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The effect of different salt concentrations of salicylic and ascorbic acids on the germination of pot marigold plant (*Calendula officinalis* L.)

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Abstract

Calendula officinalis is one of the important medicinal plants known as "Pot marigold" in the world and has become widespread in recent years due to its medicinal and economic importance. In this study, it was aimed to determine the effects of salicylic acid (SA) and ascorbic acid (AsA) application on germination and some physiological parameters of calendula (*Calendula officinalis* L.) seeds applied different salt (NaCl) concentrations. In the study, 4 different concentrations of NaCl (0, 50, 100 and 150 mM), SA and AsA applications (4 different concentrations (0, 50, 100, and 150 mM) were used for each application. Germination rate (%), germination time in the study (day), root length (cm) and stem length (cm) criteria were examined.

As a result of this research, it was determined that increasing salt concentrations had a negative effect on germination. In addition, positive effects of 50 mM and 100 mM AsA, SA applications on germination and other examined criteria were determined. It has been determined that AsA and SA applications, which will be applied at the optimum level, can have a positive effect on the germination of Calendula plant in saline conditions.

Keywords: *Calendula officinalis*; Salicylic acid (SA); Ascorbic acid (AsA); Salinity

1. Introduction

One of the most important stress factors affecting agricultural production in the world and in our country is soil salinity (Saqip et al. 2011). This effect has increased continuously and has led to a decrease in yield and quality in many products (Oral et al. 2020). It has become a major problem especially in irrigated or newly opened lands in arid and semi-arid climatic regions (Kızılgeçi et al. 2017). The main reason that triggers this situation is unconscious irrigation and evaporation (Oral et al 2020). Especially as a result of the evaporation of water from the soil, salt accumulation is observed in the soil and plant root zone (Mahajan and Tuteja 2005).

Findings regarding the role of salicylic acid in seed germination vary somewhat depending on genotype and experimental conditions. It is stated that salicylic acid is not required for germination under normal conditions, but it promotes germination by reducing oxidative damage at high salinity level (Lee et al. 2010). In addition, it is stated that salicylic acid applications have positive effects on seed germination at high and low temperatures (Korkmaz, 2005; Özdener and Kutbay, 2008; Ekinci et al. 2011). Salicylic acid (SA) is a group of plant phenolics with an aromatic ring, usually bearing a hydroxyl group or its functional derivative. Metabolic bifurcation, which leads to the formation of essential amino acids such as salicylic acid tryptophan, tyrosine and phenylalanine, also plays an important role in the biosynthesis of phytohormones with different effects in plants. Studies on salicylic acid levels of agriculturally important plant species have revealed that this compound may be dispersed in plants anytime and anywhere (Özeker 2005).

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Salicylic acid is a phenolic compound known as phytohormone that contributes to the regulation of growth and development stages such as photosynthesis, respiration, flowering and senescence, especially germination (Rivas-San Vicente and Plasencia, 2011). In addition, SA is a signal molecule that has an important role in plant defense responses against biotic and abiotic stress factors such as extreme temperature, ozone pollution, UV radiation, heavy metals, drought and salinity (Hara et al. 2012; Miura and Tada, 2014; Semida et al. .2017). It has been proven by various studies that the priming application of salicylic acid reduces the effects of salinity on plants (Jini and Joseph, 2017; Anaya et al. 2018). In recent years, it has been suggested that some plant growth regulators such as salicylic acid (SA), polyamines (PA), abscisic acid (ABA) and jasmonic acid (JA) can be used to increase salt tolerance in plants (Yoon et al. 2009; Roychoudhury et al. 2011). ; Singh and Gautam 2013; Sripinyowanich et al. 2013). Salicylic acid is a signal molecule that has an important role in plant defense responses against many pathogens (Snyman and Cronjé 2008). Salicylic acid's usage areas are to provide resistance to adverse conditions such as high and low temperature, heavy metal and frost stress, as well as salinity and drought stress (Baktır 2010; Kumlay and Eryiğit, 2011).

