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Investigation of nursery treatments on sea buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson) seed germination in the field

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Abstract

This study was conducted for the first time in nursery treatment in order to pioneer forest trees of the species *Elaeagnus rhamnoides* and also to determine the effects of different treatments on seed germination in Iran. This species is resistance for plantation. Seeds originating from Qazvin province were placed in the nursery. Treatments comprised three factors including seed pretreatments (control, cold, ice water, hot water, lime juice and Gibberellin acid), soils (control, Stockosorb, sand and compost) and irrigation (control and supplementary water). These treatments were accomplished in CRD experimental designs with five replicates. At the end (65st day) we evaluated the number and percentage of germination, the number of days until germination, the mean germination time, germination rate, germination energy, the maximum germination and germination value. Results show that the means of germination percent in control irrigation were 7.5, 23.75, 21.25, 0, 15, and 42.5. Average germination percentage of pretreatments and the same attributes with supplementary irrigation were 1.25, 43.75, 35, 1.25, 8.75, and 46.25. Mean of germination in soil factor were 0.83, 3.54, 8.12 and 8.02. Germination in all treatments and at whole levels was on average 20.52 percent. The highest germination percentage and the highest average germination were observed in the sand and in the Gibberellin treatment, respectively.

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Introduction

Researchers need on strategies to increase the success rate of afforestation and tree production and provision of appropriate application of the multipurpose seedlings due to increasing forest degradation in recent years (Ranal and Santann, 2006). Early plantation species in arid and semiarid regions is essential. The sea-buckthorn (*Elaeagnus rhamnoides* (L.) A.Nelson) is one of the pioneer and medicinal species which is famous for its stabilizing effect of soil nitrogen (Marvi Mohajer, 2005). In this study this Irano-Turanian indigenous species (Mozaffarian, 2005; Sabetti, 1993, Ghahraman, 1995) was used to examine its seed germination and establishment. The sea-buckthorn is a shrub-deciduous species, resistance to cold, drought and low coverage regions (Zhang *et al*., 2010). The *Hippophae* plants, the sea-buckthorns, are deciduous shrubs in the family *Elaeagnaceae* The exact number of species within the genus is still unclear. However, there are considered to be seven species. The male bud consists of four to six apetalous flowers, which produce wind-distributed pollen whereas, the female bud usually consists of one single apetalous flower with one ovary and one ovule (Geetha and Asheesh, 2011). The Genetic Resources of *Hippophae* are very rich. *Hippophae* includes 15 species and subspecies that are widely distributed in Europe and Asia (Lu and Ahani, 2013). The species has been used for inflammation of the mouth, stomach ulcers, radiation injuries and burns in traditional Chinese medicine and Russia (Lu, 1992). Moreover, the fruit and leaves of this species are used as anti-bacterial and antioxidant materials (Geetha *et al*., 2002). The oil extracted from the berries is used for treatment of gastritis, stomach ulcers, erosion of uterus and inflammation of genital organs. The sea-buckthorn leaves contain nutrients and bioactive substances which mainly include flavonoids, carotenoids, free and esterified sterols, triterpenols, and isoprenols. The leaves comprise an equally rich source of important antioxidants such as carotene, vitamin E, catechins, elagic acid, ferulic acid, folic acid and significant values of calcium, magnesium and potassium (Geetha and Asheesh, 2011). The bone-breaking fever virus in the blood is inhibited using substances extracted from the sea-buckthorn leaves (Jain, 2008). Total phenolic content of root and seed extracts were significantly higher than that of the leaf and stem. On the other hand, there are no significant differences between root and seed, or between leaf and stem (Michel *et al*., 2012). In order to start germination, embryos of dry seed need special conditions such as humidity, temperature and oxygen (Vilela *et al*., 2001; Kuriakose and Prasad, 2008). Using effective treatments such as hydrolyzing enzymes which increase metabolic activities in seed will change the synthesis of plant hormones such as cytokinin and tryptophan, and ultimately prepare physiological conditions for the development of the embryo (Benito *et al*., 2005 and Elsayed *et al*., 2009). These enzymes increase the resistance of plants encountering drought, cold deceases (Oliet *et al*., 2009). Furthermore, they improve the survival of seedlings (Farooq *et al*., 2006). An essential condition to produce good seedlings is edaphic nutrition, because seedling production efficiency is increased with improvement of biological, chemical, and physical characteristics (Jocobs *et al*., 2005). Seeds soaked in water or potassium nitrate solution at room have germinated 34.5% more in comparison with gibberellin and hot water treatment (Li and Wardle, 1999). In contrast, pre-soaking seeds of Sea buckthorn with KNO3 showed negative effect on seed germination comparing to the water treatment (Korekar *et al*., 2013). Mean germination percentage of pretreatment with GA3 was 37.5 in green house reproduction (Ahani *et al*., 2014). GA_3 significantly improved germination in two populations of India of *Elaeagnus rhamnoides*. mean germination time was also reduced by GA_3 treatments in all the populations as compared to control. A high degree of variation with regard to the germination percentage in different populations is recorded (Vashistha *et al*., 2013). Then this is necessary for us to find germination of native Sea buckthorn in out of green house condition. This

