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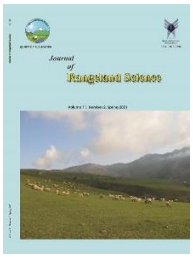

Research and Full Length Article:

Germination Enhancement and Primary Establishment of Three Medicinal Plants

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Abstract. Lack of cultivation of some Apiaceae family plants caused damage and uncontrolled exploitation of pastures and severe fluctuations in these plant markets. Therefore, efforts are essential to study the domestication of these species. For germination enhancement of three species including *Dorema aucheri* Boiss., *Echinophora cinerea* (Boiss.) Hedge & Lamond and *Ferulag oangulata* (Schlescht.) Boiss., six experiments in laboratory and greenhouse conditions were designed and performed during 2017-2018. At first, a completely randomized design factorial experiment with two factors (the first factor including 13 treatments consisted of stratification, soaking in GA_3 or KNO_3 and the second factor including stratification temperature consisted of 5, 10 and 15°C) was executed. In the second experiment, seedlings establishment was investigated in the different substrates. The results indicated that different treatments such as chilling, GA_3, KNO_3, and their integration had a low effect on *Dorema aucheri* seed germination. The effect of stratification at 4°C for 45 days on *Echinophora cinerea* (97.8%) and at 15°C for 45 days on *Ferulago angulata* (97.8%) was so clear on germination factors. The use of different growth media showed that studied species require relatively light medium and moisture during and after germination. Accordingly, mixed soil and cocopeat with 1:1 was the best substrate. It should be noted that the suitable medium for the primary growth of seeds was very similar to their natural habitats.

Key words: Chilling, *Dorema aucheri*, *Echinophora cinerea*, *Ferulago angulata*, Seed dormancy

Introduction

Iran pastures are about 94 million hectares in 1965 and according to the department of forest, rangelands and watershed management by the destruction and wrong exploitation of pastures reduced to about 86 million hectaresin 1999 (Jalili and Jamzad, 1999) and today, it is about 84 million hectares (https://frw.ir/02/Fa/default.aspx). Iran flora which was one of the richest flora changed because of these destructions. Rehabilitation of degraded rangelands and returning plant communities is associated with a lot of trouble and cost of cultivation which in this regard, awareness of the actual time of cultivation due to temperature requirements of plants and considering the temperature statistics of the area can be effective in raising the seed germination chance and avoiding the cost of re-cultivation in all crops and pastures. In other words, before planting any crop, it is necessary to examine the seed response in laboratory condition towards some physical requirements such as temperature and other factors, and after cognition, the tolerance range of plant toward these noticed factors and cultivating in the appropriate time (Cerabolini *et al.*, 2004).

Apiaceae family includes 2200 species and 250 different genera which are often observed in warm and tropical areas (Mozaffarian, 2017). This family is one of the most important plant families, which has native and important medicinal plants that are about to extinct (Christensen and Brandt, 2006). One of the important plants in this family is *Dorema aucheri* (Bilhar or Kandal in Persian language) that has many medicinal properties. Seven species have been reported in the genus *Dorema* in the flora of Iran and among them, two are endemic: *D. aucheri* Boiss, and *D. ammoniacum* D. Don (Khanahmadi *et al.*, 2012). In Iranian traditional medicine, *D. aucheri* has been employed as stimulant, calming, antispasmodic, bronchodilator, expectorant, kidney stone repellent, emmenagogue, and analgesic for visceral pain (Mozaffarian, 2004). This plant is eaten as green by some people in the west of Iran (Khanahmadi *et al.,* 2012). The side effect of *D. aucheri* ethanolic extract on albino mice has been reported. This plant has potential hepatotoxic capacities and may be related to the high prevalence of cancer in some regions of Iran (Mostafavi *et al.,* 2013).

Ferulago angulata locally called "Chavill" is another plant in this family which has many medicinal properties such as sedative and digestive and anti-tumor features (Amirghofran *et al.*, 2006; Ebrahimian *et al.*, 2013). *Ferulago* genus has nine species in Iran and most of them are very aromatic plants as valuable plants in rangelands (Mozaffarian, 2017). The *Echinophora* genus is represented in the flora of Iran to have four species including *E. orientalis* Hedge et Lamond, *E. sibthorpiana* Guss, *E. cinerea* Boiss., and *E. platyloba* DC. The two latter are endemic species (Ghani *et al.*, 2009; Mozaffarian, 2017). The Mediterranean and Middle East regions seem to be the only areas where this genus is established. *Echinophora cinerea* Boiss. is wild endemic plants that growing in some parts of Iran. This plant is locally called “Khusharizeh or Fedale” (Mozaffarian, 2004). The fresh and also dry aerial parts of the plant are used as a flavoring agent in various foods and especially in dairy products. It is also used as a medicinal plant in Iranian folk medicine (Ghani *et al.*, 2009).

