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Germination of *Carum copticum* under salinity stress as affected by salicylic acid application

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ABSTRACT

A laboratory experiment was conducted to investigate the effects of seed priming with salicylic acid (SA) on germination characteristics of ajowan (Carum copticum) under salt stress. The experimental design was completely randomized design with four SA concentrations (0, 0.00001, 0.001 and 10 mM), four salinity stress levels (0, 6, 12 and 18 dS.m^{-1}) and three replications. The seeds were soaked in SA solution for 6 hrs, and after drying, were placed in Petri dishes containing 10 ml of NaCl solution. For 21 days the germination traits were monitored and germination percentage and rate, radicle and plumule lengths and seed vigor were measured. The results showed that salt stress inhibited all of the germination characteristics measured. At high salinity (18 dS/m) all germination traits were reduced to near zero even with SA application. Therefore, under such conditions seed priming of ajowan with SA is not recommended. The 10 mM SA concentration significantly inhibited all the germination traits to almost zero; therefore, it is not recommended for seed priming of ajowan. The 0.00001 mM SA concentration significantly improved all germination characteristics of ajowan up to 12 dS.m^{-1} salt stress level. Based on the results of this study, seed priming of ajowan with low concentration of SA (0.00001 mM) is recommended under non-saline and up to 12 dS.m^{-1} salt stress conditions.

Key words: Ajowan, Apiaceae, Plant growth regulators, Plant hormone, Seed priming

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INTRODUCTION

The interest in medicinal plants for treatment of various diseases has grown widely throughout the world. The production of medicinal and herbal plants in nature is low and does not meet the growing demand in the world market. In the other hand, the fertile agricultural lands are utilized mainly for production of food and fiber crops. Marginal lands such as salt affected soils, in where conventional crop production does not produce economic yields, maybe considered for economic production of high valued medicinal and herbal plants [1]. Salinity is a major abiotic stress, especially in arid and semi-arid area of the world. It is estimated that about 77 million ha of the total cultivated land areas of the world are salt affected to various degrees [2]. Salt tolerant medicinal plants offer promising opportunities for regeneration of rangelands and economic utilization of saline soil and water resources. Therefore, study on the salt tolerance of medicinal plants such as ajowan (*Carum copticum*), is necessary for appropriate production management of such plants in saline environments.

Ajowan, a medicinal plant with white flowers and small, brownish seeds belongs to Apiaceae family. It is widely grown in India, Iran and Egypt [3]. The seeds, roots and leaves of ajowan has a wide range of use in traditional medicine throughout the world. The seeds and especially the essential oil are strongly antiseptic, antispasmodic and diuretic [4]. It has been stated that ajowan has good salt tolerance [5].

There are two general methods for improving plant growth in saline soils. They are physical methods such as leaching salts from the soil and using a drainage system, and biological methods which are based on plant physiology to improve salt tolerance [6]. Seed priming with various plant growth hormones, such as salicylic acid, is a common technique for increasing the speed and uniformity of germination and seedling emergence under stress. During priming, the seeds are usually exposed to an external water potential which is sufficient enough to prevent germination, but let biochemical and physiological processes occur before germination within the seed [7].

Salicylic acid (SA) is a naturally occurring plant hormone of phenolic nature that has diverse effects on tolerance to abiotic stresses [8]. Application of SA induced tolerance in plants to many biotic and abiotic stresses including fungi, bacteria and viruses, chilling, salinity, drought and heat [9]. It participates in regulation of physiological process in plants such as stomata closure, ion uptake and transport, inhibition of ethylene biosynthesis, transpiration, stress tolerance, membrane permeability and photosynthesis and growth [8]. Significant reduction of oxidative stress in corn plants under salt stress by external application of 0.15 ppm SA has been reported [10]. In another report, 1.4 mM concentration of SA improved salt tolerance in corn [11]. The effects of SA on physiological processes of plants depend on its concentration, type of plant, the stage of plant growth and environmental conditions; thus, it can have beneficial or inhibitory effects on plant physiological processes.

Extensive areas of agricultural lands in Iran are salt affected to various degrees. Successful germination and establishment of seedlings are essential for economic crop production in these lands. Considering the importance of germination stage for successful production of ajowan in saline agricultural lands of Iran, it is necessary to study the methods for enhancement of seed germination under salinity stress. Therefore, the main purpose of this study was to investigate the effect of different concentrations of salicylic acid on ajowan germination and seedling characteristics under salt stress. The results of this investigation can be helpful in ajowan management and irrigation at planting and germination stage, particularly under salinity stress.

