Journal of
Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

Research Article

Integrated Use of Fertilizers and Bio-Stimulant Affect the Growth and Seedling Development of Chili

Maria Abro[1], Niaz Ahmed Wahocho*[1], Maqsood Ali Wagan[1], Tahira Abbas[2], Waqas Ahmad[2], Sumreen Anjum*[3], Hube Ali[1], Ali Raza Abro[4], Safdar Ali Wahocho[1], Narjis Abro[5]

[1] *Department of Horticulture, Sindh Agriculture University Tandojam, Pakistan.*
[2] *Department of Horticulture, University of Layyah, Layyah, Pakistan.*
[3] *Institutes of Botany, University of the Punjab, Lahore, Pakistan.*
[4] *Government College University, Hyderabad, Pakistan.*
[5] *Department of Plant Pathology, Sindh Agriculture University Tandojam, Pakistan.*

ABSTRACT

NPK fertilizer, in combination with bio-stimulants, is crucial in promoting the growth and development of chili. This study investigates the individual and combined effects of NPK fertilizers and a bio-stimulant (Hook) on the growth and morphological traits of chili (*Capsicum annuum* L.) seedlings. Seven treatments were applied, including NPK at three concentrations (1.5, 2.5, and 3.5 g/L), Hook (10 ml/L as a foliar spray), and their combinations. The foliar spray of Hook was applied 15 days after germination, while NPK was applied in two split doses, one week after germination and 20 days later. Ten seeds of the Advanta Hybrid hot pepper Shehzadi were sown per pot, and growth parameters were recorded at the five-leaf and six-leaf stages. Morphological traits such as plant height, number of leaves, plant spread, seedling girth, leaf area, seedling collar, chlorophyll content, root length, and seedling weight were evaluated. The results showed significant differences in growth parameters among the treatments. NPK at a lower concentration (1.5 g/L) promoted the tallest plants and the highest leaf production at both the five-leaf and six-leaf stages. Combined treatments of NPK and Hook, particularly 1.5 g/L NPK plus Hook (T5), displayed superior growth performance compared to individual applications, indicating a synergistic effect. Seedling girth, leaf area, and chlorophyll content were also notably enhanced with moderate NPK concentrations and combined treatments. Higher concentrations of NPK (3.5 g/L) and the sole application of Hook were less effective in promoting growth. The study concludes that moderate NPK (1.5–2.5 g/L) alone or with Hook (10 ml/L) significantly improves chili seedling growth, highlights the potential of optimized NPK and bio-stimulants for sustainable production.

Keywords: Chili seedlings, Growth, NPK Fertilizer, Hook, Morphology.

INTRODUCTION

Chili (*Capsicum annuum* L.) is a vegetable and medicinal plant from the Solanaceae family, originated in Mexico and spread worldwide after the Columbian Exchange, leads to the development of many different varieties (Saleh *et al*., 2018). The *Capsicum* genus includes about 31 species, with five domesticated ones: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* (Moscone *et al*., 2007). Chili is a perennial plant grown for its fruits, which can be eaten fresh or dried (Ahmed *et al*., 2020). It is used in powdered form, made from dried pods called 'berbere,' and added to dishes as a spice and food coloring (Saleh *et al*., 2013). Chili is a good source of proteins, vitamins like A and C, and essential

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Correspondence
Niaz Ahmed Wahocho,
Sumreen Anjum
nawahocho@gmail.com,
sumreen38@gmail.com

Article History
Received: December 12, 2024
Accepted: February 23, 2025
Published: March 24, 2025

minerals such as phosphorus and iron (Ahmed et al., 2020). Chili peppers have preventive and therapeutic benefits for various health issues, including different types of cancer, rheumatism, stiff joints, bronchitis, chest colds with cough and headache, arthritis, heart rhythm problems, and digestive issues (Saleh et al., 2018).

Chilies are grown on 62 thousand hectares of land in Pakistan, producing an annual yield of 131 thousand tons. However, when compared to other agriculturally advanced countries, Pakistan's per-hectare chili yield remains relatively low (Fatima *et al*., 2024). In Pakistan, soil organic matter is decreasing over time due to intensive cultivation of high-yield crops. These crops remove essential nutrients from the soil, gradually reducing its fertility. A balance between nutrient addition and removal is essential for a sustainable cropping system. However, farmers often rely on imbalanced chemical fertilizers, mainly urea, without using organic fertilizers, which prevents sustainable agriculture and limits yields (Altaf et al., 2019; Sarwar et al., 2018*)*. Chili plants require essential nutrients like nitrogen, phosphorus, and potassium for healthy growth. Nitrogen is vital for producing amino acids, enzymes, proteins, and plant hormones like indole acetic acid. Phosphorus is crucial for DNA, RNA, phospholipids, and proteins, and it plays a role in energy transfer, enzyme activity, and the formation of compounds with sugars and alcohols. Potassium helps regulate the plant's internal pH, controls stomata opening and closing, and supports the plant's ability to cope with water stress, contributing to efficient photosynthesis. NPK fertilizers are vital for chili plant growth, with nitrogen supporting leaf and stem development, phosphorus aiding root growth and nutrient transfer, and potassium improving plant quality, stress resistance, and fruit characteristics such as color, size, and shelf life (Ahmed *et al*., 2020; Fatima *et al*., 2024). In 2014, Calvo defined bio-stimulants as substances or microorganisms, promote plant health and growth (Calvo *et al*., 2014). The Bio-stimulants are used alongside conventional production methods to modify plant physiological processes and optimize yield (Sarwar et al., 2013). They consist of substances and/or microorganisms that, when applied to plants or the rhizosphere, stimulate natural processes. This stimulation enhances nutrient uptake, improves nutrient use efficiency, boosts tolerance to abiotic stress, and elevates crop quality (Manjunath, 2024). Several research studies indicate that both organic and compound fertilizers enhance growth and production in various crops, including sugar palm, shallots, sweet corn, cabbage flowers, melons, and sorghum (Gusta *et al*., 2022). Further, examining the effects of NPK fertilizers and the bio-stimulant (Hook) on chili seedling growth, this study has been carried out with the aim of evaluating the individual and combined impacts of NPK and bio-stimulant on the growth and morphological traits of chili seedlings. This research also focuses on identifying the most effective treatments for improving chili seedling development.

MATERIALS AND METHODS

Study Area

The experiment was conducted at the Nursery of the Department of Horticulture, Sindh Agriculture University (SAU) Tando Jam, in the agro-ecological setting of Tandojam, Sindh, Pakistan, from September to November 2024. The purpose of the experiment was to evaluate the impact of fertilizers and the bio-stimulant (Hook) to identify the optimal combination for enhancing the growth and morphological traits of chili seedlings.