Most of Apiaceae species are propagated by seeds and the germination percent of commercial seeds due to dormancy is generally very low (Ebrahimian *et al.*, 2013). Various researches used different treatments for seed germination improvement of different plants for the prolonged incubation period at 5°C and stratification at 5°C for *Chaerophyllum temulum* (Vandelook *et al.*, 2007) GA_3 and nitrate for *Crithmum*

maritimum (Atia *et al.*, 2009), cold stratification at 5°C for *Osmorhiza aristata* (Walck and Hidayati, 2004), KNO_3 150 mM and Thiourea 200 µL L^{-1} for *Angelica archangelica* (Kumar Vashistha *et al.*, 2009), moist sand cold stratification for 45 days for *Dorema ammoniacum* (Irvani *et al.*, 2012), and pre-chilling and GA_3 treatment for *Ferula gummosa* (Rahnama-Ghahfarokhi and Tavakkol-Afshari, 2007).

Gholami and Askarzadeh (2007) after cultivating seeds in the soil attemperature of 5, 10, 15 and 20°C reached the conclusion that the highest germination percent of the species was at 5°C. In researches that have been done in the Apiaceae family about the effect of washing and temperature on seed germination, it can be noticed in the report by Nadjafi *et al.* (2006) that *F. gummosa* in 5°C and under washing condition had the highest percentage of germination. Growth hormones or growth regulators are involved in many aspects of plant growth and development. Thus, it's natural that seed physiologists investigate the possibilities of plant hormones interference on seed dormancy (Ebrahimian *et al.*, 2013). Researches showed that many plant hormones may be effective in stimulating germination or seed dormancy in a specific way that leads to control of the nucleic acid (Sharifi *et al.*, 2015). It is anticipated that chilling temperature and period influence the germination properties. Also, GA_3 concentrations and combination treatments have different effects on germination characteristics. On the other hand, the reaction of plant species to mention treatments differ. As well, it was guessed that different medium cultures have various efficacy in the primary growth of plants studied.

The aim of this research was to study the germination and dormancy breaking methods in laboratory and pot condition. The seeds of this plant underdeveloped embryo at the time of dispersal. Thus, the seeds of Apiaceae family have deep complex morpho-physiological dormancy (MPD). So, in this research, we applied different treatments for solving this problem. Also, the primary establishment of seedlings in order to plantlet production in the different substrates were studied.

Materials and Methods

Plant materials and study area

The study of germination including laboratory and greenhouse experiments were carried out in the Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. Mature fruits (mericarps) were collected in September 2017 from a wild population in Kohgiluyeh and Boyer-Ahmad province of Iran (30° 59′ N, 51° 29′ E). Herbarium samples were identified at Ferdowsi University of Mashhad Herbarium. Fruits that weren't used immediately in the experiments were stored dry at room temperature (about 20°C). Before starting the experiments, the seeds viability test (tetrazolium chloride test) was done. According to the tetrazolium test, more than 80 percent of seeds had potential to produce normal seedlings. Seeds were disinfected in 5% calcium hypochlorite solution for 7 min before starting the germination tests.

Laboratory experiment

In this section, for each plant, two experiments in laboratory and field conditions were designed. At first experiment, a factorial experiment based on the completely randomized design (RCD) with two factors and three replications was carried out. The first factor including 13 treatments consists of: Stratification (15, 30 and 45 days in moist sand in the refrigerator at a temperature of 5°C), Soaking in GA_3 (with 500, 1000, 1500, 2000 and 3000 ppm concentration for 80 h), Incorporation of stratification for 30 days and GA_3 (500, 1000 and 1500 ppm), Incorporation of 1000 ppm GA_3 with 3 and 4% KNO_3 percent. The second factor includes stratification temperature (5, 10, and 15°C). The experiments were designed for each plant, separately. The

seeds were placed in 16mm diameter Petri dishes (30 seeds per each) and containing a double layer of filter paper type Whatman moistened with 4 ml of distilled water or a test solution. The following experiments were carried out in a growth chamber at 16/8 h (light/dark) photoperiod conditions. After 30 days, seeds germination was stabled and the duration of seed germination test continued for 10 days more.

Greenhouse experiment

In this section, for stratification, sand was washed and sterilized. Then, seeds were mixed with sand and kept in the refrigerator at 4°C for 2 months and after that, seeds were sown in the pots with different soil treatmentsin March 2018. The pots were kept at the research greenhouse in Mashhad climate conditions (36° 18´ N, 59° 31´ E). Soil treatments contained: Soil (SO), Cattle Manure (CM), Sand (SA), and Cocopeat (CP) (Table 1).

Table 1. Different media culture for seed germination of plant studied*.

Treatments	SO+CM	SO+SA	SO+CP	SO	SO+CM	SO+SA	SO+CP	SO+CM+SA	SO+CP+SA+CM
Mix percentage	50+50	50+50	50+50	100	75+25	25+75	75+25	50+25+25	25+25+25+25

*Soil (SO), Cattle Manure (CM), Sand (SA) and Cocopeat (CP).