Ascorbic acid, along with other antioxidants, helps to minimize oxidative damage and stabilize membranes by detoxifying H_2O_2 and other AOTs. The application of ascorbic acid to plants under salt stress leads to an increase in the content of ascorbate and glutathione during the germination period (Asada, 1999). Under salt stress, ascorbic acid plays an important role in growth regulation and plant metabolism, increasing the availability of water and nutrients (Torlak 2019). In order to reduce the negative effects of salt stress on plants, the application of non-enzymatic antioxidant molecules such as ascorbic acid is considered as an alternative way (Khan et al. 2006). Ascorbic acid is found in all plants and has been reported to play an important role in reducing the negative effects of salt stress on growth and development in many agricultural plant species (Hamada, 1998). In general, the role of ascorbic acid in curing the negative effects of salt stress in plants is attributed to its activation of some enzymatic reactions (Irfan et al. 2019). Apart from that, it is known that ascorbic acid protects photosynthetic pigments and photosynthetic apparatus from the negative effects of oxidative stress and provides stabilization (Hamada, 1998).

The genus Calendula is represented by approximately 55 species in the world (The Plant List, 2013). *Calendula officinalis* is the most well-known species in the world in terms of medicine and economy. This species is a cultivar of 20-50 cm in length, with bright yellow-orange flowers, grown in many parts of the world, especially in parks and gardens, due to its beautiful appearance. It is a member of the richest family of flowering plants, Asteraceae (Çeçen, 2009).

In this study, it was aimed to determine the effect of salicylic acid and ascorbic acid applications on seed germination of calendula (*Calendula officinalis* L.) seeds applied different salt concentrations.

2. Material and methods

This research was carried out in Balıkesir University Altınoluk Vocational School Medical and Aromatic Plants Laboratory in 2022. Calendula seed of Evve variety was used as plant material. In the study, 4 different salt concentrations (0, 50, 100 and 150 mM) and 2 different priming applications (SA and AsA) and 4 different concentrations (0, 50, 100 and 150 mM) were considered in each priming application. Analytical level NaCl was used to generate salt stress. Before germination, the seeds were surface sterilized in 5% sodium hypochlorite solution for 10 minutes (Uyanık et al., 2014). Surface sterilized seeds were kept in SA and AsA solutions at different concentrations for 12 hours for priming (Nazarian, 2016) and then dried on drying papers at room conditions for 24 hours until they returned to their previous moisture content. 20 seeds were placed in petri dishes containing double-layered filter paper, and were soaked with 10 ml of solution from each of the different salt concentrations.

The data obtained from the experiment were analyzed with the TARIST statistical program according to the factorial experimental design in random plots. LSD test was used to compare the means.

3. Results

3.1. The Effect of Ascorbic Acid on Germination Rate

In the study, it has been shown that the difference between Salt doses, Ascorbic acid doses and Salt x AsA interaction in terms of germination rate of calendula seeds in different concentrations of NaCl solution should be examined at the 5% significance level (Table 1).

When the averages of salt concentrations were examined, the highest germination rate was 91.75% in 0 mM NaCl, and the lowest germination rate was 65.75% in 150 mM NaCl.

When the average of the ascorbic acid doses were examined, the highest germination rate was 88.25% in 100 mM AsA, and the lowest germination rate was 73.25% in 150 mM AsA.

When the salt x AsA interaction was examined, the highest germination rate was determined as 97.00% in the 0 mM x 100 mM interaction, and the lowest germination rate was 59.00% in the 150 mM X 0 mM interaction.

Table 1 Effect of ascorbic acid on seed germination rate (%)*

NaCL /AsA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	83.00 c	94.00 ab	97.00 a	93.00 ab	91.75 a
50 mM	79.00 cd	92.00 b	93.00 ab	88.00 bc	88.00 b
100 mM	72.00 e	90.00 b	90.00 b	76.00 d	82.00 c
150 mM	59.00 h	68.00 ef	73.00 de	63.00 fg	65.75 d
Ortalama	73.25 c	86.00 ab	88.25 a	80.00 b	

LSD salinity: 2,083; LSD AsA:2,391; LSD Interaction: 4,617

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

3.2. The Effect of Salicylic Acid on Germination Rate

In the study, it has been shown that the difference between Salt doses, Salicylic acid doses and Salt x SA interaction should be examined at a 5% significance level in terms of germination rate of calendula seeds in different concentrations of NaCl solution (Table 2).

