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Original Article

Effect of Different Times and KNO3 Concentrations on *Silybum marianum* Seedling Enhancement

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Abstract

Nowadays, the use of medicinal plants has increased and rangelands are the main sources of these plants. Excessive harvesting has resulted in degradation and reduction of diversity. Cultivation or seeding in nature could reduce the process of destruction, but the seed germination capacity of these species is limited. Methods of seed enhancement such as seed priming can improve these problems. In this research, the possibility of germination improvement of *Silybum marianum* (L.) Gaertn. was investigated by using potassium nitrate treatment. Different concentrations of 0, 1, 2 and 3 percent of the solution of KNO_3 were applied for 24 and 48 hours at 25 °C. An experiment was conducted in a factorial arrangement with a completely randomized design with three replications. The germination components (germination percentage, germination rate, seedling, root length, shoot length, seedling length, root and shoot fresh and dry weight) were measured in laboratory conditions (petri dish) and cultivation trays. Also, the leaf area index determined. Results showed significant differences in time interaction and different concentrations of KNO_3 in germination rate, shoot fresh weight, shoot dry weight, seedling dry weight and leaf area index. In the experiments designed in the petri dish, the interaction between time and various concentrations of KNO_3 affected all measured factors except shoot and seedling dry weight ($P<0.01$). The optimum treatment was 1% concentration and 24 hours, which is caused a significant increase compared to control in the studied traits.

Keywords: Priming, Germination, Cultivation, KNO_3, *S. marianum*.

Introduction

For centuries people have used plants for healing. Plant products as parts of foods or botanical potions and powders have been used with varying success to cure and prevent diseases throughout history [1]. Most of the plants are collected from their habitat. The collection of these plants from arid and semi-arid pastures can lead to degradation of the environment. Therefore, in order to reduce and prevent further degradation of these areas, the best way is cultivating and multiplication of medicine plants. One of the most important problems in the cultivation and reproduction process is germination and seeding establishment. This problem is more acute in the case of medicinal plants because of different levels of seed dormancy, germination and seedlings establishment, so the best way to overcome problems is to use seed priming techniques. The seed priming means applying seed treatment before planting, which allows the seeds to absorb water so that the early stages of germination can be done, but do not get out of the root, recently this technique has been used to increasing germination, increasing speed and germination percent [2]. Seed priming is a simple and inexpensive method for plants that cause: increasing of competitive ability, tolerate drought periods, increasing production and reduce the necessary time for growing again [3]. One of the most useful plants in cancer prevention and liver problems is *Silybum marianum*. This 2-year-old plant is an economically important from the Asteraceae family; it is renowned for the

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production of biologically important silymarin [4].Usable parts of this plant are leaves, roots and seeds [5]. It has effective chemical compounds in the treatment of cancer [6]. All parts of the leaf, fruit and seeds contain silymarin [7] and [8]. Fruits rich in silymarins, flavonoids, silibinin and seeds containing betaine, glycine trimethyl and essential fatty acids [9-12]. This plant grows well in a variety of heavy sandy and gray soils [13]. Currently, this plant is heavily pressed and harvested to produce silymarin. Research has been carried out in the field of seeds seeding in medicinal plants that can be mentioned below. By examining the priming of potassium nitrate, zinc sulfate, polyethylene 6000, gibberellic acid *Nigella Sativa* in moisture deficit conditions, the highest proline and germination percent were observed by zinc sulfate and the most soluble protein due to priming with potassium nitrate [14]. Bayat *et al.* [15] reported some the best treatment of seed priming on seedling properties of *Echinacea purpurea* (L.) Moench. Fallah hosseini *et al.* [16] reported that the use of gibberellic acid and osmo priming had a significant effect on the recycling of old seeds of *Matricaria chamomilla* L.

