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Influence of Some Physicochemical Treatments to Stimulate Seed Germination of *Agrimonia eupatoria* L. and *Clinopodium vulgare* L.

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ABSTRACT

Dormancy is an inherent limitation on germination that happens across in the life cycle of a plant. The particular goals of this research were to survey the effects of various treatments for improving seed germination of *Agrimonia eupatoria* and *Clinopodium vulgare*. These plants are known to have low seed germination. A trial was conducted with 4 replications and 13 treatments in a completely randomized design in the Seed Laboratory of Agriculture Faculty of Shirvan. The seeds were treated with three concentrations of gibberellic acid (100, 500 and 1000 ppm), three periods of dry cold stratification (10, 20 and 30 days at 2 °C), three periods of wet cold stratification (10, 20 and 30 days at 2 °C), sulphuric acid (98%) for 5 minutes, potassium nitrate (0.2%), thiourea 1 molar and distilled water as control. The seeds were monitored daily in Petri dishes for 22 days. The findings suggested that the effect of different treatments on *A. eupatoria* and *C. vulgare* seeds germination percentage were significantly ($p<0.01$) influenced. Treatments of dry cold stratification (10 and 20 days) and wet cold stratification (10 days) have the highest effect on seeds germination percentage and germination rate of *A. eupatoria*. For *C. vulgare* seeds, the highest germination percentage and germination rate was found when exposed to dry and wet cold stratification (30 days) treatments. The maximum and minimum vigor indices in *A. eupatoria* were seen under treatment of dry cold stratification for 30 days (20.5) and 100 ppm gibberellic acid (4.99), and were also seen in *C. vulgare* under treatment of potassium nitrate (11.39) and GA3 (100 ppm). The findings also revealed that sulphuric acid, potassium nitrate and thiourea on *A. eupatoria* and thiourea on *C. vulgare* did not seed germination.

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1. Introduction

Plant seed dormancy is a status of a viable seed, indicated by the prevention of germination under favorable ecological conditions demanded sufficient seed germination (Baskin and Baskin, 2004; Hilhorst, 2011). Dormancy is an adaptive trait in the natural habitat that optimizes the distribution of germination over time in a population of seeds (Fenner and Thompson, 2006; Ganjali *et al*., 2022).

Herb Biologists introduced that seed germination capacity and enhanced seedling establishment are depended on both sets of agents: intrinsic agents, including plant hormones (Gibberellic and Abscisic acid balance), proteins, chromatin-related agents (histone ubiquitination, methylation, acetylation), related genes (hormonal, epigenetics-regulating genes and maturating genes), non-enzymatic processes, seed structural and morphological components (seed coat, endosperm, pericarp), and extrinsic agents, such as temperature, light, soil nitrate, acidity, salinity (Miransari and Smith, 2014; Nee *et al*., 2017). Both biotic and abiotic agents interact and define the presence or absence of dormancy through seed growth in absorbed ripe seeds and dry seeds.

The investigation of seed germination of pharmaceutical herb species has obtained special consideration from the scientific association due to the enhanced demand for these herbs in the medicinal industry, coupled with the requirement to make illustrative crops for the production of herbs (Elhindi *et*

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al., 2016). Ecological factors such as light, temperature, soil humidity and pH influence seed germination (Ghaderi far *et al*., 2010; Ganjali *et al*., 2022). Temperature actions have a main function in determining the alternation of plant seed germination and the distribution of herbs (Guan *et al*., 2009). Germination speed commonly enhances linearly with temperature, at least within a well-determined range, and decreases harshly at higher temperatures (Alvarado and Bradford, 2002).

