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**ORIGINAL ARTICLE**

# Assessment of Seed Emergence Characteristics and Seedlings Vigor of three Populations Aromatic Medicinal Plant Species of *Anthemis pseudocotula* Boiss by using of Priming Technique and Pre-chilling

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**ABSTRACT**
*The aim was to study the effects of priming and pre-chilling on seed emergence characteristics and seedlings vigor of three populations of species of Anthemis pseudocotula Boiss., an experiment as factorial based on completely randomized design in six pre-treatments, three replications and three of populations (19320-Zanjan, 19269-Zanjan and 21071-Yazd) was carried out in the greenhouse in Research Institute of Forests and Range Lands.in 2011-2012. The pre-treatments including: osmo-priming (with 125 and 250 ppm of gibberellic acid and 0.5 and 1 % of potassium nitrate), hydro-primimng (control with distilled water) and pre-chilling (at 4°C for 14 days). Results showed that the effects of priming and pre-chilling on seed germination and emergence characteristics were significant. Comparing of seed emergence characteristics of three populations showed that population of 19320-Zanjan was higher all seed germination and emergence traits than the other two populations. The results indicated that seed priming with 250 ppm gibberellic acid improved shoot length (19.2 mm), seedling length (86.9 mm) and vigor index (66.89) in population of 19320-Zanjan. The highest emergence speed (17.94 sprout/day) and leaf area of seedling (3.32 cm$^2$) was found in primed seeds with 125 ppm gibberellic acid in population of 19320-Zanjan. In conclusion, primed seeds with gibberellic acid (osmo-priming) improved germination potential and seedlings vigor in population of 19320-Zanjan of species of Anthemis pseudocotula in greenhouse.*
***Keywords:*** *emergence speed, leaf area, osmo-priming, vigor index.*

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## INTRODUCTION

The *Anthemis pseudocotula* Boiss. is an annual and aromatic plant which is belonging to the *Asteraceae* family. This species grows natively in the north-west, west and south-west regions of Iran. The species grows to 10 cm up to 60 cm height, with multiple, green and from the base of branch stems and flowers appears in spring with white in color [13]. Also, almost medicinal plants collected from the wild type of *A. pseudocotula* which will be used as a medicinal purpose for gout, headache, carminative, indigestion, neuralgia, skin irritations, catarrhal, deodorant and sedative [14]. Priming can be more effective on poorer quality seed lots and it is an important way for enhancing of seed germination. Seed priming is defined as the uptake of water to initiate the early events of germination but not sufficient to permit radicle protrusion, followed by drying [12]. Seed priming is seen as a viable technology to enhance fast and uniform germination and emergence, high vigor of seedling and better yields [3]. priming techniques include osmo-priming, halo priming and hydro-priming. Osmo-priming is the process of soaking seeds in aerated osmotica of low water potential to control the amount of water they imbibe. Osmo-priming improved the performance of pepper seed lots having a high percentage of viable compared to non-viable seeds. Hydro-priming is the process of soaking and misting seeds in water and re-drying them before they complete germination [12]. Gibberellins are most directly implicated in the control and promotion of germination. Gibberellins promote growth by increasing extendibility of the cell wall followed by the hydrolysis of starch to sugars which reduces the potential in the cell, resulting in the entry of water into the cell causing elongation [2]. Potassium nitrate is well documented as a compound which increases the germination of photo-dormant seeds [17]. According to Bewley and Black [4], potassium nitrate raises ambient oxygen levels by making less oxygen available for citric acid cycles. The objective of this research was to determine the effects of best seed priming treatments on enhanced seed germination parameters and seedlings vigor in three populations of medicinal species of *Anthemis pseudocotula* Boiss in greenhouse condition.

## MATERIAL AND METHOD

This study was carried out in greenhouse of Gene Bank in Research Institute of Forests and Range Lands in 2011-2012. The experiment was conducted a factorial based on randomized complete design with six pre-treatments, three replications and three populations including: 19320-Zanjan, 19269-Zanjan and 21071-Yazd from species of *Anthemis pseudocotula* Boiss. The seeds were disinfested with liquid fungicides of vita wax tiram 1% for 2 min. 50 seeds were used for each pre-treatment which including: osmopriming (gibberellic acid with concentrations 125 and 250 ppm and potassium nitrate with concentrations 0.5 and 1 %), hydro-priming (distilled water as control) and pre-chilling (14 days at 4 °C).

