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Bio-Fertilizers and Bacterial Bio-Control for Root-Knot Nematode Management and Tomato Yield Enhancement

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Abstract

Agriculture faces growing population pressure for food security and sustainability. Controlling root-knot nematodes and reducing fertilizer use is crucial for environmental resources preservation. In Egypt, tomato production faces pollution and human health risks by using chemical nematicides for control nematode, so rhizobacteria can be used for controlling parasitic nematodes and bio-fertilization for reduce chemical fertilizers. This experiment was established to study the role of modified bacterial to control *Meloidogyne incognita* and improve using nitrogen fertilizer to increase tomato crop productivity. The tomatoes were cultivated in a greenhouse, 100 ml of different bacterial strains (fusant (F7), *Bacillus thuringiensis* (Bt), *Bacillus cereus* (Bc), and *Achromobacter xylosoxidans* (Ax)) were compared to a chemical nematicide (Oxamyl) applied as foliar spraying and as soil drenching at two doses after 3 and 30 days from the transplanting, where, using two levels of nitrogen fertilization (250 and 500 N/ha). Results showed that all treatments effectively reduced root-knot nematode reproduction, with F7 being more effective in reducing nematode all parameters like J2, galls, and egg masses. Soil drench achieved the best reduction compared to spraying foliage. Our result, the detection and amplification of the alkaline serine protease gene, which has the ability to improve nematode control for F7, and their parental strains (Bc and Bt), produced 1100 base pair fragments. Results clearly reported that bio-fertilizer treatments significantly improved all vegetative growth. The nitrogen treatments had improvements in yield parameters and tomato fruit quality characteristics grown under deficit nitrogen treatment. Tomato plants that received the minimum quantity of nitrogen fertilizers (250 N/ha) and were treated by F7 as a soil drench produced the highest vegetative growth, flowering, fruit yield, and fruit quality. Where, harmony yield parameters and tomato fruit quality characteristics are compared with SDS-PAGE protein banding patterns for eleven treatments leaf tomato plant treatment varieties for leaf water-soluble proteins. The plant exposed to F7 as a soil drench showed the highest number of bands (17 bands) in comparison to the lesser number of bands in the control when applied the minimum quantity of nitrogen fertilizers.

Keywords: Tomato plant, Root-knot nematode -Bio-control, Bacterial strains, Fusant F7, Deficit nitrogen, Bio-fertilizer.

1. Introduction

An increasing population is placing pressure on agriculture to provide food security. The search for natural and safe alternatives for controlling root-knot nematodes that attack crops and reduce fertilizer use is a necessity for preserving environmental resources and ensuring their sustainability. The tomato, or *Solanum lycopersicum* L., is one of the most significant vegetable crops and a favorite vegetable in Egypt, especially for local consumption and export, according to the Food and Agriculture Organization (FAO 2018; Godfray *et al.*, 2010).

To increase agricultural production, it must control the diseases and pests that reduce crop output and use chemical fertilizers as a crucial source of plant nourishment. Due to this, farmers began to believe that expanding their usage of chemical nematicides and fertilizers would result in higher crop yields, and this is regarded as an excess expense on agricultural inputs and a source of increased pollution and hazard to humans.

One of the most serious pests affecting tomato production and other vegetable crops is root-knot nematodes. *M. incognita* is one of the world's most destructive agricultural pests and one of the most economically significant nematode species (Sasanelli *et al.*, 2018). Chemical nematicides have detrimental effects on human health and the environment (Mohamed *et al*., 2021a; Mohammad *et al.*, 2022). This negative effect pushes the search for non-chemical alternatives to chemical nematicides that are more effective, eco-friendly, and safe (Forghani *et al.*, 2020; Mohammad *et al.*, 2022).

New production tools for controlling plant pathogens and improving plant growth are needed to advance sustainable agriculture practices. Many Plant Growth-Promoting Rhizobacteria (PGPRs), such as *Bacillus* spp., constitute an environmentally acceptable method of enhancing crop productivity. They have the potential to stimulate and enhance crop output through

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biocontrol of plant parasitic nematodes and biofertilization for plants (Borriss 2011; Ramezani *et al.,* 2014; Yao *et al.,* 2006). Many bacteria, such as *Bacillus* spp., can boost plant growth and yield while reducing the need for chemical fertilizers by fixing nitrogen (Al-Hawamdeh *et al*., 2024; Mohamed *et al*., 2021b; Sivasakthi *et al.,* 2014).

In agricultural production, bio-fertilizers have several advantages, including the replacement of chemicals nitrogen (N) and phosphorus (P), stimulation of plant growth through increased root formation, raising plant dry matter and plant biomass, carotenoids, chlorophyll, and antioxidant enzymes (İİlbaş 2009).

