



## Increasing tolerance to salt-dryness stress of snail medic seedlings using magnetic field and ultrasonic waves

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### ABSTRACT

Snail medic (*Medicago scutellata* L.) is among the crucial medicinal legumes that exhibit minimal growth rates during the germination stage under salt and drought stresses. The objective of this study is to identify the sensitive components of seedling growth in response to salt and drought stresses. In the first experiment, seeds were subjected to magnetic field levels of 125 and 250 mT for varying durations (6, 12, 18, and 24 h). In the second experiment, ultrasonic waves were applied for durations of 3, 6, 9, 12, and 15 min. Both experiments were conducted using various osmotic pressures (OP) induced by NaCl (-3, -6, -9, and -12 MPa) and PEG 6000 (for similar OPs) with three replications. The results indicated that seedling growth rate (SGR), weight of mobilized seed reserve (WMSR), seed reserve depletion (SRDP), and seed reserve utilization efficiency (SRUE) were significantly affected by salt and drought stresses. Moreover, the maximum seedling dry weight (SLDW) of 0.195 mg was observed in the magnetic field treatments of 125 mT during 12 h, and the SRUE of 0.665 mg seed<sup>-1</sup> was attained in ultrasonic wave treatments of 6 and 9 min. Under drought stress conditions, the maximum root length (22.84 mm), shoot length (8.09 mm), seed germination (49.93%), WMSR (0.096 mg per seed), and SRDP (43.1%) were obtained with the ultrasonic treatment of 9 min. Furthermore, the maximum SLDW of 0.09 mg was observed in the magnetic field treatment of 250 mT, and the maximum SRUE (0.516 mg seed<sup>-1</sup>) was obtained in the control treatment. For the improvement of salt and drought tolerances, priming with magnetic field and ultrasonic waves at 125 and 250 mT for 12 h and 9 min, respectively, can be efficiently used as pre-sowing treatments for snail medic seeds.

### ARTICLE

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## 1. Introduction

Snail medic is one of the most important forage crops used in the pharmaceutical and medicine industries. This plant is usually exposed to such stresses as low temperature, drought and salinity at germination, and early growth stages (Saxena, 1996; Sharafi and Ramroudi, 2021). In recent years, seed priming has been suggested as a modern strategy for managing and coping with stress. Therefore, if the priming method can improve snail medic seed germination, one can also see its final forage yield (Kataria *et al.*, 2017; Sharafi *et al.*, 2022). Leguminous crops are highly sensitive to environmental stresses and the seedling establishment is the most sensitive period of the crop growth stage (Sharififar *et al.*, 2015; Sharafi, 2020a). Therefore, the successful establishment of the crops may affect by the germination rate (Harris *et al.*, 1999). In addition, the hard seed is the way of preventing the germination of an intact viable seed in legumes. Snail medic grows in natural conditions, and therefore, shows different levels of seed dormancy. In these conditions, seed preparation and germination rates are important for the stimulation and early growth of crops (Nazari *et al.*, 2014).

Salt and drought stresses are two complex phenomena in agricultural activities. Whereas many countries are likely to encounter negative impacts of these stresses on agriculture subsidies by chemical fertilizer due to the effects of soil salinization, droughts are still considerably unpredictable (Hosseini *et al.*, 2009). On the other hand, drought and salinity management should not be treated as an isolated problem; however, they can be considered an integral and key factor in sustainable agriculture. Until now, different methods have been applied to overcome the hard seed (such as hormones, seed scarification, and stratification, etc.). Recently, with the development of new techniques such as the magnetic field (Gholami *et al.*, 2010a) and ultrasonic waves (Salemi Nasab *et al.*, 2017; Aladjadjyan, 2010), the hard seed effect can be eliminated.

Biophysical methods, including exposure to magnetic fields and ultrasonic waves, augment plant growth by enhancing cell permeability, stimulating metabolic processes, activating gene expression patterns, modulating signal transduction pathways, and eliminating the hard seed effect. These techniques elevate the energy levels within plant cells, facilitating better nutrient and water absorption, accelerating photosynthesis and respiration, and triggering changes in stress-related gene expression. Furthermore, they disrupt barriers to germination and growth, promoting uniform development without requiring genetic modification. Through these mechanisms, magnetic fields and ultrasonic waves offer promising avenues for improving plant tolerance to salt and drought stresses (Vasilevski, 2003). The main advantage of the application of the magnetic field method over traditional chemical processes is the absence of toxic residues. Yinan *et al.* (2005) and Gholami *et al.* (2010b) found out an increase in the rate of wheat seedling elongation under magnetic field conditions. The positive effect of irradiation of seeds with ultrasonic waves on seedling growth has recently been considered by Kataria *et al.* (2015).

