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Original Article

Effects of Different Treatments on Improving Seed Germination Characteristics in Medicinal Species of *Origanum vulgare* L. and *Thymus transcaspicus* Klokov

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Abstract

Seed dormancy is a serious limiting factor in revenue of an economically important species to its fullest. Because of the importance of medicinal plants in curing diseases and the shortage of natural habitats, little reproduction rate and mass cutting of trees, it is necessary to program cultivation and naturalization of medicinal plants. The objectives of this study were to investigate the effects of different treatments on seed germination in two medicinal species (Oregano and Khorasan thyme). Both species are known to have low seed germination. An experiment was performed with 9 treatments and 4 replications in a completely randomized design in the Seed Laboratory of Agriculture Faculty, Higher Education Complex of Shirvan in 2016. The treatments applied are: (1) Three concentrations of gibberellic acid (100, 500 and 1000ppm), (2) Potassium nitrate (KNO3 (0.2%)), (3) Thiourea 1 Molar, (4) Three prechilling periods (10, 20 and 30 days at 2 °C) and (5) distilled water as control. The seeds were sown in Petridishes for 15 days. Results of the analysis indicated that the effect of various treatments on the percentages of *Thymus transcaspicus* Klokov and *Origanum vulgare* L. seeds germination were highly significant ($p<0.01$). Treatments of 100 ppm gibberellic acid and cold (10 and 20 days) have the highest effect on seeds germination percentage of *T. transcaspicus* and KNO3 and cold (10 and 20 days) on *O. vulgare* seeds. The highest and lowest vigor indices in *T. transcaspicus* were seen under treatment of 100 ppm gibberellic acid (16.2) and KNO3 (2.4), and were also seen in *O. vulgare* under treatment of 1 M thiourea (10.9) and cold for 20 days (1.37).

Keywords: Pre-Germination treatment, Rangeland plants, Seed dormancy, *Origanum vulgare* L., *Thymus transcaspicus* Klokov.

Introduction

Medicinal aromatic herbs have been used traditionally as a stout source of vegetables, spices and natural drugs for many centuries [1,2]. The Lamiaceae family is one of the largest and most explicit families of flowering plants, with about 220 genera and almost 4000 species worldwide. This family has an almost cosmopolitan apportionment. Some genera like *Thymus* L., *Nepeta* L., *Origanum* Tourn. ex L., *Ziziphora* L., *Phlomis* L., *Eremostachys* Bunge, *Salvia* L. and *Lagochilus* Bunge ex Benth. have a great diversity in the Mediterranean and C/SW Asia [3]. *Origanum vulgare* L., Lamiaceae family, is widely recognized as a very whirling plant with many curative functions (diaphoretic, carminative, antispasmodic, antiseptic, tonic) being used in traditional medicine systems in many countries [4,5]. *Thymus transcaspicus* Klokov is an aromatic and medicinal plant, which it has been widely dispersed in the north of Khorasan province, Iran, and southern areas of Turkmenistan as stated by Rechinger [6]. This plant has antifungal activities According to a preceding report.

Germination is a grave stage in the life cycle of weeds, medicinal and crop plants, and often

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controls population dynamics, with capital practical inclusions [7]. Seed dormancy is the repose period of seed after physiological puberty and also an assenting mechanism to defeat stress conditions. Seeds germinate when it come in contact with moisture at optimum temperature in the attendance of oxygen. There are exceptional species, which does not germinate even under all propitious conditions, and this is because of the dormancy overcoming in the seeds. Superiority of the medicinal plants are non obedient, and they grow as wild plants. The wild nature has made the seeds dormant for their survival under inopportune conditions also this mechanism helps for their dispersal and permanent. Problem consequents when these types of dormant species were admitted for term cultivation and this need to be addressed to develop the plant stand and yield.

Knowledge about effect of different physical and chemical treatments on seed dormancy of medicinal *O. vulgare* and *T. transcaspicus* species can help us in conception of germination and to find the most appropriate factors can prevail to the natural barrier of seed dormancy in these plants. As each private plant has own specific solution for development of germination; there is a lack of information about influence physical and chemical traits on medicinal *O. vulgare* and *T. transcaspicus* plants have not been computed.

