

Effects of Different Treatments to Stimulate Seed Germination of *Salsola arbusculiformis* Drob

Ali Mohammad Asaadi^{1*}, Gholamali Heshmati² and Alireza Dadkhah³

¹ Former Ph.D. Student, Department of Rangeland Management, Faculty of Range and Watershed Management, Gorgan University of Agricultural Sciences and Natural Resources, Iran

² Professor, Department of Rangeland Management, Faculty of Range and Watershed Management, Gorgan University of Agricultural Sciences and Natural Resources, Iran

³ Associate Professor, Faculty of Agriculture, Higher Education Complex of Shirvan - Ferdowsi University of Mashhad, Iran

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ABSTRACT Germination is a critical stage in the life cycle of plants and often controls population dynamics, with major practical implications. *Salsola arbusculiformis* is one of the most important plants used to prevent soil erosion and a good fodder resource for sheep and goat in the rangelands of Iran. However, the species seems to have low seed germination, so the purpose of this study was to investigate the effects of different treatments to improve its seed germination. Three concentrations of gibberellic acid (100, 500 and 1000 ppm), mechanical scarification with sandpaper, concentrated sulphuric acid (H₂SO₄ (98%)), potassium nitrate (KNO₃ (0.2%)), thiourea one Molar, four prechilling periods (10, 20, 30 and 150 days at 2 °C) were used as study treatments and distilled water as control. The results showed that there were significant differences (p<0.05) among and within the treatments in their effects on seed germination. The effective treatments to stimulate seed germination were prechilling for 150 and 30 days; sulphuric acid and sandpaper scarification. Prechilling for 150 and 30 days increased germination by 88.4% and 85.65%, respectively, while sulphuric acid and sandpaper scarification both increased germination by 76.1% compared to the control. The results also showed that gibberellic acid, potassium nitrate and thiourea did not promote seed germination. It was inferred that the most effective treatments among the methods used for breaking seed dormancy of *Salsola arbusculiformis* were prechilling for 150 days and mechanical scarification by hand with sandpaper.

Key words: Pre-Germination treatment, Rangeland plants, Scarification, Seed dormancy, Stratification

1 INTRODUCTION

Salsola arbusculiformis Drob. is one of plant species in Iran with soil conservation, forage and ecological values in the steppe and dispersedly semi-steppe areas in North of Iran (Assadi, 2001). The investigation of various features such as its

reproduction seems to be essential for vegetation expansion projects in mentioned regions (Tavili *et al.*, 2009). The species belongs to the family of Chenopodiaceae. It has shrub form, about 20-50 (80) cm height, stem is woody with light gray bark and much branches. *Salsola arbusculiformis*

* Corresponding Author: Ph.D. Student, Department of Rangeland Management, Faculty of Range and Watershed Management, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Tel: 0583 635 3068, E-mail: am-asaadi@um.ac.ir

grows in central Asia, Iran and China (Wen *et al.*, 2014). In Iran, it grows in some provinces such as Northern Khorasan, Semnan, Azarbaijan, Tehran and Golestan (Assadi, 2001).

Dormancy breaking and germination prompt is important for proliferation and early production of important plants, especially useful rangeland plants. Because propitious environmental conditions are not always prepared for growth of plant seed in nature, seed pretreatment to break dormancy is of paramount importance. This is deliberately important for stumpy germinating seeds such as *Salsola arbusculiformis* seed. Seed germination is the necessary stage of plant rehabilitation in rangelands (Askarian, 2004; Naderi Fasarani *et al.*, 2009). Some different mechanisms are used by plants to postpone germination and protect the seeds until the favorable conditions for seedling are set. Germination requires propitious temperature, oxygen, water and lack of inhibitory materials in the environment (Ali *et al.*, 2011; Yildiztugay and Kucukoduk, 2012). Seeds of many plant species cannot germinate despite favorable environmental conditions required for germination. Main reason for this is the so-called physical seed dormancy which could be due to, hard and impenetrable seed coat and presence of premature or dormant embryo (Finch-Savage and Leubner-Metzger, 2006; Olmez *et al.*, 2008). Seed dormancy is categorized as physical, physiologic, morphologic, morpho-physiologic and combined dormancies (Baskin and Baskin, 2004). Many of plant seeds that are produced in natural conditions, such as rangelands, show different levels of seed dormancy.

