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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Impact of Salinity and Drought Stress on Germination of *Asclepias curassavica* L.

Tuzluluk ve Kuraklık Stresinin *Asclepias curassavica* L.'nin Çimlenmesine Etkisi

Işın KOCABAŞ OĞUZ[1*]

Abstract

In this study, the germination performance of *Asclepias curassavica* L. seeds under salinity and drought stress, as well as their effects on radicle and plumule, were investigated. For the salinity stress experiment, solutions were prepared using 6 different NaCl at 0, 50, 100, 150, 200 and 250 mM. To simulate drought stress, six distinct levels of water stress were created using polyethylene glycol-6000 (PEG 6000) solutions with varying water retention capacities, encompassing 0, -2, -4, -6, -8, and -9.8 bar. Germination trials were carried out with 20 seeds in each petri dish according to the random plots experimental design with four replications. In order to examine the germination performances of the seeds, the petri dishes in which the solutions to create the stress conditions were applied and the seeds were placed were tested for 15 days in a controlled germination cabinet with a constant ambient temperature of 20°C, 70% humidity, 14 hours of light, and 10 hours of dark conditions. According to the results obtained, in salinity stress applications, the germination rate of *Asclepias curassavica* L. seeds ranged from 100.00% to 5.00%; the radicle length varied between 34.34 mm and 2.65 mm; the plumule length ranged from 35.11 mm to 8.11 mm; the fresh weight of the radicle fluctuated between 9.38 mg and 0.70 mg; and the fresh weight of the plumula varied from 20.25 mg to 5.88 mg. In drought stress treatments, the germination rate of *Asclepias curassavica* L. seeds ranged from 100.00% to 65.00%; radicle length ranged from 33.86 mm to 5.89 mm; plumule length ranged from 23.86 mm to 10.04 mm; radicle fresh weight ranged from 8.95 mg to 2.53 mg; and plumule fresh weight ranged from 15.55 mg to 5.58 mg. The study indicates that low to moderate salt levels did not hinder the germination of *Asclepias curassavica* seeds, but salt stress could lead to a reduction in radicle and plumule growth, resulting in disproportionate development of the plants. Additionally, it was concluded that the germination of *Asclepias curassavica* seeds was preserved under drought stress up to a certain level, with root and shoot growth increasing compared to plants that were not exposed to stress. These findings highlight the species' ability to adapt under stress conditions.

Keywords: Germination, *Asclepias curassavica*, Salinity, Drought stress, Radicle, Plumule