Many of plant seeds that are produced in natural conditions, such as rangelands, appear different levels of seed dormancy. Different methods have been applied to defeat seed physical and physiological dormancy. These included salinity, temperature, humidity [8], light, seed scarification [9], seed stratification [10], regulatory hormones [11] and chemical compounds. Yazdanshenas *et al* studied the effect of freezing, wetting and drying, pre-chilling, nitrate potassium and sulfuric acid on germination of *Portulaca oleracea* L. and reported that pretreatments with nitrate potassium and pre-chilling had most influence on seed germination [12]. Hassani *et al* investigated the Effect of two temperatures (23 °C and 4 °C), exogenous GA3 and cytokinins on dormancy breaking and germination of Ferula assa-foetida seeds. They stated that among the treatments, cold stratification (4 °C) significantly stimulated seed breaking dormancy, while GA3 was not effective to overcome dormancy for this species [13]. Hassan Shaykhi *et al* also evaluated the effects of stratification (0, 3, 6, 9 and 12 weeks), stratification and gibberellin and stratification and nitrate potassium to overcome seed dormancy of *Kelussia odoratissima* Mozaff.. They reported that stratification and gibberellic acid (500 ppm) is the best treatment for breaking of *K. odoratissima* [14]. Tavili *et al* researched the effect of Gibberellic acid and KNO3 on germination of *Salsola rigida* and reported that pretreatment with KNO3 0.2% had most influence on seed germination [15].

The objectives of this study were to determine the influence of gibberellic acid, prechilling, KNO3 and thiourea applied on seed germination and to introduce an effective method for improving seed germination characteristics in medicinal species of *O. vulgare* and *T. transcaspicus*.

Material and Methods

Seeds of *O. vulgare and T. transcaspicus* were collected from North East Rangelands of Iran, Bojnourd. This region is located between 37° 23' to 37° 26' North latitude and 57° 7' to 57° 15' East longitudes. The area is approximately 5300 hectares with elevation ranging from 1200 m to 1900 meter. The means of precipitation is 295mm/year that maximum and minimum of precipitation occur in April and July respectively. The mean of annual temperature is 11.28 °C. The average maximum temperature is 26 °C in July and minimum temperature is -6.8 °C in January. The climate of this region with using of Emberger method is cold semi-arid. These plants generally prefer sandy loam texture, lime, alkaline pH and non-saline soils [16]. To prevail the dormancy obtruded by the hard seed coat and embryo and to obtain quick, uniform and high germination rates, 9 treatments were used. These are: Three concentrations of gibberellic acid (100, 500 and 1000ppm), Potassium nitrate (KNO_3 (0.2%)), Thiourea 1 Molar, Three prechilling periods (10, 20 and 30 days at2- 4 °C before the germination test) and distilled water as control. Seeds were cleaned and prepared. The study was conducted in the Seed Laboratory of Higher Education Complex of Shirvan in 2016. Seeds were disinfected using hyposodium chloride (2%) for 5 minutes, and then washed with distilled water several times and left to dry under room conditions. Then 25 disinfected seeds were evenly distributed between two layers of WhatmanNo.1filter paper in each of 9-cm plastic Petridish and transferred to germinator 15-25 °C for 15 days, light-to-dark cycle of 16 hours light

(1000 lux) to 8 hours dark and relative humidity 90%. The treatments were arranged in a randomized complete blocks design with four replications.

Germinated seeds of more than 2mm length were recorded daily during over 15 days (Tavili *et al*, 2009). At the end of germination test, the germination percentage, germination speed, root length, shoot length, seedling fresh and dry weight and seed vigor index were measured with using International Seed Testing Association criterion (17). Germination percentage [18], germination speed [19] and seed vigor index [8] was calculated based on the following equations:

Germination percentage: = $GP = \sum G_i / N \times 100$ (1)

Where GP is germination percentage, G_i is the number of germinated seeds and N is the number of seeds.

Germination speed: $GR = \sum S_i / D_i$ (2)

Where S_i is the number of germinated seed at each counting, D_i is the number of day until n counting and n is the number of counting.

Vigor index= Total germination percentage × Mean of plant length (mm) / 100 (3)

Plant length = root length + shoot length. (4)

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

Results

The results of the germination percentage and early seedling growth parameters were presented in Table 1, 2, 3 and 4. There were significant differences (P≤0.01) among the treatments in terms of their effects on germination percentage, germination rate, root and shoot length, seedling fresh-dry weight and vigor index.

1. Germination percentage

Analysis of variance of under study characteristics in *O. vulgare and T. transcaspicus* seeds are given in Table 1 and 2. The impression of treatments on germination showed that prechilling (10, 20 and 30 days), potassium nitrate, thiourea and gibberellic acid 500 have increased germination percentage in *O. vulgare* species. The results indicated that gibberellic acid 100and 1000 treatments had a negative influence and decreased the germination and seedling characters in comparison with the control treatment. The results of mean comparison revealed that there were significant difference (p<0.05) between prechilling for 10, 20 and 30 days, potassium nitrate, thiourea and gibberellic acid 500 (Table 2). The highest germination percentage of 92% and 80% were recorded with prechilling for 10 days and potassium nitrate, respectively. There were no significant differences (p<0.05) between prechilling for 30 days and thiourea (Table 2). Prechilling for 10, 20 and 30 days, potassium nitrate and thiourea increased germination percentage by 109.09%, 72.73%, 63.64%, 81.82% and 63.64% respectively compared to the control (Figure 1). As Table 4 shows, prechilling (10, 20 and 30 days), gibberellic acid 100 and 500 and thiourea have increased germination percentage in *T. transcaspicus* species. The results revealed that potassium nitrate and gibberellic acid 1000 treatments had a negative influence and decreased the germination and seedling characters in comparison with the control treatment. The results of mean comparison indicated that there were not any significant difference (p<0.05) between prechilling for 10 days and gibberellic acid 100 (Table 4). Prechilling for 10, 20 and 30 days, gibberellic acid 100 and thiourea increased germination percentage by 50%, 60%, 20%, 50% and 20% respectively compared to the control (Figure 1).

