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Original Article

Effect of Seed Priming and Moist Chilling on Emergence Traits of Six Populations (*Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss.) in Greenhouse Condition

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Abstract

Chamomile (*Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss.) are annual, medicinal and aromatic plant belong to Asteraceae family. This two species have special important in pharmaceutical and cosmetic industries. Current study was carried out to investigation of different pre-treatments for enhancement of seed emergence potential, rate of emergence and vigor in some populations of *Anthemis haussknechtii* and *Anthemis pseudocotula* For this purpose, a factorial experiment was conducted based on randomized complete design with three replications. The first were six populations and the second factor were six pre-treatments levels including osmopriming (0.5% and 1% of potassium nitrate (KNO_3)), hormon priming (125 ppm and 250 ppm of gibberellic acid (GA_3)), hydropriming (as control with distilled water) and moist chilling (4 $^{\circ}$C) in greenhouse conditions. The results showed significant differences between population, treatment and their interactions ($p<0.01$). Priming improved means of manty traits in some populations. In comparing between populations, 19320-Zanjan population had higher seed emergence characteristics higher than the other five populations. It was concluded that giberellic acid and potassium nitrate had higher effects on emergence characteristics than moist chilling and control. It was concluded that hormonal priming and osmopriming as physiological treatment, enhanced seed emergence characteristics and seedling vigor in some populations of *Anthemis* in greenhouse conditions.

Keywords: *Anthemis haussknechtii*, *Anthemis pseudocotula*, Emergence percentage, Hydropriming, osmopriming

Introduction

Chamomile is widely used throughout the world. Although there are numerous varieties of chamomile, the two valuable species are *Anthemis haussknechtii* and *Anthemis pseudocotula* which are from Asteraceae family. *A. haussknechtii* is annual, grass, aromatic, with have multiple stems to 5 cm to 25 cm height from the base of branches, leaves to 1.5 cm to 4.5 cm length and egg-like and flowers appears in spring with yellowish in color. It grows in north-west, west, center and south-west regions of Iran. *A. pseudocotula* is annual, herb, aromatic, with multiple stems to 10 cm to 60 cm height, with small rootlets, leaves with the sharp and more or less thin, egg-like and flowers grows in spring to white in color. This species grows in north-west, west and south-west regions of Iran

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(1&2). This two species used from medicinal properties including wounds, ulcers, eczema, The use of plants as medicine approach requires to domestically cultivated. Seed germination is a critical stage in the life of plants [4]. skin irritations, neuralgia, sciatica, rheumatic pain, hemorrhoids, mastitis and leg ulcers [3].

Gibberellins are well known plant hormones which would be used to stimulate seed germination in a wide range of plant species and the predominant active GA depends on the species [5]. GA may stimulate germination via transition from embryonic to vegetative development, which mediated by the chromatin remodeling factor PICKLE (PKL) [6]. The effect of GA_3 as a germination promoter is hypothesized to increase of germination with stratification treatment [7]. Potassium nitrate is well documented as a compound which increases the germination of photo-dormant seeds [8]. According to Bewley and Black [9], KNO_3 raises the ambient oxygen levels by making less oxygen available for citric acid cycle. Seed technology with priming has been developed and used extensively to improve germination and seedling emergence in a wide range of crop species [10]. A wide variety of priming treatment has been used to enhance seed germination. Seed priming has been proved to advance germination for many agricultural plant species [11-15]. During the priming spinach seeds, seed absorb enough water and the germination processes initiate but radical and the plumule do not emerge [16]. This method is successful in small seed plants and the most medicinal plants that have small seed and also they have great economic value because of quick and uniform emergence requirement [17]. Nowadays, various seed priming techniques have been developed, including hydro priming (soaking in water), halo priming (soaking in inorganic salt solutions), osmo priming (soaking in solutions of different inorganic with osmotic potential), thermo priming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices) and bio priming (hydration using biological compounds) [18]. Hydropriming and osmopriming are commonly used to prime seeds [19]. Hydropriming consists in soaking seeds in pure water and re-drying them before complete germination. Osmopriming is a pre-sowing treatment that consists of the incubation of seeds in an osmoticum solution [20]. Mohammadi [21] found that primed seeds with potassium nitrate showed the highest values for all traits comparing with control. By effect of potassium nitrate, increasing of value for some traits including: germination percentage, germination rate and seedling dry weight were obtained as 28.3 %, 129.4 % and 58.1 %, respectively. The plant species of Anthemis are growing as wild type in rangeland of Iran, therefore some seed of those plant might be dormant and have problem to emergence in different place of Iran. Therefore for domestication of those species, it is necessary to use seed priming method for improving of seed performance leading to faster and more synchronized seed emergence in natural habitat.

The aim of this research was to determine the effects of different seed priming treatments (osmopriming, hormonal priming, hydropriming and moist chilling to enhance seed germination parameters and seedlings growth in some populations of two medicinal species *Anthemis haussknechtii* and *Anthemis pseudocotula*.