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Öz

Bu çalışmada *Asclepias curassavica* L. türünün tuzluluk ve kuraklık stresine karşı tohumların çimlenme performansları, radikula ve plumula üzerindeki etkileri incelenmiştir. Tuzluluk stresi deneyi için çözeltiler altı farklı NaCl konsantrasyonu ile formüle edilmiştir: 0, 50, 100, 150, 200 ve 250 mM. Kuraklık stresine benzer ortam sağlamak için su tutma kapasitesi yüksek polietilen glikol-6000 (PEG 6000) çözeltisi kullanılmış ve 0, -2, -4, -6, -8 ve -9,8 bar dahil olmak üzere altı farklı su stresi derecesi oluşturulmuştur. Çimlendirme denemeleri her petri kutusunda 20 tohum olacak şekilde tesadüf parselleri deneme desenine göre dört tekrarlamalı olarak yürütülmüştür. Tohumların çimlenme performanslarını incelemek amacıyla, stres koşullarını oluşturacak olan çözeltilerin uygulandığı ve tohumların bulunduğu petri kapları; ortam sıcaklığı 20ºC'de sabit olan, %70 nem 14 saat aydınlık 10 saat karanlık koşullarda, kontrollü bir çimlendirme kabini içerisinde 15 gün süresince teste tabi tutulmuştur. Elde edilen sonuçlara göre, tuzluluk stresi uygulamalarında *Asclepias curassavica* L. tohumlarının çimlenme oranı %100.00 ile %5.00 arasında; radikula uzunluğu 34.34 mm ile 2.65 mm arasında; plumula uzunluğu 35.11 mm ile 8.11 mm arasında; radikula yaş ağırlığı 9.38 mg ile 0.70 mg arasında ve plumula yaş ağırlığı 20.25 mg ile 5,88 mg arasında değişmiştir. Kuraklık stresi uygulamalarında ise, *Asclepias curassavica* L. tohumlarının çimlenme oranı %100.00 ile %65.00 arasında; radikula uzunluğu 33.86 mm ile 5.89 mm arasında; plumula uzunluğu 23.86 mm ile 10,04 mm arasında; radikula yaş ağırlığı 8.95 mg ile 2.53 mg arasında ve plumula yaş ağırlığı 15.55 mg ile 5.58 mg arasında değişiklik göstermiştir. Çalışma, düşük ile orta tuz seviyelerinin *Asclepias curassavica* tohumlarının çimlenmesini engellemediğini, ancak tuz stresinin radikula ve plumula büyümesindeki azalmaya yol açarak bitkilerin orantısız gelişimine neden olabileceğini göstermektedir. Ayrıca, belirli bir seviyeye kadar kuraklık stresi altında *Asclepias curassavica* tohumlarının çimlenmesinin korunduğu ve strese maruz kalmayan bitkilere göre kök ve sürgün büyümesinin arttığı sonucuna ulaşılmıştır. Bu bulgular, bu türün stres koşullarında adaptasyon yeteneğini vurgulamaktadır.

Anahtar Kelimeler: Çimlenme, *Asclepias curassavica*, Tuzluluk, Kuraklık stresi, Radikula, Plumula

1. Introduction

Seed germination is the process where a plant embryo inside a seed grows, taking in water and nutrients in the right environment, and then developing roots, stems, and leaves. The growth phase is a crucial stage in the plant life cycle, affected by several stressors, including water, temperature, light, soil conditions, salt, and toxic chemicals (Wang et al., 2016; Forte et al., 2019; Begum et al., 2024). Drought, a condition defined by insufficient or irregularly distributed water resources, has detrimental effects on seed water uptake and metabolic activities. The excess salt in the soil hinders the seed's ability to absorb water and impedes proper embryo development, leading to osmotic stress. Therefore, it is crucial to consider environmental factors like drought and salinity stress in crop cultivation, as they significantly impact the success of plant germination.

It has been reported that the germination ability of seeds under salinity and drought stress conditions varies from plant to plant, even between different species of the same plant (Khan et al., 2024; Arslan et al., 2018; Mahpara et al., 2022). Although many medicinal and aromatic plants have the ability to thrive under harsh environmental conditions such as salinity and drought, various factors such as timing, frequency, amount, and duration of these environmental stresses affect the resistance of plants (Leal et al., 2013; Tatrai et al., 2016; Hâkimi et al., 2021; Jiang et al., 2021). In some studies, on the *Apocynaceae* family, which is in the group of medicinal aromatic plants, there are some studies that seed germination percentage, vitality index, root and shoot lengths decreased in salt stress (Jaleel et al., 2007; Xu et al., 2020) and are considered to be salt tolerant or moderately salt tolerant (Xu et al., 2021; Jiang et al., 2021; Li et al., 2023).