2. Germination rate

According to the results of analysis of variance of traits (Table 1, 3), germination rate were significant (p<0.01) among different treatments in both species. The results revealed that prechilling (10, 20 and 30 days), potassium nitrate, thiourea and gibberellic acid 500 treatments increased germination rate in *Origanum vulgare*. The results of mean comparison showed that there were not any significant difference (p<0.05) between treatments of prechilling 20 and 30 days and thiourea (Table 2). The highest germination rate of 4 and 1.53 were observed in the potassium nitrate and prechilling for 10 days treatments, respectively. The lowest germination speed was obtained in gibberellic acid 100 and 1000 (.6) treatment (Table 2). As Table 4 indicates, prechilling 10and 20 days, potassium nitrate and gibberellic acid 100 treatments increased germination rate in *T. transcaspicus* species. The results of mean comparison indicated that there were not any significant difference (p<0.05) between treatments of prechilling 30 days, gibberellic acid 500 ppm and thiourea with the control treatment.

3. Shoot, root and seedling length

Analysis of variance revealed that root and shoot length of seedling significantly affected ($p<0.01$) by different treatments in tow species (Table 1, 3). Seedling length of *O. vulgare* was largely influenced by Thiourea, gibberellic acid (100 and 500) and prechilling for 10 and 20 days treatments (Table 2). gibberellic acid 1000 (ppm) treatment had no effect on shoot and root length, while potassium nitrate and prechilling 20 days decreased seedling growth compared with control treatment (Table 2). As Table 4 shows, Seedling length of *T. transcaspicus* was largely affected by gibberellic acid 100 ppm treatment. The results showed that other treatments decreased seedling growth compared with control treatment (table 4).

4. Seed vigor index

The results indicated that seed vigor index of *O. vulgare and T. transcaspicus* at the various treatments were significantly different from control treatment (Table 1, 3). The impression of treatments on seed germination showed that thiourea, prechilling for 10 and 20 days and gibberellic acid (100 and 500ppm) significantly increased seed vigor index in *O. vulgare* species, while The gibberellic acid 1000 ppm and prechilling 20 days treatments had a negative effect on seed vigor index in comparison with the control treatment (Table 2). As Table 4 indicates, seed vigor index of *T. transcaspicus* species was largely affected by gibberellic acid 100 ppm, prechilling for 10 days and thiourea treatments. The results revealed that other treatments decreased seed vigor index compared with control treatment in *T. transcaspicus* species.

5. Seedling fresh and dry weight

The results indicated that seedling fresh and dry weight of *O. vulgare and T. transcaspicus* at the various treatments were significantly different from control treatment (Table 1, 3). Seedling fresh weight of *O. vulgare* was largely effected by potassium nitrate, gibberellic acid (100 and 500ppm), prechilling for 10, 20 and 30 days and Thiourea treatments (Table 2). The results showed that gibberellic acid 1000 ppm decreased seedling fresh weight compared with control treatment (Table 2).

Table 1 Variance analysis for studied properties of *Origanum vulgare* L. affected by various treatments

Variable sources	Sum of Squares	df	Mean Square	F
Germination (%)	13550.222	8	1693.778	2540.67**
Error	18	27	0.667	
Total	13568.222	35		
Germination rate	35.171	8	4.396	1177.59**
Error	0.101	27	0.004	
Total	35.272	35		
Root length	2391.440	8	298.930	1949.54**
Error	4.140	27	0.153	
Total	2395.580	35		
Shoot length	509.040	8	63.630	280.721**
Error	6.120	27	0.227	
Total	515.160	35		
Seedling length	3498.640	8	437.330	823.425**
Error	14.340	27	0.531	
total	3512.980	35		
Vigor Index	442.790	8	55.349	602.408**
Error	2.481	27	0.092	
Total	445.271	35		
Seedling fresh weight	13406.222	8	1676.778	685.545**
Error	66	27	2.444	-
Total	13472.222	35		-
Seedling dry weight	7.556	8	0.944	3.778**
Error	6.750	27	0.250	-
Total	14.306	35	-	-

** Significant difference between treatments at 1% levels

Table 2 Germination percentage, Germination rate, Root length, Shoot length, Seedling fresh-dry weight and Vigor index in *Origanum vulgare* L.under different treatments.

