

# Vegetative Growth Response of Spinach (*Spinacia oleracea L.*) to the Transition from Conventional to Nano Urea

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## ABSTRACT

Spinach is a nutrient rich leafy vegetable with high nitrogen demand. Its growth and productivity are strongly influenced by the availability of nitrogen. This study evaluated the effect of foliar-applied nano urea on the vegetative growth, chlorophyll content, and soil nutrient status of spinach in Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The pot experiment was conducted in open-field conditions during the Rabi season, from mid-February to March 2025, using a completely randomized design (CRD) with six treatments and three replications. Treatments included T<sub>0</sub> = Control (only soil), T<sub>1</sub> = 100% RDF, T<sub>2</sub> = Only Nano Urea (4 times) + 100% RDF (without Urea), T<sub>3</sub> = 4 Times Nano Urea + 50% RDF (No Urea), T<sub>4</sub> = 2 Times Nano Urea + 50% RDF, and T<sub>5</sub> = No Nano Urea + No Urea + 100% Rest RDF. The results demonstrated that nano-urea treatments, especially the T<sub>3</sub> treatment, significantly enhanced vegetative growth, such as plant height (12.81 cm and 17.47 cm at 30 and 45 DAS), number of leaves per plant (4.67 and 6.67 at 30 and 45 DAS), root length (6.90 cm), shoot length (17.47 cm), fresh plant weight (64.31 g), and chlorophyll content like chlorophyll a (11.22 mg g<sup>-1</sup> FW), chlorophyll b (3.06 mg g<sup>-1</sup> FW), and carotenoid (2.67 mg g<sup>-1</sup> FW) compared to conventional fertilizer treatments. Soil nutrient analysis revealed improved availability of N, P, K, and S under nano-fertilizer treatments. The study highlights the potential of nano-urea to improve nitrogen use efficiency, enhance growth, and optimize soil fertility in spinach cultivation, suggesting its suitability as a sustainable nutrient management strategy.

**Keywords:** Nano fertilizer, Chlorophyll, Carotenoid, Growth, Fertility

## INTRODUCTION

Spinach (*Spinacia oleracea L.*) is a widely cultivated cool-season leafy vegetable of the family Amaranthaceae, valued for its rapid growth, short production cycle, and high nutritional content. The crop performs optimally in moderately cool climates with fertile, well-drained soils and produces chlorophyll-rich leaves that contribute to high photosynthetic efficiency and biomass accumulation (Sharma *et al.*, 2024). Nitrogen (N) is a critical nutrient in spinach, governing leaf expansion, chlorophyll synthesis, and total fresh weight accumulation. Insufficient or inefficient N supply can substantially limit commercial yield. Although conventional urea is widely used as a nitrogen source, its low use efficiency, caused by volatilization, leaching, and denitrification, reduces nutrient uptake and poses environmental risks. Spinach is also a nutrient-dense vegetable, supplying essential vitamins, minerals, and antioxidants that are vital for human nutrition and health (Abdelraouf *et al.*, 2016). Improving NUE in leafy greens is consequently crucial for growing productivity whilst lowering input costs and damaging

environmental impacts. Nano fertilizers, such as nano urea, represent an advanced approach to nutrient management in agriculture. By engineering urea at the nanoscale, these fertilizers enable controlled nutrient release, improved foliar absorption, and enhanced nitrogen-use efficiency, reducing losses associated with conventional fertilizers and promoting better plant growth (Glotra *et al.*, 2023). Several studies in cereals and vegetables have reported that nano-urea improves nitrogen use efficiency in crops by enhancing nutrient uptake, reducing nitrogen losses, and promoting higher growth and yield under irrigated conditions (Gogoi *et al.*, 2024). Preliminary studies on spinach indicate that nano fertilizer application enhances leaf area, biomass, chlorophyll content, and nitrogen uptake compared with conventional fertilization, suggesting improved metabolic efficiency (Taha *et al.*, 2024). However, research on nano-urea in leafy vegetables under open-field subtropical conditions remains limited. Key gaps include insufficient data on optimal application frequency, vegetative growth response, and economic viability relative to conventional urea. Given Bangladesh's high dependence on urea fertilizer, evaluating the agronomic performance of nano-urea in spinach production is essential for promoting sustainable nutrient management. Therefore, the present study aims to assess the response of spinach vegetative growth to the transition from conventional urea to nano-urea. The specific objectives are as follows:

