Bacterially Produced Indole-3-Acetic Acid as a Biological Alternative to Commercial Plant Growth Regulators for Improving Growth, Yield, and Fruit Quality of Tomato (*Solanum lycopersicum* L.)

Wafaa Hadi Hassoon[1], Doaa Abbas Hanoon[2] , Safaa Abid Al-Rahem Mahmood [3], Ali Khudhair Yas[4].
1,2,3,4Scientific Research commission,Baghdad,Iraq
1Email: *Corresponding author's email: wafaahassoon@src.edu.iq
2Email: doaa.a.hanoon@src.edu.iq 3Email: safaa.a.mahmood@src.edu.iq 4 Email: ali.y.algburi@src.edu.iq

Abstract

A greenhouse pot experiment was conducted during the winter season of 2022 to evaluate the effects of bacterially produced indole-3-acetic acid (IAA), commercial auxins, and putrescine on growth, yield, and fruit quality of tomato (*Solanum lycopersicum* L.). The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replicates. Treatments included bacterially produced IAA obtained from *Bacillus thuringiensis* KS3 kurstaki, commercial indole-3-acetic acid (IAA) at concentrations of 0.2 and 0.4 mg L^{-1}, naphthaleneacetic acid (NAA) at 0.2 and 0.4 mg L^{-1}, putrescine at 0.2 and 0.4 mg L^{-1}, and an untreated control. Significant differences among treatments were observed for most vegetative growth, flowering, yield, and fruit quality traits. Bacterially produced IAA significantly increased plant height, chlorophyll content, number of fruits $plant^{-1}$, and yield $plant^{-1}$ compared with the control. Commercial IAA at 0.2 mg L^{-1} significantly improved leaf area, fruit set percentage, vitamin C content, and lycopene concentration, whereas commercial IAA at 0.4 mg L^{-1} produced the highest number of flowers $plant^{-1}$. Putrescine treatments showed comparatively limited effects on most studied traits under greenhouse conditions. The results indicate that bacterially produced IAA has potential as a biological plant growth regulator for improving tomato growth, productivity, and fruit quality under protected cultivation conditions.

Keywords: Indole-3-acetic acid; *Bacillus thuringiensis* KS3; PGPR; Lycopene; Tomato.

Introduction

Plant growth regulators play an important role in regulating plant growth and development through their effects on cell division, elongation, flowering, fruit set, and physiological processes. Among these regulators, auxins are considered one of the most important phytohormones involved in root development, photosynthesis, and plant productivity[1].Indole-3-acetic acid (IAA) is the major naturally occurring auxin and can be produced not only by plants but also by several soil microorganisms associated with the rhizosphere [2].However, plant responses to auxins are highly dependent on hormone concentration, plant tissue

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type, and developmental stage. While appropriate concentrations may stimulate cell division, elongation, flowering, and fruit development, excessive concentrations may produce inhibitory effects. Therefore, the physiological response to auxin application depends on both the dose applied and the developmental status of the target plant tissue [1]..Plant growth-promoting rhizobacteria (PGPR), such as *Bacillus subtilis*, *Azotobacter* spp., and *Pseudomonas fluorescens*, are known for their ability to produce phytohormones, especially IAA, which positively affects plant growth In addition to phytohormone production, PGPR promote plant growth through several other mechanisms, including biological nitrogen fixation, phosphate solubilization, siderophore production, improvement of nutrient availability, and enhancement of plant tolerance to biotic and abiotic stresses. These multiple mechanisms contribute collectively to improved plant growth, nutrient acquisition, and crop productivity [3]. The beneficial effects of IAA-producing bacteria on different crops have been widely reported, including improvements in vegetative growth, chlorophyll content, flowering, and yield components [4,5].

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops worldwide. Commercial growth regulators are commonly used to improve tomato productivity and fruit quality; however, the repeated use of synthetic compounds may increase production costs and raise environmental concerns. Therefore, attention has recently shifted toward environmentally friendly biological alternatives that can provide similar physiological effects with lower environmental impact [6].

