

RESEARCH PAPER

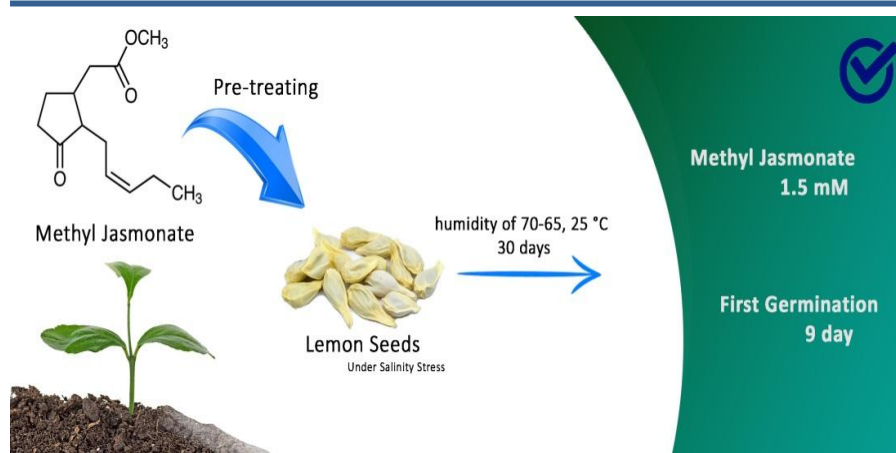
The effect of methyl jasmonate on the germination of lemon seeds under the influence of salinity stress

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Highlights

- The effect of methyl jasmonate on lemon seed germination was investigated under salinity conditions.
- This test was performed in the laboratory and factorially.
- Germination energy of primed seeds with a concentration of 1.5 mM methyl jasmonate had the highest value in the absence of stress.

Graphical Abstract



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Abstract

In order to find the best treatments to accelerate the germination of lemon seeds, a factorial study with 15 in 3 replications was conducted in a completely randomized design. In order to find superior treatments to accelerate the germination of lemon seeds, research was conducted in the Qasr Shirin Azad University of Kermanshah in 2016. Environmental stresses such as salinity reduce germination percentage and speed, reduce root and stem growth, seedling weakness and as a result non-uniformity of field cover and yield loss. The first factor includes different concentrations of methyl jasmonate (zero, 0.5 and 1.5 mM) and salicylic acid (zero, 1 and 2 mM) and the second factor includes different concentrations of sodium chloride (zero, 5 and 10 mM). Des Siemens per meter). After pre-treating the seeds with the priming solutions mentioned above, the seeds are carefully placed in petri dishes with dimensions of 9 × 10 cm in a germinator with a temperature of 25 °C and relative humidity of 65-70 for 30 days. Was placed. For each of the petri dishes, 10 ml of sodium chloride with concentrations of zero, 5 and 10 dS was used. In all experiments, the percentage of germination, the number of days required for germination, the germination of the first seed, the maximum value, the average daily germination and the germination value were calculated. Based on the results, primed seeds with a concentration of 1.5 mM methyl jasmonate that was in normal condition and also primed seeds had the highest germination percentage under salinity stress. The first germination is observed on the ninth day of the experiment. The germination energy of primed seeds with a concentration of 1.5 mM methyl jasmonate was normal under normal and stress-free conditions.

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1. Introduction

Citrus is one of the most important horticultural products that is in the second place in the world in terms of production and every year the area under cultivation and their production in the world is increasing. The amount of citrus yield per unit area depends on various causes and factors such as climate, soil type, crop care and its compatibility with the place of cultivation. Each of these factors has a significant impact on the yield and quality of citrus. Stress is the result of abnormal process of physiological processes and is caused by the influence of one or the effect of biological and environmental factors. In other words, stress is the organism being severely affected by an environmental factor that causes a decrease in its appearance, efficiency or value (Mona et al., 2017).