work aims to provide data on plantation forest species with special focus on the issues affecting the successfulness of the sea buckthorn germination in the nursery in Iran. This study was attempted to improve Iranian sea buckthorn germination percentage to reduce its mean germination time and other germinated factors.

Materials and methods

Study area

The area of this study was located in the nursery Department of Natural Resources and Watershed Management of Khorasan Razavi, Iran. In the nursery the average temperature and the humidity were 30 °C and 60%, respectively. In this work, after examining various queries (due to the lack of knowledge concerning the species name which is confused with Chinaberry), sea buckthorn fruit was prepared from the Department of Natural Resources of Qazvin (Figure 1). After winnowing the weight of 1000 grains was measured 12 grams.

Treatments

Then six pretreatments with four different irrigation treatments were implemented based on two types of soil (Table 1) to form a factorial experiment in a completely randomized design. Seeds were sown in plastic pots by using 4 types of soil including, 1) control soil (normal nursery soil); 2) control soil + water absorbent by stockosorb 0.3% (Zangoee Nasab *et al.*, 2012) ; 3) sand; and 4) control soil : litter (3:5). Nursery irrigation was performed with tap water (control) and it was supplemented with 1:5000 (Saeed Afkham Shoara, 2013). The supplementary water was made by using macro and micro elements. Four seeds and each with five replicates were sown for each treatment per pot for a total of 960 seeds. At the same depth seeds were sown on 15 Jan 2013. To disinfect the seeds the fungicide carbendazim was used with a ratio of 2 to 1000. Counting the germinated seeds was started 10 days after planting and it was continued for 35 days. Data were collected before and after the germination.

Analyses

Finally, we evaluated the number and percentage of germination, mean germination time, germination rate, germination energy, maximum germination, and germination value each calculated by the formula as follows:

Percentage of germination: The number of germinated seeds/the number of seeds planted × 100 (Panwar and Bahardwaj, 2005).

Mean germination time: Total (number of seeds germinated per day × day count)/the total number of germinated seeds during germination (Kulkarni *et al.*, 2007).

Germination value: Maximum germination × Mean germination daily (Czabator, 1962).

Germination rate (speed): The sum of the total number of germinated seeds per period/the number of days after the germination (Panwar and Bahardwaj, 2005).

Maximum germination: Cumulative germination percentages/the number of days during drilling (Czabator, 1962).

Germination energy: The number of germinated seeds in a sample with maximum germination (the highest number in a given day) (William, 2005). Furthermore, a graph was drawn for a cumulative 65-day germination period (to ensure that all seeds have been germinated).

Data were analyzed using the SAS software. Diagrams were drawn using the EXCEL software. The normality test was used using Ryan-Joiner (similar to Shapiro-Wilk) and then the homogeneity of variance was carried out using the Dunnet method. In order to determine statistical significance of differences between germination characteristics we used the

variance analysis. When differences were significance the SNK test was used to compare the means. Moreover, the grouping was applied separately for replications, pre-treatment, soil and irrigation. The Pearson correlation was used to determine the correlation between parameters using the Minitab software (Mesdaghi, 2011).