The study of three levels of magnesium priming (0, 5 and 10) and temperature (15, 25 and 35 Celsius) on sesame seeds showed that magnesium can improve germination specifications of this seed and the best temperature is 25 Celsius[17]. Application of priming of KNO_3 concentrations (2.5, 5.0, 7.5 and 10 percent) in 2, 4 and 6 hours compared to hydro priming on American cotton seeds caused a lower increasein germination percent and germination rate of Linseed [18]. Abdollahi *et al.* [19] by studying the effect of salicylic priming on wheat seed found that the use of salicylic acid increased the photosynthetic parameters and reduced the late effects of wheat planting. The effect of salicylic acid and hydro priming on *Vicia dasycarpa* Then seed were tested and increase the emergence and growth of seedlings, proline accumulation, seedling solution sugars of this species were reported [20]. Afzal *et al.* [21] by study of hydro priming, halo priming (CaCl2), salicylic acid, Ascorbate on *Vicia dasycarpa* seed in condition of treated NaCl found that Ascorbate priming, salicylic acid, halo priming and hydropriming can increase the percent of germination, germination rate, root length and shoot and seedling dry weight. Metal nanoparticles priming such as aluminum nanoparticles has a positive effect on the germination percent of seed, root and shoot growth and Biochemical Specifications of *S. marianum* [22,23]. The effect of 24 hour priming of humic acid (2 percent), potassium chloride (2 percent) and Moringa plant of leaf extract on *S. marianum* seed was studied as potted cultivation. The results indicated that the improvement of the performance of germination is contained germination percent, germination rate and seed vigor index [24]. Were investigated the reaction of concentrations of 0, 0.25 and 0.35 millimolar, potassium nitrate and drought stress using sodium chloride (0, 150 and 250 millimolar) on germination indices, seeding protein content and peroxide activity of *S. marianum* seed. The results showed that improvement of the components under treatment at less concentration (0.25 millimolar), potassium nitrate and disproportion of higher concentrations (0.35 millimolar). Marianum seed priming with the pyroxene improves the germination index under drought stress [25]. The use of Ascorbic acid (ASA) increases germination speed and morphological seedlings properties of *Tavernier acuneifolia*.

The present study evaluated the effect of the different concentrations of KNO_3 solution (1, 2 and 3 percent) and different times (24 and 48 hours) on *Silybum marianum* seedling growth enhancement.

Material and Methods

This experiment performed to determine the effect of different concentrations of potassium nitrate (KNO_3) and duration of treatment on germination parameters and leaf area index (LAI) of *S. marianum*. Concentrations of potassium nitrate (KNO_3) were 0, 1, 2, 3% and the duration of the time consists of 24 and 48 hours. The germination components consist germination percentage, germination rate, seedling, root length, shoot length, seedling length, root, shoot and seedling weight (fresh and dry). The experiment performed under laboratory conditions (Petri dish and cultivation tray). The leaf area index component examined only in the cultivation tray. The experiment performed as a factorial test based on a completely randomized design with three replications. The plant seeds prepared and disinfected with 0. 1% Mercuric acid.

The seeds placed in two different times and different concentrations of potassium nitrate and then rinsed with distilled water. Twenty-five of the

treated seeds placed in a petri dish with an 11cm diameter on a filter paper (Whatman filter paper number 42) and added 10 ml of distilled water. Transplant tray (54cm length, 33cm width, diameter of cells 35mm), prepared and sterilized. Transplant tray filled with a culture media (Sterilized by autoclave at 120 ºC for 90 minutes). The composition included 20% vermicompost, 80% of the fertile soil. Two seeds put in 10 cells and covered with fine sand. Petridish and trays of cultures placed in a germinator with photo period duration 16/8 and temperature variation ±1 degree Celsius, for 21 days [26]. After this time, germination parameters and leaf area index measured.

The number of germinated seeds was recorded per day and then germination percent was obtained with the following formula.

Equation 1:

$$RG = \sum_{i=1}^{n} \frac{ni}{n}$$

RG: Germination percentage, ni: number of germinated seeds per day, n: total number of seeds. The rate of Germination (S) was also calculated using the following equation[27].

Equation 2:

$$GS = \sum_{i=1}^{n} \frac{n}{t}$$

GS: The rate of germination, n: number of germinated seeds in time t and t: number of days since the start of the test.

The Root length and shoot length are measured with the ruler and seed vigor index is calculated with using the following equation

Equation 3:

$$SVI = (RL + SL) \times RG$$

RL: Root length, SL: Shoot length, RG: Germination percent and SVI: seed vigor index. Seeding length was obtained from the total Root length and shoot length in millimeters [28].

The leaf area index of seeding in the culturing tray was estimated using the Axio vision SE64 Rel. 4.9.1 software.

Data Analysis

In order to analyze the data used from SAS 9. 1, the analysis of variance (ANOVA) and LSD test were used for statistical analysis and values of $p \leq 0.05$ were considered as significant indicators. The graphs were drawn by excel software.

