above experiments, it can be concluded that the seed dormancy of *A. kuhsorkhense* is probably of a physiological type, and long chilling had the most positive effect on its germination.

The amount of growth stimulating substances including GA_3 in the seed is relatively low. This amount is increased with the use of stratification (Amiri and Rahemi, 2006). The increase of growth stimulating substances in seeds increases the rate of seed germination and seedling growth. The stratification reduces the amount of abscisic acid at the time of seed ripening and increases the amount of GA_3 and cytokinin. In three wild pear species, 60 days' stratification was the best treatment to eliminate seed dormancy (Akbari Mousavi and Saadat, 2006). Cold priming of seeds has a positive effect on germination components. The highest percentage of germination in the olive seeds was obtained after the complete removal of the endocarp of the fruit and the application of cold treatment for 500 h (Rostami Dastjerdi *et al*., 2014). Cold treatment at 5 to 7 °C for 30 days was the best treatment to breaking dormancy of olive seeds (Sadeghi and Aboutalebi, 2011). Chemical treatment with sulfuric acid and stratification at 4-5°C for three weeks stimulated the highest percentage of wild *Z. spina-christi* seed germination (Aboutalebi *et al*., 2012).

CONCLUSION

The cause of dormancy and lack of germination in the seeds of some tree and shrub species are both mechanical and physiological. To overcome this dormancy of the seeds, some physical treatments, especially cold and chemical treatments, are used. Chemical treatment with high concentration of sulfuric acid had a negative effect on seed germination. The most widely used chemical treatment to overcome physical dormancy is the use of sulfuric acid. Of course, the appropriate concentration of sulfuric acid to obtain a suitable result for germination depends on the type of plant species and the type of seed, especially the thickness of the coat. By removing a part of the seed coat and the appearance of lumen and macroscleroid cells, the absorption of water by the seed increases, which will lead to better germination. Treatment with sulfuric acid and stratification remove the dormancy of the embryo and increase its germination rate. Different cultivars of a species can receive different effects from treatments according to their genetic, and physiological and morphological characteristics. The results of the present research showed that the most suitable stratification treatment for most of the traits measured in this research was 50% sulfuric acid and 3 °C cold for 20 days. It is recommended that future researches be directed towards the use of compounds without any risk to overcome the dormancy of the seeds.

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Literature Cited

Aboutalebi, A., Hasanzada, H. and Hosseini Farahi, M. 2012. Effect of various treatments on seed germination characteristics of wild ziziphus (*Ziziphus spina-christi*). World Applied Science Journal, 17 (7): 900–904.

Akbari Mousavi, Z. and Saadat, Y.A. 2006. Breaking dormancy and germination of wild pear (*Pyrus* spp.) seeds. Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research, 14 (2): 92–104. (In Persian)

Amiri, J. and Rahemi, M. 2006. Evaluation of differences of gibberellic acid-like materials during cold stratification in olive seed. Science and Technology of Agriculture and Natural Resources, 10 (3): 311–319.

Arji, I., Rostami Dasgerdee, M. and Gerdekaneh, M. 2020. Comparison of acid scarification, cold stratification on seed germination of three olive cultivars. Iranian Journal of Seed Science and Technology, 9 (2): 119–132. (In Persian)

Contu, S. 2012. *Robinia pseudoacacia*. IUCN Red List of Threatened Species. e.T19891648A20138922.doi:10.2305/IUCN.UK.2012.RLTS.T19891648A20138922

Ebrahimi, A., Alaghehmandan, H., Khazaeli, Sh., Shahsavari, A. and Salehi, A. 2008. The first complete reference for organic fertilizer production quality management. Isfahan: Publications of the Danesh Pazhouhane-e-Berin Institute. p. 154. (In Persian)

Eda, K. and Hori, K. 2007. Geographical differentiation and diallel analysis of seed dormancy in barley. Euphytica, 153: 249–256.

Esvand, H.R. and Nasiri, M. 2001. Investigation of the effect of sulfuric acid on dormancy breaking and germination of *Fraxinus excelsior* and *Ailanthus altissima* seeds. Genetic Research, Improvement of Pasture and Forest Plants of Iran, 8: 95–111. (In Persian)

Everitt, J. H. 1983. Seed germination characteristics of two woody legumes (retama and twisted *Acacia*) from south Texas. Journal of Range Management, 36 (4): 411–414.

Ghassemi-Golezani, K., Chadordooz-Jeddi, A., Nasrollahzadeh, S.M. and Moghaddam, M. 2010. Effects of hydro-priming duration on seedling vigour and grain yield of pinto bean (*Phaseolus vulgaris* L.) cultivars. Notulae Botanicae Horticulture Agrofarebotanici Cluj-Napoca, 38: 109–113. (In Persian)

Heidari, M., Rahemi, M. and Daneshvar, M.H. 2008. Effect of mechanical, chemical scarification and stratification on seed germination of *Prunus scoparia* (Spach.) and *Prunus webbi*

(Spach.) Vierh. American-Eurasian Journal of Agricultural and Environmental Sciences, 3: 114–117.

Hosseini, A., Tehranifar, A., Samiei, L., Shoor, M. and Memariani, F. 2016. Investigation of the effect of different treatments on seed dormancy of *Allium kuhsorkhense* native to Khorasan. Seed Research Journal, 5 (2): 1–10. (In Persian)

Imani, A. 2011. Reproduction and recognition of fruit trees. Publication of Islamic Azad University, Abhar Branch.