Previous studies have demonstrated that bacterial-derived IAA produced by Bacillus spp. can enhance plant growth.can enhance plant growth, branching, and overall vegetative development under different growing conditions [7,8]. Although both sources may have similar physiological roles, their effectiveness may differ according to concentration, plant species, application method, and interaction with plant metabolism [9]. In addition to auxins, polyamines such as putrescine are also involved in plant growth regulation. Putrescine contributes to cell division, membrane stability, flowering, fruit development, and plant tolerance to stress conditions [10]. Therefore, comparing bacterial-derived IAA, synthetic IAA, NAA, and putrescine may help identify effective and biological alternatives for improving tomato growth and productivity. While chemically synthesized and biogenically derived indole-3-acetic acid (IAA) share an identical molecular core ($C_{10}H_9NO_2$), they diverge significantly in their structural purity and environmental stability. Synthetic manufacturing yields a highly homogenous product (≥98% purity) but carries risks of industrial solvent or catalyst residues; furthermore, in its pure form, synthetic IAA is highly labile, undergoing rapid photo-oxidation and thermal degradation. Conversely, biogenic IAA is naturally embedded within a complex organic matrix containing biosynthetic precursors and cellular antioxidants. Although this biological matrix introduces organic impurities that complicate absolute purification, it simultaneously imparts superior operational stability, shielding the auxin from oxidative stress and facilitating a controlled, gradual release through stable ester- or amide-linked conjugates[9]. Previous studies have demonstrated that bacterial-derived IAA produced by Bacillus spp. can enhance plant growth,

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branching, and overall vegetative development under different growing conditions [7,8].. Therefore, the use of B. thuringiensis KS3 in the present study was based on its potential as an IAA-producing bacterium. However, limited information is available regarding the comparative effectiveness of bacterial-derived IAA, synthetic auxins, NAA, and putrescine on tomato growth and fruit quality under Iraqi greenhouse conditions.

Recent studies have demonstrated that foliar application of NAA can improve reproductive growth, fruit yield, and quality characteristics in tomato plants through enhanced fruit set and assimilate partitioning [12]. Furthermore, exogenous application of indole-3-acetic acid (IAA) has been shown to improve plant growth through enhanced photosynthetic efficiency, antioxidant activity, and nutrient utilization [13].Therefore, the present study was designed to assess the significance of the intact bacterial culture of *B. thuringiensis* KS3 as a whole bio-inoculant, in comparison with its cell-free crude extract, alongside commercial IAA, NAA, and putrescine, in terms of growth performance, yield components, and fruit quality attributes of tomato plants under Iraqi greenhouse conditions to identify sustainable alternatives for tomato production.