Plant growth and yield in many parts of the world are limited by numerous living and non-living environmental stresses, and among non-living stresses, salinity stress has caused extensive damage to plants worldwide (Arteca, 2013). High soil salinity is one of the factors limiting crop yields around the world, which is one of the main problems in the agricultural sector, especially in arid and semi-arid regions (Ayers and Hayward, 1948). Meanwhile, Iran, with its hot and dry climate, is no exception. More than half of its arable land (about 27 million hectares) is composed of saline and sodium soils. Therefore, in order to make optimal use of these lands and saline water resources, increasing the salinity tolerance of plants with higher production capacity is an important corrective approach (Khan, 1975). The adverse effects of salinity stress are not only on one plant growth stage but also can be different according to the intensity of stress, type of stress, plant resistance, different growth stages, type of plant tissue and organs. Jasmonates play a regulatory role in growth and response to environmental stresses. In this regard, jasmonic acid is used as a gene encoder of inhibitory proteins such as threonine, smutinin, hydroxyproline and proline proteins, as well as enzymes involved in flavonoid biosynthesis (Chavan, 2014; Vick and Zimmerman, 1984).

2. Materials and Methods

2.1. Environmental factors affecting citrus growth and production

Citrus trees are grown in a wide climatic range between latitudes 40 degrees north and south of the equator, but the main commercial citrus production areas appear to be tropical areas above 20 degrees north to south (Matthews and Ghanem, 2021). In general, the most suitable temperature for citrus growth is between 30-32 °C and plant growth is severely reduced at temperatures below 12 and above 40 °C. Soil temperature less than 15 °C limits water uptake and more than 32 °C reduces root growth. Suitable soil for citrus cultivation should be sandy loam and completely permeable to water and should not contain growth-limiting factors such as high concentrations of salts and toxic elements.

The best soil acidity for citrus is between 5.5-5.5. Citrus fruits are sensitive to salinity and up to 1.7 mM, no reduction in yield is observed. In salinity of 3.3 mM (milli molar), the salinity of citrus is reduced by 25%, in salinity of 4.8 mM by 50% and salinity of 8 mM by 100%. Citrus fruits need a lot of water because they are evergreen and due to the climatic conditions of the cultivated areas. In citrus, the sap never stops moving, and in addition, transpiration occurs most of the year. In areas with an annual rainfall of about 1,200 mm, the plant does not need irrigation as long as it is regularly distributed in all seasons (Vanderklift et al., 2020).

2.2. Environmental factors affecting seed germination

In order for the seed to germinate, the internal factors of the seed and the environmental conditions must be suitable. Internal factors include: seed viability, having adequate nutrient stores until the newly germinated plant is able to provide the necessary nutrients through photosynthesis and can use seed storage materials. Usually, the higher the amount of seed storage material, the higher the seedling growth. Wrinkled, small and immature seeds usually do not have enough nutrients (Zahra et al., 2020).

2.3. Seed germination

Germination is defined as the emergence of seedlings from seed and the initiation of a variety of anabolic and catabolic activities, including respiration, protein synthesis, and the movement of stored nutrients after

water uptake. According to the definition of seed breakers, germination is the emergence and development of essential embryonic structures that show the ability of seeds to produce natural plants under favorable conditions in nature (Huber et al., 1996; Yang et al., 2021).

2.4. Methyl jasmonat

Methyl jasmonate is widely used in plants, invertebrates and algae as a plant growth regulator and causes many reactions in plants (Schaller et al., 2004). Jasmonic acid and methyl jasmonate belong to the group of jasmonates, these substances are directly and indirectly involved in plant reactions to adverse conditions. Internal jasmonates are hormonal substances that promote aging, but external jasmonates act as a stress reliever. Methyl jasmonate increases plant resistance to physical and chemical damage. Methyl jasmonate-treated tomatoes and peppers increase the transcription of some stress proteins that increase cold tolerance and decay stability. Jasmonate disrupts photosynthesis by increasing the level of abscisic acid in the leaves and eventually reducing the enzyme Rubisco and chlorophyll, resulting in ROS activity and lipid peroxidation. Due to the use of jasmonic acid on peanuts, it has been seen that the level of malondialdehyde in the roots and leaves of this plant has increased. Also, the external use of jasmonic acid in pickled potatoes stimulates tuberculosis in this plant 2000 (Koshioka et al., 1998).