Until now, many studies have been conducted on breaking seed dormancy (or eliminating the hard seed effect) in forage plants such as snail medic. Therefore, the main objective of this study is to assess the effect of the magnetic field and ultrasonic waves on the seedling growth of snail medic under salt-drought stress.

## 2. Materials and Methods

### 2.1. Experimental setup

Four experiments were separately conducted at the greenhouse plant production of Arak University (Arak, Iran) to evaluate the effect of the magnetic field and ultrasonic wave under salt-drought stress.

### 2.1.1. Magnetic field

Magnetic field treatment was provided by different magnetic field induction ( $M_1$  and  $M_2$ ) and the exposure times ( $T_1$ - $T_4$ ). The magnetic fields generated by ring magnets with different magnetic induction levels with an external diameter of 7.5 cm, an internal diameter of 3 cm, and height of 1 cm for  $M_1$  and height of 1.5 cm for  $M_2$ . The magnet was placed at the top of the vessel to generate different magnetic time exposure. Besides, each roll containing 20 seeds was placed into the hole of the magnet for the end of the exposure time. The times  $T_1$ - $T_4$  were obtained by exposing the seeds to each magnetic field induction for different times, as follows: (1) Exposure to 125 mT:  $T_1$  (4 h),  $T_2$  (12 h),  $T_3$  (18 h),  $T_4$  (24 h). (2) Exposure to 250 mT:  $T_5$  (4 h),  $T_6$  (12 h),  $T_7$  (18 h),  $T_8$  (24 h). Each filter paper with seeds was rolled and placed in a vessel containing distilled water. After five hours, when the seeds were soaked, each roll was subjected to magnetic treatment. All the vessels containing rolls with seeds were labeled with numbers and randomly located to perform the experiments. The distance between the two vessels was considered at least 50 cm, to avoid the effect of each magnet on the other vessels around. Label numbers were not related to the applied magnetic field, so scores of germinated seeds and other measurements were blind (Florez *et al.*, 2007).

### 2.1.2. Ultrasonic waves

In this section, the treatments were exposed to ultrasonic waves at different times of 3, 6, 9, 12, and 15 min. The wave frequency was 42 kHz (CD4820-model: Digitaltrasonic). For ultrasonic wave exposure, all seeds were soaked in cotton fiber-containing distilled water and placed in the ultrasonic apparatus. After the wave's exposure, all seeds were placed on a filter paper moistened with 10 ml of distilled water in sterilized pots. These times were selected based on several treatments and experiments (Gholami *et al.*, 2010a, b; Salemi Nasab *et al.*, 2017).

### 2.1.3. Traits evaluation

In the first experiment, 20 seeds were weighed in five replications ( $W_1$ ), dried at 104 °C for 24 h then re-weighed ( $W_2$ ). Seed water content was calculated as  $[(W_1 - W_2) / W_2]$ . The initial seed dry weight was calculated using seed water content and  $W_1$  (Soltani *et al.*, 2006). Twenty seeds per dish were used for each treatment. It should be mentioned that the germination tests were performed according to the guidelines issued by the International Seed Testing Association (ISTA 2004).

The pots were placed in greenhouse at 22 °C with a relative humidity of 65 percent and a photoperiod of 14/10 h. After ten days, the seedling dry weight (SLDW) was obtained after oven drying at 70 °C. The weight of mobilized seed reserve (WMSR) was calculated as the dry weight of the original seed minus the dry weight of the seed remnant. The conversion efficiency of the mobilized seed reserve into plant tissue was estimated by dividing the seedling dry weight (SLDW) by the weight of the mobilized seed reserve. The ratio of utilized seed reserve to initial seed dry weight was considered as the seed reserve depletion percentage (SRDP).

## 2.2. Statistical analysis

The results of each experiment were subjected to an analysis of variance (ANOVA) to detect differences among the mean values of the parameters ( $p > 0.05$ ). Mean values were compared using the Duncan test to detect differences among the parameters of treated seedlings and control.