Table 2 Effect of salicylic acid on seed germination rate (%)*

NaCL /SA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	84.00 bc	90.00 ab	93.00 a	73.00 ef	85.00 a
50 mM	82.00 cd	87.00 b	87.00 b	67.00 f	80.75 b
100 mM	76.00 de	83.00 c	79.00 d	58.00 gh	74.00 c
150 mM	54.00 gh	57.00 h	62.00 g	51.00 ı	56.00 d
Ortalama	74.00 b	79.25 a	80.25 a	62.25 c	

LSD $_{salinity}$: 3,267; LSD $_{SA}$:1,182; LSD $_{Interaction}$.: 3,543

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

When the averages of salt concentrations were examined, the highest germination rate was found at 85.00% with 0 mM NaCl, and the lowest germination rate at 56.00% with 150 mM NaCl.

When the average of salicylic acid doses were examined, the highest germination rate was 80.25% in 100 mM SA, and the lowest germination rate was 62.25% in 150 mM SA.

When the salt x SA interaction was examined, the highest germination rate was determined as 93.00% in the 0 mM x 100 mM interaction, and the lowest germination rate was 51.00% in the 150 mM x 150 mM interaction.

3.3. Effect of Ascorbic Acid on Germination Time

In the study, it has been shown that the difference between Salt doses, Ascorbic acid doses and Salt x AsA interaction in terms of germination time of calendula seeds in different concentrations of NaCl solution should be examined at the 5% significance level (Table 3).

When the average salt concentrations were examined, the highest germination time was found at 0 mM NaCl with 4.66 days, and the lowest germination time was found at 150 mM NaCl with 6.04 days.

When the average of ascorbic acid doses were examined, the earliest germination time was 5.22 days in 50 mM AsA, and the latest germination time was 5.72 days in 0 mM AsA.

When the salt x AsA interaction was examined, the earliest germination time was determined as 4.08 days in the 0 mM x 100 mM interaction, and the latest germination time was determined as 6.20 days in the 150 mM x 0 mM interaction.

Table 3 Effect of ascorbic acid on seed germination time (days)*

NaCL /AsA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	5.03 ef	4.26 g	4.08 h	5.27 de	4.66 d
50 mM	5.58 cd	5.09 ef	5.32 d	5.16 e	5.29 c
100 mM	6.06 ab	5.55 cd	5.83 c	5.98 b	5.86 b
150 mM	6.20 a	5.98 b	5.94 bc	6.02 ab	6.04 a
Ortalama	5.72 a	5.22 c	5.29 c	5.61 ab	

LSD $_{salinity}$: 0,128; LSD $_{AsA}$:0,235; LSD $_{Interaction}$.: 0,159

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

3.4. Effect of Salicylic Acid on Germination Time

In the study, it has been shown that the difference between Salt doses, Salicylic acid doses and Salt x SA interaction in terms of germination time of calendula seeds in different concentrations of NaCl solution should be examined at a 5% significance level (Table 4).

When the averages of salt concentrations were examined, the earliest germination time was 5.12 days at 0 mM NaCl, and the latest germination time was 6.31 days at 150 mM NaCl.

When the average of salicylic acid doses were examined, the earliest germination time was determined as 5.50 days at 50 mM SA, and the latest germination days were determined as 6.11 days at 150 mM SA.

When the salt x SA interaction was examined, the earliest germination time was determined as 4.86 days in the 0 mM x 50 mM interaction, and the latest germination time was 6.56 days in the 150 mM x 150 mM interaction.