Researchers working on drought stress in the *Apocynaceae* family have stated that the plant's germination rate, radicle length, and fresh weight increase depending on the severity of the drought. Plants under stress protect their root system development by showing a morphological drought avoidance mechanism, and thus plant growth continues and adapts to semi-arid or arid environments (Leal et al., 2013; Wu et al., 2022). *Asclepias curassavica* L., a member of the *Apocynaceae* family, is a perennial plant with medicinal and fragrant properties that maintains its green color throughout the year. Commonly referred to as milk flower, blood flower, or Mexican butterfly flower, this plant is characterized by its spiny stem and symmetrically arranged leaves (Majewska and Altizer, 2019; Anonymous, 2025). *Asclepias curassavica* L. is usually attractive with its bright red or orange flowers. During the summer months, these flowers attract a diverse range of insect and butterfly species, thus contributing to the reproductive cycle and playing a vital role within the plant's ecosystem. This pharmaceutically important plant with anti-inflammatory (Alonso-Castro et al., 2021), antimicrobial (Hemavani and Thippeswamy, 2012), and antioxidant (Raja et al., 2005) properties is used in the treatment of diarrhea, dysentery, and chronic rheumatism. It is also used in the treatment of cancer and many lung diseases (Zheng et al., 2019; Shelke and Bhot., 2019).

This study examined the impact of drought and salinity stress on the germination of *Asclepias curassavica* L. seeds, through which the plant’s responses and potential for environmental adaptation were sought to be understood. This knowledge is expected to be an important basis for determining strategies for the development of plant varieties resistant to environmental stresses in agricultural practice, ecosystem management, and plant breeding.

2. Materials and Methods

This research, conducted at the Akdeniz University Faculty of Agriculture, Department of Field Crops Laboratory, utilized *Asclepias curassavica* seeds as plant material and was carried out in October 2021. In order to determine the effects of salinity and drought stress on the germination characteristics of the plant, two independent experiments were carried out according to the random plots experimental design. In each germination trial, seeds of *Asclepias curassavica* were subjected to six different levels of stress conditions. Germination trials were carried out with 20 seeds in each petri dish according to the random plots experimental design with four replications. A total of 24 petri dishes were used for one experiment and 48 pieces with 9 cm petri dishes were used for 2 separate experiments. Six salinity stress studies were conducted using NaCl as the salt source. The solutions contained 0, 50, 100, 150, 200, and 250 mM. Using polyethylene glycol-6000 (PEG 6000: Merck, Germany), 6 different drought stress trials were also conducted with solutions of 0, -2, -4, -6, -8 and -9.8 bar water potentials. The impact of these stresses on the germination characteristics of *Asclepias curassavica* seeds was investigated.

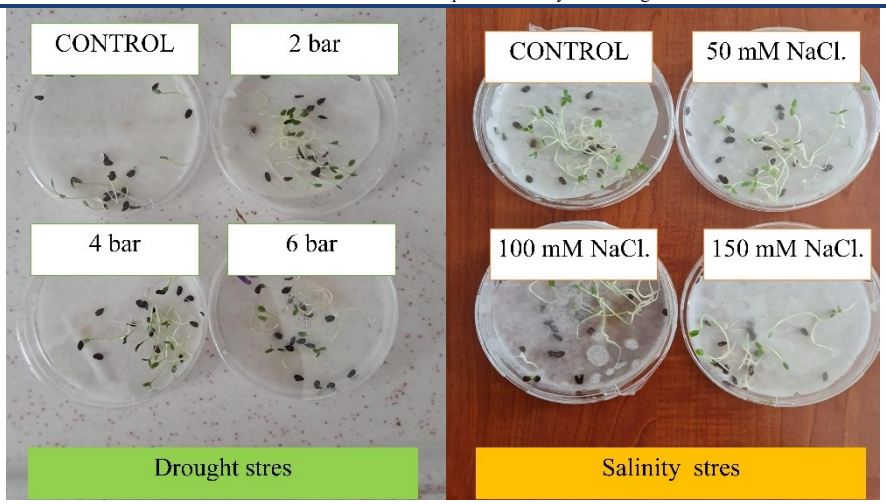

Figure 1. A general picture looks at the end of the experiment for both stress factors.