Discussion

Experiments in Cultivation Tray

The study of the variance analysis table was the effect of duration (24 and 48 hours), the placement of treated seeds KNO_3 showed a significant difference in Germination rate, seeding fresh weight, shoot dry weight, seeding dry weightand leaf area index in 1% level and seed vigor index in 5%. Different concentrations of KNO_3 (1, 2and 3 percent) resulted in a significant difference at 1% level in seed vigor index, Germination rate, shoot fresh weight, seed dry weight, and seed vigor index and affectedseed length and shoot dry weight in 5% level. The reaction of time and different concentrations of KNO_3 on germination rate, shoot fresh weight, shoot dry weight, seed dry weight and leaf area index at 1% level were effective (Table 1).

The reaction of time and various concentrations of KNO_3 indicated a significant in increase in 1% concentration and 24 hours' time compared to control in termsof Germination rate, shoot fresh weight, shoot dry weight, seed dry weight and leaf area index. Increasing the time and concentration of KNO_3 caused a significant reduction in the measured components. In factors of shoot and root dry weight, concentrations of 1 and 2% in the 24 hours' time showed the same effect. The lowest rate in the concentration of 3% and in the time was 48 hours (Fig. 1, 2 and 3). Experiments in petri dish

The result of the analysis of variance of factors germination components under the influence of different KNO_3 times showed a significant difference in all components except shoot and root dry weight at 1% percent level. Also, these 2 components are the only components under different concentrations of KNO_3 and reaction effects of concentration and time did not show significant differences at 1% level (Table 2).

Comparison of mean values showed significant differences in germination rate, shoot length and seed length, root fresh weight, shoot and seeding and root dry weight in 24 hours treatment and concentration 1% comparison with control. Increasing concentration and time of KNO_3 caused a significant decrease in all traits. The concentration of 3% in 48 hours in all components was significantly decreased (Fig. 4, 5, 6, 7 and 8).

Table 1 Analysis of variance (ANOVA) of the *Silybum marianum* (L.) Gaertn. seed germination components in cultivating tray

Change Resources	DF	Germination	Seed vigor index	Germination Rate	Root length (mm)	Shoot Length (mm)	Seedling Length (mm)	Root Fresh Weight (mm)	Shoot Fresh Weight (mg)	Seedling Fresh Weight (mg)	Root Dry Weight (mm)	Shoot Dry Weight (mg)	Seedling Dry Weight (mg)	Leaf Area Index (LAI)
time	1	130. 7 ^{ns}	9873. 9 *	15. 44 **	0. 482 ^{ns}	0. 107 ^{ns}	0. 135 ^{ns}	0. 012 ^{ns}	0. 002 **	0. 007 ^{ns}	0. 0006 ^{ns}	0. 0011**	0. 003 **	50. 30 **
Various concentrations KNO_3	3	392. 4 ^{ns}	38164. 3 **	44. 04 **	0. 314 ^{ns}	0. 314 ^{ns}	0. 821*	0. 011 ^{ns}	0. 0002 **	0. 010 ^{ns}	0. 00006 ^{ns}	0. 00007 *	0. 0002 **	6. 108**
Various concentrations KNO_3 *time	3	172. 0 ^{ns}	5756. 6^{ns}	37. 80 **	0. 063 ^{ns}	0. 281 ^{ns}	0. 303 ^{ns}	^{ns}0. 006	0. 0008**	0. 009 ^{ns}	0. 00007 ^{ns}	0. 0001**	0. 0004 **	6. 407 **
Error	16	127. 5	2043. 1	0. 0001	0. 140	0. 107	4. 027	0. 005	0. 0003	0. 088	0. 00004	0. 0002	0. 0004	0. 327
Coefficient of variation (CV)		12. 98	9. 999	0. 459	14. 14	14. 36	10. 17	12. 63	3. 963	43. 57	14. 25	23. 75	17. 25	5. 710

* : significant difference 0. 05, **: significant difference0. 01, ns: no significant difference

Table 2 Analysis of variance (ANOVA) of *Silybum marianum* (L.) Gaertn. seed germination components in Petri Dish

