



# Enhancing the emergence characteristics of three populations of mountain Thyme (*Thymus kotschyanus*) using different priming methods

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## Original Research

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## Abstract:

The aim of this research was to determine the emergence ability of seeds and seedling vigor of three populations of Mountain thyme (*Thymus kotschyanus*) using different priming methods. A factorial experiment was conducted based on a Completely Randomized Design (CRD) with three replications in Research Institute of Forest and Rangeland, Tehran, Iran in 2016. In this experiment, factor A was: three populations of Tehran, Gazvin and Zanzan, and factor B was seven levels of priming such as: osmopriming [PolyEthylene Glycol (PEG) 0.6 and 0.9 MPa], hormonal priming [Gibberellic Acid (GA 250 and 500 ppm], matrix priming (perlite), Hydro priming (distilled water) and control (without priming). The primed seeds and control were sown in 2 kg capacity pots at a depth of 1 cm and the pots were kept in greenhouse conditions (the temperature of 20 – 30 °C during the day and 5 – 12 °C at night). The emergence traits including the percentage and speed of emergence, root and shoot length, seedling length, seedling vigor index, seedling fresh and dry weight, Root to Shoot length ratio (RS) and seedling Dry Matter (DM%) were measured. The statistical analyses and comparison of means were done using SAS 9.1 software. Tukey test method was used to compare the mean of data ( $p < 0.05$ ). The results of analysis of variance showed a significant effect of population ( $p < 0.05$ ) for speed of emergence and plant DM%. The effect of priming treatments was significant for all traits and the priming by population interaction effect was significant for the traits of speed of emergence, root shoot length ratio and plant dry weight. The Tehran population with average values of emergence percentage (64.98%), and speed of emergence (4.98 sprouts/day) had the highest emergence appearance and speed of emergence, respectively. In comparison, between treatments, the highest value of speed of emergence (5.40) was obtained using PEG 0.6 MPa. The highest stem and seedling's length with values of 65.7 and 31.4 mm were related to hormonal priming GA (250 and 500 ppm), respectively. The highest fresh seedling weight (39.44 mg) was obtained by hydropriming. According to the results, it was concluded that the Osmopriming (PEG) and hormonal priming (GA) were effective priming methods for seedling establishment.

**Keywords:** Thyme; Hormonal priming; Osmopriming; Matrix priming; Seed vigor index

## Introduction

The genus of thyme belongs to the family Lamiaceae and has 70 species. In Iran, the word thyme is commonly used for three genera, (*Thymus*, *Ziziphora* and *Zataria*), which belonged to the mint family. These three genera have different botanical characteristics (Mozaffarian, 2007). The plants of this genus grow as a wild type and in the form of

thick bushes on dry slopes and between boulders in different Mediterranean countries such as France, Portugal, Spain, Greece and some parts of Asia. Also, they grow in altitudes upper than 1200 m in the mountain area in many parts of Iran (Jamzad, 2009). These plants have many therapeutic uses, especially in traditional medicine, and are also grown as ornamental plants in some areas. One of the characteristics of this plant is its woody roots and uneven branches. It

easily penetrates hard ground and rocks and keeps the plant, which has many stems compressed together. Therefore, it causes well fixation of the plant (Jamzad, 2009).

The thyme species belongs to the mint family, which is one of the largest plant families. The *Thymus kotschyanus* is a woody plant, short, cushion 6 to 12 cm high, with many branches, with dormant hairs and dense with a white flower cup. The flower type is a dense cluster. The flowering time is from late spring to mid-summer. The type of fruit is achene (Jamzad, 2009). The main components of essential oil in thyme include thymol (73.9%) and carvacrol (6.7%) (Jamzad, 2009).

This thyme species is an important medicinal plant, highly recommended due to a variety of therapeutic properties of its essential oils. The infusion and decoction of thyme plant was reported as a conditioner, flavoring, anti-cough, anti-spasm, expectorant, anti-flatulent, anti-microbial and antifungal (Nickavar et al., 2004).