Statistical analysis

Germinated seeds were counted at the two-day interval (appearance radicle). Germinated seeds were discarded after counting. The germination parameters including final germination percent (GP), germination rate (GR) and mean germination time (MGT) were determined according to the equations 1, 2 and 3, respectively (Schelin *et al.*, 2003; Irvani *et al.*, 2012).

$$GP=\frac{\text{number of germinated seeds}}{\text{number of viable seeds initiated}}\times 100 \quad \text{(Equation 1)}$$

$$GR=\sum_{n-1}^{30}\frac{Gt}{Dt} \quad \text{(Equation 2)}$$

Where Gt is the number of germinated seeds after t days (Dt)

$$MGT=\frac{\sum FiNi}{n} \quad \text{(Equation 3)}$$

Where Fi is day during germination period (between 0 and 30 day), Ni is number of germinated seeds per day and n is the total number of germinated seeds in the treatment.

Aalysis of variance in the first experiment was performed using a factorial design with two factors and three replications and the second experiment was conducted on a completely randomized design with nine treatments and three replications. The LSD test was used to detect significant differences among the treatments with the probability of 95% ($P<0.05$).

Results

Laboratory experiment

Echinophora cinerea Boiss.

The relative influence of physical and chemical different treatments and growth regulators in the removal of dormancy and improved seed germination is shown in Table 2. Based on the results of variance analysis, effect of treatments and temperature and the interaction of these two factors on measured variables had a significant difference statistically ($p\leq0.05$).

According to means comparison performed (Table 2), the highest percentage of germination (97.8 and 95%) was obtained in the moist chilling treatment for 45 days at 5°C and 15°C, respectively. After that, treatment with 30

days moist chilling at 15°C was the best treatment with 77.8% germination. The best GA_3 concentration for seed germination was 2000 ppm in comparison with other concentrations. The effect of temperature on germination seems a bit complicated and at temperatures of 5° and 15°C in moist chilling treatment (45 days) can see more than 95% germination. Without considering temperature factor, maximum germination (81.5%) was seen on 45-day stratification and other treatments including GA_3 2000 ppm, 30-day stratification+GA_3 1000 ppm, and 30-day stratification alone were classified in the second group. It seems that the best chilling requirement for the seeds of this plant is 45 days and by reducing this period, it decreases germination, but germination more than 75% in 30 days of moist chilling treatment at a temperature of 15°C seems to indicate low temperature and relatively high frequency which has a positive effect on seed germination. The effect of GA_3 on germination was less affected by temperature and more affected by its concentration and it seems that the use of growth regulators up to 2000 ppm concentration has a positive effect on germination. Incorporation using GA_3 and moist chilling could have a positive impact on seed germination.

Ferulago angulate (Schltdl.) Boiss.

Based on the results of variance analysis, the effect of different treatments and germination temperature and the interaction of these two factors on measured variables were significant ($P \leq 0.05$). The effect of different treatments on seed germination on *F. angulata* was shown in Table 3. Based on the results, the highest percentage of germination was observed at 45 days moist chilling treatment at 15°C temperature. Thirty-day moist chilling treatment doesn't have significant differences to these treatments but most importantly, it is the effect of 5 and 15°C temperatures on 30 and 45 days treatments. At 5°C, we are seeing higher germination in 30-day treatments compared to 45 days and 30-day germination was indicated at 5°C supply seed chilling requirement. But in 45-day treatment, the temperature has less impact on germination. Even in this temperature, the seeds which have received chilling treatment for 15 days had acceptable germination. It can be seen in 5 and 15°C good germination in treated seeds with moist chilling but at 10°C germination, it is much less. The use of other treatments causes to improve germination irregularly.

Dorema aucheri Boiss.

Based on the analysis of variance, the effect of treatments, germination temperature and the interaction of these two factors on measured variables were significantat 5% (Data not shown). Based on means comparison results, the highest percentage of germination (20%) was obtained for seeds placement in stratification in the sand for 30 days and subsequently placement at a 10°C temperature. However, germination was low and it seems that more chilling is required. Totally, regardless of temperature, the highest seed germination (11.1%) was reached in 30 days of stratification and GA_3 1000 ppm+KNO_3 4% was more effective than other treatments. Also, the maximum germination rate (0.053) and mean germination time (26.9) were observed in 30-day stratification treatment. According to the current results, it can be realized that the best period of the chilling requirement for this plant is 30 days and after chilling requirement supplying, seeds should placed at the optimum temperature (about 10°C). In this case, remarkable result is GA_3 deterrent effect when it is used in conjunction with the 30-day cold.

Table 2. The effect of incorporated different treatment of stratification, GA_3 and KNO_3 and incubation temperature on germination percentages, germination rate and mean germination time of *Echinophora cinerea**.

