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Effect of Fertilizer Application on Biochemical and Physiological Parameters of Salt-Stressed Tomato (*Solanum lycopersicum* L.) Plants

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Abstract

Tomato (*Solanum lycopersicum* L.) is a vital horticultural product that significantly contributes to human nutrition because of its high levels of antioxidants, vitamins, and other health-promoting compounds. It is considered a moderately salt-tolerant species; however, excessive soil salinity is one of the main abiotic stresses limiting its productivity worldwide. High soil salt levels negatively impact seed germination, root and shoot growth, leaf expansion, and overall plant health, ultimately causing severe yield reductions. Salinity stress also leads to ion toxicity, osmotic imbalance, and oxidative damage by stimulating the enhanced production of reactive oxygen species (ROS). As a result, plants show increased levels of proline, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and total sugars, which are markers of stress but also indicate cellular damage. To counteract these harmful effects, proper nutrient management strategies are essential. This study evaluated the exogenous foliar application of DAP fertilizer on tomato seedlings under salt stress. The results showed that additional N and P significantly enhanced seedling growth parameters—including root and stem length, leaf area, and chlorophyll content—while reducing oxidative damage by limiting excess proline, MDA, and H_2O_2 levels. Overall, the findings underscore the effectiveness of DAP fertilizer in alleviating salt stress in tomato seedlings. This approach provides a scientific basis for developing sustainable fertilization practices and offers practical benefits to growers in saline-prone areas by improving seedling establishment and productivity.

Keywords

Solanum lycopersicum L., Salt stress, Fertilizer, Proline, Total sugar content

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Gübre Uygulamasının Tuz Stresine Maruz Kalan Domates (*Solanum lycopersicum* L.) Bitkilerinin Biyokimyasal ve Fizyolojik Parametreleri Üzerine Etkisi

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Özet

Domates (*Solanum lycopersicum* L.), yüksek antioksidan, vitamin ve diğer sağlık yararları sağlayan bileşikleri nedeniyle insan beslenmesine önemli katkı sağlayan hayati bir bahçe bitkisidir. Orta derecede tuz toleranslı bir tür olarak kabul edilir; ancak aşırı toprak tuzluluğu, dünya çapında verimliliğini sınırlayan başlıca abiyotik streslerden biridir. Yüksek toprak tuzu seviyeleri, tohum çimlenmesini, kök ve sürgün gelişimini, yaprak genişlemesini ve genel bitki sağlığını olumsuz etkileyerek sonuçta ciddi verim düşüşlerine neden olur. Tuz stresi ayrıca reaktif oksijen türlerinin (ROS) artan üretimini uyararak iyon toksisitesine, ozmotik dengesizliğe ve oksidatif hasara yol açar. Sonuç olarak, bitkilerde stresin göstergeleri olan ancak aynı zamanda hücresel hasarı da gösteren prolin, malondialdehit (MDA), hidrojen peroksit (H_2O_2) ve toplam şeker seviyelerinde artış görülür. Bu zararlı etkileri ortadan kaldırmak için uygun besin yönetimi stratejileri şarttır. Bu çalışma, tuz stresi altındaki domates fidelerinde DAP gübresinin yapraktan uygulanmasının etkisini değerlendirmiştir. Sonuçlar, ilave N ve P'nin, kök ve gövde uzunluğu, yaprak alanı ve klorofil içeriği de dahil olmak üzere fide büyüme parametrelerini önemli ölçüde iyileştirdiğini ve aşırı prolin, MDA ve H_2O_2 seviyelerini sınırlayarak oksidatif hasarı azalttığını göstermiştir. Genel olarak, bulgular DAP gübresinin domates fidelerinde tuz stresini hafifletmedeki etkinliğinin altını çizmektedir. Bu yaklaşım, sürdürülebilir gübreleme uygulamalarının geliştirilmesi için bilimsel bir temel sağlamakta ve fide gelişimini ve verimliliğini artırarak tuzlu topraklara eğilimli bölgelerdeki yetiştiricilere pratik faydalar sunmaktadır.

