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The Effect of Soil Salinity on the Early Seedling Stage of Black eyed Peas

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ABSTRACT

Background: Inadequate and unbalanced nutrition is a significant problem today. The aim should be to prevent this by changing plant production methods. Black eyed peas is used as an important protein source in human nutrition. Black eyed peas is a legume that can adapt to adverse conditions and is resistant to hot and dry environments. Black eyed peas is a plant with low soil selectivity and high adaptability, yielding the best results in well-drained soils. Soil salinity, a critical global challenge in crop production, inhibits water uptake in plants, causes nutrient imbalances and has a negative effect on plant growth and yield by havinge an adverse effect on the microbial activity in soil. Therefore, determining the salinity tolerance of different species and varieties is of great importance in planning crop production in areas with salinity problems. The goal of this study was to assess the effect of different soil salinity levels on black eyed peas during the early seedling stage.

Methods: NaCl doses of 0, 50, 100, 150, 200, 250, 300, 350, 400 and 450 mM were applied when the black eyed pea plant were during the germination stage. The study was set up in a random full blocks trial design with 10 replicates and conducted as a pot experiment.

Result: The study determined macro-mineral intake (ppm and %), relative water content (%), water holding capacity (%), tolerance index (%), nodule number (number per plant) and nodule weight (mg per plant). The variance analysis revealed statistically significant differences between salt doses in almost all of the examined traits and indicated that soil salinity had a negative impact on plant growth in general.

Key words: Black eyed peas, Early seedling, Plant, Soil salinity.

INTRODUCTION

The effects of climate change include water and soil salinity, soil pollution and water scarcity (Shokat and Grobkinsky, 2019; Ingrao *et al.,* 2023). Climate change, rising sea level, drought and agricultural irrigation cause increased salinization (Negacz *et al.,* 2022). The quality and sustainability of water and soil resources are crucial for human life. The deterioration of irrigation water in terms of quantity and quality, climate change and environmental pollution are making it increasingly difficult to obtain quality irrigation water from nature (Tunç and Kaman, 2022).

Water used for irrigation exhibits different properties depending on the contained salts. Salts formed as a result of the breakdown and decomposition of rocks and soil particles may contain soil minerals such as lime and gypsum. These salts are transported through the soil with irrigation water, reaching the plant root zone where they accumulate. Salinity problems arise if these salts cannot be removed from the plant root zone by rainfall and leaching (Ertaş and Öztürk, 2020). Rising salinity in irrigation water or soils represents a serious environmental threat to agricultural systems (Srivastava, 2020). The decline in arable farmland and rapid population growth increase stress on plants and accelerate the extinction of species (Shokat and Grobkinsky, 2019; Ingrao *et al.,* 2023). Salt stress, which has a detrimental effect on plant growth and development during the germination and early seedling growth, affects plants at the anatomical, molecular and morphological levels (Fan *et al.,* 2020; Chavarria *et al.,* 2020). Soil-derived stress conditions represent a major constraint on productivity and plant growth worldwide (Liu *et al.,* 2023).

A wide range of factors such as improper irrigation, environmental conditions, erosion and impermeable soil layers contribute to the salinization and degradation of soils. Given these challenges and the costs associated with improving saline soils, identifying and cultivating plant species well-suited to these conditions is of critical importance (Kadioglu, 2021). Minerals (such as Na, Mg, K, Ca, SO_4, HCO_3, CO_3, Cl and NO_3) present in the soil solution cause salinity (Deliboran and Savran, 2015). Salinity negatively affects water uptake, growth, seed germination and plant biology (Kadioglu, 2022; Kaya, 2021). As salt concentration increases, germination time increases and shoot and root length decrease (Kadıoğlu, 2021;

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Demiroglu and Özkan, 2020). Previous studies have shown that different salinity levels negatively influence plant grow, dry biomass and stem and shoot growth (Hosseini *et al*., 2006; Akbari *et al*., 2007; Prakash *et al.,* 2021).

