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Greenhouse Treatments on *Elaeagnus rhamnoides* Seed Germination

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ABSTRACT
This study was for the first time conducted in a greenhouse in order to pioneer forest trees (*Elaeagnus rhamnoides*) and to determine the effects of different treatments on seed germination in Iran. This species has drought tolerance for plantation. For this purpose, seeds originating in Qazvin were placed in the greenhouse. Treatments included 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 supplement water). These treatments were accomplished in CRD experimental designs with five replicates. At the end (31st day) number and percentage of germination, number of days until germination, mean germination time, germination rate, germination energy, maximum germination, germination value were calculated. Average germination percentage of pretreatments was 3.75, 43.75, 17.5, 1.25, 15 and 37.5, respectively. Mean of germination in soil factor were 1.04, 5.21, 22.7 and 11.3, respectively. Germination in control irrigation and same attributes were 8.75, 33.75, 27.5, 3.75, 11.25 and 37.5 in supplement irrigation. Germination in all treatments and at whole levels was on average 20.1%. The highest germination percentage was observed in the sand. The highest average germination was seen in Gibberellin treatment.

Key words: Reproduction, sea-buckthorn, *Hippophae*, treatment

INTRODUCTION
Research is needed 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 is inevitable (Ranal and Santana, 2006). Leading to early plantation species in arid and semiarid regions is essential and one of the pioneer species and medicinal values of sea-buckthorn is stabilizing effect of soil nitrogen (Mohajer, 2006). Iran-Turanian indigenous species of sea-buckthorn of our country (Mozaffarian, 2004; Sabeti, 1976; Ghahraman, 1995) are examined in this study for germinating investigation. Sea-buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson) multiuse species is shrubs deciduous species, resistant to cold, drought and low coverage regions (Zhang *et al.*, 2010). *Hippophae,* the sea-buckthorns, are deciduous shrubs in the family Elaeagnaceae. The exact number of species in the genus *Hippophae* 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 (Suryakumar and Gupta, 2011).

In traditional Chinese medicine and the former Soviet Union for inflammation of the mouth, stomach ulcers, radiation injuries and burns have been used (Rongsen, 1992). Anti-bacterial and antioxidant support and protection of the natural seeds of this plant species is recommended. Methanol fruit and leaves of the plant are also antioxidant and help prevent cell necrosis (Suryakumar *et al.*, 2002). The oil extracted from berries is used for treatment of gastritis, stomach ulcers, erosion of uterus and inflammation of genital organs. Sea-buckthorn leaves contain nutrients and bioactive substances which mainly include flavonoids, carotenoids, free and esterified sterols, triterpenols and isoprenols. The leaves are an equally rich source of important antioxidants including carotene, vitamin E, catechins, elagic acid, ferulic acid, folic acid and significant values of calcium, magnesium and potassium (Suryakumar and Gupta, 2011). Bone-breaking fever virus in the blood of substances extracted from sea-buckthorn leaves is inhibited. Total phenolic content of root and seed extracts were significantly higher than leaf and stem extracts. No significant differences were seen between root and seed, or between leaf and stem (Michel *et al.*, 2012). Seeds soaked in water or potassium nitrate solution at room in comparison with Gibberellin and hot water emerged 34.5% minimum in higher percentages (Li and Wardle, 1999). Opposite to earlier research to KNO_3 pre-soaking treatment showed negative effect on seed germination of *Hippophae* rhamnoides compared to water treatment (Korekar *et al.*, 2013).

Frankia is a coral-like and special group of actinorhizal bacteria (Microsymbionts) that forms nitrogen-fixing root nodules on *Hippophae* trees (Schmidt, 2007). Embryos of dry seed need special conditions in order to start germination such as humidity, temperature and oxygen (Vilela and Ravetta, 2001; Kuriakose and Prasad, 2008). Treatment increases hydrolyser of metabolic enzymes in the embryo of the seed by the use of hormones such as cytokinin synthesis and tryptophan and ultimately conditions will be met for the development and proliferation of fetal (Benito *et al.*, 2005; Elsayed *et al.*, 2008). These enzymes increase the resistance of plants encountering drought and cold and deceases (Oliet *et al.*, 2009) and improves the survival of seedlings (Farooq *et al.*, 2006). The condition necessary to produce good seedlings is edaphic nutrition, because with biological, chemical and physical improvement of characteristics, seedling production efficiency is increased (Jacobs *et al.*, 2005). The purpose of this study is the introduction of sea-buckthorn to evaluate the actual germination in the greenhouse and in the field which is generated at later stages. In this study, germination was presented at the nursery greenhouse.