Material and Methods

Bacterial isolate belong to *B. thuringiensis* KS3 kurstaki was pre- isolated from soil and characterized in 2017 and used extensively as a biological control agent[14]. For Indole-3-acetic acid production, MS broth was prepared and supplemented with 0.3% tryptophan, the precursor for indoles production. Medium was inoculated with *B. thuringiensis* KS3 and incubated at 30C for 24h using shaker incubator at 120 rpm.To prepare cell free extract, culture was centrifuged for 15 min at 7000 rpm using cooling centrifuge[15]. While, bacterial containing treatment was prepared from whole culture**.**indole-3-acetic acid concentration was quantified using the standard curve of commercial indole-3-acetic acid serial dilutions.**High performance Liquid Chromatography HPLC was applied to separate, identify and quantify** indole-3-acetic acid in culture supernatant**.** Agilent ZORBAX Eclipse plus C18: 4.6 ×150 mm. 5 µm system was setup and outfitted with a C18 reverse column and a UV-VIS detector calibrated at 254 nm. Pure IAA standard solution was injected for predetermined concentration and purity of bacterial extract [12].Bacterial cultures of *B.thuringiensis* KS3 spp. were obtained from the Nitrogen Fixation Laboratory, Microbiology Division, Department of Food Sciences and Biotechnology, Agricultural Research Center, Scientific Research Commission, Baghdad, Iraq. The ability of the bacterial isolate to produce indole-3-acetic acid (IAA) was determined using Salkowski's colorimetric method according to Gordon and Weber [16]. Similar approaches have been used for screening IAA-producing bacterial isolates from Iraqi soils [17]. The Salkowski assay was used as a preliminary screening method for IAA production. Confirmation of IAA identity was subsequently performed using HPLC analysis.A greenhouse pot experiment was conducted during the winter season of 2022 at the Agricultural Research Center, Scientific Research Commission, Baghdad, Iraq, to evaluate the effect of bacterial-derived IAA and commercial growth regulators on the growth and productivity of tomato (*Solanum lycopersicum* L.). The experiment was arranged according to a Randomized Complete Block Design (RCBD) with three replicates. The pots were arranged according to the Randomized Complete Block Design (RCBD) within the greenhouse.Although

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the experiment was conducted under protected conditions, slight gradients in light intensity, temperature, relative humidity, and air movement were expected along the length of the greenhouse. Therefore, blocking was used to reduce experimental error associated with these environmental variations and to improve the precision of treatment comparisons.Plastic pots with a capacity of 10 L were filled with a growth medium consisting of 75% loamy soil and 25% peat moss. The soil mixture was air-dried, sieved, and thoroughly mixed before filling the pots. The growth medium had approximately the following properties: pH 7.1 and electrical conductivity (EC) 2.0 dS m^{-1}.

Tomato seeds of the cultivar Wijdan (*'Wijdan' is a hybrid cultivar with an indeterminate growth habit)* were obtained from local agricultural markets in Baghdad, Iraq. Seeds were sown on January 15, 2022, with 4–5 seeds per pot. After germination and seedling emergence, thinning was carried out to maintain one healthy seedling per pot. The seedlings were maintained under greenhouse conditions with regular irrigation and standard agricultural practices throughout the growing period.

The bacterial suspension used for spraying contained approximately 1×10^8 CFU mL^{-1}. Foliar spraying treatments were applied after the appearance of the first true leaf. The experiment included nine treatments as follows:

T0: Untreated control
T1: Bacterial-derived IAA with *Bacillus* bacteria (bacterial count 1×10^8, concentration of IAA 1 mg L^{-1}).
T2: Bacterial-derived IAA without bacteria (concentration of IAA 1 mg L^{-1}).
T3: Commercial IAA at 0.2 mg L^{-1}
T4: Commercial IAA at 0.4 mg L^{-1}
T5: Naphthaleneacetic acid (NAA) at 0.2 mg L^{-1}
T6: NAA at 0.4 mg L^{-1}
T7: Putrescine at 0.2 mg L^{-1}
T8: Putrescine at 0.4 mg L^{-1}

The concentrations of 0.2 and 0.4 mg L^{-1} were selected based on preliminary observations and preliminary trials conducted before the experiment to identify effective concentrations for tomato growth under greenhouse conditions.

Plants were sprayed three times at 15-day intervals using a hand sprayer until complete wetting of the leaves. The first spray was applied after the appearance of the first true leaf. Control plants were sprayed with sterile distilled water.Foliar treatments were applied using a hand sprayer until full leaf wetting. Tween 20 was added as a surfactant at a concentration of 0.1% (v/v) to improve leaf coverage and ensure uniform application of the spray solutions.Vegetative growth parameters included plant height (cm), number of compound leaves $plant^{-1}$, total leaf area per plant (cm^2 $plant^{-1}$), and total chlorophyll content (mg g^{-1} FW). Total leaf area per plant was determined using ImageJ software and expressed as the cumulative leaf area of all leaves on each sampled plant. total chlorophyll content (mg g^{-1} FW). Total chlorophyll content was determined using fresh leaf tissue (0.5 g), which was homogenized in 10 mL of 80% acetone. The extract was filtered and the absorbance was measured at 663 and 645 nm using a spectrophotometer. Total chlorophyll concentration was calculated according to the standard equations and expressed on a fresh weight basis.Total chlorophyll