The study aimed to determine relative water content, macro-mineral uptake, water holding capacity, tolerance index, nodule number and nodule weight in black eyed pea plants during the early seedling stage at different salt concentrations. Stress factors cause a slowdown in the growth and development stages of plants, impair their metabolic functions and ultimately lead to plant mortality.

MATERIALS AND METHODS

Experimental design

The experiment was conducted as a pot trial with ten replicates in a growth chamber (Mikrotest, -20 to +70°C) randomized block design to examine the effect of soil salinity during germination and the early seedling stage of black eyed peas. Soil sieved through a 4 mm sieve was used as the plant growing medium in the study. The research was conducted at an agricultural research institute between 2025 and 2026. The methods used are listed below:

Germination tests

Ten diverse salt concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400, 450 mM NaCl) were applied to the black eyed pea seeds in the study, which was set up for 10 replicates according to the random full blocks trial design. Seeding was done manually, with 8 seeds per pot (2.5 kg soil pot^{-1}). Growth chamber at 25°C with a 16 h light and 8 h dark photoperiod. The pots were thinned when the second true leaves appeared on the plants, leaving 4 plants per pot. Salt doses were applied gradually at 2-day intervals. Considering the severe salt damage, the plants were harvested 16 days after the first salt application (Önal and Altun, 2021). Seed germination times were determined according to the principles specified in ISTA (ISTA, 1996; Kumar *et al.,* 2023). In addition, the number of nodules per plant^{-1} and the nodule weight (mg≤ plant^{-1}) were determined (Önal and Altun, 2021).

Determination of tolerance ındices

Length measurements were performed on both roots and shoots, with root length defined as the distance between the root crown and root tip and shoot length as the distance between the root crown and shoot tip (Tenikecier and Gençtan, 2013). Following length determination, fresh biomass of roots and shoots were recorded. Dry matter content was assessed after oven-drying the samples at 70°C for 48 h (Tenikecier and Ateş, 2018). The retention capability of the shoot (RCS) was computed using the equation established by Clarke (1986).

$$\text{RCS (mg)} = \frac{\text{SFM - SDM}}{\text{SDM}} \times 100$$

SFM: Shoot fresh matter.
SDM: Shoot dry matter.

The relative water content (RWC) (%) was estimated following Ateş and Tekeli (2007) by using to formula:

$$\text{RWC (\%)} = \frac{\text{SFM - SDM}}{\text{SFM}} \times 100$$

The tolerances index (TI) was measured by Kargbo (2019) by using the formula:

$$\text{TI} = \frac{\text{SDM in salinity stress}}{\text{SDM in control}} \times 100$$

Chemical analyses

The total nitrogen content of plant shoot samples prepared for analysis was measured by the “Micro-Kjeldahl Method” after wet digestion with H_2SO_4 (AOAC, 1996). Macro and micro mineral content (P, K, Ca, Na, Mg, Fe, Cu, Zn, Mn, Cl) was determined using a Perkin Elmer (Optima 2100) Model ICP-OES device after wet digestion with an $HClO_4$ (AOAC, 1996) mixture. The K+/Na+ ratios of the extracts were calculated. Each sample underwent duplicate analysis.

Statistical analysis

The two-year data were analyzed using analysis of variance (ANOVA) under the Random Full Blocks Trial design with IBM SPSS Statistics v.26. Differences among treatments were valuated and grouped using the LSD multiple comparison test at $p<0.05$.

RESULTS AND DISCUSSION

This research assessed the effects of salinity treatments on germination performance, physiological characteristics and macro-mineral uptake of black-eyed pea seeds. Germination rates ranged from 14% to 72%, with the highest value determined with a salt concentration of 50 mM (Fig 1). Average germination times was influenced by salt concentrations. The quickest average germination times were recorded with 100 and 150 mM salt concentrations, at 6.59 and 6.54 days, respectively. However, the uppermost concentration levels (400 mM and 450 mM) were associated with the slowest germinations (Fig 1).