Different methods have been applied to overcome seed physical and physiological dormancy. These included salinity, temperature, humidity (Sarmadnia, 1997), light, seed scarification (Suleiman *et al.*, 2008; Soltanipoor *et al.*, 2010; Alderete- Chavez *et al.*, 2010

Khafef *et al.*, 2011; Zare *et al.*, 2011; Saberi *et al.*, 2011), seed stratification (Rehman and Park, 2000; Walck *et al.* 2002; Sharifi and Pouresmael, 2006; Eisvand *et al.*, 2006), regulatory hormones (Yamauchi *et al.*, 2004; Keshtkar *et al.*, 2008; Aliloo and Mustafavi, 2014) and chemical compounds. Tavili *et al.* (2009) studied the effect of Gibberellic acid and KNO₃ on germination of *Salsola rigida* and reported that pretreatment with KNO₃ 0.2% had most influence on seed germination. Sadeghi *et al.* (2009) also evaluated the effects of mechanical scarification (sanding), chemical scarification with acids (dilution in concentrated sulphuric acid for 10, 15 or 20 minutes), soaking of seed in gibberellic acid (0.05% GA3) and prechilling at 4°C for 4, 6 or 10 weeks to overcome seed dormancy of *Rubia tinctorum*. They reported that mechanical scarification (sanding) and chemical scarification (treatment with acid for 15 minutes) were efficient in promoting germination. For practical purposes, mechanical scarification is highly recommended. Hassani *et al.* (2009) investigated the effect of two temperatures (23°C and 4°C), exogenous gibberellic acid and cytokinins on dormancy breaking and germination of *Ferula assafoetida* seeds. They reported that among the treatments, cold stratification (4°C) significantly stimulated seed breaking dormancy, while gibberellic acid was not effective to overcome dormancy for this species. The goal of this study was to determine the effect of different treatments on seed germination and to introduce an effective method for breaking seed dormancy of *Salsola arbusculiformis*.

2 MATERIAL AND METHODS

Seeds of *Salsola arbusculiformis* were collected in December 2014 from arid regions of Northeast of Iran, Garmeh and Jajarm. This region is located between 37° 18' to 37° 24'

North latitudes and 56° 20' to 56° 37' East longitudes. The area is approximately 20000 ha with elevation ranging from 1280 to 1600 m. Annual means of precipitation is 229 mm that maximum and minimum of precipitation occur in April and July, respectively. The mean of annual temperature is 12.9 °C. The climate of this region with using of Emberger method is cold arid. The soil texture, electrical conductivity and pH are loamy and sandy-loam, 210 (µS cm⁻¹) and 8.15 respectively (Asaadi *et al.*, 2014).

To overcome the dormancy imposed by the hard seed coat and embryo and to achieve rapid, uniform and high germination rates, 12 treatments were applied. These are: (1) Three concentrations of gibberellic acid (100, 500 and 1000 ppm), (2) Mechanical scarification with sandpaper, (3) Concentrated sulphuric acid (H₂SO₄ (98%)) for 5 minutes, (4) Potassium nitrate (KNO₃ (0.2%)), (5) Thiourea 1 Molar, (6) Four prechilling periods (10, 20, 30 and 150 days at 2- 4 °C before the germination test) and (7) distilled water as control. Seeds were cleaned and prepared.

The study was conducted in the Seed Laboratory of Natural Resources Faculty, Complex Higher Education of Shirvan. Seeds were disinfected using hyposodium chloride (2%) for 5 minutes, and then washed with distilled water several times and left to dry under room conditions. Then 20 disinfected seeds (seed water content about 12% and weight of 1000 seed about 10.25 g) were evenly distributed between two layers of Whatman No.1 filter paper in each of 9-cm plastic Petri dish and transferred to incubator 8°C (Cardinal temperature) for 15 days. The treatments were arranged in a randomized complete blocks design with three replications.