Experimental Design, Plant Material and Treatment Details

The study investigated different concentrations of NPK fertilizer applied through soil application and the bio-stimulant (Hook) at a concentration of 10 ml/L as a foliar spray. The experimental treatments included NPK at 1.5 g/L of water (T1), NPK at 2.5 g/L of water (T2), and NPK at 3.5 g/L of water (T3). The bio-stimulant (Hook) was applied as a foliar spray (T4). Additional combined treatments included T5 (T1 + T4, soil + foliar application), T6 (T2 + T4, soil + foliar application), and T7 (T3 + T4, soil + foliar application) (Table 1 and Figure 1).

Advanta Hybrid hot pepper Shehzadi chili seeds were used as plant material. A completely randomized design (CRD) was employed, consisting of three replications. Each replication included ten seeds sown in pots filled with sandy and silt soil. The pots had dimensions of 17.5 cm in width and 21.5 cm in length. The foliar spray of bio-stimulant (Hook) was applied 15 days after germination using a hand sprayer during the morning hours. NPK was applied in two split doses, the first application occurring one week after germination and 20 days after first split. Intercultural practices, including irrigation and weeding, were carried out as standard practices to support the growth and development of chili seedlings. The seedlings were grown under a shade house, providing a combination of shade and light to create suitable conditions for young chili seedlings. The average temperature during the experiment ranged from 39°C during the day to 25°C at night.

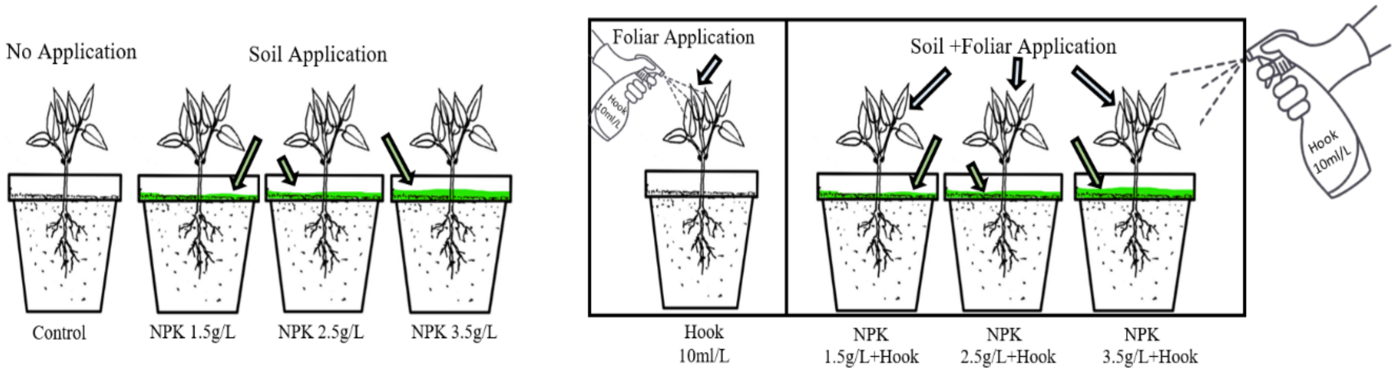

Figure 1. Schematic diagram illustrating different application method and doses.

Assessment of Growth and Morphological Traits

Growth and morphological traits of chili seedlings were assessed at two stages. At the five-leaf stage, plant height (PH) and the number of leaves (NOL) were evaluated under both control and treatment conditions. At the six-leaf stage, the remaining traits were measured, including plant height (PH), number of leaves (NOL), plant spread (PS), seedling girth (SG), leaf area (LA), seedling collar (SC), chlorophyll content (CC), root length (RL), and seedling weight (SW). Plant height (PH) was measured by randomly selecting seedlings and recording the distance from the ground level to the tip of the main stem using a measuring scale. The number of true leaves (NOL) was recorded manually for each seedling. Plant spread (PS) was measured by recording the maximum width of the plant canopy from one end to the other using a measuring scale. Seedling girth (SG) was measured at three locations like top, middle, and base of the stem using a vernier caliper. The average of these three measurements was calculated to ensure precision. Leaf area (LA) was calculated using the formula proposed by (Dheebakaran and Jagannathan, 2009): Leaf Area = Length × Width × 0.74. Seedling collar (SC) was measured at the point where the stem meets the roots using a vernier caliper to determine the collar diameter. Chlorophyll content (CC) was assessed using a portable chlorophyll meter (SPAD meter). SPAD readings were taken from three different locations on the leaf top, middle, and bottom. The average of these readings was recorded as the chlorophyll content. Before measuring root length (RL), the roots of tagged seedlings were washed to remove soil particles. Excess water was removed by gently pressing the roots with a tissue paper sheet. The longest root from each seedling was selected and measured using a measuring scale. Seedling weight (SW) was determined by gently uprooting the seedlings and washing the roots to eliminate any attached soil. The fresh weight of the entire seedling, including both root and shoot, was measured using a digital weighing balance.

Statistical Analysis

One-way analysis of variance was used by applying using Statistix software, version 8.1 and significant differences among different treatments were evaluated using least significant difference analysis test at 0.05 probability.

RESULTS

All phenotypic traits at the five-leaf and six-leaf stages across all treatments exhibited continuous and significantly wide variations.

Impact of Different Concentrations of NPK Fertilizer and Bio-stimulant on Plant Height (PH)

The mean plant height at the six-leaf stage was significantly greater across all treatments, with values of 5.36, 7.46, 8.68, 9.50, 9.13, 9.98, 10.27, and 10.63cm, respectively (Table 2). In comparison, the corresponding mean heights at the five-leaf stage were notably lower, recorded as 4.75, 5.00, 6.06, 6.18, 6.89, 6.99, 7.74, and 7.95 cm, respectively (Table 2).

Table 1. List of different treatments with doses.

Treatments	Concentration	Application
T0	Control	No application
T1	NPK @ 1.5 g/L of water	Soil application
T2	NPK @ 2.5 g/L of water	Soil application
T3	NPK @ 3.5 g/L of water	Soil application

T4	Hook @10 ml/L of water	Foliar Application
T5	T1 + T4	Soil + Foliar Application
T6	T2 + T4	Soil + Foliar Application
T7	T3 + T4	Soil + Foliar Application

Table 2. Basic statistics of Seedling traits at five-leaf and six-leaf stages grown under different treatments.