A progressive increase in salt concentration negatively influenced shoot and root lengths, as well as root fresh weight. The measured parameters varied as follows: shoot length 56.58-70.42 cm, root length 57.75-73.42 cm and root fresh weight 0.56-1.18 mg. The highest root fresh weight, root length and shoot length were determined at 0 mM and the lowest values were determined at 450 mM (Fig 2). The root dry weight ranged between 0.65 and 0.88 mg. The shoot fresh weights of black-eyed pea varied between 4.47 and 5.77 mg at different salt concentrations. The maximum shoot fresh weight was recorded under control conditions (0 mM), whereas the minimum value was obtained at the highest salt concentration (450 mM).

Increasing salinity levels adversely affected the relative water content and water-holding capacity of the shoots. The highest relative water content and water-holding capacity of the shoots were obtained at a salt concentration of 0 mM (9.37 mg, 47.74%). The lowest values were determined at the highest salt concentration (450 mM) (5.05 mg and 34.72%). The tolerance index was lowest at 450 mM (80.17) and highest at 0 mM (143.34) (Fig 3).

The number and weight of black eyed pea nodules were negatively affected by salt concentration; as salt concentration increased, the number and weight of nodules decreased. The number and weight of nodules reached their highest values at 0 mM (4.71-9.34) and their lowest values at 450 mM (0.38-0.21).

All examined mineral averages showed significant statistical changes at different salt concentrations ($p<0.01$).

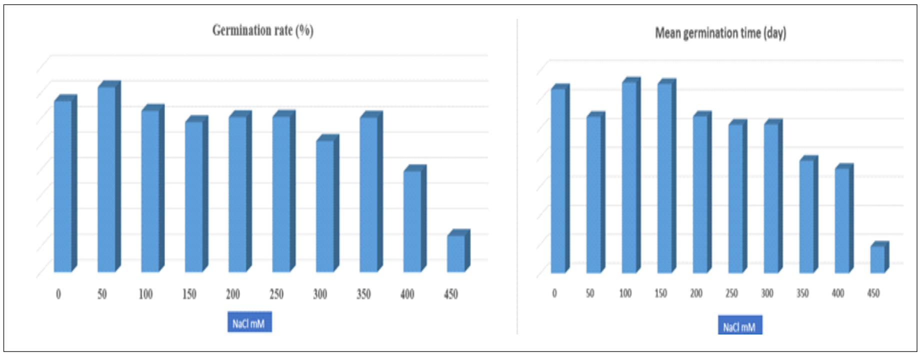

Fig 1: Germination rate and mean germination time of black-eyed pea.

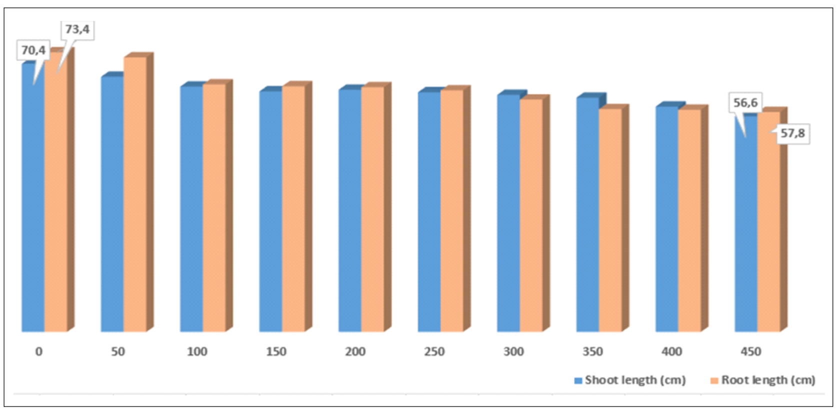

Fig 2: Shoot length and root length of black-eyed pea.