Table 4 Effect of salicylic acid on seed germination time (days)*

NaCL /SA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	5.14 f	4.86 ı	4.93 h	5.56 de	5.12 d
50 mM	5.72 cd	5.08 gh	5.64 d	6.03 bc	5.62 c
100 mM	6.18 b	5.84 c	6.03 bc	6.28 b	6.08 b
150 mM	6.32 ab	6.23 b	6.13 bc	6.56 a	6.31 a
Ortalama	5.84 b	5.50 c	5.68 b	6.11 a	

LSD $_{salinity}$: 0,191; LSD $_{SA}$:0,263; LSD $_{Interaction}$: 0,173

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

3.5. The Effect of Ascorbic Acid on Root Length

In the study, it has been shown that the difference between Salt doses, Ascorbic acid doses and Salt x AsA interaction in terms of root length of calendula seeds in different concentrations of NaCl solution should be examined at a 5% significance level (Table 5).

When the average salt concentrations were examined, the highest root length was found with 2.19 cm in 0 mM NaCl, and the lowest root length was found at 1.17 cm in 150 mM NaCl.

When the average of ascorbic acid doses were examined, the highest root length was determined as 2.08 cm in 100 mM AsA, and the lowest root length was 1.43 cm in 0 mM AsA.

When the salt x AsA interaction was examined, the highest root length was determined as 2.56 cm in the 0 mM x 100 mM interaction, and the lowest root length was 0.87 cm in the 150 mM X 0 mM interaction.

Table 5 Effect of ascorbic acid on root length (cm)*

NaCL /AsA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	1.88 c	2.21 b	2.56 a	2.12 bc	2.19 a
50 mM	1.73 d	2.08 bc	2.23 b	1.81 cd	1.96 ab
100 mM	1.24 gh	1.78 d	1.97 c	1.42 ef	1.60 b
150 mM	0.87 i	1.36 efg	1.53 e	0.91 ıi	1.17 c
Ortalama	1.43 d	1.86 b	2.08 a	1.57 c	

LSD $_{salinity}$: 0,262; LSD $_{AsA}$:0,127; LSD $_{Interaction}$: 0,292

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

3.6. Effect of Salicylic Acid on Root Length

In the study, it has been shown that the difference between Salt doses, Salicylic acid doses and Salt x SA interaction in terms of root length of calendula seeds in different concentrations of NaCl solution should be examined at 5% significance level (Table 6).

When the average salt concentrations were examined, the highest root length was found with 1.91 cm in 0 mM NaCl, and the lowest root length was found at 0.94 cm in 150 mM NaCl.

When the average of salicylic acid doses were examined, the highest root length was 1.74 cm at 100 mM SA, and the lowest root length was 1.20 cm at 0 mM SA.

When the salt x SA interaction was examined, the highest root length was determined as 2.08 cm in the 0 mM x 100 mM interaction, and the lowest root length was 0.40 cm in the 150 mM X 0 mM interaction.

Table 6 Effect of salicylic acid on root length (cm)*

NaCL /SA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	1.74 d	2.00 b	2.08 a	1.83 c	1.91 a
50 mM	1.71 de	1.84 c	2.02 ab	1.76 cd	1.83 ab
100 mM	0.96 ı	1.36 f	1.63 e	1.55 ef	1.38 c
150 mM	0.40 i	1.07 h	1.24 g	1.03 hı	0.94 d
Ortalama	1.20 c	1.57 b	1.74 a	1.54 b	

LSD $_{salinity}$: 0,133; LSD $_{SA}$:0,148; LSD $_{Interaction}$: 0,063

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

3.7. The Effect of Ascorbic Acid on Length

In the study, it has been shown that the difference between Salt doses, Ascorbic acid doses and Salt x AsA interaction in terms of stem length of calendula seeds in different concentrations of NaCl solution should be examined at a 5% significance level (Table 7).