Many of herb seeds that are produced in natural habitat conditions, such as rangeland ecosystems, exhibit various levels of seed dormancy. The international seed testing association (ISTA) and the Association of Official Seed Analysts (AOSA) have proposed several techniques for germination experiments of many herb species. It is well known that various techniques like cold stratification, scarification (chemical and physical), various solutions (including: KNO3, abscisic acid (ABA), thiourea, nitric acid, ethanol, glycol, polyethylene etc), temperature and light alternatives are used to release dormancy (Rezvani *et al*., 2018). Investigates display that many of the herb hormones, including gibberellin, cytokinin, ethylene, auxin and abscisic acid, may regulate nucleic acids efficiency improvement of seed germination and promote dormancy (Chiwocha *et al*., 2005).

The genus Agrimonia is herbaceous and consists of 16 species distributed in the Southern and Northern temperate zones of the world (Granica *et al*., 2015). *A. eupatoria* L. belongs to the family Rosaceae. The species is widespread throughout Afghanistan, Iran, Europe, Africa and North America. In Iran, this plant grows in provinces such as Mazandaran, Northern Khorasan, Gilan, Ghazvin, Lorestan, Azerbaijan, Ardabil, Tehran and Golestan (Masjedi *et al*., 2016). *A. eupatoria* is used in the treatment of liver diseases. Recent researches led to the finding of antioxidant, antiviral and anti-inflammatory properties as well as increased metabolism. Nowadays, more study is focused on herb health profits related to MS disease. The herbal tea of this medicinal herb is efficient in the disposal of gallstones and the treatment of diarrhea (Zargari, 1989; Najafpour Navaei, 2017). *C. vulgare* (Lamiaceae family) is erect, up to 45 cm tall perennial herbaceous plant. Aerial parts of *C. vulgare* are utilized in traditional medicine for the therapy of gastric ulcers, diabetes and cancer (Nasar-Eddin *et al*., 2019).

As there is lack of data on seed germination necessities of *A. eupatoria* and *C. vulgare*. The goals of the present survey were to define the influence of various treatments on seed germination and to introduce an efficient technique for progressing seed germination traits in medicinal plants of *A. eupatoria* and *C. vulgare*.

2. Materials and methods

2.1. Seed collection

The ripe Seeds of *A. eupatoria* and *C. vulgare* were gathered from arid areas of the Northeast Rangelands of Iran, Akhardagh in Bojnourd.

2.2. Study area of species

The geographical coordinates of the survey area are 37° 23' to 37° 26' North latitude and from 57° 7' to 57° 15' East longitudes. The study site is nearly 5500 hectares with a minimum and maximum elevation of 1300 m to 1700 m above sea level. The annual average rainfall is 295mm. The highest and lowest rainfall occurs in April and July, respectively. The annual average temperature is 11.28 °C. The average maximum recorded temperature of the warmest month (July) is 26 °C and the minimum recorded temperature of the coldest month is -6.8 °C which occurred in January. According to the Emberger method, the climate of this area is cold and semi-arid. These herbs usually prefer lime, sandy loam texture, non-saline and alkaline pH soils (Asaadi, 2016).

2.3. Germination experiment

In order to determine the best effective method to overcome seed dormancy, 13 various treatments were applied. The seeds were treated with three levels of concentrations of gibberellic acid (100, 500 and 1000ppm), concentrated sulphuric acid (98%) for 5 minutes, potassium nitrate (0.2%), thiourea 1 molar, three periods of wet, cold stratification (10, 20 and 30 days), three periods of dry cold stratification (10, 20 and 30 days at 2- 4 °C before the germination test) and distilled water as a control treatment. Before the experiment, all the seeds were cleaned and prepared. The survey was carried out in the Laboratory of Shirvan Agriculture Faculty in 2020. The seeds were sterilized using sodium hypochlorite solution (2%) for 5 minutes, and rinsed with distilled water several times and left to dry under room conditions. For the seed germination

experiments, 20 seeds were placed in plastic petri dishes (90 mm diameter) with two layers of Whatman No.1 filter paper and plates were transferred to germinator 18 °C for 22 days. The treatments were arranged in a completely randomized design with four replications.

