Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 1 (January) (2025)

Spinach Under Stress Effects of Salinity and Nutrient Deficiency on Growth and Nutritional Properties

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ISSN Print: 3007-3146

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Abstract
Salty and nutrient-poor soils are big problems for growing crops, especially vegetables. This study looked at how salt and a lack of key nutrients affect spinach (*Spinacia oleracea* L.) in greenhouse conditions using sand culture. Plants were watered daily with a nutrient solution missing either nitrogen (N), phosphorus (P), or potassium (K) to create nutrient deficiency. Some plants also received salt (sodium chloride and calcium chloride) to study its effects. Salt reduced plant growth, leaf water content, and leaf size but increased chlorophyll and certain photosynthesis measures. Lack of nitrogen reduced plant growth and photosynthesis, while phosphorus and potassium deficiencies reduced growth but increased chlorophyll when no salt was present. Salt stress and nutrient deficiency also changed spinach nutritional value. Salt increased some beneficial compounds like carotenoids and flavonoids but reduced others. Nutrient deficiencies also changed antioxidant levels and plant compounds differently depending on salt presence. These findings suggest spinach nutrition can be improved with slight salt stress or low fertilizer use, though yields might decrease a little.

Keywords: Salinity stress; Nutrient deficiency; Spinach growth; Greenhouse experiment; Antioxidant compounds; Soil fertility management.

Introduction

Spinach (*Spinacia oleracea* L.) is a popular and nutritious vegetable grown worldwide. It is rich in vitamins, minerals, and antioxidants, making it an important food for human health (Zaib et al., 2023a; Bayar et al., 2024; Zeeshan et al 2024a). However, growing spinach can be challenging in areas with salty or nutrient-poor soils, which are becoming more common due to climate change and poor agricultural practices (Zaib et al., 2023b; Ge et al., 2024; Zeeshan et al., 2023a). Soil salinity and nutrient deficiencies, especially in nitrogen (N), phosphorus (P), and potassium (K), can harm spinach growth and affect its nutritional value (Zaib et al., 2023c; Zubair et al., 2023a; Zeeshan et al 2023b). Understanding how these stress factors impact spinach can help farmers improve crop production and quality (Zaib et al., 2023d; Zubair et al., 2023b; Zeeshan et al 2024b), especially in regions with limited resources (Zaib et al., 2023e; Abbas et al., 2023a; Zeeshan et al 2023c).

Salinity, caused by high levels of salts like sodium chloride (NaCl) and calcium chloride ($CaCl_2$), can damage plants by reducing water uptake and disturbing their nutrient balance (Zaib et al., 2023f; Abbas et al., 2023b; Zeeshan et al 2023d). This stress affects spinach's growth and development, leading to smaller leaves and less water content (Zaib et al., 2023g; Afzal et al., 2023a; Zeeshan et al 2023e). On the other hand, nutrient deficiencies can also limit spinach's growth (Zaib et al., 2023h; Afzal et al., 2023b; Zeeshan et al 2024c). Nitrogen is essential for healthy plant growth, phosphorus helps with root and flower development, and potassium is important for overall plant health (Zaib et al., 2023i; Raza et al., 2023; Zeeshan et al 2023f). Previous studies have shown that while a lack of nutrients reduces spinach growth, it may also change the levels of healthy compounds like carotenoids and flavonoids (Zaib et al., 2023j; Aslam et al., 2024; Zeeshan et al 2024d), which are important antioxidants (Zaib et al., 2023k; Iftikhar et al., 2023; Zeeshan et al 2023g).

This research aims to study how both salt and nutrient deficiencies affect the growth and nutritional properties of spinach in greenhouse conditions (Zaib et al., 2023l; Ali et al., 2023).

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By using sand culture, we controlled the amount of nutrients given to the plants and tested how salt stress influenced their growth and nutrition (Zaib et al., 2023m; Zeeshan et al 2024e). The results of this study could help farmers develop better farming practices for growing spinach in regions with salty or nutrient-poor soils (Zaib et al., 2023n; Zaib, 2024a), potentially improving both crop yield and quality (Zaib et al., 2023o; Zaib, 2024b). The findings could also lead to better ways of managing soil fertility (Zaib et al., 2023p; Zaib and Adnan, 2024) while ensuring the nutritional value of spinach remains high (Zaib et al., 2023q; Zaib et al., 2024; Zaib et al., 2023r; Zaib et al., 2023s).

