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RESPONSE OF TOMATO (*SOLANUM LYCOPERSICUM* L.) TO BIOFERTILIZERS AND BIOSTIMULANTS IN VEGETATIVE GROWTH AND NUTRITIONAL STATUS

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SUMMARY

The effects of biofertilizer and biostimulant application on tomato (*Solanum lycopersicum* L.) were this study's focus for evaluation. The experiment examined the irrigation of rhizobacteria in different combinations, including B0 (plants irrigated with water only); B1 (plants treated with inoculant containing *A. chroococcum* (8.8×10^9) with 10 g plant^{-1}; B2 (plants treated with inoculant containing *B. subtilis* (7.5×10^9) with 10 g plant^{-1}; and B3 (plants treated with *A. chroococcum* in 10 g plant^{-1}) plus *B. subtilis* (10 g plant^{-1}) in tomato (*Solanum lycopersicon* L.). The use of biostimulant under the trade name "Deflan," which contained organic matter (18.4%), amino acids (10%), total nitrogen (3%), and organic nitrogen (3%), had three levels (0, 0.250, and 500 mg l^{-1}). The combination of both *A. chroococum* and *B. subtilis* at 10 g plant^{-1} had an influential effect, which reversed an increase in root and vegetative growth, specifically the content of macroelements. A biostimulant spray significantly affected all parameters, especially the 500 mg l^{-1} dose. Furthermore, a solidarity effect markedly appeared, which raised all vegetative parameters, especially the biofertilizer treatment *A. chroococcum* (10 g plant^{-1}) plus *B. subtilis* (10 g plant^{-1}) with spraying biostimulant (500 mg l^{-1}). These results will contribute favorably to providing evidence for desirable effects from the interaction between biofertilizers and biostimulant spraying on tomato plant development.

Keywords: Tomato (*Solanum lycopersicum* L.), biofertilizers, rhizobacteria, azotobacter, *Bacillus*, foliar application, biostimulants

Key findings: The application of rhizobacteria and foliar treatment with biostimulant increased vegetative traits and mineral content of leaves, especially the mixture between *A. chroococcum* and *B. subtilis* (10 g plant^{-1}) in tomato (*Solanum lycopersicum* L.). Furthermore, foliar application of the biostimulant at a concentration of 500 mg l^{-1} boosted all the parameters under study.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important crop in Iraq, and its cultivation is under a wide range of production systems. Considered a valuable crop vegetable after the potato in many countries worldwide, it is also one of the essential crops due to its nutrient content, being a source of carbohydrates, proteins, and antioxidants (Heuvelink, 2005).

The increase in world population has led to growing food demands; therefore, interest in greatly raising production regardless of quality arose, which led to an excessive use of chemical fertilizers, especially when cultivating vegetable crops compared with other crops. This is due to the short growing season and the high production and consumption of their fruits, causing an elevation in adverse consequences of excessive use of chemical fertilizers on health and the environment by the rise of nitrates and oxalates (Tilman *et al*., 2002; Cordell *et al*., 2009). It is forthcoming that the amount of chemical fertilizers used will increase in the course of time if the same conventional methods are continuous in their implementation to expand food production to complement the growing world population (Baligar *et al*., 2001).

The importance of biofertilizers in reducing mineral fertilizers' use on the one hand and increasing the costs of crop production (Vessey, 2003) also points to their major part in nitrogen fixation and boosting the nutritional conditions of plants. They activate in expanding the capability of P absorption, as it is given that the absorption of K and more elements correlated to enhancing the nutritional balance for the N and P uptake, thereby improving vegetative and flowering of plants (Adesemoye *et al*., 2008; Haifaa *et al*., 2022).

Azotobacter (*Azotobacter chroococcum*) is one of the non-symbiotic N-fixing bacteria in non-leguminous crops, which has become a widely used biofertilizer, in addition to its importance in the secretion of some hormones, enzymes, vitamins, and growth regulators. This beneficial effect may be due to an improved root growth and development, consequent increased in water and nutrient absorption, and resistance of plant pathogenic bacteria (Rao, 1986).