Treatment	Total germination (%)				Germination rate				Mean germination time (day)			
	5°C	10°C	15°C	Means	5°C	10°C	15°C	Means	5°C	10°C	15°C	Means
15 day Stratification	26.7^{kl}	24.5^{kl}	57.8^{c-f}	36.3^{DE}	0.037^{cd}	0.028^{d}	0.098^{bc}	0.054^{BC}	27.7^{d-i}	36.1^{a-f}	10.2^{kl}	24.7^{CDE}
30 day Stratification	60.0^{cde}	26.7^{kl}	77.8^{b}	54.8^{B}	0.038^{cd}	0.029^{d}	0.047^{bcd}	0.038^{C}	26.2^{e-i}	34.5^{a-f}	20.9^{g-k}	27.2^{BCD}
45 day Stratification	97.8^{a}	51.1^{e-h}	95.5^{a}	81.5^{A}	0.065^{bcd}	0.031^{d}	0.180^{a}	0.093^{A}	13.4^{i-l}	31.4^{ag}	5.5^{l}	17.4^{E}
GA_3 500 ppm	48.9^{e-h}	6.7^{m}	51.1^{e-h}	35.6^{DE}	0.028^{d}	0.031^{d}	0.041^{bcd}	0.034^{C}	35.1^{a-f}	32^{a-g}	23.9^{f-j}	30.3^{BC}
GA_3 1000 ppm	31.1^{i-l}	26.7^{kl}	33.3^{ijk}	30.4^{EF}	0.025^{d}	0.028^{d}	0.044^{bcd}	0.033^{C}	42.7^{ab}	36^{a-f}	23.4^{f-j}	34.0^{AB}
GA_3 1500 ppm	42.2^{ghi}	45.7^{fgh}	47.7^{fgh}	45.2^{C}	0.028^{d}	0.029^{d}	0.033^{d}	0.030^{C}	35.1^{a-f}	34.5^{a-f}	31.8^{a-g}	33.8^{AB}
GA_3 2000 ppm	53.3^{d-g}	66.7^{bc}	57.8^{c-f}	59.3^{B}	0.024^{d}	0.032^{d}	0.180^{a}	0.079^{AB}	40.4^{a-d}	30.2^{b-g}	12.8^{jkl}	27.8^{BCD}
GA_3 3000 ppm	40.0^{hij}	26.7^{kl}	33.3^{ijk}	33.3^{E}	0.026^{d}	0.029^{d}	0.025^{d}	0.027^{C}	43.7^{a}	34.5^{a-f}	41.1^{abc}	39.7^{A}
30 day Stratification+ GA_3 500 ppm	57.8^{c-f}	20.0^{l}	46.7^{fgh}	41.5^{CD}	0.060^{bcd}	0.031^{d}	0.068^{bcd}	0.053^{BC}	16.5^{h-l}	34.7^{a-f}	15.1^{ikl}	22.1^{DE}
30 day Stratification+ GA_3 1000 ppm	60.0^{cde}	64.5^{cd}	42.2^{ghi}	55.6^{B}	0.037^{d}	0.028^{d}	0.036^{d}	0.034^{C}	27.1^{e-i}	34.7^{a-f}	28.7^{c-h}	30.2^{BC}
30 day Stratification+ GA_3 1500 ppm	40.0^{hij}	53.3^{d-g}	31.1^{i-l}	41.5^{CD}	0.036^{d}	0.034^{d}	0.101^{b}	0.058^{BC}	27.8^{d-i}	28.8^{c-h}	7.4^{l}	21.3^{DE}
GA_3 1000 ppm+KNO3 (%3)	28.9^{jkl}	26.7^{kl}	22.2^{kl}	25.9^{F}	0.026^{d}	0.027^{d}	0.033^{d}	0.029^{C}	41.8^{abc}	37.6^{a-e}	34.4^{a-f}	38.0^{A}
GA_3 1000 ppm+KNO3 (%4)	24.5^{kl}	26.7^{kl}	26.7^{kl}	25.9^{F}	0.028^{d}	0.032^{d}	0.035^{d}	0.032^{C}	35.6^{a-f}	32.4^{a-g}	29.2^{c-h}	32.4^{AB}
Means	47^{A}	35.9^{B}	47.9^{A}	*	0.036^{B}	0.030^{B}	0.071^{A}	*	31.9^{A}	33.6^{A}	21.9^{B}	*

*Different letters in each column show significant differences at $P \leq 0.05$ (LSD).

Table 3. The effect of incorporated different treatment of stratification, GA_3 and KNO_3 and incubation temperature on germination percentages, germination rate and mean germination time of *Ferulago angulata**.