### 3. Results

#### 3.1. Growth parameters

In order to evaluate the effect of magnetic field intensity on seed germination of snail medic affected by salinity, such characteristics as the root length, seedling dry weight, weight of mobilized seed reserve, and seed reserve utilization rate were significant. The simple effects of the exposure time and salinity levels were significant for all characteristics. In addition, the interaction effects of the magnetic field with exposure time for root length, shoot length, seed germination percentage, seedling dry weight, weight mobilized seed reserve, and seed reserve depletion percentage, there was a significant intensity  $\times$  salinity stress for seed germination percentage and seedling dry weight, time exposure  $\times$  salinity for seedling dry weight, and also magnetic field intensity  $\times$  time exposure  $\times$  salinity interaction for root length and seed reserve depletion percentage, respectively (Table 1).

As can be seen in Table 2, the magnetic intensity for seed germination rate, seedling dry weight, the weight of mobilized seed reserve, and seed reserve utilization efficiency was significant. The effect of exposure time for all of the characteristics except seed germination rate, the effect of drought levels, and interaction effects of intensity  $\times$  exposure time for all of the traits were significant. In addition, the interaction effect between intensity  $\times$  drought for seed germination percentage, seed germination rate, seedling dry weight, and weight of mobilized seed reserve were significant; whereas, exposure time  $\times$  drought was not significant. There was also a significant difference between intensity  $\times$  time exposure  $\times$  drought stress for seed germination percentage, seed germination rate, seedling dry weight, the weight of mobilized seed reserve, seed reserve utilization efficiency and seed reserve depletion percentage (Table 2).

In addition, the interaction effect of ultrasonic waves and salinity levels was significant for root length, shoot length, root to shoot ratio, seedling growth percentage, and seedling growth rate. Furthermore, the analysis of drought stress showed obvious differences, so that the effects of ultrasonic waves and drought levels were significant on all traits (except for root to shoot ratio and seed reserve utilization efficiency). Interaction effects of ultrasonic waves with drought levels were significant for root length, shoot length, seed germination percentage, and seed germination rate and seedling dry weight (Table 3).

Root length, shoot length, seed germination percentage, and rate decreased considerably with increasing the magnetic field and ultrasonic waves. The maximum root length was observed in treatments of T2 (57.01 mm), T5 (56.54 mm), and T10 (54.76 mm), respectively. This result for shoot length was different because T1 and control treatments had the highest length of 33.73 and 31.49 mm, respectively. Furthermore, the maximum seed germination rate (100 %) and seed reserve depletion percentage (64.24 %) occurred in the control treatment, however, it produced fragile seedlings (SLDW: 0.088 mg). The seeds were affected by the magnetic field and ultrasonic waves at different levels (Table 4).

The direct relationship between seed germination percentage and seed growth rate was observed in the control treatment; however, this relationship with seedling dry weight was strong. The relationship between the seed germination percentage and the seed germination rate was constant, while the magnetic field and ultrasonic wave levels increased progressively. Therefore, the highest seed germination rate, seedling dry weight, and weight of mobilized seed reserve occurred in treatments T5 (0.02 mm d<sup>-1</sup>, 0.82 mg, and 0.34 mg seed<sup>-1</sup>). Based on the results of seed germination percentage and rate, it was found that the seed reserve utilization efficiency has a direct relationship with them. On the other hand, the maximum SRUE was observed in treatment T11 (0.87 mg. mg. seed<sup>-1</sup>) (Table 4).

Kordas (2002) investigated the effect of electromagnetic fields on spring wheat. It was observed that the seeds affected by the electromagnetic fields had significantly longer shoot lengths than control seeds so the magnetic field treatments significantly increased the shoot length ratio by 27%. Martinez *et al.* (2009) showed that lentil seeds, chickpeas, treated with

125 mT magnetic field for 10 min, significantly increased shoot and plant length compared to the control treatment.