2.5. Seed stress and seed germination

Decreased ambient water potential and ion toxicity are the most important factors that affect germination in the saline environment (Xue et al., 2021). Water is the most important factor in initiating the processes related to germination and early survival of seedlings after emergence (Ni and Bradford, 1993). Since the water potential depends on the presence of salts, under high salinity conditions, the accumulation of salts causes a more negative osmotic potential of the soil and leads to a reduction in water inflow into the seed, during which germination is impaired (Santo et al., 2017). The presence of water is essential for hydrolysis processes, and during these processes, seed storage materials, including fats, proteins, and carbohydrates, are converted into simpler materials and transferred to the embryonic axis for use (Kaur et al., 2000; Seneviratne et al., 2019). Reduction of water potential in salinity conditions leads to increased production of abscisic acid. Abscisic acid is a natural antagonist of gibberellins (Seneviratne et al., 2019), and is able to prevent their effects by inhibiting the activity of enzymes involved in the biosynthesis of gibberellins (Partheeban et al., 2017).

Gibberellins play the most important role in controlling and accelerating germination processes (Seneviratne et al., 2019). High levels of abscisic acid in seeds increase the sensitivity of seeds to reduced water potential and this also reduces seed vigor in germination (Malabarba et al., 2021). Decreased seed respiration can also be one of the adverse effects of salinity on seed germination. Since increased respiration is the most important metabolic change since the onset of seed germination processes (Seneviratne et al., 2019), the presence of high concentrations of ions due to high salinity damages and reduces the electron transfer system on mitochondrial membranes. 1984). Increased seed respiration after the dewatering phase provides the energy needed for germination-related activities (Malabarba et al., 2021).

3. Results and Discussion

This experiment was performed to investigate the germination characteristics of lemongrass seeds in November 2016 in the horticultural laboratory of Islamic Azad University, Qasr Shirin branch. After preparing the sweet fruit from the gardeners of Shiraz region, the seeds were carefully separated so that no damage was done to the seeds, and washed to remove the remnants of the flesh of the fruits. In all experiments, seeds that appeared to be uniform in size and growth strength were selected to be more accurate and to minimize error as much as possible. Before performing the experiment, the tetrazolium test with a concentration of 0.1% was used for 2 hours to determine the seed's beauty.

The seeds were disinfected before treatment with 3% sodium hypochlorite solution and after rinsing with distilled water; they were superficially dried on paper and then dried at room temperature ($\pm 25 \pm 2^\circ\text{C}$) for 24 hours. They were completely dried. In order to increase the germination percentage of seeds, two factorial experiments were performed in a completely randomized design with 3 replications. In the first experiment; the first factor was different concentrations of methyl jasmonate (0.5 and 1.5 mM) and the second factor was different concentrations of sodium chloride at three levels (zero, 5 and 10 dS/m). In the second experiment, the first factor of different concentrations of methyl jasmonate (0.5 and 1.5 mM) control treatment without the use of the solution and the use of distilled water was considered as zero concentration. In order to test the germination of the treated seeds, the seeds were placed in petri dishes (30 seeds per petri dish) containing a filter paper. In the second test, 10 ml of the appropriate polyethylene glycol solutions were added. To create drought stress levels, 6000 polyethylene glycol according to Mitchell and Kaufman (1973) formula was used as follows:

$$S = - (1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T$$

Where C is the concentration of polyethylene glycol (in grams per liter), T is the temperature (in degrees Celsius) and S is the osmotic potential (in terms of charge) (Lanteri et al., 1993). In order to avoid the negative effects of water evaporation, the amount of evaporated water was determined by weighing each petri dish and was compensated through distilled water. The cultures were kept at $20 \pm 5^\circ\text{C}$ in complete darkness. Germination in this experiment was considered as root and stem outlet at least 5 mm. After sowing the seeds, the number of germinated seeds per experimental unit was counted daily to estimate the germination rate in each experimental unit, and this was done until there was a change in the number of germinated seeds in each experimental unit for three consecutive days not found.