Table 1. Pretreatments of Seeds

Treatment	Characteristics
Ice water	16 days in 0°c and every 5 days set to 24 hours in 30°c
Cold	20 days in 4°c
Lime water	5% lime in water at 8 hours
Gibberellin acid	500mg per litter for 40 hours
Hot water	water at 90°c by 15 minutes
Control	pure Seed

Results and discussion

Germination

All of the data taken (240) were analyzed by the GLM procedure. So powerful parametric test method even for the data analysis of variance was performed (Mansoorfar, 2006). Average germination percentage of pretreatments was respectively 7.5, 23.75, 21.25, 0, 15, and 42.5 in control irrigation and same attributes were 1.25, 43.75, 35, 1.25, 8.75, and 46.25 in supplement irrigation. Germination percentage of days, the division of irrigation, soil types, and treatments are presented in Figure 2. Results of traits in all repeats by irrigation types are presented in Table 2. Relationship between indices: The indexes of germination are correlated with each other Table 3. Statistical parameters are presented in Table 4.

Germination rates were near zero in control soil, only in supplementary irrigation in pre-treated with cold and ice water little germination was observed. In hot water and lime water pretreatments, germination observed in all soils and two irrigations were minimal. Pretreatment of germinating gibberellin and cold in sand (3) and compost (4) showed the highest germination rate. There was no significant difference between the supplementary irrigation with control water.

Mean comparison

Comparisons of results SNK mean separation at 5% level replication, irrigation, soil and pretreated are as follows: 1) Grouping all iterations for all parameters in group A was the one, 2) Grouping for irrigation: a group for all indices except days to germination index was in the 2 group, 3) Grouping soils: all were classified in four groups except germination energy and germination values were in the 3 groups and 4) Grouping in Pretreatments: in Table 5 it is indicated that some groups have similarities.

Fig. 1. Sea buckthorn berry (Left), seeds in pots in winter season

Table 2. Results of all of repeats and pretreatments by irrigation types

Soil / Traits	Supplement irrigation				Normal irrigation			
	1	2	3	4	1	2	3	4
Number of germination	7	23	41	39	1	13	37	36
Percent of germination	3.55	11.67	20.81	19.79	0.50	6.59	18.78	18.27

Mean of germination percentage in all of treatments was obtained 20.52%. The earliest germination was seen on 47th day and the final germination of seeds was observed on 65th day. Mean of germination in soil factor in all of treatments were 0.83, 3.54, 8.12 and 8.02 respectively.

Table 3. Correlation Pearson results between traits

Traits	1	2	3	4	5	6	7
1. Number of germination	1						
2. Percent of germination	0.99**						
3. Day to germination	0.72**	0.72**					
4. Mean time to Germination	0.99**	0.99**	0.75**				
5. Rate of germination	0.78**	0.78**	0.45**	0.72**			
6. Energy of germination	1	0.99**	0.72**	0.99**	0.78**		
7. Maximum of germination	0.78**	0.78**	0.45**	0.72**	1	0.78**	
8. Value of germination	0.82**	0.82**	0.38**	0.81**	0.81**	0.82**	0.81**

** significant at 0.01 level

In this study the correlation between indexes is significant. If the correlation coefficient is near 1, the population is likely to be normal.

Table 4. Statistics characteristics of traits

Trait	Mean	Standard Error	Determination coefficient	Significance	Root MSe
Number of germination	0.82	0.08	0.61	0.0001	0.84
Percent of germination	20.52	1.94	0.62	0.0001	21.02
Day to germination	23.5	1.68	0.71	0.0001	15.89
Mean time to Germination	0.22	0.02	0.61	0.0001	0.22
Rate of germination	0.01	0.002	0.53	0.0001	0.02
Energy of germination	2.85	0.32	0.43	0.0001	4.14
Maximum of germination	0.33	0.04	0.53	0.0001	0.51
Value of germination	0.22	0.04	0.42	0.0001	0.52