**Table 1 The results of ANOVA test for magnetic field intensity on seed germination of snail medic during salinity stress**

SOV	RL	SL	R/S	SGP	SLDW	WMSR	SRUE	SRDP
Magnetic Field Intensity (MFI)	1206.64*	0.63 <sup>n.s</sup>	17.64 <sup>n.s</sup>	556.8 <sup>n.s</sup>	0.77**	0.11**	0.93*	0.034 <sup>n.s</sup>
Time (T)	2477.36**	1048.96**	60.17*	2645.29**	0.194**	0.2**	0.84*	0.28**
MFI×T	1555.8**	199.75*	50.44 <sup>n.s</sup>	1806.27**	0.213**	0.15**	0.74 <sup>n.s</sup>	0.16**
Salinity (S)	7822.26**	1216.54**	127.17**	10500.02**	0.4**	0.01 <sup>n.s</sup>	1.67**	0.39**
MFI×S	165.64 <sup>n.s</sup>	139.19 <sup>n.s</sup>	17.43 <sup>n.s</sup>	365.17**	0.09**	0.006 <sup>n.s</sup>	0.33 <sup>n.s</sup>	0.027 <sup>n.s</sup>
T×S	308.11 <sup>n.s</sup>	77.76 <sup>n.s</sup>	26.82 <sup>n.s</sup>	319.03 <sup>n.s</sup>	0.022**	0.006 <sup>n.s</sup>	0.35 <sup>n.s</sup>	0.035 <sup>n.s</sup>
MFI×T×S	170.2*	80.57 <sup>n.s</sup>	15.65 <sup>n.s</sup>	244.7 <sup>n.s</sup>	0.038 <sup>n.s</sup>	0.009 <sup>n.s</sup>	0.35 <sup>n.s</sup>	0.047*
Error	290.69	80.36	25.88	326.11	0.02	0.011	0.3	0.027
CV	13.78	12.06	14.16	16.1	13.42	10.17	19.57	11.51

n.s.: not significant; (\*) and (\*\*) represent significant difference over control at  $p < 0.05$  and  $p < 0.01$ , respectively.

Abbreviations: RL: Root Length, SL: Shoot Length, R/S: Root/shoot, SGP: Seedling Germination Percentage, SLDW: Seedling Dry Weight, WMSR: Weight of Mobilized Seed Reserve, SRUE: Seed Reserve Utilization Efficiency, SRDP: Seed Reserve Depletion Percentage.

**Table 2 The results of ANOVA test for magnetic field intensity on seed germination of snail medic during drought stress**

SOV	RL	SL	R/S	SGP	SGR	SLDW	WMSR	SRUE	SRDP
Magnetic Field Intensity (MFI)	3.28 <sup>n.s</sup>	3.31 <sup>n.s</sup>	0.32 <sup>n.s</sup>	1.7 <sup>n.s</sup>	0.002*	0.362**	0.076*	0.26*	0.035 <sup>n.s</sup>
Time (T)	567.56**	177.83*	11.86*	2443.32**	0.0 <sup>n.s</sup>	0.142*	0.124**	0.23**	0.126*
MFI×T	352.55*	185.97*	12.7*	2715.45**	0.001**	0.272**	0.154**	0.22**	0.399**
Drought (D)	3116.48**	1093.57**	26.75**	11858.5**	0.006**	0.849**	0.241**	1.66**	1.35**
MFI×D	187.25 <sup>n.s</sup>	91.7 <sup>n.s</sup>	2 <sup>n.s</sup>	936.97*	0.001**	0.093*	0.048*	0.097 <sup>n.s</sup>	0.089 <sup>n.s</sup>
T×D	113.27 <sup>n.s</sup>	32.88 <sup>n.s</sup>	6.32 <sup>n.s</sup>	353.61 <sup>n.s</sup>	0.0 <sup>n.s</sup>	0.04 <sup>n.s</sup>	0.019 <sup>n.s</sup>	0.081 <sup>n.s</sup>	0.053 <sup>n.s</sup>
MFI×T×D	189.24 <sup>n.s</sup>	84.93 <sup>n.s</sup>	5.24 <sup>n.s</sup>	783.2**	0.001*	0.082*	0.042**	0.092*	0.098*
Error	131.22	59.08	5.78	357.77	0.0	0.043	0.014	0.056	0.046
CV	11.88	14.26	17.88	11.11	28	13.15	9.83	10.84	6.77

n.s.: not significant; (\*) and (\*\*) represent significant difference over control at  $p < 0.05$  and  $p < 0.01$ , respectively.

Abbreviations: RL: Root Length, SL: Shoot Length, R/S: Root/shoot, SGP: Seedling Germination Percentage, SGR: Seedling Growth Rate, SLDW: Seedling Dry Weight, WMSR: Weight of Mobilized Seed Reserve, SRUE: Seed Reserve Utilization Efficiency, SRDP: Seed Reserve Depletion Percentage.