2.4. Measured characteristics

A seed was considered germinated when the radical emergence of more than 2 mm in length had grown (Tavili *et al*., 2009). The seeds germination was monitored daily for 22 days. At the final of the seed germination experiment, the seed germination percentage, germination rate, shoot and root length, seed vigor index and seedling fresh and dry weight were calculated by the International Seed Testing Association criterion (Asaadi, 2017). Based on the following equations, the seed germination parameters were recorded:

Germination Percentage = GP = (GS / TS) × 100 (1)

Where GP is germination percentage, GS is the number of germinated seeds and TS is the total number of seeds (Abdul-baki, 1973).

Germination speed: GS= Σ Si / Di (2)

Where Si is the number of germinated seeds at each counting, Di is the number of days until n, and n is the number of counting (Finch-Savage and Leubner-Metzger, 2006).

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

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

2.5. Statistical analysis

The SPSS program was used for the statistical analyses of the obtained data. The means were compared by Duncan's multiple range tests at $p \leq 0.05$.

3. Results and discussion

In this study, the obtained finding of the seed germination and early seedling growth parameters were illustrated in Tables 1-4. Maximum germination percentages in non-treated *A. eupatoria* and *C. vulgare* seeds were 10% and 30.67%, respectively. There were significant ($P \leq 0.01$) influenced between various treatments in terms of their effects on germination percentage, germination speed, vigor index, shoot and root length, seedling fresh weight and seedling dry weight. No germination was recorded by soaking in H2SO4 (98%) for 5 min, thiourea 1 molar and KNO3 0.2% on *A. eupatoria* and thiourea on *C. vulgare* where germination was failed.

The findings of the analysis of variance for final germination percentage in *A. eupatoria* and *C. vulgare* seeds displayed that there was a statistically significant difference among the treatments (Tables 1 and 3). The influence of treatments on germination indicated that dry and wet cold stratification (10, 20 and 30 days) and concentrations of gibberellic acid 100, 500 and 1000 ppm have enhanced germination percentage in *A. eupatoria* species, while the sulphuric acid (98%), KNO3 0.2% and thiourea 1 molar treatments have no significant effect. The findings of the mean comparison demonstrated that there were not any significant ($p<0.05$) influenced between dry cold stratification for 10 and 30 days and wet prechilling 10 days (Table 2). The most germination percentage of 56.7%, 53.3% and 45% were found with dry cold stratification for 10 days and 30 days and wet cold stratification for 10 days, respectively (Table 2). Dry cold stratification for 10, 20 and 30 days, wet cold stratification for 10, 20 and 30 days, and gibberellic acid concentrations 100, 500 and 1000 ppm enhanced germination percentage by 50%, 60%, 20%, 50% and 20% respectively compared to the control (Fig.1).

According to Table 4 results, wet cold stratification (10, 20 and 30 days), Dry cold stratification (10, 20 and 30 days), gibberellic acid (100, 500 and 1000 ppm) and KNO3 0.2% have enhanced germination percentages in *C. vulgare* species. The findings suggested that thiourea 1 molar treatments had a negative influence the germination and seedling characteristics in comparison with the distilled water treatment. The findings of the mean comparison showed that there were not any significantly influenced ($p<0.05$) between cold stratification for 10 days and gibberellic acid 100 (Table 4). Dry cold stratification for 30 days, wet cold stratification for 30 days and gibberellic acid 1000 ppm enhanced germination percentage by 160.84%, 139.09%, and 121.71%, respectively, compared to the control (Fig. 1).

