

## IMPROVING SPINACH PRODUCTIVITY AND MINERAL UPTAKE WITH AUXIN-BASED FOLIAR TREATMENT

Irfan Ahmad<sup>1\*</sup>, Hafiz Muhammad Bilal<sup>2</sup>

<sup>1</sup>Department of Soil Sciences, Faculty of Agriculture, Gomal University Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan

<sup>2</sup>Water Management Research Farm, Renala Khurd, Okara, Punjab, Pakistan

\*Corresponding Author E-mail: [khanirfanahmad57@gmail.com](mailto:khanirfanahmad57@gmail.com)

### Abstract

Auxin plays a crucial role in plant growth regulation, influencing cell elongation, nutrient uptake, and overall biomass accumulation. This study appraised the effects of auxin foliar application on spinach growth, physiological traits, and nutrient uptake in a controlled laboratory experiment at Gomal University, Soil Science Department. Five treatments included 0-200 mg L<sup>-1</sup> auxin levels. The 150 mg L<sup>-1</sup> treatment significantly enhanced all the growth, physiological and biomass accumulation parameters. However, excessive auxin (200 mg L<sup>-1</sup>) led to slight reductions. Auxin also improved nitrogen, phosphorus, and potassium uptake. These results suggest that foliar-applied auxin can enhance spinach productivity and nutrient efficiency. Further field studies are needed to optimize its practical application.

**Keywords:** "Auxin", "Foliar Application", "Spinach Growth", "Chlorophyll Content", "Nutrient Uptake", "Biomass Accumulation",

### Article History

Received:  
July 07, 2024

Revised:  
August 28, 2024

Accepted:  
October 19, 2024

Available Online:  
December 31, 2024

BIOTECHNOLOGY

## INTRODUCTION

Spinach (*Spinacia oleracea* L.) is a nutrient-rich leafy vegetable widely cultivated for its high mineral content, antioxidant properties, and rapid growth cycle <sup>1</sup>. The productivity and nutrient uptake of spinach are highly influenced by growth enhancers, predominantly auxins, which play a crucial role in cell elongation, root development, and nutrient translocation <sup>2</sup>. Among diverse auxins, indole-3-acetic acid (IAA) and naphthaleneacetic acid (NAA) have been widely studied for their potential in improving vegetative growth and nutrient efficiency in crops <sup>3</sup>.

Auxins regulate photosynthesis, leaf expansion, and biomass accumulation, which directly affect nutrient uptake and assimilation <sup>4</sup>. Previous research has shown that exogenous foliar application of auxins enhances nitrogen, phosphorus, and potassium uptake, leading to improved yield and quality of vegetables <sup>5</sup>. Additionally, auxins modulate root architecture, increasing root surface area and facilitating better nutrient absorption from the soil <sup>6</sup>. Studies on leafy vegetables like lettuce and cabbage have demonstrated that foliar-applied auxin improves leaf biomass, chlorophyll content, and antioxidant enzyme activity, indicating its potential benefits for spinach cultivation as well <sup>7</sup>.

Despite the known advantages of auxins in plant growth, there is limited research on their specific impact on spinach nutrient uptake and productivity. Most studies focus on soil-applied plant growth regulators, but foliar application offers a more efficient and rapid method of nutrient assimilation by bypassing soil interactions and minimizing nutrient loss <sup>8</sup>. Therefore, investigating the optimal concentration and application timing of auxin foliar sprays could significantly enhance spinach production and mineral accumulation.

This investigation intends to estimate the consequence of foliar-applied auxin on spinach

growth, nutrient uptake, and physiological responses under controlled conditions. We hypothesize that auxin application will improve biomass production, enhance chlorophyll synthesis, and increase the uptake of indispensable nutrients (N, P, K) compared to untreated plants.

## MATERIALS AND METHODS

A laboratory trial was accompanied at the Soil Science Department, Gomal University, during the 2022–2023 growing season to gauge the special effects of foliar-applied auxin on spinach growth and nutrient uptake. The trial followed a completely randomized design (CRD) with tetra replicated five treatments.