Treatment	Germination%	Germination rate	Root length(mm)	Shoot length(mm)	Seedling length(mm)	Vigor Index	Seedling fresh weight (mg)	Seedling dry weight (mg)
KNO3 0.2%	80 b	4 b	4.1 b	1.9 a	6 b	2.4 b	67 b	4.5 a
GA3 100 ppm	36 c	0.6 c	16.6 c	12 b	28.6 c	5.148 c	26 c	2 c
GA3 500 ppm	56 d	0.93 d	14.2 d	11 c	25.2 d	7.056 d	49 d	3 b
GA3 1000 ppm	36 c	0.6 c	3.4 e	5.6 d	9 a	1.62 a	3 e	2 c
Thiourea 1 molar	72 e	1.2 e	26 f	4.3 e	30.3 e	10.908 e	21 f	2 c
Prechilling (10 days)	92 f	1.53 f	17.1 c	3 f	20.1 f	9.246 f	46 g	3 b
Prechilling (20 days)	76 g	1.26 e	2.4 g	1.2 g	3.6 g	1.368 a	30 h	2 c
Prechilling (30 days)	72 e	1.2 e	21 h	2.4 af	23.4 h	8.424 g	40 i	2.25 bc
Control	44 a	0.73 a	5.6 a	2.4 af	8 a	1.76 a	10 a	2 c

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

Table 3 Variance analysis for studied properties of *Thymus transcaspicus* Klokov affected by various treatments

Variable sources	Sum of Squares	df	Mean Square	F
Germination (%)	3971.556	8	496.444	744.667**
Error	18	27	0.667	
Total	3989.556	35		
Germination rate	3.129	8	0.391	104.771**
Error	0.101	27	0.004	
Total	3.230	35		
Root length	6887.680	8	860.960	3798.353**
Error	6.120	27	0.227	
Total	6893.8	35		
Shoot length	637.449	8	79.681	519.659**
Error	4.140	27	0.153	
Total	641.589	35		
Length seedling	8238.240	8	1029.780	1678.229**
Error	16.567	27	0. 614	
total	8254.807	35		
Vigor Index	728.716	8	91.089	986.43**
Error	2.493	27	0.092	
Total	731.209	35		
Seedling fresh weight	15768	8	1971	173.912**
Error	306	27	11.333	
Total	16074	35		
Seedling dry weight	94.222	8	11.778	39.750**
Error	8.000	27	0.296	
Total	102.222	35		

** Significant difference between treatments at 1% levels

Table 4 Germination percentages, Germination rate, Root length, Shoot length, Seedling fresh-dry weight and Vigor index in *Thymus transcaspicus* Klokov under different treatments.

Treatment	Germination%	Germination rate	Root length (mm)	Shoot length	Seedling length	Vigor Index	Seedling fresh weight (mg)	Seedling dry weight (mg)
KNO3 0.2%	33.33 b	1.66 b	9.7 b	2.1 b	11.8 b	1.95 b	55 b	3.5 de
GA3 100 ppm	60 c	1 c	40.8 c	13.2 c	54 c	16.2 c	71 c	5 c
GA3 500 ppm	44 d	0.73 a	21d	12.2 d	33.2 d	7.3 d	50 b	3 e
GA3 1000 ppm	36 e	0.6 d	11.8 e	10.2 e	22 e	3.96 e	43 d	2 f
Thiourea 1 molar	48 f	0.8 a	38 f	3.8 f	41.8 f	10.03 a	53 b	5 c
Prechilling (10 days)	60 c	1 c	39.8 g	3.5 fg	43.3 g	12.99 f	104 e	7 a
Prechilling (20 days)	64 g	1.06 c	6.6 h	2 b	8.6 h	2.75 g	51 b	6 b
Prechilling (30 days)	48 f	0.8 a	24 i	3 g	27 i	6.48 h	23 f	2 f
Control	40 a	0.73 a	43.2 a	5.4 a	48.6 a	9.72 a	66 a	4 d