Findings of analysis variance indicated that there was a significantly influenced between experimental treatments for germination rate in both species (Table 1, 3). The findings revealed that dry cold stratification for 10 and 30 days and wet cold stratification for 10 days treatments enhanced the germination rate in *A.*

eupatoria. The findings of the mean comparison revealed that there were not any significantly ($p<0.05$) influenced between treatments of wet cold stratification (10 and 20 days), dry cold stratification (20 days) and gibberellic acid concentrations (100, 500 and 1000 ppm) with the distilled water treatment (Table 2). The most rate of germination was found for treatments of dry cold stratification for 10 and 30 days (1.27 Seed/day), which enhanced more than 496% compared to distilled water treatment. Based on Table 4 results, gibberellic acid 500 and 1000 ppm, dry cold stratification 10 and 30 days, wet cold stratification (10, 20 and 30 days) treatments enhanced the germination rate in *C. vulgare* species. The findings of the mean comparison revealed that there were not any significantly influenced ($p<0.05$) between treatments of potassium nitrate, gibberellic acid 100 ppm and dry cold stratification 20 days, with the distilled water treatment.

Table 1. Results of variance analysis for surveyed characteristics of *A. eupatoria* affected by different treatments.

Source of variation	Sum of Squares	df	Mean Square	F
Germination (%)	17630.769	12	1469.231	17.020**
Error	3366.667	39	86.325	
Total	20997.436	51		
Germination rate	9.474	12	.789	18.566**
Error	1.658	39	.043	
Total	11.132	51		
Root length	26770.813	12	2230.901	19**
Error	4579.108	39	117.413	
Total	31349.921	51		
Shoot length	575.953	12	47.996	32.855**
Error	56.973	39	1.461	
Total	632.926	51		
Seedling length	34676.500	12	2889.708	21.691**
Error	5195.696	39	133.223	
total	39872.196	51		
Vigor Index	1416.143	12	118.012	10.167**
Error	452.687	39	11.607	
Total	1868.830	51		
Seedling fresh weight	21311.179	12	1775.932	18.432**
Error	3757.688	39	96.351	
Total	25068.867	51		
Seedling dry weight	1054.568	12	87.881	12.489**
Error	274.426	39	7.037	
Total	1328.995	51		

Mean in each column followed by same letters are not significantly different at the P 0.05.

Table 2. Effect of various treatments on germination percentages, germination rate, root length, shoot length, vigor index and seedling fresh-dry weight in *A. eupatoria*.

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%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Thiourea 1 molar	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
H2so4 98%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
GA3 100 ppm	21.666^{bc}	0.343^{b}	44.77^{cd}	6.666^{bc}	51.44^{c}	4.987^{abc}	42.48^{bcd}	7.241^{b}
GA3 500 ppm	23.333^{bc}	0.399^{b}	46.50^{cd}	6.066^{b}	52.566^{cd}	6.320^{bc}	45.99^{cde}	9.556^{bc}
GA3 1000 ppm	30^{c}	0.482^{b}	57.30^{cde}	6.443^{bc}	63.743^{cde}	8.617^{c}	40.67^{bcd}	9.256^{b}
Wet cold (10 days)	45^{d}	0.997^{c}	26.56^{b}	6.406^{bc}	32.967^{b}	7.680^{bc}	27.906^{b}	5.605^{b}
Wet cold (20 days)	16.666^{bc}	0.258^{ab}	47.71^{cd}	8.663^{d}	56.373^{cd}	4.60^{abc}	50.353^{de}	6.313^{b}
Wet cold (30 days)	18.333^{bc}	0.364^{b}	62.00^{de}	8.17^{cd}	70.17^{de}	7.483^{bc}	58.313^{e}	14.886^{d}
Dry cold (10 days)	56.667^{d}	1.27^{c}	26.75^{b}	6.026^{b}	32.78^{b}	9.329^{c}	29.003^{b}	6.025^{b}
Dry cold (20 days)	21.666^{bc}	0.383^{b}	43.50^{c}	7.0^{bcd}	50.50^{c}	6.033^{bc}	40.183^{bcd}	5.771^{b}
Dry cold (30 days)	53.333^{d}	1.269^{c}	65.52^{e}	10.729^{e}	76.254^{e}	20.495^{d}	59.61^{e}	13.29^{cd}
Control	10^{ab}	0.213^{ab}	51.80^{cde}	7.266^{bcd}	59.066^{cde}	2.855^{ab}	32.696^{bc}	7.713^{b}

Mean in each column followed by same letters are not significantly different at the P 0.05.