Protoplast fusion is regarded as a crucial tool for genetic recombination. By combining genes from different microorganisms to create strains with desired characteristics, the improved strains are used to increase the efficiency of plant nematode control and reduce chemical fertilizer use (Mohamed *et al.,* 2021; Soliman *et al.,* 2020). Mohamed *et al.* (2021) found that protoplast fusion of *B. cereus* (Bc) and *B. thuringiensis* (Bt) produced ten stable bacterial fusants with stronger nematicidal activity than parental strains. In vitro, bacterial F7 achieved the highest juvenile mortality (J2) of 98.3%. F7 showed the greatest reduction in the galls and egg masses by 77.18 and 72.35%, respectively, in vivo. Also, in pot trials, F7 had the largest significant increase in eggplant parameters. F7 was found to be more capable of fixing atmospheric nitrogen than its bacterial parents. Soliman *et al*. (2023) found that Mutant No. 1 of *B. cereus*, developed through UV-induced mutation, significantly increased the mortality rate of *M. incognita* juveniles. This mutant had the strongest impact in reducing nematode infection and promoting tomato plant growth. These results align with the observation that plants treated with Mutant No. 1 showed the highest number of protein bands in soluble-protein electrophoretic

patterns, both at the end of the application and one month later. Geng *et al*. (2016) discovered that the serine protease produced by *B. firmus* is a novel biocontrol agent against root-knot nematodes. This enzyme was shown to degrade multiple intestinal and cuticle-associated proteins, effectively breaking down the nematode's physical defenses and contributing to its control.

This study aimed to replace chemical fertilizers, which pose risks to human health and the environment, with more effective, eco-friendly, and safe bio-fertilizers. Additionally, it explored the potential of bio-control strategies against *M. incognita*, focusing on bacterial strains carrying the alkaline serine protease gene, known for inducing plant resistance. The study also examined the impact of bio-fertilizers on tomato leaf protein profiles using SDS-Protein Electrophoresis, highlighting their influence on plant growth and yield. These findings will enhance our understanding of the mechanisms by which bacteria combat plant-parasitic nematodes.

2. Materials and Methods

2.1. Bacterial strain source and growth conditions

The bacterial bio-agents *B. thuringiensis* subsp *tenebrionis* (Bt) strain (El-Kawokgy *et al*., 2004), *B. cereus* NRC12 (Bc) under accession number MW548408 in the GeneBank (USA), F7 fusant between Bc:Bt were obtained from previous studies (Mohamed *et al*., 2021) and *Achromobacter xylosoxidans* under accession number LC214968.1 obtained from (Soliman *et al*., 2019). Luria-Bertani (LB) medium (Sigma-Aldrich company) was used to cultivate different bacterial strains (Davis *et al.,* 1980).

2.2. PCR amplification of protease genes

Primers were developed utilizing the protein sequence of a serine protease found in the Uniprot database (https://www.uniprot.org/uniprotkb/A0A316Y3T2/entry) and the gene sequence corresponding to the alkaline serine protease, which was imported into GenBank under the entry PWN77930.1 (https://www.ncbi.nlm.nih.gov/protein/PWN77930.1). The design of the primers was conducted using Primer3 Plus software (https://www.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi). The forward primer, F, possesses the nucleotide sequence 5'-CATAAAGTTAATATTGTTCTGATGTCACTG -3'. The reverse primer, R, consists of the nucleotide sequence 5'-ATATGAAATTGTATTGCCATCTTTATAGC -3 .'

To initiate the experiment, a single colony of *B. cereus* strain NRC12 was cultivated in LB broth media at 37 °C with 180 rpm shaking for 18 hours using a shaking incubator (Thermoscientific, USA). Genomic DNA was isolated using the GeneJET Genomic DNA Purification Kit (Thermoscientific, USA) following the manufacturer's instructions.

2.3. PCR amplification and agrose gel electrophoresis

For PCR-based amplification of the alkaline serine protease gene, a DNA thermal cycler (Perkin Elmer GeneAmp PCR System 9600, Waltham, MA, USA), was employed according to the provided protocol. The PCR reaction was set up using a 25 µL reaction mixture containing 1X Taq buffer (with $MgCl_2$), 0.2 mM dNTPs, 0.5 µM of each primer, 1.25 U Taq DNA polymerase (Thermo Fisher Scientific, USA), and 50 ng of template DNA, following the standard PCR protocol. The annealing temperature used for the PCR reaction was set at 62 °C, based on the calculated melting temperature (Tm) of the primer pairs using the nearest-neighbor thermodynamic model. For agarose gel electrophoresis, a 1.5% (w/v) agarose gel was prepared in 1X TAE buffer, and electrophoresis was carried out at 100V for 45 minutes. The PCR products were visualized under UV light after staining with ethidium bromide/sybr safe.