Treatments	Five-leaf stage		Six-leaf stage	
	Plant Height (cm)	Number of Leaf	Plant Height (cm)	Number of Leaf
T0	7.95	6.66	9.98	7.22
T1	7.74	6.00	10.63	8.00
T2	6.99	5.77	10.27	7.33
T3	5.00	4.27	7.46	5.77
T4	4.75	3.44	5.36	4.22
T5	6.89	4.66	9.13	6.88
T6	6.06	4.44	8.68	6.33
T7	6.18	4.72	9.05	6.77
S.E.±	1.4536	0.7678	2.0537	1.1733
$LSD_{0.05}$	3.0814	1.6276	4.3537	2.4872

A statistical difference was observed in plant height (PH) between all individual and combined treatments of NPK and Hook at the five-leaf and six-leaf stages. The highest PH at the five-leaf stage was recorded in T_0 (7.95 cm), which was statistically similar to T_1 (7.74 cm) (1.5 g/L NPK) and T_2 (6.99 cm) (2.5 g/L NPK). The PH recorded in T_5 (6.89 cm) (1.5 g/L NPK + 10 ml/L Hook) was comparable to T_7 (6.18 cm) (3.5 g/L NPK + 10 ml/L Hook) and T_6 (6.06 cm) (2.5 g/L NPK + 10 ml/L Hook). Lower PH values were observed in T_3 (5.00 cm) (3.5 g/L NPK) and T_4 (4.75 cm) (10 ml/L Hook), suggesting that higher NPK concentrations and the sole application of Hook were less effective at this stage (Table 2 and Figure 2)

At the six-leaf stage, PH was highest in T_1 (10.63 cm) (1.5 g/L NPK), followed by T_2 (10.27 cm) (2.5 g/L NPK) and T_0 (9.98 cm) (control). The PH observed in T_5 (9.13 cm) (1.5 g/L NPK + 10 ml/L Hook) was statistically comparable to T_7 (9.05 cm) (3.5 g/L NPK + 10 ml/L Hook) and T_6 (8.68 cm) (2.5 g/L NPK + 10 ml/L Hook). Less PH was recorded in T_3 (7.46 cm) (3.5 g/L NPK) and T_4 (5.36 cm) (10 ml/L Hook), indicating that higher NPK concentration and the sole application of Hook were less effective. The synergistic effect of 1.5 g/L NPK combined with Hook (T_5) showed better PH (9.13 cm) than the combined applications of 2.5 g/L NPK (T_6) (8.68 cm) and 3.5 g/L NPK (T_7) (9.05 cm). The results indicate that moderate concentrations of NPK (1.5–2.5 g/L) were more effective than higher concentrations, and the combined application of NPK and Hook produced better results than the individual application of either treatment (Figure 2).

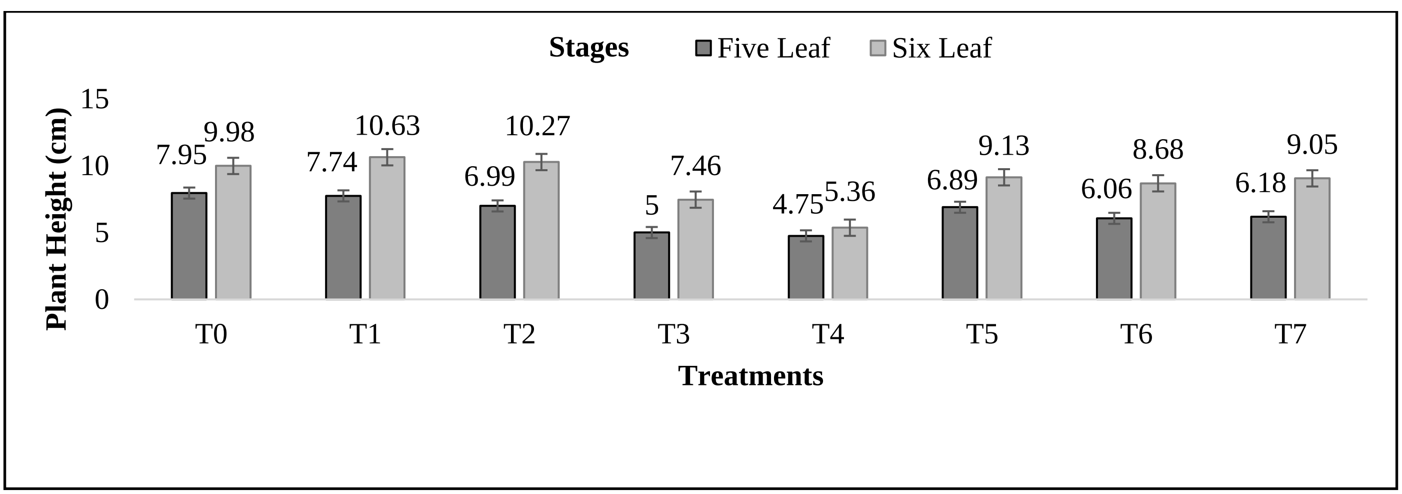

Figure 2. Effect of different concentration of NPK fertilizer and bio-stimulant on Plant height.

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Impact of Different Concentrations of NPK Fertilizer and Hook on Number of Leaf (NOL)

The number of leaves (NOL) showed significant variation among all treatments at the five-leaf and six-leaf stages. The mean values of NOL at the six -leaf stage were higher (8, 7.33, 5.77, 4.22, 6.88, 6.33, and 6.77, respectively) as compared to the five-leaf stage (6, 5.77, 4.27, 3.44, 4.66, 4.44, and 4.72, respectively). At the five-leaf stage, the highest NOL was recorded in T_1 (6) (1.5 g/L NPK), followed by T_2 (5.77) (2.5 g/L NPK) and T_5 (4.66) (1.5 g/L NPK + 10 ml/L Hook). Lower NOL was recorded in T_6 (4.44) (2.5 g/L NPK + 10 ml/L Hook) and T_7 (4.72) (3.5 g/L NPK + 10 ml/L Hook). The minimum NOL (3.44) was observed in T_4 (10 ml/L Hook), followed by T_3 (4.27) (3.5 g/L NPK), indicating that the sole application of bio-stimulant-or higher concentrations of NPK resulted in fewer leaves (Table 2 and Figure 3).

At the six-leaf stage, the highest NOL was observed in T_1 (8) (1.5 g/L NPK), followed by T_2 (7.33) (2.5 g/L NPK) and T_5 (6.88) (1.5 g/L NPK + 10 ml/L Hook). The NOL recorded in T_7 (6.77) (3.5 g/L NPK + 10 ml/L Hook) was comparable to T_6 (6.33) (2.5 g/L NPK + 10 ml/L Hook), showing that the combined application of NPK with Hook improved NOL compared to individual applications. Lower NOL was observed in T_3 (5.77) (3.5 g/L NPK) and T_4 (4.22) (10 ml/L Hook), indicating that the higher concentration of NPK and the sole application of Hook were less effective. The outcomes showed that the highest NOL was recorded in T_1 (8) at the six-leaf stage, followed by T_2 (7.33) and T_5 (6.88), while the lowest NOL was observed in T_4 (4.22) (10 ml/L Hook) and T_3 (5.77) (3.5 g/L NPK).