Kaviani, B. and Sedaghathoor, Sh. 2020. Effect of osmopriming, hydropriming treatments and salt stress on seed germination and some morphologic and physiologic characteristics of *Calendula officinalis* L. Journal of Seed Researches, 10 (1): 61–71.

Kouchekey, A. and Sadrabadi Haghighi, R. 2000. Evaluation of seed vigour of four alfalfa cultivars by accelerated aging and conductivity test. Biaban, 5: 25–44. (In Persian)

Lee, S.S. and Kim, S. 2000. Total sugars, α-amylase activity, and germination after priming of normal and aged rice seeds. Korean Journal of Crop Science, 45: 108–111.

Mahmoodi, A., Bijanzadeh, E. and Shekari, M.R. 2013. Different seed dormancy breaking methods on germination of forest plants *Prspopis juliflora* and *Acacia victoriae.* Desert Ecosystem Engineering Journal, 3 (4): 35–42. (In Persian)

Mirzajani Fathkouhi, R., Eslami, A. and Kaviani, B. 2022. Effect of harvest time, cold stratification and sulfuric acid on seed germination of three varieties of olive (*Olea europaea* L.). Journal of Plant Production, 29 (2): 241–263.

Muhmmad, S. and Amusa, A. 2003. Effects of sulphuric and hot wate treatments on seed germination tamarind (*Tamarinus indica* L.). African Journal of Biotechnology, 2(9): 276–279.

Nejadsahebi, M., Khaleghi, E. and Moallemi, N. 2007. The effects of different acid and hot water scarification treatments on the germination parameters of the forest torch and floss seeds. Journal of Agricultural Sciences and Natural Resources, 4 (1): 100–109. (In Persian)

Norouzi Haroni, N. and Tabari Kouchaksaraei, M. 2014. The effect of hydropriming, halopriming and boiling water on seed germination of black locust (*Robinia pesudoacasia* L.). Ecology of Iranian Forest, 2 (3): 76–88. (In Persian)

Rehman, S., Loescher, R.N. and Harris, P.J.C. 1999. Dormancy breaking and germination of *Acacia saliciina* seeds. Seed Sciences and Technology, 27: 553–557.

Rezvani, H., Asghari, J. and Ehteshami, S. 2012. Investigation of prechilling and scarification (chemical and physical) treatments in break of wild mustard (*Sinapis arvensis*) seed dormancy. Iranian Journal of Seed Science and Technology, 2 (2): 147–159. (In Persian)

Rostami, A.A. and Shahsavar, A. 2009. Effects of seed scarification on seed germination and early growth of olive seedlings. Journal of Biological Science, 9: 825–828.

Rostami Dastjerdi, M., Arji, I. and Gerdkaneh, M. 2014. Comparison of scarification with acid, stratification and elimination of endocarp on germination of three olive varieties. 2th International Congress on Sustainable Agriculture and Natural Sciences, Tehran, Iran, 13 p. (In Persian)

Sadeghi, B. and Aboutalebi, A. 2011. Comparison of the effect of chemical treatments, complete endocarp removal and cold stratification on olive seed germination. The 5th Natural Conference on New Ideas in Agriculture, Shiraz, Iran. (In Persian)

Sadrabadi Haghighi, R. 2014. Effects of accelerated aging and scarification of seed coat with hydrochloric acid on seed germination characteristics of chickpea c.v. Kaka. Journal of Crop Ecophysiology, 28: 485–498. (In Persian)

Sedghi, M., Nemati, A., Amanpour-Balneji, B. and Gholpori, A. 2010. Influence of different priming materials on germination and seedling establishment of milk thistle (*Silybum marianum*) under salinity stress. World Applied Science Journal, 11 (5): 604–609.

Shahi Gharahlar, A., Zamani, Z. and Khdivi Khub, A. 2009. Effects of sulfuric acid and moist chilling on dormancy breaking of some subgenus *Cerasus* wild genotyps. Proceeding of 6th Iranian Horticultural Science Congress, Faculty of Agricultural Science, Gilan University. (In Persian)

Shahmoradi, Sh., Chaeichi, M., Mozaffari, J., Mazaheri, V. and Sharifzadeh, F. 2014. Breaking seed dormancy in *Hordeum spontaneum* L. plant. Plant Research Journal, 27 (5): 872–884. (In Persian)

Sladonja, B., Marta, S. and Julia, G. 2015. Review on invasive tree of heaven *(Ailanthus altissima* (Mill.) Swingle) *conflicting values:* Assessment of its ecosystem services and potential biological threat. Environmental Management, 56 (4): 1009–1034.

Tadoos, M., Samarah, N. and Alqudah, A.M. 2012. Effect of different pre-sowing seed treatments on the germination of *Leucaena leucocephala* (Lam.) and *Acacia farnesiana* (L.). New Forests, 42 (3): 397–407.

Thomas, P.A. 2016. Biological flora of the British Isles: *Fraxinus excelsior*. Journal of Ecology, 104 (4): 1158–1209.

Yari, A., Akbari, M. and Nazeri, R. 2011. The effect of different chemical and physical treatments on the germination of *Robinia pseudoacacia* seeds. Journal of Agricultural Sciences and Natural Resources, 4 (4): 15–30. (In Persian)

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