Germinated seeds of more than 2 mm length were counted each day over 15 days (Tavili *et al.*, 2009) and the germination percentage, germination speed, root length, shoot length

and seed vigor index were measured. Germination percentage (Camberato and Mccarty, 1999), germination speed (Maguire, 1962) and seed vigor index (Sarmadnia, 1997) was calculated based on the following (Eq. 1):

$$\text{Germination percentage} = GP = \sum G_i / N \times 100 \quad (1)$$

Where *GP* is germination percentage, *G_i* is the number of germinated seeds and *N* is the number of seeds (Eq. 2):

$$\text{Germination speed: } GR = \sum S_i / D_i \quad (2)$$

Where *S_i* is the number of germinated seed at each counting, *D_i* is the number of day until *n* counting and *n* is the number of counting (Eqs. 3 and 4):

$$\text{Vigor index} = \text{Total germination percentage} \times \text{Mean of plant length (mm)} / 100 \quad (3)$$

$$\text{Plant length} = \text{Root length} + \text{Shoot length.} \quad (4)$$

Statistical Package for the Social Science (SPSS) was used for data analysis and Duncan's Multiple Range Test (DMRT) was used for the means comparisons (SPSS Inc., 2007).

3 RESULTS

The results of the germination percentage and early seedling growth parameters were presented in Tables 1 and 2. There was significant differences (*p*<0.05) among the treatments in terms of their effects on germination percentage, germination rate, root and shoot length and vigor index.

3.1 Germination percentage

The trend of cumulative germination in response to improved seed germination treatments were different (Figure 1). Analysis of variance of under study characteristics in *S.*

arbusculiformis seeds are given in Table 2. The influence of treatments on germination showed that prechilling (10, 20, 30 and 150 days), sulphuric acid (98%) and scratching with sandpaper have increased germination percentage in *S. arbusculiformis* species, while the gibberellic acid 100, 500 and 1000 ppm treatments have no significant effect. The results showed that potassium nitrate and thiourea treatments had a negative influence and decreased the germination and seedling characters in comparison with the control treatment.

The results of mean comparison showed that there were not any significant difference ($p < 0.05$) between prechilling for 150 and 30 days, sulphuric acid and sandpaper (Table 1). The highest germination percentage of 88% and 86.7 % were recorded with prechilling for 150 days and 30 days, respectively. There were no significant differences ($p < 0.05$) between prechilling for 10 and 20 days; also among prechilling 10 days, sulphuric acid (98%) and scratching with sandpaper treatments, no significant difference was observed (Table 1). Prechilling for 150 and 30 days, sulphuric acid and sandpaper scarification increased germination percentage by 88.4%, 85.65%, 76.1% and 76.1% respectively compared to the control (Figure 2).

3.2 Germination rate

According to the results of analysis of variance of traits (Table 2), germination rate was significant ($p < 0.01$) among different treatments. The results showed that sulphuric acid, prechilling for 150 days, sandpaper and prechilling for 30 days treatments increased germination rate. The results of mean comparison showed that there were not any significant difference ($p < 0.05$) between

treatments of prechilling (10 and 20 days), gibberellic acid (100, 500 and 1000 ppm), potassium nitrate and thiourea with the control treatment (Table 1). The highest germination rate of 5.5 and 5.27 were observed in the sulphuric acid and prechilling for 150 days treatments, respectively. The lowest germination speed was obtained in thiourea (1.62) treatment (Table 1).

3.3 Shoot and root length

Analysis of variance showed that root and shoot length of seedling significantly affected ($p < 0.01$) by different treatments (Table 2). Shoot and root length of *S. arbusculiformis* were largely influenced by prechilling for 150 days treatment (Table 1). Prechilling (10, 20 and 30 days), gibberellic acid (100, 500 and 1000 ppm), potassium nitrate, sulphuric acid (98%) and scratching with sandpaper treatments had no effect on shoot and root length, while thiourea decreased seedling growth compared with control treatment (Table 1).

3.4 Seed vigor index

The results indicated that seed vigor index of *Salsola arbusculiformis* at the various treatments were significantly different from control treatment (Table 2). The influence of treatments on seed germination showed that prechilling for 150 days, scratching with sandpaper and prechilling for 150 days significantly increased seed vigor index in *S. arbusculiformis* species, while the prechilling (10 and 20 days), gibberellic acid (100, 500 and 1000 ppm), potassium nitrate and sulphuric acid treatments had no significant effect compared with control treatment (Table 1). The thiourea treatment had a negative effect on seed vigor index in comparison with the control treatment (Table 1).