Twenty uniform-size seeds were placed in petri dishes with two layers of germination paper. Then, 10 ml of solution containing different levels of NaCl (Arslan and Aydınoğlu, 2018) was applied to each petri dish for salinity stress experiments. Similarly, in drought stress experiments, 10 ml of each solution, comprising varying concentrations of PEG-6000, were applied to each petri dish. Finally, to prevent evaporation, the petri dishes containing the seeds were covered with parafilm. Petri dishes were prepared and incubated in a germination chamber under a photoperiod of 14 hours light/10 hours dark, 70% humidity, and a temperature of 20 °C. The Petri dishes remained in the germination chamber for a 15day period (Gheidary et al., 2017). In the experiment, seeds were considered to have germinated if their root length exceeded 2 mm, and daily observations were made at a consistent time each day (Bıçakçı et al., 2020). Germination rates (%) were calculated by counting the germinated seeds at the end of the 15th day (Scott et al., 1984). On the fifteenth day of germination, the lengths of radicles and plumules were measured using a digital caliper, while their fresh weights were determined with an analytical balance. *Figure 1* shows a photo of the plants after their fifteen-day evaluation.

The effects of salinity and drought stress conditions on the germination characteristics of *Asclepias curassavica* were evaluated independently of each other in accordance with the experimental plan. The statistical evaluation of the research data included the application of analysis of variance according to the randomized complete block design with four replications. Duncan multiple comparison test was used to determine the groups. The licensed SPSS software package (SPSS Statistics Base v 23) was utilized for this study (SPSS, 2017).

3. Results and Discussion

The germination response of *Asclepias curassavica* to different levels of salinity and drought stress was examined in this research. Germination was observed at 0, 50, 100, 150 and 200 mM NaCl concentrations under salinity stress and at 0, -2, -4 and -6 bar water potential levels under drought stress. It was determined that germination could not be achieved in *Asclepias curassavica* seeds at a salinity stress level of 250 mM NaCl and -8 to -9.8 bar drought stress levels.

3.1. Germination rate

The germination rates of *Asclepias curassavica* varied from 18.75% to 87.50% under under different levels of salinity stress. The germination rate of 0, 50, 100, 150, and 200 mM NaCl doses was determined to be 87.50, 85.00, 85.00, 52.50, and 18.75%, respectively (*Figure 2*). Under NaCl-induced salinity stress, germination percentage of *Asclepias curassavica* seeds was similar to the control group at the lowest NaCl doses of 50 and 100 mM. As salinity stress increased, there was a gradual negative decrease in seed germination from NaCl, especially in the high concentration range (150-200 mM) and this reduction was statistically significant ($p<0.01$; *Figure 2*). Variations in seed germination response to salinity were observed among *Apocynaceae* (oleander) family members. For example, seeds of *Apocynum venetum* (50 mmol/L) showed a slight increase in germination rates at the lowest salt stress treatment (50 mmol/L), with a gradual decrease with increasing NaCl concentration (Li et al., 2023). Jiang et al. (2021) found that salt concentrations of up to 200 mM did not significantly impact the germination of

Apocynum venetum and *Apocynum pictum* seeds; however, germination rates decreased dramatical above this threshold. In other studies, it was reported that germination decreased in *Catharanthus roseus (*Jaleel et al., 2007; Rezaee et al., 2012) and *Apocynum venetum* (Xu et al., 2020) seeds exposed to salt. The increased salt concentration inhibits seed germination by disrupting the osmotic balance, thereby restricting water absorption and preventing the physiological processes necessary for germination (Çakmakçı and Dallar, 2019). Excessive salinity negatively impacts seed imbibition and germination due to decreased osmotic potential in the surrounding water (Aslan et al., 2016). Cytosolic accumulation of sodium and chloride ions can result in ion toxicity and the suppression of cell division and expansion (Zhang et al., 2010). Through osmotic effects, ion toxicity or a combination of the two, water absorption and cellular structure of the seed can be affected, inhibiting or delaying the germination process. In addition, salinity may negatively impact seed germination through the reduction of germination stimulants, such as gibberellins, an elevation in abscisic acid, and alterations to membrane permeability and water dynamics within the seed (Uçarlı, 2020).