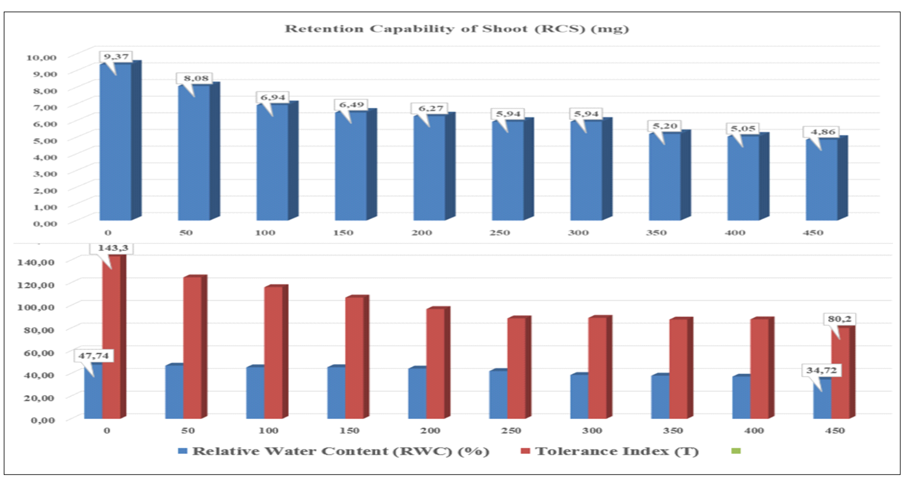

Fig 3: Retention capability, relative water content and tolerance index of black-eyed pea shoots.

Nitrogen levels varied in response to increasing salinity. However, the highest nitrogen contents were determined at a concentration of 150 mM (0.29%) and the lowest at 0 mM (0.17%) (Fig 4). Salt concentrations had not significantly impacted the Ca and P contents of the black eyed pea seedlings (Fig 4).

K, Mg, Na and Cl concentrations ranged from 0.059-0.72%, 0.39-0.66%, 0.30-0.70% and 0.10-0.28%, respectively (Fig 5). The lowest Cl concentration (0.10%) was recorded under control conditions (0 mM), while the highest (0.30%) was observed at 450 mM. The highest Mg contents were associated with 50 mM and 100 mM salt concentrations. As expected, Cl contents increased with salt concentrations (Fig 5). The highest K contents were determined at 0 mM, 50 mM, 100 mM and 150 mM (0.72%, 0.71%, 0.71% and 0.71%), respectively. The K^+/Na^+ ratio peaked at 0 mM (1.28) and reached its minimum at 450 mM (0.97), where the lowest K content (0.59%) was also recorded (Fig 5).

Improper irrigation practices, inadequate drainage and the high salt content in the irrigation water in semi-arid and arid regions leads to the accumulation of salts in the soils. Soluble salts present in the soil are transported upward by capillary action during irrigation and accumulate at or near the root zone. Salinity causes serious yield losses in plants. When salinity cannot be eliminated or soil remediation is not economically feasible, it is necessary to identify and cultivate plant species fitting for these conditions. In our study, relative water content (%), macro-mineral uptake (ppm and %), water holding capacity (%), tolerance index (%), nodule number (number per plant) and nodule weight (mg per plant) in black eyed peas were examined during the early seedling stage under saline conditions. Four salt concentrations were applied to ten soybean varieties (0 mM, 50 mM, 100 mM and 200 mM) in a study examining soybean germination and seedling development. It was determined that increasing salt concentration affected soybean germination and seedling development, root length decreased proportionally with the salt dose and shoot development was inhibited at increasing salt concentrations. Additionally, on the seventh day of germination, the germination rates were determined as 42.22%, 33.98%, 27.04% and 15.56%, while the water content was 72.7%, 66.4%, 64.35% and 62.73% (Pavli *et al.*, 2021). Oyetunji and Imade (2015) reported that the application of 50, 100 and 150 mM NaCl in black eyed peas reduced plant height compared to the control group, while Abeer *et al.* (2015) reported that the application of