**Table 3 The results of ANOVA test for ultrasonic waves on seed germination of snail medic during salt-drought stress**

SOV	RL (mm)	SL (mm)	R/S	SGP (%)	SGR (mm.d <sup>-1</sup> )	SLDW (mg)	WMSR (mg.seed <sup>-1</sup> )	SRUE (mg.mg.saeed <sup>-1</sup> )	SRDP (%)
<b>Salinity</b>									
Ultrasonic (U)	153.17**	325.97**	132.11*	6561.13**	0.0**	0.008*	0.004**	0.75**	0.014**
Salinity (S)	3195.28**	588.64**	229.5**	16431.17**	0.001**	0.016**	0.014**	1.33**	0.133**
U×S	406.72**	108.35**	203.5**	1237.8**	0.0**	0.004 <sup>n.s</sup>	0.002 <sup>n.s</sup>	0.228 <sup>n.s</sup>	0.036 <sup>n.s</sup>
Error	70.08	23.37	47.6	148.73	0.0	0.003	0.001	0.218	0.023
CV	7.84	10.52	19.58	19.97	8.82	20.53	12.41	15.27	12.41
<b>Drought</b>									
Ultrasonic (U)	1254.53**	157.35**	1481.1 <sup>n.s</sup>	7327.87**	0.0**	0.002**	0.018*	3.42 <sup>n.s</sup>	0.434*
Drought (D)	1646.87**	237.22**	2015.1 <sup>n.s</sup>	11875.9**	0.001**	0.006**	0.049**	3.25 <sup>n.s</sup>	1.87**
U×D	405.5**	58.02**	2119.9 <sup>n.s</sup>	1632.84**	0.0**	0.001**	0.009 <sup>n.s</sup>	2.12 <sup>n.s</sup>	0.225 <sup>n.s</sup>
Error	8.76	6.22	1855.07	74.12	0.0	0.0	0.009	1.99	0.206
CV	13.79	17.89	45.9	13.02	8.9	13.43	16.34	6.29	16.2

n.s.: not significant; (\*) and (\*\*) represent significant difference over control at  $p < 0.05$  and  $p < 0.01$ , respectively.

Abbreviations: RL: Root Length, SL: Shoot Length, R/S: Root/shoot, SGP: Seedling Germination Percentage, SGR: Seedling Growth Rate, SLDW: Seedling Dry Weight, WMSR: Weight of Mobilized Seed Reserve, SRUE: Seed Reserve Utilization Efficiency, SRDP: Seed Reserve Depletion Percentage.

**Table 4 The mean comparison of magnetic field and ultrasonic waves on seed germination of snail medic**

Treatment		RL (mm)	SL (mm)	SGP (%)	SGR (mm.d <sup>-1</sup> )	SLDW (mg)	WMSR (mg.seed <sup>-1</sup> )	SRUE (mg.seed <sup>-1</sup> )	SRDP (%)
Control	C	48.3b	31.49ab	100a	0.02a	0.088f	0.13b	0.699c	64.24a
M 125 (mT)	T <sub>1</sub>	43.64c	33.73a	63.3d	0.016a	0.053f	0.12b	0.49e	59.33ab
	T <sub>2</sub>	57.01a	21.63c	60d	0.016a	0.093f	0.126b	0.73bc	61.3a
	T <sub>3</sub>	37.23d	10e	46.46ef	0.006c	0.47bc	0.063c	0.38f	46.66c
	T <sub>4</sub>	17.93g	5.65f	30gh	0.01b	0.04f	0.003f	0.53e	41d
M 250 (mT)	T <sub>5</sub>	56.54a	29.73b	63.3d	0.02a	0.82a	0.34a	0.7c	55.33b
	T <sub>6</sub>	43.92c	17.46cd	50e	0.013ab	0.57b	0.09bc	0.76b	43.3cd
	T <sub>7</sub>	52.05ab	16.39cd	23.32i	0.01b	0.3d	0.045c	0.72bc	39.2d
	T <sub>8</sub>	21.58f	9.92e	46ef	0.02a	0.48bc	0.013de	0.65cd	52.3b
Ultrasonic	T <sub>9</sub>	31.74e	6.75f	86.6b	0.016a	0.066f	0.055c	0.548e	27.38e
	T <sub>10</sub>	54.76a	21.17c	93.3ab	0.018a	0.068f	0.107b	0.751b	46.22c
	T <sub>11</sub>	27.2ef	9.84e	70.31c	0.013ab	0.23e	0.114b	0.87a	56.2b
	T <sub>12</sub>	2.83h	0.86g	35.6g	0.012b	0.008g	0.023d	0.392f	11.34f
	T <sub>13</sub>	1.03h	0.01g	11j	0.011b	0.001g	0.053c	0.046g	26.22e

In each column, there is no significant difference between treatments with common letters according to Duncan test at a significance level of 0.05.