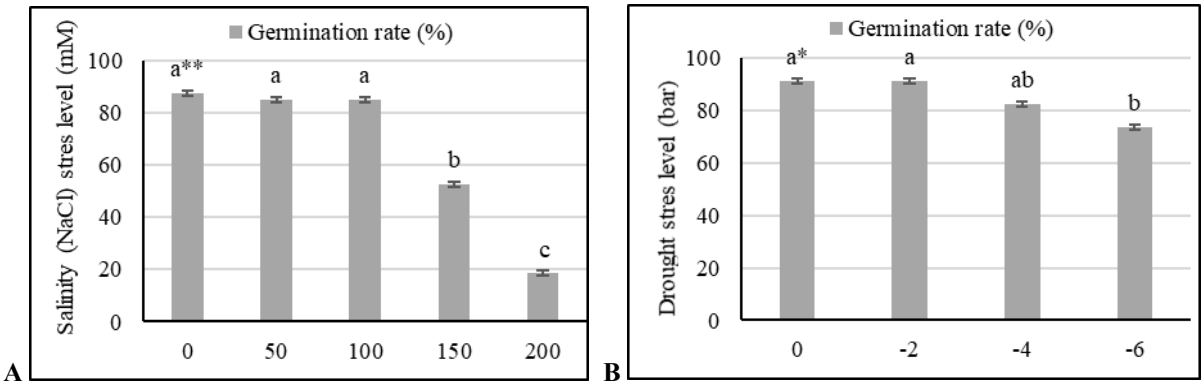

Figure 2: Impact of (A) salinity stress and (B) drought stress on germination rate (%) of Asclepias curassavica. Each column represents the mean of a specific treatment. **The means were significantly different at p≤0.01, and *they differed at the p≤0.05 level.

The effect of increasing drought stress levels on the germination of *Asclepias curassavica* seeds was found to be statistically significant at the p≤ 0.05 level, and it was determined that germination was not achieved at -8 and -9.8 bar drought stress levels. The germination rate of 0, -2, -4, and -6 bar was determined to be 91.25, 91.25, 83.50, and 73.75%, respectively. Moderate drought stress was found to have no negative impact on the germination of *Asclepias curassavica* seeds. In studies investigating the seed germination characteristics and physiological changes of *A. venetum,* which is in the same family as *Asclepias curassavica,* under drought stress, it was shown that the germination rate was promoted under moderate drought severity for *A. venetum,* and then it was determined that germination decreased and stopped as the drought severity increased (Jiang et al., 2021; Wu et al., 2022).

Salinity and drought conditions can induce osmotic stress within plant cells, resulting in turgor pressure loss. These conditions may result in membrane disorganisation, loss of activity or alteration of protein structure, inhibition of photosynthesis, metabolic dysfunction, and damage to cellular structures (Krasensky and Jonak, 2012). As a result, both stressors stimulate stress responses in plants

3.2. Length of the radicle and fresh weight

The averages of radicle length and radicle fresh weight obtained under varying levels of salinity stress conditions for *Asclepias curassavica* range from 31.36 to 4.39 mm and 7.93 to 2.37 mg, respectively. The radicle lengths for NaCl concentrations of 0, 50, 100, 150, and 200 mM were measured at 31.36, 21.53, 14.89, 7.54, and 4.39 mm, respectively. For NaCl concentrations of 0, 50, 100, 150, and 200 mM, the radical fresh weights were found to be 7.93 mg, 5.04 mg, 3.96 mg, 3.86 mg, and 2.37 mg, respectively. A statistically significant decrease (p< 0.01) in radicle length and fresh weight was observed with increasing salinity stress (*Figure 3*).