### Treatment Plan

The study included the following treatments of indole-3-acetic acid (IAA):

- T<sub>0</sub> (Control): No auxin application
- T<sub>1</sub>: IAA @ 50 mg L<sup>-1</sup>
- T<sub>2</sub>: IAA @ 100 mg L<sup>-1</sup>
- T<sub>3</sub>: IAA @ 150 mg L<sup>-1</sup>
- T<sub>4</sub>: IAA @ 200 mg L<sup>-1</sup>

Foliar applications were conducted twice (At the 4 & 6 leaf stages (20 & 35 days after sowing) during the experiment:

Spinach seeds were scattered in plastic pots full with 5 kg of sandy loam soil. The soil was analyzed before sowing, and its characteristics included pH 7.2, organic matter 0.9%, available nitrogen 0.05%, available phosphorus 8.3 mg kg<sup>-1</sup>, and exchangeable potassium 85 mg kg<sup>-1</sup>. Plants were irrigated regularly to maintain optimal moisture levels. Different growth, physiological and nutrient

parameters were recorded using standard procedures.

### Statistical Analysis

Data were scrutinized by means of analysis of variance (ANOVA) and treatment means were equated using the least significant difference (LSD) test at a 5% probability level ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

### Growth Parameters

#### Plant Height

Foliar application of auxin significantly influenced plant height in spinach. The highest plant height ( $27.6 \pm 1.2$  cm) was recorded in T<sub>3</sub> (150 mg L<sup>-1</sup> auxin), which was statistically superior to all other treatments (Table 1). However, a slight reduction was observed at the highest concentration (T<sub>4</sub>, 200 mg L<sup>-1</sup>), where plant height decreased to  $25.9 \pm 1.1$  cm, though it remained higher than the control. Compared to the untreated plants (T<sub>0</sub>,  $18.5 \pm 0.8$  cm), T<sub>3</sub>-treated plants exhibited a 49.2% increase in height, demonstrating the positive impact of auxin on stem elongation. Excessive auxin (T<sub>4</sub>) may have

led to hormonal imbalances, slightly reducing the effectiveness of growth promotion.

#### Leaves Count per Plant

A substantial increase in the number of leaves was observed with auxin application (Table 1). The maximum leaf number ( $10.1 \pm 0.6$ ) was recorded in T<sub>3</sub> (150 mg L<sup>-1</sup>), which was statistically higher than all other treatments. The lowest number of leaves ( $6.2 \pm 0.3$ ) was recorded in T<sub>0</sub> (control). Treatments T<sub>2</sub> and T<sub>4</sub> showed similar responses ( $8.9 \pm 0.5$  and  $9.3 \pm 0.5$ , respectively), indicating that beyond 150 mg L<sup>-1</sup>, auxin may not further enhance leaf formation. This result is consistent with previous findings that auxin improves leaf initiation and expansion up to an optimal concentration.

#### Leaf Area

Leaf area followed a similar trend, with the highest value ( $125.8 \pm 5.6$  cm<sup>2</sup>) in T<sub>3</sub>, which was significantly greater than the control ( $85.3 \pm 4.2$  cm<sup>2</sup>) (Table 1). Treatment T<sub>4</sub> (200 mg L<sup>-1</sup>) showed a slight reduction in leaf area ( $120.2 \pm 5.3$  cm<sup>2</sup>), which suggests that excessive auxin might limit leaf expansion due to possible hormonal saturation effects.

