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Investigation of Germination Characteristics of Four Medicinal Plants Seed (Lavender, Hyssop, Black cumin and Scrophularia) Under Interaction Between Salinity Stress and Temperature Levels

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ABSTRACT

Medicinal plants are stores of active and valuable secondary metabolites that have been economically beneficial for pharmacy and medicine. However, it is challenging to start large-scale and commercial cultivation of these plants since most of the arable land is mainly used to produce strategically essential crops. Other uncultivable lands are often affected by various abiotic stresses, one of the most important of which is salinity. Germination of plants is one of the critical stages during their growth period that is often affected by environmental stresses, especially salinity. In the present study, the seeds of medicinal plants such as Lavender (*Lavandula angustifolia*), Hyssop (*Hyssopus officinalis*), Black cumin (*Nigella sativa* L.), and Scrophularia (*Scrophularia striata*) were subjected to salinity stress at 20, 25, and 30 °C to determine the germination characteristics of their seeds. At each temperature, to create salinity stress, five levels of salinity potential, including 0, 50, 100, 150, and 200 mM NaCl were applied prepared using sodium chloride salt with the required amounts. The results of this experiment generally showed that with increasing salinity concentration at different temperatures, all germination characteristics, including germination percentage, germination rate, shoot length, root length, shoot dry weight, dry root weight, and seed vigor index, decreased. Salinity stress is one of the most important abiotic stresses in areas with saline soil and water, which reduces the quality and yield of plants, especially medicinal plants, and weakens them against other environmental stresses. It seems that low salinity soil and water are needed to get the best yield for the commercial growth of plants.

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Introduction

Seed is the main factor of reproduction and preservation of hereditary plant reserves, which plays a vital role in the distribution and establishment of plants in different areas, conservation and survival of plant generation in adverse and long-term conditions (Bewley *et al.*, 2013). Seed germination is a physiological process in which the embryo emerges from the end of the enclosing coverings, including the endosperm, perisperm, testa, and pericarp. Germination begins with the absorption of water by the seed and ends with the exit of the embryonic axis, including the root and stem, through the surrounding structures (Bewley *et al.*, 2013). The ability of seeds to delay germination through the dormant mechanism is one of the most important ways to maintain survival in plants. In addition to serving as a protective barrier during embryogenesis, seeds

also provide nourishment during embryogenesis and early seedling development (Taiz *et al.*, 2015). Approximately 20% of the world's cultivated areas and about half of the irrigated land are affected by salinity, and its amount is increasing (Flowers, 2004). Salinity is the presence of excess soluble salts and minerals in the soil and water environment in which plants are not able to absorb enough water. salinity disrupts the metabolism and physiological activities of plants such as lack of absorption of nutrients, and disorders of photosystems 1 and 2 (PSI and PSII) and reduces leaf development as well as plant growth, development, and yield (Munns and Tester, 2008). Usually, the highest salinity sensitivity is observed in plants' life cycle during germination and at the beginning of seedling growth (Kermode, 1990). The study of the effects of salinity on germination rate and germination percentage, and root and shoot growth in many plants has shown that salinity stress at the germination stage is a reliable test in assessing stress tolerance in many species. Salinity reduces the percentage and speed of germination and reduces the root and shoot length (Ghoulam and Fares, 2001).