MATERIALS AND METHODS

This experiment was conducted at the Plant Physiology Laboratory of Shahid Chamran University. The experimental design was completely randomized in a factorial arrangement with three replications. Treatments consisted of four salt stress levels of 0 (control), 6, 12 and 18 $dS.m^{-1}$ and four salicylic acid concentrations of 0 (control), 0.00001, 0.001and 10 mM. The pH of salicylic acid and control solutions were adjusted to 6.5.

After disinfection, ajowan seeds were immersed for 6 hours in every solution of salicylic acid and control in the dark at 25 °C. At the end of this period, the seeds were removed and dried. 15 seeds were placed on a filter paper in a clean and sterilized Petri dish, and 10 ml of NaCl solution was added to cover the seeds. Petri dishes were transferred to a germinator set at 25 °C and 60% relative humidity. They were inspected daily and the number of germinated seeds (>2 mm radicle) were counted. After 21 days, the seeds were taken out of the Petri dishes, and their radicle and plumule lengths were measured. Germination percentage (GP), germination rate (GR) and seed vigor index (VI) was also calculated according to the following equations [12].

$$GP = \frac{N_f}{N} \qquad GR = \sum \frac{N_i}{i} \qquad VI = \frac{SL \times GP}{100}$$

N, N_f and N_i are total number of seeds, number of seeds germinated and seeds germinate at the day i, respectively, and SL is radicle length plus plumule length.

The data were statistically analyzed by MSTATC statistical software. The comparison between treatments were performed by Duncan's multiple range test (DMRT) at 5% probability level.

RESULTS AND DISCUSSION

The results of analysis of variance showed that the effects of salicylic acid (SA) concentrations, salinity stress and their interaction on all germination traits measured were highly significant (Table 1).

Table 1- Analysis of Variance test results for the characteristics measured in ajowan experiment

Source of variation	df	Germination percentage	Germination rate†	Radicle length (cm)	Plumule length (cm)	Seed vigor
Replication	3	109.73 ^{ns}	0.13^{ns}	0.02 ^{ns}	0.08 ^{ns}	8.51 ^{ns}
Salicylic acid (A)	3	**10822.75	**90.76	21.85**	**12.45	4050.57**
Salinity stress (B)	3	**8127.44	**62.58	**7.76	**4.46	**1295.53
A×B	9	**1598.96	**12.01	3.11**	1.36**	542.48**
Error	45	92.92	0.50	0.06	0.04	12.73
CV		29.20	24.94	24.38	25.63	27.32

NS: Not significant ***: Significant at 0.01 probability level.*
† Number of seeds germinated per day

Germination percentage

Salt stress significantly reduced germination percentage (GP) of ajowan at all SA concentrations (Figure 1). Reduced GP by salinity is mainly due to reduced water potential gradient between the seed and the surrounding environment, which results in impaired enzyme synthesis that is required for germination [13]. At low salinity level (6 dS dS.m^{-1}) both 0.001 and 0.00001 mM SA concentrations prevented significant GP reduction as compared to the control (Figure 1).

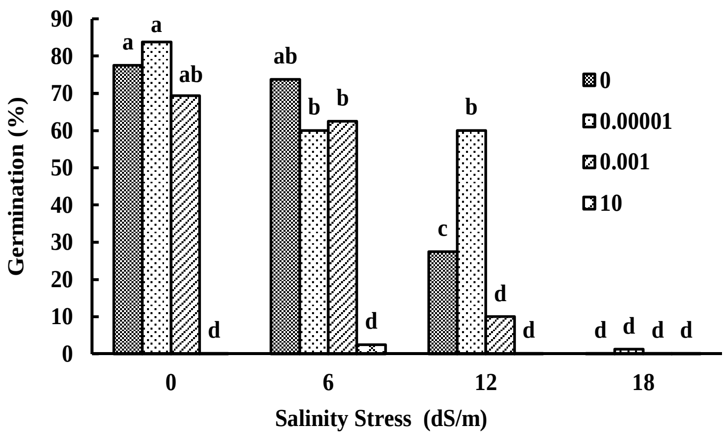

Figure 1- Effects of different concentrations of salicylic acid (mM) on ajowan germination percentage at different salinity stress levels
Columns with at least a letter in common are not significantly different according to Duncan's Multiple Range test at 5% probability level.