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

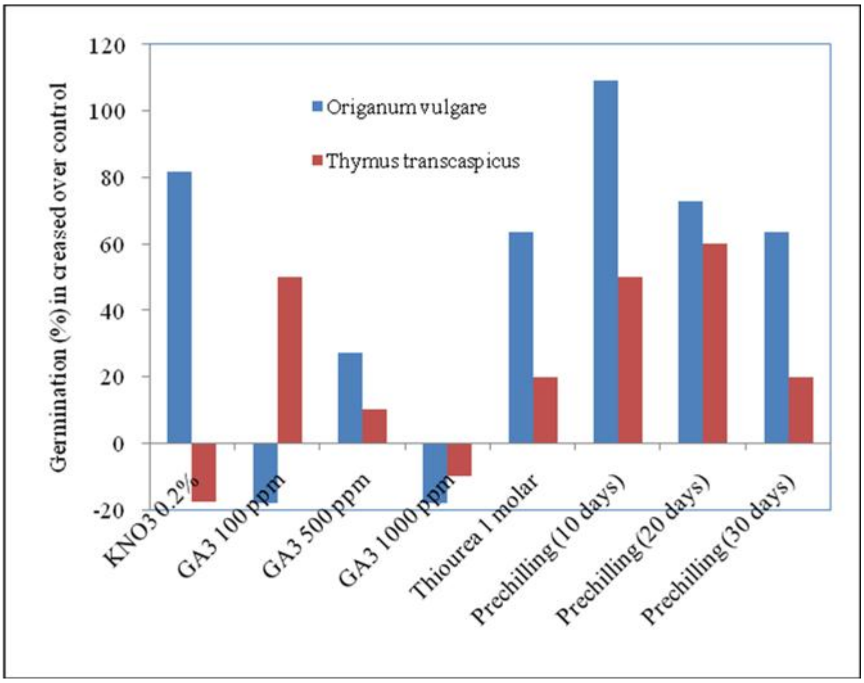

Fig. 1 Germination (%) of *Origanum vulgare* L. and *Thymus transcaspicus* Klokov seeds at different treatments over control

As Table 4 shows, prechilling 10 days and gibberellic acid 100 treatments increased seedling fresh weight in *T. transcaspicus* species, while other treatments had a negative effect on seedling fresh weight in comparison with the control treatment. The influence of treatments on seed germination indicated that potassium nitrate, gibberellic acid (500ppm) and prechilling for 10 days significantly increased seedling dry weight in *O. vulgare* species, while the prechilling (20 and 30 days), gibberellic acid (100 and 1000 ppm) and Thiourea treatments had no significant effect compared with control treatment (Table 2). The results from different treatments on *T. transcaspicus* seeds revealed that prechilling (10 and 20 days), gibberellic acid (100 ppm) and Thiourea treatments have had a positive effect on seedling dry weight of mentioned species, while the effect of potassium nitrate was not significantly different (Table 4).

Discussion

According to the obtained results, prechilling stratification was the most effective treatments for amendment of seed germination properties in two medicinal species. The most common type of seed dormancy is endogenous physiological dormancy. It is the kind of dormancy that is relieved by periods of chilling stratification [20]. Baskin *et al*.

[21] and Walck *et al*. [22] reported that Erythorium and Osmorhiza species possess a degree of physiological dormancy that can be broken with application of proper cold stratification periods. They believed that this requirement for cold stratification is related to ecological distribution of seeds. Seasonal variations in environmental conditions are liable for controlling the cycles of growth and dormancy in plants and for the timing of seed germination, eventually by means of hormone-like materials [23]. In some plant chill of winter is conducted to increasing of GAs and changes in hormonal balances is caused ready for germination [24].The chilling process could also exert its effect through changing membrane penetrability [25].Seeds of *O. vulgare* and *T. transcaspicus* species, belong to cold semi-arid climate, and thus grow better in this region. Hence, this may express that they could have developed a kind of physiological dormancy in the form of ecological adaptation that we can break by using prechilling treatments.

Prechilling stratification is a standard procedure that plays a serious role in providing the stimulant required to overcome dormancy. The action of low temperatures in ending dormancy may be: to promote a fall in the level of inhibitors, or to enhance the seeds capacity for production of high levels of promotive hormones [13]. It can be attributed that at low temperature more oxygen dissolves in water and therefore more oxygen is available for embryo [26]. It has been reported by other researchers that cold stratification is successful in ending dormancy and accelerating the germination of dormant seeds. Tavili *et al* found that prechilling (4 °C for 10 days) was the most effective treatment on seed germination of *Descurainia sophia* and *Plantago ovata* species [27]. Sharifi and Pouresmael reported that stratification at 4 °C was effective in breaking seed dormancy of *Bunium persicum* and that increasing the duration of stratification resulted in enhanced germination percentage [28]. In another research, Naderi Fasarani *et al* evaluated the effects of prechilling on seed dormancy of *Limonium iranicum* and perceived that using prechilling for 7 days at 0-5 °C enhanced germination rate [29]. Eisvand *et al* also reported that stratification of imbibed seeds of *Astragalus siliguosus* amended germination percentage as well as germination rate [30]. Rehman and Park revealed that prechilling increased germination of *Koelreuteria paniculata* Laxm by up to 44 and 45% after 60 and 90 days, respectively [31].

O. vulgare seeds were released from physiological dormancy in 0.2% concentration of KNO3; this could be because of the effect of KNO3 on the seed membrane. Other study has shown that Potassium nitrate play a regulatory role in breaking seed dormancy of many species [32, 33, 34, 35, 12].