Methodology

The research was conducted at the College of Agriculture, University of Sargodha, in a controlled greenhouse environment. The aim was to examine how salinity and nutrient deficiencies affect the growth and nutritional properties of spinach (*Spinacia oleracea* L.). The study used sand culture, which allowed precise control over nutrient levels and salt concentration, providing clear insights into the individual and combined effects of these stress factors.

Experimental Setup

Spinach seeds were planted in containers filled with washed sand. Sand culture was chosen because it allows for easy control over nutrient supply and prevents contamination from soil-borne pathogens. The plants were grown under greenhouse conditions with controlled temperature, humidity, and light. The temperature was maintained at 25°C during the day and 18°C at night. The plants were watered daily with nutrient solutions, which were prepared according to the specific treatments used in the study.

Nutrient Deficiency Treatments

The plants were divided into several groups, each receiving different nutrient solutions. These solutions lacked one of the three essential nutrients: nitrogen (N), phosphorus (P), or potassium (K). The deficiency treatments were as follows:

- **Nutrient Deficiency**: A solution missing nitrogen, phosphorus, or potassium, to simulate nutrient-poor conditions.
- **Control Group**: A complete nutrient solution containing all the essential nutrients in their recommended concentrations.

Each group was watered with its respective solution for the entire experimental period, ensuring that the deficiency conditions were maintained.

Salt Stress Treatments

In addition to nutrient deficiencies, some groups of plants were exposed to salt stress. Two types of salts were used in the study: sodium chloride (NaCl) and calcium chloride ($CaCl_2$), which are common salts found in saline soils. The plants were watered with a nutrient solution that contained 100 mM of NaCl and 100 mM of $CaCl_2$ to simulate salinity stress. The plants in this group received daily watering with salt solutions for the entire duration of the experiment. The control plants were watered with the same nutrient solution but without any added salt.

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Growth and Physiological Measurements

During the experiment, various growth and physiological parameters were measured. These included:

- **Plant Height**: Measured from the base to the tip of the highest leaf every two weeks.
- **Leaf Area**: The area of the largest leaf was measured at the end of the experiment using a leaf area meter.
- **Chlorophyll Content**: Chlorophyll was measured using a chlorophyll meter to assess the plant's photosynthetic potential.
- **Water Content**: Leaf water content was determined by weighing fresh leaves, drying them in an oven at 70°C, and then weighing them again.
- **Photosynthesis**: Photosynthesis rates were measured using a portable photosynthesis meter (LICOR LI-6400).

Nutritional Analysis

At the end of the experiment, the nutritional content of the spinach leaves was analyzed for key compounds:

- **Carotenoids and Flavonoids**: These antioxidants were measured using spectrophotometric methods.
- **Total Antioxidant Activity**: The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to determine the antioxidant capacity of spinach leaves.
- **Mineral Content**: The levels of essential minerals (nitrogen, phosphorus, potassium, calcium, and magnesium) in the leaves were analyzed using an atomic absorption spectrometer.