Anahtar kelimeler

Solanum lycopersicum L.,
Tuz stresi,
Gübre,
Prolin,
Toplam şeker içeriği

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an edible vegetable that is a rich source of antioxidants, phytochemicals, antimicrobials, and anti-inflammatory compounds [1]. Tomatoes are a moderately salt-tolerant crop cultivated all around the world, but most of the tomato growing regions are found in semi-arid and arid climates where the salinity is a major problem.[2]. High soil salinity in tomatoes leads to various issues, including poor germination, yellowing of mature leaves, and stunted growth.

By 2050, there will likely be 10 billion people on the planet, and in order to feed them, food and feed supplies must rise by 70% [3]. Crop plants undergo morphological, physiological, and biochemical alterations as a result of a variety of biotic and abiotic pressures brought on by climate change [4]. Moreover, these stresses, especially salinity, significantly reduce crop yield and quality. According to FAO (2018) [5], more than 20 km2 of land in arid and semiarid regions becomes exposed to soil salinization each day. Globally, soil salinity directly impacts 5% of the total arable land, ultimately decreasing crop growth and yield. High salt levels in soil alter its physical properties, reducing porosity, hydraulic capacity, and water-holding ability. It also causes hormonal imbalances in plants by lowering cytokinin and gibberellin levels while increasing abscisic acid levels. Additionally, it severely hampers morphophysiological processes by disrupting osmotic and ionic balance in plant cells, leading to nutrient and metabolite imbalances across membranes and hindering cell growth [6, 7]. Long-term salt stress exposure causes major impairments in photosynthesis, Rubisco activity, and stomatal conductance, which directly decrease internal CO2 levels. Plants exposed to salt stress either excrete excess salt or store it in cellular vacuoles. They adapt through numerous physiological and biochemical changes to counteract the negative effects of salt stress [8]. These adaptations may include regulation of growth and development, ROS detoxification, ionic homeostasis, and osmotic adjustment by osmoprotectants [9]. Producing fruits and vegetables of desired quality requires ever-changing strategies that incorporate an increasing understanding of their nutritional value, and health-promoting roles. Current fruit and vegetable production methods, often based on maximizing product volume or weight, are crucial for fertilization [10]. Phosphorus (P) is an essential component of metabolism and regulates the functioning of various pathways involved in the biosynthesis of secondary plant products, many of which are nutraceuticals [11]. Nitrogen (N) is a macronutrient essential for plant growth and development [12]. Although N is a crucial nutrient for plants, it is generally not directly available to plants in agricultural ecosystems. Fertilizer use facilitates N uptake, and only 50% is recovered by the plant, with the remainder lost through soil deposition and atmospheric evaporation [13]. S. lycopersicum requires nitrogen and phosphorus to reach maximum seedling size. DAP fertilizer is applied to tomato leaves to provide rapid nitrogen and phosphorus uptake, especially during periods when the plant has high nutrient demand. Foliar application helps improve photosynthesis, growth, and fruit set more quickly than soil application. The recommended dose is generally 1–2 kg DAP per 1000 L of water (about 100–200 g per 100 L). Since it cannot directly bind N elements from the air, DAP (Diammonium Phosphate) fertilizer, which is rich in nitrogen and phosphorus, is used.

The purpose of this study was to investigate how seedling development in S. lycopersicum cultivated under salt stress was affected by exogenous foliar nitrogen (N) and phosphorus (P) administration. The study determined the morphological, physiological, and biochemical changes caused by salt stress and evaluated the extent to which nitrogen and phosphorus application could mitigate these negative effects. N and P supplementation in the early development stages of tomato seedlings increased root and shoot growth, leaf area, and chlorophyll content. These evaluations will contribute to the development of sustainable fertilization strategies to reduce yield and quality losses under salt stress conditions. The findings may provide advantages to tomato producers during the cultivation process by increasing the seedling establishment rate. The results will provide a scientific basis for the effectiveness of foliar N and P application in tomato cultivation, especially in regions where salinity is prevalent.