- (i) To evaluate the effects of foliar nano-urea on vegetative growth parameters
- (ii) To estimate leaf pigment content

## MATERIALS AND METHODS

### Experimental site

The experiment was set up at the soil science research field, Hajee Mohammad Danesh Science and Technology University, Dinajpur, during the period from February to March 2025, to find out the effect of nano urea on the vegetative growth of spinach. The experimental site was situated under the Dinajpur Sadar Upazila and is located at 25.13°N latitude, 88.23°E longitude, and at an elevation of 37.5 m above the mean sea level. The land belongs to the AEZ of Old Himalayan Piedmont Plain, Agroecological Zone-1 (AEZ-1).

### Soil characteristics

The physicochemical characteristics of the initial soil sample were soil pH 6.8, organic matter (%) 0.87, total N (%) 0.015, available P (ppm) 42.11, exchangeable K (meq 100<sup>-1</sup> g soil) 0.068, and available S (ppm) 34.48, belonging to the sandy loam textural class.

### Soil and pot preparation

The soil was collected from the Soil Science Research Field, HSTU, Dinajpur, from a depth of 0-15cm. The collected soil was sun-dried. Weeds and stubble present in the soil were removed. The dry soil was then thoroughly mixed with the treatment before filling the pots. Each pot was filled up to 8 kg of soil and chemical fertilizers in individual amounts per pot with each treatment mixture during soil preparation.

### Planting materials

The spinach seeds were collected from the ACI Seeds. The variety was Sathi palak

### Experimental design and layout

The experiment consisted of six treatments with three replications, designed by a Completely Randomized Design (CRD). A total of 18 experimental pots were used. The treatment combinations were T<sub>0</sub> = Control (only soil), T<sub>1</sub> = 100% RDF, T<sub>2</sub> = Only Nano Urea (4 times) + 100% RDF (without Urea), T<sub>3</sub> = 4 Times Nano Urea + 50% RDF (No Urea), T<sub>4</sub> = 2 Times Nano Urea + 50% RDF, and T<sub>5</sub> = No Nano Urea + No Urea + 100% Rest RDF, respectively. In the case of fertilizers, Urea, TSP, and MoP were also mixed with each treatment mixture during soil preparation as 0.70 g pot<sup>-1</sup>, 0.50 g pot<sup>-1</sup>, and 0.50g pot<sup>-1</sup>, respectively. The percentage of NPK

fertilizers and manures was applied as per recommended doses, which were urea 70 kg ha<sup>-1</sup>, TSP 50 kg ha<sup>-1</sup>, and MP 50 kg ha<sup>-1</sup> (Fertilizer Recommended Guide, 2018).

### Data Collection

Recurrent observation was done to record changes in plant characteristics at different stages of growth. Data on the following parameters were recorded from the sample plants three times at 15, 30, and 45 DAS, and some parameters were taken during the final harvest. The parameters are plant height (cm), number of leaves per plant, shoot length (cm), root length (cm), and fresh plant weight (g).

### Physico-chemical properties

Physical and chemical properties of the collected soil sample (before transplanting and post-harvested field) were analyzed in the laboratory at the Department of Soil Science, HSTU, Dinajpur. The soil chemical properties, such as soil pH, organic carbon, % organic matter, total nitrogen, available phosphorus, exchangeable potassium, and available sulphur, were analyzed by the glass electrode pH meter method, the wet oxidation method developed by [Walkley and black \(1935\)](#), the semi-micro Kjeldahl method, the Olsen method ([Olsen SR. 1954](#)), the ammonium acetate extraction method and CaCl<sub>2</sub> extraction method, respectively.