MATERIALS AND METHODS

This study is under way in the greenhouse nursery Department of Natural Resources and Watershed Management of Khorasan Razavi, situated in IRAN. In the Nursery greenhouse, the average temperature is 30°C and the humidity is 60%. In this study, after examining various queries (due to lack of knowledge about the species and the same name confused with Chinaberry) Qazvin fruit measures were taken by the Department of Natural Resources (Fig. 1). After winnowing the weight of 1000-grain got 12 g. The six pre-treatment and four different irrigation treatments based on 2 types of soil (Table 1) to form a factorial experiment in a completely randomized design was implemented. Seeds in plastic pots by 4 types of soil: (1) Control soil (current soil of nursery), (2) Control soil+water absorbent by stockosorb 0.3% (Nasab *et al.*, 2012), (3) Sand and (4) Control: Litter (3:5) were sown. Nursery irrigation with tap water (control) and supplemented with 1:5000 was performed. Supplement water had macro and

Fig. 1(a-b): (a) Sea-buckthorn berry and (b) Seedlings in pots

Table 1: Pretreatments of seeds

Treatment	Characteristics
Control	Pure seed
Cold	20 days in 4°C
Ice water	16 days in 0°C and every 5 days set to 24 h in 30°C
Hot water	water at 90°C by 15 min
Lime water	5% lime in water at 8 h
Gibberellin acid	500 mg L^{-1} for 40 h

micro elements. Five replicates for each treatment and 4 seeds pot^{-1} for a total of 960 seeds were sown. At the same depth seeds were sown on 15 Jan 2013. In the beginning, the experiment was to determine the characteristics of seed treatments. For disinfection of seed fungicide carbendazim was used a ratio of 2-1000. Counting the germinated seeds began 10 days after planting. Germination continued till 35 days and the number of germinated seeds was counted. Data was recorded before and after germination. At the end, number and percentage of germination, mean germination time, germination rate, germination energy, maximum germination and germination value were calculated.

Percentage of germination: No. of germinated seeds/No. of seeds planted *100 (Panwar and Bhardwaj, 2005).

Mean germination time: Total No. of seeds germinated day^{-1}*day count/total No. of germinated seeds during germination (Kulkarni *et al.*, 2007).

Germination value: Maximum germination*mean germination daily (Czabator, 1962).

Germination rate (speed): Total No. of germinated seeds day^{-1} to start counting the day count germination (Panwar and Bhardwaj, 2005).

Maximum germination: Cumulative germination percentages/No. of days during drilling (Czabator, 1962).

Germination energy: Until the maximum number of seeds that germinate (germination, the highest number in a given day) has germinated.

The other characters are calculated, the graph of the cumulative 31 day germination period (to ensure all seed germination) were drawn.

Statistical analysis: Data analysis using SAS software and EXCEL software were drawn AS diagrams. The normality test data using Ryan-Joiner (similar to Shapiro-Wilk) and homogeneity of variance with Dunnet method was used. In case of a significant treatment effect for mean comparison test and the SNK grouping for replication, pre-treatment, soil irrigation was the dissociation. To determine the correlation between parameters and Pearson correlation test was used Minitab software (Mesdaghi, 2011).

RESULTS

Repeat for all of the data taken in comparing the two modes without removing zeros (240) and removing zero (97) were analyzed. The data were normalized in the first case and the second case, the number and percentage germination were normal. So powerful parametric test method even for the data analysis of variance was performed (Mansourfar, 2007). Average germination percentage of pretreatments was, respectively 3.75, 43.75, 17.5, 1.25, 15 and 37.5 in control irrigation and same attributes were 8.75, 33.75, 27.5, 3.75, 11.25 and 37.5 in supplement irrigation. Germination percentage of days, the division of irrigation, soil types and treatments are presented in Fig. 2. Results of traits in all repeats by irrigation