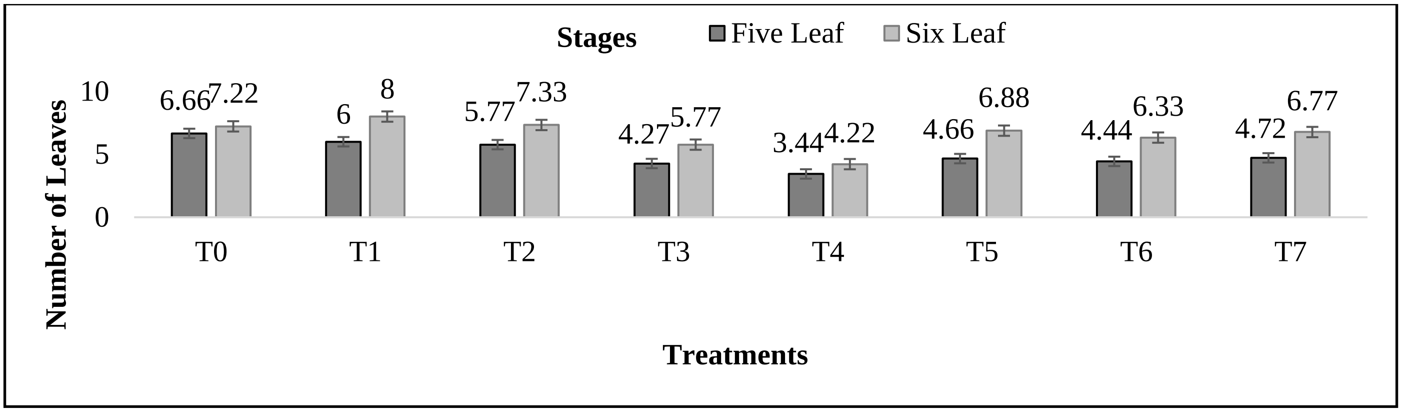

Figure 3. Effect of different concentration of NPK fertilizer and Hook on Number of leaves.

Regulation of bio-stimulant (Hook) and Different Concentrations of NPK Fertilizer on Seedling Girth (SG)

The seedling girth (measured in millimeters) showed variation across different treatments. The highest seedling girth was observed in T2 (2.5 g/L NPK) with a mean of 1.76 mm, followed closely by T1 (1.5 g/L NPK) and T5 (1.5 g/L NPK + 10 ml/L Hook), with means of 1.68 mm and 1.69 mm, respectively. These treatments, involving moderate to low concentrations of NPK combined with or without the bio-stimulant Hook, resulted in the thickest seedlings. On the other hand, the T3 (3.5 g/L NPK) treatment showed a lower mean girth of 1.38 mm, indicating that the highest concentration of NPK did not contribute to the maximum seedling girth. Similarly, the T4 (10 ml/L Hook) treatment, which involved only the bio-stimulant, had the lowest seedling girth of 0.82 mm, suggesting that the bio-stimulant alone was less effective in promoting seedling growth compared to NPK treatments (Figure 4).

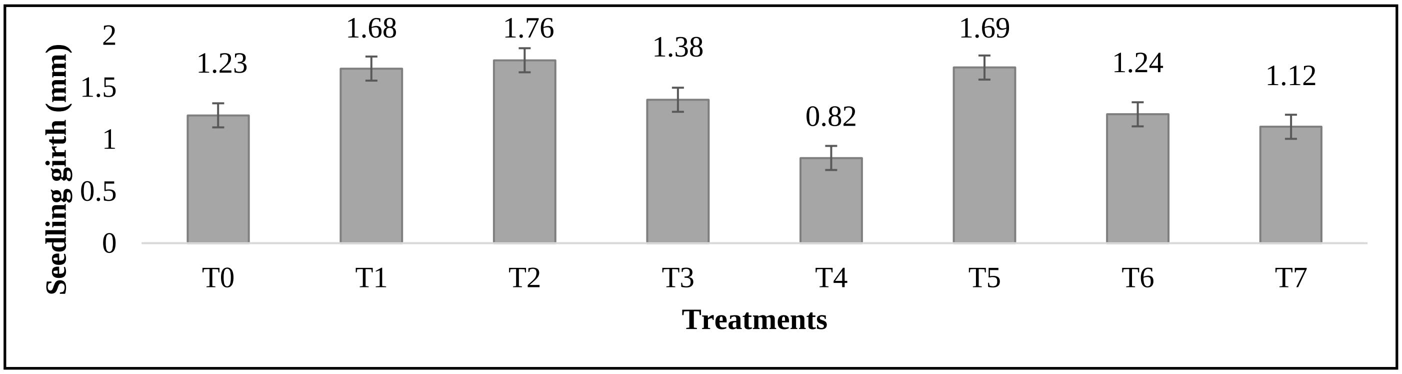

Figure 4. Effect of different concentration of NPK fertilizer and Hook-on seedling girth (mm).

Lastly, the combined treatments of T6 (2.5 g/L NPK + 10 ml/L Hook) and T7 (3.5 g/L NPK + 10 ml/L Hook) showed lower mean seedling girth values (1.24 mm and 1.12 mm, respectively) compared to the treatments that included only NPK. This suggests that the addition of Hook with higher concentrations of NPK may not have a synergistic effect on seedling girth development.

Impact of Bio-stimulant (Hook) and Different Concentrations of NPK Fertilizer on Leaf Area (LA).

The application of different treatments significantly influenced the leaf area (cm²) of chili plants. Among the treatments, T2 (2.5 g/L NPK) produced the largest leaf area, with a mean of 5.76 cm², followed by T3 (3.5 g/L NPK) at 5.45 cm² and T1 (1.5 g/L NPK) at 5.42 cm². These results indicate that NPK fertilizer alone had a pronounced effect on promoting leaf expansion. In contrast, T4 (10 ml/L Hook), which involved only the bio-stimulant, resulted in the smallest leaf area, with a mean of 1.48 cm², suggesting that the bio-stimulant alone was less effective in promoting leaf growth compared to NPK treatments. The combined treatments showed an intermediate effect on leaf area. T7 (3.5 g/L NPK + 10 ml/L Hook) had a mean leaf area of 4.62 cm², followed by T5 (1.5 g/L NPK + 10 ml/L Hook) with 4.5 cm², and T6 (2.5 g/L NPK + 10 ml/L Hook) with 3.96 cm². These values were higher than the leaf area observed for T4 but lower than those recorded for NPK-only treatments. This pattern suggests that while the addition of the bio-stimulant to NPK may have some effect, it did not result in a larger leaf area than NPK alone (Figure 5).