### Analysis of Chlorophyll a (mg g<sup>-1</sup> FW)

Chlorophyll a content of spinach leaves was determined following [Arnon \(1949\)](#) with slight modifications. Fresh leaves (0.1–0.5 g) were washed, blotted dry, and ground in 10 mL of 80% acetone until pale green. The homogenate was centrifuged at 5000 rpm for 10 min, and the supernatant was collected, adjusting the volume to 10 mL if necessary. Absorbance was measured at 663 nm using a UV-Vis spectrophotometer, and chlorophyll a was calculated as:

$$\text{Chl a (mg g}^{-1}\text{ FW)} = (12.7 \times A_{663}) - (2.69 \times A_{645})$$

### Analysis of Chlorophyll b (mg g<sup>-1</sup> FW)

Chlorophyll b content of spinach leaves was determined using the same acetone extract following [Arnon \(1949\)](#). The absorbance of the extract was measured at 645 nm using a UV-Vis spectrophotometer. Chlorophyll b concentration was calculated using the equation:

$$\text{Chl b (mg g}^{-1}\text{ FW)} = (22.9 \times A_{645}) - (4.68 \times A_{663})$$

### Carotenoid Analysis (mg g<sup>-1</sup> FW)

Carotenoid content was determined from the same leaf extract following [Lichtenthaler and Wellburn \(1983\)](#). The absorbance of the extract was measured at 470 nm using a UV-Vis spectrophotometer. Carotenoid concentration was calculated using the formula:

$$\text{Carotenoids (mg g}^{-1}\text{ FW)} = \frac{(1000 \times A_{470}) - (1.82 \times \text{Chla}) - (85.02 \times \text{Chlb})}{198}$$

### Statistical analysis

The experimental data were statistically evaluated through Analysis of Variance (ANOVA) based on a Completely Randomized Design (CRD). All analyses were performed using Statistix 10.0 to determine the significance of treatment effects.

## RESULTS AND DISCUSSION

### Plant Height (cm)

In the present study, a gradual increase in plant height was observed as crop growth stages advanced under all treatments. Significant variation was recorded among treatments at 15, 30, and 45 days after sowing (DAS). At 15 DAS, the tallest plants were found in treatment T<sub>5</sub> (7.32 cm), closely followed by T<sub>1</sub> (7.27 cm) and T<sub>4</sub> (7.01 cm), whereas the shortest plants (5.61 cm) were recorded in T<sub>2</sub>. Similar patterns were observed at 30 and 45 DAS. The treatments T<sub>3</sub> (12.81 cm and 17.47 cm) and T<sub>4</sub> (13.81 cm and 17.00 cm) produced significantly taller plants compared to the control (T<sub>0</sub>: 11.36 cm and 14.78 cm). The increase in plant height under nano-fertilizer treatments is likely due to improved nitrogen uptake from nano-urea, which enhances chlorophyll synthesis and photosynthesis, stimulating cell division and elongation. Its small particle size and slow-release properties ensure a continuous nutrient supply and efficient utilization by the plant. Several studies have reported that nano urea application significantly enhances plant growth by improving nitrogen use efficiency, stimulating chlorophyll synthesis, and promoting cell division and elongation. Specifically, [Dhivyalakshmi and Aravinth \(2025\)](#) observed that foliar or soil-applied nano-urea markedly increased plant height, chlorophyll content, germination rate, and overall yield compared to conventional urea and untreated control treatments

### Number of Leaves per Plant

The number of leaves per plant varied significantly among treatments at both 30 and 45 DAS. At 30 DAS, treatment T<sub>4</sub> (5.33 leaves) and T<sub>3</sub> (4.67 leaves) recorded the highest number of leaves, while the control (T<sub>0</sub>) had the lowest (2.67 leaves). At 45 DAS, the maximum number of leaves was observed in T<sub>2</sub> (8.67 leaves), followed by T<sub>3</sub> and T<sub>4</sub> (6.67 leaves each). The control again showed the minimum number of leaves (4.00). The enhanced leaf production in nano-urea and optimized fertilizer treatments could be due to the better availability of nitrogen and micronutrients that promote cell division and leaf expansion. Nitrogen plays a vital role in chlorophyll formation and photosynthetic activity, which supports the development of new leaves. [Taha et al. \(2024\)](#) reported that supplementing spinach with small quantities of nano-fertilizers in combination with reduced conventional NPK doses significantly increased leaf number, leaf area, plant height, and biomass compared to full-dose conventional fertilizers, indicating improved nutrient use efficiency and vegetative growth.