Phosphorus is evidently one basic macroelement necessary for the plant's life, being called the key of life because of its direct role in most processes, as these processes within plant cells cannot take place without it. Phosphorus participates in the storage and distribution of energy compounds and enzymatic cofactors, even though the amount of phosphorus in soil (both organic and inorganic) is high, but most of it is insoluble (Holford, 1997). Therefore, the plant must depend on phosphate-solubilizing microorganisms. One of the best-known phosphate-solubilizing bacteria is the *Bacillus,* which tends to secrete phosphatase enzymes and organic acids to make the environment surrounding the roots acidic in nature to facilitate the conversion process of inorganic phosphorus into $H2PO4^-$ and $HPO4^-$ ions (Bhattacharyya and Jha, 2012).

Knowingly, foliar feeding of amino acids is necessary for supplying plants with their requirements, in addition to its major motivational role, which has a decisive influence in increasing growth, flowering, and yield, as well as resisting external conditions caused by environmental stresses (Kowalczyk *et al*., 2008). In this regard, the presented study aimed to examine the influence of microorganisms (Azotobacter, Bacillus), amino acids, and their combinations on several vegetative traits and macro-elements of tomato plants.

MATERIALS AND METHODS

This study began implementation from September 2020 to June 2021 in the greenhouse of the Agricultural Research Center, Abu-Graib District, Baghdad, Iraq. It sought to investigate the response of tomato crops to fertilization with biofertilizers (PGPR) and foliar application of biostimulant. Tomato seeds' planting commenced on September 20, 2020, in the nursery of the research fields. When the seedlings reached five true leaves, their relocation to the greenhouse occurred on November 5, 2020.

Experiment design and treatments

The study, as implemented in a greenhouse, had a randomized complete block design with 12 treatments, with each treatment containing 10 plants (experimental units) with three replicates, and each replicate had 120 plants. The application of chemical fertilizers at 50% of recommended doses (120-160-120 kg ha^{-1}) took place for all plants. The experiment consisted of two factors. The first factor is soil application with biofertilizer (PGPR), which includes B0 (a group of tomato plants irrigated only with water without any PGPR); B1 (plants treated with microbial inoculant containing *A. chroococcum* (8.8×10^9) with 10 g $plant^{-1}$; B2 (plants treated with microbial inoculant containing *B. subtilis* (7.5×10^9) with 10 g $plant^{-1}$; and B3 (plants treated with *A. chroococcum* (10 g $plant^{-1}$) + *B. subtilis* (10 g $plant^{-1}$). The loading of Azotobacter and Bacillus proceeded on sterilized peat moss at a 1 kg rate, with peat moss for each liter of the liquid broth being added twice, during the sowing of seeds and when transferring seedlings to the greenhouse (45 days) at 10 g $seedlings^{-1}$. The second factor included foliar application with biostimulant under the commercial name "Deflan," which contained organic matter (18.4%), amino acids (10%), total nitrogen (3%), and organic nitrogen (3%). Spraying continued in three concentrations—D0 (spraying with water only), D1 (250 mg l^{-1}), and D2 (500 mg l^{-1})—with plants sprayed four times. The first spray occurred after two weeks of planting in the greenhouse. The second time was after four weeks of planting, the third time was when flowers started, and the final spraying was when the fruits were set, with foliar feeding achieved early morning until complete wetness, with the addition of 0.1% Tween 20 surfactant. Some properties of soil are available in Table 1.

Vegetative and leaves' mineral content sustained assessment in June 2021, comprising plant height (cm), stem diameter (mm), leaves per plant (leaf $plant^{-1}$), and leaf area (dm^2), according to Watson and Watson (1953). Meanwhile, measuring dry weight (g $plant^{-1}$) included vegetative characteristics, while chemical characteristics represented by determining chlorophyll content (mg per 100 g fresh weight) employed Goodwin's (1976) method, and nitrogen, phosphorus, and potassium (%) followed Bhargava and Raghupathi (1999).

Statistical analysis

The results, as statistically analyzed, used the statistical program Genstat, with the individual differences and combinations between them achieved by using the F-test and least significant differences test at a 5% probability.

RESULTS

Plant height

The results illustrated the soil application with biofertilizers, especially B3 (Azotobacter + Bacillus) showed an increase in plant height (269.47 cm) compared with the lowest values in the treatment of B0 (227.38 cm) (Table 2).

Table 1. Properties of the experimental soil.

pH	EC (1:1) ds/m	CEC C mol/L	OM g/kg	Sand g/kg	Loam g/kg	Clay g/kg	N mg/kg	P mg/kg	K mg/kg	CaCO3 g/kg	Fe mg/kg	Zn mg/kg
7.2	2.36	28.40	13	243	557	189	64.4	4.48	217.8	179.0	3.16	1.57
Microorganism Communities												
Azotobacter (cfu g^{-1} soil)				Bacillus (cfu g^{-1} soil)				Soil texture				
1.04×10^3				0.68×10^3				Loam clay				

Table 2. Impact of biofertilizers (B), biostimulant (D), and their interactions on vegetative traits in tomato.