Table 1 Germination percentage, Germination rate, Shoot length, Root length and Vigor index in *Salsola arbusculiformis* under different treatments

Treatment	Germination %	Germination rate (Seeds per day)	Shoot length (mm)	Root length (mm)	Vigor index
Control	46.70 ^d	2.23 ^{cd}	14.6 ^{def}	5.7 ^{cd}	4.74 ^{ef}
Prechilling (150 days, 2-4°C)	88.00 ^a	5.27 ^a	28 ^a	10.2 ^a	16.81 ^a
Prechilling (30 days, 2-4°C)	86.70 ^a	2.88 ^{bc}	11.7 ^{cde}	5.7 ^{cd}	7.54 ^d
Prechilling (20 days, 2-4°C)	66.77 ^c	1.80 ^d	9.9 ^c	4.7 ^{bc}	4.87 ^{ef}
Prechilling (10 days, 2-4°C)	73.44 ^{bc}	2.07 ^{cd}	12.2 ^{cde}	5.9 ^{cd}	6.64 ^d
GA3 100 ppm	48.91 ^d	2.27 ^{cd}	11.1 ^{cd}	5.1 ^{bc}	3.96 ^f
GA3 500 ppm	42.22 ^{de}	1.81 ^d	16.1 ^{ef}	5.9 ^{cd}	4.30 ^{ef}
GA3 1000 ppm	44.45 ^d	1.72 ^d	13.9 ^{cdef}	5.9 ^{cd}	4.40 ^{ef}
Thiourea 1 molar	20.01 ^f	1.62 ^d	2.6 ^b	3.5 ^b	0.60 ^b
H2SO4 (98%)	82.23 ^{ab}	5.50 ^a	10.2 ^{cd}	5.6 ^{cd}	6.49 ^{de}
Sandpaper	82.22 ^{ab}	3.55 ^b	18 ^f	7.4 ^d	10.44 ^c
KNO3 0.2%	33.34 ^e	1.90 ^d	12.9 ^{cde}	5.7 ^{cd}	3.12 ^f

Means within a column followed by the same letter are not significantly different by Duncans' multiple range tests (P > 0.05)

Table 2 Variance analysis for studied properties of *Salsola arbusculiformis* affected by various treatments

Variable Sources	Sum of Squares	df	Mean Square	F
Germination (%)	23447.512	11	2131.592	40.644**
Error	1888.049	36	52.446	
Total	25335.561	47		
Germination speed	84.495	11	7.681	25.570**
Error	10.812	36	0.3	
Total	95.307	47		
Root length	1.155	11	0.105	6.439**
Error	0.587	36	0.016	
Total	1.742	47		
Shoot length	15.743	11	1.431	18.112**
Error	2.845	36	0.079	
Total	18.588	47		
Vigor Index	746.929	11	67.903	33.135**
Error	73.775	36	2.049	
Total	820.703	47		