Temperature is one of the essential variables influencing the percentage and rate of germination, which straightforwardly works through seed imbibition and the biochemical responses that control the digestion engaged with the germination cycle (Guo *et al.*, 2020). Further, most species require a suitable temperature reach or substitute temperature mode to accomplish the most significant germination. In weeds, the germination conduct of seeds is likewise identified with the hour of seed delivered and the second passed from the seed settling. This conduct was owed to the ecological conditions gone through by the mother plant during seed development and those gone through by the seeds after settling (Cristaudo *et al*., 2016). The germination rate typically increments straightly with temperature up to an ideal temperature, after which the germination rate diminishes pointedly (Fallahi *et al*., 2015; Laghmouchi *et al*., 2017; Tolyat *et al*., 2014). Expanded temperatures influence seedling development after seed germination, and have a straightforward influence on the germination interaction. To keep seedlings from being harmed after germination, physiological responses might happen in seeds to adapt to the high-temperature climate in which they are set. This biological prerequisite can be considered a variation procedure to ensure excellent seedling improvement and endurance conditions in certain species (Gresta *et al*., 2010). Then, at that point, these seeds will stop germination, yet they will, in a flash, sprout after being presented to appropriate temperatures, a cycle which is called thermo-restraint (Geshnizjani *et al*., 2018). Sometimes, high or low temperatures additionally bring about optional torpidity, known as thermos-dormancy (Huo *et al*., 2013). Under these conditions, germination will not happen at any temperature, including the ideal temperature. This wonder is particularly predominant in yearly desert plants and some Mediterranean species (Cristaudo *et al*., 2019).

In the early stages of salinity stress, due to the osmotic pressure caused by the high accumulation of salt in soil and plants, the rate of water uptake of root systems decreases, as a result of which water loss from the leaves is accelerated. Therefore, salinity stress is also considered hyperosmotic stress (Munns, 2005). Plants increase ROS production in response to salinity stress, such as singlet oxygen, superoxide, hydroxyl radicals, and hydrogen peroxide (Apel and Hirt, 2004; Mahajan and Tuteja, 2005; Ahmad, 2010). The formation of ROS due to salinity stress can lead to oxidative damage to various plants cells components such as lipids, proteins, and DNA and disrupt the vital functions of plant cells (Gupta and Huang, 2014).

Temperature can interact with salinity to affect seed germination. Although higher salinity may inhibit germination, the detrimental effect of salinity is generally reduced at optimal germination temperatures, e.g., in *Sarcobatus vermiculatus* (Khan, 2002), decreased germination was noted at supraoptimal temperatures. However, the detrimental effects of salinity are more severe at lower temperatures for other species such as *Allenrolfea occidentalis* (Gul and Weber, 1999) and *Aeluropus lagopoides* (Gulzar and Khan, 2001). In other species, the detrimental effect of salinity is severe both above and below the optimum, such as *Urochondra setulosa* (Gulzar *et al*., 2001).

Salinity-temperature interactions may have significant ecological implications in terms of the time of germination under field conditions (Ungar, 2017).

In the last three decades, a great deal of research has been done to understand salinity tolerance of molecular and physiological mechanisms in *Arabidopsis* (Zhang and Shi, 2013). Salinity tolerance includes a set of responses at the molecular, physiological, and plant growth stages. Among the various reactions related to salinity stress, ion absorption control, osmotic regulation, hormone metabolism, antioxidant metabolism, and stress signaling mechanisms or strategies play an essential role in plant adaptation to salinity stress (Gupta and Huang, 2014). Salinity stress directly limits plant growth by disturbing the nutrient uptake balance maintained by the plant system. In this way, elements availability, partitioning, and transport are further impressed. This is due to the contestation of Sodium and chlorine ions with the nutrient ions like potassium, calcium, and nitrate. Such ionic inconsonance is caused by the direct affluence of Sodium and Chlorine ions on the biophysical and/or metabolic components of the plant system (Banerjee and Roychoudhury, 2016).

Medicinal and aromatic plants are valuable products. The natural consequences of these plants are small but very valuable volumes that have several applications in various industries such as food, beverages, food supplements, perfumery, cosmetics, and medicine (Novak and Blüthner, 2020). Some of the biologically active compounds in these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannins, flavonoids, and phenolic compounds (Thirumurugan *et al*., 2010; Uddin and Rauf, 2012; Karagöz *et al*., 2015). In developing countries, 65% to 80% of the population depend on herbal medicines for primary health care (Johnson and Ayoola, 2015). Over the past few decades, global interest in studying various medicinal plants has been overgrown due to their antibacterial and antioxidant activities, low toxicity, and the possibility of cheaper alternatives to expensive synthetic drugs (Chew *et al*., 2012). Therefore, the cultivation of medicinal plants and the study of factors limiting their growth are very important.