The priming technique is a pre-sowing treatment in which the seed absorbs water in a controlled manner so that the metabolic processes before germination are allowed to occur in the seed but the root has not appeared (Armin et al., 2010). Seed priming is a physiological method that accelerates the process of seed germination (Nascimento and Aragao, 2004). Priming improves the seed germination percentage, speed and uniformity of germination in unfavorable conditions in the field. This technique has been developed in order to reduce germination time (high-speed germination), uniform germination and better seedling growth for many agricultural and horticultural plants (Farooq et al., 2006).

Common methods for priming treatments include:

- Hydropriming: soaking seeds in distilled water without using chemicals. This method has an important effect on the germination of seeds compared to seeds without soaking and this technique increases the yield in field conditions (Carrillo-Reche et al., 2018).
- Osmopriming: soaking seeds in solutions with low osmotic potential such as polyethylene glycol solution, glycerol, sorbitol, mannitol, and mineral salts that regulate water absorption (Lutts et al., 2016).
- Halopriming: immersion of seeds in salt solutions including NaCl, KNO<sub>3</sub>, CaCl<sub>2</sub> and CaSO<sub>4</sub>, which facilitate germination and emergence of seedlings in adverse environmental conditions (Robledo, 2020).
- Matrix priming with solid materials: treatment of seeds with a bed of solid materials including materials with low osmotic pressure is done under the conditions of controlled hydration and makes it possible for the seeds to absorb water (Paparella et al., 2015). These materials are perlite, vermiculite, zeolite, polypropionate gel, soft coal, calcium

silicate, sawdust, charcoal and granulated clay.

- Hormonal priming: seeds are soaked in growth-regulating hormonal solutions such as gibberellic acid, kinin, salicylic acid, polyamines, which stimulate the growth and development of seedlings (Jabari and Jabari, 2023).

- Biopriming: seed treatment involves the act of seed by hydration and adding a biological treatment in the form of inoculation such as live bacteria (Mahmood et al., 2016). In this seed priming method, plant growth is stimulated by *rhizobacteria* such as *Azotobacter*, *Pseudomonas*, *Bacillus*, *Agrobacterium*, fungicides and biological agents.

- Fertilizer priming (Nutri priming): soaking seeds in a solution containing fine and coarse nutrients, which is a synergistic action of chemicals with water, and is a cost-effective and environmentally friendly method (Rehman et al., 2018).

- Nano priming: it is a method to improve the germination, emergence, establishment and growth of seedlings using nanoparticle materials based on metals and carbon (Panda and Mondal, 2020; Mazhar et al., 2022). In nano-priming, the seeds do not dry before planting, and it is different from other priming methods.

In a research work, the effects of seed priming on germination characteristics of *Achillea millefolium* seed were studied under different aging treatments (Rasoolzadeh et al., 2020). The result showed that all the factors and their interaction had significant impact on germination traits.

The aim of this research was to use the priming techniques for evaluating seed emergence capacity, seedling growth performance and seedling vigor of three populations of the species (*T. kotschyanus*) in the greenhouse conditions.

## Materials and methods

The seeds of three populations were collected in 2003. Name of province, geographical characteristics and initial quality of the seeds including purity, moisture content, thousand weights of three populations (*Thymus kotschyanus*) were presented in Table 1. In order to study seed emergence and seedling vigor of three populations of *Thymus kotschyanus*, a factorial experiment was conducted based on a Completely Randomized Design (CRD) with three replications in the greenhouse of Research Institute of Forests and Rangeland, Tehran, Iran in 2015 – 2016. Factor A had three populations of (*T. kotschyanus*) from Tehran, Qazvin and Zanzan provinces. Factor B had seven levels of priming treatments such as osmopriming [PolyEthylene Glycol (PEG) 0.6 and 0.9 MPa], hormonal priming [Gibberellic Acid (GA) 250 and 500 ppm], Matrix priming (Perlite 1%), hydropriming (distilled water) and control without priming.