Table 7 Effect of ascorbic acid on stem length (cm)*

NaCL /AsA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	1.22 d	1.56 b	1.72 a	1.43 bc	1.48 a
50 mM	1.03 def	1.30 cd	1.44 b	1.09 de	1.22 b
100 mM	0.76 fg	1.08 de	1.13 d	0.81 fg	0.95 c
150 mM	0.42 ı	0.76 fg	0.63 h	0.55 h	0.59 d
Ortalama	0.86 c	1.18 ab	1.23 a	0.97 b	

LSD $_{salinity}$: 0,163; LSD $_{AsA}$:0,046; LSD $_{Interaction}$: 0,138

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

When the averages of salt concentrations were examined, the highest stem length was 1.48 cm in 0 mM NaCl, and the lowest stem length was 0.59 cm in 150 mM NaCl.

When the average of ascorbic acid doses were examined, the highest stem length was 1.23 cm in 100 mM AsA, and the lowest stem length was 0.86 cm in 0 mM AsA.

When the salt x AsA interaction was examined, the highest stem length was determined as 1.72 cm in the 0 mM x 100 mM interaction, and the lowest stem length was 0.42 cm in the 150 mM x 0 mM interaction.

3.8. The Effect of Salicylic Acid on Stem Length

In the study, it has been shown that the difference between salt doses, Salicylic acid doses and Salt x SA interaction should be examined at the 5% significance level in terms of stem length in NaCl solution with different concentrations of calendula seeds (Table 8).

When the averages of salt concentrations were examined, the highest stem length was 1.78 cm in 0 mM NaCl, and the lowest stem length was 0.92 cm in 150 mM NaCl.

When the average of salicylic acid doses were examined, the highest stem length was found to be 1.60 cm at 100 mM SA, and the lowest stem length was 1.07 cm at 0 mM SA.

When the salt x SA interaction was examined, the highest stem length was determined as 1.92 cm in the 0 mM x 100 mM interaction, and the lowest stem length was 0.58 cm in the 150 mM x 0 mM interaction.

Table 8 Effect of salicylic acid on stem length (cm)*

NaCL /SA	**0 mM**	**50 mM**	**100 mM**	**150 mM**	**Ortalama**
0 mM	1.64 bc	1.84 ab	1.92 a	1.71 b	1.78 a
50 mM	1.24 ef	1.76 b	1.85 ab	1.47 de	1.58 b
100 mM	0.83 hı	1.24 ef	1.53 d	1.33 e	1.23 c
150 mM	0.58 i	0.96 h	1.11 g	1.02 gh	0.92 d
Ortalama	1.07 d	1.45 b	1.60 a	1.38 c	

LSD $_{salinity}$: 0,148; LSD $_{SA}$:0,092; LSD $_{Interaction}$: 0,083

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

4. Discussion

In addition to the regulation of stress physiology in plants, ascorbic acid (AsA) has an important effect during the germination period (Arrigoni et al. 1997; Noctor and Foyer, 1998; Conklin, 2001). Afzal et al. (2006); Bassuony et al. 2008; Mohsen et al. 2013; Erkoyuncu and Yorgancılar (2020) reported in their research that AsA has a beneficial effect in various development stages of plants grown in salty environments at appropriate concentrations. It shows parallelism with the results of the criteria examined in this study.

In this study, the negative effects of increasing SA concentrations on the criteria examined in parallel with the rising salt concentration were determined. Farahbakhsh (2012); Jam et al. (2012); Soliman et al. (2016) in their research, it is possible to increase the performance of plants against salt stress with the determined optimum salicylic acid applications. Ramanujam et al. (1998), Mendoza et al. (2002), Tari et al. (2002) El-Tayeb (2005) and Erkoyuncu and Yorgancılar (2020) found that low concentrations of salicylic acid promote flowering and positively affect vegetative growth. Salicylic acid increases the germination of seeds by reducing oxidative damage in high salt stress (Lee et al. 2010). The results obtained in this study are in agreement with the literature.

5. Conclusion

As a result of this research, it was determined that increasing salt concentrations had a negative effect on germination. In addition, positive effects of 50 mM and 100 mM AsA, SA applications on germination and other examined criteria were determined. It has been determined that AsA and SA applications, which will be applied at the optimum level, can have a positive effect on the germination of Calendula plant in saline conditions.

Compliance with ethical standards

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Disclosure of conflict of interest

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