Materials and Methods

For this purpose, mature seeds were collected from Sanandaj, Baneh, Divandareh, Zanjan and Yazd in spring 2011. The equal number of seeds were sown in pods. This study was carried out in greenhouse of Gene Bank in Research Institute of Forests and Range Lands in 2011. The experiment was conducted by a factorial based on randomized complete design with six pre-treatment, three replications, The six populations including: 17021-Sanandaj, 9796-Baneh and 26044-Divandareh of *Anthemis haussknechtii* and 19320-Zanjan, 19269-Zanjan and 21071-Yazd of *Anthemis pseudocotula*. The seeds were disinfested with liquid fungicides of vitawax tiram 1 % for 2 min. 50 seeds were treated by different treatments including: moist chilling at 4 $^{\circ}$C for 2 weeks, gibberellic acid (125 ppm and 250 ppm) as hormon priming, potassium nitrate (0.5% and 1%) as osmopriming and distilled water (control) as hydropriming and those treated seeds placed in petri dishes (9 cm). Then, these Petri dishes were placed in a germinator for 24 hours at 20±2 $^{\circ}$C in the dark condition and seed were air dried until the moisture level comes back to its original in room temperature. After this time, the primed of seed samples were sown in pots (with ratio 1:1:1 of soil, sand and soil leaf were filled) with three replicates of 50 seeds. In the greenhouse

experiment. The pots kept in temperature 20-30 °C and 10,000 lux of light during the day and the temperature range 5-12 °C were at night. Percentage and emergence rate of seeds after 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21th days, were recorded. Seedlings growth was complete for 50th days. The emergence characteristics including: emergence percentage, speed or rate of emergence, length of rootlet and shootlet, seedling length, ratio of rootlet length by shootlet length, vigor index, fresh weight and dry weight of seedlings, seedlings dry to fresh weight ratio and leaf area were evaluated during 50th days of experiment. The seed emergence percentage was calculated according to total number of emerged seedlings in numbering final day ISTA [22] and speed of emergence was calculated according to [Kotowski,23; Maguire, 24].

- According, the speed of germination were calculated by flowing equation: Speed of emergence (sprout/day):

$$G.S = \frac{\sum n}{\sum n\,(n \times DN)} \times 100$$

After 50th days of start of the experiment, length of rootlet (mm) and shootlet (mm) (that including 5 seedlings per pot on random) was measured according to Lekh and Kairwal [25]. Then seedlings were dried in oven for 24 hours at 80 °C and their weight was measured by a Scale with precision 0.001.

Vigor index was calculated according to Abdulbaki and Anderson [26] that their values obtained from

$$Vi = \frac{\%\,Gr \times MSH}{100}$$

follow equations:

Where:

VI=vigour index

%G r=final germination percentage

MSH=mean seedling height

After 50th days of start of the experiment, leaf area seedlings (cm^2) (that including 3 leaf per pot on random) was measured according to leaf area evaluation system with leaf area meter.

Data given in percentages were subjected to arcsine transformation before statistical analysis. Statistical analysis was carried out using SAS software. The differences between the means were compared using Duncan test ($P<0.05$). Finally determined, the best treatment for enhanced seed germination characteristics and seedling vigor of *Anthemis* populations.

Results

Result of analyze variance showed significant differences between population, pre-treatment and their interactions for all traits (Table 1).

Seed Emergence and Rate of Emergence

Comparing of emergence percentage and emergence rate of six populations showed that population 19320-Zanjan with average values of 74% and 14.57 (sprout/day) had higher the emergence percentage and emergence rate than other populations,respectively (Table 2). The highest emergence percentage value (89%) was detected for population of 19320-Zanjan in *A. pseudocotula* using 125 ppm concentration gibberellic acid (GA_3) and potassium nitrate of 0.5% concentration (KNO_3). The minimum emergence percentage was observed by effect of 1% of potassium nitrate and control for population of 26044-Divandareh in *A. haussknechtii* (No seedlings emerged) (Fig. 1). The highest of emergence rate as 18 (sprout/day) obtained in population of 19320-Zanjan using 125 ppm of gibberellic acid compare to other treatment (Fig. 2).

Seedling Length Characteristics

Result of comparisons of rootlet shootlet and seedling length of six populations showed that 19320-Zanjan population with average values of 58.81 mm, 15.98 mm and 73.92 mm, had higher rootlet, shootlet and seedling length, respectively than other populations (Table 2). Result indicated that using gibberellic acid of 125 ppm, the population 19320-Zanjan *A. pseudocotula* with average value of 67.3 mm produced higher rootlet length than other treatment (Fig. 3).