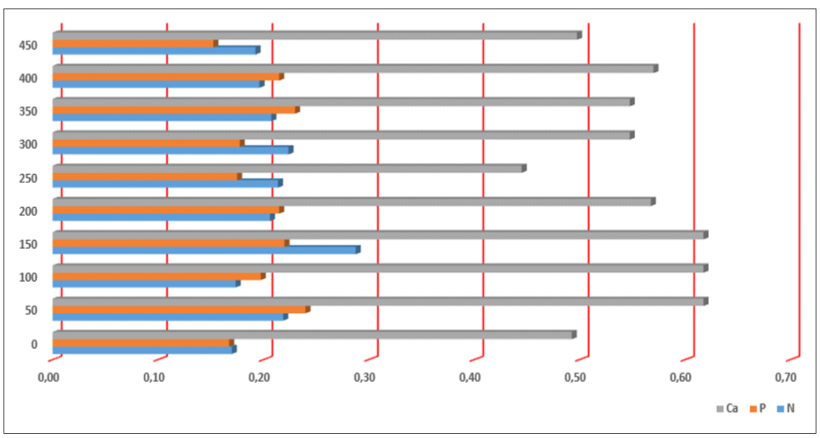

Fig 4: Nitrogen, calcium and phosphorus content of black-eyed pea shoots.

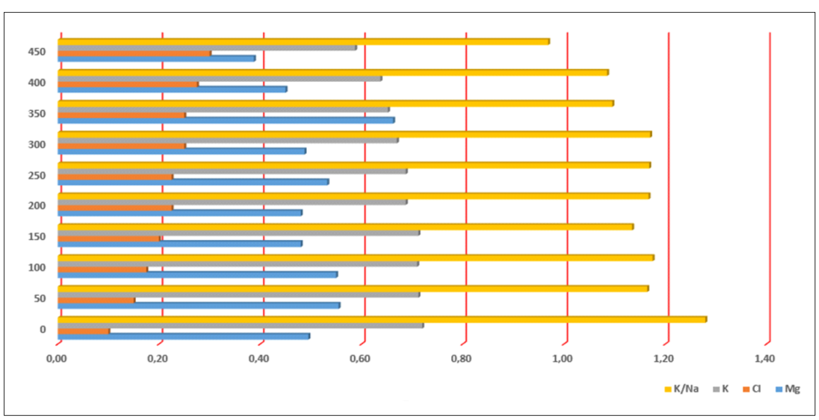

Fig 5: K/Na^+, potassium, chlorine and magnesium of black-eyed pea shoots.

200 mM salt reduced plant height compared to the control group. Özkorkmaz and Yılmaz (2017) reported that salt stress reduced stem fresh weight in black eyed peas seedlings. Some morphological changes occurring in plants as a result of salinity stress cause a decrease in shoot and root length and limited rooting (Mısra *et al.,* 1995; Evers *et al.,* 1997). The effect of salt stress on plants has been reported to vary depending on the applied dose, the time elapsed after exposure to salt (Hasanuzzaman *et al.,* 2013) and the varieties (Önal, 2011). Taffouo *et al.* (2010) stated that the salt dose reduced both the stem and leaf dry weight in cowpeas. As a result of a study conducted on different black eyed peas varieties, it was determined that above-ground dry weight decreased as salt doses increased (Wilson *et al.,* 2006). A study conducted on black eyed peas reported that the number of nodules, root age and dry weight decreased as the salt dose increased (Padilla *et al.,* 2010). It was determined that root age and dry weight, above-ground age and plant height, dry weight, number of leaflets on the plant, stem diameter, leaf area, nodule formation and development decreased as the salt dose increased (Önal and Altun, 2021). Studies indicate that plants exposed to salt stress exhibit reduced characteristics such as root, stem and shoot growth, with a corresponding decrease in yield (Köşkeroğlu, 2006). In the study conducted on Persian alfalfa, the tolerance index was determined to range between 0.97 and 1.03 at 100 mM and between 0.40 and 0.48 at 150 mM (Topçu *et al*., 2024). The data obtained from the black eyed peas trial conducted are similar to the results of other studies reported in the literature. These data show that increasing salt concentrations have a negative impact on germination and early stages in different plant species.