Biostimulant (D)	Biofertilizers (B)				Means
	B0 = Control	B1 = Azoto. (10 g plant $^{-1}$)	B2 = Bacil. (10 g plant $^{-1}$)	B3 = Azot. + Bacil	
Plant height (cm)					
D0 = Control	218.83	225.67	233.10	249.00	231.65
D1 = 250 mg l^{-1}	228.47	249.77	260.67	268.67	251.89
D2 = 500 mg l^{-1}	234.83	254.50	268.10	290.73	262.04
Means	227.38	243.31	253.96	269.47	
	B	D	B × D		
$LSD_{0.05}$	3.36	2.91	5.83		
Stem diameter (mm)					
D0 = Control	9.80	11.56	12.06	14.23	11.91
D1 = 250 mg l^{-1}	10.50	13.40	13.74	15.36	13.25
D2 = 500 mg l^{-1}	11.10	14.13	14.36	16.46	14.01
Means	10.46	13.03	13.39	15.35	
	B	D	B × D		
$LSD_{0.05}$	0.45	0.39	0.79		
Leaves plant $^{-1}$					
D0 = Control	29.90	31.80	32.60	35.00	32.32
D1 = 250 mg l^{-1}	33.00	35.17	36.13	38.67	35.74
D2 = 500 mg l^{-1}	33.27	36.53	37.83	43.00	37.66
Means	32.06	34.50	35.52	38.89	
$LSD_{0.05}$	B	D	B × D		

Foliar spraying with biostimulant revealed that D2 (500 mg l^{-1}) achieved the highest values (262.04 cm), while D0 treatment registered 231.65 cm, as indicated by the results of the study. The treatment interaction provided a significant rise in plant height, with B3D2 achieving the topmost values (290.73 cm) compared with the lowest values with B0D0, which amounted to 218.83 cm.

Stem diameter

The findings detailed an increment appeared in this trait. It suggests that fertilization by biofertilizers significantly affected stem diameter, especially B3 which gave a value of 15.35 mm in comparison with B0 giving 10.46 mm (Table 2). However, the biostimulant foliar spray recorded the highest value, especially in treatment D2 (14.01 mm) compared with D0, which registered a lowest value (11.91 mm). Regarding the interaction between the studied factors, B3D2 achieved the premier value (16.46 mm) versus the control B0D0 at 9.80 mm.

Leaves per plant

Results showed an increase in the number of leaves from the soil application of biofertilizers, where the topmost number of leaves was 38.89 leaves plant^{-1} at treatment B3 compared with the least number at 32.06 leaves plant^{-1} at B0. Concerning the biostimulant, D2 achieved the highest value (37.66 leaves plant^{-1}), while D0 gave 32.32 leaves plant^{-1} (Table 2). The interaction treatment B3D2 provided the maximum value (43.00 leaves plant^{-1}), whereas B0D0 gave a value of 29.90 leaves plant^{-1}.

Leaf area

The outcomes indicated that biofertilizers appeared with an increment in the area of the leaves, which rose with an increase in concentration. B3 achieved the highest values with a significant difference from other treatments, which reached 153.56 dm^2 compared with B0, which gave 137.50 dm^2. On the biostimulant, D2 obtained the broadest

Table 3. Impact of biofertilizers (B) and biostimulant (D) and their interactions on growth traits and chlorophyll content in tomato.