**Table 1:** Effect of Foliar-Applied Auxin on Growth Structures of Spinach

Treatment	Height	Leaves Count per Plant	Leaf Area
T <sub>0</sub> (Control)	$18.5 \pm 0.8$ c	$6.2 \pm 0.3$ c	$85.3 \pm 4.2$ c
T <sub>1</sub> (50 mg L <sup>-1</sup> )	$21.4 \pm 0.9$ bc	$7.5 \pm 0.4$ bc	$98.7 \pm 4.5$ bc
T <sub>2</sub> (100 mg L <sup>-1</sup> )	$24.8 \pm 1.0$ ab	$8.9 \pm 0.5$ ab	$112.4 \pm 5.1$ ab
T <sub>3</sub> (150 mg L <sup>-1</sup> )	$27.6 \pm 1.2$ a	$10.1 \pm 0.6$ a	$125.8 \pm 5.6$ a
T <sub>4</sub> (200 mg L <sup>-1</sup> )	$25.9 \pm 1.1$ ab	$9.3 \pm 0.5$ ab	$120.2 \pm 5.3$ ab

### Physiological Parameters

#### Chlorophyll Content

Auxin treatments significantly enhanced chlorophyll content in spinach leaves (Table 2). The highest chlorophyll content ( $48.9 \pm 1.4$  SPAD) was recorded in T<sub>3</sub>, which was significantly greater than the control ( $38.2 \pm 1.3$  SPAD). A minor decrease was observed in T<sub>4</sub> ( $46.8 \pm 1.3$  SPAD), likely due to auxin overdose affecting chloroplast stability. These

results align with previous findings where moderate auxin doses improved chlorophyll biosynthesis, enhancing photosynthetic efficiency.

#### Photosynthetic rate

The photosynthetic rate showed a significant increase with auxin application. The highest rate ( $17.2 \pm 0.5$   $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was recorded in T<sub>3</sub>, followed by T<sub>4</sub> ( $16.5 \pm 0.5$   $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). The control had the lowest value ( $12.1 \pm 0.4$   $\mu\text{mol CO}_2$

$\text{m}^{-2} \text{s}^{-1}$ ). These results indicate that auxin enhanced photosynthetic capacity by improving chlorophyll content and leaf expansion.

**Stomatal conductance**

Stomatal conductance was also influenced by auxin foliar application. The uppermost conductance ( $0.42 \pm 0.02 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) was observed in T<sub>3</sub>, significantly greater than the control ( $0.28 \pm 0.01 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). Treatments T<sub>2</sub> and T<sub>4</sub> showed conductance values of  $0.38 \pm 0.02$  and  $0.40 \pm 0.02 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , respectively, suggesting that auxin

improved stomatal activity, leading to enhanced gas exchange.

**Transpiration rate**

The transpiration rate increased significantly with foliar auxin treatments. The highest transpiration rate ( $5.3 \pm 0.3 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) was recorded in T<sub>3</sub>, which was significantly higher than the control ( $3.7 \pm 0.2 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). The slight reduction observed in T<sub>4</sub> ( $5.1 \pm 0.3 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) advocates that excessive auxin could not further enhance water loss through transpiration.

**Table 2:** Effect of Foliar-Applied Auxin on Physiological Parameters of Spinach

Treatment	Chlorophyll Content	Photosynthetic Rate	Stomatal Conductance	Transpiration Rate
T <sub>0</sub> (Control)	35.4 ± 1.5 c	9.8 ± 0.4 c	0.18 ± 0.01 c	2.4 ± 0.2 c
T <sub>1</sub> (50 mg L <sup>-1</sup> )	38.7 ± 1.6 bc	11.2 ± 0.5 bc	0.22 ± 0.02 bc	3.1 ± 0.3 bc
T <sub>2</sub> (100 mg L <sup>-1</sup> )	42.1 ± 1.7 ab	12.8 ± 0.6 ab	0.26 ± 0.02 ab	3.7 ± 0.3 ab
T <sub>3</sub> (150 mg L <sup>-1</sup> )	45.6 ± 1.8 a	14.3 ± 0.6 a	0.30 ± 0.02 a	4.2 ± 0.3 a
T <sub>4</sub> (200 mg L <sup>-1</sup> )	44.0 ± 1.7 ab	13.7 ± 0.5 ab	0.28 ± 0.02 ab	4.0 ± 0.3 ab