GenBank submission

The alkaline protease sequences were submitted to GenBank under the accession number OR030044 https://www.ncbi.nlm.nih.gov/nuccore/OR030044.1/

2.4. Multiple sequence alignment and phylogenetic analysis

Molecular Evolutionary Genetics Analysis (MEGA) software version MEGA 11 was used for multiple sequence alignment analysis. ClustalW was employed for sequence alignment with default gap opening and extension penalties. A neighbor-joining (NJ) phylogenetic tree was constructed using the p-distance model with pairwise deletion for gaps/missing data. The statistical reliability of the phylogenetic tree was assessed using a bootstrap analysis with 100 replicates (Mahmoud *et al.,*

2021). The tree was midpoint-rooted, and branch lengths were measured in the number of substitutions per site.

2.5. Field experiment

Site description

A field experiment was conducted during the winter season on the 1st of October 2020 in the National Research Centre farm, El-Noubaria region, Beheira Governorate, north of Egypt. The experimental site was located at latitude: 30°15"N, longitude: 30°47"E (Figure 1). The experimental soil was analyzed to test the physical and chemical properties. The soil texture was 90.5% sand, 6.22% clay and 3.28% silt; the soil pH and EC were 7.95 and 2.18 dS m^{-1} respectively. The soluble cations in the soil (Ca^{++}, Mg^{++}, Na^{+} and K^{+}) were 6.02, 3.97, 9.44 and 2.37 mmol L^{-1}, respectively. The soil soluble anions (CO_3^{--}, HCO_3^{-}, Cl^{-} and SO_4^{--}) were 0, 0.64, 12.9 and 8.26 mmol L^{-1}, respectively (Sims, 1996).

Figure 1. Experimental site (Google map, Satellite) at which the field experiment was conducted

Tomato seeds (*S. lycopersicum* Mill. cv. CH7) were transplanted into a greenhouse with sandy soil in the October 1st 2020. A 10.5 m^2 (3 x 3.5m) plot with five rows was used in this experiment. Tomato seedlings were cultivated 0.5 m apart on one side of the irrigation line (21 plants per plot). Two doses of all treatments were applied after 3 and 30 days from the transplant date. One hundred milliliter of bacteria was applied as foliar spraying and as soil drenching around the plant root (10^{9} CFU/ml). According to Ali and El-Ashry's (2021), vydate was used at the recommended rates of 0.2 ml/plant and 12.5 kg/fed in Egypt. Horticulture techniques for growing tomato plants and the fertilizers were applied according to the Egyptian Ministry of Agriculture and Land Reclamation (150 units of P_2O_5 and 200 units of K_2O/ha), while nitrogen fertilizer was given in accordance with the experimental treatments.

2.6. Experimental treatments

Tomato seedlings (35 days of age) were exposed to foliar spraying and soil drench of bacterial suspension treatments, for controlling nematode diseases and improving tomato growth and productivity. In this study, the experiment included two fertilization groups (250 and 500 N/hectare), each group contained two sub-groups of bacterial suspension addition, whether (foliar spraying or soil drenching). Each sub-group underwent six treatments

Fusant (F7), *Bacillus thuringiensis* (Bt), *Bacillus cereus* (Bc), *Achromobacter xylosoxidans* (Ax), xamyl as a chemical nematicide marketed as "Vydate", and a control).

All nitrogen treatments (and other mentioned fertilizers P and K) were applied with a drip irrigation system during the season, while bacteria treatments were applied as foliar spraying and soil drenching at two times (after two days and one month of cultivation date). Nitrogen fertilizer was applied in the form of ammonium nitrate (NH_4NO_3) (33.5 N).

2.7. Measurements and Data analysis

After 65 days from cultivation (flowering stage), three tomato plants were randomly selected from each plot to assess the following characteristics: plant height (cm), number of branches and leaves, fresh and dry leaf weight (g), flowering and fruit yield, and the number of clusters per plant.

Tomato yield was evaluated by harvesting fully red-ripe fruits over five collection periods, beginning three months after planting. The total marketable yield, fruit count, fruit yield per plant (g), and overall fruit yield (tons/ha) were recorded.

For fruit quality analysis, tomato samples were randomly selected from each experimental plot during the middle of the harvest period. Measurements included average fruit weight (g), fruit diameter (cm), and total soluble solids (TSS), determined using a hand refractometer (Atago, U.S.A.).

2.8. Determination of nitrogen, phosphorus, and potassium percentages in tomato leaves:

The percentage of nitrogen was determined using the Kjeldahl method (A.O.A.C., 1990). Phosphorus content was assessed colorimetrically using the NH4-Metavanadate method, as described by Motsara and Roy (2008). Potassium percentage was measured using a flame photometer, following A.O.A.C. (1990) guidelines. Additionally, soil-accessible nitrogen (mg/kg) was analyzed according to the method outlined by Wolf and Beegle (2011).