Treatment	Total germination (%)				Germination rate				Mean germination time (day)			
	5°C	10°C	15°C	Means	5°C	10°C	15°C	Means	5°C	10°C	15°C	Means
15 day Stratification	73.3^{a-d}	24.5^{j-n}	97.8^{a}	65.2^{AB}	0.037^{d-g}	0.028^{e-h}	0.066^{a}	0.044^{ABC}	27.1^{e-l}	36.1^{a-f}	15.1^{l}	26.1^{CDE}
30 day Stratification	86.7^{ab}	26.7^{i-m}	95.5^{a}	70.0^{AB}	0.056^{ab}	0.030^{e-h}	0.066^{a}	0.050^{A}	17.9^{jkl}	34.7^{b-g}	15.1^{l}	22.5^{DE}
45 day Stratification	77.8^{abc}	51.1^{d-i}	97.8^{a}	75.5^{A}	0.049^{bcd}	0.031^{e-h}	0.062^{ab}	0.048^{AB}	20.1^{i-l}	31.4^{d-i}	16.0^{kl}	22.5^{DE}
GA_3 500 ppm	11.1^{lmn}	6.7^{mn}	33.3^{h-l}	17.0^{F}	0.023^{gh}	0.031^{e-h}	0.033^{e-h}	0.029^{E}	48.0^{a}	32.0^{d-i}	30.8^{d-j}	37.0^{A}
GA_3 1000 ppm	6.7^{mn}	26.7^{i-m}	24.5^{j-n}	19.3^{F}	0.029^{e-h}	0.029^{e-h}	0.033^{e-h}	0.030^{DE}	34.0^{b-h}	36.0^{a-f}	30.6^{d-j}	33.5^{ABC}
GA_3 1500 ppm	11.1^{lmn}	46.7^{e-j}	6.7^{mn}	21.5^{F}	0.024^{fgh}	0.029^{e-h}	0.038^{def}	0.030^{DE}	45.3^{abc}	34.5^{b-g}	26.0^{f-l}	35.3^{AB}
GA_3 2000 ppm	0.0^{n}	66.7^{b-f}	20.0^{k-n}	28.9^{DEF}	0.000^{i}	0.033^{e-h}	0.042^{cde}	0.025^{E}	0.0^{m}	30.2^{d-j}	26.0^{f-l}	18.7^{E}
GA_3 3000 ppm	24.5^{j-n}	26.7^{i-m}	33.3^{h-l}	28.2^{EF}	0.025^{fgh}	0.030^{e-h}	0.037^{def}	0.031^{DE}	40.6^{a-d}	34.7^{b-g}	27.1^{e-l}	34.1^{AB}
30 day Stratification+ GA_3 500 ppm	26.7^{i-m}	26.7^{i-m}	17.8^{k-n}	23.7^{F}	0.029^{e-h}	0.030^{e-h}	0.025^{fgh}	0.029^{E}	35.0^{a-g}	33.6^{c-h}	40.2^{a-e}	36.3^{A}
30 day Stratification+ GA_3 1000 ppm	37.8^{g-k}	42.2^{f-k}	42.2^{f-k}	40.7^{DE}	0.027^{fgh}	0.034^{e-h}	0.052^{bc}	0.038^{CD}	38.2^{a-f}	29.7^{d-j}	22.1^{g-l}	30.0^{A-D}
30 day Stratification+ GA_3 1500 ppm	62.2^{b-g}	31.1^{h-m}	37.8^{g-k}	43.7^{CD}	0.034^{e-h}	0.030^{e-h}	0.028^{e-h}	0.031^{DE}	29.0^{d-k}	35.1^{a-g}	36.0^{a-f}	33.4^{ABC}
GA_3 1000 ppm+KNO3 (%3)	55.5^{c-h}	51.1^{d-i}	68.9^{b-e}	58.5^{BC}	0.037^{def}	0.028^{e-h}	0.062^{ab}	0.042^{BC}	27.5^{d-l}	35.5^{a-f}	21.3^{h-l}	28.1^{BCD}
GA_3 1000 ppm+KNO3 (%4)	48.9^{d-j}	40.0^{g-k}	33.4^{h-l}	40.7^{DE}	0.033^{e-h}	0.030^{e-h}	0.022^{h}	0.029^{E}	29.5^{d-j}	34.1^{b-h}	46.9^{ab}	36.9^{A}
Means	40.2^{AB}	35.9^{B}	46.8^{A}	*	30.2^{AB}	33.6^{A}	27.2^{B}	*	0.031^{B}	0.031^{B}	0.044^{A}	*

*Different letters in each column show significant differences at $P \leq 0.05$ (LSD).

Greenhouse experminnt

Dorema aucheri Boiss.

According to variance analysis results, the treatment impact was significant on measured parameters ($p \leq 0.05$). The best condition to start seed germination in substrate containing soil and cocopeat obtained a 1:1 ratio (Table 4). After 12 days of starting culture seeds, maximum germination was observed in substrate containing soil and cocopeat with 1:1 ratio (14.7%) and soil+sand 3:1 ratio (14%). Seeds germination in the different mediums was low after partial supplying of their chilling requirement like germination in the petri dish.

According to results, the seeds of this plant for germination need the relatively light culture medium. In addition, it requires the continuous presence of moisture in the substrate during germination. This condition in a substrate containing soil and cocopeat with the 1:1 ratio is available for seeds. In general, it seems that for optimal germination, the seeds of this plant need a relatively light substrate adding compounds that cause to retaining continuous soil moisture (e.g. super absorbent). The number of produced seedling after 40 days of starting test was harmonized with the best treatments related to germination (15 seedlings survive in soil and cocopeat substrate with 1:1 ratio). The obtained results of means comparison showed that the greatest amount of fresh and dry weight (0.14 and 0.030 g, respectively) in a substrate containing soil, sand, fertilizer, and cocopeat was obtained equally (Table 4). The highest seedling fresh weight (g) and seedling fresh weight (g) were in the mixed substrate (SO+CP+SA+CM) with the same ratio.