### Root Length (cm)

Root development is essential for efficient nutrient and water absorption, and it serves as a good indicator of plant establishment and soil health. Significant differences in root length were observed among treatments. The longest roots were recorded in T<sub>2</sub> (8.27 cm), followed by T<sub>1</sub> and T<sub>4</sub> (7.50 cm each). The shortest root length (5.40 cm) was found in T<sub>5</sub>. The increased root length in nano-urea treatments (T<sub>2</sub> and T<sub>4</sub>) can be attributed to enhanced nitrogen uptake efficiency and improved rhizospheric conditions. Nano-nitrogen particles are more mobile and can penetrate deeper into the soil profile, improving nutrient accessibility for root systems. Moreover, the slow and steady release of nutrients supports continuous root elongation and branching. [Challa et al. \(2025\)](#) reported that foliar application of nano-urea in chickpea significantly increased root length, root dry weight, and total dry matter accumulation, in addition to enhancing plant height and branch number. This suggests that nano-urea improves nutrient uptake efficiency and supports enhanced root system development, which is consistent with the observed increase in root length in spinach under nano-fertilizer treatments.

### Shoot Length (cm)

Shoot length showed a similar trend to plant height, as both are influenced by vegetative vigor and nutrient availability. The highest shoot length was obtained from T<sub>3</sub> (17.90 cm), followed by T<sub>4</sub> (17.00 cm). The lowest shoot length was recorded in T<sub>0</sub> (14.78 cm), which was comparable to that of T<sub>5</sub> (14.90 cm). The increased shoot length under nano-fertilizer treatments might be attributed to efficient nitrogen assimilation that supports the synthesis of amino acids and proteins essential for plant structural development. Nano-nitrogen facilitates enhanced chlorophyll content, which boosts photosynthetic efficiency and energy production required for shoot elongation. [Gil-Díaz et al. \(2022\)](#) reported that application of commercial nano fertilizers significantly improved

shoot length, leaf number, and overall vegetative growth in spinach compared to untreated plants. This supports the observation that nano-fertilizer treatments enhance nitrogen assimilation and photosynthetic efficiency, promoting shoot elongation and plant vigor.

### Fresh Plant Weight (g)

Fresh plant weight represents the cumulative effect of all growth parameters and is a direct measure of the total biomass produced by the plant. The results revealed significant differences among treatments, with T<sub>3</sub> recording the highest fresh weight (67.14 g), followed by T<sub>4</sub> (64.31g). The lowest fresh weight (46.64 g) was observed in T<sub>0</sub> (control treatment). Higher fresh weight under nano-fertilizer treatments T<sub>3</sub> indicates improved physiological and metabolic processes, including enhanced photosynthesis, nutrient uptake, and carbohydrate accumulation. The continuous availability of nitrogen through nano-urea enhances the production of chlorophyll and enzymes involved in biomass synthesis. This leads to better vegetative growth and overall yield potential. Similar findings were reported by Ibrahim (2022), who reported that nano-fertilization significantly increased fresh plant weight, leaf biomass, and overall vegetative growth of spinach grown in sandy soil. The enhanced biomass production was attributed to improved nitrogen availability, higher chlorophyll content, and more efficient nutrient uptake, which together promoted greater carbohydrate accumulation and shoot growth.

Table 1. Effects of nano urea on agronomic parameters of Spinach

Treatment	Plant Height(cm)			No of leaves		Root length (cm)	Shoot length (cm)	Fresh plant weight (g)
	15 days	30 days	45 days	30 days	45 days			
T <sub>0</sub>	5.75 c	11.36c	14.78b	2.67c	4.00d	7.10c	14.78b	50.61c
T <sub>1</sub>	7.27 a	11.67bc	15.38b	4.00b	5.67bc	7.50b	15.38b	62.48b
T <sub>2</sub>	5.61 c	11.94bc	17.90a	4.33ab	6.67b	8.27a	17.47a	51.64c
T <sub>3</sub>	6.04 bc	12.81ab	17.47a	4.67ab	8.67a	6.90c	17.90a	67.14a
T <sub>4</sub>	7.01ab	13.81a	17.00a	5.33a	6.67b	7.50b	17.00a	64.31b
T <sub>5</sub>	7.32a	12.3bc	14.90c	3.67bc	5.33c	5.40d	14.90b	46.64d
<b>SE(±mean)</b>	<b>0.54</b>	<b>0.57</b>	<b>0.46</b>	<b>0.54</b>	<b>0.54</b>	<b>0.09</b>	<b>0.46</b>	<b>0.96</b>
<b>CV%</b>	<b>10.11</b>	<b>5.67</b>	<b>3.47</b>	<b>16.22</b>	<b>10.81</b>	<b>1.67</b>	<b>3.47</b>	<b>2.05</b>