Table 3. Results of variance analysis for surveyed characteristics of *C. vulgare* affected by different treatments.

Source of variation	Sum of Squares	df	Mean Square	F
Germination (%)	20764.444	11	1887.677	34.069**
Error	1994.667	36	55.407	
Total	22759.111	47		
Germination rate	28.433	11	2.585	45.812**
Error	2.031	36	.056	
Total	30.464	47		
Root length	4338.884	11	394.444	49.393**
Error	287.491	36	7.986	
Total	4626.375	47		
Shoot length	35.103	11	3.191	22.911**
Error	5.014	36	0.139	
Total	40.117	47		
Seedling length	5077.139	11	461.558	46.932**
Error	354.043	36	9.835	
total	5431.182	47		
Vigor Index	537.301	11	48.846	34.829**
Error	50,488	36	1.402	
Total	587.790	47		
Seedling fresh weight	9662.392	11	878.399	22.258**
Error	1420.693	36	39.464	
Total	11083.086	47		
Seedling dry weight	349.221	11	31.747	7.518**
Error	152.020	36	4.223	
Total	501.241	47		

** Significant at the P 0.01 levels.

Table 4. Effect of various treatments on germination percentages, germination rate, root length, shoot length, vigor index and seedling fresh-dry weight in *C. vulgare*.

Treatment	Germination%	Germination rate	Root length (mm)	Shoot length (mm)	Seedling length (mm)	Vigor Index	Seedling fresh weight (mg)	Seedling dry weight (mg)
Thiourea 1 molar	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
KNO3 0.2%	52^{cd}	1.272^{bc}	40.90^{g}	2.90^{b}	43.80^{g}	11.39^{de}	8.03^{ab}	3.93^{bc}
GA3 100 ppm	46.67^{c}	1.383^{bcd}	29.99^{de}	3.10^{b}	33.09^{de}	7.73^{c}	9.27^{abc}	2.67^{ab}
GA3 500 ppm	$60d^{e}$	1.664^{d}	35.32^{f}	2.81^{b}	38.14^{f}	11.30^{de}	17.93^{cd}	2.43^{ab}
GA3 1000 ppm	68^{ef}	2.665^{fg}	27.77^{bcd}	3.01^{b}	33.78^{def}	11.34^{de}	15.53^{bcd}	1.67^{ab}
Wet cold (10 days)	66.67^{ef}	2.041^{e}	24.60^{bc}	3.23^{b}	27.84^{bc}	9.21^{c}	25.07^{d}	8.1^{d}
Wet cold (20 days)	66.67^{ef}	2.735^{g}	33.59^{ef}	3.32^{b}	36.90^{ef}	12.27^{e}	36.43^{e}	7.73^{d}
Wet cold (30 days)	73.33^{fg}	2.345^{ef}	23.35^{b}	2.80^{b}	26.16^{b}	9.54^{cd}	42.67^{e}	7.8^{d}
Dry cold (10 days)	49.33^{cd}	1.562^{cd}	28.43^{cd}	3.25^{b}	31.68^{cd}	7.78^{c}	18.80^{cd}	7.43^{d}
Dry cold (20 days)	46.67^{c}	1.095^{b}	30.93^{de}	3.07^{b}	34.00^{def}	7.91^{c}	42.40^{e}	3.17^{ab}
Dry cold (30 days)	80^{g}	2.516^{fg}	25.00^{bc}	3.05^{b}	28.06^{bc}	11.16^{de}	43.70^{e}	6.87^{cd}
Control	30.67^{b}	1.113^{b}	27.83^{bcd}	2.84^{b}	30.67^{bcd}	4.77^{b}	21.86^{d}	3.53^{b}

Mean in each column followed by same letters are not significantly different at the P 0.05.