2.9. SDS-Protein Electrophoresis

To evaluate the induction of systemic resistance (ISR) in tomatoes against nematode infection and assess the role of nitrogen fixation in improving plant yields, 1 g of tomato leaf samples from different treatments (at a rate of 250 N/ha) was used for protein analysis. Protein profiling was conducted using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), following the method of Laemmli (1970). Sample preparation and water-soluble protein extraction were performed according to Stegmann (1979). Gel images were captured and analyzed using Gel-Pro Analyzer V.3 with the Gel Doc Bio-Rad System.

2.10. Cluster analysis to protein profiles of treated plants using NTSYS methods

The SDS-PAGE results were compiled and encoded in binary form for analysis. The data were then processed using the NTSYSpc v2.10e statistical software. Statistical analysis was performed following the method of El-Kawokgy *et al.* (2015), with a 5% probability level used to determine the least significant difference (LSD) values.

2.11. Initial soil sample collection

Soil samples were randomly collected from the experimental plots to determine the initial population of second-stage juveniles (J2s). One week before planting,

five sub-samples were taken from each plot at a depth of 15–30 cm. These sub-samples were thoroughly mixed, and a 250 g aliquot of the composite soil sample was used for nematode extraction. The nematodes were identified as *Meloidogyne incognita* based on the perennial pattern morphology of mature females in tomato roots, observed under a light microscope (Eisenback, 1985).

To assess nematode damage, J2s were extracted from 250 g of soil using the sieving and Baermann technique (Barker, 1985). The extracted juveniles were then counted using a Hawksley slide under a light microscope, and the J2 population in soil was calculated according to Puntener (1981).

For root analysis, tomato root systems were gently washed with tap water. The reduction percentages in root galls and egg masses (5 g of root sample) were calculated and indexed on a 1–10 scale, following the method of Sharma *et al*. (1994).

2.12. Experimental design and statistical analysis

The experiment was conducted with three replicates using a split-plot design. The major plots received nitrogen treatments, while the sub-plots were subjected to bacteria treatments. A total of 24 treatments were included in each replicate. All data collected were directly analyzed using the Mstatic (M.S.) software, and analysis of variance (ANOVA) was performed. Significant differences between means were determined using Duncan's Multiple Range Test (DMRT) at the $P < 0.05$ level. The comparison between means of different treatments was based on the methodology described by Snedecor and Cochran (1982). Means followed by the same alphabetical letter were considered not statistically different at the 5% significance level according to Duncan (1955).

3. Results

The *alkaline protease* gene ASP16 from strains Bc and Bt, as well as its F7, were utilized for the amplification process. The amplification resulted in fragments with a length of 1100 base pairs, which were visualized on an agarose gel (Figure 2).

The resulting amino acid sequence of the alkaline protease gene comprised 359 amino acids. Further analysis using BLASTp and phylogenetic methods revealed a strong association between our alkaline protease ASP16 and the S8 family peptidases of *B. cereus* (Figure 3).

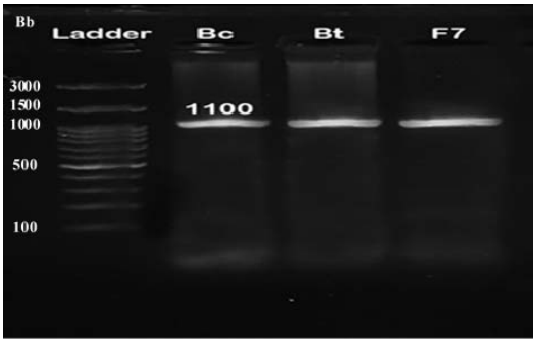


Figure 2. Agarose gel of the amplified protease gene. Line 1, DNA ladder (GeneRuler100 Bp, Thermo Fisher Scientific Inc., US), line 2,3 and 4 1100 bp band of the protease gene in Bc, Bt and F7 respectively.

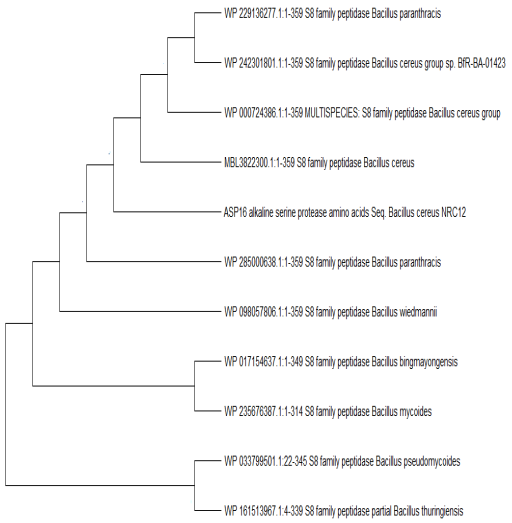


Figure 3. Neighbor-Joining phylogenetic analysis of ASP16 alkaline protease and those of *Bacillus*.