** Significant difference between treatments at 1% levels

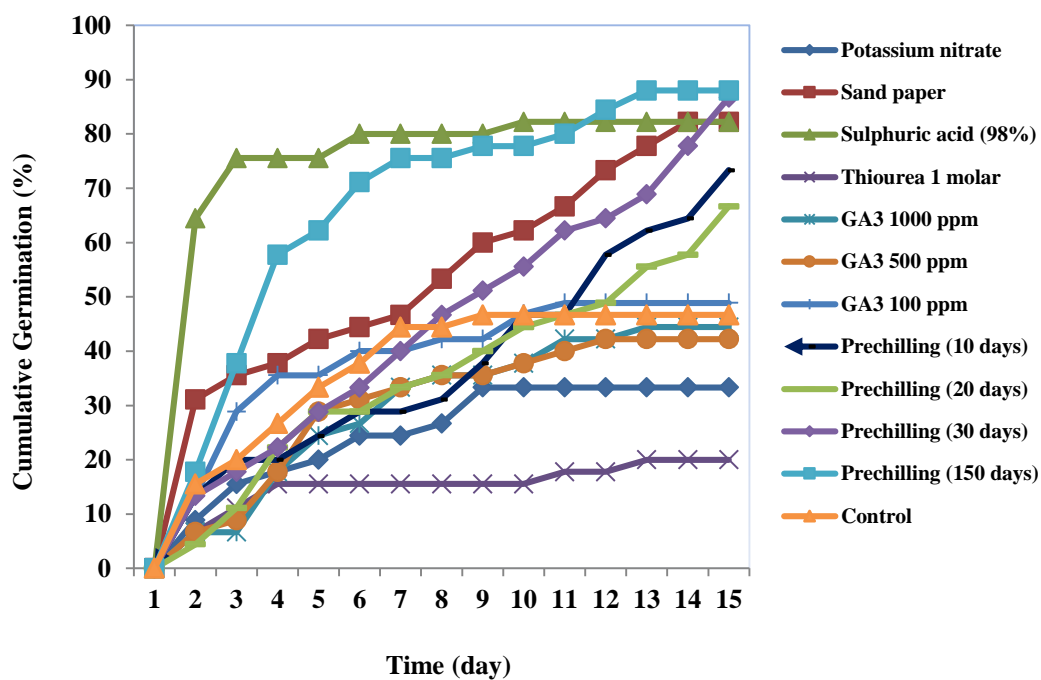


Figure 1 Trend of cumulative germination percentage for *Salsola arbusculiformis* under different treatments of seed dormancy breaking

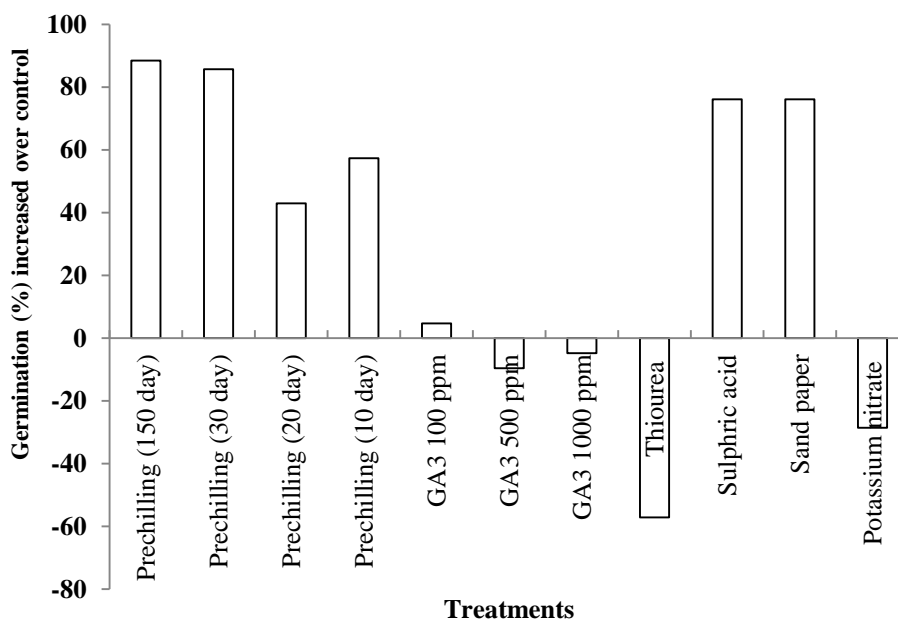


Figure 2 Germination (%) of *Salsola arbusculiformis* seeds at different treatments over control

4 DISCUSSION

It was stated that the onset of embryo dormancy is relevant with compilation of growth inhibitors while breaking of dormancy is pertinent with a shift in the balance of growth increasers that overcome the effect of inhibitors (Keshtkar *et al.*, 2008). Baskin *et al.* (1995) and Walck *et al.* (2002) reported that *Erythorium* and *Osmorhiza* species possess a degree of physiological dormancy that can be broken with application of suitable cold stratification periods. They believed that this requirement for cold stratification is related to ecological distribution of seeds. Seeds of *Salsola arbusculiformis*, belong to cold arid climate, and thus grow better in these areas. Hence, this may suggest that they could have developed a kind of physiological dormancy in the form of ecological adaptation that we can break by using prechilling treatments.

Germination with prechilling stratification and other treatments was compared. Our results indicated that prechilling stratification had a significant effect on seed dormancy and that the germination percent enhanced with increasing stratification periods. It can be attributed that at low temperature more oxygen dissolves in water and therefore more oxygen is available for embryo (Young and Young, 1992). Prechilling stratification is a standard procedure used to enhance the germination of dormant seeds. It has been used for various dormant seeds and has been reported to successfully mitigate endogenous dormancy. Sharifi and Poursmael (2006) found that stratification at 4°C was effective in breaking seed dormancy of *Bunium persicum* and that increasing the duration of stratification resulted in enhanced germination percentage. In another research, Naderi Fasarani *et al.* (2009) evaluated the effects of prechilling on seed dormancy of *Limonium iranicum* and observed that using prechilling for 7 days at 0-5°C increased germination rate. Eisvand *et al.*, (2006) also

reported that stratification of imbibed seeds of *Astragalus siliguosus* improved germination percentage as well as germination rate. Rehman and Park (2000) reported that prechilling increased germination of *Koelreuteria paniculata* Laxm by up to 44 and 45% after 60 and 90 days, respectively.