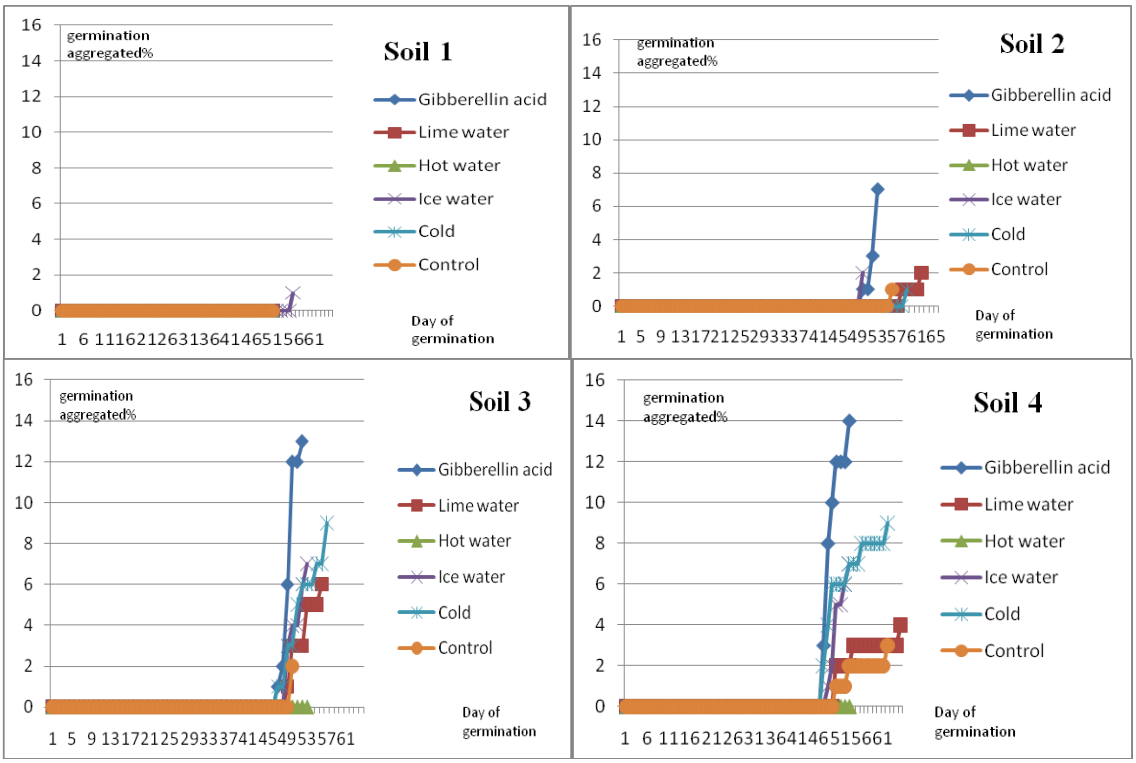

Fig. 2. Percentage of germination in control irrigation (1, 2, 3, 4 are soil treatments) Pretreatments of seeds are shown by color of curves

Figure 2 showed the aggregated germination compared with day of germinated seeds for all repeats of pretreatments by soil treatment for control irrigation divided.

Table 5. SNK test grouped for pre treatment

Trait	Group
Number of germination	A (6) ،B(2-3) ،C(5-1) ،D (4-1)
Percent of germination	A (6) ،B(2-3) ،C(5-1) ،D (4-1)
Day to germination	A (6-3-2) ،B(5) ، C(1)، D(4)
Mean time to Germination	A (6) ،B(2-3) ،C(5-1)، D(4-1)
Rate of germination	A (6-2-3) ،B(5-1-4)
Energy of germination	A (6-3-2) ،B(5-3-2) ،C(2-1-4)
Maximum of germination	A (6-2-3) ،B(5-4-1)
Value of germination	A (6-2) ،B(3-2) ،C(5-4-3-1)

A, B, C and D are groups. 1, 2, 3, 4, 5 and 6 are pretreatments.