**Table 1.** Name of province, geographical characteristics and initial quality of the seeds including: Purity, moisture content, thousand weights of three populations (*Thymus kotschyanus*).

Origin	Code	Elevation (m)	Latitude	Longitude	Moisture%	Purity%	1000 Seed weight (g)
Qazvin	17901	1800	36°26'00"	50°10'00"	7.5	60	0.23
Tehran	18803	2000	33°15'00"	48°30'00"	6.0	92	0.28
Zanzan	19266	1900	36°37'40"	47°36'31"	5.5	100	0.23

The osmotic solution (PEG 0.6 and 0.9 Mpa) was prepared according to (Michel and Kaufmann, 1973). The seeds of populations were transferred into solution treatments for overnight, then seed samples were dried at room temperature for 24 h. The primed seeds were sown in 2000 mL pots (with ratio 1:1:1 of soil, sand and compost) with three replicates. In each pot, 25 seeds were sown in 1 cm depth. The pots were kept in a glasshouse with the temperature of 20 – 30 °C during the day and 5 – 12 °C at night using light illumination of 6000 – 10000 lux and the relative humidity (RH) ranged from 50 to 60%

. Then 45 days after the plants grow in the pots, The seedling characteristics including emergence percentage, speeds of emergence, shoot length (mm), root length (mm), seedling length (mm), seedling dry weight (mg), seed vigor index and seedling dry matter content (DM%) were collected. At the end of the experiment, seedling dry weight was measured using five seedlings per pot, weighted and averaged according to (Lekh and Khairwal, 1993). The seedlings were then dried in an oven for 24 h at 80 °C and weighted as seedling dry weight (mg).

The emergence percentage was calculated according to the total number of emerging seedlings on the final day (ISTA, 2009).

The final emergence percentage and speed of emergence were calculated according (Maguire, 1962) to the following equations

$$E\% = \frac{\sum E}{N} \times 100$$

E% = Percentage of emergence,

E: Emerged seeds number

N: Total seeds number

$$SP = \frac{\sum n}{\sum (n \times DN)} \times 100$$

where:

SP = speed of emergence

n is the number of seeds germinated on day DN,

Dn is the number of days from sowing, corresponding to n,

The vigor index was measured according to (Abdul-Baki and Anderson, 1973) from following formula.

$$Vi\% = \frac{G\% \times MSL}{100}$$

where:

Vi = Seedvigor index

G% = final germination percentage

MSL = Seedling length mean

### Statistical analysis

In analysis of variance, the effects of seed priming on the seed emergence ability and seedling vigor of three populations of Mountain thyme were studied using a factorial experiment based on a RCD with three replications. Means comparison test was done by Tukey method ( $P \leq 5\%$ ). All statistical analyses were conducted using SAS software.

## Result

### Analysis of variance

The analysis of variance in a factorial experiment showed that there was no significant difference between the populations for all the traits except speed of germination and seedling DM%. So, there was a significant difference in the speed of germination and seedling DM% ( $P \leq 5\%$ ). The effect of priming treatment was significant ( $P \leq 1\%$ ) for the traits of shoot length, root/shoot length, fresh weight, dry weight and dry matter, but for the remaining traits, it was significant ( $P \leq 5\%$ ). The interaction between populations and treatments was significant ( $P \leq 1\%$ ) for speed of emergence, root/shoot length ratio, seedlings dry weight and seedling dry matter, but for vigor index, there was a significant difference ( $P \leq 5\%$ ) (Table 2).

### The main effect of populations

Results of means comparison among three populations showed that the population of Tehran had significantly higher mean values of emergence percentage (64.95%), speed of germination (4.89), vigor index (40.04) and seedling DM (13%) than the other populations (Table 3).