Change resources	DF	Germination	Seed vigor index	Germination Rate	Root length (mm)	Shoot Length (mm)	Seedling Length (mm)	Root Fresh Weight (mg)	Shoot Fresh Weight (mg)	Seedling Fresh Weight (mg)	Root Dry Weight (mg)	Seedling Dry Weight (mg)	Seedling Dry Weight (mg)
time	1	1066. 7 **	761281. 3 **	272. 9 **	10. 28 **	19. 78 **	58. 58 **	0. 003 **	0. 001 **	0. 009 **	0. 0005 **	0. 003 ^{ns}	0. 002 ^{ns}
Various concentrations KNO_3	3	4902. 8 **	1977290. 6 **	116. 9 **	20. 36 **	26. 20 **	92. 68 **	0. 002 **	0. 002 **	0. 0066 **	0. 0001 **	0. 013 ^{ns}	0. 015 ^{ns}
Various concentrations KNO_3 * time	3	633. 9 **	291149. 9 **	73. 65 **	1. 986 **	2. 665 **	8. 926 **	0. 001 **	0. 0005 **	0. 001 **	0. 00006 **	0. 00004 ^{ns}	0. 0002 ^{ns}
Error	16	119. 8	23846. 4	5. 757	0. 154	2. 468	0. 617	0. 00002	0. 00002	0. 00004	0. 000005	0. 007	0. 007
Coefficient of variation (CV)		19. 60	18. 49	16. 64	6. 814	5. 207	5. 903	3. 834	3. 763	2. 778	5. 543	139. 7	83. 39

* : significant difference 0. 05, **: significant difference0. 01, ns: no significant difference

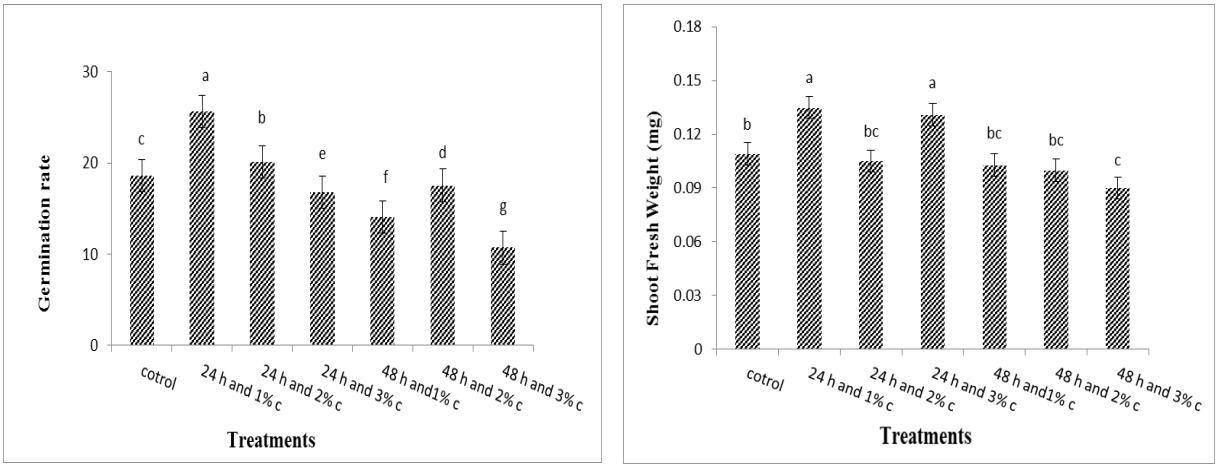

Fig. 1 Comparison of time effect and different concentrations of KNO_3 on germination rate and shoot fresh weight (mg) of *Silybum marianum* (L.) Gaertn. seed in cultivating tray (h: hours and c: concentration)