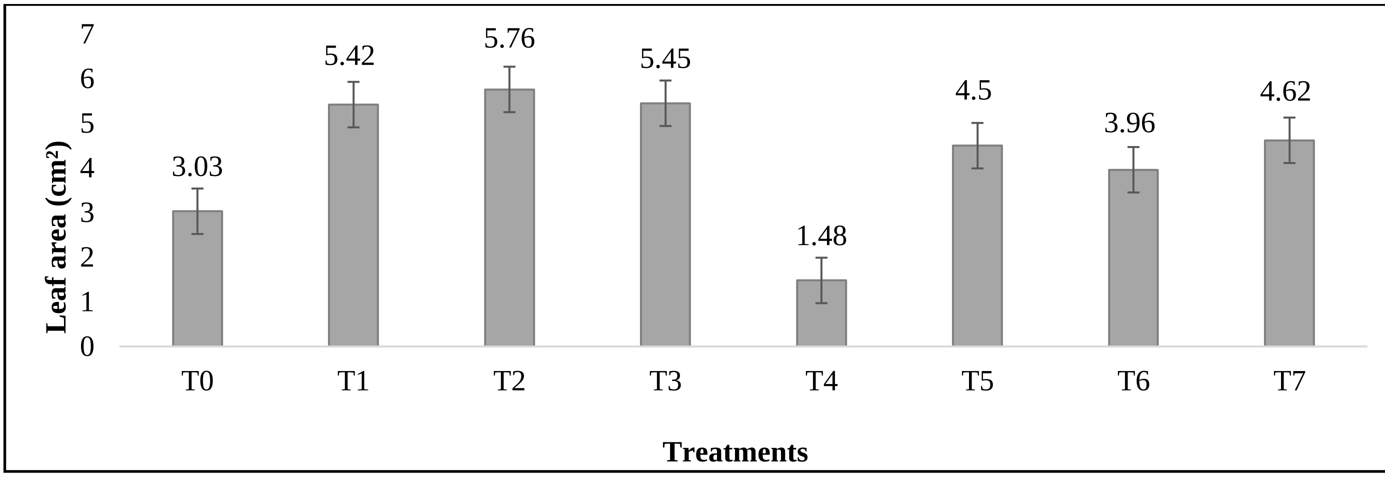

Figure 5. Effect of different concentration of NPK fertilizer and Hook on Leaf area.

Impact of Bio-stimulant (Hook) and NPK Fertilizer Levels on Plant Spread (PS).

The plant spread (measured in centimeters) showed significant variation across different treatments. The highest plant spread was observed in T2 (2.5 g/L NPK), with a mean of 10.75 cm, followed closely by T5 (1.5 g/L NPK + 10 ml/L Hook) with a mean of 10.27 cm. These treatments, involving NPK fertilizer, resulted in the largest plant spread, indicating that NPK alone, and even when combined with the bio-stimulant Hook, had a positive effect on the lateral growth of the plants (Figure 6).

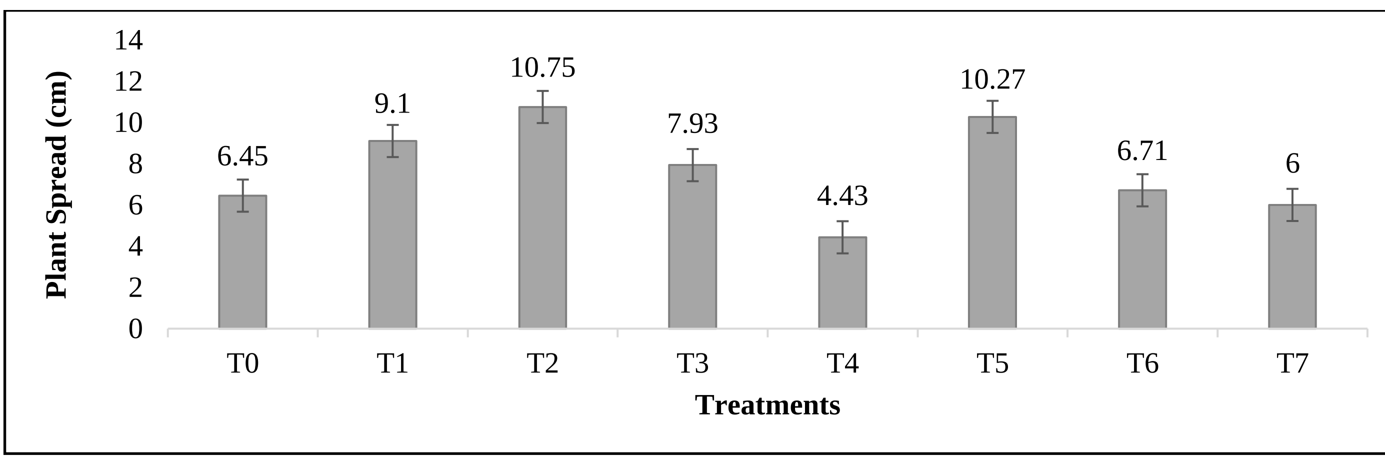

Figure 6. Effect of different concentration of NPK fertilizer and Hook on Plant spread.

https://doi.org/10.55627/agribiol.003.01.1169

In contrast, T4 (10 ml/L Hook), which involved only the bio-stimulant, showed the lowest plant spread with a mean of 4.43 cm, suggesting that the bio-stimulant alone did not significantly enhance plant spread. The treatments combining Hook with higher concentrations of NPK, such as T6 (2.5 g/L NPK + 10 ml/L Hook) and T7 (3.5 g/L NPK + 10 ml/L Hook), showed intermediate values for plant spread, with means of 6.71 cm and 6 cm, respectively. While the bio-stimulant Hook improved plant spread slightly, the best results were observed with NPK alone, especially at 2.5 g/L.

Role of Bio-stimulant (Hook) and NPK Fertilizer Concentrations in Seedling Collar Thickness

The seedling collar diameter, which is an indicator of seedling growth, showed varying responses to different treatments. The treatment with 1.5 g/L NPK (T1) showed the highest collar diameter of 2.20 mm, followed closely by 2.5 g/L NPK (T2) with 2.12 mm. These values suggest that NPK fertilizers positively impacted seedling growth, promoting larger collar diameters. The combination of 1.5 g/L NPK and Hook (T5) also resulted in a relatively larger collar diameter (1.97 mm) compared to the bio-stimulant Hook alone (T4), which had the smallest collar size at 1.43 mm. When Hook was combined with higher concentrations of NPK (T6, T7), the collar diameters were slightly larger than Hook alone but still lower than the NPK-only treatments. Overall, NPK treatments, particularly at 1.5 g/L and 2.5 g/L, proved to be more effective in promoting seedling collar growth compared to Hook or its combination with NPK (Figure 7).