**Germination test**

The seeds were imbibed with 10 ml of osmotic solution of potassium nitrate (0.5 and 1 %), solution of gibberellic acid (125 and 250 ppm) and distilled water in petri dishes (9 cm). Then, these petri dishes were put in an a dark growth chamber 24 h and then seed samples were air dried until the moisture level comes back to its original in room temperature. After this time, the prime of seed samples were planted and 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, these 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 speed of seeds after 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21$^{th}$ days, were recorded. Seedlings growth was complete for 50$^{th}$ days. The germination characteristics including: emergence percentage, emergence speed, length of rootlet and shootlet, seedling length, vigor index, dry weight of seedlings and leaf area of seedling were evaluated during 50$^{th}$ days of experiment.

**Emergence percentage:** In current research, emergence percentage was calculated according to total number of emerged seedlings in numbering final day [7].

**Emergence speed:** Speed of emergence (sprout/day) was calculated according to Maguire [11] and Kotowski [9] that by following equation.

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

**Vigor index:** Vigor index was measured according to Abdulbaki and Anderson [1] that their values obtained from follow formula.

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

Where:

Vi = vigor index

%Gr = final germination percentage

MSH = mean seedling height

**Length of rootlet and shootlet:** After 50$^{th}$ days of start of the experiment, length of rootlet (mm) and shootlet (mm) (that including five seedlings per pot on random) was measured according to Lekh and Kairwal [10].

**Dry weight of Seedlings:** Then seedlings were dried in oven for 24 h at 80 °C and their weight (mg) was measured by a scale with precision 0.001.

**Leaf Area of seedling:** After 50$^{th}$ days of start of the experiment, leaf area seedlings (cm$^2$) (that including three leaf per pot on random) was calculated according to leaf area evaluation system with 4SHTAS-2357. GATEHOUSE made in UK model with 80 HZ and with using WinDias 2,0 software.

**Statistical analysis**

A factorial experiment on the basis of randomized complete design was conducted with three replications, six pre-treatments and three population from species of *Anthemis pseudocotula*. 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).

## RESULTS AND DISCUSSION

In this experiment, simple effects (between pre-treatments and between three populations) and mutual interactions (effect of pre-treatment on population) of all factors were significant (P<0.01) on all traits (Table 1). Comparison of mean emergence characteristics of three populations showed that population of 19320-Zanjan was higher on all seed emergence traits than other two populations (Table 2).

Table 1. Mean square of seed emergence characteristics under the effect of different pre-treatments on three populations of *A. pseudocotula* in greenhouse condition

| | | Mean Square | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SOV | df | Emergence | Emergence speed | Rootlet length | Shootlet length | Seedling length | Vigor index | Dry weight of seedling | Leaf area |
| Population | 2 | 15147.83 ** | 796.77 ** | 258.85 ** | 119.45 ** | 517.62 ** | 10181.92 ** | 13912.19 ** | 11.1 ** |
| Pre-treatment | 5 | 411.45 ** | 3.86 ** | 96.59 ** | 26.56 ** | 133.24 ** | 334.34 ** | 2394.81 ** | 0.88 ** |
| Population×pre-treatment | 10 | 510.23 ** | 29.42 ** | 237.93 ** | 7.06 ** | 261.09 ** | 252.18 ** | 1051.62 ** | 1.2 ** |
| Error | 34 | 5.75 | 0.41 | 5.74 | 0.94 | 3.89 | 1.55 | 2.25 | 0.0001 |
| CV | | 5.62 | 9.13 | 4.33 | 6.97 | 2.91 | 4.62 | 3.01 | 1.18 |

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

Table 2. Mean comparison seed emergence characteristics of three populations of *A. pseudocotula* in greenhouse condition

| Name of population | Emergence (%) | Emergence speed (sprout/day) | Rootlet length (mm) | Shootlet length (mm) | Seedling length (mm) | Vigor index | Dry weight of seedling (mg) | Leaf area ($cm^2$) |
|---|---|---|---|---|---|---|---|---|
| 19320-Zanjan | 74 a | 14.57 a | 58.81 a | 15.98 a | 73.92 a | 54.01 a | 69.5 a | 2.06 a |
| 19269-Zanjan | 37.22 b | 4.42 b | 55.66 b | 11.02 c | 64.37 b | 17.44 b | 61.99 b | 0.71 b |
| 21071-Yazd | 16.75 c | 2.05 c | 51.26 c | 14.71 b | 64.91 b | 9.38 c | 18.03 c | 0.69 c |