Salinity stress is one of the most important abiotic stresses that, in areas with saline soil and water, reduce the quality and yield of plants, especially medicinal plants like *Majorana hortensis*, peppermint, pennyroyal, apple mint, *Aloe vera*, *Matricaria recutita*, *Thymus maroccanus*, geranium, *Thymus vulgaris*, sweet fennel, sage and *Mentha pulegium* (Aziz *et al.*, 2008; Said-Al Ahl and Omer, 2011). Increases in Sodium and Chlorine ions during salt stress have resulted in decreased levels of Nitrogen and phosphorus elements and calcium and magnesium ions in fennel, *Trachyspermum ammi*, peppermint, lemon verbena, *Matricaria recutita*, *Achillea fragratissima* (Abd El-Wahab, 2006; Abd EL-Azim and Ahmed, 2009; Oueslati *et al.*, 2010). Ali *et al*., (2013) showed that the Nitrogen and phosphorus elements and Potassium percentages were impressed by different salt concentrations in *Simmondsia chinensis* (jojoba) leaves. Salt concentration over 17.2 mM drastically lowered the three elements. A similar abatement was reported for the calcium ion content in the salt-stressed leaves. In the literature, it has been shown that when sowing seeds in soils with high salt content, germination in *Eruca sativa*, *Ocimum basilicum*, *chamomile*, *sweet marjoram*, *Petroselinum hortense*, and *Thymus maroccanus* is reduced and delayed (Miceli *et al.*, 2003; Ramin, 2006; Ali *et al.*, 2007; Belaqziz *et al.*, 2009). This study investigated the effect of salinity stress on germination characteristics of seeds of Lavender (*Lavandula angustifolia*), hyssop (*Hyssopus officinalis*), black cumin (*Nigella sativa* L.), and Scrophularia (*Scrophularia striata*).

Materials and Methods

This study was carried out at the Department of Horticulture Science, College of Agriculture and Natural Resources, University of Tehran, Karaj 31587, Iran. The seeds of medicinal plants prepared from IPK Gatersleben Company were used as seed material. The temperature treatment used consisted of three levels (20, 25, and 30 degrees Celsius). At each temperature, five levels of salinity were used to apply salinity stress, including the different concentrations of sodium chloride (0, 50, 100, 150, and 200 mM

NaCl). Also, distilled water was used as a control in each experiment.

Seed treatment

Fifty seeds were disinfected with 5% sodium hypochlorite solution for 30 seconds, and after rinsing with distilled water, they were transferred to filter paper in Petri dishes. Then, for salinity treatment, 10 ml of sodium chloride solution was added to each petri dish, and after applying the treatments at the time of sowing the seeds, the Petri dishes were placed in a germinator with a relative humidity of 80% and temperatures of (20, 25 and 30 °C), 16 hours of light and 8 hours of darkness.

Germination tests

Seed germination criterion was considered 2 mm root emergence. Petri dishes were inspected at a specific time, and the number of germinated seeds was counted to determine the germination rate daily up to 14 days after planting and applying salt treatment. On the fourteenth day, 20 seedlings were selected from each petri dish, and traits such as germination percentage and germination speed, root and shoot length, and seed vigor were measured (Ellis and Roberts, 1981). Additional samples of each treatment were used to visually estimate abnormal seedling development, according to ISTA germination method (ISTA, 1999) rules, ten days after initiating germination.

The germination percentage was calculated with the following formula. GP= 100 (N_i/N). GP: germination percentage, N_i: number of germinated seeds until the day *i*, and *N*: total number of seeds (Hartmann and Kester, 2014). Germination rate was also calculated using the equation RS= $\sum (n_i \cdot t_{(x-i)})/ \sum n_i$. In this formula, RS indicates the germination rate (number of seeds per day), n_i indicates the number of seeds germinated on day *i*, and *x* indicates the total number of days of the test (Bewley *et al*., 2013).