Echinophora cinerea Boiss.

According to the results obtained in Table 5, the substrate containing cocopeat and soiland a substrate containing a combination of all three forms with equal proportion provided the best conditions for the start of germination. After 12 days of starting the test in substrate containing soil and sand, it was observed that maximum germination is likely because of proximity to the plant habitat conditions. Finally, the highest number of the produced seedling was observed in substrate containing soil+cocopeat and soil+sand. The highest seedling fresh weight (g) and seedling dry weight (g) were obtained in soil media and SO+CM+SA mix with 2:1:1 ratio respectively.

Ferulago angulata Boiss.

Ferulago angulata seeds sowing in the different substrates after supplying chilling requirements showed that substrate containing soil+sand and soil+cocopeat caused better conditions to start seeds germination (Table 6). But after 12 days of stating seeds cultivation in a wide range, there were no significant differences and only in the heavy medium like 100 percent soil, the low germination of seeds was seen. After 38 days of starting tests, the highest number of seedlings was obtained in a medium containing soil and cattle manure. The soil and cocopeat substrate showed the highest seedling fresh weight while the highest seedling dry weight was obtained in the SO+CP+SA+CM substrate.

Table 4. *The effect of different substrate on seed germination and growth characteristics of* Dorema aucheri*.

Treatment	Seed germination after 6 day (%)	Seed germination after 12 day (%)	Number of seedling after 40 day	Seedling fresh weight (g)	Seedling dry weight (g)
SO+CM (1:1)	0.0^{d}	1.3^{d}	1.0^{d}	0.07^{fg}	0.008^{ef}
SO+SA (1:1)	5.7^{c}	12.3^{ab}	9.0^{b}	0.06^{g}	0.006^{f}
SO+CP (1:1)	11.0^{a}	14.7^{a}	15.0^{a}	0.10^{d}	0.012^{d}
SO	1.7^{d}	3.0^{d}	2.3^{cd}	0.09^{e}	0.010^{de}
SO+CM (3:1)	0.7^{d}	3.3^{d}	1.0^{d}	0.11^{cd}	0.011^{e}
SO+SA (1:3)	1.3^{d}	14.0^{ab}	12.7^{ab}	0.12^{bc}	0.017^{c}
SO+CP (3:1)	8.0^{b}	12.3^{ab}	13.3^{a}	0.12^{b}	0.021^{b}
SO+CM+SA (2:1:1)	2.0^{d}	7.7^{c}	5.0^{c}	0.08^{f}	0.016^{c}
SO+CP+SA+CM (1:1:1:1)	6.0^{bc}	10.7^{bc}	11.3^{ab}	0.14^{a}	0.030^{a}

*Different letters in each column show significant differences at $P \leq 0.05$ (LSD). Soil (SO), Cattle Manure (CM), Sand (SA) and Cocopeat (CP).

Table 5. The effect of different substrate on seed germination and growth characteristics of *Echinophora cinerea**.

Treatment	Seed germination after 6 day (%)	Seed germination after 12 day (%)	Number of seedling after 40 day	Seedling fresh weight (g)	Seedling dry weight (g)
SO+CM (1:1)	2.7^{c}	6.7^{c}	4.0^{cd}	0.21^{bcd}	0.030^{bc}
SO+SA (1:1)	4.3^{bc}	11.7^{ab}	3.0^{d}	0.19^{d}	0.038^{ab}
SO+CP (1:1)	12.7^{a}	14.0^{a}	$14^{.0a}$	0.22^{bc}	0.041^{ab}
SO	2.7^{c}	10.0^{bc}	6.0^{cd}	0.25^{a}	0.045^{ab}
SO+CM (3:1)	4.7^{bc}	11.0^{ab}	5.0^{cd}	0.24^{ab}	0.004^{d}
SO+SA (1:3)	6.3^{b}	14.3^{a}	12.3^{ab}	0.21^{cd}	0.035^{abc}
SO+CP (3:1)	4.3^{bc}	12.7^{ab}	13.0^{ab}	0.14^{e}	0.021^{c}
SO+CM+SA (2:1:1)	5.3^{b}	9.3^{bc}	7.0^{cd}	0.22^{bcd}	0.049^{a}
SO+CP+SA+CM (1:1:1:1)	12.0^{a}	11.3^{ab}	8.3^{bc}	0.21^{cd}	0.050^{a}

*Different letters in each column show significant differences at $P \leq 0.05$ (LSD). Soil (SO), Cattle Manure (CM), Sand (SA) and Cocopeat (CP).

Table 6. The effect of different substrate on seed germination and growth characteristics of *Ferulago angulata**.

