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## Research Article

# Effect of Macronutrient Levels on Growth and Development of Spinach (*Spinacia oleracea* L.)

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## ABSTRACT

Spinach (*Spinacia oleracea* L.) is a nutrient-dense leafy vegetable of global importance, valued for its health-promoting properties. However, its growth and productivity are often constrained by inadequate nutrient management practices. To address this, a study was conducted from November 2022 to February 2023 at the Nursery Department of Horticulture, Sindh Agriculture University, Tandojam, with the objective of evaluating the response of spinach growth to varying levels of macronutrients. The experiment was laid out in a Completely Randomized Design (CRD) with three replications. Two spinach varieties, namely Sindhi spinach and English spinach, were cultivated in pots, and macronutrients were applied as treatments, including N<sub>1</sub> = Control N<sub>2</sub> = NPK (2g L<sup>-1</sup>) N<sub>3</sub> = Calcium Nitrate (0.5g L<sup>-1</sup>) and N<sub>4</sub> = NPK (2g L<sup>-1</sup>) + Calcium Nitrate (0.5g L<sup>-1</sup>). The results of the present study revealed that English spinach consistently outperformed Sindhi spinach across growth parameters, while the combined application of NPK and calcium nitrate produced the most favorable outcomes. Overall, the study demonstrates that integrated nutrient management, particularly NPK with calcium nitrate, significantly enhances spinach growth and variety performance.

**Keywords:** Macronutrients, Spinach, Nutrient combination, Calcium Nitrate, Growth

## INTRODUCTION

Spinach (*Spinacia oleracea* L.), a member of the family Chenopodiaceae, is one of the most popular leafy vegetable crops grown and consumed worldwide. It is valued for its high nutritional content and versatility in culinary use (Zhou *et al.*, 2018). Originating from southwestern Asia, spinach has spread to many regions across the globe, including Europe, North America, Australia, and parts of Africa. It is widely appreciated for its health-promoting properties and forms an essential part of diets across cultures. Spinach is an excellent source of vital vitamins and minerals, particularly vitamins A and C, iron, and calcium. It is also low in fat and calories and high in dietary fiber, making it a health-promoting food suitable for a wide range of nutritional needs (Umar *et al.*, 2007). In terms of culinary use, spinach can be consumed raw in salads or cooked in a variety of dishes, such as soups, pastas, omelets, and as filling in pastries and pies (Max *et al.*, 2016). Beyond its dietary applications, spinach has been historically recognized for its medicinal properties. It contains potent antioxidant and anti-inflammatory compounds that may help lower the risk of chronic illnesses such as cardiovascular diseases and cancer. Due to its dense nutrient profile and broad adaptability, spinach has earned global importance both as a food crop and a medicinal plant (Isaza *et al.*, 2025).



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Spinach is a cool-season annual crop, typically propagated via direct seed sowing, either through broadcasting or hill sowing in well-prepared dry soil (Vignesh *et al.*, 2012). It is a shallow-rooted crop, relatively easy to cultivate and manage, particularly in controlled environments such as nurseries or greenhouses. In Egypt and other parts of the world, it is considered one of the most important leafy vegetables due to its high market value and demand. As with many green leafy vegetables, spinach is packed with antioxidants and bioactive compounds and is often referred to as one of "nature's anti-aging wonders" due to its role in combating degenerative diseases and promoting healthy digestion (Ahmadi *et al.*, 2010; Rabie *et al.*, 2014).

In African regions, including rural and urban areas, spinach is widely grown by smallholder farmers, both for subsistence and income generation (Kibirige *et al.*, 2013). Its short growing cycle, low input requirements, and adaptability to small-scale farming make it an ideal crop for enhancing household food security. However, productivity among smallholder spinach growers often remains below potential due to limited access to improved agricultural inputs and nutrient management practices (Aisha *et al.*, 2013). For instance, spinach yields from smallholder farms can be as low as 1500 bundles per hectare, compared to 1700 bundles per hectare on commercial farms (Abu *et al.*, 2011). Improving spinach production under such conditions requires a better understanding of nutrient requirements and cost-effective fertilization strategies.

Macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) are essential for plant development. Nitrogen supports vegetative growth, phosphorus aids in root and seed development, and potassium enhances overall plant resilience and quality (Gianquinto *et al.*, 2013; Ullah *et al.*, 2024). Deficiencies in any of these macronutrients can severely limit crop performance. Proper macronutrient management is, therefore, a cornerstone of modern agronomic practices (Joshi & Bhamburdekar, 2015). Research has shown that combined applications of NPK fertilizers with secondary nutrients like calcium nitrate can significantly boost vegetative and root growth in many crops (Sheikhi & Ronaghi, 2012; El-Saady, 2016, Chachar *et al.*, 2024).

While chemical fertilizers are effective in supplying essential nutrients and boosting yields, their overuse can lead to increased production costs, soil degradation, and environmental pollution (Adesemoye *et al.*, 2009). One common alternative explored globally is the use of organic amendments such as sewage sludge, which can enhance soil fertility and crop productivity if properly treated and managed. However, concerns exist regarding the presence of pathogens and heavy metals in untreated sludge, which can lead to long-term environmental and health hazards (Zhang *et al.*, 2017). The accumulation of heavy metals in soil and plants can be influenced by factors such as soil pH, organic matter content, and microbial activity, and may pose serious risks including neurological and developmental disorders and even carcinogenic effects in humans (Arora *et al.*, 2008). Given the increasing demand for nutrient-rich crops, optimizing fertilization strategies for spinach is essential. The specific effects of NPK and calcium-based fertilizers on spinach under local agro-climatic conditions remain underexplored. This study, therefore, evaluates macronutrient treatments on two spinach varieties to identify effective strategies for enhancing germination, growth, and root development.

## MATERIALS AND METHODS

The present research study has been conducted during November 2022 to February 2023 at the SAU Nursery, Department of Horticulture, Sindh Agriculture University, Tandojam to investigate the response of spinach (*Spinacia oleraceae* L.) growth under various levels of nutrients. Pot experiments were selected to provide uniform growth conditions, minimize variability from heterogeneous field soils, and allow precise control of nutrient application and water management. Seeds of two varieties were sown in pots (12) containing media soil + silt in the ratio of 1:1 after germination seedling were thinned out and kept two per pot three pots per treatment per variety were maintained for experiment. The details of the experiment are as under:

### Experimental design

The experiment was laid out in a factorial Completely Randomized Design (CRD) with three replications. Two factors were evaluated: spinach varieties (Factor A) and nutrient combinations (Factor B). Factor A included two varieties, Sindh spinach ( $V_1$ ) and English spinach ( $V_2$ ). Factor B comprised four nutrient combinations:  $N_1$  = Control,  $N_2$  = N.P.K (2g L<sup>-1</sup>),  $N_3$  = Calcium Nitrate (0.5g L<sup>-1</sup>) and  $N_4$  = N.P.K (2g L<sup>-1</sup>) + Calcium Nitrate (0.5g L<sup>-1</sup>).

### Soil and Media Properties

The potting mixture (soil + silt, 1:1) was analyzed before sowing. The soil was sandy clay loam with pH 7.6, electrical conductivity 1.3 dS m<sup>-1</sup>, organic matter 0.65%, available nitrogen 28.4 mg kg<sup>-1</sup>, available phosphorus 6.8 mg kg<sup>-1</sup>, and available potassium 115.6 mg kg<sup>-1</sup>.

### Environmental Conditions

The experiment was conducted under natural field conditions in the SAU nursery area. Average temperature during the experimental period ranged from 14–28 °C, with relative humidity between 55–70%. Day length varied between 10–11 hours, and plants were irrigated with canal water as required to maintain adequate soil moisture.

### Data collection and methodology

The data on seed germination and growth-related attributes were recorded for further statistical analysis. The seed germination (%), germination index (GI), plant height (cm), leaves per plant, leaf length (cm), leaf width (cm), leaf weight (g), fresh biomass of root (g) and root depth (cm) were recorded.

#### Seed germination (%)

Seed germination percentage was recorded by counting the number of germinated seeds out of total number of seeds sown and expressed as a percentage.