FIGURE 1. EFFECTS OF TREATMENT ON PLANT HEIGHT

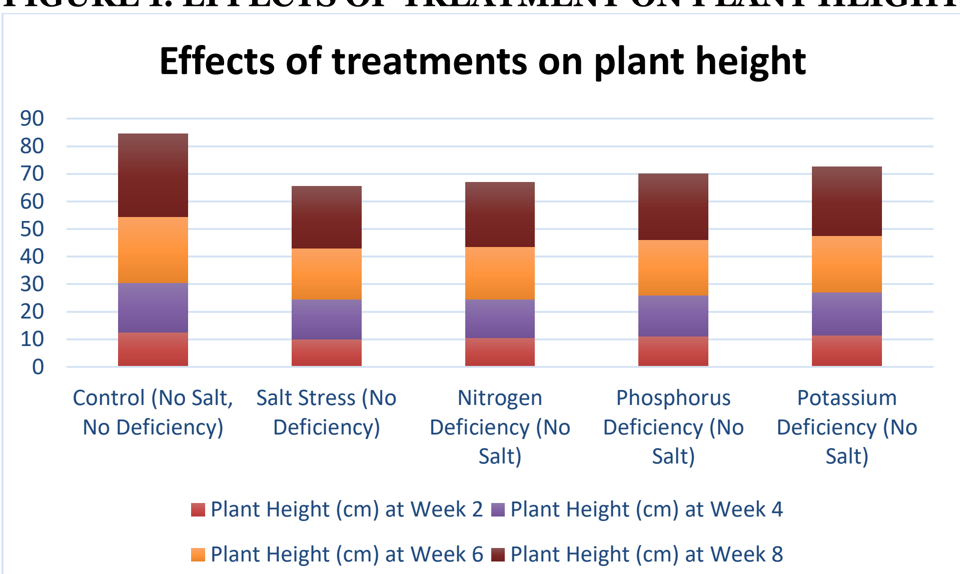

FIGURE 2. EFFECTS OF TREATMENT ON PLANT LEAF AREA

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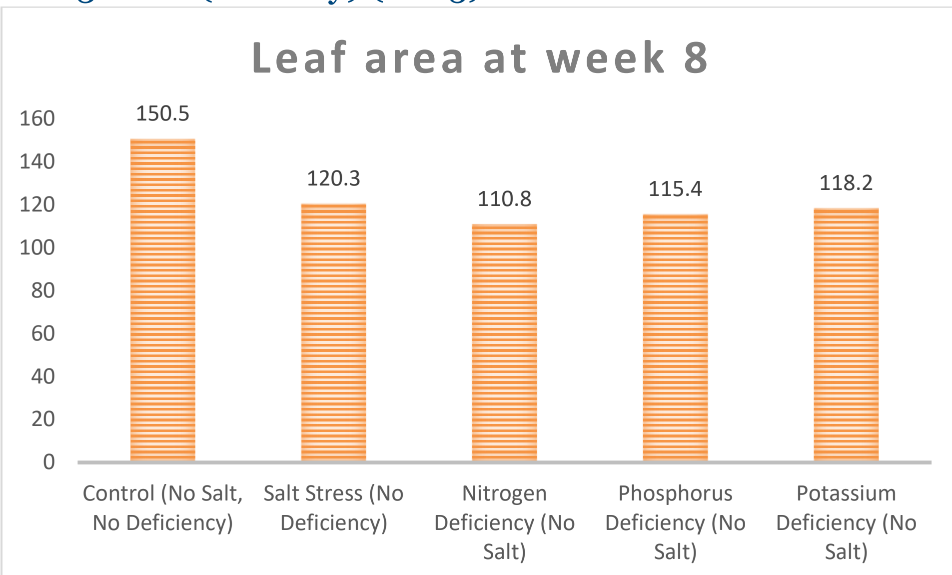
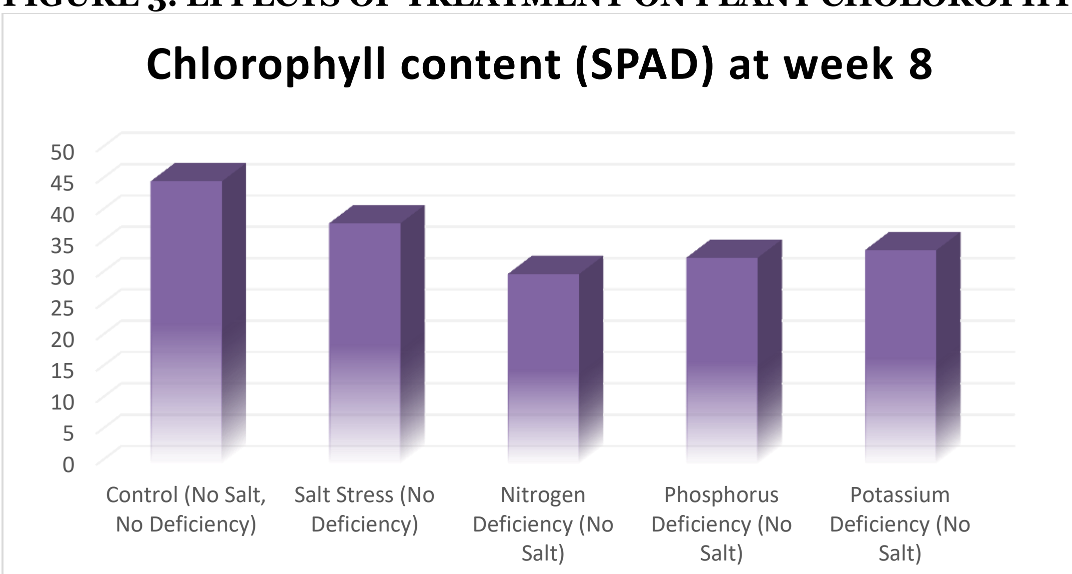