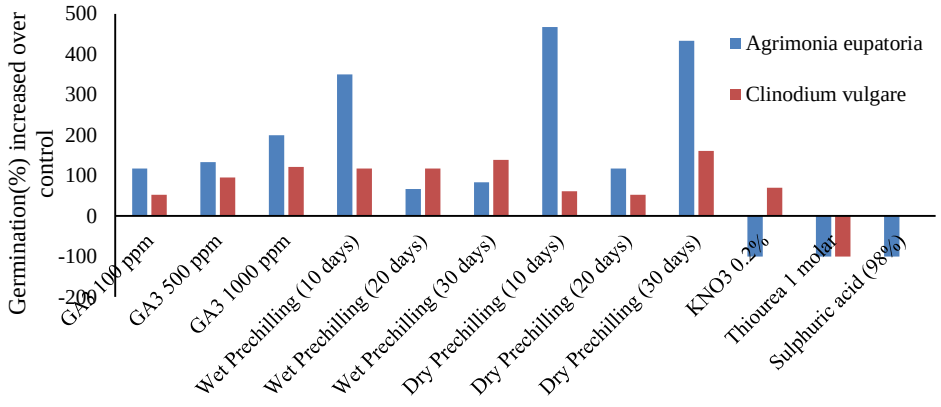

Figure 1.Germination percentage of *A. eupatoria* and *C. vulgare* seeds at different treatments over control.

The findings of the experiment revealed that the effect of experimental treatments on the root and shoot length of seedling were significant in both species (Table 1, 3). Root and shoot length of *A. eupatoria* were largely affected by dry cold stratification for 30 days of treatment (Table 2). Dry cold stratification (10 and 20 days), wet cold stratification (10 and 20 days) and gibberellic acid (100 and 500 ppm) treatments had no effect on the shoot and root length (Table 2). According to Table 4 results, the seedling length of *C. vulgare* was largely influenced by potassium nitrate, gibberellic acid 500 ppm and wet cold stratification 20 days treatments. The findings indicated that other treatments declined seedling growth compared with the control (Table 4).

The findings indicated that seed vigor index of *A. eupatoria* and *C. vulgare* at the various treatments were significantly different from the control treatment (Table 1, 3). The impression of treatments on seed germination showed that dry cold stratification for 10 and 30 days and gibberellic acid (1000 ppm) significantly enhanced seed vigor index in *A. eupatoria* species, while the gibberellic acid 100 and 500 ppm and wet cold stratification 20 days treatments had no effect on seed vigor index in comparison with the control treatment (Table 2). The highest value of the vigor index (20.495) was related to dry cold stratification (30 days) treatment. The results revealed that experimental treatments enhanced the seed vigor index compared with distilled water treatment in *C. vulgare* species (Table 4).

The findings of this experiment showed that seedling fresh and dry weight of *A. eupatoria* and *C. vulgare* at the various treatments were significantly different from the control treatment (Table 1, 3). Seedling fresh weight of *A. eupatoria* was largely affected by dry cold stratification for 30 days and wet cold stratification for 20 and 30 days treatments (Table 2). Dry cold stratification for 30 and 20 days, wet cold stratification for 30 and 20 days, gibberellic acid 1000, 500 and 100 ppm enhanced seedling fresh weight by 82.35%, 22.91%, 78.37%, 54.02%, 24.41%, 40.68% and 29.95% respectively compared to the control. As Table 2 shows, dry cold stratification 30 days, wet cold stratification for 30 days and gibberellic acid (500 and 1000 ppm) treatments enhanced seedling dry weight in *A. eupatoria* species, while other treatments had a negative effect on seedling dry weight in comparison with the distilled water treatment. As Table 4 shows, dry cold stratification (20 and 30 days) and wet cold stratification (20 and 30 days) treatments enhanced seedling fresh weight in *C. vulgare* species, while other treatments had a negative effect on seedling fresh weight in comparison with the distilled water treatment.