Treatment	Seed germination after 6 day (%)	Seed germination after 12 day (%)	Number of seedling after 40 day	Seedling fresh weight (g)	Seedling dry weight (g)
SO+CM (1:1)	0.7^{c}	5.3^{b}	7.0^{d}	0.14^{d}	0.019^{d}
SO+SA (1:1)	4.0^{ab}	13.3^{a}	14.7^{ab}	0.12^{f}	0.026^{c}
SO+CP (1:1)	6.3^{a}	13.0^{a}	14.7^{ab}	0.24^{a}	0.033^{b}
SO	0.7^{c}	4.3^{b}	6.0^{d}	0.10^{h}	0.016^{d}
SO+CM (3:1)	4.3^{ab}	11.0^{a}	17.0^{a}	0.16^{c}	0.023^{c}
SO+SA (1:3)	5.0^{a}	12.7^{a}	11.7^{bc}	0.11^{g}	0.018^{d}
SO+CP (3:1)	6.0^{a}	13.7^{a}	11.7^{bc}	0.13^{e}	0.002^{e}
SO+CM+SA (2:1:1)	2.0^{bc}	5.0^{b}	8.3^{cd}	0.16^{c}	0.023^{c}
SO+CP+SA+CM (1:1:1:1)	6.3^{a}	12.3^{a}	14.7^{ab}	0.23^{b}	0.050^{a}

*Different letters in each column show significant differences at $P \leq 0.05$ (LSD). Soil (SO), Cattle Manure (CM), Sand (SA) and Cocopeat (CP).

Discussion

Laboratory experiment

In spite of using different treatments and various temperatures, the significant increase was not observedin the germination of *D. aucheri.* Sharifi *et al.* (2015) reported *D. aucheri* seeds stratification for 6 months at 4°C lead to increase seed germination over 90%. The positive effect of nanopriming with selenium on seed germination and root growth of *D. aucheri* was reported recently (Abedi, 2020). The low seed germination in the present study can be attributed to various factors such as seed prematurity and maybe hidden infection of seeds to insect larvae (Irvani *et al.*, 2012). The highest seed germination of *D. ammoniacum* was observed after 45 days of chilling at 4°C (Irvani *et al.*, 2012). The study of phenological stages of this plant

also expresses the chilling requirement for seed germination (Kazemi *et al.,* 2011). The results indicated chilling for 30 and 45 days had a severe impact on *E. cinera* and *F. angulata*. Moist chilling positive effect on *Cyclocarya paliurus* (Fang *et al.*, 2006), *Ferula gummosa* (Nadjafi *et al.*, 2006), *D. ammoniacum* (Irvani *et al.*, 2012) and various *Nepeta* species (Asgari *et al.*, 2015) plants have been reported.

Application of GA_3 and KNO_3 was accompanied by 10% germination in 5°C temperature, which indicates that these two compounds were not been able to provide seed chilling requirement as much as 30 days chilling on *D. aucheri*. Irvani *et al.* (2012) and Mellati *et al.*(2010) reported the lack of effect of GA_3 on seed germination on their researches. Unlike *D. aucheri*, gibberellic acid had positive effects on *E. cinera* and *F. angulata* seeds germination. Although using gibberellic acid and potassium nitrate was more effective in *F. angulata* seeds germination, the role of growth regulators was studied in germination improving and removing seed dormancy in different species and in some cases, it increased seed germination. Keshtkar *et al.* (2008) also reported that *Ferula ovina* and *F. gummosa* seeds treatment with gibberellic acid and low temperature caused germination; meanwhile, no germination was observed in untreated seeds. They reported that the germination of *Ferula* increased by increasing of gibberellic acid concentration, and the increase was greater when higher concentrations of gibberellic acid were associated with low temperature. It is obvious that the role of cold requirement is more effective than gibberellic acid in breaking seeds dormancy.

Analysis of the effect of germination temperature on three species indicated that the best temperature for dormancy breaking was 15°C. Ghasemi-Arian (2009) indicated that temperature is effective in germination percent of *Dorema ammoniacum*, so the highest germination percent (94.2%) of this plant was obtained at 4°C. In the present study, the chilling requirement of seeds was not removed earlier and this reason caused germination at 4°C. This indicates the chilling requirement was funded during the germination of seeds. Zia and Khan (2004) expressed that it seems 5°C or slightly less had the greatest impact on seed dormancy for plants which grow on cold climate. Nadjafi *et al.* (2006) reported daily washing of *Ferula* seeds at 5°C temperature for 14 days caused the breaking of their dormancy and the highest germination percent and germination rate. Mellati *et al.* (2010) reported that more than 8°C temperature risecauses a sharp decrease in germination percent and also, germination rate of *Ferula* seeds in both washed and non-washed treatment. Ghadiri and Bagherani (2000) indicated the best temperature for germination of Licorice was 15 and 25°C. In another study, it was found that seed treatment Candle Coma (*Dorema ammoniacum*) at a temperature of 3°C and using washed treatment had the highest amount of green seed. It seems to improve germination in these types of seeds; further investigation and usage of other treatments like washable and newer seeds are needed.