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

**Seed emergence percentage (SEP):**

In the results, comparing of emergence percentage of three populations showed that the emergence percentage of 19320-Zanjan population as 74 % was higher and it was lower as 16.75 % in 21071-Yazd population of species of *Anthemis pseudocotula* (Table 2). Highest increase in emergence percentage (89 %) was observed after priming with 0.5 % potassium nitrate and 125 ppm gibberellic acid ($GA_3$) in population of 19320-Zanjan than other pre-treatments but the minimum emergence percentage (11 % and 12 % respectively) was observed after priming with 1 % potassium nitrate and pre-chilling (4 °C) of population of 21071-Yazd in species of *Anthemis pseudocotula* (figure 1). Priming with potassium nitrate and gibberellic acid improved the time to germination and seed germination percentage. According to Tilki [19], the best emergence percentage for *Arbutus unedo* was 84 % at 300 ppm gibberellic acid. This result agrees with Khaninejad *et al*., [8], they reported that best germination percentage of caper seeds with 250 ppm gibberellic acid and 0.8 % potassium nitrate whereas in the present study the highest emergence percentage was 89 % at 125 ppm gibberellic acid and 0.5 % potassium nitrate.

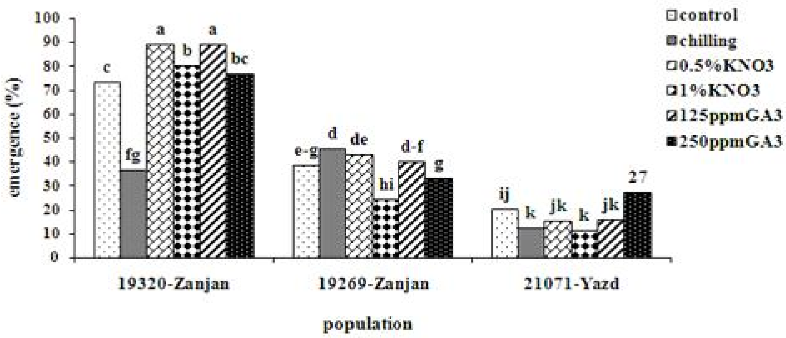


**Figure 1.** Mean comparison of the effect of pre-treatment on seed emergence percentage of three populations of *Anthemis pseudocotula* in greenhouse (Duncan 0.05).

**Seed emergence speed (SES):**

Results showed that the highest speed of emergence was obtained from 19320-Zanjan population (14.57 sprout/day) while the speed of emergence of population of 21071-Yazd significantly decreased (2.05 sprout/day) in species of *Anthemis pseudocotula* (Table 2). Seed priming with 125 ppm gibberellic acid ($GA_3$) had a negative effect on emergence speed (17.94 sprout/day) of population of 19320-Zanjan compare with other pre-treatments. Seed priming with 1% potassium nitrate of population of 19269-Zanjan, 125 ppm gibberellic acid, 0.5 and 1% potassium nitrate in population of 21071-Yazd had minimum effect on emergence speed (1.65, 1.59, 1.47 and 1.52 sprout/day respectively) in species of *Anthemis pseudocotula* (figure 2). Germination, speed and vigor are important indicators of seed quality and influenced on seed viability. Seed priming enhances the speed of metabolism, which influenced which will be increasing speed of germination and emergence. Sedghi *et al*. [15] fou
nd that seed primed with gibberellic acid increases the speed and percentage of emergence in comparison with other treatments of medicinal pumpkin (*Cucurbita pepo* L.). They concluded that gibberellic acid induces the generation of the hydrolytic enzymes responsible for endosperm degradation and causes faster germination and emergence.