Potassium nitrate (KNO_3) decreased the germination and seedling attributes in comparison with the control in *T. transcaspicus* plant. Potassium nitrate was very effective in breaking seed dormancy of many species [36], and it has been stated as being a growth-regulating material in Salvia species [37]. However, Potassium nitrate treatment was unable to break seed dormancy in *T. transcaspicus* in the present study. This could be due to its excessively hard seed coat. Tavili *et al* reported that using 0.1 and 0.2% KNO3 did not have huge effect on germination of *Plantago ovate* [27]. Derkan and Karssen explained that seed reaction against KNO3 is related to sensitivity of seed [38]. Mensah and Ekeke revealed that potassium nitrate was ineffective in breaking seed dormancy of *Senna obtusifolia* [39].

This research showed that 100 and 500ppm GA3 treatments significantly increased germination of *T. transcaspicus* seeds (figure 1). These results seem to confirm this assumption which probably the main cause for the dormancy of *T. transcaspicus* is related to hormone balances in embryo. With increasing GA3 concentration, the germination and seedling attributes is decreased. Gibberellins are concerned with release from dormancy, and have been shown to increase in amount during winter chilling and after dormancy-breaking photoperiodic treatments [40, 41]. In seed germination, GA3 cause an increase in RNA polymerase activity and in RNA level in hazel embryos and this is preceded by an increase in the reactivity of DNA in supporting RNA transcription. GA3 may therefore act directly or indirectly on the DNA, making it more available for transcription and allowing the various enzymes responsible for growth to be produced in lag phase of germination [25, 42].

The results revealed that there was no significant difference in seed germination and seedling attributes between the control and the gibberellic acid (100 and 1000ppm) different concentrations in *O. vulgare* species. So, addition of gibberellic acid did not raise germination. The lack of gibberellic acid effectiveness in stimulating seed germination might be referred to the following possibilities: (i) a negative effect of gibberellic acid on the level of some enzymes activity (glutamate-oxaloacetate transaminase, pyruvate kinase and malate dehydrogenase) [43], (ii) consumption of nucleotides in the synthesis of nucleic acid [44] (iii) and/or the production of a proteinaceous germination inhibitor [45]. Our results are consistent with findings of the previous studies on *Ferula assa-foetida* L. [13], *Heracleum mantegazzianum* Sommier & Levier [46], *Thlaspi arvense* L., *Descurainia sophia* (L.) Webb ex Prantl and *Malcolmia africana* (L.) R.Br. [47], *Echinacea purpurea* (L.) Moench[48] and *Sophora alopecuroides* L.[43].

The thiourea treatment increased the sprouting and seedling attributes in comparison with the control in *O. vulgare* species. Compared to the control treatment, the thiourea chemical treatment stimulated the germination of *T. transcaspicus* seeds, and the effect of thiourea on germination was statistically significant. Thiourea prevail definite types of dormancy, such as deep embryo-dormant in both plant seeds [49]. Similar results regarding the effects of thiourea on the germination were recorded in some other species [50, 51, 52]. This instigative effect of thiourea on seeds germination can be attributed to a reduction of the restraint effect of seed coat and its cytokinin activity in overcoming inhibition.

Conclusions

The results of this research indicated that the seeds of *O. vulgare* and *T. transcaspicus* are temperature dependent for germination. The usage of the prechilling stratification terminated the seed dormancy in two plants, and is the best effective stimulator for the germination and conservation of these very important medicinal plants. Based on the analysis of the obtained results, KNO3 (0.2%) and Thiourea 1 Molar affected on the germination percentage of *O. vulgare* seeds. For the *T. transcaspicus* seeds, GA3 (100 and 500 ppm) and Thiourea 1 Molar treatments increased seed germination. In general, the present investigate suggests chilling stratification as an economic and easily enforceable procedure in seed germination over costly plant growth regulators and associated technicalities for the field establishment of uniform plant population in both species.