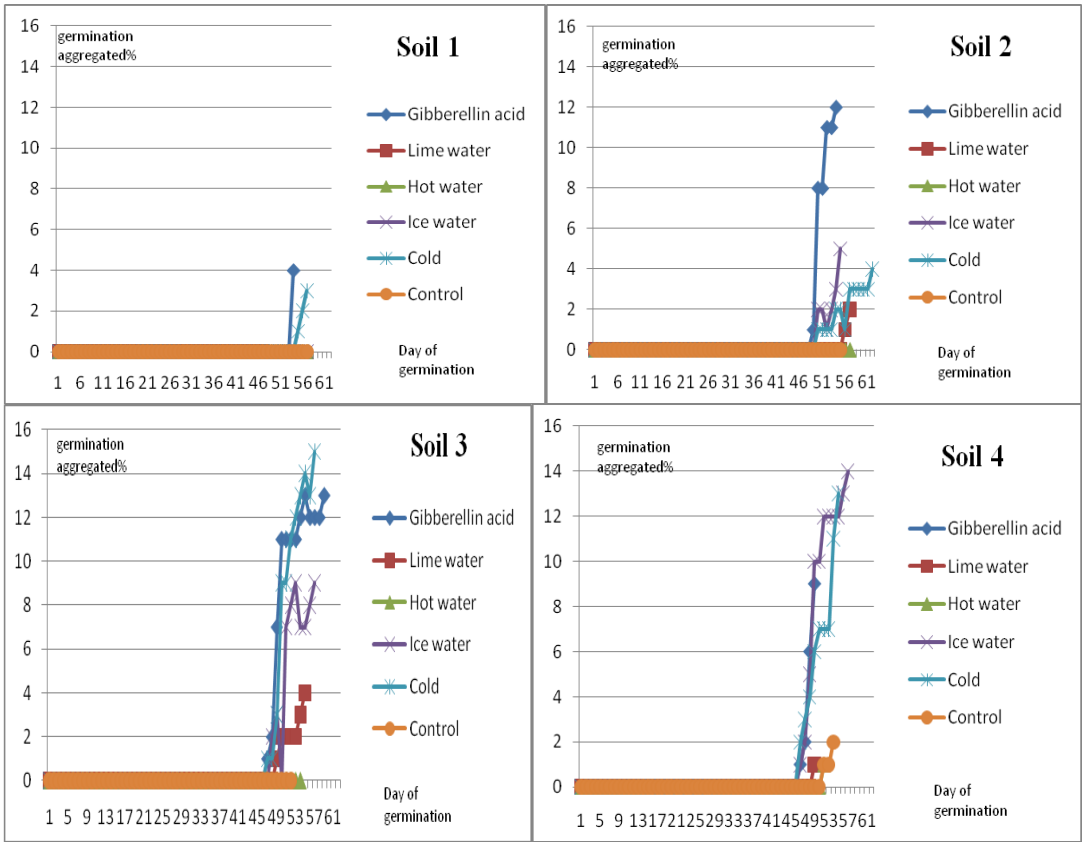

Fig. 3. Percentage of germination in supplementary irrigation (1, 2, 3, 4 are soil treatments) Pretreatments of seeds are shown by color of curves

Figure 3 showed the aggregated germination in comparison to day of germinated seeds for all repeats of pretreatments by soil treatment for supplementary irrigation divided. In control soil (type 1) there has been more germination than in control irrigation.

The least square mean difference test for germination percentage showed the significant difference between soil types 3 and pretreatment type 2, 3, 5 and 6; in addition between soil types 4 and pretreatment type 2, 3, 5 and 6.

In supplementary irrigation LS mean showed the significant difference between soil types 1 with pretreatment type 6; also between soil types 2 and pretreatment type 2, 3, 5 and 6. Furthermore between soil types 3 and pretreatment type 2, 3, 5 and 6. Results showed significant difference between soil types 4 and pretreatment type 2, 3 and 6.

Final assessment of germination in both irrigation in soil types 3 and 4 was significant. Least squares means for interactive effects have been shown in table 6.

Table 6. Least squares means for effects of IRR×SOIL×PTRT (Pr > |t|)

Trait	Control irrigation*	Supplement irrigation*
Number of germination	S2P6, S3P2356,S4P236	S2P36,S3P12356,S4P236
Percentage of germination	S2P6, S3P2356,S4P236	S2P36,S3P12356,S4P236
Day to germination	S2P56,S3P12356, S4P236	S2P356, S3P12356, S4P2346
Mean time to Germination	S2P6, S3P2356, S4P236	S2P36, S3P12356, S4P236
Rate of germination	S2P6, S3P2356, S4P236	S2P36, S3P12356, S4P236
Energy of germination	S3P2356, S4P2	S2P3, S3P12356, S4P236
Maximum germination	S2P6, S3P2356, S4P236	S2P36, S3P12356,S4P236
Value of germination	S3P256, S4P236	S2P3, S3P236, S4P26

*Soil type: S, Pretreatment: P, For example S2P56 means: between soil type 2 and pretreatments 5 and 6 are significant difference.

Least squares means for mutual effect of pretreatments and soil in traits of germination between soils types 3, 4 and pretreatments 2, 3, 5 were significant. Then the best treatment is gibberellins and the best soil is sand, but soil types 4 and ever pretreatment except 4 are good.

The results can be seen in Table 5 which indicated that most of the parameters measured (except value of germination) in the sand soil and control pretreatment in supplement irrigation has been significant.