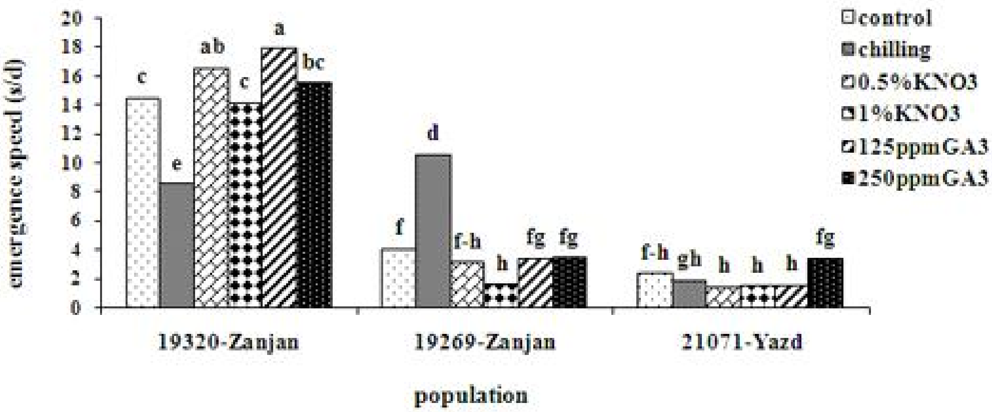


**Figure 2.** Mean comparison of the effect of pre-treatment on seed emergence speed of three populations of *Anthemis pseudocotula* in greenhouse (Duncan 0.05).

**Rootlet length (RL):**

As Table 2 shows, the rootlet length of 19320-Zanjan population as 58.81 mm was higher and it was lower as 51.26 mm for 21071-Yazd population. This also showed, the most rootlet length was (66.6 mm and 67.3 mm respectively) for population of 19269-Zanjan after seed primed of with 1 % potassium nitrate. Population of 19320-Zanjan with 125ppm gibberellic acid compare with other pre-treatments but minimum rootlet length was 37.4 mm by pre-chilling (4°C) in population of 19269-Zanjan (figure 3). The rootlet and seedling growth increase during seed priming. Sedghi *et al*. [16] reported that seed priming had significant effect on germination percentage, germination speed and rootlet and shootlet length in two medical plants including marigold and sweet fennel.

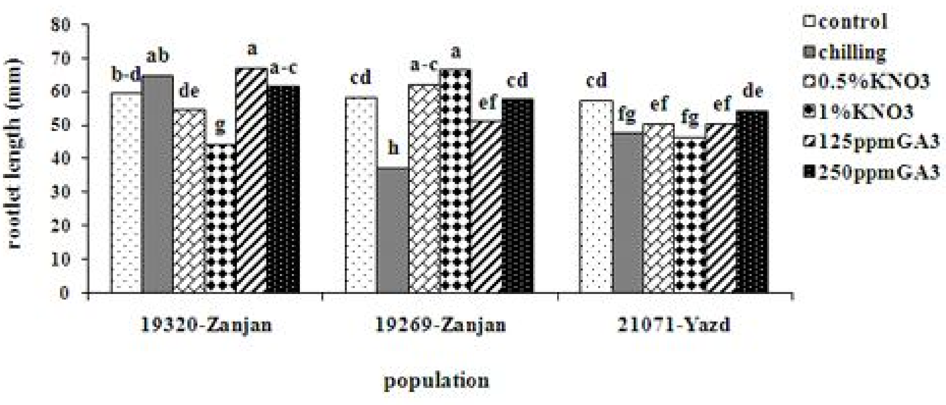


**Figure 3.** Mean comparison of the effect of pre-treatment on rootlet length of three populations of *Anthemis pseudocotula* in greenhouse (Duncan 0.05).

**Shootlet length (ShL):**

Comparison of mean showed that the highest shootlet length of 15.98 mm was achieved from population of 19320-Zanjan when it was decreased as 11.02 mm in population of 19269-Zanjan of species of (Table 2). The highest shootlet length (19.2 mm) found in seed primed with 250 ppm concentration of gibberellic acid in population of 19320-Zanjan compare with other pre-treatments while the minimum shootlet length was 9.9 mm with pre-treatment of distilled water (as control) in population of 19269-Zanjan (figure 4). Seed primed with gibberellic acid caused increasing of shootlet length so that the results of the present study are in agreement with observations of Zareh et al. [20] who showed that shootlet length of wheat was increased by seed priming with gibberellic acid.