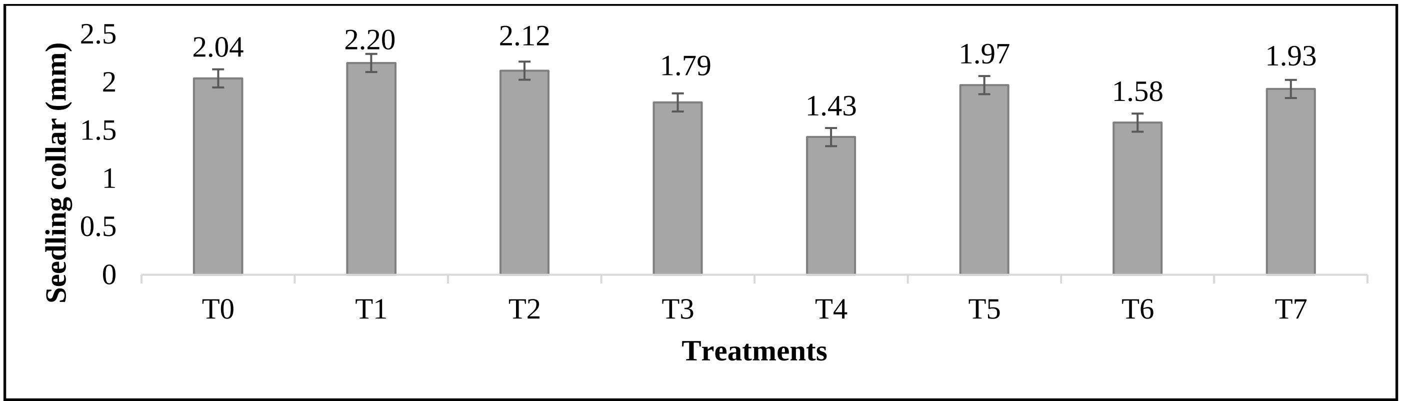

Figure 7. Effect of different concentration of NPK fertilizer and Hook on Seedling collar.

Influence of Bio-stimulant (Hook) and NPK Fertilizer Levels on Chlorophyll Content (SPAD)

The chlorophyll content, measured through SPAD readings, showed significant variation among the treatments. The highest chlorophyll content was recorded in the combination of 2.5 g/L NPK and 10 ml/L Hook (T6) at 49.36, followed by 3.5 g/L NPK and 10 ml/L Hook (T7) at 47.78. This indicates a synergistic effect when NPK is combined with Hook. Among the NPK-only treatments, 1.5 g/L NPK (T1) had a notable impact, recording 45.66, while 2.5 g/L NPK (T2) reached 42.05. However, the 3.5 g/L NPK (T3) had a comparatively lower mean of 32.58. The control (T0) recorded 35.46, serving as a reference for comparison. The lowest chlorophyll content was observed in Hook alone (T4) at 31.36 (Figure 8).

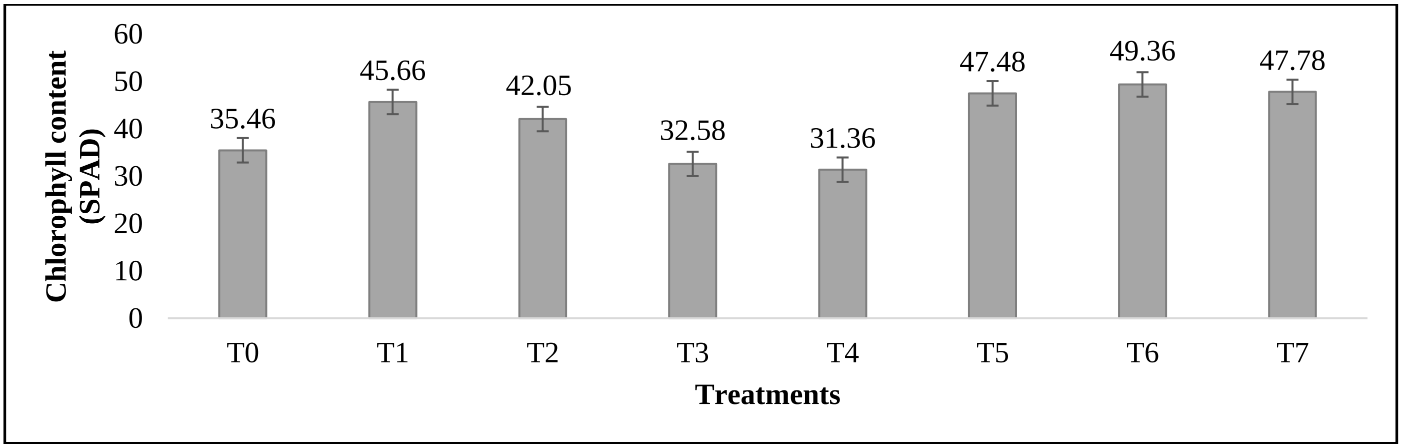

Figure 8. Effect of different concentration of NPK fertilizer and Hook on Chlorophyll content SPAD.

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The results suggest that Hook individual had less effect compared to when it was combined with NPK, which gave better results. These findings emphasize the effectiveness of combined treatments, particularly T6, in boosting chlorophyll content.

Root Length Performance Under Bio-stimulant (Hook) and Different NPK Fertilizer Concentrations

The root length of seedlings showed notable variation among the different treatments. Each treatment demonstrated a distinct influence on root development, reflecting the varying effects of NPK concentrations and the bio-stimulant on root growth. The longest root length (4.66 cm) was recorded in both the control (T0) and T1 (1.5 g/L NPK), indicating that a lower concentration of NPK was as effective as the control in promoting root elongation (Figure 9). Among the NPK-only treatments, T2 (2.5 g/L NPK) and T3 (3.5 g/L NPK) showed shorter root lengths of 3.74 cm and 3.09 cm, respectively, suggesting that higher NPK concentrations may have a limiting effect on root growth. The individual application of the bio-stimulant Hook (T4) produced the shortest root length (2.41 cm), reflecting its limited influence on root elongation when used alone. The synergistic effects of NPK and Hook showed mixed results. T5 (1.5 g/L NPK + 10 ml/L Hook) resulted in a root length of 3.96 cm, which was better than T6 (2.5 g/L NPK + 10 ml/L Hook) at 2.80 cm and T7 (3.5 g/L NPK + 10 ml/L Hook) at 3.33 cm. This indicates that the combined effect of NPK and Hook is more effective at lower NPK concentrations for promoting root growth.