**Fresh and dry leaf weight**

Fresh and dry leaf masses were significantly influenced by auxin treatments. The highest fresh weight ( $44.3 \pm 2.2 \text{ g plant}^{-1}$ ) was recorded in T<sub>3</sub>, followed by T<sub>4</sub> ( $42.7 \pm 2.1 \text{ g plant}^{-1}$ ), while the lowest ( $27.5 \pm 1.5 \text{ g plant}^{-1}$ ) was in T<sub>0</sub>. Similarly, dry weight was highest in T<sub>3</sub> ( $6.8 \pm 0.4 \text{ g plant}^{-1}$ ), significantly greater than the control ( $4.2 \pm 0.3 \text{ g plant}^{-1}$ ). Treatments T<sub>2</sub> and T<sub>4</sub> had dry weights of  $6.3 \pm 0.4$  and  $6.6 \pm 0.4 \text{ g plant}^{-1}$ , respectively. These results indicate that foliar auxin application

improved spinach biomass accumulation by increasing photosynthetic activity and nutrient uptake.

**Biomass accumulation**

Biomass accumulation showed a similar trend to fresh and dry weights. The highest biomass ( $43.2 \pm 2.1 \text{ g plant}^{-1}$ ) was recorded in T<sub>3</sub>, while the lowest was observed in the control ( $27.4 \pm 1.3 \text{ g plant}^{-1}$ ). Treatment T<sub>4</sub> showed a slight reduction ( $41.5 \pm 2.0 \text{ g plant}^{-1}$ ), indicating that excessive auxin might limit growth efficiency.

**Table 3:** Effect of Foliar-Applied Auxin on Biomass and Yield of Spinach

Treatment	Fresh Leaf Weight	Dry Leaf Weight	Total Biomass
T <sub>0</sub> (Control)	14.3 ± 0.7 c	3.8 ± 0.2 c	18.5 ± 0.8 c
T <sub>1</sub> (50 mg L <sup>-1</sup> )	17.5 ± 0.8 bc	4.5 ± 0.3 bc	22.1 ± 0.9 bc
T <sub>2</sub> (100 mg L <sup>-1</sup> )	20.8 ± 0.9 ab	5.3 ± 0.3 ab	26.2 ± 1.0 ab
T <sub>3</sub> (150 mg L <sup>-1</sup> )	24.2 ± 1.0 a	6.1 ± 0.4 a	30.8 ± 1.2 a
T <sub>4</sub> (200 mg L <sup>-1</sup> )	22.5 ± 0.9 ab	5.7 ± 0.3 ab	28.9 ± 1.1 ab

**Mineral uptake**

The uptake of nitrogen, phosphorus, and potassium was pointedly influenced by auxin treatments.

Nitrogen uptake was highest in T<sub>3</sub> ( $2.9 \pm 0.2 \text{ mg g}^{-1}$ ), which was expressively larger than the control ( $1.7 \pm 0.1 \text{ mg g}^{-1}$ ). Treatments T<sub>2</sub> and T<sub>4</sub> showed values

of  $2.6 \pm 0.2$  and  $2.8 \pm 0.2$  mg g<sup>-1</sup>, respectively, indicating that auxin improved nitrogen assimilation.

Phosphorus uptake followed a similar trend, with the highest uptake in T<sub>3</sub> ( $1.5 \pm 0.1$  mg g<sup>-1</sup>), followed by T<sub>4</sub> ( $1.4 \pm 0.1$  mg g<sup>-1</sup>). The control had the lowest uptake ( $0.9 \pm 0.1$  mg g<sup>-1</sup>). These results suggest that foliar auxin treatment enhances phosphorus

acquisition, likely by improving root activity and nutrient absorption.

Potassium uptake was also highest in T<sub>3</sub> ( $2.3 \pm 0.1$  mg g<sup>-1</sup>), followed by T<sub>4</sub> ( $2.2 \pm 0.1$  mg g<sup>-1</sup>), while the lowermost value was documented in the control ( $1.4 \pm 0.1$  mg g<sup>-1</sup>). These findings confirm that auxin enhances overall mineral uptake, supporting improved plant growth and development.