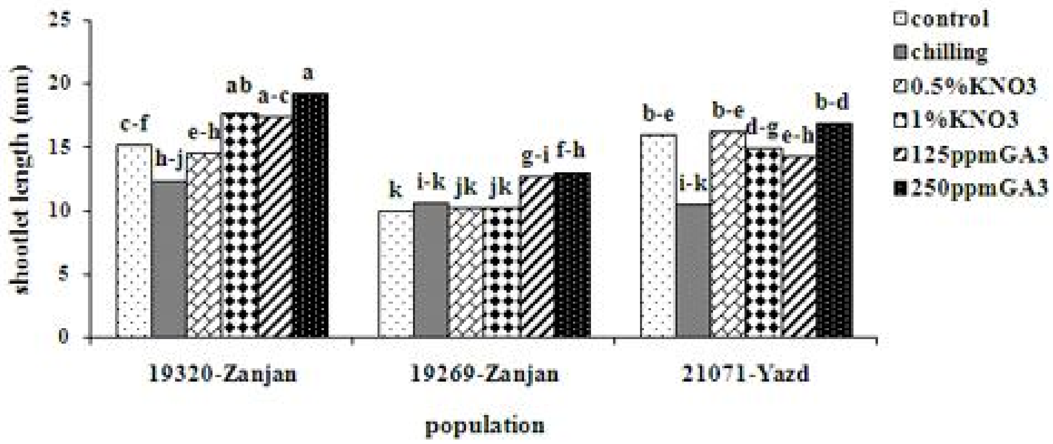


**Figure 4.** Mean comparison of the effect of pre-treatment on shootlet length of three populations of *Anthemis pseudocotula* in greenhouse (Duncan 0.05).

**Seedling length (SL):**

The result is shown in Table 2. The seedling length in population of 19320-Zanjan was as 73.92 mm was higher than other two populations in species of *Anthemis pseudocotula*. Pre-treated seeds with 250 ppm gibberellic acid showed the highest seedling length as 86.9 mm) which is belonging to the population of 19320-Zanjan and it was higher than other pre-treatments.The minimum seedling length (47.9 mm ) by pre-chilling (4 °C) treatment was observed in population of 19269-Zanjan (figure 5). Dissanayake *et al*. [6] who reported that priming treatment increased the seedlings length compared with non-seed primed.

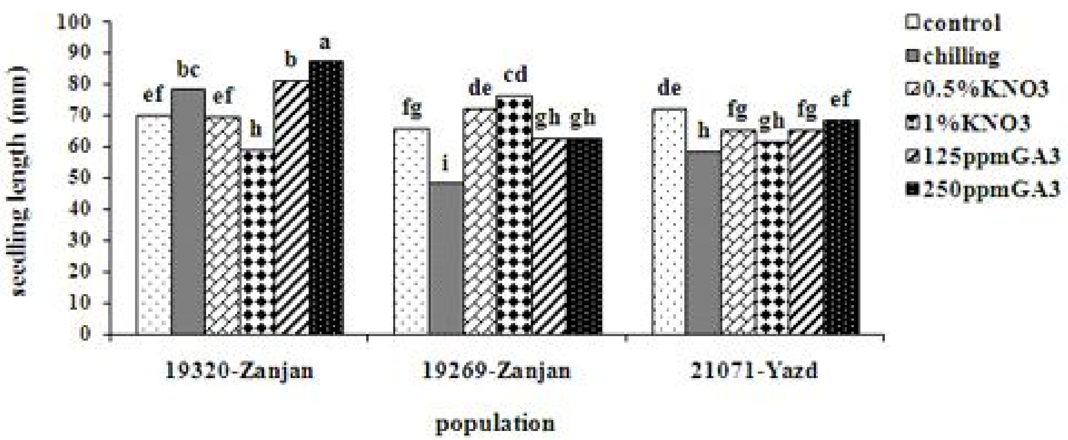


**Figure 5.** Mean comparison of the effect of pre-treatment on seedling length of three populations of *Anthemis pseudocotula* in greenhouse (Duncan 0.05).