C: Control, M 125, 250: Magnetic field 125, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>: 4, 12, 18, 24, Magnetic field 250, T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub>, T<sub>8</sub>: 4, 12, 18, 24 respectively. U: Ultrasonic T<sub>9</sub>, T<sub>10</sub>, T<sub>11</sub>, T<sub>12</sub>, T<sub>13</sub>: 3, 6, 9, 12, 15 min, respectively.

Abbreviations: RL: Root Length, SL: Shoot Length, R/S: Root/shoot, SGP: Seedling Germination Percentage, SGR: Seedling Growth Rate, SLDW: Seedling Dry Weight, WMSR: Weight of Mobilized Seed Reserve, SRUE: Seed Reserve Utilization Efficiency, SRDP: Seed Reserve Depletion Percentage

Magnetic field treatment enhances plant growth through free radicals involved in chemical reactions and increased the activity of proteins and enzymes. In this research, seed germination was accelerated by the ultrasonic wave and magnetic field, and a significant difference with control treatment was observed. The effect of drought stress using polyethylene glycol (PEG) on seed germination of the snail medic showed a drastic inhibition of the germination percentage and rate (Iqbal *et al.*, 2012; Sharafi, 2020b).

Magnetic fields affect both the activity of ions and the polarization of dipole molecules in living cells. The magnitude of the effect of the electromagnetic field on seed germination and seedling growth depends on the time and type of seeding and seed. Similar to oak and cotton, low intensity of electromagnetic field increased the germination rate, whereas increased electromagnetic field intensity did not change the germination of these two seeds; however, it was observed that increasing the exposure time of electromagnetic field increases the germination percentage and shoot fresh weight (Podleony *et al.* 2003). Also, the creation of 125 mT for exposure times of 1, 11, 21, and 21 min to 24 hours increased the length and weight of seedlings, respectively (Martinez *et al.* 2009a). According to the results, it can be generally concluded that the intensity of the ultrasound waves is much more important than the exposure time to ultrasonic waves. Using 700 kHz ultrasound in radish seeds, Shimomura (1990) reported that these waves increased the germination rate and increased the root length by 13 to 16 percent. In addition, an increase in germination percentage in barley seeds was reported after ultrasonic treatment (Yaldagard *et al.*, 2008).

### 3.2. Seed Reserve Mobilization in response to salt-drought stress

Seed reserve mobilization was determined for both treatments under salt-drought stress. The results showed that there is a significant difference in seed reserve mobilization between the treated and the control seeds in response to salinity levels of -3 MPa and -6 MPa, in addition to the magnetic field and ultrasonic wave treatments. Seed reserve mobilization was 0.49 mg seed<sup>-1</sup> in drought stress under the magnetic intensity of 250 mT and exposure time T<sub>5</sub>. Whereas, after exposure to ultrasonic wave, it was reduced to 20% (Figure 1A, 1B).