After performing the experiment and measuring the traits in the laboratory, the germinated seeds related to each treatment were transferred to the pot and after the plants reached the 6-8 leaf stage, the following traits were evaluated. In the greenhouse, no salinity stress was applied. After 30 days and in the last days of the experiment, from the soil surface to the end of the plant length was measured in centimeters based on the length of the main stem. Simultaneously with measuring the shoot length, the root length was evaluated by a ruler in centimeters. On the last day of the experiment, all grown seedlings related to each treatment were used to evaluate the proline content. The seedlings were wrapped in aluminum foil and placed in a flask containing liquid nitrogen to deliver a heat shock and stop all plant activity at the same time. The samples were then transferred to the laboratory in liquid nitrogen and kept at -20°C until measurement. The content of free proline in seedlings was determined based on the method of Beitz et al. (1973) as follows:

Prepare solution Standard samples: To have a stock of 100 ppm of proline, dissolve 0.01 g of proline in distilled water and make 100 ml with distilled water. Then standards of 8, 16, 24, 32 and 40 ppm of these solutions were made. Distilled water was used to prepare the standard ppm 0 and the control sample. **Ninhydrin solution:** Dissolve 1.25 g of ninhydrin acid powder in 30 ml of glacial acetic acid and add 20 ml of 6 M phosphoric acid. 0.1 g of seedling samples were completely pulverized in liquid mortar in a porcelain mortar and 10 ml of 3% sulfosalicylic acid was added to them and the samples were also crushed in acid (acid is added step by step). The prepared samples were poured into 15 ml Falcon, and then centrifuged at 4°C for 15 minutes to separate the excess material from the solution. The amount of 2 ml of the sample extract and at the same time 2 ml of the standard samples and distilled water as standard 0 ppm and the control were poured separately into 15 ml Falcons. To all Falcons (whether Falcons containing stem extracts or Falcons containing standard specimens) 2 ml of ninhydrin acid solution was prepared and then 2 ml of glacial acetic acid was added.

The samples were placed in a hot water bath at 100°C for 1 hour, then the samples were immediately placed in ice. About 4 ml of toluene was added to the samples and mixed with a high-speed vortex for 20 seconds. From the two phases created, the upper phase, which is red brick, contained proline and was used to determine the concentration of this phase. From this phase, enough was poured into the cuvette of the model

spectrophotometer (Spectrophotometer, Model: V-530, JASCO, Japan) and the absorption rate was read at 520 nm. To determine the proline concentration, the standard curve was plotted by the device using standard solutions and the device based on this curve given the concentration of unknown samples in ppm. After determining the concentration of proline per gram of fresh weight, according to the ratio of dry weight to wet weight, the concentration of proline in terms of ppm per gram of dry weight was calculated.

3.1. Results of analysis of variance

Based on the results of this experiment, the effect of priming solutions on germination percentage, germination rate, germination index, mean germination time, seed vigor index, germination energy, the tensile value was determined (Asgedom and Becker, 2001). The seedling length was significant at the level of 1% probability. Also, examination of shoot length, root length, peroxidase enzyme and proline content in plants transferred to the greenhouse showed that the use of priming solutions had a significant effect on the level of one percent probability. The effect of salinity stress on germination percentage, germination rate, germination index, mean germination time, seed vigor index, germination energy, germination value and seedling length at 1% probability level means it was. Also, the study of traits in plants transferred to the greenhouse showed that shoot length, root length, peroxidase content and proline content were affected by salinity stress and had a significant difference in the level of one percent probability (Basker and Hatton, 1987).

Interaction of priming solutions and different concentrations of sodium chloride showed germination percentage, germination index and seedling length at 1% probability level and germination rate, mean germination time and germination energy were significantly different at 5% probability level, but seed vigor index and tensile value were not affected by the interaction of priming solutions and different concentrations of sodium chloride (Table 1). The study of traits in plants transferred to the greenhouse showed that the interaction of priming solutions and different concentrations of sodium chloride on shoot length, root length, proline content and peroxidase content were significantly different at the level of 1% probability.

Table 1. Analysis of variance of different traits under priming solutions and salinity stress in the laboratory.