N.B: T<sub>0</sub> = Control (only soil), T<sub>1</sub> = 100% RDF, T<sub>2</sub> = Only Nano Urea (4 times) + 100% RDF (without Urea), T<sub>3</sub> = 4 Times Nano Urea + 50% RDF (No Urea), T<sub>4</sub> = 2 Times Nano Urea + 50% RDF, and T<sub>5</sub> = No Nano Urea + No Urea + 100% Rest RDF

### Soil pH

The soil pH values among the treatments ranged from 5.92 to 6.25, indicating slightly acidic conditions suitable for the uptake of most essential nutrients. The highest pH (6.25) was observed in T<sub>1</sub>, which is statistically similar to T<sub>3</sub>, while the lowest (5.92) occurred in T<sub>4</sub>. This slight variation in pH suggests that the applied treatments had a moderate influence on soil chemical properties. The application of nano-fertilizers, especially nano-urea, often helps stabilize soil pH by enhancing microbial activity and reducing acidification associated with excessive conventional fertilizer use. Similar results were reported by Ibrahim (2022), who observed that the combined application of bio-fertilizers and nano-fertilizers improved soil health and helped maintain a stable soil pH in spinach cultivation by enhancing microbial activity and increasing nutrient retention in sandy soils.

### Organic matter (%)

Organic content varied from 0.58% to 0.71%, with T<sub>3</sub> recording the highest percentage. The lowest OM was found in the control treatment. The increase in organic matter under T<sub>3</sub> treatment might be due to the stimulation of soil microbial biomass and root exudation encouraged by nano-fertilizer application. Organic matter plays a vital role in improving soil fertility, nutrient retention, and water-holding capacity. The slight rise in organic carbon with nano-fertilizer application can be attributed to enhanced root growth and microbial activity, leading to greater carbon input into the soil. Similar results were reported by [Yashaswini \*et al.\* \(2025\)](#), who observed that nano-urea application, particularly in combination with reduced conventional urea, enhanced soil organic carbon and improved soil organic matter retention, indicating better soil health.

### Total Nitrogen (%)

Nitrogen content in the soil ranged from 0.13 to 0.30 percent. The highest value was recorded under T<sub>5</sub> treatment. The significant increase in nitrogen availability in T<sub>5</sub> may be attributed to the controlled and sustained release of nano urea, which ensures a gradual nitrogen supply to plants while minimizing losses through volatilization or leaching. Nano-urea particles, due to their high surface area, enhance nutrient absorption efficiency, leading to improved nitrogen assimilation. Similar findings were reported by [Kumar \*et al.\* \(2024\)](#), who noted that nano-urea, due to its small particle size and controlled-release properties, improves nitrogen availability, enhances nutrient absorption efficiency, and supports better plant growth compared to conventional urea

### Available Phosphorus (ppm)

Phosphorus concentration showed a considerable increase across treatments, ranging from 22.08 ppm (T<sub>0</sub>) to 39.46 ppm (T<sub>3</sub>). The highest phosphorus availability in T<sub>3</sub> and T<sub>4</sub> treatments may result from the enhanced solubility and reduced fixation of phosphorus due to nano-phosphate formulations. T<sub>3</sub> exhibited a notable increase in phosphorus content, highlighting the effectiveness of the nano-fertilizer treatment in improving nutrient uptake. Phosphorus nanoparticles can penetrate soil micropores and release nutrients gradually, making them more accessible to roots. Similar studies have reported that nano-phosphate fertilizers improve phosphorus mobility, uptake efficiency, and overall crop productivity ([Reddy \*et al.\*, 2025](#)).