#### Germination index (GI)

Germination index was calculated on the basis of germination per day. The germination index was calculated by using the following formula used by Association of Official Seed Analysts (1983).

$$GI = \sum \frac{Gt}{Dt}$$

Whereas, Gt = Number of seeds germinated on day t and Dt = Number of days after sowing (day t)

#### Plant height (cm)

Five plants of each variety per treatment were selected at random from pots and their height was measured from ground surface to the top with foot scale and the average tallness was worked out in cm at the time of harvest.

#### Number leaves per plant

For the parameter of leaves per plant, 5 plants were randomly selected from each treatment within every replication. The number of leaves per plant was counted visually, and the mean values were calculated to represent the average for each variety under the respective treatments.

#### Leaf length (cm)

Leaf length was measured in centimeters using a foot scale, placed along the midrib from the leaf apex to the base. Observations were recorded for each variety under the respective treatments.

#### Leaf width (cm)

Leaf width was measured in centimeters at the top, middle, and bottom portions of each leaf from randomly tagged plants in each treatment, after which the mean value was calculated.

#### Leaf weight (g)

The fresh weight of individual leaves was determined in grams. Five plants per variety were randomly selected and tagged in each treatment, and the mean leaf weight was calculated.

#### Fresh root biomass (g)

At harvest, roots from five randomly selected plants per treatment were carefully excised, washed with water to remove adhered soil particles, air-dried at room temperature, and weighed using an electronic balance. Data were expressed in grams, and average values were computed.

#### Root depth (cm)

Following harvest, roots of the selected plants were washed to remove soil residues, air-dried at room temperature, and measured for depth (cm) using a scale.

### Statistical analysis

The experimental data were statistically analyzed using Statistix 8.1 software (Analytical Software, 2006). Analysis of variance (ANOVA) was performed according to the factorial design of the experiment to determine the significance of varietal differences, nutrient treatments, and their interactions. Where the F-test indicated significant differences, Least Significant Difference (LSD) test at 5% probability level ( $p \leq 0.05$ ) was employed to separate and compare treatment means. This approach ensured reliable interpretation of treatment effects on the measured growth and yield parameters of spinach.

## RESULTS

The present research was carried out in 2022-23 at the SAU Nursery Department of Horticulture, Sindh Agriculture University, Tandojam. The data and analysis of variance on the studied parameters are presented in Tables 1 to 5.

#### Seed Germination (%)

Seed germination data for Sindhi and English spinach varieties exposed to different nutrient combinations are summarized in Table 1. ANOVA indicated a significant impact of nutrient treatments ( $P < 0.05$ ) and varieties ( $P < 0.05$ ), whereas the nutrient  $\times$  variety interaction was non-significant ( $P > 0.05$ ). The greatest seed germination (95.54%) was

observed with the combined application of NPK 2 g L<sup>-1</sup> + calcium nitrate 0.5 g L<sup>-1</sup> (N<sub>4</sub>), followed closely by calcium nitrate alone (93.35%). The control (84.60%) and NPK alone (82.86%) recorded the lowest germination percentages. Between varieties, English spinach showed a significantly higher mean germination (95.29%) compared to Sindhi spinach (82.88%). Although the interaction effect was non-significant, both varieties responded consistently, with English spinach performing better across all treatments.

#### **Germination index (GI)**

Germination index data for Sindhi and English spinach varieties under different nutrient combinations are also presented in Table 1. ANOVA revealed a highly significant effect of varieties ( $P < 0.01$ ), while nutrient treatments and the nutrient  $\times$  variety interaction were non-significant ( $P > 0.05$ ). Across nutrient treatments, the highest germination index (1.28) was recorded with calcium nitrate (N<sub>3</sub>), followed by NPK + calcium nitrate (0.73) and the control (0.67). NPK alone (0.76) resulted in the lowest germination index. With respect to varieties, Sindhi spinach exhibited a significantly higher germination index (1.34) than English spinach (0.37), reflecting greater uniformity and speed of germination. The non-significant interaction indicated that both varieties showed similar response patterns across nutrient treatments.