### The main effect of treatments

The effect of treatment was significant for all the traits ( $P \leq 5\%$ ) (Table 4). For seedling emergence, the higher value of 82.22% was observed in control, but it had no significant difference with PEG 0.6 Mpa. For speed of emergence, all the treatments were statistically similar to the control and a lower value of 3.35 was obtained in PEG 0.9 Mpa. In contrast, for root length, the higher value of 35.99 mm was obtained at PEG 0.9 Mpa, which was significantly higher than other treatments (Table 4). For shoot length, the higher values of 31.70 and 31.77 mm were obtained in GA 250 and

**Table 2.** Analysis of variance of seed priming technique on the seed emergence characteristics of three populations (*Thymus kotschyanus*).

S.O.V.	DF	MS									
		Emergence %	Speed of Emergence	Root Length	Shoot length	Root/shoot length ratio	Seedling length	Vigor index	Fresh weight	Dry weight	Seedling DM%
Population (P)	2	609.08	7.71*	6.28	1.49	0.11	9.85	234.34	89.89	0.97	0.10*
Treatment (T)	6	1266.6*	5.66*	46.63*	53.01**	1.24**	119.63*	377.36*	314.87**	3.76**	0.61**
P×T	12	798.08	6.04**	20.49	10.54	0.53**	29.70	341.75*	105.52	2.38**	0.06
Error	42	457.24	2.40	17.57	13.48	0.17	49.31	186.84	66.95	0.69	0.03
CV%		35.51	34.71	12.90	12.90	11.42	11.52	37.05	24.45	21.43	15.32

\*, \*\* = Significant at 5%, and 1% probability levels, respectively.

**Table 3.** Means of the speed of emergence and seedling DM% in three populations *Thymus kotschyanus* in a pot experiment.

Population	Emergence %	Speed of emergence Sprout/day	Root length mm	Shoot length mm	Root/shoot length ratio	Seedling length mm	Vigor index	Fresh weight mg/p	Dry weight mg/p	Seedling DM%
Qazvin	54.36b	3.80b	32.50a	28.74a	61.24a	1.15a	33.39b	31.61a	3.66a	0.12a
Tehran	64.95a	4.98a	33.04a	28.40a	61.43a	1.18a	40.04a	33.09a	4.09a	0.13a
Zanjan	61.33a	4.61ab	31.94a	28.22a	60.16a	1.14a	37.24a	35.70a	3.88a	0.11b

Dissimilar letters in each column mean significant difference at the 5% level using Tukey method.

GA 500 ppm, respectively, which were significantly higher than control and other treatments (Table 4). A similar trend was observed for seedling length and the higher values of 65.34 and 65.26 mm were obtained in GA 250 and GA 500 ppm, respectively.

For root shoot length ratio (RS), the higher value of 1.39 was obtained at PEG 0.9 Mpa, which was significantly higher than all the treatments, indicating that increasing root length may improve seedling establishment in the field. For the vigor index, the higher value of 49.90 was obtained in control. However, there were no significant differences between GA 500 ppm and PEG 0.9 Mpa and control.

For seedling fresh weight, the higher values of 42.14 and 39.14 mg were obtained in control and hydropriming, respectively. For seedling dry weight, the higher values of 4.79, 4.16 and 4.33 mg were obtained in control, hydropriming and PEG 0.6 Mpa, respectively that were significantly higher than other treatments (Table 4). For seedling DM%, the higher values of 0.17 and 0.13 were obtained in PEG 0.6 and PEG 0.9 Mpa, which were significantly higher than

all other treatments (Table 4), indicating osmopriming may improve seedling establishment by increasing the seedling DM% in a pot experiment.

### The population by treatment interaction effects

The population by treatment interaction effects was significant for speed of germination, root/shoot length ratio, vigor index and seedling dry matter weight ( $P \leq 5\%$ ) (Table 2). Indicating that the populations had different responses to application priming treatments. The means of the population by treatment interaction effects were compared only for significant traits (figures 1 to 4).