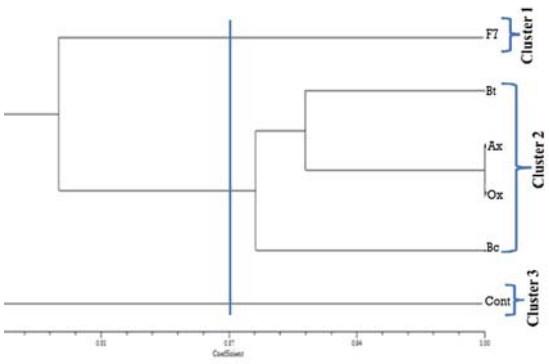
3.1. Protein band expression in tomato leaves following foliar spray and soil drench treatments

According to the results of SDS-PAGE protein banding patterns for leaf water-soluble proteins from eleven tomato plant treatment varieties (Table 1), the majority of the extracted proteins migrated in the range from 24 to 191 kDa, with 18 bands; seven of these bands were monomorphic at M.W. 134, 110, 98, 74, 60, 47, and 37 kDa, while eleven of the bands were polymorphic.

Overall, the soil drench treatment resulted in the highest number of protein bands across all treatments, compared to both the foliar spray and the control. For instance, the control plants expressed 7 bands, while plants treated with F7 via foliar spray and soil drench expressed up to 17 bands. The number of protein bands in tomato plants ranged from a low of 8 bands in the control to a high of 18 bands in plants treated with F7. The protein bands were ranked as follows, from highest to lowest: soil drench treatments produced 12 bands in Bt and Bc, 15 bands in Bt, 11 bands in Bc and Ax, and 10 bands in Oxamyl, while foliar spray treatments resulted in 10 bands in Ax and Oxamyl.

Table 1. The electrophoretic water-soluble protein patterns for tomato plant leaves when spraying foliage and drenching the soil

No	MW	Spraying foliage					Soil drench					
	KD	F7	Bt	Bc	Ax	Oxamyl	F7	Bt	Bc	Ax	Oxamyl	Control
1	191	+	-	-	-	-	+	-	-	-	-	-
2	170	+	+	+	+	+	+	+	+	+	+	-
3	152	+	-	+	-	-	+	+	-	+	-	-
4	134	+	+	+	+	+	+	+	+	+	+	+
5	124	+	-	-	-	-	+	-	-	-	-	-



6	110	+	+	+	+	+		+	+	+	+	+	+
7	98	+	+	+	+	+		+	+	+	+	+	+
8	80	+	+	+	+	+		+	+	+	+	+	-
9	74	+	+	+	+	+		+	+	+	+	+	+
10	68	+	+	-	-	-		+	+	-	-	-	-
11	60	+	+	+	+	+		+	+	+	+	+	+
12	47	+	+	+	+	+		+	+	+	+	+	+
13	43	+	-	-	-	-		+	-	-	-	-	-
14	42	-	-	-	-	-		-	+	+	-	-	-
15	37	+	+	+	+	+		+	+	+	+	+	+
16	33	+	-	+	-	-		+	+	-	-	-	-
17	28	+	+	+	+	+		+	+	+	+	+	-
18	24	+	+	-	-	-		+	+	-	-	-	-
Total Bands		17	12	12	10	10		17	15	11	11	10	7

3.2. Cluster analysis of protein profiles of treated plants using NTSYS

The findings of a numerical analysis of the whole-cell protein profiles of treated plants using an arithmetic averages algorithm and an unweighted pair group approach are displayed in (Figures 4 and 5). Figure 4 reveals the presence of three clusters: the first includes the protein profile of plants treated with cell suspension of F7; the second includes plants treated with Bt in its own subcluster separated from the subcluster that includes plants treated with Ax (*A. xylosoxidans*) and Ox (Oxamyl), where they have the same protein profile with a similarity of 100%; and the third cluster includes the protein profile of the control treatment. However, in case spraying foliage trial protein profiles in treated plants displayed different similarity compared to that in trial of soil drainage (Figure 5) revealed three clusters. Interestingly, the protein profile in plants treated with Ox was the same as in those treated with Ax and different from those treated with Bc and Bt. Our findings implied that the expressed proteins in plants were different as a response to different external factors and this behavior is logical and that depends on the interaction between plant biological system and external environmental factors.

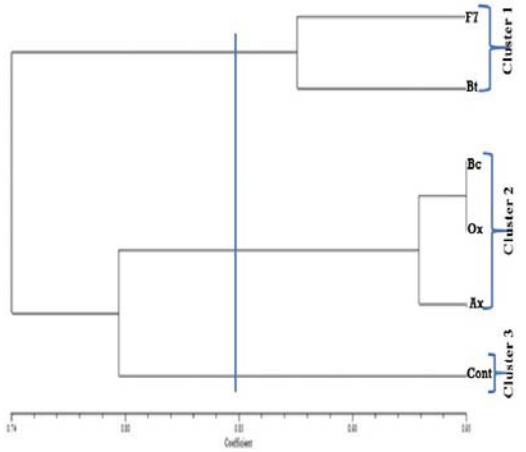


Figure 4. Dendrogram of the SDS-PAGE profiles of the total proteins of the treated plants in the case of the soil drench trial. The dendogram was created using the unweighted pair group approach and an arithmetic averages algorithm.