Biostimulant (D)	Biofertilizers (B) B0 = Control	B1 = Azoto. (10 g plant $^{-1}$)	B2 = Bacil. (10 g plant $^{-1}$)	B3 = Azot. + Bacil	Means
Leaf area (dm ^{2})					
D0 = Control	134.00	142.67	141.80	144.87	140.83
D1 = 250 mg l^{-1}	138.90	147.80	146.60	151.00	146.07
D2 = 500 mg l^{-1}	139.60	154.00	145.33	164.80	150.93
Means	137.50	148.16	144.58	153.56	
	B	D	B × D		
$LSD_{0.05}$	1.60	1.39	2.78		
Dry weight (g plant^{-1})					
D0 = Control	142.69	145.43	144.15	147.53	144.95
D1 = 250 mg l^{-1}	146.49	151.41	149.50	157.53	151.23
D2 = 500 mg l^{-1}	147.60	154.50	152.16	167.16	155.35
Means	145.59	150.44	148.60	157.41	
	B	D	B × D		
$LSD_{0.05}$	2.28	1.98	3.96		
Chlorophyll content (mg 100 gm $^{-1}$ fresh weight)					
D0 = Control	197.31	203.24	200.73	212.84	203.53
D1 = 250 mg l^{-1}	202.77	214.51	205.71	227.25	212.56
D2 = 500 mg l^{-1}	208.44	237.59	214.24	247.12	226.85
Means	202.84	218.45	206.89	229.07	
	B	D	B × D		
$LSD_{0.05}$	1.95	1.69	3.38		

value (150.93 dm ^{2}) compared with the narrowest value at D0 (140.83 dm^2) (Table 3). From another side, B3D2 registered the maximum value (164.80 dm ^{2}) when compared with B0D0, which was at 134.00 dm ^{2}.

Dry weight

The biofertilizers achieved a superior effect on the dry matter, and this effect manifested in B3 achieving the highest value (157.41 g) compared with B0, which gave the lowest value (145.59 g) (Table 3). Biostimulants spray showed an improvement, as D2 obtained the supreme value (155.35 g) versus D0's 144.95 g. The overlap between treatments gave a marked effect on this characteristic, as B3D2 achieved 167.16 g, while the lowest value (142.69 g) was with B0D0.

Chlorophyll content

Biofertilizers had a substantial effect on the chlorophyll content (Table 3). The B3 treatment attained the highest value (229.07 mg 100 g^{-1} fresh weight) in comparison with B0, which registered 202.84 mg 100 g^{-1} fresh weight. The results also showed spraying with biostimulant affected this characteristic, where D2 achieved the ultimate value, reaching 226.85 mg 100 g^{-1} fresh weight, compared with the lowest value from D0, which amounted to 203.53 mg 100 g^{-1} fresh weight. The combination between studied factors exhibited a similar effect to that of the individual treatments. B3D2 acquired the highest value for the chlorophyll content with a value of 247.12 mg 100 g^{-1} fresh weight, while the minimum value came from the control.

Nitrogen (%)

Using biofertilizers caused an increase in nitrogen content, where B3 registered 1.65% in comparison with B0, which reached only 1.39% (Table 4). Similarly, biostimulants spray gave a higher percentage of nitrogen. D2 achieved 1.58%, whereas 1.47% was evident in D0. The interactive effect among different treatments of biofertilizers and biostimulant was also notable, having the maximum value of nitrogen content (1.74%) for B3D2, and the lowest value resulted in B0D0, which reached 1.37%.

Phosphorus (%)

The results indicated remarkable differences among biofertilizer treatments, biostimulant concentrations, and their interaction (Table 4). More phosphorus content materialized when tomato plants received treatment with B3, which had a significant effect versus other treatments, while less content of phosphorus appeared in B0. Similarly, among biostimulant concentrations, the maximum content was evident in D2, which registered 0.52% compared with the minimum content in D0 (0.44%). Moreover, B3D2 achieved the highest value (0.63%), while the lowest value emerged in B0D0 (0.34%).

Potassium (%)

It is noteworthy that biofertilizer treatments, especially B3, registered the maximum content of potassium (2.24%), while the minimum value was in B0 (2.07%) (Table 4). Similarly, more content of phosphorus (2.20%) was evident when spraying the plants with D2. The interaction of both biofertilizer treatments and biostimulants was also meaningful, with the most interaction value observed in B3D2 compared with the control.

DISCUSSION

The biofertilizers (Azotobacter, Bacillus, and their interaction) improved the vegetative growth traits, i.e., plant height, stem diameter, leaf number and area, vegetative dry weight, leaves per plant, and chemical content

Table 4. Impact of biofertilizers (B) and biostimulant (D) and their interactions on leaf mineral content (N, P, and K) in tomato.