At 12 dS dS.m^{-1} salinity stress level, the low SA concentration (0.00001 mM) significantly increased GP as compared to control, and was not significantly different than GP of control at 6 dS dS.m^{-1}. The stimulating effects of SA on GP of fennel and cress has been reported [14, 15]. SA prevents oxidative damages during germination and thus, can improve germination process [16]. The 10 mM SA concentration reduced GP to almost zero at all salinity levels. Although GP of 0.00001 mM SA was highest at high salinity stress level (18 dS dS.m^{-1}), but there was no significant differences among different SA concentrations at this stress level. SA causes an increase in auxin and cytokinin hormones in plants, which may enhance or reduce germination [17]. High concentrations of auxins usually reduce germination, while low concentrations usually enhance it. Therefore, different concentrations of SA by affecting hormone production in plants, will exhibit different effects on germination process of seeds as observed in this experiment.

Germination rate

Germination rate (GR) is one of the most important indicators for evaluation of plant tolerance. The effects of salt stress, SA concentration and their interaction on GR of ajowan were highly significant (Table 1). Increasing salt stress levels significantly reduced GR of ajowan at all SA concentrations (Figure 2).

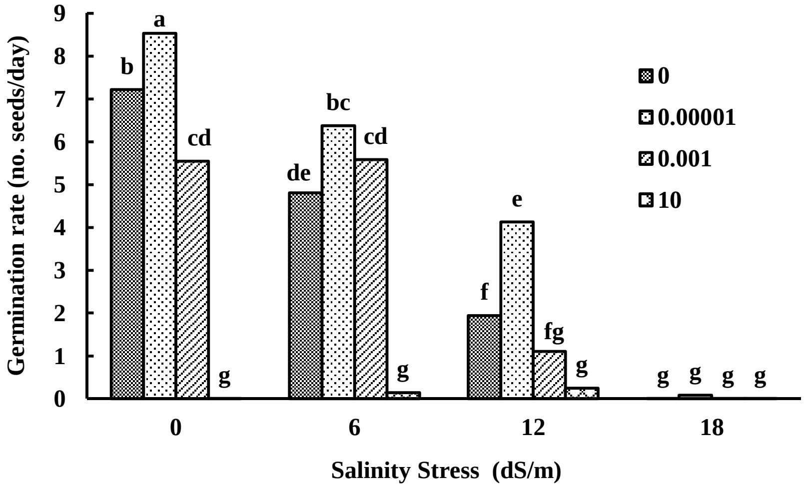

Figure 2- Effects of different concentrations of salicylic acid (mM) on ajowan germination rate at different salinity stress levels
Columns with at least a letter in common are not significantly different according to Duncan's Multiple Range test at 5% probability level.

GR of ajowan at 0.00001 mM SA concentration was significantly higher than control and other SA concentrations at 0 to 12 dS.m^{-1} salinity levels. Although the GR of 0.00001 SA concentration was highest among all treatments at 18 dS.m^{-1} stress level, the differences were not significant (Figure 2). The 10 mM SA concentration reduced GR to almost zero at all salinity levels. GR of canola was increased at 1.5 mM SA and decreased thereafter [18]. Primed sunflower seeds with SA have been reported to show more germination rate under 4 dS.m^{-1} salt stress condition [19].

Radicle length

Increasing salt stress levels significantly reduced radicle length of ajowan at all SA concentrations, while the 10 mM SA concentration reduced radicle length to almost zero at all salinity levels (Figure 3). High salinity stress by reducing water potential or by increasing the concentrations of toxic minerals in plant, can reduce radicle length [6]. Reduction in radicle length have been also observed in thyme and fennel under salinity stress [20, 21]. Under non-saline condition (0 dS.m^{-1}), 0.00001 and 0.001 mM SA concentrations significantly increased ajowan radicle length as compared to the control (Figure 3). But at 6 dS.m^{-1} both were significantly lower than control and there was no significant differences between these two concentrations. At 12 dS.m^{-1} salinity stress level, radicle length of 0.00001 mM SA concentration was significantly higher than control and other concentration levels. Increased radicle lengths of fox berry seedlings with the used of SA at high levels of salinity and drought stresses has also been reported [22].