#### **Plant height (cm)**

Plant height data for Sindhi and English spinach varieties under different nutrient combinations are summarized in Table 2. ANOVA indicated a highly significant effect of nutrient treatments ( $P < 0.001$ ) and nutrient  $\times$  variety interaction ( $P < 0.001$ ), whereas varietal differences were non-significant ( $P > 0.05$ ). The interaction effect showed that English spinach under control (N<sub>1</sub>) produced the tallest plants (21.13 cm), while Sindhi spinach under the same treatment reached only 8.43 cm. Conversely, Sindhi spinach under NPK + calcium nitrate (N<sub>4</sub>) attained 16.50 cm, substantially taller than English spinach (8.60 cm) under the same treatment. The lowest plant height (6.60 cm) was observed in English spinach with calcium nitrate (N<sub>3</sub>), while Sindhi spinach under the same treatment grew to 11.33 cm. Across nutrient treatments, the highest mean height was observed in N<sub>1</sub> (14.78 cm), followed by N<sub>4</sub> (12.55 cm), while the lowest mean was recorded in N<sub>3</sub> (8.96 cm). Between varieties, mean plant height was almost similar (Sindhi: 11.61 cm; English: 12.40 cm), indicating that overall varietal differences were not significant.

#### **Number of leaves per plant**

Number of leaves per plant for Sindhi and English spinach varieties under different nutrient combinations is also presented in Table 2. ANOVA revealed a significant nutrient  $\times$  variety interaction ( $P < 0.01$ ), while nutrient treatments alone showed a marginal effect ( $P = 0.0577$ ) and varietal differences were non-significant ( $P > 0.05$ ). The interaction effect showed that Sindhi spinach under NPK + calcium nitrate (N<sub>4</sub>) produced the maximum number of leaves (9.00), while under control conditions (N<sub>1</sub>), it produced the lowest (3.33). English spinach, on the other hand, produced the maximum number of leaves under control conditions (7.33), while the lowest was noted with calcium nitrate (N<sub>3</sub>: 4.00). Across nutrient treatments, the overall highest mean number of leaves (7.50) was observed with NPK + calcium nitrate (N<sub>4</sub>), followed by the control (5.33), while the lowest (5.00) was recorded under NPK alone and calcium nitrate. Between varieties, English spinach (5.83) and Sindhi spinach (5.58) did not differ significantly, showing that the varietal main effect was not pronounced.

#### **Leaf Length (cm)**

The findings related to Leaf length (cm) of Sindhi and English spinach varieties under varying nutrient combinations are presented in Table 3. Analysis of variance (ANOVA) revealed that varietal differences were significant ( $P < 0.01$ ), and the nutrient  $\times$  variety interaction was highly significant ( $P < 0.001$ ). In contrast, the main effect of nutrient treatments alone was not statistically significant ( $P > 0.05$ ). The interaction effect showed that English spinach under control conditions (N<sub>1</sub>) produced the longest leaves (11.76 cm), whereas Sindhi spinach under the same treatment exhibited the shortest (3.50 cm). In contrast, Sindhi spinach responded positively to NPK + calcium nitrate (N<sub>4</sub>), producing 7.46 cm leaves compared to only 6.10 cm in English spinach. The lowest overall leaf length was observed in Sindhi spinach under control (3.50 cm). Across nutrient treatments, mean values showed the highest leaf length in N<sub>1</sub> (7.63 cm), followed by N<sub>4</sub> (6.78 cm), whereas the lowest was in N<sub>3</sub> (5.81 cm). Between varieties, English spinach (7.82 cm) had significantly longer leaves than Sindhi spinach (5.35 cm), indicating a strong varietal effect.

English spinach showed greater leaf length in the control treatment than in fertilized treatments, likely due to varietal differences in growth strategy. Under limited nutrients, English spinach invested more in leaf elongation, whereas fertilization promoted allocation to leaf number, width, and biomass. Similar trade-offs between leaf size and overall vegetative growth under nutrient supply have been reported in leafy vegetables (Roy, 2007; Popat *et al.*, 2009; Ahmadi *et al.*, 2010; Ali *et al.*, 2013). El-Saady (2016) also noted that NPK effects vary across traits, supporting the trait-specific response observed here.

Table 1. Seed germination (%) and germination index of spinach varieties under nutrient combinations.