SC	DF	Average of squares (MS)							
		GP	GR	GI	AGT	SVI	GE	VT	SL
Types of priming (A)	4	1210.35 **	27.50 **	166.22 **	12.02 **	4.85 **	0.01 **	420.96 **	5.74 **
Salinity stress (B)	2	10657.40 **	37.95 **	2394.46 **	75.26 **	15.55 **	0.07 **	1837.26 **	7.30 **
A × B	8	245.20 **	0.73 *	99.77 **	1.07 *	0.30 ^{ns}	0.0006 *	27.76 ^{ns}	0.69 **
						0.28	0.0003	17.62	0.09
Test error	30	12.97	0.31	30.93	0.46	-	-	-	-
Coefficient of variation (%)	-	5.18	8.65	1.91	6.74	9.09	7.17	6.12	5.15

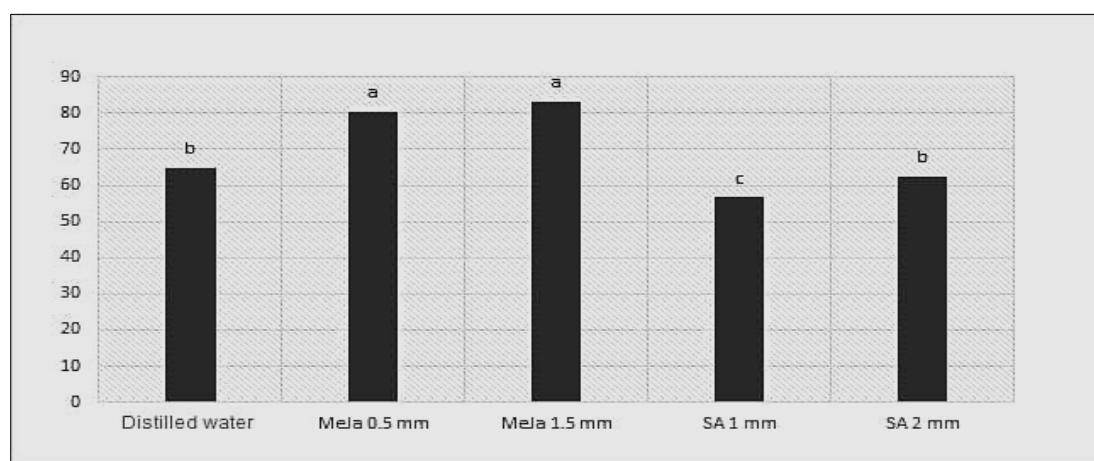
NS: Non-significant, * and ** significant at 5 and 1%, respectively. SC: Sources Change, DF: Degrees of freedom, GP: Germination percentage, GR: Germination rate, GI: Germination index, AGT: Average germination time, SVI: Seed vigor index, GE: Germination energy, VT: The value of tension, SL: Seedling length.

3.2. Germination percentage

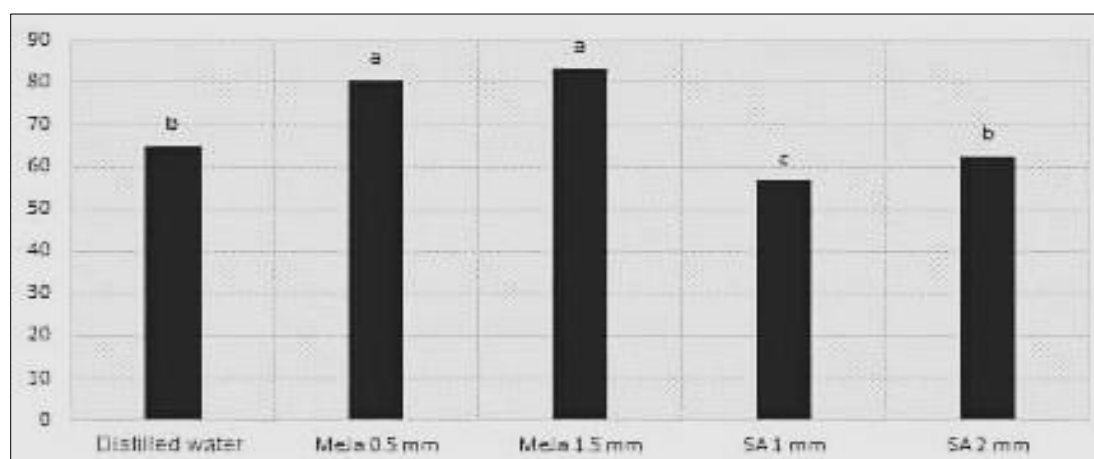
Based on the results of this study, it was found that priming of lemongrass seeds by methyl jasmonate with concentrations of 0.5 and 1.5 mM increased germination percentage and had the highest (83.11%) value. In other treatments, the germination percentage was lower than the mentioned treatments (Artola et al., 2003). Hydropriming (prime water distillation) (Table 2, Fig. 1).