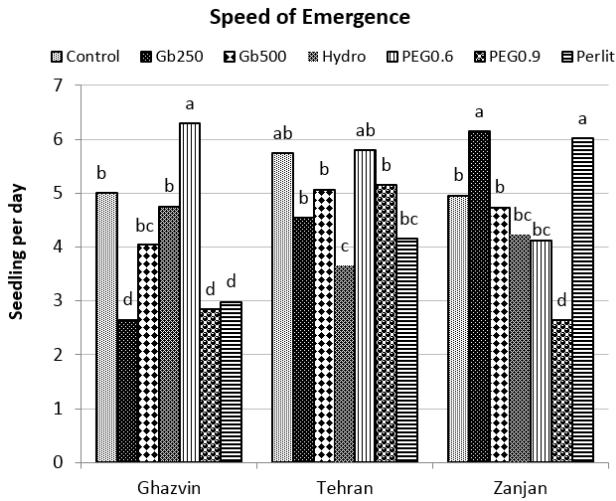
### Speed of emergence

The higher mean value of speed of emergence (6.29 sprouts/day) was observed in population Qazvin with the effect of PEG 0.6 Mpa, and the lower value (2.65 sprouts/day) was related to the GA 250 ppm (figure 1). The population of Tehran had a higher speed of emergence (5.84 sprouts/day) using PEG 0.6 than the other treatments. The population of

**Table 4.** Effect of the priming treatments on mean seed emergence characteristics averaged over three populations of *Thymus kotschyanus* in a pot experiment.

Treatments	Emergence %	Speed of emergence Sprout/day	Root length mm	Shoot length mm	Seedling length mm	RS	Vigor index	Fresh weight mg/p	Dry weight mg/p	Seedling DM%
Control	82.22a	5.57a	32.87ab	27.23b	60.09ab	1.22b	49.90a	42.14a	4.79a	0.12bc
GA 250 ppm	50.39b	4.04ab	33.64ab	31.70a	65.34a	1.07c	34.40b	28.89bc	2.78c	0.09d
GA 500 ppm	59.11b	4.62ab	33.49ab	31.77a	65.26a	1.06c	38.71ab	34.02ab	3.51bc	0.10cd
Hydro priming	53.33b	4.21ab	32.11ab	28.88ab	60.99ab	1.12bc	32.57b	39.44a	4.16ab	0.11cd
PEG 0.6 Mpa	68.89ab	5.40a	30.08b	26.22b	56.30b	1.15bc	38.71ab	25.47c	4.33ab	0.17a
PEG 0.9 Mpa	48.44b	3.35b	35.99a	26.00b	62.00ab	1.39a	30.85b	29.90bc	3.89b	0.13b
Perlite1%	59.11b	4.05ab	29.27b	27.38b	56.65b	1.08bc	33.09b	34.41ab	3.67b	0.11cd

Dissimilar letters in each column mean significant difference at the 5% level using Tukey method  
GA = Gibberellic Acid, PEG = Polyethylene glycol, RS = root/shoot length ratio.



**Figure 1.** Mean of priming methods on the speed of emergence of three populations *Thymus kotschyanus*.

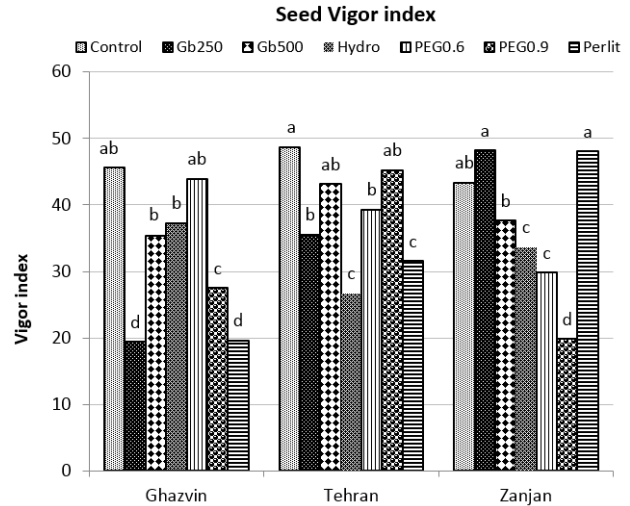
Zanjaan had a higher speed of emergence (6.14 sprouts/day) using both GA 250 and perlit than the other treatments. The minimum value of the speed of emergence with a value of 2.60 sprouts/day was obtained in PEG 0.9 with (figure 1).