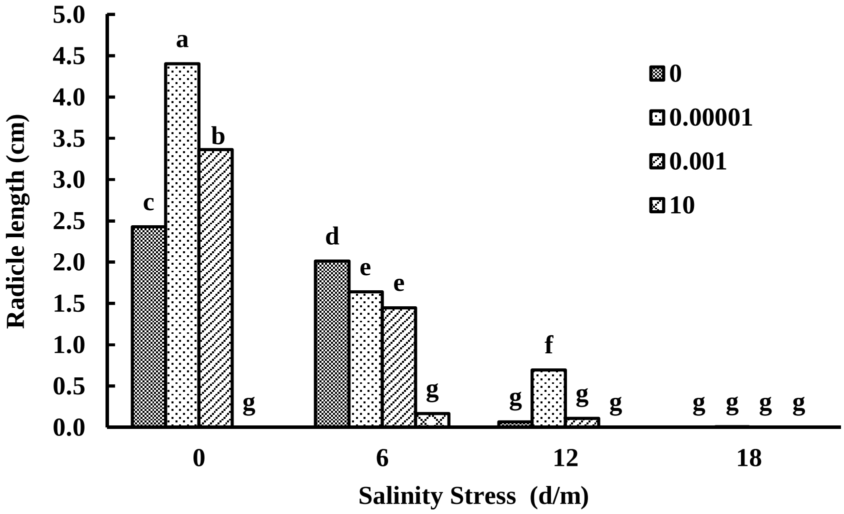

Figure 3- Effects of different concentrations of salicylic acid (mM) on ajowan radicle length at different salinity stress levels
Columns with at least a letter in common are not significantly different according to Duncan's Multiple Range test at 5% probability level.

Plumule length

Increasing salt stress levels significantly reduced plumule length of ajowan at all SA concentrations, while the 10 mM SA concentration reduced plumule length to almost zero at all salinity levels (Figure 4).

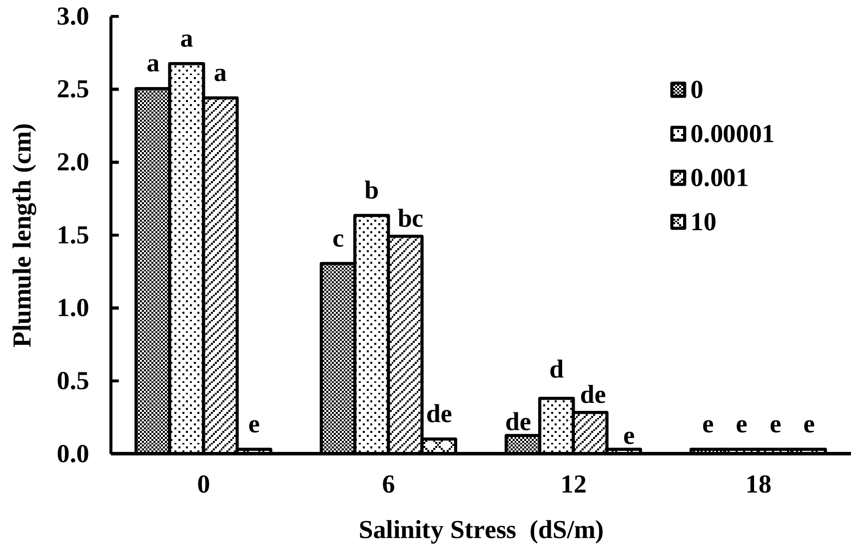

Figure 4- Effects of different concentrations of salicylic acid (mM) on ajowan plumule length at different salinity stress levels
Columns with at least a letter in common are not significantly different according to Duncan's Multiple Range test at 5% probability level.

Plumule length of ajowan in 0.00001 mM SA concentration was higher than all other treatments at non-saline and all salinity stress levels, but it was only at 6 $dS.m^{-1}$ salt stress levels that were significantly higher than the control. Different concentrations of SA on the growth of lentil seedlings have been evaluated, and it was found that only 0.15 mM concentration significantly increased plumule length [23]. It was reported that 1 mM SA concentration significantly increased and higher concentrations decreased fennel plumule length [21].