Some researchers reported that root growth and weight (Rezaee et al., 2012), which are growth indicators, decreased after NaCl treatment in the seeds of periwinkle flower (*Catharanthus roseus*), which belongs to the same family as *Asclepias curassavica.*

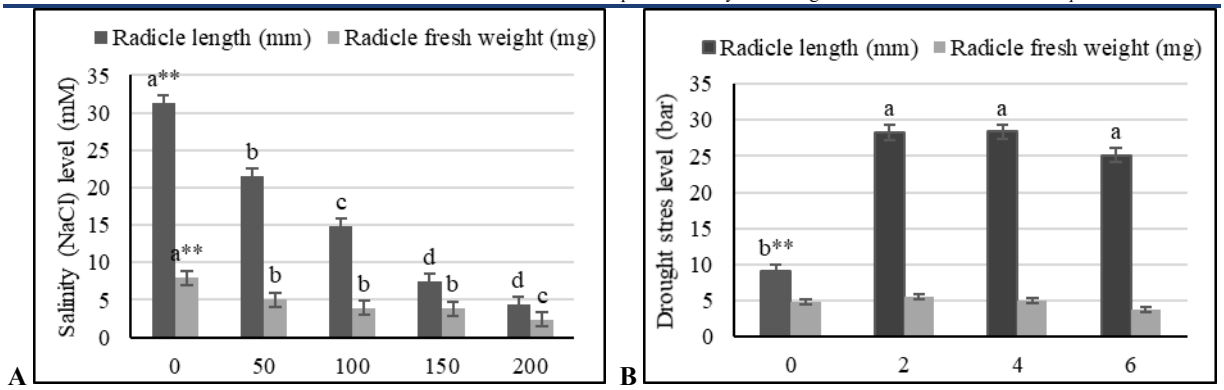

Figure 3: Impact of (A) salinity stress and (B) drought stress on radicle length (mm) and radicle fresh weight (mg) of Asclepias curassavica. Each column represents the mean of a specific treatment. **The means were significantly different at p≤0.01

There was a statistically significant increase in radicle length with increasing drought stress ($p<0.01$) compared to control treatments, but the increase in radicle fresh weight with increasing drought stress (between 0-6 bar) did not result in statistically significant differences (*Figure 3*). Radicle length was positively influenced by moderate drought stress, whereas radicle fresh weight was less responsive. Maximum mean radicle lengths were obtained under −4 bar (28.38 mm), −2 bar (28.27 mm), and −6 bar (25.13 mm) drought levels, while the minimum (8.99 mm) was observed in the control (0 bar) treatment. It was determined that radicle elongation of *Asclepias curassavica* seeds was encouraged up to -6 bar drought stress, and germination and root development were not observed in applications exceeding -8 bar. Findings indicate that moderate drought conditions may stimulate radicle elongation and radicle fresh weight in *Asclepias curassavica.* Research indicates that moderate drought conditions can stimulate radicle elongation in some species belonging to the *Apocynaceae* family (Wu et al., 2022). The response mechanisms of plants to drought stress are highly intricate and involve various changes in genes and metabolites (Tardieu and Tuberosa, 2010).

3.3. Length of the plumule and fresh weight

A significant decrease in plumule length of *Asclepias curassavica* plant was observed with the increase of stress ($p<0.01$), and plumule length was not observed at 200 mM NaCl treatments. Maximum plumule length (30.40 mm) was observed in control treatments; conversely, the minimum plumule length (9.09 mm) was recorded in treatments containing 150 mM NaCl. In a similar study conducted by Jaleel et al. (2007) reported that shoot elongation of *Catharanthus roseus* seeds diminished with rising salinity stress levels. It was emphasized that salt stress may result in diminished shoot growth by inhibiting the translocation and assimilation of photosynthetic products.

The shoot fresh weight of 0, 50, 100, and 150 mM of NaCl doses was determined to be 15.60, 18.56, 14.25, and 6.68 mg, respectively. The application of 50 mM NaCl, the lowest stress level, led to an increase in shoot fresh weight, which was later followed by a significant decrease in shoot fresh weight as salinity levels increased ($p<0.01$; *Figure 4*). Low levels of NaCl application are thought to cause an increase in the plumule fresh weight of *Asclepias curassavica* plants as a response to salt stress, decrease water absorption due to the osmotic effect with increasing salinity, cause a lack of nutrition due to ionic imbalance, and decrease many metabolic activities.