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content was calculated according to Arnon [18] using the following equation:

$$Total\ chlorophyll\ (mg\ g-1\ FW) = 20.2 * A645 + 8.02 * A663$$

Flowering parameters included number of flowers plant^{-1} and fruit set percentage. Fruit set percentage was assessed by counting the total number of flowers and the number of fruits formed on five flower clusters per plant.Fruit set percentage was calculated using the following equation:

$$Fruit\ set\ \% =) Number\ of\ fruits / Number\ of\ flowers\ (\times 100$$

Vitamin C content was determined according to Hassoon [19]. Lycopene concentration was estimated according to Suwanaruang [20] using the following $Lycopene\ (mg/kg) = A503 \times 137.4$

Yield parameters included number of fruits plant^{-1} and yield plant^{-1} Yield per plant was determined as the cumulative fresh weight of fruits harvested from each plant during all harvests throughout the production period and was expressed as kg plant^{-1}. At fruit maturity, six randomly selected plants from each treatment were used for yield determination Since each treatment consisted of ten pots distributed among three replicates, two plants were randomly selected from each replicate, giving a total of six plants per treatment for yield assessment.

. Fruit number and total fruit yield per plant were recorded for yield estimation.The obtained data were statistically analyzed using GenStat software. Treatment means were compared using Duncan's multiple range test at $p \leq 0.05$.

Results and Discussion

Vegetative Growth Parameters

The results presented in Figure 1 showed significant differences among treatments for plant height, number of compound leaves, leaf area, and total chlorophyll content ($p \leq 0.05$). In general, treatments containing bacterial-derived IAA improved vegetative growth characteristics compared with the untreated control.Plant height ranged from 140 cm in the control treatment to approximately 195 cm in treatments T1, T2, and T3, which did not differ significantly from each other (Figure 1A). Similarly, total chlorophyll content increased from 1.50 mg g^{-1} FW in the control treatment to 2.40 mg g^{-1} FW in T2, which recorded the highest value among all treatments (Figure 1D).The number of compound leaves was significantly affected by the treatments, increasing from approximately 55 leaves plant^{-1} in the control treatment to 65 leaves plant^{-1} in T4, which recorded the highest value (Figure 1B). Treatments T1, T2, T3, and T5 did not differ significantly from T4.

Leaf area was also significantly influenced by the treatments. The highest leaf area was recorded in T2 (331 cm² plant^{-1}), whereas the control treatment showed the lowest value (297 cm² plant^{-1}). Treatments T7 and T8 recorded relatively low leaf area values of approximately 300 and 302 cm² plant^{-1}, respectively (Figure 1C).