Figure 5. Dendrogram of the SDS-PAGE profiles of the total proteins of the treated plants in the spraying foliage trial. The dendogram was created using the unweighted pair group approach and an arithmetic averages algorithm.

3.3. The effect of bacterial strains on the reduction of nematode parameters on tomato plants

The data presented in Table 3 highlight the evaluation of modified F7 and its parental strains (Bt::Bc) in comparison with *A. xylosoxidans* (a nitrogen-fixing bacterium) and Oxamyl (a nematicide) for controlling *M. incognita* using foliar spray and/or soil drench application methods. Overall, the results demonstrate that all treatments, when compared to the controls, effectively reduced root-knot nematode reproduction.

Among the treatments, the nematicidal effects of F7 were more pronounced, showing greater effectiveness than the parental strains and control in reducing all nematode parameters, including the number of J2 juveniles in soil, galls, and egg masses (untreated plants). The highest percentage reduction in J2s in soil was observed with F7 (95.45%), followed by Oxamyl (87.98%) and Bc (87.79%) under foliar spray application. For soil drench application, the reductions were even higher with F7 (96.24%), followed by Oxamyl (89.37%), Ax (89.03%), Bc, and Bt.

Oxamyl showed greater reduction in all nematode parameters compared to *B. cereus*, as compared to the untreated control (Table 3). Specifically, galls in 5g of root were significantly reduced by F7, Bt, Bc, Ax, and Oxamyl by 66.75%, 29.90%, 41.39%, 40.91%, and 52.39%, respectively, when applied as a foliar spray. The reduction for the soil drench application was 75.60%, 38.04%, 46.17%, 47.39%, and 55.26%, respectively.

The trend for egg mass reduction followed a similar pattern, with a few exceptions. In general, F7 was the most effective in reducing all nematode-related parameters compared to the other treatments, and soil drench application was found to provide the best reduction in nematode infestations compared to foliar spray.

Table 2. The nematicidal effect of bacterial strains on the percentage reduction of *M. incognita* parameters on tomato plants

Treatments	Applications	Nematode parameters							
		No. J2 in soil	% Reduction	No. galls / roots	% Reduction	GI**	No. egg-masses / root	% Reduction	EI**
F7	Spraying foliage	92[b]	95.45	25.50 [g]	66.75	5	6.75 [h]	88.41	3
Bt		268[b]	86.80	64.75 [c]	29.90	7	17.25 [cd]	81.50	4
Bc		248[b]	87.79	56.25 [d]	41.39	7	13.75[ef]	84.15	4
Ax		262[b]	86.99	55.00 [d]	40.91	7	13.25[fg]	86.99	4
Oxamyl		242[b]	87.98	46.75 [e]	52.39	6	11.75 [g]	83.94	4
F7	Soil drench	76[b]	96.24	34.75 [f]	75.60	6	14.25[f]	95.51	4
Bt		248[b]	87.84	73.25 [b]	38.04	8	22.75 [b]	85.98	5
Bc		234[b]	88.56	61.25 [c]	46.17	7	19.50[bc]	88.82	5
Ax		224[b]	89.03	61.75 [c]	47.39	7	16.00 [de]	89.23	5
Oxamyl		217[b]	89.37	49.75 [e]	55.26	6	19.75[bc]	90.45	5
Control		2079[a]		104.50 [a]		9	123.00 [a]		9

The values represent the mean of five replicates. According to Duncan's Multiple Range Test, means that are followed by the same letter (s) are not statistically different.

** Gall index –GI, Egg masses index =EI, R % = % Reduction.* Bt: *B. thuringiensis* Bc: *B. cereus* Ax: *A. xylosoxidans*

3.4. Vegetative growth

The effect of nitrogen treatments (250 and 500 N/ha) and bacterial treatments (F7, *B. thuringiensis* (Bt), *B. cereus* (Bc), *A. xylosoxidans* (Ax), Oxamyl, and control) on vegetative growth characteristics (plant length (cm), the branch number, leaf number, fresh weights of leaves (g), and dry weights of leaves (g) of tomato plants are shown in Table (3). Tomato plants treated with 250 and 500 N/ha with fusant, F7, as a soil drench produced the highest plant length (174.70 cm and 171.30 cm, respectively), had no statistically significant differences between them, while the control treatment produced the shortest plant lengths (110.30 cm). While the maximum significant branch number values for tomato plants were noticed with 500 N/ha with fusant and F7 as a soil drench treatment (6.67) and the lowest values were observed with the control treatment (1.6), as for the effect of the interaction between nitrogen and bacteria treatments on leaf number, fresh and dry weights of leaves per plant were in the same