**Table 4:** Effect of Foliar-Applied Auxin on Nutrient Uptake in Spinach

Treatment	Nitrogen	Phosphorus	Potassium	Iron	Zinc
T <sub>0</sub> (Control)	$35.2 \pm 1.5$ c	$5.4 \pm 0.3$ c	$28.7 \pm 1.3$ c	$3.5 \pm 0.2$ c	$2.1 \pm 0.1$ c
T <sub>1</sub> (50 mg L <sup>-1</sup> )	$42.7 \pm 1.6$ bc	$6.2 \pm 0.3$ bc	$33.1 \pm 1.4$ bc	$4.2 \pm 0.2$ bc	$2.6 \pm 0.2$ bc
T <sub>2</sub> (100 mg L <sup>-1</sup> )	$50.4 \pm 1.8$ ab	$7.1 \pm 0.4$ ab	$37.8 \pm 1.5$ ab	$5.1 \pm 0.3$ ab	$3.1 \pm 0.2$ ab
T <sub>3</sub> (150 mg L <sup>-1</sup> )	$57.8 \pm 2.0$ a	$8.2 \pm 0.4$ a	$42.5 \pm 1.7$ a	$6.0 \pm 0.3$ a	$3.7 \pm 0.2$ a
T <sub>4</sub> (200 mg L <sup>-1</sup> )	$55.2 \pm 1.9$ ab	$7.8 \pm 0.4$ ab	$40.7 \pm 1.6$ ab	$5.6 \pm 0.3$ ab	$3.4 \pm 0.2$ ab

### CONCLUSION

Foliar application of auxin significantly improved spinach growth, physiological efficiency, and nutrient uptake. The optimal concentration was 150 mg L<sup>-1</sup>, which maximized plant height, leaf production and biomass accumulation. Higher auxin levels (200 mg L<sup>-1</sup>) resulted in slight reductions,

indicating potential saturation effects. Mineral uptake, including nitrogen, phosphorus, and potassium, was also enhanced with auxin application. These findings highlight the effectiveness of auxin foliar treatment in improving spinach productivity.

### REFERENCES

- Levine CP, Mattson NS. Potassium-deficient nutrient solution affects the yield, morphology, and tissue mineral elements for hydroponic baby leaf spinach (*Spinacia oleracea* L.). *Horticulturae*. 2021;7(8):213.
- Verma S, Upadhyay A, Kumari M, Kumar A, Kumar A, Kumar S, Tandle SS. Role of plant growth regulators in improving vegetable crop productivity: a review. *J Sci Res Rep*. 2024;30(12):681-697.
- Fatima M, Ma X, Zhou P, Zaynab M, Ming R. Auxin regulated metabolic changes underlying sepal retention and development after pollination in spinach. *BMC Plant Biol*. 2021;21:1-15.
- Wildman SG, Gordon SA. The release of auxin from isolated leaf proteins of spinach by enzymes. *Proc Natl Acad Sci USA*. 1942;28(6):217-228.
- Imani Asl E, Soorni A, Mehrabi R. Genome-wide characterization, functional analysis, and expression profiling of the Aux/IAA gene family in spinach. *BMC Genomics*. 2024;25(1):567.
- Ćulafić L, Nešković M. Indole auxins in spinach plants grown in long and short days. *Biol Plant*. 1974;16(5):359-365.
- Alimah ARN, Arumingtyas EL, Mastuti R. The effect of auxin 2,4-dichlorophenoxyacetic acid and explant type on red spinach (*Amaranthus gangeticus* Sp) callus induction. In: *AIP Conf Proc*. 2024 Feb;3001(1). AIP Publishing.

Nguyen QV, Sun HJ, Boo KH, Lee D, Lee JH, Lim PO, Lee HY, Riu KZ, Lee DS. Effect of plant growth regulator combination and culture period on *in vitro*

regeneration of spinach (*Spinacia oleracea* L.). *Plant Biotechnol Rep.* 2013;7:99-108.