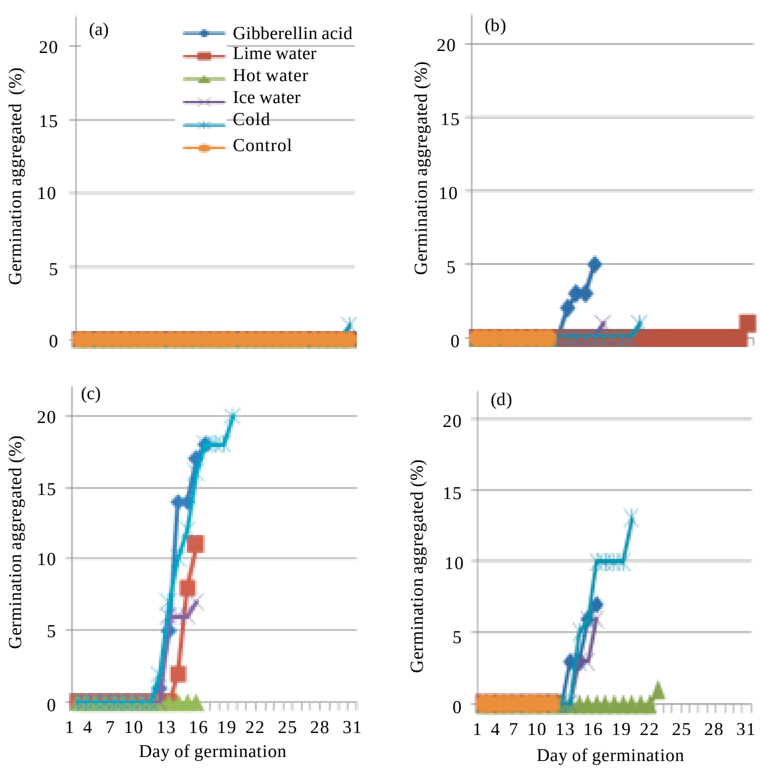

Fig. 2(a-d): Percentage of germination in control irrigation (a, b, c and d are soil treatments). Pretreatments of seeds are shown by symbols and color of curves

Table 2: Results of all of repeats by irrigation types

	Soil irrigation							
	Supplement				Normal			
Traits	1	2	3	4	1	2	3	4
No. of germination	4..00	17.0	50.0	27.0	1.00	8.00	59.0	27.00
Percent of germination	3.33	14.2	41.7	22.5	0.83	6.67	49.2	22.50
Rate of germination	0.32	1.41	3.86	2.1	0.03	0.56	4.75	2.03
Energy of germination	3.33	7.5	28.30	16.7	0.83	4.17	31.70	10.00

Table 3: Correlation pearson results between traits

Groups	Traits	1	2	3	4	5	6	7
1	No. of germination	1						
2	Percent of germination	1						
3	Day to germination	0.74**	0.74**					
4	Mean time to germination	0.97**	0.97**	0.79**				
5	Rate of germination	0.98**	0.98**	0.69**	0.93**			
6	Energy of germination	0.74**	0.74**	0.54**	065**	0.78**		
7	Maximum germination	0.98**	0.98**	0.69**	0.93**	1	0.78**	
8	Value of germination	0.94**	0.94**	0.54**	0.92**	0.93**	0.66**	0.93**

**Significant at 0.01 level

Table 4: Statistics characteristics of traits

Trait	Mean	Standard error	Determination coefficient	Significance	Root MSe
No. of germination	0.80	0.08	0.69	0.0001	0.75
Percent of germination	20.10	1.95	0.69	0.0001	18.78
Day to germination	5.37	0.45	0.57	0.0001	5.12
Mean time to germination	0.05	0.005	0.67	0.0001	0.05
Rate of germination	0.06	0.006	0.70	0.0001	0.06
Energy of germination	2.62	0.32	0.44	0.0001	4.13
Maximum germination	1.54	0.15	0.70	0.0001	1.47
Value of germination	0.27	0.036	0.66	0.0001	0.36

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.

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.

DISCUSSION AND CONCLUSION

Data were not normal because of zero data (59% of total data), so we removed zeros. After removing zeros, coefficient of variation was significantly reduced but most indicators showed a decrease in the coefficient of determination. Similarly, standard deviation and standard error remained stable and even increased in some parameters. Least square mean deletion of zeros is empty for most of the indicators (and associated points are worthless). Assessed data showed that the root mean square error in all indicators increased when we had no zeros considered. Due to this we selected data with zeros. In this study, the correlation between indexes is significant. If the correlation coefficient is near 1, the population is likely to be normal.

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

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 showed the significant difference between control irrigation in number and percentage of germination with soil types 3, 4 and showed the significant difference between supplementary irrigation with soil types 2, 3 and 4.