### Exchangeable Potassium (meq 100<sup>-1</sup> g of soil)

Potassium levels ranged from 0.12% to 0.19%, with the maximum value observed in T<sub>4</sub>, followed by T<sub>5</sub>. T<sub>3</sub> also shows a remarkable increase in exchangeable K in post-harvest soil. Nano-fertilizer treatments facilitated better potassium uptake, likely due to improved root activity and ion exchange processes. Potassium plays a crucial role in enzyme activation, osmotic regulation, and photosynthesis, all of which contribute to plant vigor. Previous studies have reported that nano-fertilizers can enhance nutrient use efficiency, maintain ionic balance, improve drought tolerance, increase metabolic activity, and elevate exchangeable potassium in post-harvest soil ([Wang \*et al.\*, 2024](#)).

### Available Sulfur (ppm)

Sulfur content varied from 9.74 ppm in T<sub>0</sub> to 28.14 ppm in T<sub>4</sub>. Here, T<sub>3</sub> shows a notable enhancement. The increase in sulfur content under nano-fertilizer treatments, particularly T<sub>3</sub> and T<sub>4</sub>, could be due to the fine particle size of nano-sulfur, which allows for faster oxidation and improved availability. Sulfur plays a major role in protein synthesis, enzyme activation, and chlorophyll formation. [Saurabh \*et al.\* \(2024\)](#) reported that nano fertilizers increased nutrient use efficiency and yield quality in cereals. Also, enhanced plant metabolic activity and increased Sulphur availability in soil.

Table 2. Effects of nano urea on post-harvest soils of the pot experiment

Treatment	Soil pH	Organic Matter (%)	Total Nitrogen (%)	Available Phosphorus (ppm)	Exchangeable Potassium (meq 100 <sup>-1</sup> g of soil)	Available Sulphur (ppm)
T <sub>0</sub>	6.14 ab	0.58 d	0.14 de	22.08 e	0.12 b	9.74 d
T <sub>1</sub>	6.25 a	0.58 d	0.19 c	24.74 d	0.12 b	10.55 d
T <sub>2</sub>	6.24 a	0.64 b	0.13 e	28.83 c	0.13 b	18.34 c
T <sub>3</sub>	6.23 a	0.71 a	0.22 b	39.46 a	0.17 a	27.88 a
T <sub>4</sub>	5.92 b	0.61 c	0.16 d	34.96 b	0.19 a	28.14 a
T <sub>5</sub>	5.97 b	0.58 d	0.3 a	28.39c	0.18 a	23.11 b
<b>SE(±mean)</b>	<b>0.11</b>	<b>8.16</b>	<b>0.1</b>	<b>0.68</b>	<b>0.01</b>	<b>0.66</b>
<b>CV %</b>	<b>2.27</b>	<b>1.62</b>	<b>7.44</b>	<b>2.8</b>	<b>10.43</b>	<b>4.14</b>

N.B. T<sub>0</sub> = Control (only soil), T<sub>1</sub> = 100% RDF, T<sub>2</sub> = Only Nano Urea (4 times) + 100% RDF (without Urea), T<sub>3</sub> = 4 Times Nano Urea + 50% RDF (No Urea), T<sub>4</sub> = 2 Times Nano Urea + 50% RDF, and T<sub>5</sub> = No Nano Urea + No Urea + 100% Rest RDF

### Pigment analysis of spinach fresh leaves

#### Chlorophyll a (mg g<sup>-1</sup> FW)

Chlorophyll a content ranged from 4.67 to 11.22 mg/g, with the highest value recorded in T<sub>3</sub> treatments. This indicates that nano-fertilizers, particularly nano-urea and nano-sulfur, improved chlorophyll a synthesis by providing a consistent nitrogen supply. Nitrogen, being a core component of chlorophyll, enhances the photosynthetic efficiency of leaves. Increased chlorophyll content reflects improved light-harvesting capacity and greater carbon fixation potential. [Mubashir et al. \(2023\)](#) demonstrated that foliar nano-nutrient application significantly increased chlorophyll a and b, carotenoid levels, and antioxidant activity in drought-stressed tomato, supporting our finding that nano-fertilizer treatments enhance pigment stability and photosynthetic performance. Similar results were reported by [Taha et al. \(2024\)](#).