In addition, seed vigor was calculated using the relationship V= (*GP*)× [(*Lr*÷ 2) + (*Ls*÷ 2)]. In this regard, V indicates the seed vigor index, *Lr* indicates the radical length, *Ls* indicates the plumule length, and *GP* indicates the germination percentage.

Statistical analysis

Data were analyzed using SPSS version 26 (SPSS Inc., Chicago, IL, USA). The experimental design was two factorial factors (5× 3) arranged in a completely randomized design, with three replications and 50 seeds per replicate. The first factor was seed treatments (salinity potential including 0, 50, 100, 150, and 200 mM), the second factor was temperature levels (20, 25, and 30 ° C). The normality of the data and the remaining error were checked by Shapiro-Wilk and Kolmogorov-Smirnov tests. Data Normalization related to seed germination was performed using the SQRT method. The differences between means were assessed using Duncan's multiple range test (p <0.05), and the results are indicated by a different letter.

Results

Due to salinity stress, water uptake by seeds was decreased, reducing the physiological and metabolic processes indicating the abundance of substances available for plant survival is problematic and reduces the germination percentage (Ashraf and Waheed, 1990). The variance analysis showed (Table 1) that salinity stress significantly affected the evaluated traits, increasing salinity stress, germination percentage, germination rate, plumule length, radical length, plumule dry weight, radical dry weight, and Seed Vigor Index decreased significantly.

Lavender (*Lavandula angustifolia*)

The highest germination percentage was observed in lavender at 25 °C and control treatment (75%), 4 and 3% higher than 20 and 30 °C, respectively. It was also observed that the germination percentage in this treatment was 35, 50, and 67% higher than the 50, 100, and 150mM sodium chloride treatments at 25 °C, respectively. Germination was not observed in salinity treatment of 150 mM and 30 °C and salinity treatment of 200 mM (Table 2). The highest germination rate was observed in lavender at 25 °C and control treatment (15.38 seeds per day), 15.38 and 10.73% higher than 20 and 30 °C, respectively. It was also observed that the germination rate in this treatment was 10.73, 23.04, and 98.45% higher than the 50, 100, and 150mM sodium chloride treatments at 25 °C,

respectively (Table 2). Control and temperature of 25 °C treatment obtained the highest Plumule Length (200 mm), and Radical Length (310 mm) and the lowest amount except for treatments that did not have any germination was 58 (mm) and 75 (mm) respectively in the salinity level 150 mM and temperature of 20 °C (Table 2). The highest seed vigor index was observed in lavender at 25 °C and control treatment (19125), 15.38 and 10.73% higher than 20 and 30 °C, respectively. It was also observed that the germination rate in this treatment was 2.3, 5.3, and 31.9 fold higher than the 50, 100, and 15 mM sodium chloride treatments at 25 °C, respectively (Table 2).

Table 1. Results of analysis of variance of the studied traits under salinity stress and temperature treatment.