2. MATERIAL AND METHOD

2.1. Plant Material and Treatment of Salt Stress

S. lycopersicum seeds were obtained commercially, and the Rio Grande (standard) variety was selected for the study because it does not exhibit high salt tolerance, allowing the effects of elevated salinity stress to be more clearly observed. Four S. lycopersicum seeds were sown in each of the 10-liter pots; the commonly accepted average dimensions are 27–30 cm in height, 28–32 cm in upper diameter, and 20–24 cm in base diameter using a peat, perlite, and soil mixture (2:1:1), and seedling growth lasted 21 days (Table 1). DAP fertilizer (2%) was applied to the tomato seedlings' growth three times on the first day at 1-hour intervals. In the subsequent period, the exogenous application was repeated three more times at three-day intervals. Concurrently with the DAP treatments, the seedlings were also exposed to NaCl solutions at concentrations of 50 and 70 mM. At the end of the 14-day treatment period, the seedlings were harvested. Tissue samples collected from the harvested seedlings were stored at −80 °C for subsequent biochemical analyses.

Table 1 The treatment numbers refer to the specific fertilizer and salt application doses used to evaluate their effects on plant growth.

Treatment No	Treatment
1	Control
2	2% DAP
3	50 mM NaCl
4	70 mM NaCl
5	50 mM NaCl+2% DAP
6	70 mM NaCl+2% DAP

2.2. Quantification of Chlorophyll Content

Plant samples were weighed to 0.2 g and homogenized on ice in the dark using 10 mL of cold 80% acetone. Chlorophyll a and chlorophyll b, and total chlorophyll (a+b) in the extract were measured with a spectrophotometer at 663 and 645 nm wavelengths according to the method of Arnon (1949) [14].

ChlorophyIIa (mg/L)=12.7xA_(663-) 2.69xA_645
ChlorophyIIb (mg/L)=22.9xA_(645-) 4,68xA_663
Total chlorophyII (mg/L)=20.2xA_(645-) 8.02xA_663

2.3. Determination of Malondialdehyde and H_2O_2 Content

MDA content was measured using the method of Dhindsa and Matowe (1981) [15]. 0.2 g of leaf samples were homogenized with a 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The samples were incubated in a water bath at 100 °C for 60 min. The supernatant fraction was measured at 532 and 600 nm. Calculations were performed based on the formula reported by Hodges et al. (1999) [16].

MDA (nmol/mL)=([[(A532-A600)-[(A440-A600)x(0.0571)]])/(15700]x10^6)

H_2O_2 content was measured using the method of Sergiev et al. (1997) [17]. After weighing 0.2 g of leaf material, it was centrifuged at 15,000 rpm for 10 minutes after the addition of 4 mL of trichloroacetic acid. 2 mL of the supernatant, 0.8 mL of KH2PO4 (pH 7.0), and 1.6 mL of KI were added. The absorbance of the products of the leaf samples was measured at 390 nm using the standard chart with H2O2 solutions [18].

2.4. Determination of Soluble Sugar and Proline Contents

0.1 gram of leaves was homogenized and 5 mL of 2.5 N cold HCl was added. Centrifugation was performed at 5000 rpm for 5 min. 2 mL of supernatant was transferred to a glass tube and 2 mL of DNSA (3,5-dinitrosalicylic acid) solution was added. As a blank, 2 mL of DNSA and 2 mL of 2.5 N HCl were added to an empty tube. After this treatment, it was incubated in a water bath at 98 °C for 60 min. The mixture was kept in an ice water bath until it cooled completely. For each sample, 100 µL per well was added to the 96-well plate in triplicate. Samples were measured on a NanoDrop at a wavelength of 550 nm [19].