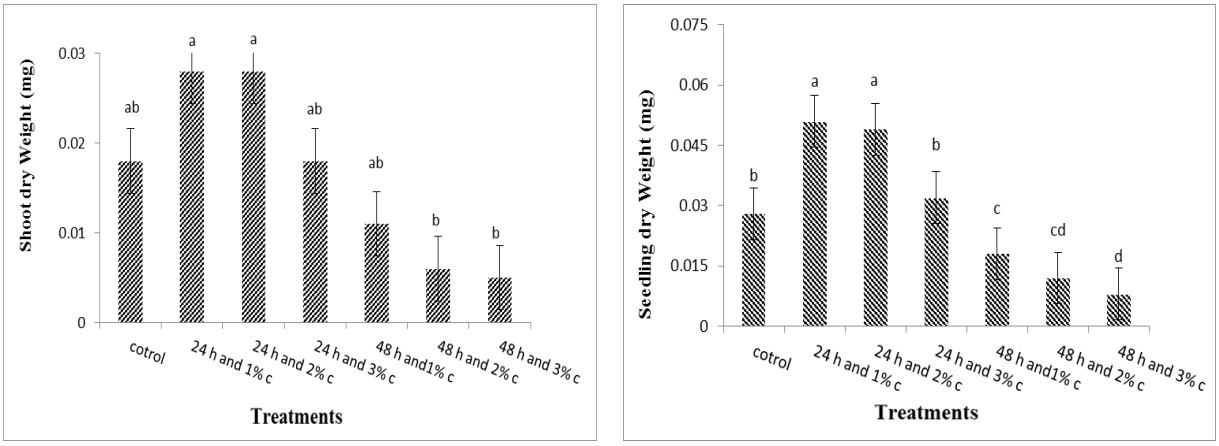

Fig. 2 Comparison of time effect and different concentrations of KNO_3 on seedling and shoot dry weight of *Silybum marianum* (L.) Gaertn. in cultivating tray (h: hours and c: concentration)

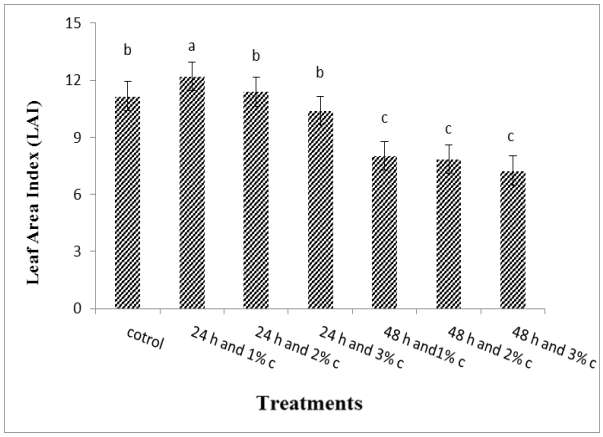

Fig. 3 Comparison of time effects and different concentrations of KNO_3 on Leaf Area Index *Silybum marianum* (L.) Gaertn. seed in cultivating tray (h: hours and c: concentration)

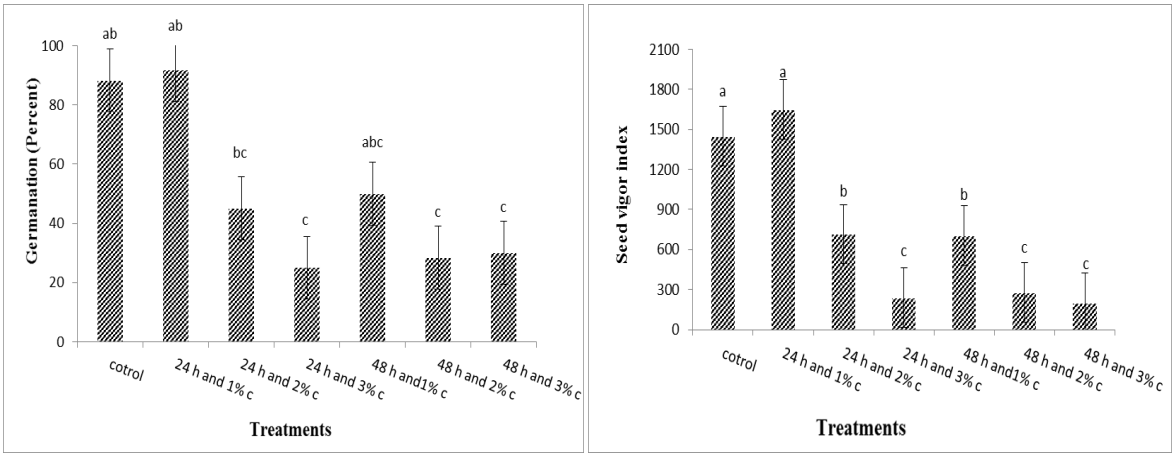

Fig. 4 Comparison of time effect and different concentrations of KNO_3 on germination (percent) and Seed Vigor Index of *Silybum marianum* (L.) Gaertn. seed in petri dish (h: hours and c: concentration)