Table 2. Analysis of variance of different traits under priming solutions and salinity stress under greenhouse conditions.

Average of squares (MS)				
Sources change	Shoot length	Root length	Peroxidase enzyme	Proline content
Types of priming (A)	10.19 **	6.73 **	307.73 **	4.06 **
Salinity stress (B)	19.09 **	12.62 **	2232.23 **	29.49 **
A × B	1.71 **	1.13 **	41.56 **	0.54 **
Test error	0.25	0.16	4.38	0.05
Coefficient of variation (%)	5.33	5.33	5.36	5.36

**Fig. 1.** The effect of different concentrations of priming solutions on the germination percentage of lemongrass seeds.

In the study of the effect of different concentrations of sodium chloride on the germination percentage of limousine seeds, it was found that seeds that were under severe salinity stress with a concentration of 10 dS/m sodium chloride had the lowest germination percentage and seeds that Normal and zero concentrations of sodium chloride were the highest They had germination percentage (Fig. 2).

**Fig. 2.** The effect of different concentrations of sodium chloride on germination.

3.2.1. percentage of lemongrass seeds

In the study of the interaction of different concentrations of priming solutions and sodium chloride, it was found that the highest germination percentage was related to seeds that were primed with 0.5 and 1.5 mM methyl jasmonate and were in normal condition (Table 3).

Table 3. Interaction of concentration of priming solutions and sodium chloride on germination percentage.

Sodium chloride concentration	Lemon seed germination percentage		
	Zero	5 Desmins per meter	10 Desi Siemens per meter
Priming solutions			
Distilled water	80.67 cd	77.66 d	36.33 h
Methyl jasmonate 0.5 mM	98.67 a	84.33 c	58.33 g
Methyl jasmonate 1.5 mM	100.00 a	85.00 c	64.33 fg

3.2.2. Seed vigor index

The results of this experiment showed that the highest index of seed vigor belonged to the seeds that were primed by methyl jasmonate with a concentration of 1.5 mM. In other treatments, seed vigor index decreased (Fig. 3).

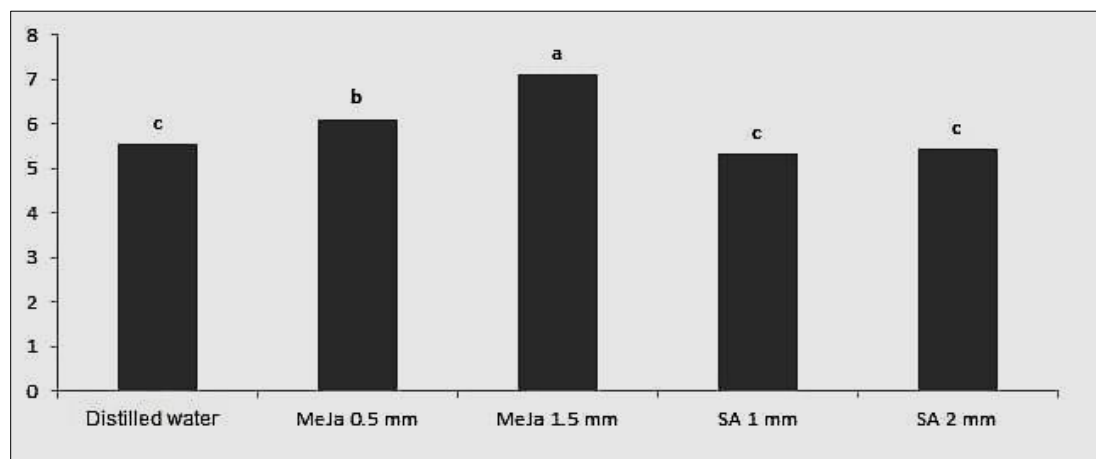


Fig. 3. Optimal Seed Index.

3.3. Germination energy

The results of this experiment showed that the highest germination energy was related to seeds that were primed by methyl jasmonate with a concentration of 1.5 mM. Priming the seeds with other solutions reduced germination energy compared to the above treatment. The results of this experiment showed that the highest acidity value belonged to the seeds that were in normal conditions. By applying salinity stress and increasing the concentration of sodium chloride to 5 and 10 dS/m, the stress value decreased (Fig. 4).