Using 0.5% of potassium nitrate, the shootlet length of population of 17021-Sanandaj of *A. haussknechtii* was 20 mm and it was higher than other treatment. Minimum of shootlet length was obtained by 1% of potassium nitrate and control on population of 26044-Divandareh of *A. haussknechtii* (Fig. 4).

For seedling length the higher values of 86.9 mm was obtained using 250 ppm of gibberellic acid in population of 19320-Zanjan compare with other treatment and the minimum seedling length was related to 1% of potassium nitrate and control for population of 26044-Divandareh of *A. haussknechtii* (Fig. 5).

Table 1 Mean square of seed emergence characteristics under the effect of different pre-treatments on six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse condition

SOV	df	MS										
		Emergence	Emergence speed	Rootlet length	Shootlet length	Seedling length	Rootlet /shoot let	Vigor index	Fresh weight of seedling	Dry weigh t of seedling	Dry/fresh	Leaf area
Population (P)	5	9990.8 **	489.39 **	5700.29 **	415.75 **	8023.01 **	18.13 **	6390.58 **	19875.03 **	10092.38 **	0.191 **	7.5**
Pre-treatment (T)	5	189.46 **	6.68 **	293 **	69.71 **	618.13 **	6.03 **	243.94 **	2000.85 **	1193.08 **	0.038 **	0.89 **
P × T	25	510.77 **	13.51 **	417.44 **	33.11**	535.7 **	4.7 **	181.33 **	1859.61 **	1033.81 **	0.074 **	0.76 **
Error	70	10.4	0.27	4.74	0.75	5.68	0.1	1.16	3.18	2.13	0.0001	0.0001
CV		10.53	12.25	5.27	7.51	4.57	8.71	6.21	2.84	3.95	2.06	1.59

^{ns}, **, * Respectively non significant of 1 and 5 percent of probability

Table 2 Mean comparison seed emergence characteristics of six populations of *A Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse condition

Name of population	Emergence (%)	Emergence speed (sprout/day)	Rootlet length (mm)	Shoot let length (mm)	Seedling length (mm)	Rootlet /shoot let	Vigor index	Fresh weight of seedling (mg)	Dry weight of seedling (mg)	Dry/fresh ratio	Leaf area (cm^2)
19320-Zanjan	74 a	14.57 a	58.81 a	15.98 a	73.92 a	4.05 b	54.01 a	105.8 a	69.5 a	0.65 a	2.06 a
19269-Zanjan	37.22 b	4.42 b	55.66 b	11.02 c	64.37 b	5.42 a	17.44 b	94.94 b	61.99 b	0.61 b	0.71 c
21071-Yazd	16.75 d	2.05 d	51.26 c	14.71 b	64.91 b	3.67 c	9.38 d	31.87 e	18.03 e	0.52 d	0.69 d
17021-Sanandaj	30.83 c	2.41 c	42.23 d	15.17 b	55.1 c	2.81 e	15.88 c	70.53 c	35.6 c	0.53 c	0.36 e
9796-Baneh	12.83 e	1.32 e	25.39 e	8.95 d	37.17 d	3.13 d	4.07 e	47.95 d	23.89 d	0.52 d	1.02 b
26044-Divandareh	12.15 e	0.71 f	14.52 f	3.45 e	17.37 e	2.77 e	3.59 e	25.36 f	12.73 f	0.35 e	0.3 f

Means of each columns followed by the same letters had no significant differences (P<0.01) based on Duncan method

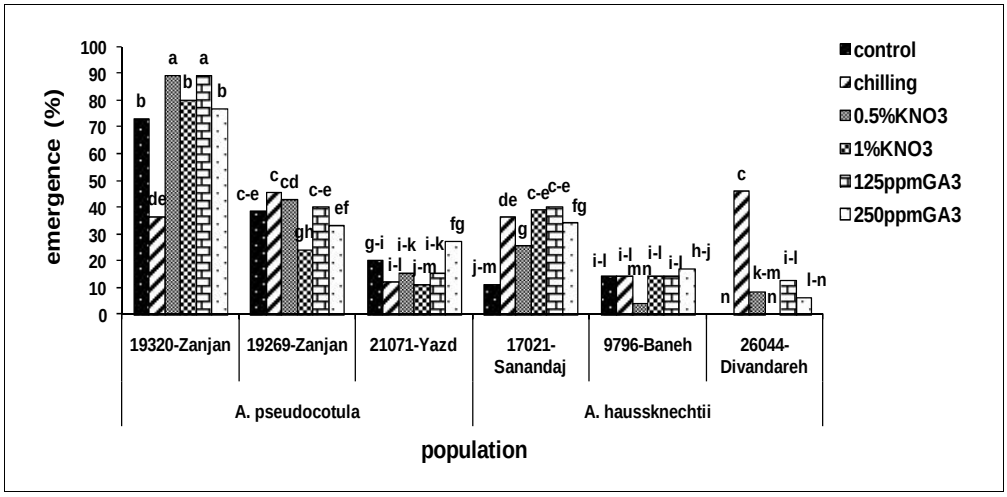

Fig. 1 Mean comparison of the effect of pre-treatment on seed emergence percentage of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