Nutrients	Seed germination (%)			Germination index		
	Varieties		Mean	Varieties		Mean
	Sindhi Spinach	English Spinach		Sindhi Spinach	English Spinach	
N <sub>1</sub> = Control	73.33	95.87	84.60 c	0.95	0.39	0.67
N <sub>2</sub> = NPK 2g L <sup>-1</sup>	73.80	91.92	82.86 d	1.33	0.19	0.76
N <sub>3</sub> = Calcium Nitrate 0.5g L <sup>-1</sup> )	90.23	96.47	93.35 b	2.06	0.51	1.28
N <sub>4</sub> = NPK 2g L <sup>-1</sup> + Calcium Nitrate 0.5g L <sup>-1</sup>	94.16	96.93	95.54 a	1.05	0.41	0.73
Mean	82.88 b	95.29 a		1.34 a	0.37 b	
<i>Level of Significance</i>	<i>Nutrients (N)</i>	<i>Varieties (V)</i>	<i>N x V</i>	<i>Nutrients (N)</i>	<i>Varieties (V)</i>	<i>N x V</i>
<i>Standard Error (SE)</i>	3.4581	2.4452	4.8904	0.2094	0.1480	0.2961
<i>LSD @ 5%</i>	10.367	7.3311	14.662	0.6277	0.4438	0.8877
<i>Probability</i>	0.0470	0.0024	0.1777	0.1815	0.0003	0.3294

Table 2. Plant height (cm) and number of leaves per plant of spinach varieties under nutrient combinations.

Nutrients	Plant height (cm)			Number of leaves per plant		
	Varieties		Mean	Varieties		Mean
	Sindhi Spinach	English Spinach		Sindhi Spinach	English Spinach	
N <sub>1</sub> = Control	8.43 f	21.13 a	14.78 a	3.33 e	7.33 b	5.33
N <sub>2</sub> = NPK 2g L <sup>-1</sup>	10.20 e	13.30 c	11.75 c	4.00 d	6.00 c	5.00
N <sub>3</sub> = Calcium Nitrate 0.5g L <sup>-1</sup> )	11.33 d	6.60 g	8.96 d	6.00 c	4.00 d	5.00
N <sub>4</sub> = NPK 2g L <sup>-1</sup> + Calcium Nitrate 0.5g L <sup>-1</sup>	16.50 b	8.60 f	12.55 b	9.00 a	6.00 c	7.50
Mean	11.61	12.40		5.58 b	5.83 a	
<i>Level of Significance</i>	<i>Nutrients (N)</i>	<i>Varieties (V)</i>	<i>N x V</i>	<i>N</i>	<i>V</i>	<i>N x V</i>
<i>Standard Error (SE)</i>	0.6506	0.4601	0.9201	0.6872	0.4859	0.9718
<i>LSD @ 5%</i>	1.9507	1.3794	2.7587	2.0603	1.4568	2.9136
<i>Probability</i>	0.0001	0.2413	0.0000	0.0577	0.7208	0.0071

Table 3. Leaf length (cm) and leaf width (cm) of spinach varieties under nutrient combinations.

Nutrients	Leaf length (cm)			Leaf width (cm)		
	Varieties		Mean	Varieties		Mean
	Sindhi Spinach	English Spinach		Sindhi Spinach	English Spinach	
N <sub>1</sub> = Control	3.50 f	11.76 a	7.63	2.20 f	4.60 b	3.40
N <sub>2</sub> = NPK 2g L <sup>-1</sup>	4.80 e	7.46 b	6.13	2.10 f	4.06 c	3.08
N <sub>3</sub> = Calcium Nitrate 0.5g L <sup>-1</sup> )	5.66 d	5.96 d	5.81	2.80 e	3.36 d	3.08
N <sub>4</sub> = NPK 2g L <sup>-1</sup> + Calcium Nitrate 0.5g L <sup>-1</sup>	7.46 b	6.10 c	6.78	5.46 a	2.16 f	3.81
Mean	5.35 b	7.82 a		3.14	3.54	
<i>Level of Significance</i>	<i>Nutrients (N)</i>	<i>Varieties (V)</i>	<i>N x V</i>	<i>Nutrients (N)</i>	<i>Varieties (V)</i>	<i>N x V</i>
<i>Standard Error (SE)</i>	0.6416	0.4537	0.9073	0.3371	0.2383	0.4767
<i>LSD @ 5%</i>	1.9235	1.3602	2.7204	1.0158	0.7183	1.4366
<i>Probability</i>	0.2367	0.0014	0.0004	0.4608	0.2629	0.0002

Table 4. Fresh biomass of root (g) and leaf weight (g) of spinach varieties under nutrient combinations.