Proline content was measured by the method suggested by Rodriguez and Redman (2005) [20]. 0.4 g of leaves were homogenized by adding 7 mL of cold 3% aqueous sulfosalicylic acid and centrifuged at 5000 rpm at 4 °C for 15 min. 2 mL of the extract, 2 mL of acid-ninhydrin, and 2 mL of acetic acid were added to each of the empty glass tubes. The samples were incubated at 98 °C for 1 h and placed in an ice water bath to complete the reaction. 4 mL of toluene was used for extraction from this reaction mixture. As a blank, it was measured at 520 nm using ninhydrin, acetic acid, and toluene as blanks. Finally, the proline concentration was determined using a calibration curve.

2.5. Statistical analysis

A single S. lycopersicum Rio Grande (standard) variety was used for each analysis. A total of 18 pots were planted, three for each treatment. Each pot was considered an experimental unit. At least three replicates were performed for each analysis. Two-way analysis of variance (ANOVA) was used for data analysis. Duncan's multiple range test was applied for subsequent multiple comparisons. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 26). $P<0.05$ was set as the significance level for statistical analysis.

3. RESULTS

In the present study, the application of DAP fertilizer to *S. lycopersicum* exposed to salt stress affected the morphological parameters (root, stem, and leaf length of tomato plant growth. Compared to the control (4.06; 4.56; 3 cm), root, leaf, and stem length decreased under salt stress; however, root, leaf, and stem length increased with fertilizer and combinations thereof. The best results were obtained with 50 mM NaCl+Fertilizer (8; 6.66; 6.5 cm) in root, stem, and leaf length (Figures 1 and 2).

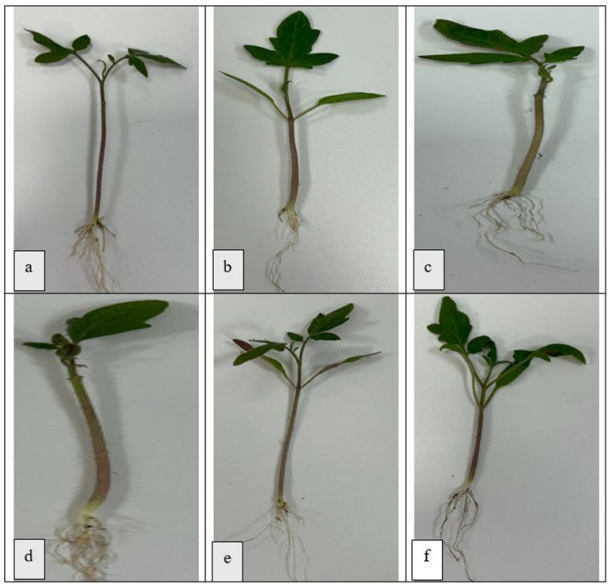

Figure 1. Growth status in *S. lycopersicum* treated with DAP and salt. The numbers on the pictures refer to the treatment
a: Control, b:2% DAP, c: 50 mM NaCl, d: 70 mM NaCl, e: 50 mM NaCl+2% DAP, f: 70 mM NaCl+2% DAP

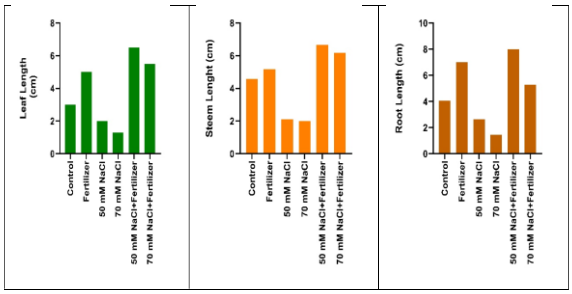

Figure 2. Effect of DAP and salt on root, leaf, and shoot growth in *S. lycopersicum.*

In our study, the amount of MDA, an oxidative stress biomarker, and the level of H_2O_2, a reactive oxygen species (ROS), increased under salt stress compared to the control, whereas both parameters decreased in the DAP and DAP+salt treatments. When the MDA content is compared with the control group (0.036 nmol/g FW), the best results were obtained with the Fertilizer treatment (0.013 nmol/g FW) and 70 mM NaCl+Fertilizer (0.024 nmol/g FW), respectively.