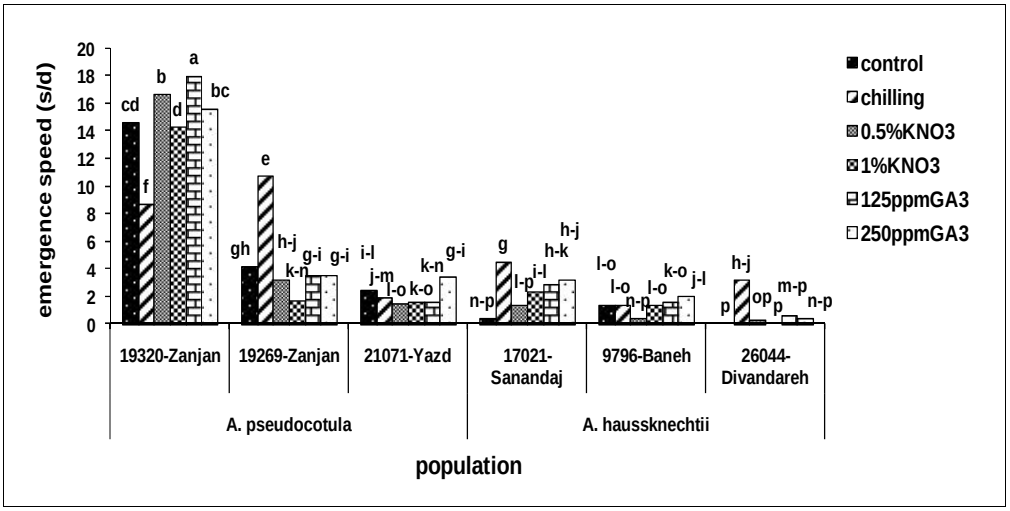

Fig. 2 Mean comparison of the effect of pre-treatment on seed emergence speed of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

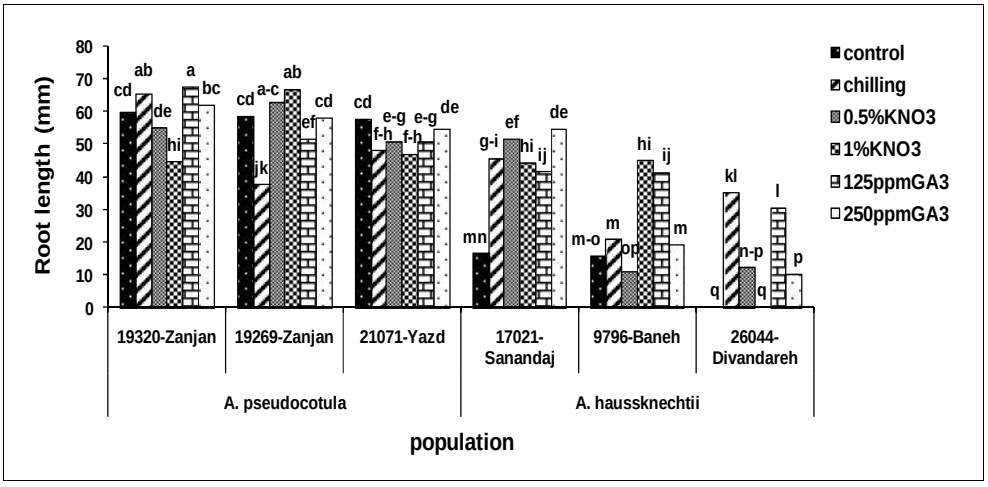

Fig. 3 Mean comparison of the effect of pre-treatment on rootlet length of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

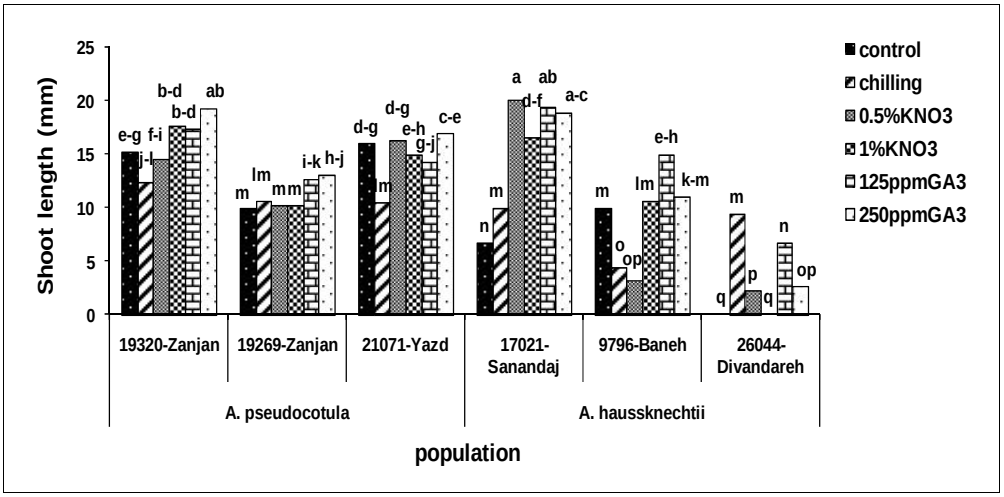

Fig. 4 Mean comparison of the effect of pre-treatment on shootlet length of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