The main inhibition to water and oxygen permeation inside the *Salsola arbusculiformis* seeds was the presence of a layer of water impenetrable lignified palisade cells (Finch-Savage and Leubner-Metzger, 2006). Similarly, this inhibition type was reported in *Sphaeralcea munroana* species (Kildisheva *et al.*, 2011) and five *Acacia* species (Venier *et al.*, 2012). Physical dormancy was observed in *Salsola arbusculiformis* seeds due to this type of seed coat. In seeds untreated germination was low. In our study, it was observed that mechanical scarification by hand with sandpaper was quite effective in increasing germination of *Salsola arbusculiformis* seeds and 82.22% germination percentage was achieved. Lignified palisade cell layer in the seeds was damaged after sandpapering and germination occurs due to water penetration (Yildiztugay and Kucukoduk, 2012). Similarly, it was reported that mechanical scarification by hand with sandpaper was an effective method in breaking seed dormancy of *Medicago scutellata* and *Medicago polymorpha* (Khaef *et al.*, 2011), *Prosopis koelziana* and *Prosopis juliflora* (Zare *et al.*, 2011) and *Citrullus colocynthis* (Saber *et al.*, 2011).

Chemical scarification techniques were found to be quite effective in breaking seed dormancy of *Salsola arbusculiformis*. In sulphuric acid treatment, seed germination percentage and germination rate value were enhanced when compared to the control. It was found that sulphuric acid (H₂SO₄) treatment was the most effective chemical scarification technique in breaking seed dormancy of this species. Our results confirmed by findings of

the previous studies on *Prosopis koelziana* and *Prosopis juliflora* (Zare et al., 2011), *Sophora alopecuroides* (Aliloo and Mustafavi, 2014), *Sphaerophysa kotschyana* (Yildiztugay and Kucukoduk, 2012), *Foeniculum vulgare* and *Abutilon fruticosum* (Soltanipoor et al., 2010), *Capparis sponosa* (Suleiman et al., 2008), *Crotalaria retusa* (Alderete- Chavez et al., 2010) and *Gleditschia caspica* (Zoghi et al., 2011). The hard and thick seed coat of *Salsola arbusculiformis* were soften and cracks due to sulphuric acid treatment and consequence seed germination increased.

Based on the results of present study, it can be suggested that sulphuric acid increased the germination percentage and germination rate, but excessive acid abrasion caused injury to the embryo structure and as a result poor seedlings were obtained. This finding consented with those found by Rana and Nuatiyal (1989), Aliero (2004), Makkizadeh et al. (2006), Sadeghi et al. (2009).

The results indicated that there was no significant difference in seed germination and seedling traits between the control and the gibberellic acid different concentrations. So, addition of gibberellic acid did not promote germination. The lack of gibberellic acid effectiveness in stimulating seed germination might be referred to the following possibilities: (i) a negative effect of gibberellic acid on the level of some enzymes activity (glutamate-oxaloacetate transaminase, pyruvate kinase and malate dehydrogenase) (Aliloo and Mustafavi, 2014), (ii) consumption of nucleotides in the synthesis of nucleic acid (EL-Dengawy 1997) (iii) and/or the production of a proteinaceous germination inhibitor (Balouchi and Modarres-Sansvy, 2006). Our results are consistent with findings of the previous studies on *Heracleum mantegazzianum* (Moravcova et al., 2007), *Dorema ammoniacum* (Irvani et al., 2011), *Ferula ovina* and *Ferula gummosa* (Keshtkar et

al., 2008) and *Sophora alopecuroides* (Aliloo and Mustafavi, 2014).