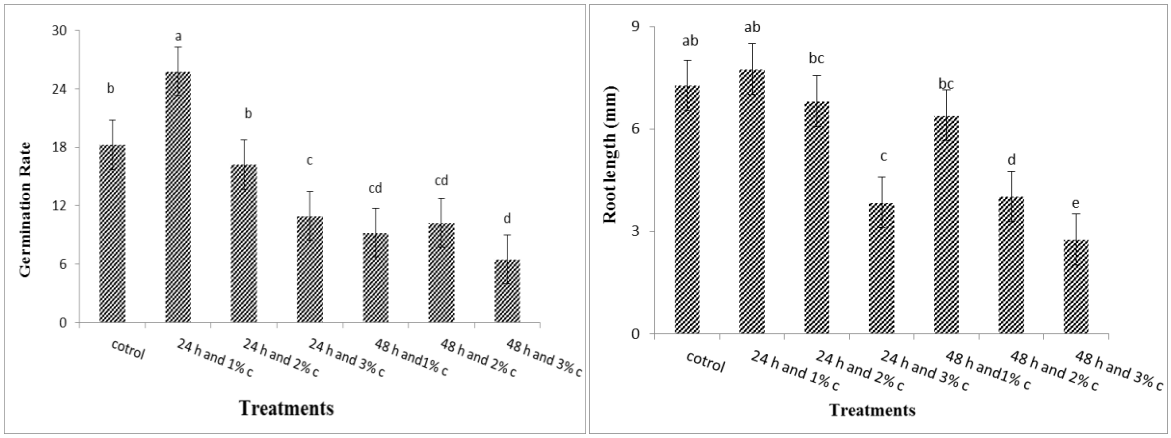

Fig. 5 Comparison of time effect and different concentrations of KNO_3 on germination rate and Root Length (mm) of *Silybum marianum* (L.) Gaertn. seed in the petri dish (h: hours and c: concentration)

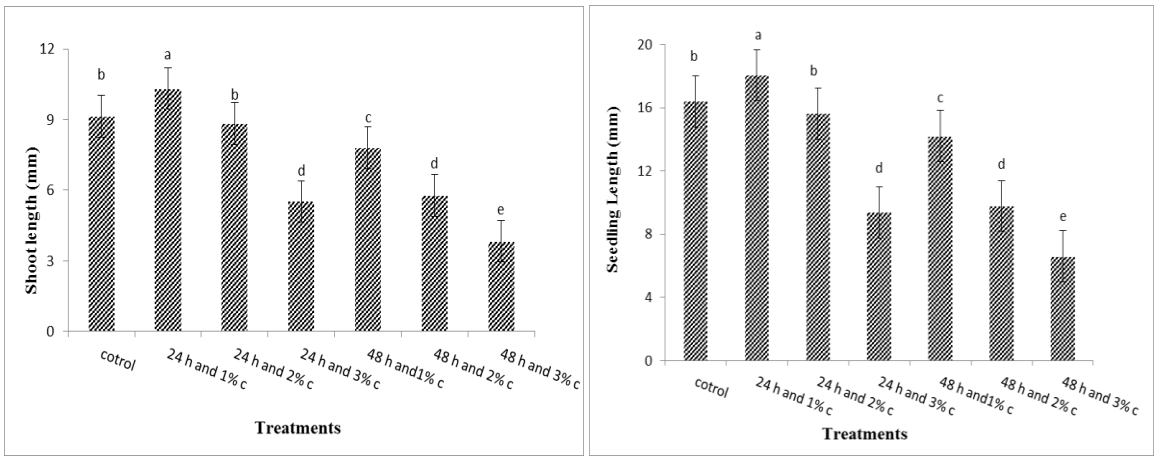

Fig. 6 comparison of time effect and different concentrations of KNO_3 on the shoot and seedling length (mm) of *Silybum marianum* (L.) Gaertn. seed in the petri dish (h: hours and c: concentration)