Table 3. Effect of the interaction of nitrogen deficiency and bacterial treatments on vegetative growth of tomato plants

Nitrogen dose	Treatments	Application	Plant length (cm)	Number of branches / plant	Number of leaves/ plant	Fresh weights of leaves/ plant (g)	Dry weights of leaves/ plant (g)
250 N/ha	Fusant F7	Foliar spraying	155.30^{fg}	4.00^{def}	80.00^{gh}	639.30^{gh}	39.05^{cd}
	Bt		151.00^{ghi}	3.00^{ghi}	70.67^{i}	541.60^{i}	36.50^{fg}
	Bc		142.30^{kl}	3.00^{ghi}	57.33^{lm}	390.70^{kl}	34.37^{hi}
	Ax		151.00^{ghi}	3.00^{ghi}	71.66i	543.00^{i}	36.55^{fg}
	Oxamyl		140.00^{l}	2.00^{ikl}	54.67^{m}	355.90^{lm}	33.39^{i}
	Control		102.70^{o}	1.33^{l}	30.68^{o}	194.10^{p}	30.33^{j}
	Fusant F7	Soil drench	171.30^{ab}	5.33^{bc}	144.70^{b}	1204.00^{b}	41.29^{b}
	Bt		159.30^{def}	4.00^{def}	86.67^{f}	706.00^{fg}	35.87^{g}
	Bc		164.30^{cd}	4.33^{de}	105.71^{d}	988.90^{d}	37.65^{ef}
	Ax		159.00^{ef}	4.00^{def}	86.33^{f}	703.30^{fgh}	35.83^{g}
	Oxamyl		147.70^{hij}	3.00^{ghi}	61.00^{kl}	447.90^{jk}	35.41^{gh}
	Control		110.30^{mn}	1.67^{kl}	33.00^{o}	219.00^{op}	31.04^{j}
500 N/ha	F7	Foliar spraying	158.30^{ef}	4.67^{cd}	85.00^{fg}	725.10^{ef}	39.67^{c}
	Bt		154.80^{fg}	4.33^{de}	77.33^{h}	632.30^{h}	38.14^{de}
	Bc		146.00^{ijk}	4.00^{def}	64.33^{jk}	475.10^{ij}	35.73^{g}
	Ax		155.00^{fg}	4.33^{de}	77.67^{h}	634.70^{gh}	38.50^{cde}
	Oxamyl		143.70^{jkl}	3.33^{fgh}	61.66^{kl}	445.40^{jk}	34.13^{i}
	Control		106.30^{no}	2.67^{hij}	35.68^{no}	280.60^{no}	31.52^{j}
	F7	Soil drench	174.70^{a}	6.67^{a}	153.0^{a}	1284.00^{a}	42.47^{a}
	Bt		163.00^{cde}	5.67^{b}	94.00^{e}	792.30^{e}	37.65^{ef}
	Bc		167.20^{bc}	5.33^{bc}	111.3^{c}	1074.00^{c}	39.44^{c}
	Ax		163.30^{cde}	5.66^{b}	93.00^{e}	792.70^{e}	37.99^{de}
	Oxamyl		151.70^{gh}	3.66^{efg}	67.66^{ij}	531.60^{i}	36.35^{g}
	Control		113.20^{m}	2.34^{ijk}	40.00^{n}	307.30^{mn}	33.54^{i}

Means with the same letter(s) are not statistically ($P < 0.05$) different from each other. F7: fusant, Bt: *B. thuringiensis,*Bc: *B. cereus,* Ax: *A. xylosoxidans* trend., where the highest significant values were achieved with 500 N/ha with, F7 as a soil drench treatment (153.0, 1284.00 g, and 42.47 g, respectively), followed by 250 N/ha with, F7 as a soil drench, while the lowest values were observed with 250 N/ha with control plants as foliar spraying (30.00, 194.10 g, and 30.33 g).

As for the effect of nitrogen treatments (250 and 500 N/ha) on the vegetative growth parameters of tomato plants was clear in (Fig. 6 A-E.) Tomato plants which received the minimum quantity of nitrogen fertilizers (250 N/ha) produced the highest significant plant length values, and the other above mentioned vegetative growth characteristics. While tomato plants treated by, F7, as a soil drench achieved the most significant plant length values, branch numbers, leaf numbers, fresh and dry weights of leaves (g), and followed by Bc as a soil drench treatment. While tomato plants grown in control treatments had the lowest values of vegetative growth characteristics (Fig. 7A-E.).

3.5. Flowering and fruit yield

The data presented in Table 4 illustrate the effects of nitrogen treatments (250 N/ha and 500 N/ha) and bacterial treatments (F7, Bt, Bc, Ax, Oxamyl, and the control) on flowering and fruit yield characteristics of tomato plants, including cluster number, fruit number per plant, fruit yield (g/plant), and fruit yield (ton/ha).