The process of seed germination is complex, which begins with the imbibition of water, followed by a short remain, and finishes with the synthesis and activation of enzymes. Germination is regulated by many interactions of hormonal and ecological agents, and this is feasible only when appropriate conditions are met (Aticia *et al*., 2003). Germination happens as a consequence of the partial presence of the cotyledons in the seeds, which permits the proceeding of hydrolysis whereby hormones enhance nucleic acid metabolism and protein combination, are released (Uwaegbute, 1996). Seed dormancy is a physiological phenomenon that many range land plants, crops, and weeds face (Ganjali *et al*., 2022). These phenomena can help herbs adapt to various habitats and climates and survive in various ecosystems (Rezvani *et al*., 2018).

According to the received findings, cold stratification was the best efficient treatment for improvement of seed germination characteristics in both pharmaceutical plant species. Both dry and wet prechilling (10, 20 and 30 days) increased seed germination of A. eupatoria about 467%, 117%, 433%, 350%, 67%, 83.3% and C. vulgare about 61%, 52%, 161%, 117%, 117%, 139% compared with the control, respectively (Fig. 1). Across all vegetation regions on terrain, physiological dormancy is the highest current type. Seeds with physiological dormancy have wholly developed embryos with a physiological prohibiting mechanism for germination (Baskin and Baskin, 2020). It is a sort of dormancy that is removed by courses of cold stratification (Finch-Savage and Leubner-Metzger, 2006).

The positive effects of dry and wet prechilling on seed germination of various vegetation species were formerly introduced by Moradi et al. (2018) and Wartidiningsih et al. (1994). It has been stated by other investigators that prechilling stratification is successful in relieving dormancy and precipitating the germination of dormant seeds. Kheloufi et al. (2020) reported that cold stratification (5°C for 120 days) was the highest effective treatment for seed germination of

Ziziphus lotus species. Asaadi (2017) found that cold stratification (10 and 20 days) at 4 °C was effective in removing seed dormancy of Thymus transcaspicus and Origanum vulgare seeds. Naderi Fasarani et al. (2009) appraised the effects of cold stratification on seed dormancy of Limonium iranicum and stated that applying cold stratification for 7 days at 0-5 °C enhanced germination speed. Eisvand et al. (2006) also indicated that stratification is effective in the stimulation seeds of Astragalus siliguosus. Similar to these findings, a positive effect at low temperatures in causing dormancy removal has also been presented in Ferula gummosa seeds by Nadjafi et al. (2006). Their findings displayed that cold stratification of dormant seeds at 5 ˚C for a period of 14 days resulted in the most germination index (0.45 seed per week) and percentage (26.1%) of Ferula gummosa.

The prechilling stratification may change the hormonal equilibrium of the seed and augment germination through the increase of cytokinin and gibberellic acid activity and/or the decrease of abscisic acid (Copeland and McDonald, 2001). The wet prechilling prepares adequate humidity to actuate the hydrolytic enzymes that make seeds provide to germinate once they are moved to the warm temperature.

Seasonal changes in ecological statuses are responsible for regulating the periods of development and dormancy in herbs and for the timing of seed germination, finally by using hormone-like substances (Bewley and Black, 1985). The cold stratification process could also apply its result by varying membrane permeability (Villiers, 1978). Seeds of A. eupatoria and C. vulgare species belong to chilly semi-arid climates and thus grow better in this area. Therefore, this may declare that they could have developed a type of physiological dormancy in the form of environmental conformity that we can release through cold stratification treatments.