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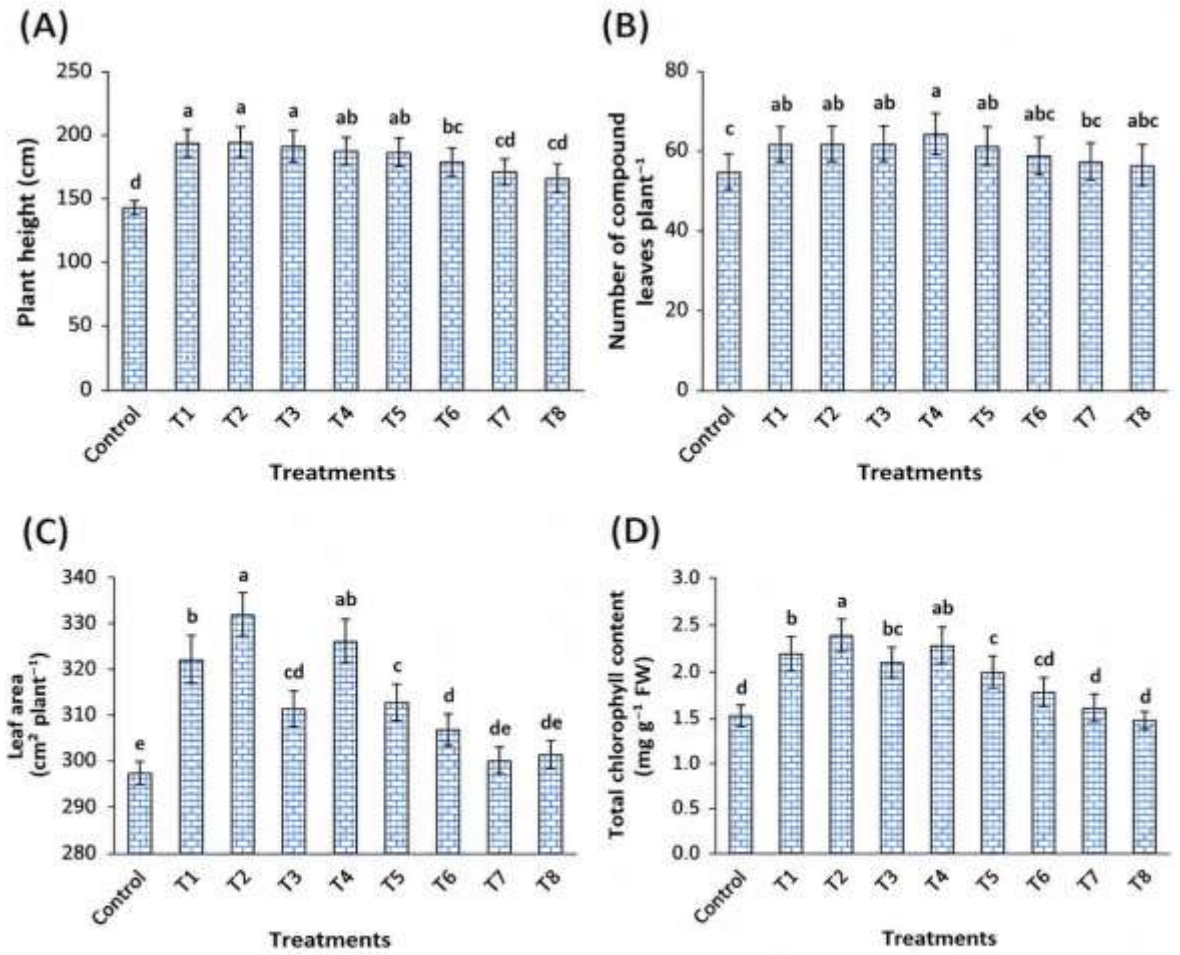

Figure 1. Effect of bacterial-derived IAA and commercial growth regulators on vegetative growth parameters of tomato plants: (A) plant height, (B) number of compound leaves plant^{-1}, (C) leaf area (cm^2 plant^{-1}), and (D) total chlorophyll content (mg g^{-1} FW).
Means followed by the same letters are not significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

Flowering Parameters

The flowering characteristics of tomato plants were significantly affected by the applied treatments compared with the untreated control ($p \leq 0.05$). Treatment T4 produced the highest number of flowers (86.7 flowers plant^{-1}), whereas the control treatment recorded the lowest value (54.3 flowers plant^{-1}).

(Figure 2A). Fruit set percentage also increased significantly under the applied treatments. Treatment T3 recorded the highest fruit set percentage (65.78%), while the control treatment showed the lowest percentage (48%) (Figure 2B).