In day to germination and mean time to germination between soil types 2, 3 and 4 with control and supplementary irrigation there was no significant differences.

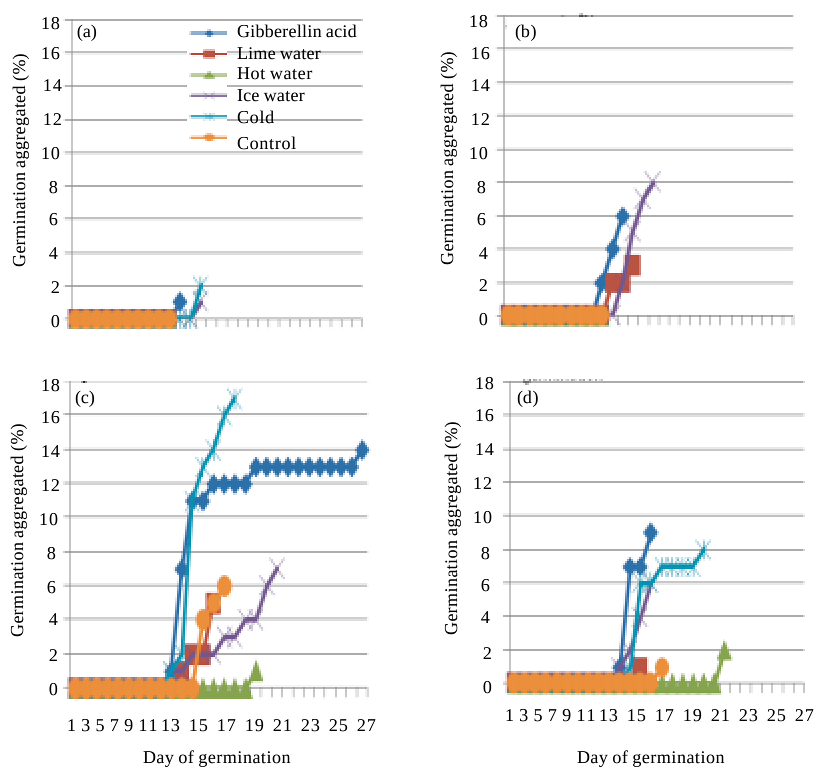

Fig. 3(a-d): Percentage of germination in supplementary irrigation (a, b, c and d are soil treatments). Pretreatments of seeds are shown by symbols and color of curves

Table 5: SNK test group d

Trait	Group
No. of germination	A (6-2), B(3), C(5-1), D (4-1)
Percent of germination	A (6-2), B(3), C(5-1), D (4-1)
Day to germination	A (6-3-2), B(1-4-5)
Mean time to germination	A (6-2), B(3), C(5-4-1)
Rate of germination	A (6-2), B(3), C(5-1), D (4-1)
Energy of germination	A (6-5-3-2), B(5-4-1)
Maximum germination	A (6-2), B(3), C(5-1), D (4-1)
Value of germination	A (6-2), B(3-5), C(5-4-1)

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

Table 6: Least squares means for effects of IRR×Soil×PTRT (pr>|t|)

Trait	Control irrigation*	Supplement irrigation*
No. 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

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

Rate of germination showed significant relationship between supplementary irrigation in soil types 2, 3, 4 and between control irrigation with soil types 3 and 4. Germination energy has been shown in soil types 3 and 4 for control irrigation and in soil types 2, 3 and 4 with supplementary irrigation. Maximum germination in soil types 3 and 4 in control irrigation and in supplementary irrigation with soil types 2, 3 and 4 were significant. 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.

Least squares means for mutual effect of priming treatments and soil in number of germination between soils types 2 and pretreatments 3 and 6, soils types 3 and pretreatments 1, 2, 3, 5 and 6, soils types 4 and pretreatments 2, 3 and 6 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 (10th day) and highest for the treatment of hot water to start germination (21st day) was observed. Furthermore, supplemental irrigation caused faster initial germination through gibberellin treatment (10th day) and the maximum term to initiate germination in hot water treatment was on (21st day). Percentage, vigor and germination rate are the main factors affecting seed germination and plant establishment (Pedersen *et al.*, 1993). 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. 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.* (2007) 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 study 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 (Aref *et al.*, 2011) 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 (Zeynali *et al.*, 2009). 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.*, 2011) 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. 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. This research was the first study on multispecies and valuable plant (sea-buckthorn in Iran).

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