According to the findings acquired in this experiment, the gibberellic acid stimulates 100, 500 and 1000 ppm, significantly enhancing the germination percentage of A. eupatoria and C. vulgare seeds. This study indicated that 100, 500 and 1000 ppm GA3 treatments increased seed germination of A. eupatoria about 117%, 133%, 200% and C. vulgare about 52%, 96% and 121% compared with the control, respectively. Considering the findings obtained in this research, A. eupatoria and C. vulgare species probably exhibit a combination of physiological dormancy. These findings seem to confirm this assumption which is probably the main cause for the dormancy of A. eupatoria and C. vulgare are related to hormone balances in embryos.

Considering the findings obtained in this research, A. eupatoria and C. vulgare species presumably show a combination of physiological dormancy. These findings appear to admit this presumption which likely the basic reason for the dormancy of A. eupatoria and C. vulgare are associated with hormone equilibriums in embryos. The seed germination and seedling properties are enhanced by enhancing the concentration of gibberellic acid. GA3 stimulates connected with release from dormancy and have been indicated to augment in amount during the winter cold and after dormancy-releasing photoperiodic treatments (Atwater, 1980; Gonzalez- Melero, 1997; Rezvani *et al*., 2018). In seed germination (imbibitions) of plant species, GA3 reason an increment in RNA polymerase activity and in RNA amount in hazel embryos and this is preceded by an increment in the reactivity of DNA in patronizing RNA transcription. GA3 may hence act directly or indirectly on the DNA, making it more accessible for transcription and permitting the different enzymes liable for an increase to be produced (Villiers, 1978; Baskin and Baskin, 1999).

In C. vulgare application of KNO3 promoted overall seed germination. This could be due to the action of KNO3 on the seed membrane. Another survey has indicated that potassium nitrate play a regulatory role in releasing seed dormancy of many plant species (Khajeh-Hosseini *et al*., 2018).

In our research, Seeds of A. eupatoria indicated no response to different treatments of H2SO4 (98%) for 5 min, thiourea 1 molar and KNO3 0.2% since H2SO4 (98%) for 5 min, thiourea 1 molar and KNO3 0.2% failed to rupture the seed coat and its attraction. Thiourea has been known to improve germination by decreasing the preventative effect of the seed coat in Prunus avium L. seeds (Çetinbaş and Koyuncu, 2006) and sulphuric acid is directed to the weakening of the seed coat cells, stimulating germination and emergency of the radicle across the endosperm and the seed coat (Gharehmatrossian *et al*., 2014). Similarly, KNO3 was highly efficient in releasing dormancy of many plant species (Previero *et al*., 1996), and it has been presented as an entity of a growth-promoting material in Salvia

species (Yücel, 2000). Three of these chemicals were incapable of releasing dormancy in A. eupatoria seeds in the current survey. This could be due to its extremely strict seed coat. Similar to our findings, other researchers have found that Alstromeria ligtu species with potassium nitrate (Nasri *et al*., 2014); Rhynchosia capitata species with thiourea and potassium nitrate (Ali *et al*., 2011), Ducrosia anethifolia with sulphuric acid (Ghavam *et al*., 2018) was not germination.

4. Conclusion

Based on the present research findings, this experiment showed that the seeds of A. eupatoria and C. vulgare are temperature related for germination. The employment of the dry and wet cold stratification consummated the seed dormancy in two pharmaceutical plant species and is the most efficient stimulator for the germination and preservation of these very significant pharmaceutical herbs. Generally, the current assay suggests chilling stratification as a low-cost, quick and easily enforceable method in seed germination over expensive herb development regulators and affiliated technicalities in both species. According to the findings, sulphuric acid, thiourea 1 molar and KNO3 (0.2%) on A. eupatoria and thiourea on C. vulgare did not seed germination.

Conflict of interests

The author states that there are no conflicts of interest.

Ethics approval and consent to participate

No human or animals were used in the present survey.

Consent for publications

The author read and approved the final manuscript for publication.

Availability of data and material

All the information is embedded in the manuscript.

Informed consent

The authors declare not to use any patients in this research.

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