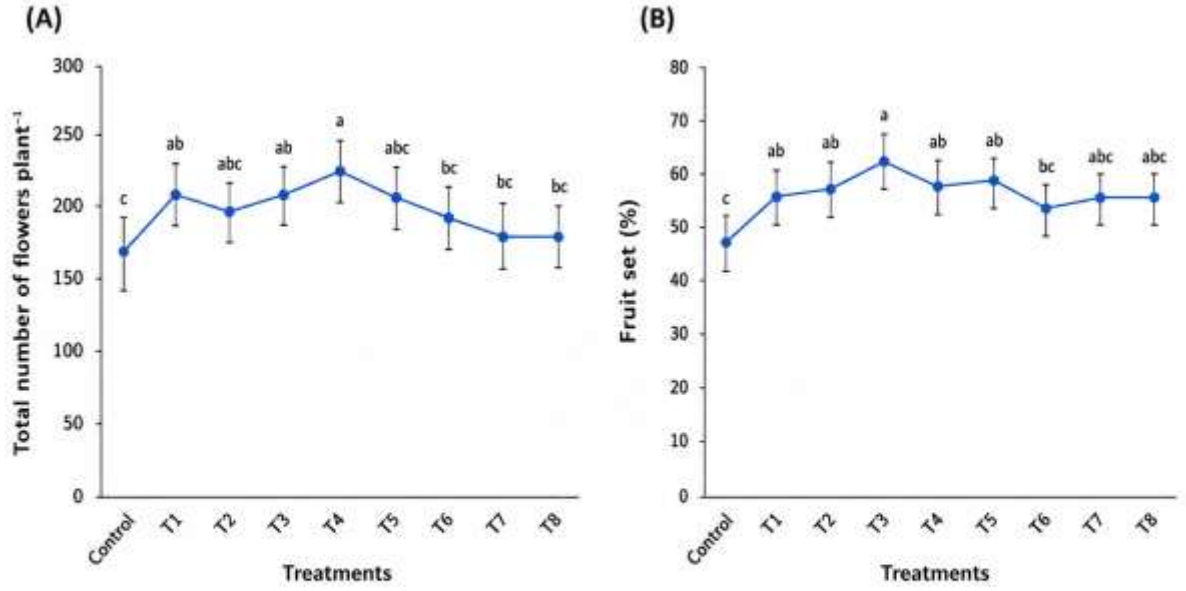

Figure 2. Effect of bacterial-derived IAA and commercial growth regulators on flowering characteristics of tomato plants: (A) total number of flowers plant^{-1} and (B) fruit set (%).
Means followed by the same letters are not significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.

Fruit Quality Parameters

Vitamin C content was significantly affected by the applied treatments ($p < 0.05$). The highest vitamin C concentration was recorded in T3 (22.33 mg 100 g^{-1} fresh weight), which did not differ significantly from T2 (21.85 mg 100 g^{-1} fresh weight), T4 (21.67 mg 100 g^{-1} fresh weight), and T5 (20.67 mg 100 g^{-1} fresh weight). In contrast, the control treatment recorded the lowest vitamin C content (15.00 mg 100 g^{-1} fresh weight).

.Lycopene concentration was also significantly influenced by the treatments. Treatment T3 recorded the highest lycopene concentration (2.13 mg 100 g^{-1} fresh weight), followed by T5, whereas the lowest value was observed in the control treatment.

Table 1. Effect of bacterial-derived IAA, commercial auxins, and putrescine on vitamin C and lycopene content of tomato fruits.

Treatment	Vitamin C (mg 100 g^{-1} FW)	Lycopene (mg 100 g^{-1} FW)
Control	15.00 c	0.83 d
T1	20.00 ab	1.63 abc
T2	21.85 a	1.83 ab
T3	22.33 a	2.13 a
T4	21.67 a	1.40 bcd
T5	20.67 ab	2.03 a
T6	20.00 ab	1.03 d
T7	17.00 bc	1.37 bcd
T8	16.33 bc	1.10 cd

Different letters indicate significant differences among treatments according to Duncan's multiple range test at $p<0.05$.