Potassium nitrate (KNO₃) and thiourea decreased the germination and seedling traits in comparison with the control. Thiourea has been known to stimulate germination by reducing the preventive effect of the seed coat in *Prunus avium* L. (Cetinbaş and Koyuncu, 2006). Similarly, potassium nitrate was very effective in breaking seed dormancy of many species (Previero et al., 1996), and it has been stated as being a growth-regulating substance in *Salvia* species (Yucel, 2000). However, both chemical treatments (Thiourea and Potassium nitrate) were unable to break seed dormancy in *Salsola arbusculiformis* in the present study. This could be due to its excessively hard seed coat. Ali et al. (2011) revealed that thiourea and potassium nitrate were ineffective in breaking seed dormancy of *Rhynchosia capitata*.

5 CONCLUSION

In conclusion, the most effective treatments among the methods of breaking seed dormancy of *Salsola arbusculiformis* seeds were found to be prechilling for 150 and 30 days, sulphuric acid (98%) and mechanical scarification by hand with sandpaper treatment. Though, sulphuric acid increased germination percentage and germination rate, but excessive acid abrasion might be injured the embryo structure and poor seedlings were obtained. The results indicated that gibberellic acid, thiourea and potassium nitrate were less effective in breaking the seed dormancy of *Salsola arbusculiformis*.

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تأثیر تیمارهای مختلف بر تحریک جوانه‌زنی بذر گونه جامه‌در

علی محمد اسعدی^{۱*}، غلامعلی حشمتی^۲ و علیرضا دادخواه^۳

۱- دانش‌آموخته دکترای علوم مرتع، گروه مرتعداری، دانشکده مرتع و آبخیزداری، دانشگاه کشاورزی و منابع طبیعی گرگان، ایران

۲- استاد گروه مرتعداری، دانشکده مرتع و آبخیزداری، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، ایران

۳- دانشیار دانشکده کشاورزی مجتمع آموزش عالی شیروان- دانشگاه فردوسی مشهد، ایران

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چکیده جوانه‌زنی بذر مرحله‌ای مهم در چرخه زندگی گیاهان است و کسب اطلاعات لازم از آن، برای مهار پویایی جمعیت و ارائه راه‌کارهای مدیریتی مهم است. گیاه جامه‌در، یکی از گیاهان مهم در زمینه جلوگیری از فرسایش خاک و یک منبع علوفه‌ای مناسب برای دام‌ها در مراتع ایران و جوانه‌زنی بذر آن کم است. بنابراین هدف از این تحقیق، بررسی اثر تیمارهای مختلف افزایش جوانه‌زنی بذر جامه‌در بود. آزمایشی در قالب طرح کاملاً تصادفی در ۱۲ تیمار و ۴ تکرار شامل اسید جیبرلیک در سه غلظت (۱۰۰، ۵۰۰ و ۱۰۰۰ میلی‌گرم در لیتر)، خراش‌دهی مکانیکی با کاغذ سنباده، اسید سولفوریک (۹۸ درصد)، نیترات پتاسیم (۰/۲ درصد)، تیوره یک مولار و سرمادهی (۱۰، ۲۰، ۳۰ و ۱۵۰ روز در دمای ۲ درجه سانتی‌گراد) و شاهد (آب مقطر) بود. نتایج آزمایش نشان داد که جوانه‌زنی بذر به طور معنی‌داری تحت تأثیر تیمارها قرار گرفت. پیش تیمار سرمای ۱۵۰ و ۳۰ روزه؛ خراش‌دهی با اسید سولفوریک و کاغذ سمباده موثرترین روش‌های تحریک جوانه‌زنی بذر بودند. تیمارهای سرمای ۱۵۰ و ۳۰ روزه درصد جوانه‌زنی بذر را به ترتیب ۸۸/۴ و ۸۵/۶۵ درصد و هم‌چنین خراش‌دهی با اسید سولفوریک و کاغذ سمباده، هر دو درصد جوانه‌زنی بذر را ۷۶/۱ درصد نسبت به تیمار شاهد (آب مقطر) افزایش دادند. هم‌چنین نتایج نشان داد که تیمارهای اسید جیبرلیک، نیترات پتاسیم و تیوره تأثیری بر جوانه‌زنی بذر جامه‌در نداشتند. از این آزمایش می‌توان نتیجه‌گیری کرد که موثرترین تیمارها برای شکستن خواب بذر گیاه جامه‌در پیش تیمار سرمای ۱۵۰ روزه و خراش‌دهی مکانیکی با کاغذ سمباده بوده است.

کلمات کلیدی: تیمار پیش جوانه‌زنی، چینه بندی، خراش‌دهی، خواب بذر، گیاهان مرتعی