Plants require 14 plant nutrients to complete a full life cycle. These elements are classified as macro and micro nutrients based on their quantities in plant dry matter. N, P, S, K, Ca and Mg (“Nitrogen, phosphorus, sulfur, potassium, calcium and magnesium respectively”) are macronutrients (de Bang *et al*., 2021). Plant nutrients have various functions within plant physiology as components of inorganic compounds or as ions (Kathpalia and Bhatia, 2018). Plant growth in holomorphic soils are inhibited by the first osmotic stress phase following ionic toxicity caused by the accumulation of Cl^- and Na^+ ions, resulting in oxidative stress and nutrient deficiency (Arzani *et al.,* 2016). Sodium ions (Na^+) are not essential for plant growth. Excessive sodium causes toxicity in most plant species. The uptake of other plant nutrient elements such as K^+, Ca^{2+} and Mg^{2+} under saline conditions is often inhibited by high Na^+ levels in plant tissues, resulting in nutrient deficiency (Assaha *et al*., 2017). Nutrient deficiency inhibits biosynthesis, thereby hindering plant growth (Pagare *et al*., 2015). Potassium deficiency occurs as a result of high salt (NaCl) intake. An increase in NaCl leads to an increase in Cl^- and Na^+ and a reduction in Mg^{2+}, K^+ and Ca^{2+} levels in plants (Khan, 2001). Salt stress directly damages plants and disrupts metabolic processes (Akhtar *et al.,* 2015). Salinity enhances the content of Na^+, Ca^{2+} and Cl- in *Vicia faba* L. and the ratio of K^+/Na^+ decreases (Gadallah, 1999). A positive correlation is observed between Na^+ and Cl- concentrations, while a negative correlation is evident between Na^+ and K^+ concentrations in both roots and leaves. The concentration of Mg^{2+} remains unaltered in both leaves and roots, irrespective of changes in the Na^+ concentration. Similarly, the concentration of Ca^{2+} exhibits no variation with the Na^{2+} concentration in leaves; however, it demonstrates an inverse relationship in the roots (Ferreira *et al*., 2001). Salt stress directly damages plants by inducing ionic stress and disrupting ionic homeostasis. The accumulation of Na^+ in plants under salt stress perturbs metabolic processes, particularly in environments characterized by low Na^+ and high K^+ and Ca^{2+} concentrations (Akhtar *et al*., 2015). The results of trials and studies conducted with different species indicate that the early development of plants is important for producing strong seedlings. Kadam (2021) declared that root and shoot length, leaf area, number of leaves, fresh and dry weight, leaf succulence, leaf thickness, relative water content (RWC) and 100 seed weight were affected by salinity and revealed that higher salt concentrations lead to the reduction in plant height in Crotalaria species. The results of our study on black eyed peas show that increased salt concentration during the early seedling stage has a negative effect on development. Although identifying salt-tolerant genotypes at germination is insufficient alone, varieties that tolerate salinity during this critical stage generally retain their resistance throughout subsequent growth.

CONCLUSION

The black eyed peas [*Vigna unguiculata* (L.) Walp], belonging to the Fabaceae family, is a drought-tolerant plant that can grow in sandy, clayey and other soil types. It shows moderate tolerance to salinity. The findings of this study demonstrate that saline conditions adversely affect both germination and seedling development in black-eyed peas. Germination, nodule number and weight decreased at salt concentrations of 400 mM and 450 mM. The tolerance index was measured at 0-450 mM. Analysis of the study results in terms of macro minerals revealed an increase in Na and Cl content with increasing salt concentrations. Understanding the response of black eyed peas development to salinity in its early stages, along with climate change and irrigation water quality, is important. For early-stage growth, cultivation in saline environments up to 300 mM may be recommended.

Disclaimers

The views and conclusions expressed in this article are solely those of the author and do not necessarily represent the views of their affiliated institutions. The author are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The author declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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