Yield Parameters

Significant differences were observed among treatments for both number of fruits plant^{-1} and yield per plant ($p \leq 0.05$) (Figure 3). The highest number of fruits plant^{-1} was recorded in T2 (78 fruits plant^{-1}), which differed significantly from the control treatment (48 fruits plant^{-1}).

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Treatments T1, T3, and T4 did not differ significantly from T2, whereas T7 produced the lowest value among the treated plants.

Similarly, yield per plant was significantly affected by the treatments. Treatment T2 produced the highest yield (8.0 kg plant^{-1}), while the lowest yield was recorded in the control treatment (4.6 kg plant^{-1}), followed by T7 (4.9 kg plant^{-1}). Treatments T1 and T3 did not differ significantly from T2 according to Duncan's Multiple Range Test.

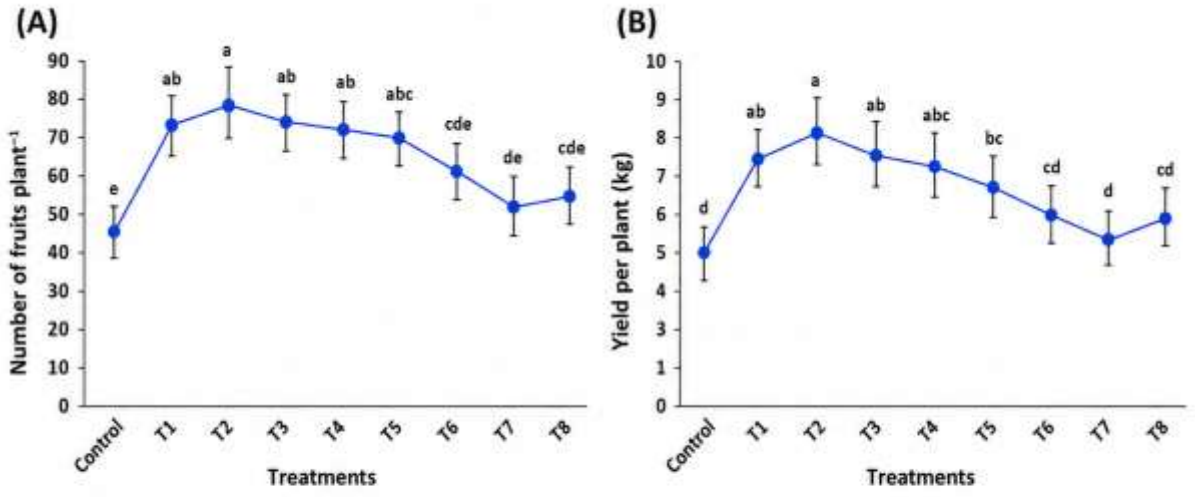

Figure 3. Effect of bacterial-derived IAA and commercial growth regulators on yield parameters of tomato plants: (A) number of fruits plant^{-1} and (B) yield per plant (kg).

Discussion

Beyond its well-documented entomopathogenic activity through the production of insecticidal crystal proteins, the Iraqi isolate *Bacillus thuringiensis* KS3 kurstaki demonstrated the ability to synthesize indole-3-acetic acid (IAA) through a tryptophan-dependent pathway under optimized culture conditions. The isolate produced 1 mg L^{-1} of IAA after 24 h of incubation, and its identity was confirmed by HPLC analysis through the detection of the characteristic IAA peak. The ability of *B. thuringiensis* to express plant growth-promoting traits has been reported previously, including phytohormone production and other metabolites involved in plant growth enhancement [11]. The successful detection of IAA production in the present study confirms that the local isolate possesses the metabolic capacity required for potential biofertilizer applications. These findings are consistent with the concept that *B. thuringiensis* can function not only as an entomopathogen but also as a plant growth-promoting bacterium (PGPB) with dual agricultural functions [21].The present study demonstrated that bacterial-derived IAA and commercial growth regulators significantly improved vegetative growth, flowering, fruit quality, and yield of tomato plants compared with