**Vigour index (VI):**

Based on the results, the highest vigor index of 54.01 was achieved for population of 19320-Zanjan when the lowest vigor index of 9.38 was observed for population of 21071-Yazd in species of *Anthemis pseudocotula* (Table 2). Higher amount of vigor index (66.89) was recorded from osmo-primed seeds with 250 ppm gibberellic acid pre-treatment in population of 19320-Zanjan which was higher than other pre-treatments while lower amount of this parameter (2.82) was recorded by hydropriming of distilled water (as control) pre-treatment in population of 19269-Zanjan (figure 6). Seed vigor is another which affecting seed germination and seedling growth, high seed vigor increase the potential of further growth of and dry weight seedling. The present study, seed priming improved seedling vigor and effect of gibberellic acid of 250 ppm on vigor index and it was similar with result of Chauhan *et al*. [5] because they reported that seed primed with gibberellic acid caused an increase in seed germination and seedling vigor.

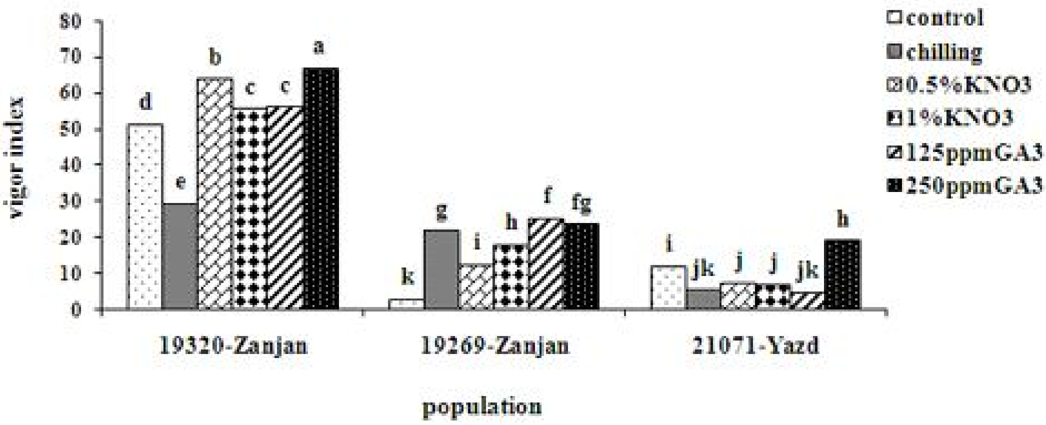


**Figure 6.** Mean comparison of the effect of pre-treatment on vigor index of three populations of *Anthemis pseudocotula* in greenhouse (Duncan 0.05).

**Seedling dry weight (SDW):**

According to the results shown in Table 2, the highest seedling dry weight of 69.5 mg was revealed for population of 19320-Zanjan whereas the lowest seedling dry weight of 18.03 mg was recorded for population of 21071-Yazd in species of *Anthemis pseudocotula*. The maximum dry weight of seedling (108.62 mg and 111.2 mg respectively) was observed from 1 % potassium nitrate in populations of 19320-Zanjan and 19269-Zanjan, respectively which were higher than other pre-treatments whereas the minimum dry weight of seedling (5.95 mg) was observed from pre-chilling (4 °C) treatment in population of 21071-Yazd (figure 7). Increasing dry weight of seedling might be due to synchronized germination and improved DNA, RNA synthesis during seed treatments and also the increasing shootlet and rootlet lengths by osmo-priming may be due to nuclear replication in rootlet and shootlet. The highest dry weight of seedling of 25 mg was achieved when the seeds of Caper (*Capparis spinosa* L.) were treated with 100 ppm gibberellic acid treatment and 1000 ppm potassium nitrate [8].