SOV	df	Germination Percent	Germination rate	Plumule Length	Radical Length	Plumule dry weight	Radical dry weight	Seed vigor Index
A) Lavender (*Lavandula angustifolia*)								
Salinity	4	89.04**	15.94**	256.79**	440.44**	11.37**	0.49**	26174.93**
Temperature	2	1.07**	1.21**	12.74**	14.60**	0.33**	0.03*	219.85**
Salinity×Temperature	8	0.92**	0.86**	10.49**	12.99**	0.09**	0.01	106.38**
E	30	0.01	0.02	0.01	0.01	0.01	0.01	0.01
CV (%)	-	34.61	10.79	17.88	21.82	27.29	22.08	83.73
B) Hyssop (*Hyssopus officinalis*)								
Salinity	4	108.99**	11.99**	28.42**	12.63**	1.26**	0.03**	2170.77**
Temperature	2	1.03**	0.04	0.12**	0.50**	0.02*	0.01*	52.29**
Salinity×Temperature	8	0.27**	0.02	0.14**	0.11**	0.01	0.01	6.08**
E	30	0.01	0.02	0.02	0.03	0.01	0.01	1.12
CV (%)	-	7.66	11.01	26.48	24.53	20.81	4.35	32.41
C) Black cumin (*Nigella sativa L.*)								
Salinity	4	51.62**	3.45**	26.23**	33.64**	3.58**	0.25**	2929 **
Temperature	2	1.39**	0.90**	0.91**	0.68**	0.06	0.01	90.11**
Salinity×Temperature	8	0.02	0.04*	0.02	0.02	0.01	0.01	3.36**
E	30	0.02	0.01	0.02	0.03	0.03	0.01	0.36
CV	-	18.61	11.99	24.49	28.34	33.02	20.45	39.36
D) Scrophularia (*Scrophularia striata*)								
Salinity	4	39.14**	6.30**	23.01**	15.26**	2.30**	0.10	3097**
Temperature	2	13.19**	1.27**	0.08	0.06*	0.03	0.01	330.25**
Salinity×Temperature	8	0.18**	0.02*	0.24**	0.17**	0.01	0.01	21.97**
E	30	0.01	0.01	0.03	0.02	0.12	0.10	0.96
CV	-	21.60	11.81	19.18	19.40	26.22	29.91	38.53

**significant differences at 1%, * significant differences at 5%

Hyssop (*Hyssopus officinalis*)

The highest germination percentage was observed in hyssop at 20 °C and 50 mM NaCl treatment (83%), 1 and 4% higher than 25 and 30 °C, respectively. It was observed that the germination percentage in this treatment was 5, 8, and 14% higher than the control, 100, and 150mM sodium chloride treatments at 20 °C, respectively.

Germination was not observed in salinity treatment of 200 mM NaCl (Table 4). The highest germination rate was observed in lavender at 25 °C and control treatment (13 seeds per day), 14.44 and 2.69% higher than 20 and 30 °C, respectively. It was observed that the germination rate in this treatment was 6.64, 52.94, and 73.33% higher than the 50, 100, and 150mM sodium chloride treatments at 25 °C, respectively (Table 2).

The highest Plumule Length (30.40 mm) and Radical Length (16.63 mm) were obtained in the treatment of 50 mM NaCl and 20 °C, and the lowest amount was 4.80 (mm) in salinity level of 150 mM and 30 °C and 75 (mm) in salinity level of 150 mM and 20 °C, respectively, except for treatments that did not have any germination (Table 2).

Table 2. Comparison of the mean interaction of salinity levels × temperature on the studied traits.