Nutrients	Fresh biomass of root (g)			Leaf weight (g)		
	Varieties		Mean	Varieties		Mean
	Sindhi Spinach	English Spinach		Sindhi Spinach	English Spinach	
N <sub>1</sub> = Control	0.37 d	0.76 a	0.56 c	5.31 e	12.95 a	9.13
N <sub>2</sub> = NPK 2g L <sup>-1</sup>	0.65 a	0.72 b	0.68 a	3.21 g	9.73 b	6.47
N <sub>3</sub> = Calcium Nitrate 0.5g L <sup>-1</sup>	0.55 c	0.53 d	0.54 d	4.10 f	8.28 c	6.19
N <sub>4</sub> = NPK 2g L <sup>-1</sup> + Calcium Nitrate 0.5g L <sup>-1</sup>	0.63 b	0.68 c	0.65 b	7.46 d	7.79 d	7.62
Mean	0.55 b	0.67 a		5.02 b	9.68 a	
<i>Level of Significance</i>	<i>Nutrients (N)</i>	<i>Varieties (V)</i>	<i>N x V</i>	<i>Nutrients (N)</i>	<i>Varieties (V)</i>	<i>N x V</i>
<i>Standard Error (SE)</i>	0.0724	0.0512	0.1024	0.7582	0.5361	1.0722
<i>LSD @ 5%</i>	0.2171	0.1535	0.3070	2.2731	1.6073	3.2147
<i>Probability</i>	0.4789	0.1146	0.2362	0.0559	0.0000	0.0175

Table 5. Root depth (cm) of spinach varieties under nutrient combinations.

Nutrients	Varieties		Mean
	Sindhi Spinach	English Spinach	
N <sub>1</sub> = Control	10.00 g	16.43 b	13.21 b
N <sub>2</sub> = NPK 2g L <sup>-1</sup>	10.74 f	12.00 e	11.37 d
N <sub>3</sub> = Calcium Nitrate 0.5g L <sup>-1</sup>	12.06 e	12.36 d	12.21 c
N <sub>4</sub> = NPK 2g L <sup>-1</sup> + Calcium Nitrate 0.5g L <sup>-1</sup>	19.46 a	15.10 c	17.28 a
Mean	13.06	13.97	
<i>Level of Significance</i>	<i>Nutrients (N)</i>	<i>Varieties (V)</i>	<i>N x V</i>
<i>Standard Error (SE)</i>	1.1284	0.7979	1.5958
<i>LSD @ 5%</i>	3.3831	2.3922	4.7844
<i>Probability</i>	0.0094	0.4339	0.0300

### Leaf width (cm)

The data regarding leaf width (cm) of spinach varieties under nutrient treatments are also presented in Table 3. ANOVA showed a highly significant effect of nutrient × variety interaction ( $P < 0.001$ ), whereas the main effects of nutrients ( $P > 0.05$ ) and varieties ( $P > 0.05$ ) were non-significant. The interaction results indicated that Sindhi spinach with NPK + calcium nitrate (N<sub>4</sub>) produced the widest leaves (5.46 cm), while English spinach under the same treatment had much narrower leaves (2.16 cm). Conversely, English spinach under control conditions (4.60 cm) was wider than Sindhi spinach (2.20 cm). The narrowest leaves overall were recorded in Sindhi spinach treated with NPK alone (2.10 cm). Across nutrient treatments, the widest mean leaves were observed in N<sub>4</sub> (3.81 cm), followed by the control (3.40 cm), while N<sub>2</sub> and N<sub>3</sub> recorded narrower leaves (3.08 cm each). Mean varietal comparison revealed slightly wider leaves in English spinach (3.54 cm) compared to Sindhi spinach (3.14 cm), though this difference was not statistically significant.