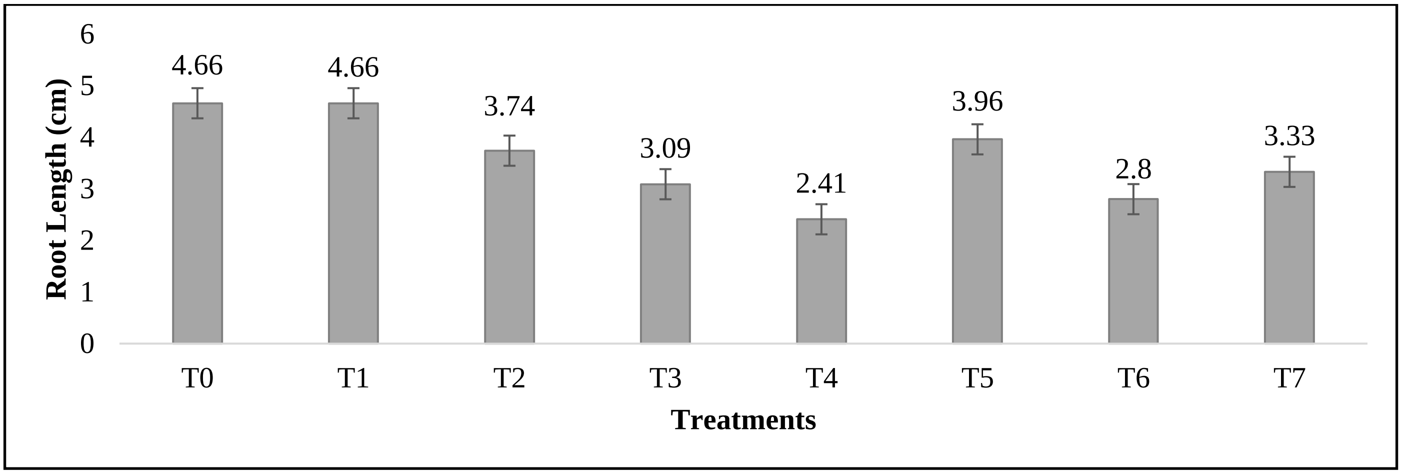

Figure 9. Effect of different concentration of NPK fertilizer and Hook Root Length.

Impact of Bio-stimulant (Hook) and Different NPK Fertilizer Concentrations on Seedling Weight

The results show the seedling weight under different treatments, with the highest weight observed in T1 (1.5 g/L NPK) at 0.84 g, followed by T2 (2.5 g/L NPK) at 0.69 g and T3 (3.5 g/L NPK) at 0.59 g. The lowest seedling weight was found in T4 (10 ml/L Hook) at 0.18 g, indicates a significant reduction with Hook alone. The synergistic treatments applications of NPK and Hook, such as T5 (1.5 g/L NPK + 10 ml/L Hook), showed a weight of 0.59 g, similar to T3. T6 (2.5 g/L NPK + 10 ml/L Hook) and T7 (3.5 g/L NPK + 10 ml/L Hook) had lower weights of 0.46 g and 0.31 g, respectively, suggesting that Hook's impact did not enhance seedling weight when combined with higher NPK concentrations (Figure 10).

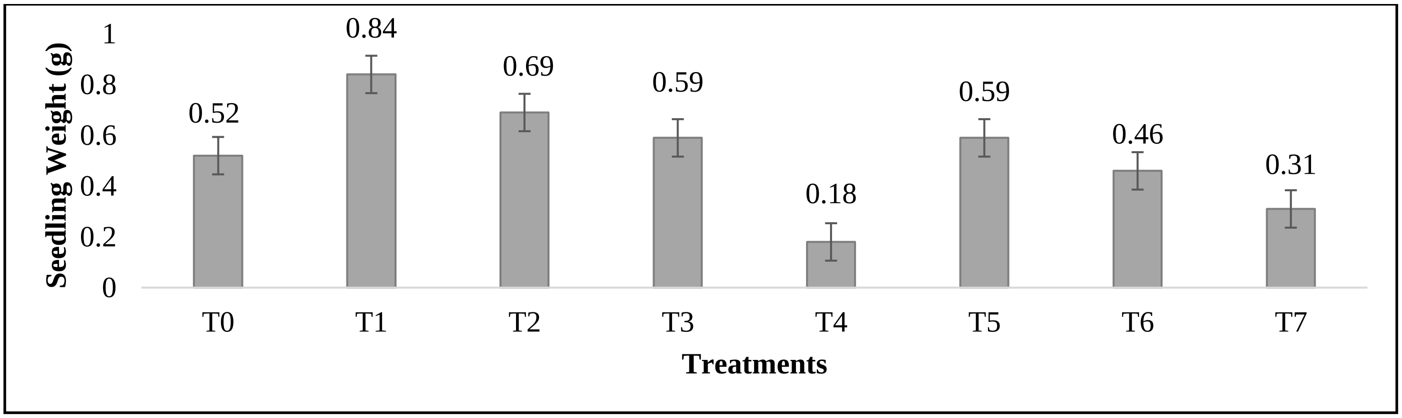

Figure 10. Effect of different concentration of NPK fertilizer and Hook Seedling Weight.

DISCUSSION

Scientific application of fertilizers and hormones is a key approach enhancing crop development, protecting the environment, and promoting sustainable agriculture (Fatima *et al*., 2024). Plant height (PH) is a key factor influencing overall plant growth and yield. According to the findings of this study, PH was observed remarkably higher in control at the five-leaf stage but declined by following the application of various treatments (Table 2, Figure 2). At the six-leaf stage, PH increased across all treatments, demonstrating a positive response to their application but, decreased by the application of NPK @ 3.5 g/L of water and Hook in the present study (Figure 2). The highest PH was achieved with NPK at 1.5 g/L of water, likely due to the optimal nutrient availability that supported balanced vegetative growth without causing excessive soil osmotic pressure. At this concentration, the plants may have efficiently absorbed nutrients, promoting enhanced cell division, elongation, and overall structural development, results in increased plant height. In contrast, higher concentrations of NPK might have created adverse conditions, such as increased osmotic stress, reducing water uptake and hindering growth (Fatima *et al*., 2024). Similar to plant height (PH), significant variations were observed in the number of leaves (NOF) across different treatments at both the five-leaf and six-leaf stages. At the five-leaf stage, NOF was higher in the control but decreased following the application of various treatments (Table 2, Figure 3). At the six-leaf stage, all treatments showed an increase in NOF, indicating a favorable response to the applied nutrients and bio-stimulants. A reduction in NOF was noted with the application of NPK @ 3.5 g/L of water and Hook in this study (Figure 3). The maximum NOF was recorded with NPK at 1.5 g/L of water, which can be linked to an optimal nutrient balance supporting robust vegetative growth and leaf expansion. This favorable outcome might be attributed to improved chlorophyll synthesis and photosynthetic efficiency, which are essential for healthy leaf development. Nitrogen plays a crucial role in chlorophyll production and supports protein synthesis. An adequate supply of nitrogen, combined with balanced plant nutrition, promotes an increase in the number of leaves per plant (Arthanawa *et al*., 2022).