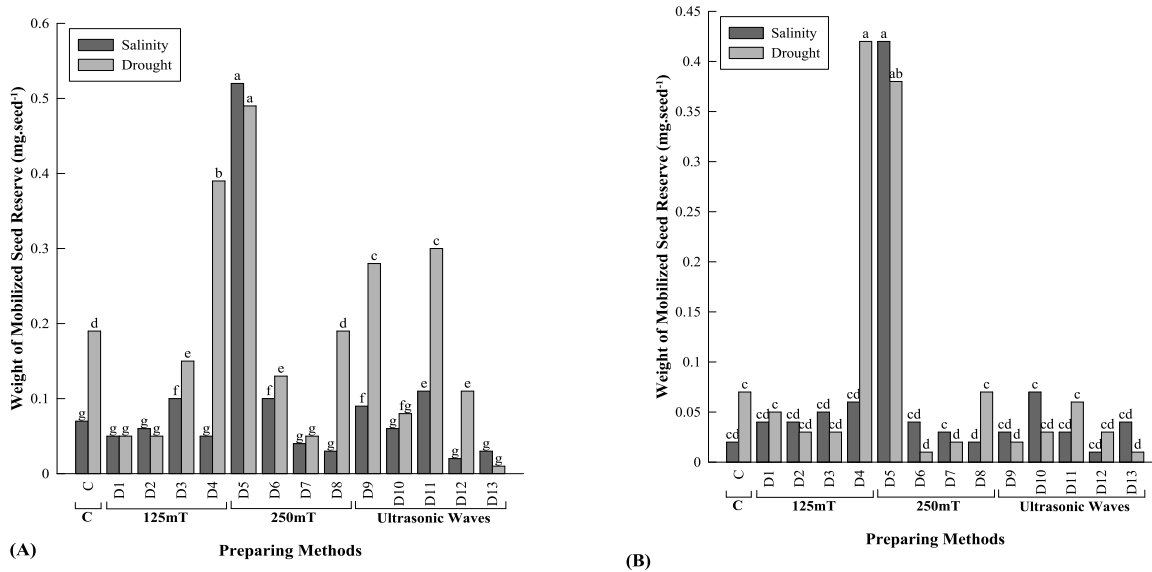


Figure 1 The effect of magnetic field and ultrasonic wave levels on weight of mobilized seed reserve of snail medic under -3 MPa (A), -6 MPa (B) of salt-drought stress.

The results of seed reserve utilization efficiency indicated the same trends as shown for seed reserve mobilization. The significant difference was shown in both treatments of magnetic field and ultrasonic wave under salt-drought stress. Magnetic field of 250 in treatment T5 under salinity level of -3 MPa had the highest seed reserve to 0.9 as compared to level of -6 MPa (Fig. 2A, B).

Various researchers have suggested that the positive effects of exposed seeds in a magnetic field on various growth parameters are highly affected by drought stress, which leads to increased ion and water uptakes, and ultimately acceleration of chemical processes. The seeds exposing to the magnetic field become more swollen due to the increase in water uptake, and therefore, the auxin hormone becomes more active (Meiqiang *et al.*, 2005). In addition, respiration is increased and more energy is used to increase the germination rate; therefore, the tolerance of stress in plants during growth will be uniform and continuous. In the present study, although with increasing the drought stress at magnetic field intensity of 125 mT, negative effects on germination rate were observed, at the exposure time to ultrasonic waves for 6 and 9 min under salt-drought stress, some germination traits are improved.

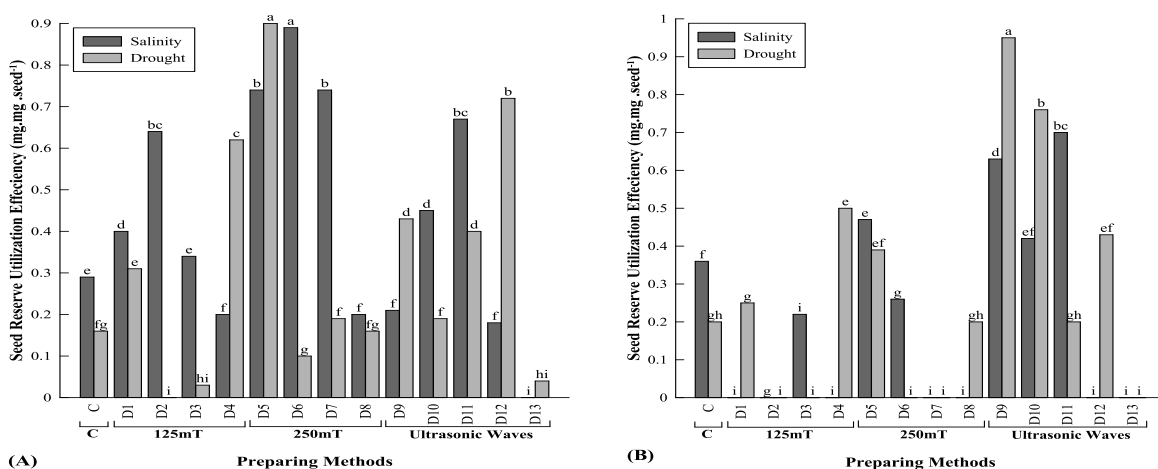


Figure 2 The effect of magnetic field and ultrasonic wave levels on seed reserved utilization efficiency of snail medic under -3 MPa (A) and -6 MPa (B) of salt-drought stress.