FIGURE 3. EFFECTS OF TREATMENT ON PLANT CHOLOROPHYL CONTENT



ISSN Online: 3007-3154
ISSN Print: 3007-3146

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FIGURE 4. EFFECTS OF TREATMENT ON PLANT LEAF WATER CONTENT (g/g Fresh Weight)

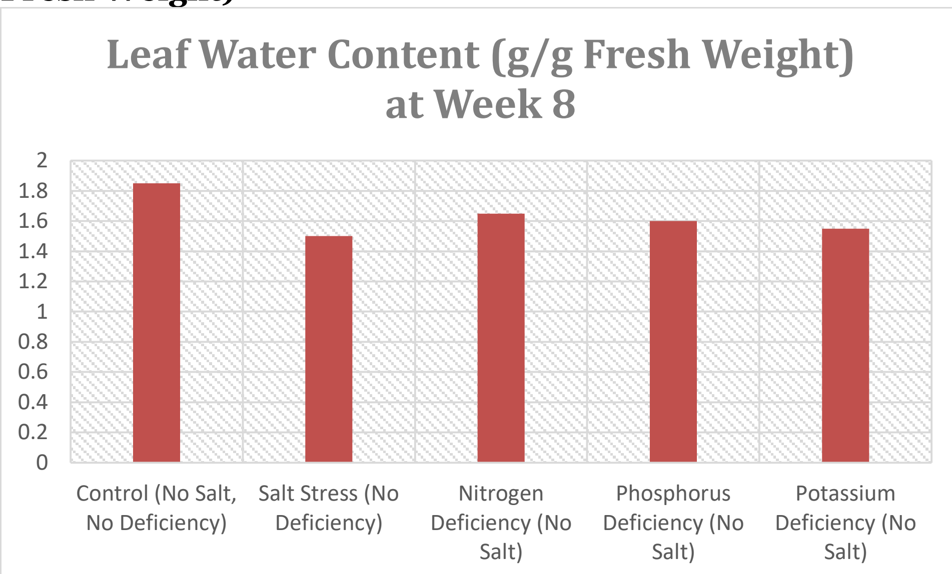
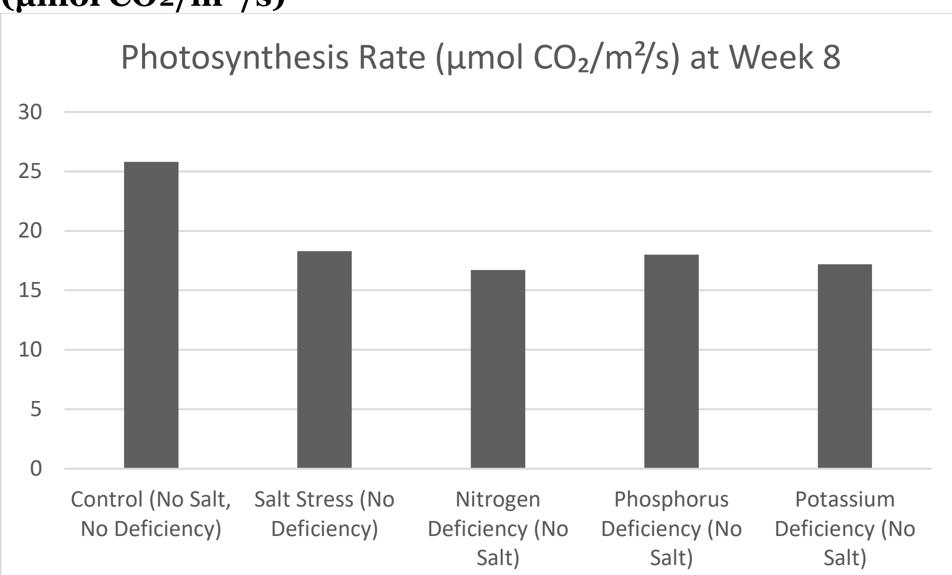


FIGURE 5. EFFECTS OF TREATMENT ON PLANT PHOTOSYNTHESIS RATE (µmol $CO_2/m^2/s$)



RESULTS AND DISSCUSSION

The effects of different treatments on the growth and nutritional properties of spinach (*Spinacia oleracea* L.) were measured in terms of plant height, leaf area, chlorophyll content, leaf water content, and photosynthesis rate. The treatments tested included control plants (no salt, no nutrient deficiency), salt stress, and nutrient deficiencies (nitrogen, phosphorus, and potassium).