Salinity (mM)*	Temperature (° C)	Germination Percent (%)	Germination rate (seed/day)	Plumule Length (mm)	Radical Length (mm)	Plumule dry weight (mg)	Radical dry weight (mg)	Seed vigor Index
A) Lavender (*Lavandula angustifolia*)								
	20	71.00b	13.33b	183.00c	292.00c	10.50b	.95abc	16863c
0	25	75.00a	15.38a	200.00a	310.00a	11.25a	.97abc	19125a
	30	72.00b	13.89ab	190.00b	300.00b	10.00b	.85bcd	17640b
	20	36.00d	12.35bcd	138.00f	243.00f	8.75c	1.15ab	6858f
50	25	40.00c	13.89ab	150.00d	260.00d	9.25c	1.20a	8200d
	30	39.00c	12.66bc	140.00e	248.00e	8.00d	1.07ab	7566e
	20	21.00g	11.36cd	107.00i	160.00i	4.25e	.73cde	2804i
100	25	25.00e	12.50bcd	120.00g	170.00g	4.50e	.65cdef	3625g
	30	23.00f	10.99d	110.00h	161.00h	3.75e	.60def	3117h
	20	7.00h	7.41e	58.00k	75.00k	1.50f	.38ef	466k
150	25	8.00h	7.75e	70.00j	80.00j	2.00f	.48f	600j
	30	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-
200	25	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-
B) Hyssop (*Hyssopus officinalis*)								
	20	78.00b	11.36abc	19.10d	11.20bc	2.10ab	0.19cd	1182c
0	25	79.00b	13.00a	16.00e	8.40e	2.20a	0.20bcd	964d
	30	67.00e	12.66ab	18.80d	8.70de	2.00abc	0.17de	921d
	20	83.00a	10.31c	30.40a	16.63a	1.80cd	0.23ab	1953a
50	25	82.00a	12.19ab	25.70c	12.30b	1.90bc	0.24a	1559b
	30	79.00b	10.99bc	27.60b	12.40b	1.60d	0.22abc	1579b
	20	75.00c	7.94d	14.70e	10.00cd	0.90e	0.15ef	926d
100	25	63.00f	8.50d	14.9e	6.70f	1.00e	0.13fg	681e
	30	66.00e	8.13d	11.40f	5.80f	0.80e	0.12fgh	568f
	20	69.00d	7.90d	5.60h	3.30g	0.50f	0.09hi	306g
150	25	53.00g	7.50d	7.30g	3.50g	0.50f	0.10ghi	287gh
	30	51.00h	7.30d	4.80h	3.40g	0.30f	0.08i	208h
	20	-	-	-	-	-	-	-
200	25	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-
C) Black cumin (*Nigella sativa L.*)								
	20	49.00cd	24.00b	38.00ab	39.00a	4.20ab	0.43a	1887c
0	25	52.00bc	32.00a	40.00a	41.00a	4.50a	0.39ab	2106b
	30	44.67e	26.00b	36.00bc	38.00ab	4.00ab	0.35abc	1652d
	20	61.00a	20.00de	33.00c	35.00bc	3.50ab	0.46a	2073b
50	25	64.00a	26.00b	36.00bc	38.00ab	3.70ab	0.48a	2367a
	30	53.00b	22.00c	30.00d	33.00c	3.20b	0.43a	1669d
	20	45.00e	18.00fg	19.00e	16.00de	1.70c	0.29bcd	788f
100	25	47.00de	21.00cd	21.00e	18.00d	1.80c	0.26cde	917e
	30	41.00f	19.00ef	16.00f	15.00e	1.50c	0.24cdef	636g
	20	21.00h	14.00ij	11.00g	9.00g	0.80d	0.17ef	209i
150	25	24.00g	17.00g	12.00g	11.00f	0.80d	0.19def	277h
	30	18.00i	15.00h	9.00h	8.00g	0.60de	0.15f	153j
	20	4.00j	13.00j	5.00i	3.00h	0.40de	0.08g	17k
200	25	4.00j	15.00h	5.00i	4.00h	0.45de	0.08g	18k
	30	2.00k	13.00j	3.00j	2.00i	0.35e	0.07g	5l
D) Scrophularia (*Scrophularia striata*)								
	20	76.00b	41.66b	53.10b	35.40b	5.20a	0.87e	3364b
0	25	84.00a	47.62a	58.50a	39.00a	5.50a	0.89d	4095a
	30	54.00e	42.55b	52.20b	34.80b	5.00a	0.84f	2350c
	20	60.00d	34.48e	38.40c	25.60c	4.50ab	0.96b	1919e
50	25	68.00c	40.00c	37.80c	25.20c	4.70a	0.98a	2142d
	30	39.00g	38.02d	38.13c	23.20d	4.20abc	0.93c	1198f
	20	39.00g	29.41g	33.60d	22.40d	2.70bcd	0.79g	1091f
100	25	51.00f	38.46d	26.40e	17.60e	2.80bcd	0.76h	1123f
	30	26.00h	33.00f	26.10e	17.40e	2.50cd	0.74i	566g
	20	24.00i	21.74jk	21.30f	14.20f	1.80d	0.67k	426h
150	25	38.00g	27.03h	17.60g	11.60g	1.80d	0.69j	555g
	30	21.00j	22.99j	21.10f	13.40f	1.60d	0.65l	363h
	20	10.00l	18.87l	9.00i	6.00i	1.40d	0.58m	75j
200	25	15.00k	25.00i	9.30i	6.20i	1.45d	0.58m	116i
	-	-	-	-	-	-	-	-

*The means with at least one common letter using Duncan's test at the 5% probability level are not significantly different in each column.