**Root shoot length ratio**

The higher mean value of root/shoot length ratio (RS) (1.31) was observed in Qazvin population using PEG 0.9, and the lower value of this population was related to the perlit with a value of 0.96 (figure 2). The population of Tehran had the higher RS with a value of (1.4) using PEG 0.9 than the other treatments. The population of Zanjaan had higher RS values of (1.23, 1.19 and 1.19) with effect of PEG 0.9, PEG 0.6 and perlit, respectively, than the other treatments (figure 2).

**Seed vigor index**

The higher mean values of vigor index (47.62 and 46.94) were observed in population Qazvin in control and PEG 0.6, respectively, but the lower value was related to GA 250 ppm as 19.41 (figure 3). The maximum vigor index

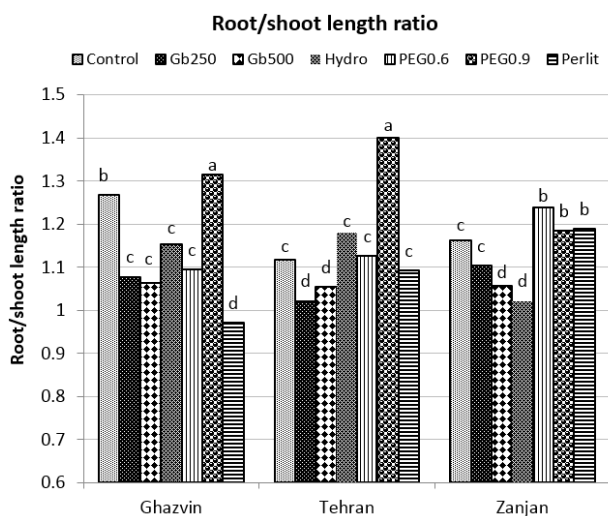


**Figure 3.** Mean of priming methods on the seed vigor index in three populations *Thymus kotschyanus*.

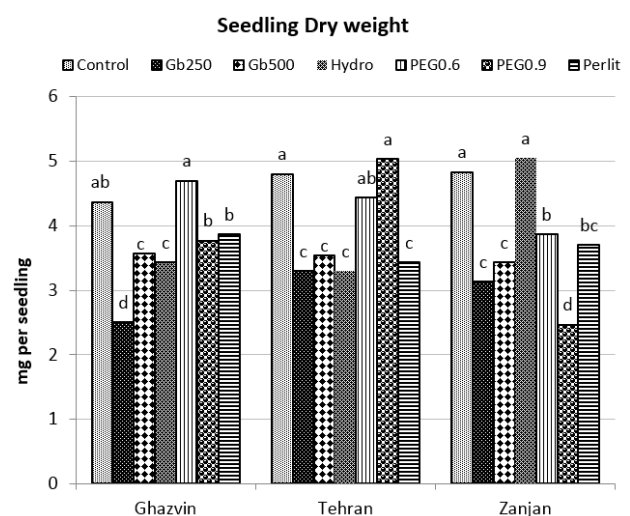
with the value of 48.69 was observed in control treatment in population of Tehran, but the lower value (26.75) was related to the primed seed using hydropriming (figure 3). The population of Zanjaan had higher mean values of vigor index (48.23 and 48.03) by application of GA 250 ppm and perlit, respectively, but its lower value of 19.85 was related to PEG 0.9 (figure 3).

**Seedling dry weight**

The higher value of seedling dry weight (4.63 mg) was obtained in the Qazvin population using PEG 0.6. Pa, but its lower values given as 2.40 mg were related to hydropriming and GA 250 ppm (figure 4). The population of Tehran had the higher value of seedling dry weight (5.07 mg) using hydropriming (figure 4). The higher value of seedling dry weight of the population of Zanjaan with a value of 5.05 mg was obtained with the effect of hydropriming, but its lower value as 2.47 mg was related to the PEG 0.6 Pa (figure 4).