### Fresh biomass of root (g)

Fresh root biomass data for Sindhi and English spinach varieties under nutrient combinations are summarized in Table 4. The analysis of variance revealed that the effects of nutrients, varieties, as well as their interaction were all non-significant ( $P > 0.05$ ), indicating similar responses of both varieties to the nutrient treatments. English spinach under control (N<sub>1</sub>) produced the highest root biomass (0.76 g), while Sindhi spinach under the same treatment had only 0.37 g. Sindhi spinach with NPK (N<sub>2</sub>) produced the maximum root biomass for this variety (0.65 g), whereas English spinach recorded its highest biomass (0.72 g) under NPK. The lowest overall biomass (0.37 g) was observed in Sindhi spinach under control conditions. In case of nutrients the maximum fresh biomass of root (0.68 g) was recorded in N<sub>2</sub> (NPK 2g L<sup>-1</sup>) while the minimum fresh biomass of root (0.54 g) was recorded in N<sub>3</sub> (Calcium Nitrate 0.5g L<sup>-1</sup>). Across treatments, mean values showed slightly higher root biomass in English spinach (0.67 g) compared to Sindhi spinach (0.55 g).

### Leaf weight (g)

The results related to leaf weight (g) of both spinach varieties under nutrient combinations are also presented in Table 4. The analysis of variance indicated that the effect of varieties and nutrient  $\times$  variety interaction ( $P < 0.05$ ) was significant, whereas the effect of nutrients alone was non significant. The interaction results showed that English spinach under control conditions ( $N_1$ ) produced the highest leaf weight (12.95 g), followed by NPK (9.73 g). Sindhi spinach, however, recorded the maximum leaf weight (7.46 g) with NPK + calcium nitrate ( $N_4$ ), while its lowest was observed with NPK alone (3.21 g). The smallest overall leaf weight was found in Sindhi spinach under NPK (3.21 g). Across nutrient treatments, the highest mean leaf weight was observed in the control (9.13 g), followed by NPK (6.47 g), while the lowest was recorded with calcium nitrate (6.19 g). Between varieties, English spinach (9.68 g) had significantly higher leaf weight compared to Sindhi spinach (5.02 g), confirming a strong varietal effect.

### Root depth (cm)

The effect of different nutrient combinations on root depth of spinach varieties (Sindhi and English Spinach) and analysis of variance is presented in Table 5. The analysis of variance revealed that nutrients ( $P < 0.01$ ) and the nutrient  $\times$  variety interaction ( $P < 0.05$ ) had significant effects on root depth, whereas the varietal effect was non-significant ( $P > 0.05$ ). The interaction results showed that Sindhi spinach responded most positively to the combined application of NPK + calcium nitrate ( $N_4$ ), producing the maximum root depth of 19.46 cm, which was also the highest overall among all treatments. In contrast, the shallowest roots were observed in Sindhi spinach under control conditions (10.00 cm). For English spinach, the deepest roots (16.43 cm) were recorded in the control treatment, while NPK + calcium nitrate (15.10 cm) and calcium nitrate alone (12.36 cm) produced comparatively moderate root depths. These findings indicate that the two varieties responded differently to nutrient treatments. Across treatments, the combined application of NPK + calcium nitrate ( $N_4$ ) produced the deepest roots on average (17.28 cm), significantly higher than control (13.21 cm), calcium nitrate (12.21 cm), and NPK alone (11.37 cm). NPK alone produced the shallowest average root depth across both varieties. In case of varieties, English spinach (13.97 cm) showed slightly deeper roots on average compared to Sindhi spinach (13.06 cm).

## DISCUSSION

Macronutrients like NPK and calcium nitrate enhance physiological processes and crop yield in spinach, especially when applied in combination, offering maximum returns to growers (Jiskani, 2005; Chachar *et al.*, 2024). In this study, the combined application of NPK (2 g L<sup>-1</sup>) and calcium nitrate (0.5 g L<sup>-1</sup>) significantly enhanced growth and yield parameters compared to individual applications or the control. These findings are consistent with El-Saady (2016), who reported that a balanced NPK ratio of 2:1:1 applied at 125 kg/fed resulted in the highest values for plant height, leaf area, fresh biomass, and total yield in two growing seasons.