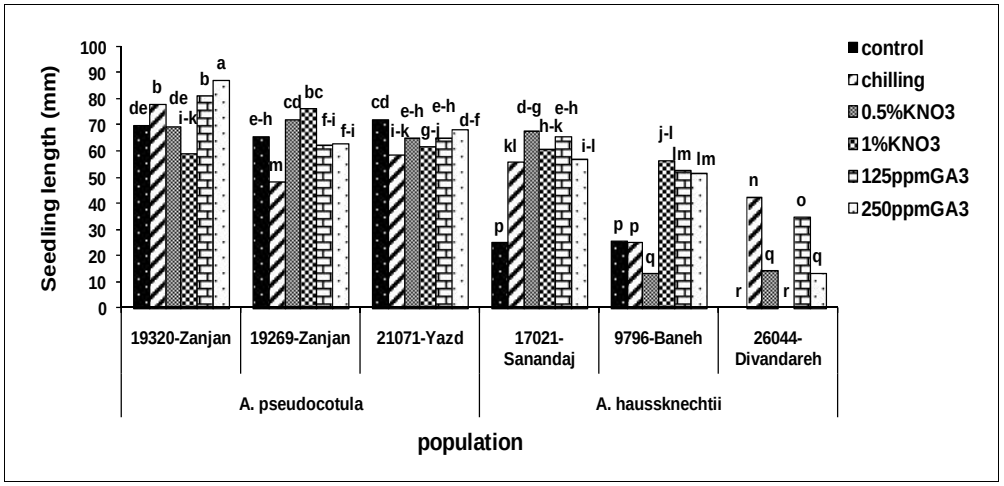

Fig. 5 Mean comparison of the effect of pre-treatment on seedling length of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

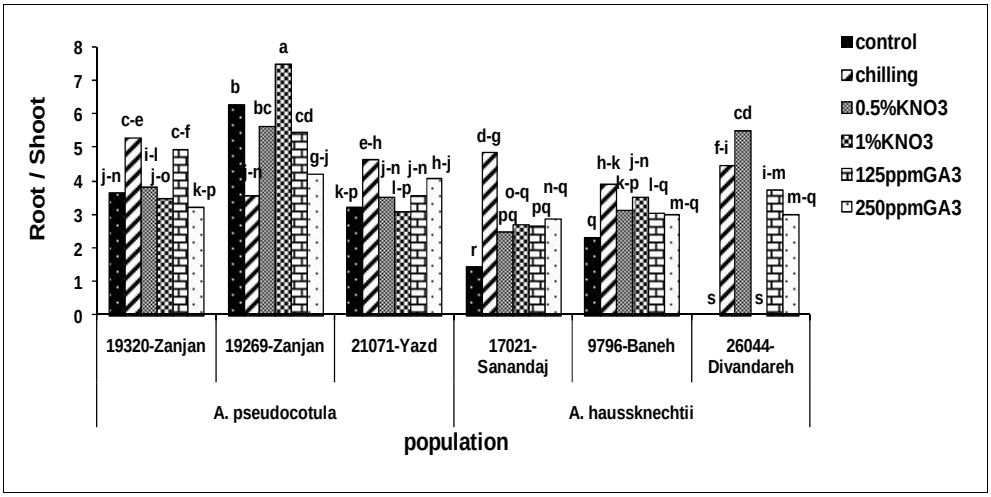

Fig. 6 Mean comparison of the effect of pre-treatment on root shoot length Ratio of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

The root shoot length Ratio of 19269-Zanjan population of *A. pseudocotula* as 5.42 was higher than other populations (Table 2). Using 1 % of potassium nitrate, had increased root shoot length Ratio as 7.45 in population of 19269-Zanjan of *A. pseudocotula* which was higher than other treatments but minimum root shoot length Ratio was obtained by 1% of potassium nitrate and distilled water for population of 26044-Divandareh of *A. haussknechtii* (No seedlings emerged) (Fig. 6).

Vigour Index

Mean comparison of vigor index of six population showed that Vigor index of 19269-Zanjan population of *A. pseudocotula* as 54.01 was higher than other populations (Table 2). In comparison between treatment on six populations the maximum vigor index as 66.89 was by 250 ppm of gibberellic acid on population of 19320-Zanjan of *A. pseudocotula* . The minimum value of vigour index was related to 1% of potassium nitrate and control on population of 26044-Divandareh of *A. haussknechtii* (Fig. 7).