Greenhouse experiment

The autecology study of *Dorema aucheri* plants showed that normal soil texture coupled with a lot of pebbles has the highest germination prescent (Kazemi *et al.,* 2011). In total, more efforts should be done to improve the germination of these species and subsequently examine the possibility of germination in different media. Ginwal *et al.* (2005) in *Jatropha curcas* (*Euphorbiaceae*) observed the highest percentage of germination and seed germination rate in vermiculite and sand. Also, it was stated that Cockscomb (*Celosia* spp.) seeds had better germination in peat compared to soil medium and also, peat caused to increase 22 percent of germination compared to soil that may be the reason of this reduced sensitivity to

high pH, soil texture, high moisture and lack of enough oxygen to the occurrence of germination in the Cockscomb.

It seems that the seeds of *F. angulata* plant for germination need to have a light-medium. Investigating the soil habitats of this plant confirms that oamy and sandy loamy, non-saline and shallow lime soil are suitable for this plant (Jahantab *et al.*, 2012).

Conclusion

The results of all these studies confirm that the seed of Apiaceae family requires pre-chiling to improve germination and in this connection, the use of some substances such as gibberellic acid and potassium nitrate also had a positive impact. It should be noted that the role of cold is much more effective and clearer than other treatments. These results indicate that the seed of these plant species has a morpho-physiological type of dormancy (Vandelook *et al.,* 2007; Asgari *et al.,* 2015).

One way to restore pastures and development of seed cultivation of these plants is seeding improvement. If planting time was coincided with the time of germination and seed requirements to be provided especially after planting chilling, the chances of germination increase (select the best planting date according to the seed requirements). The role of temperature has been proved in the seed germination of these plants by numerous studies. Studying the requirements of seed germination provides production management and cultivating development in other areas. So, with these requirements, plants can be easily cultivated in these areas artificially. Since all three species are mostly distributed in cold temperate areas, to omit their dormancy needs low temperature.

In this research, different treatments were applied to improve seed germination but results were not remarkable about Bilhar (*Dorema aucheri*), so it is recommended that other studies should be done in this field such as the use of new and old seeds (to assess the after-ripening seeds), the use of alternating hot and cold temperatures, study of germination and growth of small embryo of these plants intissue culture. It seems that the seeds of these plants have a primary dormancy and lack of hard shell and we should search the main reason for their low germination in physiological factors and inhibitors substances (A combination dormancy: morpho-physiological). Finally, this study showed that 30 days' stratification is enough to reduce the level of germination inhibitors and seeds problemis related to small and narrow embryos.

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تقویت جوانه‌زنی و استقرار اولیه سه گیاه دارویی ارزشمند ایران

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چکیده. عدم کشت و کار برخی از گونه‌های خانواده Apiaceae باعث خسارت و بهره‌برداری بدون کنترل از مراتع و نوسانات شدید در بازارهای این گیاهان شده است. بنابراین تلاش برای اهلی‌سازی این گونه‌ها ضروری است. به منظور افزایش جوانه‌زنی در سه گونه شامل بیلهر یا کندل کوهی (*Dorema aucheri*)، خوشاریزه علوفه‌ای یا کوهی (*Echinophora cinerea*) و چویل سه‌پاره یا گوشه‌دار (*Ferulago angulata*) شش آزمایش در شرایط آزمایشگاه و گلخانه در طی سالهای ۱۳۹۶ و ۱۳۹۷ طراحی و اجراء شد. در ابتدا، آزمایش فاکتوریل در قالب طرح کاملاً تصادفی با دو فاکتور (فاکتور اول دربرگیرنده ۱۳ تیمار شامل سرمادهی مرطوب، غوطه‌وری در جیبرلیک اسید و نیترات پتاسیم و فاکتور دوم شامل دماهای ۵، ۱۰ و ۱۵ درجه سانتیگراد سرمادهی مرطوب) اجرا شد. استقرار دانهالها در طی آزمایش دوم در محیط کشت‌های مختلف بررسی شد. نتایج نشان داد، تیمارهای مختلف جوانه‌زنی مثل سرمادهی، GA_3، KNO_3 و تلفیق آنها تاثیر کمی در جوانه‌زنی بذور بیلهر (۲۰ درصد) داشتند. تاثیر سرمادهی مرطوب با دمای ۴ درجه سانتیگراد به مدت ۴۵ روز در خوشاریزه (۹۷/۸ درصد) و دمای ۱۵ درجه سانتیگراد به مدت ۴۵ روز در چویل (۹۷/۸ درصد) بر جوانه‌زنی این دو گونه مشهود بود. استفاده از محیط کشت‌های مختلف نشان داد، که گونه‌های مورد مطالعه نیاز به محیط کشت نسبتاً سبک و مرطوبت در طی جوانه‌زنی و بعد از آن دارند. براین اساس بهترین محیط کشت جهت جوانه‌زنی و استقرار گونه مخلوط خاک و کوکوپیت با نسبت یک به یک بود. باید یادآور شد که محیط کشت مناسب برای رشد اولیه بذرها بسیار شبیه شرایط رویشگاهی این گیاهان در مراتع بود.

کلمات کلیدی: بیلهر، تیره چتریان، خواب بذر، خوشاریزه، چویل، سرمادهی