When H2O2 is compared with the control (0.036 µmol/mL), the most effective concentrations are 70 mM NaCl+Fertilizer (0.016 µmol/mL) and 50 mM NaCl+Fertilizer (0.020 µmol/mL), respectively in reducing oxidative damage (Figure 3).

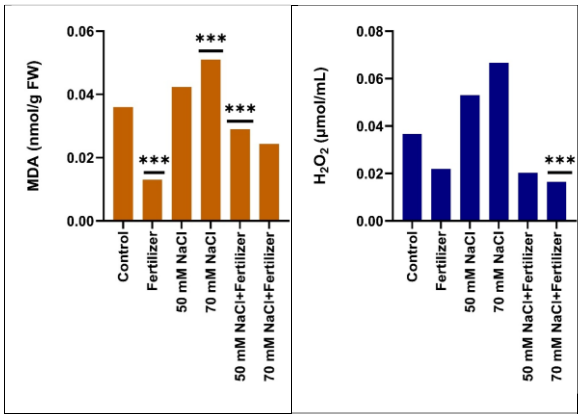

Figure 3. Effect of DAP and salt on MDA and H_2O_2 levels in S. lycopersicum. FW – Fresh weight

S. lycopersicum the accumulation of proline under salinity stress is widely recognized as a physiological response to osmotic imbalance and ionic toxicity induced by NaCl, serving as a compatible osmolyte that helps stabilize proteins and membranes under stress conditions. Salinity treatments have been shown to increase proline content in tomato tissues as part of the plant's adaptive mechanism to maintain cellular homeostasis under high salt environments, rather than simply reflecting a reduction in stress due to fertilization. For example, proline levels typically rise as salinity increases, indicating activation of stress-response pathways and proline biosynthesis enzymes, which may be modulated differently depending on nutrient supply and stress severity. In this study, proline levels increased with increasing salt stress compared to the control, and decreased with the application of fertilizer and its combination. When the amount of proline was compared to the control (0.331 µmol/g FW), the lowest proline accumulation was in Fertilizer (0.24 µmol/g FW), 70 mM NaCl+ Fertilizer (0.291 µmol/g FW), and 50 mM NaCl+ Fertilizer (0.297 µmol/g FW), respectively. Total sugars decreased with increasing salt stress compared to the control, and increased with fertilizer and its combination. When the amount of total sugars was compared to the control (1.443 mg/g FW), the highest accumulation was in Fertilizer (1.715 mg/g FW) and 50 mM NaCl Fertilizer (1.684 µmol/g FW). (Figure 4).

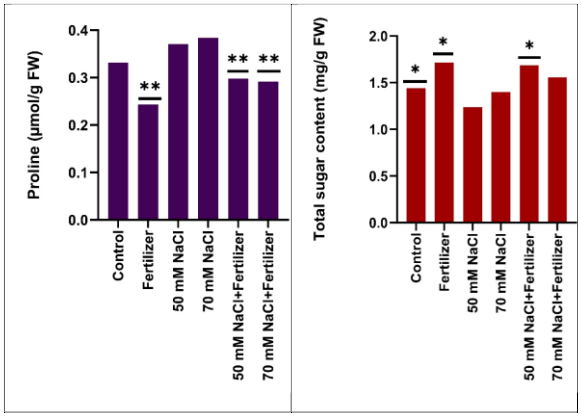

Figure 4. Effect of DAP and salt on total sugar content and proline levels in *S. lycopersicum*. FW – fresh weight

Our results show that the amount of chlorophyll decreased with increasing salt stress compared to the control, while the amount of chlorophyll increased with fertilizer and salt treatment. As a result, when the amount of chlorophyll a, b and total chlorophyll was compared with the control (22.2,11.65, and0.72 mg/g FW, respectively), the highest chlorophyll content was found in 70 mM NaCl+Fertilizer (31.16, 14.89, and 2.86 mg/g FW, respectively) (Figure 5).