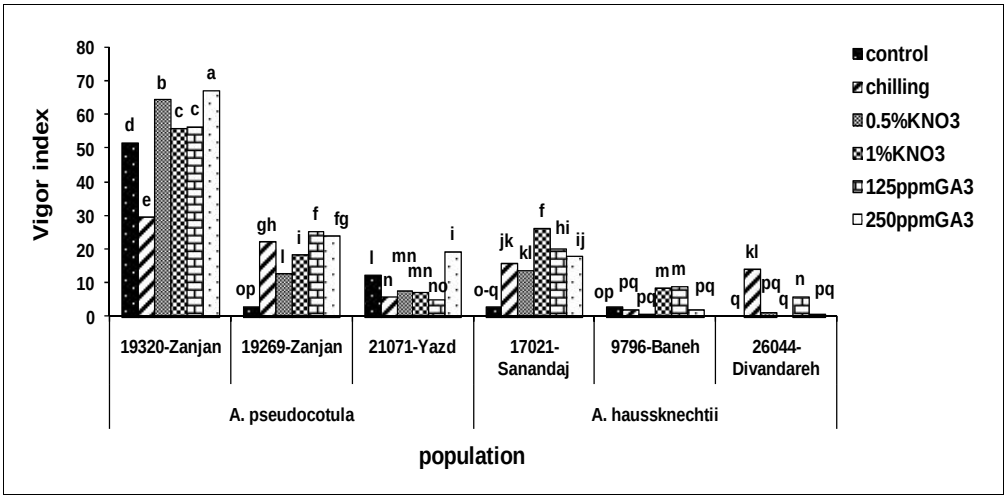

Fig. 7 Mean comparison of the effect of pre-treatment on vigor index of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

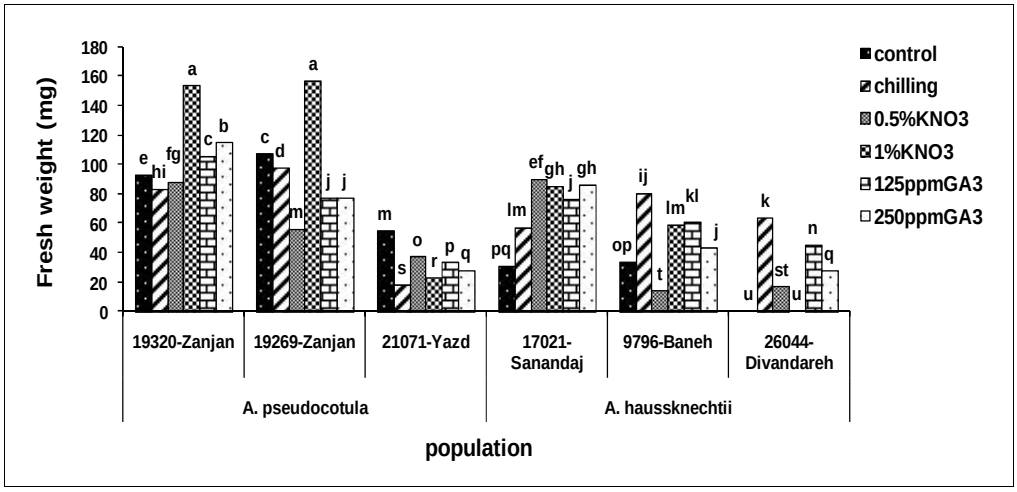

Fig. 8 Mean comparison of the effect of pre-treatment on fresh weight of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

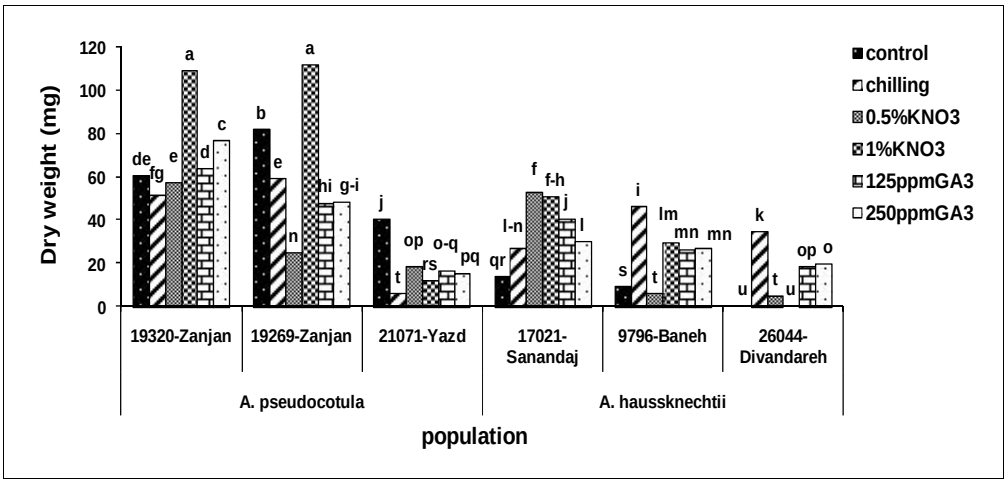

Fig. 9 Mean comparison of the effect of pre-treatment on dry weight of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