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the untreated control. The observed increases in plant height, leaf number, leaf area, chlorophyll content, fruit set, and yield may be attributed to the well-established role of auxins in promoting cell division, cell elongation, and plant developmental processes [1,12]. Auxins also influence chloroplast development and photosynthetic efficiency, which may contribute to improved plant growth and productivity.The superior performance of T2 may be attributed to the physiological effects of bacterially produced IAA present in the biological preparation. Since T2 did not contain living bacterial cells, its effect should be interpreted as a response to the bacterial IAA preparation rather than to the direct activity of *Bacillus* spp. or bacterial colonization. The differences observed between bacterially produced IAA and commercial IAA may be related to differences in stability, composition, and the possible presence of additional biologically active metabolites in the bacterial filtrate. These compounds may act synergistically with IAA and contribute to the observed growth responses [9,21]. Similar improvements in vegetative growth following the application of IAA-producing microorganisms have been reported previously [6,7,8].

The increase in chlorophyll content and leaf area observed in treated plants may have enhanced photosynthetic efficiency and assimilate production, resulting in improved vegetative growth and reproductive performance. Greater photosynthetic activity may increase the availability of photoassimilates required for flower formation, fruit development, and yield accumulation. Similar findings have been reported in studies involving plant growth-promoting microorganisms and growth regulators [5,6,7].Flowering and fruit set were significantly affected by the applied treatments. Auxins play a central role in reproductive development by stimulating cell division and cell expansion within the ovary after pollination [12]. In addition, auxins may reduce flower and young fruit abscission by maintaining hormonal balance and enhancing sink strength, thereby improving fruit retention and fruit set percentage [1,12].The substantial increase in yield observed under the IAA treatments may be attributed to the combined improvement of vegetative growth, flowering, fruit set, and fruit retention. Plants receiving bacterial-derived IAA exhibited higher chlorophyll content, larger leaf area, increased flower production, and improved fruit set percentage, all of which contributed to greater assimilate production and allocation to developing fruits.The increase in vitamin C and lycopene content observed in tomato fruits under IAA treatments may be associated with enhanced physiological activity and improved assimilate production. Increased chlorophyll content and leaf area likely improved photosynthetic efficiency, resulting in greater carbohydrate accumulation and translocation to developing fruits. Furthermore, auxins may stimulate metabolic pathways involved in the synthesis of antioxidant compounds, contributing to higher vitamin C and lycopene concentrations in treated fruits [12,20].Although auxins and putrescine differ in their physiological mechanisms, both are recognized as plant growth regulators capable of influencing plant growth and development. Therefore, the comparison conducted in the present study focused on their overall effects on vegetative growth, flowering, yield, and fruit quality rather than on their specific modes of action.Overall, the results indicate that bacterially produced IAA has considerable potential as a biological alternative to conventional growth regulators for improving tomato growth, productivity, and fruit quality under

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greenhouse conditions. However, further studies are required to evaluate economic feasibility, environmental impact, and long-term sustainability under commercial production systems.

Conclusion

The present study demonstrated that bacterially produced indole-3-acetic acid (IAA) significantly improved vegetative growth, flowering performance, fruit quality, and yield of tomato plants under greenhouse conditions. Treatment T2 (bacterially produced IAA without bacterial cells) produced the best overall response for several growth and yield parameters, whereas commercial IAA was more effective in improving fruit set percentage, vitamin C content, and lycopene concentration. The results indicate that bacterially produced IAA can be considered a promising biological growth regulator for enhancing tomato productivity and fruit quality. Further studies are required to quantify bacterial IAA concentrations and to evaluate its effectiveness under different environmental conditions and commercial production systems.

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