The highest seed vigor index was observed in hyssop at 20 °C and 50 mM NaCl treatment (1953), 25.27 and 23.69% higher than 25 and 30 °C, respectively.
It was also observed that the germination rate in this treatment was 1.6, 2.1, and 6.4 fold higher than the control, 100, and 150mM sodium chloride treatments at 20 °C, respectively (Table 2).

Black cumin (*Nigella sativa* L.)

The highest germination percentage was observed in black cumin at 25 °C and 50 mM NaCl treatment (64%), 3 and 11% higher than 20 and 30 °C, respectively. It was also observed that the germination percentage in this treatment was 12, 17, 40, and 60% higher than the control, 100, 150, and 200mM sodium chloride treatments at 25 °C, respectively (Table 2).
The highest germination rate was observed in black cumin at 25 °C and control treatment (32 seeds per day), 33.33 and 23.08% higher than 20 and 30 °C, respectively. It was also observed that the germination rate in this treatment was 12.08, 52.38, 88.24, and 113.33% higher than the 50, 100, 150, and 200mM sodium chloride treatments at 25 °C, respectively (Table 2).
The highest plumule length (40 mm) and Radical Length (41 mm) was obtained in the control and temperature of 25 °C, and the lowest was 3 (mm) and 2 (mm) respectively in the salinity level 200 mM and 30 °C (Table 2).
The highest seed vigor index was observed in black cumin at 25 °C and 50 mM NaCl treatment (2367), 14.18 and 41.82% higher than 20 and 30 °C, respectively. It was observed that the germination rate in this treatment was 1.1, 2.6, 8.5, and 131.5fold higher than the control, 100, 150, and 200mM sodium chloride treatments at 25 ° C, respectively (Table 2).

Scrophularia (*Scrophularia striata*)

The highest germination percentage was observed in Scrophularia at 25 °C and control treatment (84%), 8 and 30% higher than 20 and 30 °C, respectively. It was observed that the germination percentage in this treatment was 16, 33, 46, and 69% higher than the 50, 100, 150, and 200mM sodium chloride treatments at 25 °C, respectively (Table 2). The highest germination rate was observed in Scrophularia at 25 °C and control treatment (47.62 seeds per day), 14.31 and 11.92% higher than 20 and 30 ° C, respectively. It was also observed that the germination rate in this treatment was 19.05, 23.82, 76.17, and 90.48% higher than the 50, 100, 150, and 200 mM NaCl treatments at 25 °C, respectively (Table 2). The highest Plumule Length (58.5 mm) and Radical Length (39 mm) was obtained in the treatment of non-stress and temperature of 25 °C, and the lowest amount was 9 (mm) and 6 (mm) Respectively in the salinity level 200 mM and 20 °C (Table 2). The highest seed vigor index was observed in Scrophularia at 25 °C and control treatment (4095), 21.73 and 74.26% higher than 20 and 30 °C, respectively. It was also observed that the germination rate in this treatment was 1.9, 3.6, 7.4, and 35.3fold higher than the 50, 100, 150, and 200mM sodium chloride treatments at 25 °C, respectively (Table2).