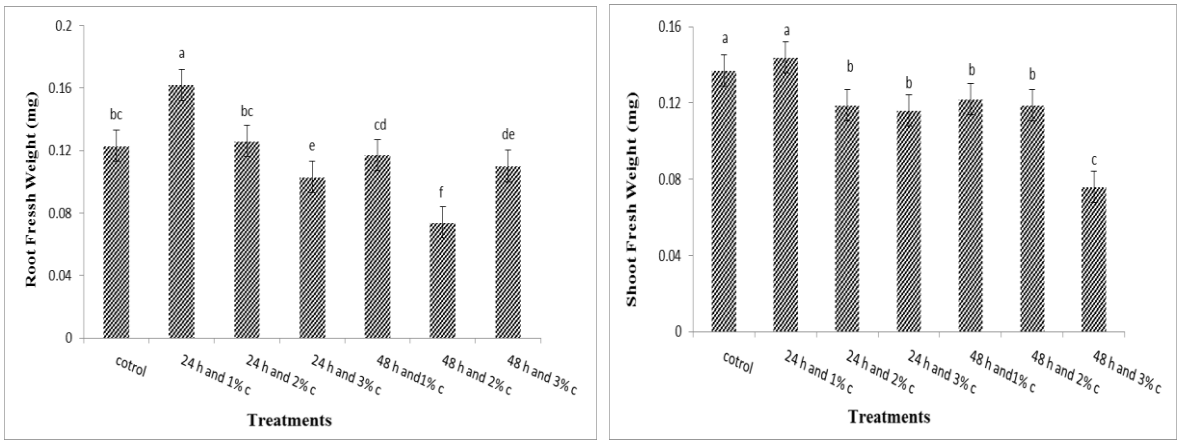

Fig. 7 Comparison of time effect and different concentrations of KNO_3 on root and root fresh weight (mg) of *Silybum marianum* (L.) Gaertn. seed in the petri dish (h: hours and c: concentration)

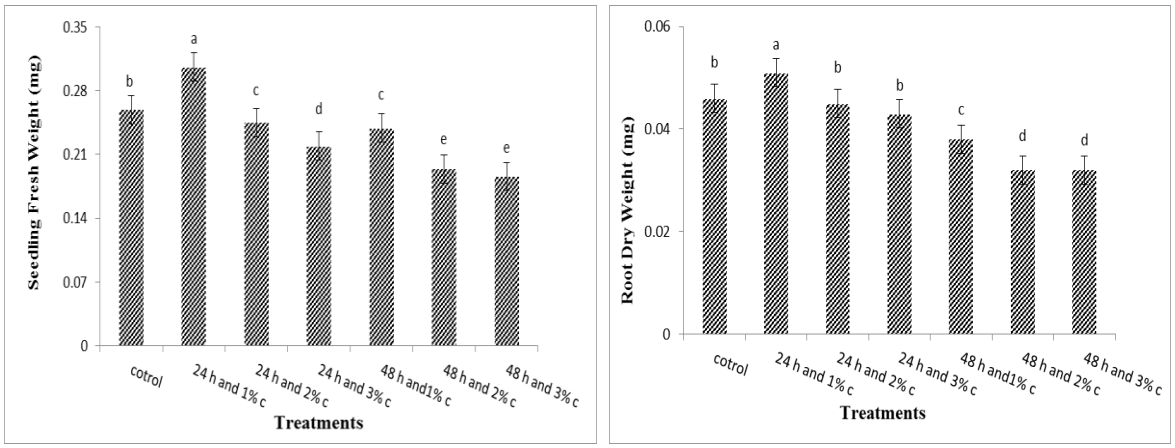

Fig. 8 Comparison of time effect and different concentrations of KNO_3 on seedling fresh weight and root dry weight (mg) of *Silybum marianum* (L.) Gaertn. seed in the petri dish (h: hours and c: concentration)

Reaction of time and different concentrations of KNO_3 on germination rate and leaf area index in cultivation tray and germination rate components, shoot length and seeding, root fresh weight, shoot and seeding and shoot dry weight in petri dishes had highest the rate 24 hours and the concentration of 1%. The most significant reduction was observed in the control at 3% concentration and 48 hours. In all of the studied components, the increase in concentration in this experiment did not decrease and didn't have a positive effect.

Seeds under priming treatment at low and controlled concentrations can achieve the optimal effect on germination components, and higher concentrations may not have a positive effect on priming. for example, we can point to the effects of reaction of concentrations of 0, 0.25 and 0.35 millimolar potassium Nitrate and dry stress using calcium chloride (0, 150 and 250 millimolar) on germination indexes, content of seeding protein and seed peroxidase activity, *S. marianum* that [18] studied and the result of this study showed improvement of the components under treatment with less concentration (0.25 millimolar) of potassium nitrate and disproportion of higher concentrations (0.35 millimolar). According to the results, seed placement time in priming solutions can also be effective on the result. In this study, the more time placement in different concentrations reduced the components that were consistent with [15] considering all the results, it can be concluded that the optimum treatment for seeding priming of *S. marianum* is placement 24 hours with concentrations of 1% KNO_3.

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