**Figure 2.** Mean of different priming method on the root shoot length ratio (RS) of three populations: *Thymus kotschyanus*.



**Figure 4.** Mean of priming methods on the seedling dry weight in three populations *Thymus kotschyanus*.

## Discussion

The effect of treatment of three populations of *Thymus kotschyanus* showed that the percentage and speed of emergence of the Qazvin population increased using PEG 0.6 MPa. Also, the speed of emergence increased in the population of Zanjan with the effect of perlite treatment. The speed of emergence is known as a suitable indicator of establishment success (Harris et al., 2001). The reasons for increasing the speed of germination are DNA, RNA and protein synthesis during priming (Bray, 1995). In a study, Afzal et al. (2008) performed hydropriming, osmotic and matrix priming on the seeds of *Brassica napus*. They found an increase in germination at the time it reached 50% germination and the average time of germination decreased. The effect of treatments on the seedling length and vigor index of the populations was determined that gibberellic acid increased the seedling length and vigor index of the Zanjan population. Research on the germination characteristics of yarrow plants proved that GA treatment increased the germination percentage, stem length, root length, seedling length and seed germination index (Tavili et al., 2009). Our research work was also in agreement with the result of Mirzaei et al. (2022). They treated the seed of a Yarrow plant with GA (250 and 500 ppm) and found that seed germination improved and the best results for seed quality parameters were obtained with GA (500 ppm). Also, our result was similar to the results of (Dissanayake et al., 2010). They investigated the effects of osmotic priming on the seeds of the grey plant (*Parthenium argentatum*), and concluded that priming increased the percentage of germination from 36% to 47%, and as a result, improved seedling emergence, root length, stem length, dry matter of seedlings and the establishment of seedlings from 3.3 seedlings to 5.7 per square meter.

The effect of the treatment on the fresh and dry seedlings of three populations showed that the fresh weight of Zanjan population increased by hydropriming. The seedling dry weight of Qazvin and Tehran populations increased using PEG treatment. In a study, Bonome et al. (2006) conducted an experiment on *Brachiaria brizantha* CV. Marandu grass for pasture improvement using osmotic priming of polyethylene glycol 6000 Daltons, potassium nitrate and a mixture of both substances. The osmotic pressures  $-0.9$ ,  $-1.1$  and  $-1.4$  MPa were applied for 12, 24, 48, 72 hours, and they found that osmopriming improved the seedling growth. Similarly, our result was in agreement with Alizadeh et al. (2020). They conducted the effect of seed priming including polyethylene glycol, auxin, zeolite, hydropriming treated on the seeds of three populations of (*Thymus daenensis*). They found that both matrix priming (zeolite), and hydro priming methods induced seedlings fresh and dry weight. In another research, Alizadeh et al. (2022) investigated the effect of priming methods including hydro, osmopriming and hormonal priming for germination characteristics of the deteriorated seeds of *Tanacetum parthenium* and they proved that the effect of osmopriming (PEG) and hormonal priming (GA) cause increasing seedlings fresh and dry weigh.

## Conclusion

- The results of this research showed that priming methods, especially osmopriming (PEG 0.6 MPa and 0.9 MPa) and hormonal priming (GA 500 ppm) as inducer treatments were important in improving the speed of emergence and reducing the average time of germination.
- Also, the seedling's performance including length of root and shoot and the seedling's dry weight were increased using osmo and hormonal priming.
- According to this research work, the priming treatments, especially osmo and hormonal priming had improved seedling emergence characteristics of *Thymus kotschyanus* populations. Therefore, the osmopriming and hormonal priming methods were recommended for seed priming of the crops with proper concentration before cultivation in the field.

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### Authors contributions

Authors have contributed equally in preparing and writing the manuscript.

### Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

### Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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