Regardless of the type of soil in conventional irrigation, rapid germination, early in treatment, gibberellin, cold and ice water (47th day) and highest for the treatment of hot water to start germination was observed. Furthermore supplemental irrigation caused faster initial germination through gibberellins treatment (47th day) and the maximum term to initiate germination was in hot water pretreatment. Percentage, vigor and germination rate are the main factors affecting seed germination and plant establishment (Pederson *et al*, 1993). Comparison of the obtained means with those of the control type showed that most of the parameters in cold and gibberellin treatments were different. The highest germination percentage was observed in the treatment of cold and gibberellins which is the same as result of Sasani *et al.*, 1386 in humid cold and gibberellin in *Carum carvi.*

Cold treatment for seed germination of *Calloginum* was the most significant for germination (Ren and Tao, 2004). Treatment of *Rubia tinctorum seeds by hot water showed* contradictory results to our study. Hot water, Wear paper and sulfuric acid cause increasing of germination percentage in *Rubia tinctorum* (Farhoudi *et al.*, 2006). Result of study in Acacia by hot water lead to increasing of germination (Ibrahim Mohamed *et al.*, 2004) which might be for the hard seed coat. To overcome dormancy, Scarification treatments and layering of hot and cold water and acid is effective in the short term (Ertekin and Kirdar, 2010) but in our study hot water did not gave good results. Conventional treatments were used in this study that the gibberellin and cold pretreatment, soil sandy and soil with compost is the best answer and supplemental irrigation had little effect on germination. It seems that the type of seed

dormancy is Thermo dormancy because of Thermal fluctuations (Heating and cooling swing) lead to germination improvement. Hot water treatment makes seed recession; so seeds were permeable and did not have physical dormancy (Schmidt, 2007). Lines that begin to germinate at low temperatures are able to be useful if planted late in autumn (Zeinali *et al*., 2010). Removing barriers to seed which stop water and nutrients to reach the seed will increase its metabolic function. The more the embryo nutrient rich reserves, the faster the germination will be. (Marcano *et al*., 2005; Kuriakose and Prasad, 2008). The seeds require a cold period to break the dormancy that is naturally found within them, this is easily achieved by placing the prepared bag of seeds and compost mix in the fridge (4 Celsius or 39F) for around 12 weeks. It is quite possible for the seeds to germinate in the bag at these temperatures when they are ready to do so, if they do, just remove them from the bag and carefully plant them up (Tree Seed Online Ltd, 2013).

The effect of moist chilling treatment-by itself- on breaking seed dormancy was remarkable in this plant as germination increased up to 89%. Effect of combined chilling and gibberellin treatment was not so remarkable. On the other hand, other applied treatments had no effects on breaking seed dormancy in Rheum ribes which indicates that the type of seed dormancy in Rheum ribes is not physical or is due to the accumulation of inhibitory substance in seed coat. We might conclude that the reason had been physiological dormancy of the seeds (Nabaei *et al*., 2011). Seven seeds pretreatments; soaking in boiling water, 98% sulfuric acid for 5, 10 and 15 min, chilling, potassium nitrate and 0.2 and control of the Zygophyllum species was done and germination after 14 days were measured. Comparison means showed the best result obtained by hormone (potassium nitrate) pretreatment (Soltanipour *et al*., 2012) and in our study hormone had the best result. Gibberellin, Thiourea and potassium nitrate are THE same hormones approximately, that research on doze of these hormones is suggested for future studies. In the present study the effect of cold and gibberellin treatments on survival was significant. The plant hormone stimulates the absorption of nutrients and increases crop yields and increased enzyme activity and metabolism of carbohydrates, proteins, organic acids, and mineral elements in plant tissue and by this way makes plant vigorous and increases growth and survival rate (Zhao and Liu, 2009). Determination coefficient is a measure of the ability to model and predict the parameters of an appropriate assessment that this study was good.

Conclusion

Result of this study and other research could be announced that Gibberellin and cold pretreatments and light sandy soil with compost and litter via physiological conditions improvement by overcoming embryo dormancy, will facilitate germination of Sea buckthorn seeds. In our study hormone had the best result. Factors affecting the germination of seeds increase the chances of seedling in terms of quality and quantity as well as the establishment of forest areas. The highest germination percentage was observed in the sand soil. Then we conclude for better reproduction using of sand soil and Gibberellin treatment for seed can ascendant our success. This research was the first study on multispecies and valuable plant Sea buckthorn, in Iran. Further studies for habitats of our country are suggested.

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