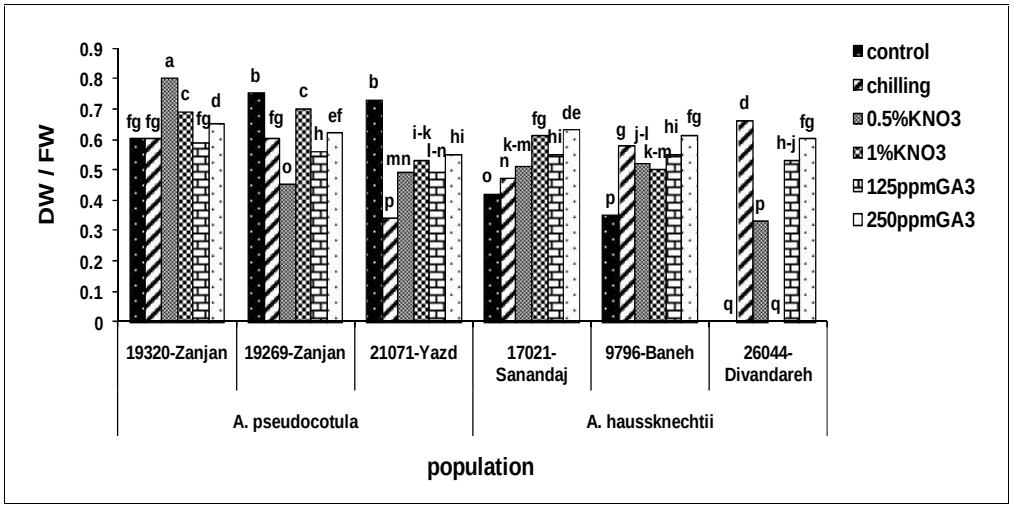

Fig. 10 Mean comparison of the effect of pre-treatment on ratio of dry weight by fresh weight of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

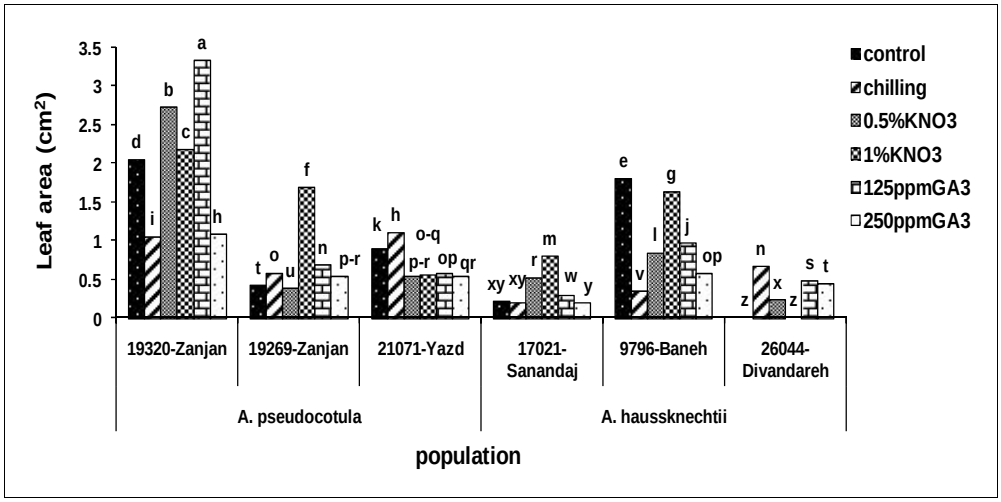

Fig. 11 Mean comparison of the effect of pre-treatment on leaf area of six populations of *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. in greenhouse (Duncan 0.05).

Seedlings Fresh and Dry Weight

Fresh and dry weight of Seedlings and dry to fresh weight ratio of seedlings in 19320-Zanjan population of *A. pseudocotula* was 105.8 mg, 69.5 mg and 0.65, respectively that its mean values was higher than other populations (Table 2). Maximum Fresh and dry weight of Seedlings (152.9 mg and 156.5 mg) respectively were obtained using 1% of potassium nitrate on population of 19320-Zanjan in *A. pseudocotula* compare with other treatment. The minimum Fresh and dry weight of seedlings were obtained using 1% of potassium nitrate and control in population of 26044-Divandareh (Fig. 8 and 9).
Also, the maximum dry to fresh weight ratio of seedlings was 0.8 using potassium 0.5 % potassium nitrate on population of 19320-Zanjan of *A. pseudocotula* compare with other treatments and the minimum values was obtained for 1% potassium nitrate and control on population of 26044-Divandareh of *A. haussknechtii* (Fig. 10).