Plants usually contain iron-containing cells that have a particular influence on plant growth. The reason for the difference in response to the magnetic field in plants depends not only on the magnetic field level but also on the physiological growth stage in which the plant is tested. It is concluded that the magnetic field can increase plant growth by increasing such elements as Mg and Ca. On the other hand, increasing the contents of ions can increase the nutritional value of plants. The magnetic field affects the spread of biological particles in the solvent through the Lorentz and Maxwell forces. Lorentz force affects the release of such particles as plasma proteins. The orientation of the ferromagnetic particles and the oscillations of the coupled radicals are considered the mechanism of the magnetic field effect. Under the influence of ultrasound due to the heat generation, there are many biochemical changes in the tissues, such as increasing the rate of chemical reactions, increasing the rate of release of substances, breaking down of substances such as enzymes, and the destruction of microorganisms (Li *et al.* 2005; Majd and Shabrangi 2009). In general, the results indicated clearly that a decline in seedling dry weight in response to drought and salinity is a consequence of the decline in the weight of mobilized seed reserve (seed reserve depletion percentage), not the conversion efficiency of the mobilized reserve to seedling tissue. This is in agreement with Soltani *et al.* (2006) findings on chickpea. They showed that the reduction of seedling dry weight was a result of a decrease in seed reserve mobilization.

#### 4. Conclusion

The results of this study revealed a direct correlation between the decline in seedling dry weight and the decline in the weight of mobilized seed reserve. The ultrasonic waves' treatments, had significant effects on seedlings resistance to salinity and drought. Furthermore, the use of ultrasonic waves produced higher germination percentage and more vigorous seedlings under drought and saline conditions. In terms of germination and seedling growth, the differences among treatments increased with the increase in stress intensity. This advantage led to greater seed reserve utilization. Moreover, prepared seeds resulted in longer cotyledon length and more improvement in vigorous seedlings. The maximum of seed germination (47.2%) was indicated in the control treatment; the maximum seedling dry weight (0.195 mg) was identified in magnetic field treatment; and the maximum of seed reserves utilization efficiency (0.665 mg.seed<sup>-1</sup>) was obtained in ultrasonic waves treatments. In the drought experiment, the maximum root length (22.84 mm), shoot length (8.09 mm), seed germination (49.93 %), the weight of mobilized seed reserve (0.096 mg.seed<sup>-1</sup>), and seed reserve depletion percentage (43.1%) was measured in ultrasonic treatment; the maximum of seedling dry weight (0.09 mg) was found in magnetic field treatment, and the maximum of SRUE (0.516 mg mg seed<sup>-1</sup>) was observed in the control treatment.

The results showed that the seed preparation methods in both salinity and drought experiments have a significant impact on the root length, shoot length, germination percentage, and seedling dry weight. In the salinity experiment, the weight of mobilized seed reserve and seed reserve depletion percentage were affected by preparation methods. According to the obtained results, more studies should be done on the activity of enzymes or hormones affecting the dynamics of seed reserves encountering other environmental stresses will be useful in both soil and pot mediums.

#### Conflict of interest

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. In addition, I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all of us. I confirm that I have given due consideration to the protection

of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing I confirm that I have followed the regulations of our institutions concerning intellectual property.

### Abbreviation

ANOVA	Analysis of variance	RL	Root length
GR	Germination rate	R/S	Root/shoot
h	Hour	SGP	Seedling germination percentage
ISTA	International Seed Testing Association	SGR	Seedling growth rate
kHz	Kilo Hertz	SLDW	Seedling dry weight
M1	Magnetic field 125 mT	SOV	Source of variance
M2	Magnetic field 250 mT	SRDP	Seed reserve depletion
MFI	Magnetic Field Intensity	SRUE	Seed reserve utilization efficiency
min	Minute	SL	Shoot length
MPa	Mega Pascal	T1	Exposure times (4 h)
mT	Milli Tesla	T2	Exposure times (12 h)
OP	Osmotic pressures	T3	Exposure times (18 h)
n.s	not significant	T4	Exposure times (24 h)
PEG	Poly ethylene glycol	WMSR	Weight of mobilized seed reserve

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