References

1. Salehi P, Sonboli A, Eftekhar F, Nejad Ebrahimi S, Yousefzadi M. Essential oil composition, antibacterial and antioxidant activity of oils and various extracts of Ziziphora clinopodioides subsp rigida (Boiss). Biol Pharm Bull. 2005;28:1892-1896.
2. Aliakbarlu J, Farnaz Shameli F. In vitro antioxidant and antibacterial properties and total phenolic contents of essential oils from Thymus vulgaris, T. kotschyanus, Ziziphora tenuior and Z. clinopodioides. Turk J Bioch. 2013;38:425-431.
3. Naghibi F, Mosaddegh M, Mohammadi Motamed S, Ghorbani A. Labiatae Family in folk Medicine in Iran: from Ethnobotany to Pharmacology, Iranian J. Pharmaceutical Res. 2005;2:63-79.
4. Sagdic O, Kusçu A, Ozcan M, Ozçelik S. Effects of Turkish spices extracts at various concentrations on the growth of *Escherichia coli* O157:H7. Food Microbiol. 2002;19:473-480
5. Sahin F, Gulluce M, Daferera D, Sokmen A, Polissiou M, Agar G, Ozer H. Biological activities of the essential oils and methanol extract of *O. vulgare* ssp. v*ulgare* in the Eastern Anatolia region of Turkey. Food Cont. 2004;15:549-557.
6. Rechinger KH. Satureja. In: Cavaleiro C, Salgueiro LR, Antunes T, eds. Flora Iranica. Graz, Austria: Sevinate. 1980.
7. Keller M, Kollmann J. Effects of seed provenance on germination of herbs for agricultural compensation sites. Agriculture, Ecos. Environ. 1999;72:87-99.
8. Sarmadnia, G.H. Seed technology, Mashhad Univ. Press. 1997;288.
9. Jun R, Tao L. Effects of different presowing seed treatments on germination of 10 Calligonum species. Forest Eco. Manag. 2004;195:291-300.
10. Lindig CR, Lara-Cabrera S. Effect of scarification and growing media on seed germination of *Crotalaria pumila* (Ort.). Seed Sci. Technol. 2004;32:231-234.
11. Yamauchi Y, Ogawa M, Kuwahara A, Hanada A, Kamiya Y, Yamaguchi S. Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. Plant Cell. 2004;16:367-378.
12. Yazdanshenas H, Nasiri M, Tavili A. Effect of Different Physicochemical Treatments on Seed Dormancy of Medicinal Herbs (*Portulaca oleracea* L.). Res. J Medi Plant. 2015;9:72-80.
13. Hassani SB, Saboora A, Radjabian T, Fallah- Husseini H. Effects of Temperature, GA3 and Cytokinins on breaking seed dormancy of *Ferula assa-foetida*. Iranian J Sci Techno Transaction. 2009;33:75-85.
14. Hassan shaykhi A, Majd Nassiry B, Ataei Kachouei M. Effect of some Treatments on seed dormancy, germination and antioxidant enzymes of Kelussia odoratissima Mozaff. Seeds. Cercetări Agronomice în Moldova. 2015;162:79-90.