Leaf Area of Seedling

In comparison between treatments for leaf area result showed that, population 19269-Zanjan with average value of 2.06 cm^2 had higher than other populations (Table 2). Mmaximum leaf area of seedling as 3.32 cm^2 were obtained using 125 ppm of gibberlic acid on population of 19320-Zanjan of *A. pseudocotula* than other treatments but the minimum value was obtained by 1% potassium nitrate and control on population of 26044-Divandareh in *A. haussknechtii* (Fig. 11).

Discusion

The result of this experiment indicated that 19320-Zanjan population had higher mean values for all of traits than the other five populations (Table 2). The highest enhancement of seed emergence percentage and emergence rate achieved by 0.5% KNO_3 and 125 ppm GA_3 priming on 19320-Zanjan population compare control and other treatment (Fig. 1 and 2). This result agree with Khaninejad, *et al*. [27], they reported that best germination percentage of caper seeds with 250 ppm GA_3 and 8000 ppm KNO_3. In addition, it was reported that the best germination percentage for the seeds of *Sabal palmetto* plant was obtained using 1% KNO_3 and 500 ppm GA_3, respectively [28]. According to Tilki [29], the best germination percentage for *Arbutus unedo* was 84% at 300 $mg.L^{-1}$ GA_3, whereas in the present study the highest emergence percentage was 89% at 125 $mg.L^{-1}$ GA_3. Paparozzi, *et al*. [30], reported for soaking seeds of *Penstemon digitalis* Nutt. ex Sims cv. Husker Red in 1000 $mg.L^{-1}$ GA_3 for 24 hours which increased the rate and number of germinating seeds. Similar to result of this research, Bocian and Holubowicz [31] showed that seed priming with KNO_3 for 6 hours and 12 hours improved seed germination of tomato and reduced mean germination time.
Additionally, Amri [32] treated seeds of *Terminalia sericea* with GA_3 (400 ppm) and confirmed that higher percentage of germination (67%) obtained compared with the control. Based on current research results, effect of 125 ppm and 250 ppm GA_3 were effective in increasing rootlet and seedling length and vigor index of 19320-Zanjan population compare with control (Fig. 5 and 7). Effect of 250 ppm gibberellic acid on Seedling length and vigor index was similar with result of Rahmanpour and Majd [33] because they reported that using of gibberellic acid increase of rootletlet and shootlet length (seedling length) and vigor index. Liopa-Tsakalidi and Barouchas [34] confirmed this result, because they reported that the seeds germination of chervil plant seed which treated with GA3 concentrations 200 ppm, 500 ppm and 1000 ppm was significantly higher than the corresponding one in H_2O treatment. On the other hand, Dissanayake, *et al*. [35], reported that priming treatment increased the seedlings length and fresh weight seedling compared with non-primed ones. With regard of increasing of 1% KNO_3 on fresh and dry weigh of seedlings of populations of 19320-Zanjan and 19269-Zanjan (Fig. 8 and 9). This result coordinated with result of Khaninejad, *et al.* [36] reported, the highest seedling dry weight of 25 mg wusing100 ppm gibberellic acid treatment and 1000 ppm potassium nitrate. Shanmugavalli, *et al*. [37] in sorghum soaked using 0.5% and 1% potassium nitrate (KNO_3) improve germination up to 44%. The highest shootlet and rootlet, dry weights were found when seeds soaked for 24 hours in 200 $mg.L^{-1}$ GA_3 [35].
The present results indicated that 125 ppm of gibberellic acid caused increasing leaf area of 19320-Zanjan population compared with control and other treatment (Fig. 11). In an experiment, Mung bean seed primed with PEG (-0.5 MPa osmotic potential) and distilled water (0 MPa osmotic potential) showed the PEG (-0.5 MPa osmotic potential) caused highest leaf area compare

with control [38]. Alvarado, *et al*. (12) greater plant dry mass and leaf area of seedlings obtained from primed seeds of tomato (*Lycopersicon esculentum* Mill.).

Conclusion

The results indicated that seed emergence characteristics of 19320-Zanjan population was higher than five population (Table 2). Totally, the results revealed that both osmopriming (potassium nitrate) and hormonapriming (gibberellic acid) techniques improved seed emergence characteristics of populations of 19320-Zanjan, 19269-Zanjan of *A. pseudocotula* and 17021-Sanandaj of *A. pseudocotula*. The priming techniques accelerated germination physiological process and enhanced seed emergence characteristics and seedling vigor of some populations of *A. haussknechtii* and *A. pseudocotula*.

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