Seed vigor

Seed Vigor Index (SV) is one of the valuable characteristics for evaluation of seedling tolerance under stress conditions. Thus, the SA treatments with higher SV may be recommended for increasing the salt tolerance at germination stage [24]. Increasing salt stress levels significantly reduced SV of ajowan at all SA concentrations (Figure 5).

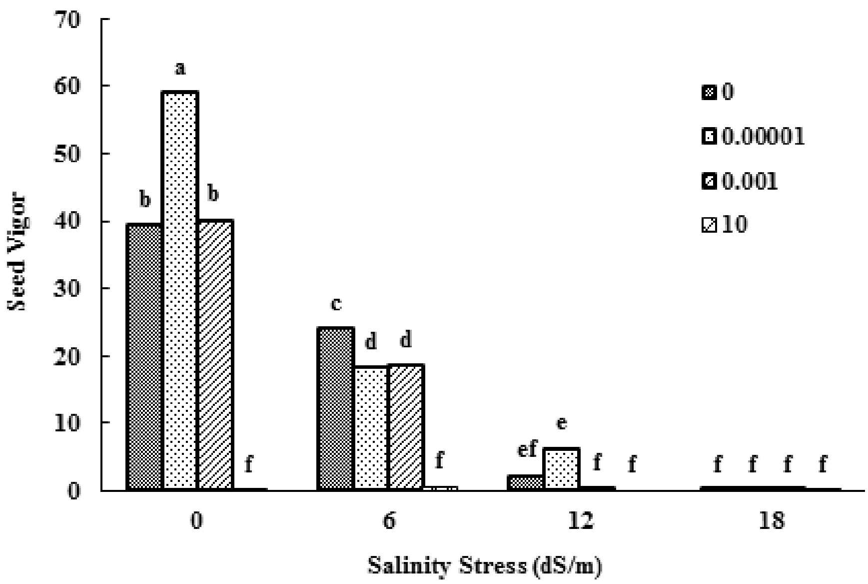

Figure 5- Effects of different concentrations of salicylic acid (mM) on ajowan seed vigor at different salinity stress levels
Columns with at least a letter in common are not significantly different according to Duncan's Multiple Range test at 5% probability level.

These results are consistent with the results of Habibi and Abdoli [14] with cress plant. The 10 mM SA concentration reduced SV to almost zero at all salinity levels (Figure 5). Under non-saline condition (0 $dS.m^{-1}$), 0.00001 mM SA concentration significantly increased ajowan SV as compared to the control and other SA concentrations, but there was no significant difference between control and 0.001 mM concentration (Figure 5). All SA concentrations significantly reduced SV at 6 $dS.m^{-1}$ salt stress level, but there was no significant difference between 0.00001 and 0.001 mM SA concentrations, although SV of ajowan in 0.00001 mM was highest at 12 $dS.m^{-1}$, but it was not significantly different than control. However, it was significantly higher than the other two SA concentrations (Figure 5). SA primed soybean seeds have been reported to exhibit more vigor under salt stress [25]. Ghanbari et al. [24] also reported that 1 and 2 mM SA concentrations significantly increased seed vigor of radish at 4 $dS.m^{-1}$ salinity stress level, but increasing SA concentrations to 3 mM had no significant effect on salinity tolerance of radish.

CONCLUSION

The overall results of this experiment showed the inhibitory effects of salt stress on germination indices of ajowan. However, seed priming with SA improved and enhanced germination traits under various stress levels, thus, reducing some of the inhibitory effects of salt stress. Under high salinity stress conditions (18 $dS.m^{-1}$), none of the SA concentrations enhanced any of the germination traits. Therefore, under such conditions seed priming of ajowan with SA is not recommended.

The 10 mM SA concentration significantly inhibited all the germination traits to almost zero; therefore, it is not recommended for seed priming of ajowan. The 0.00001 mM SA concentration had the most beneficial and positive effects on germination traits of ajowan under salt stress condition. This concentration significantly improved all germination characteristics of ajowan up to 12 $dS.m^{-1}$ salt stress level. Based on the results of this study, seed priming of ajowan with low concentration of SA (0.00001 mM) is recommended under non-saline and up to 12 $dS.m^{-1}$ salt stress conditions. This concentration is very low; therefore, seed priming practices with this level seems to be also an economical and environmentally friendly practice.

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