Asclepias curassavica plumule length and plumule fresh weight values obtained under different levels of drought stress conditions ranged from 21.77 to 11.76 mm and 11.48 to 7.04 mg, respectively. At the lowest levels of drought stress application, -2 bar (21.77 mm) and -4 bar (20.00 mm) applications resulted in an increase in plumule length; however, plumule elongation decreased when the drought stress level was increased to -6 bar (13.13 mm), and no measurable plumule length could be detected after -8 bar (*Figure 4*).

Drought stress treatments of 0 (10.30 mg), -2 (11.48 mg), and -4 (10.93 mg) bar exerted similar effects on the plumule fresh weight of *Asclepias curassavica;* however, the -6 bar (7.04 mg) treatment resulted in a statistically significant decrease ($p<0.01$). It was determined that a limited degree of drought stress treatment would exert a favourable influence on the plumule length and fresh weight of *Asclepias curassavica (Figure 4*).

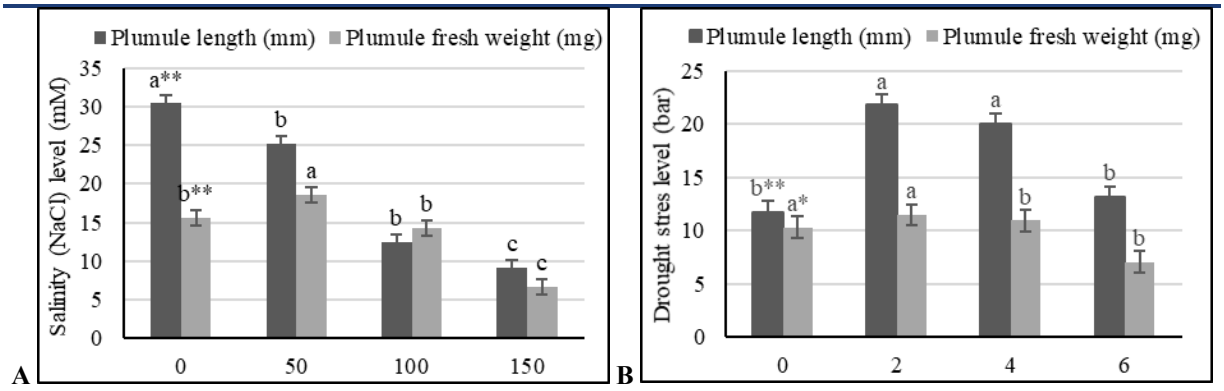

Figure 4: Impact of (A) salinity stress and (B) drought stress on plumule length (mm) and plumule fresh weight (mg) of Asclepias curassavica. Each column represents the mean of a specific treatment. **The means were significantly different at p≤0.01, and *they differed at the p≤0.05 level.

4. Conclusions

In the study, although germination of *Asclepias curassavica* seeds was not decreased in low and medium salt treatments, it is thought that the decrease in root and shoot growth will lead to disproportionate development of the plant under increasing stress conditions and will be significantly affected. The findings demonstrated that the germination of *Asclepias curassavica* seeds could be sustained under conditions of drought stress up to a certain threshold, and that the growth of roots and shoots exhibited an increase in comparison to plants that were not subjected to such conditions. This observation suggests that this plant possesses a high degree of tolerance to drought stress and the capacity to adapt to stress-inducing environments. The seed germination properties of *Asclepias curassavica* under conditions of salinity and drought stress were examined in this study; its findings will be a key resource for future investigations.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

The authors of the article have no conflicts of interest.

Authorship Contribution Statement

Concept: Kocabaş Oğuz, I.; Design: Kocabaş Oğuz, I.; Data Collection or Processing: Kocabaş Oğuz, I.; Statistical Analyses: Kocabaş Oğuz, I.; Literature Search: Kocabaş Oğuz, I.; Writing, Review and Editing: Kocabaş Oğuz, I.

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