On the other hand, higher concentrations of NPK, particularly in combination with Hook, might have triggered nutrient competition or reduced hormonal equilibrium, negatively affecting leaf formation. These observations underscore the importance of precise nutrient management to optimize leaf production and ensure healthy plant development. NPK treatment promotes vegetative growth. An optimal phosphorus (P) dose enhances NOF, while excessive P can reduce the number of leaves per plant (Akram *et al*., 2017). In the current study, effect of NPK at higher dose 3.5 g/L in a lower number of leaves (NOF), this suggests that an excess of nitrogen (N) does not consistently promote vegetative growth, leading us to hypothesize that too much nitrogen may impair the plants' ability to effectively utilize it (Chen *et al*., 2018). The seedling girth (SG) varied across different treatments, with the highest girth observed in T2 (2.5 g/L NPK) and the lowest in T4 (10 ml/L Hook). Treatments involving moderate to low concentrations of NPK, with or without the bio-stimulant Hook, promoted thicker seedlings, while the highest concentration of NPK (T3) resulted in a lower girth. The T4 treatment, which included only the bio-stimulant, showed the lowest seedling girth, indicating that the bio-stimulant alone was less effective in promoting growth compared to NPK treatments. Similarly, the combined treatments of NPK and Hook (T6 and T7) also resulted in lower seedling girth than treatments with only NPK (Figure 4). These results can be explained by the absorption and movement of NPK through the plant's vascular system, where it stimulates cell elongation and division. This process leads to increased Plant height, seedling girth and leaf area (Singh and Singh, 2023), especially when moderate concentrations of NPK are used. However, the combination of higher NPK concentrations with Hook may interfere with the plant's growth processes, potentially limiting the enhancement of seedling girth.

The application of different treatments significantly influenced the leaf area (cm²) of chili plants. The largest leaf area was observed in T2 (2.5 g/L NPK), while the smallest was recorded in T4 (10 ml/L Hook). These findings suggest that NPK fertilizer alone had a substantial impact on promoting leaf expansion, whereas the bio-stimulant alone was less effective. Combined treatments exhibited intermediate effects, indicating that while the addition of the bio-stimulant to NPK had some influence, it did not surpass the effectiveness of NPK alone (Figure 5). According to Ali, this could be attributed to moderate leaf area development during the early growth stages when various soil nutrients have yet to be fully absorbed and utilized by the plant (Ali *et al*., 2020). Plant spread, an important measure of lateral growth, also showed significant variation across treatments. The greatest spread was observed with NPK alone, particularly in the treatment with 2.5 g/L NPK, highlighting its strong effect on plant growth. In contrast, the treatment with Hook alone resulted in the smallest plant spread, indicating that the bio-stimulant had limited impact on lateral growth. When Hook was combined with NPK, the results were intermediate, suggesting that while the bio-stimulant contributed to some extent, the most notable plant spread was achieved with NPK alone (Figure 6). Variations in seedling collar diameter

were evident across the treatments, reflecting their influence on seedling growth. The treatment with 1.5 g/L NPK (T1) yielded the thickest collar, emphasizing the beneficial role of NPK in enhancing growth. In contrast, the bio-stimulant Hook alone (T4) resulted in the thinnest collar, suggesting limited effectiveness in this regard. The combination of 1.5 g/L NPK with Hook (T5) showed improvement over Hook alone, but treatments with NPK alone demonstrated superior outcomes overall. Chlorophyll content, an essential indicator of photosynthetic efficiency, was assessed under each treatment. The highest chlorophyll content was observed in T6 (2.5 g/L NPK + 10 ml/L Hook), indicating a synergistic effect of NPK and Hook. Among the NPK-only treatments, T1 (1.5 g/L NPK) had a notable impact, while the lowest chlorophyll content was recorded in T4 (10 ml/L Hook), which involved Hook alone. These results emphasize the effectiveness of combined treatments in boosting chlorophyll levels (Figure 8). According to Rather, the enhancement of vegetative growth can be attributed to the stimulatory effects of nano micronutrients on chlorophyll production, photosynthesis, mitochondrial respiration, and hormone biosynthesis (Rather *et al*., 2022; Sarwar et al., 2022).
Root length and seedling weight exhibited significant variation across the different treatments, reflecting the effects of different NPK concentrations and the bio-stimulant Hook on growth. The longest roots and highest seedling weight were observed in T1 (1.5 g/L NPK), indicating that lower NPK concentrations support optimal growth. Higher NPK levels (T2 and T3) hindered root elongation, while Hook alone (T4) showed limited impact. Among combined treatments, T5 (1.5 g/L NPK + Hook) promoted better root development and comparable seedling weight to T3, highlighting the synergistic effect of Hook with lower NPK concentrations. According to Fatima, the combined application of NPK fertilizer enhances growth by activating enzymes for hormonal synthesis and supporting chlorophyll production, photosynthesis, and mitochondrial respiration. Root length and diameter significantly contribute to chili yield, with combined NPK treatments outperforming others in promoting root development (Fatima *et al*., 2024). The results of this study indicate that the applied NPK fertilization levels significantly enhanced chili vegetative growth parameters compared to the control. These findings align with those reported by (Ahmed *et al*., 2020) for chili and (Ahmed *et al*., 2017) for celery and chili. Additionally, Marschner (1995) highlighted that nitrogen (N), phosphorus (P), and potassium (K) play a crucial role in various physiological and biochemical processes, further supporting the importance of these nutrients in plant development**.**

CONCLUSIONS

In conclusion, different concentrations of NPK and their combinations with the bio-stimulant Hook significantly influenced the morphological traits and chlorophyll content of chili seedlings. When applying fertilizers and growth enhancers, it is crucial to consider their appropriate type, application method, and the seedling's developmental needs for optimal early-stage growth. Our study demonstrates that while all treatments had varying effects on seedling traits, the application of NPK at 2.5 g/L showed the most promising results for leaf area and chlorophyll content when combined with Hook, whereas 1.5 g/L of NPK yielded the best outcomes for plant height, root length, and seedling weight. Conversely, higher concentrations of NPK and Hook alone exhibited limited effectiveness in enhancing growth. Therefore, the combined application of 1.5 g/L of NPK with Hook can be recommended for balanced seedling growth and improved chlorophyll levels. The findings of this study provide valuable insights into the response of chili seedlings to different nutrient treatments and offer a practical approach for producing robust seedlings with enhanced growth, photosynthetic efficiency, and structure.

ACKNOWLEDGEMENTS

This research study was conducted without any external funding support. The authors sincerely thank the research team for their unwavering support, expert guidance, and invaluable contributions throughout this study.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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