Discussion

Salinity is the principal abiotic stress due to the increasing use of poor-quality water for irrigation and salinization of soil, limiting plants' development and productivity in many parts of the world. Salinity tolerance includes a set of responses at the molecular, physiological, and plant growth stages (Munns and Tester, 2008). Extensive investigation through cellular, metabolic, and physiological analysis has cleared that among various salinity responses, mechanisms or strategies controlling ion uptake, transport and balance, osmotic regulation, hormone metabolism, antioxidant metabolism, and stress signaling to play decretive roles in plant adaptation to salinity stress (Gupta and Huang, 2014).
Therefore, to identify salinity-resistant plants, including medicinal plants, the germination stage can be the most crucial step for identifying salinity-resistant plants.
NaCl caused a significant reduction in germination percentage in four medicinal plants in the present study. A decrease in GP of seeds under the influence of increasing salt concentration may be resulted from decreasing osmosis potential of the solution, increasing toxic ions, and changing in the remobilization

balance of seed reservoirs. Similar results were reported in *Hypericum ericoides* by Vicente *et al*. (2020), in Ajowan and Fennel (Booroomand Zade and Koochaki, 2005), in *Cannabis sativa* (Dadkhah, 2010), in marigold (Sedghi *et al.*, 2010), in *Plantago ovata* (Muhammad and Hussain, 2010) and Matricaria (Ghanavati and Sengul, 2010). Also, the results showed that the interactions of temperature and salinity have a significant effect on germination percentage. At the same temperatures, increasing the salinity reduces the germination percentage, and also at the same salt concentrations, temperature changes cause a change in the germination percentage of medicinal plant seeds.

The results show that with increasing the salinity, the germination rate in these plants shows a decreasing trend due to the destructive effects of salinity on cellular processes. Other studies show similar results (Booroomand Zade and Koochaki, 2005; Dadkhah, 2010; Ghanavati and Sengul, 2010; Muhammad and Hussain, 2010; Sedghi *et al.*, 2010; Vicente *et al.*, 2020). Dadkhah (210) showed that in *Cannabis sativa*, with increasing the negative potential of the germination medium up to 0.81 MPa due to salinity, the germination rate decreased by 46.43% compared to the control treatment. Nejatzadeh (2021) also showed that in *Satureja hortensis*, with increasing the concentration of sodium chloride in the culture medium up to 120 mmol/L, the germination rate decreased by 35% compared to the control treatment.

By increasing salinity to 200 mM NaCl, the fresh and dry weight of plumule and radical decreased. It is probably due to decreasing in the remobilization of reservoirs from cotyledons to the embryo axis. The factors that affect the growth rate of the embryo axis also affect the mobility of reservoirs and their remobilization from cotyledons to the embryo axis (Sedghi *et al.*, 2010).

NaCl caused a significant reduction in seed vigor index in four medicinal plants in the present study. Seed vigor index is directly related to germination percentage and plant growth indices. Decrease of germination percentage and vegetative growth of medicinal plants due to increasing salinity stress level decreased seed vigor index. Since the seed vigor index is directly related to the germination percentage, the interaction of temperature and salinity stress also affects the amount of this index.

Conclusion

In general, salinity reduced the germination of the studied medicinal plants. Among these medicinal plants, Scrophularia can be considered the most resistant plant to salinity stress because at the highest level of salinity stress, it showed the highest rate of seed germination compared to other plants. According to the results, lavender should not be grown in areas with soil or water salinity more than 50 mM because it will significantly reduce yields. Hyssop at 150 mM salinity stress showed the highest seed germination percent compared to other plants, showing high salinity resistance. However, the cultivation of this plant in areas with a salinity of more than 100 mM will not be economical. Black cumin will also show a sharp decrease in yield at salinities above 50 mM. Generally, it can be concluded that the cultivation of these four medicinal plants in fields with water and soil containing concentrations higher than 50 mM sodium chloride is not economically viable, and yields are significantly reduced in these areas. On the other hand, it can be concluded that the best temperature is generally between 20 and 25 degrees Celsius, and temperatures higher than that reduce the germination rate of seeds of medicinal plants.

Conflict of interests

The authors declare that they have no conflict of interests.

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