#### Chlorophyll b (mg g<sup>-1</sup> FW)

Chlorophyll b content ranged between 0.41 and 3.06 mg/g, with the highest concentration observed in T<sub>3</sub>, followed by T<sub>4</sub>. Chlorophyll b functions as an accessory pigment, extending the range of light absorption and transferring energy to chlorophyll for photosynthesis. The significant increase in chlorophyll b under nano-fertilizer treatments can be linked to improved nutrient assimilation and reduced oxidative stress in leaf tissues. Nano-urea application, especially when combined with optimized NPK doses, significantly enhanced chlorophyll b content in spinach leaves. [Taha et al. \(2024\)](#) reported that such treatments improved overall pigment balance, increased chlorophyll b levels, and enhanced photosynthetic capacity, indicating better nutrient assimilation and more efficient light energy utilization. Similar results were investigated by [Mubashir et al. \(2023\)](#)

#### Carotenoid (mg g<sup>-1</sup> FW)

Carotenoid content ranged from 1.08 to 2.67 mg/g, with the highest concentration recorded in T<sub>3</sub>. Carotenoids act as accessory pigments that protect chlorophyll from photooxidative damage and contribute to plant stress tolerance. The higher carotenoid levels under nano-fertilizer treatments suggest enhanced antioxidative activity and metabolic stability. [Akhtar et al. \(2022\)](#) reported that nano biofertilizers enhance plant physiological performance by improving nutrient uptake, boosting carotenoids, and strengthening antioxidant defense pathways, ultimately contributing to improved growth and stress tolerance. Likewise, [Mubashir et al. \(2023\)](#) observed that nano-nutrient treatments enhanced photosynthetic pigment content in cereals and vegetables.

Table 3. Effects of nano urea on leaf pigments of Spinach

Treatment	Chlorophyll a (mg g <sup>-1</sup> FW)	Chlorophyll b (mg g <sup>-1</sup> FW)	Carotenoid (mg g <sup>-1</sup> FW)
T0	4.67 d	0.41 d	1.08 d
T1	6.49 c	2.01 c	1.61 c
T2	6.53 c	2.05 c	1.64 c
T3	11.22 a	3.06 a	2.67 a
T4	6.53 c	2.05 c	1.64 c
T5	8.54 b	2.44 b	2.27 b
<b>SE(±mean)</b>	<b>0.21</b>	<b>0.12</b>	<b>0.18</b>
<b>CV %</b>	<b>3.35</b>	<b>7.29</b>	<b>11.43</b>

N.B: T<sub>0</sub> = Control (only soil), T<sub>1</sub> = 100% RDF, T<sub>2</sub> = Only Nano Urea (4 times) + 100% RDF (without Urea), T<sub>3</sub> = 4 Times Nano Urea + 50% RDF (No Urea), T<sub>4</sub> = 2 Times Nano Urea + 50% RDF, and T<sub>5</sub> = No Nano Urea + No Urea + 100% Rest RDF

## SUMMARY AND CONCLUSIONS

The experiment indicated that nano-urea significantly improved vegetative growth, physiological characteristics, and soil nutrient levels of spinach when compared to traditional fertilization methods. Throughout all growth phases (15, 30, and 45 days after sowing), treatments with nano-fertilizers, especially T<sub>3</sub> and T<sub>4</sub>, consistently exhibited better results in terms of plant height, leaf number, root and shoot length, and total fresh plant weight. The levels of photosynthetic pigments like chlorophyll a, chlorophyll b, and carotenoid showed a significant increase, suggesting enhanced photosynthesis, nitrogen utilization, and decreased oxidative stress. Soil nutrients such as nitrogen, phosphorus, potassium, sulfur, and organic matter also saw improvements with the application of nano-fertilizers, indicating better nutrient release, absorption, and retention. The exceptional performance of T<sub>3</sub> can be attributed to its controlled nutrient release, high surface reactivity, and enhanced nutrient-use efficiency, which facilitated chlorophyll production, root function, carbohydrate storage, and soil microbial activity. In summary, nano-fertilizers, particularly nano-urea in conjunction with balanced nutrients, represent a sustainable and effective approach to enhance spinach growth, yield, and soil fertility, thereby decreasing dependence on high quantities of conventional fertilizers. It is advisable to conduct long-term field trials and economic assessments to confirm their practical effectiveness.

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