15. Tavili A, Safari B, Saberi M. Investigation of compiration effective use of acid gibberelin and KNO3 on improve germination characteristics *Salsola rigida*. J Rangeland. 2009;3:272-280.
16. Asaadi AM. Study of Ecological Characters of Ziziphora Clinopodioides L. Medicinal Plant in North East Rangelands of Iran. Inter J Agric Biosci, 2016;5:373-377.
17. Anonymous. Hand Book for Seedling Evaluation. International Seed Testing Association
(ISTA), Zurich, Switzerland. 2012.
18. Camberato J, Mccarty B. Irrigation water quality: part I. Salinity. South CarolinaTurfgrass Foundation New. 1999;6:6-8.
19. Abdul-baki A, Anderson JD. Vigor determination in soybean seed by multiplication. Crop Sci. 1973;3:630-633.
20. Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. New Phytologist, 2006;171:501-523.
21. Baskin CC, Mayer E, Baskin JM. Two type of morpho physiological dormancyin seeds of two genera (*Erythorium* and *Osmorhiza)* with an Arcto- Tertiary distribution pattern. Amer. J. Bot. 1995;82:293-298.
22. Walck JL, Hidayati SN, Okagami N. Seed germination echophysiology of Asian species *Osmorhiza aristata* (Apiaceae): Comparison with its North American cogenera and implications for evolution of type of dormancy. Am J Bot. 2002;89:829-835.
23. Bewley JD, Black M. Seeds physiology of development and germination. plenum press, New York. 1985;221.
24. Copeland L.O. and McDonald M.B. Principals of seed science and technology third edition. Chapman and Hall New york. 1995.
25. Villiers TA. Dormancy and the survival of plants Edward Arnold publishers limited. London. 1978;71.
26. Young JA, Young CG. Seeds of Woody Plants in North America. Dioscorides Press, Portland, Oregon. 1992.
27. Tavili A, Pouzesh H, Farajolahi A, Zare S, Zare Chahooki M. The effect of different treatments on improving seed germination characteristics in medicinal species of *Descurainia sophia* and *Plantago ovata*, African J. Biotechnology. 2010;9:6588-6593.
28. Sharifi M, Pouresmael M. Breaking seed dormancy in *Bunium persicum* by stratification and chemical substances. Asian. J. Plant Sci. 2006;5:695-699.
29. Naderi Fasarani A, Bassiri M, Roshan nazar B, Tavili A. Investigation on different treatments effects on seed germination of *Limonium iranicum*. Rangeland, 2009;3:456-464.
30. Eisvand HR, Arefi HM, Tavakol-Afshari R. Effect of various treatments on breaking seed dormancy of *Astragalus siliquosus*. Seed Sci Technol. 2006;34:747-752.
31. Rehman S, Park IH. Effect of scarification, GA and chilling on the germination of goldenraintree (*Koelreuteria Paniculata* Laxm.) seeds. Sci Hortic. 2000;85:319-324.
32. Gashi B, Abdullai K, Mata V, Kongjika E. Effect of gibberellic acid and potassium nitrate on seed germination of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae*, African J. Biotechnol 2012;11:4537-4542.
33. Li WQ, Liu XJ, Khan MA, Kamiya Y, Yamaguchi S. Hormonal and environmental regulation of seed germination in flixweed (*Descurainia sophia*). Plant Growth Regul. 2005;45:199–207.
34. Karimmojeni H, Rashidi B, Behrozi D. Effect of different treatments on dormancy breaking and germination of perennial pepperweed (*Lepidium latifolium*) (Brassicaceae), Australian J. Agri Engineering. 2011;2:50-55.
35. Raisi A, Nabavi Kalat SM, Sohani Darban AR. The Study Effects of Stratification, Temperature and Potassium Nitrate on Seed Dormancy Breaking *Ferula assa- foetida*, World Applied Sci. J. 2013;21:379-383.
36. Previero CA, Martins L, Fonseca RHA, Groth D. Effect of storage of guinea grass (*Panicum maximum* Jacq.) on treatment to break dormancy. Revista Brasileira de Sementes. 1996;18:143-148.
37. Yucel, E. Effects of different salt (NaCl), nitrate (KNO3) and sulphuric acid (H2SO4) concentrations on the germination of some salvia species seeds. Seed Sci Techno. 2000;28:853-860.
38. Derkan MPM, Karssen CM. Effect of light and temperature on seed dormancy and gibberellins-simulated germination in *Arabidopsis thaliana*: studies with gibberellins deficient and in sensitive mutants. Physiol Plant. 1993;89:360-368.
39. Mensah S, Ekeke C. Effects of Different Pretreatments and Seed Coat on Dormancy and Germination of Seeds of Senna obtusifolia (L.) H.S. Irwin & Barneby (Fabaceae). Inter J Biol. 2016;8:77-84.
40. Atwater BR. Germinations dormancy and morphology of the seeds of herbaceous ornamental plants. Seed Sci Technol. 1980;8:523-573
41. Gonzalez- Melero JA, Perez- Gareia F, Martinez-Laborde JB. Effect of temperature, scarification and gibberellic acid on the seed germination of three shrubby species of coronilla. Seed Sci. technol. 1997;25:167-175.
42. Baskin C.C. and Baskin J.M. Seed ecology, dormancy and germination A modern synthesis. Am J Bot. 1999;86:903-905
43. Aliloo AA, Mustafavi SH. Do physicochemical pre-treatments can alleviate germination and dormancy of *Sophora alopecuroides* seeds? Azarian J Agri. 2014;1:6-10.
44. EL-Dengawy EFA. Physiological and biochemical studies on seed dormancy and germination process in deciduous fruit trees. Ph.D. Thesis, Mansoura Univ., Egypt. 1997.
45. Balouchi HR, Modarres- Sansvy AM. Effect of gibberellic acid, prechilling, sulphuric acid and

potassium nitrate on seed germination and dormancy of annual medics. Pakistan J Biol Sci. 2006;9:2875-2880.

46. Moravcova L, Pysek P, Krinke L, Pergl J, Perglova I, Thompson K. Seed germination, dispersal and seed bank in *Heracleum mantegazzianum*. CAB International,Wallingford, UK. 2007;74-91.
47. Karimmojeni H, Taab A, Rashidi B, Bazrafshan AH. Dormancy breaking and seed germination of the annual weeds *Thlaspi arvense, Descurainia sophia* and *Malcolmia Africana* (Brassicaceae), J Plant Protection Res. 2014;54:179-187.
48. Karimian Fariman Z, Azizi M, Noori S. Seed Germination and Dormancy Breaking Techniques for *Echinacea purpurea* L. J Biol Environ Sci. 2011;5:7-10.
49. Hartmann HT, Kester DE, Davies FJ, Geneve RL. Plant Propagation Principles and Practices. Sixth Edition. New Jersey, Prentice Hall. 1997.
50. Agrawal PK, Dadlani M,. Techniques in Seed Science and Technology. Second Edition. South Asian Publishers New Delhi International Book Company Absecon Highlands.1995;109-113.
51. Jana BR, Das B. Effect of dormancy breaking chemicals on flowering, fruit set and quality in Asian pear (*Pyrus pyrifolia* L.), African J Agri Res. 2014;9:6-60.
52. Ikram RM, Tanveer A, Ata Z, Saqib M. Dormancy studies on *Euphorbia dracunculoides* and *Astragalus* spp.: Major weeds of arid areas. Planta Daninha, Viçosa-MG. 2014;32:747-753.