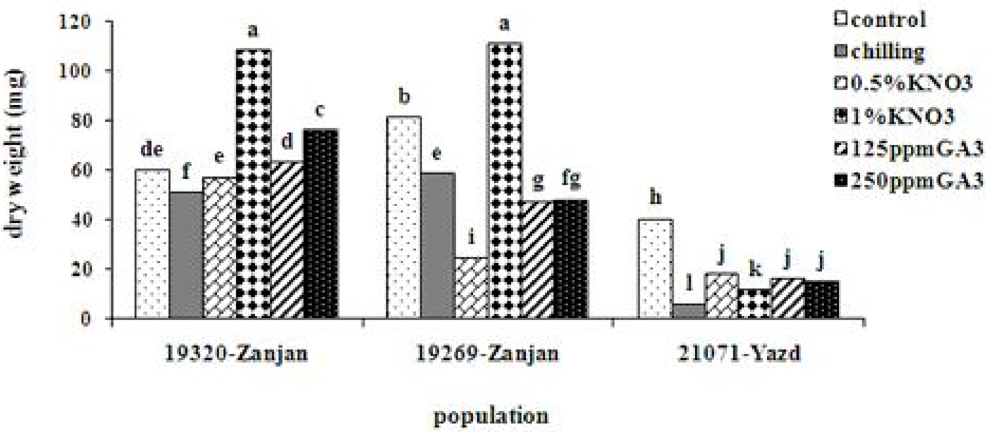


**Figure 7.** Mean comparison of the effect of pre-treatment on dry weight of seedling of three populations of *Anthemis pseudocotula* in greenhouse (Duncan 0.05).

**Seedling leaf area (SLA):**

Mean comparisons of simple effect indicated that the highest and lowest seedling leaf area were obtained for population of 19320-Zanjan and population of 21071-Yazd as 2.06 $cm^2$ and 0.69 $cm^2$, respectively in species of *Anthemis pseudocotula* (Table 2). Higher amount of leaf area of seedling (3.32 $cm^2$) was found from osmoprimed seeds with 125 ppm gibberellic acid in population of 19320-Zanjan compare with other pre-treatments while lower amount of this parameter was found from 0.5 % potassium nitrate (0.38 $cm^2$) pre-treatment in population of 19269-Zanjan (figure 8). Application of gibberellic acid have prompted the metabolic activities due to seedling growth. Seeds which were treated with gibberellic acid have a higher seedling growth and leaf area, moreover it can enhance the photosynthesis. The highest leaf area of seedling (391.18 cm2) was recorded for seed primed with distilled water and 25 ppm gibberellic acid in Oil Pumpkin [18].

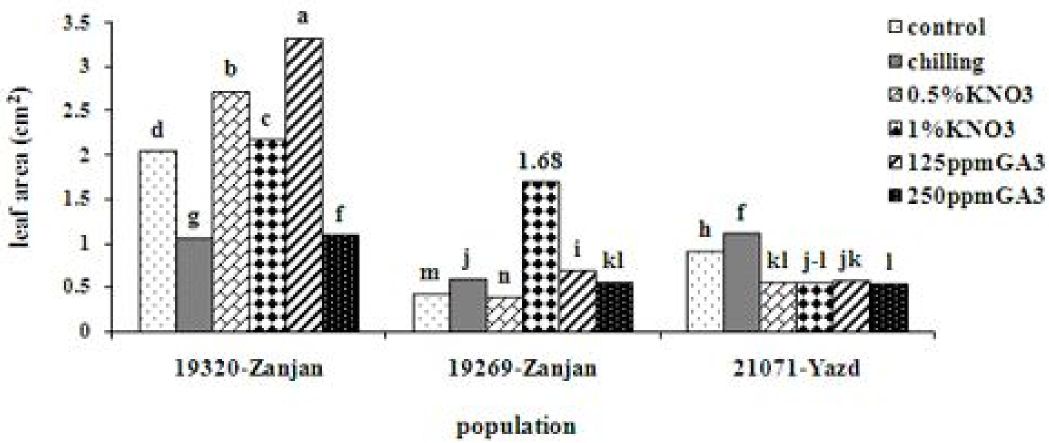


**Figure 8.** Mean comparison of the effect of pre-treatment on leaf area of seedling of three populations of *Anthemis pseudocotula* in greenhouse (Duncan 0.05).

## CONCLUSION

The highest improvement in seed germination and emergence was achieved by priming with 125 and 250 ppm gibberellic acid. Our results indicated that seed emergence characteristics population of 19320-Zanjan was higher than the other two populations (Table 2).

Totally, the results described here revealed that osmo-priming (gibberellic acid) technique improved seed germination and emergence characteristics population of 19320-Zanjan. It was concluded that priming as physiological treatment, enhanced seed germination potential and seedlings vigor population of 19320-Zanjan compare with other population in species of *Anthemis pseudocotula* in greenhouse conditions.

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