The synergistic effect of balanced nutrient application is well-supported by earlier research. Roy (2007) and Popat *et al.* (2009) demonstrated that NPK fertilizers stimulate vegetative growth through enhanced photosynthesis, chlorophyll synthesis, and meristematic activity. Similarly, Ahmadi *et al.* (2010) and Ali *et al.* (2013) explained that nitrogen promotes vegetative growth, phosphorus supports root development, and potassium regulates stomatal function and stress tolerance. Our findings corroborate these mechanisms, as spinach treated with NPK + calcium nitrate showed greater leaf number, leaf width, root elongation, and biomass accumulation than other treatments.

The role of calcium nitrate was also evident. Calcium strengthens cell walls and improves root elongation, while nitrate enhances nitrogen uptake efficiency. Patil and Biradar (2001) found that spinach treated with NPK (20-20-20 + TE) and calcium showed greater plant height and nutrient uptake, which aligns with our observations. Likewise, Lovatt (2005) emphasized that foliar sprays of NPK during early growth stages reduce flower drop and improve final yield, highlighting the importance of nutrient timing and formulation.

Balanced nutrient supply also influences chemical composition. El-Saady (2016) reported that a 2:1:1 NPK ratio increased chlorophylls, carotenoids, vitamin C, crude protein, and essential minerals (N, P, K, Ca). Optimal NPK nutrition therefore enhances both physiological performance and nutritional quality. Although nitrate accumulation is a concern in leafy vegetables, El-Saady (2016) observed that the 2:1:1 NPK ratio moderated nitrate content by improving conversion of inorganic nitrogen to proteins, a mechanism also supported by Stagnari *et al.* (2007) and Zeka *et al.* (2014). While our study did not measure nitrate accumulation, this remains a limitation that should be addressed in future work to ensure food safety. Yield improvements observed here also mirror the findings of Smith and Heinrich (2011), Zikalala (2014), and Nemadodzi (2015), who reported substantial yield increases with balanced mineral fertilization. The stimulatory effects of nitrogen, phosphorus, and potassium on metabolic pathways and biosynthesis of essential plant compounds appear to be critical drivers of enhanced spinach performance.

Nevertheless, the study has certain limitations. It was conducted in pots under nursery conditions for a relatively short period, which may not fully represent field environments. Pot experiments allow precise control of nutrients but may not capture soil heterogeneity, root system expansion, or longer-term responses. Future research should therefore include multi-season field trials to validate these findings under variable agro-ecological conditions.

In summary, the present findings reinforce the importance of integrated nutrient management in optimizing spinach production. The combined application of NPK (2 g L<sup>-1</sup>) and calcium nitrate (0.5 g L<sup>-1</sup>) improved vegetative and root traits in both varieties, with English spinach showing superior performance. These results are consistent with several prior studies (Roy, 2007; Ahmadi *et al.*, 2010; El-Saady, 2016; Ali *et al.*, 2013; Solangi *et al.*, 2015), highlighting the broad relevance of this nutrient strategy. However, considerations of nitrate accumulation and the limitations of pot-based, short-term studies should guide future investigations.

## CONCLUSION

The different nutrient combinations significantly influenced the growth and development of spinach. Among the treatments, the combined application of NPK (2 g L<sup>-1</sup>) and calcium nitrate (0.5 g L<sup>-1</sup>) (N<sub>4</sub>) was the most effective, resulting in superior vegetative growth in both English and Sindhi spinach varieties. These findings suggest that this nutrient combination provides an optimal balance for enhancing spinach productivity. For farmers, applying NPK with calcium nitrate at the recommended concentrations can improve number of leaves per plant, biomass, and overall crop performance, particularly in short-cycle leafy vegetable production. Integrating this nutrient strategy into routine fertilization practices can increase yield and quality while potentially reducing the need for multiple separate nutrient applications. However, care should be taken to monitor nitrate levels in leaves, and further field-based validation is recommended to optimize application timing and rates under local soil and environmental conditions.

## AUTHOR CONTRIBUTIONS

Ghulam Rasool Aamur; Conducted research and data collection, Tanveer Fatima Miano: Conceptualization, designing, supervision, write up and finalizing the manuscript, Muzafaruddin Chachar: Analysis of data and arrangement of tabulated data, Memoona Islam Majeedano, Maira Raqeeb Tunio and Sana Shazia Jiskani: Write-up and interpretation of data, Raheem Ullah: Data collection and statistical analysis, Zaheer Ahmed Chachar: Collection of data, citations and Reference management.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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