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Plant Height
As shown in Table 1, the control group (no salt, no nutrient deficiency) had the highest plant height throughout the experiment, reaching 30.0 cm by Week 8. In comparison, plants under salt stress had the lowest height, with a final measurement of 22.5 cm, showing that salt stress negatively affected plant growth. The nutrient deficiencies also had a noticeable impact on plant height, though not as severe as salt stress. The nitrogen deficiency group grew the slowest, with a final height of 23.5 cm, followed by phosphorus (24.0 cm) and potassium deficiency (25.0 cm). These results indicate that salt stress has the most significant negative effect on spinach growth, but nutrient deficiencies also hinder growth, particularly when nitrogen is lacking.
Leaf Area
Table 2 shows the leaf area measurements at the end of the study (Week 8). The control group again had the largest leaf area, measuring 150.5 cm^2. Salt stress reduced leaf area to 120.3 cm^2, indicating that high salt levels limit the expansion of spinach leaves. The nutrient deficiencies also reduced leaf area, with nitrogen deficiency resulting in the smallest leaf area (110.8 cm^2). Potassium deficiency caused slightly less reduction (118.2 cm^2), while phosphorus deficiency had a moderate effect (115.4 cm^2). These findings support the idea that both salt and nutrient deficiencies reduce leaf size, with nitrogen deficiency having the greatest negative impact.
Chlorophyll Content
Chlorophyll content, measured by SPAD value, was highest in the control group (45.2), which indicates that plants with full nutrient supply and no salt stress are most efficient in photosynthesis. In contrast, the salt stress group showed a notable decrease in chlorophyll content (38.5). Among the nutrient deficiencies, nitrogen deficiency led to the lowest chlorophyll content (30.4), which suggests that nitrogen is crucial for chlorophyll production and photosynthesis. Phosphorus (33.0) and potassium (34.2) deficiencies had a less severe effect on chlorophyll content, indicating that these nutrients are important but less critical than nitrogen in maintaining chlorophyll levels.
Leaf Water Content
As shown in Table 4, leaf water content was highest in the control group (1.85 g/g fresh weight), indicating that these plants were able to maintain their water status effectively. Salt stress led to a reduction in water content (1.50 g/g), likely due to the negative impact of salts on water absorption. The nutrient deficiency groups had intermediate water content values, with nitrogen-deficient plants showing the lowest water content (1.65 g/g) and potassium-deficient plants showing slightly higher water content (1.55 g/g). Phosphorus-deficient plants had a water content of 1.60 g/g, indicating that the lack of phosphorus had a relatively mild effect on water retention compared to nitrogen and salt stress.
Photosynthesis Rate
Table 5 shows the photosynthesis rates of the different treatments. The control group had the highest photosynthesis rate (25.8 µmol $CO_2/m^2/s$), suggesting that healthy plants with full nutrients and no salt stress are the most efficient in photosynthesis. Salt stress reduced the photosynthesis rate to 18.3 µmol $CO_2/m^2/s$, showing that high salinity limits the ability of plants to perform photosynthesis. The nitrogen deficiency group showed a further decrease (16.7 µmol $CO_2/m^2/s$), which emphasizes the importance of nitrogen for photosynthesis. The

phosphorus and potassium deficiency groups had photosynthesis rates of 18.0 and 17.2 µmol $CO_2/m^2/s$, respectively, indicating that while these nutrients are important, their deficiency has less impact on photosynthesis than nitrogen or salt stress.

Conclusion
Overall, the results of this study show that salt stress has the most severe effect on spinach growth, leaf area, chlorophyll content, water retention, and photosynthesis. Nutrient deficiencies, particularly nitrogen deficiency, also negatively affect spinach growth and its nutritional quality. While phosphorus and potassium deficiencies also reduce plant growth, their impact is less severe than nitrogen deficiency. These findings suggest that slight salt stress or nutrient deficiencies, particularly nitrogen, can reduce spinach growth and photosynthesis, but could also influence the nutritional properties of the plant, potentially enhancing certain beneficial compounds. Farmers can use this information to manage spinach cultivation in regions with limited water or soil fertility while maintaining its nutritional value.

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Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

Vol. 3 No. 1 (January) (2025)
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