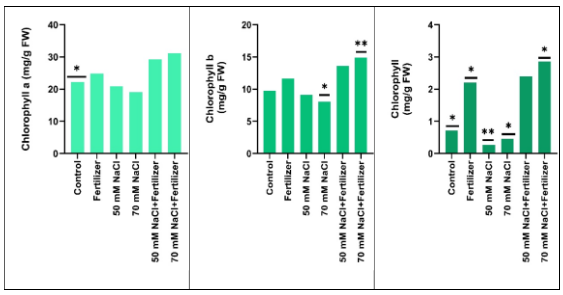

Figure 5. Effect of DAP and salt on Chlorophylla, Chlorophyll b, and Chlorophyll content accumulation in *S. lycopersicum*. FW – fresh weight

4. DISCUSSION AND CONCLUSION

Zhang et al. (2020) [21] showed a positive effect of mineral fertilization on plant height in kiwifruit (Actinidia chinesis). DAP significantly increased the yield and quality of tomato seedling establishment under salinity. This can be expressed by mineral nutrition, which

is considered the most important production factor for crop yield after water availability [22].

In order to boost their tolerance and defend themselves against environmental stress, plants raise their MDA and H_2O_2 concentration. These parameters are commonly used as indicators for evaluating stress tolerance among genotypes [23]. Our investigations have shown that increasing salt concentrations induce excessive production of reactive oxygen species (ROS), leading to oxidative stress and membrane damage unless efficiently scavenged by the plant's antioxidant defense system.This depends on the plant variety, stress factor, and stress intensity. Plants exposed to salt stress accumulate compatible solutes in the cytoplasm and vacuoles to maintain ionic balance [24]. The most important feature of osmolytes, one of these substances, is the preservation of cell structure and maintaining osmotic balance through balanced water flow [25]. Previous studies have shown a significant correlation between the response to abiotic stresses, especially salinity, and ROS in plants. ROS metabolism and antioxidant defense system were analyzed in four tomato cultivars subjected to salt stress and grown under in vitro conditions [26]. An increase in H_2O_2 levels as a result of salinity stress has been reported in various plant genotypes. A significant increase in H_2O_2 levels was observed in tomatoes, especially in susceptible cultivars, under salinity stress [27].

Chlorophylls are ubiquitous and essential components of photosynthetic membranes in plants [32]. The formation of photosynthetic pigments depends largely on the available nitrogen concentration in plants [33]. Higher amounts of chlorophyll and carotenoids may be associated with higher nitrogen availability provided by fertilizer, which stimulates the chlorophyll biosynthesis process [34]. Our results show that the amount of chlorophyll decreased with increasing salt stress compared to the control, while the amount of chlorophyll increased with fertilizer and salt treatment.

In this study, the effects of exogenously sprayed nitrogen and phosphorus applications on the development of tomato seedlings exposed to salt stress were investigated. Salt stress resulted in increased root length, decreased leaf and stem length, and decreased chlorophyll content compared to the control. Salt stress also led to increased levels of MDA and H_2O_2, biomarkers of oxidative stress. Exogenous foliar application of DAP fertilizer helped reduce oxidative stress by reducing MDA and H_2O_2 levels. Furthermore, by maintaining ion balance and supporting osmoprotectant accumulation, plant stress tolerance increased. These findings suggest that the negative effects of salt stress can be partially mitigated through fertilization (DAP) strategies. Exogenous foliar DAP application has the potential to reduce yield and quality losses by increasing seedling establishment rates in areas with widespread salinity. In conclusion, the study contributes scientifically to the development of sustainable and effective fertilization methods in tomato cultivation.

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