	Biofertilizers (B)				
Biostimulant (D)	B0 = Control	B1 = Azoto. (10 g plant $^{-1}$)	B2 = Bacil. (10 g plant $^{-1}$)	B3 = Azot. + Bacil	Means
N (%)					
D0 = Control	1.37	1.51	1.42	1.58	1.47
D1 = 250 mg l^{-1}	1.42	1.59	1.46	1.63	1.52
D2 = 500 mg l^{-1}	1.39	1.65	1.54	1.74	1.58
Mean	1.39	1.58	1.47	1.65	
	B	D	B × D		
LSD at 5%	0.022	0.014	0.031		
P (%)					
D0 = Control	0.34	0.43	0.47	0.53	0.44
D1 = 250 mg l^{-1}	0.36	0.46	0.54	0.56	0.48
D2 = 500 mg l^{-1}	0.39	0.48	0.58	0.63	0.52
Mean	0.36	0.46	0.53	0.57	
	B	D	B × D		
LSD at 5%	0.021	0.018	0.037		
K (%)					
D0 = Control	2.0	2.09	2.10	2.15	2.09
D1 = 250 mg l^{-1}	2.07	2.13	2.13	2.23	2.14
D2 = 500 mg l^{-1}	2.14	2.14	2.19	2.34	2.20
Mean	2.07	2.12	2.14	2.24	
	B	D	B × D		
LSD at 5%	0.020	0.013	0.027		

LSD = Differences between means at P = 5%.

(chlorophyll, N, P, and K), which were effective indicators of biofertilizers' efficiency. This may be ascribable to the vital role of microorganisms in increasing uptake and absorption of micro- and macronutrients.

Azotobacter can perform nitrogen fixation, offering some of the plant's requirements for this essential nutrient, being considered the basic ingredient of chlorophyll (DNA, RNA), amino acids, and protein (Abdel-Fattah *et al*., 2013; Bhardwaj *et al.*, 2014). All of these may participate in increasing the vegetative dry weight of the plant and enhancing all vegetative parameters. Furthermore, its role contributes to improving the root system's growth and raising its density due to its production of some growth regulators and chelating substances for microelements, such as iron, as reflected in boosting the size of the vegetative system (Haifaa *et al.*, 2022). In addition to the role of Bacillus in facilitating the dissolution and processing of phosphorus to plant roots, its role is pivotal in processing nitrogen, which is an important element for the formation of proteins, nucleic acids, etc.

Moreover, Bacillus is crucial in chelating the iron element and preparing it for the rhizosphere's uptake by the roots through the production of siderophores (Hayat *et al.*, 2010; Nadeem *et al.*, 2012). Additionally, the role of Azotobacter and Bacillus participates in their secretion of many important biochemical compounds like vitamins and plant hormones, such as auxins, cytokinins, and gibberellins, which perform vital roles in regulating and stimulating growth (Wani *et al.*, 2013; Glick, 2014).

Therefore, the changes that occur in the levels of these hormones are likely to play an influential role in regulating the development of growing plants, as auxins are vital and essential in the elongation and expansion of cells and stimulation of cell division at the meristematic apex of the plant. Thus, they cause an increase in plant height and cell division of the cambium cells by stimulating the activity and effectiveness of the vascular cambium cells, which results in enhancing the stem diameter (Taiz and Zeiger, 2010; Su *et al.*, 2011; Hayder and Al-Falahy, 2023).

The role of biostimulants under the trade name "Deflan" in the improvement of vegetative growth traits (plant height, stem diameter, leaf number and area, and shoot dry weight) could be due to the essential components of the nutrient solution in seedling growth. Biostimulants play a supportive role as a result of their containing amino acids. The effect could be increasing the construction of secondary compounds, activating the plant's enzymatic system, increasing the ability of cell division and elongation, raising the efficiency of carbon metabolism, and manufacturing carbohydrates and proteins, which contribute to boosting vegetative growth (Hildebrandt *et al.*, 2015). Another reason could refer to its significant catalytic role in vital processes and participation in the construction of protein, consequently stimulating the process of photosynthesis and building carbohydrates (Bender, 2012; Al-Falahy, 2021; Hussein and Al-Falahy, 2021).

CONCLUSIONS

This study attempt sought to highlight on the importance of *A. chroococcum* and *B. subtilis,* individually or in their mixture, with a biostimulant under the trade name "Deflan," containing organic matter (18.4%), amino acids (10%), total nitrogen (3%), and organic nitrogen (3%)). From this study, we can conclude the synergistic effect of the mixture of rhizobacteria *A. chroococcum* (10 g plant^{-1}) + *B. subtilis* (10 g plant^{-1}) and foliar application with biostimulants has achieved the best results for vegetative traits of tomato plants and mineral contents.

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