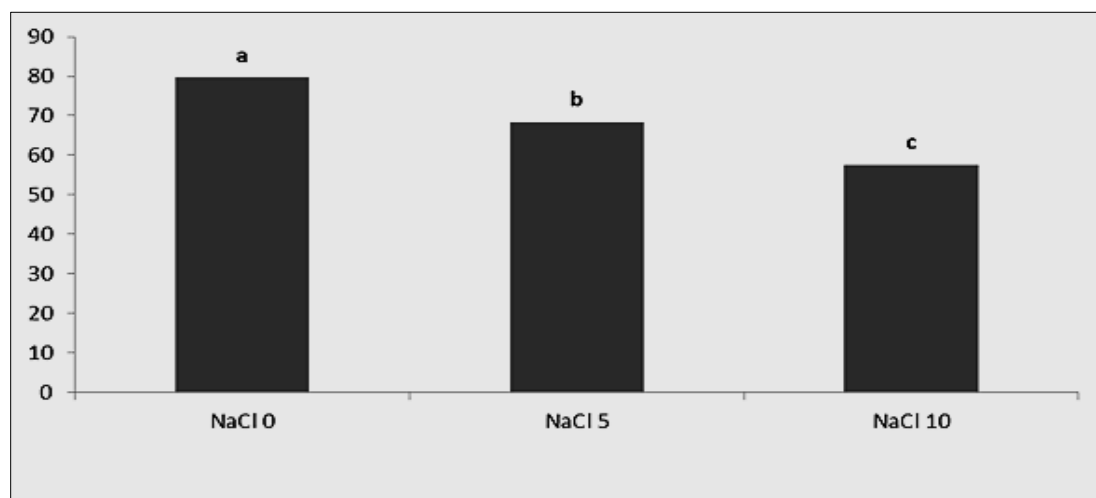


Fig. 4. The effect of different concentrations of sodium chloride on the germination value of lemongrass seeds.

4. Conclusion

Environmental stresses such as salinity reduce germination percentage and speed, reduce root and stem growth, seedling weakness and as a result non-uniformity of field cover and yield loss. During priming, the seeds are usually exposed to the potential of external water. The amount of this water is so small that it does not cause germination but allows a series of physiological and biochemical processes to occur before seed germination, which can include: reduction of inhibitors, breakage of storage materials. And a gradual increase in enzymes necessary to break down the endosperm (García et al., 1995).

When primed seeds are placed in a suitable germination environment, they germinate faster than unprimed seeds. Acceleration of germination in primed seeds can be caused by increasing the activity of degrading enzymes such as alpha-amylase, increasing the level of bioenergy charge in the form of increasing the amount of ATP, increasing DNA and RNA synthesis, increasing the number and at the same time improving yield. Be mitochondria. According to Korkmaz et al., Seed preparation with methyl jasmonate stimulates the production of free polyamine in plant tissues, so methyl jasmonate and polyamine act as a synergy, and seed preparation with methyl jasmonate percent and increases the rate of germination and emergence of seedlings (Korkmaz et al., 2004; Korkmaz et al., 2007) .

There have been reports of increasing the percentage and rate of germination with methyl jasmonate in watermelon seeds.

Examination of the results of this experiment showed that the highest seed vigor index was related to seeds that were primed with a concentration of 1.5 mM methyl jasmonate and were in normal condition. Based on the results obtained from the salinity stress test, it was found that the highest stress value was related to seeds that were primed by methyl jasmonate with concentrations of 0.5 and 1.5 mM and were in normal conditions and did not differ significantly from each other. . Based on the results obtained from the drought stress test, it was determined that the highest stress value belonged to the seeds that were primed by methyl jasmonate with a concentration of 1.5 mM and were in normal condition. The results of this experiment showed that salinity stress restricts germination and seedling growth. Stress leads to a decrease in germination percentage by increasing the average germination time and decreasing the germination rate. To counteract these changes, the plant increases the activity of antioxidants such as peroxidase and the amino acid proline. Seed pretreatment can act as a technique to increase plant resistance to salinity and drought. The pretreatment reduces the effects of stress by increasing the activity of antioxidants and proline concentration, thereby improving germination rate and percentage.

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