The results show that tomato plants treated with 500 N/ha and F7 as a soil drench produced the highest significant values for number of clusters per plant (36.00), followed by 250 N/ha with F7 as a soil drench. The lowest values for clusters per plant were observed in the control treatment (6.00).

In terms of fruit number per plant and fruit yield (ton/ha), the maximum significant values were achieved with 500 N/ha and F7 as a soil drench (60.67 fruits per plant and 43.61 ton/ha). This was followed by 500 N/ha with Bc as a soil drench (58.33 fruits per plant and 41.29 ton/ha) and 250 N/ha with F7 as a soil drench (57.50 fruits per plant and 41.02 ton/ha), with no significant differences between these treatments. The control treatment produced the lowest values for fruit number per plant and fruit yield (ton/ha) (36.67 fruits per plant and 30.88 ton/ha).

For fruit yield per plant (g/plant), tomato plants treated with 250 N/ha and 500 N/ha with Fusant F7 as a soil drench produced the highest significant values (2821.00 g and 2734.00 g), followed by 500 N/ha with Bc as a soil drench (2655.00 g). The minimum values were found with 250 N/ha and the control treatment (2059.00 g).

Regarding the effect of nitrogen treatments on flowering and fruit yield characteristics, data shown in Figures 8A–D indicate that these characteristics were negatively affected by higher nitrogen fertilizer rates. The 250 N/ha treatment showed significant superiority in all the mentioned characteristics compared to the 500 N/ha treatment.

As for the effect of bacteria treatments on flowering and fruit yield characteristics, Figures 9A–D show that all bacteria treatments positively influenced these characteristics in both soil drench and foliar application methods when compared to the control. The highest significant values for cluster number, fruit number per plant, fruit yield (g/plant), and fruit yield (ton/ha) were achieved with F7 as a soil drench, followed by Bc as a soil drench treatment.

Table 4. Effect of the interaction of nitrogen deficiency and bacterial treatments on flowering and fruit yield of tomato plants

Nitrogen dose	Treatments	Application	Number of clusters/ plant	Number of fruits/ plant	Fruit yield (g/plant)	Fruit yield (ton/ha)
250 N/ha	F7	Foliar spraying	17.33^{fg}	45.83^{fg}	2498.00^{de}	89.18^{ef}
	Bt		16.00^{gh}	45.00^{gh}	2491.00^{de}	88.94^{ef}
	Bc		12.00^{j}	40.83^{jk}	2290.00^{g}	81.75^{g}
	Ax		16.00^{gh}	45.00^{gh}	2489.00^{de}	89.49^{ef}
	Oxamyl		10.00^{k}	40.00^{jk}	2275.00^{g}	81.23^{g}
	Control		5.83^{l}	36.00^{l}	2016.00^{h}	71.30^{i}
	F7	Soil drench	34.00^{b}	57.50^{b}	2734.00^{ab}	97.63^{bc}
	Bt		20.00^{e}	47.50^{ef}	2500.00^{de}	89.23^{ef}
	Bc		27.67^{c}	54.17^{c}	2580.00^{cd}	92.11^{de}
	Ax		20.00^{e}	47.50^{ef}	2503.00^{de}	89.73^{ef}
	Oxamyl		13.00^{ij}	41.67^{ij}	2302.00^{fg}	82.18^{g}
	Control		6.00^{l}	36.67^{l}	2059.00^{h}	73.49^{i}
500 N/ha	F7	Foliar spraying	18.67^{ef}	48.33^{e}	2591.00^{cd}	94.51^{cd}
	Bt		17.67^{fg}	48.33^{e}	2585.00^{cd}	94.80^{cd}
	Bc		14.33^{hi}	44.00^{gh}	2378.00^{fg}	87.66^{f}
	Ax		18.00^{f}	48.67^{e}	2584.00^{cd}	95.53^{bcd}
	Oxamyl		11.33^{jk}	43.33^{hi}	2403.00^{ef}	87.20^{f}
	Control		7.667^{l}	39.67^{jk}	2074.00^{h}	77.23^{h}
	F7	Soil drench	36.00^{a}	60.67^{a}	2821.00^{a}	103.79^{a}
	Bt		22.33^{d}	51.00^{d}	2564.00^{cd}	94.65^{cd}
	Bc		29.33^{c}	58.33^{b}	2655.00^{bc}	98.27^{b}
	Ax		22.67^{d}	51.00^{d}	2560.00^{cd}	95.25^{bcd}
	Oxamyl		14.33^{hi}	44.33^{gh}	2375.00^{fg}	88.27^{f}
	Control		7.00^{l}	39.33^{k}	2113.00^{h}	79.21^{gh}

Means with the same letter(s) are not statistically ($P < 0.05$) different from each other. F7: fusant, Bt: *B. thuringiensis*, Bc: *B. cereus,* Ax: *A. xylosoxidans*

3.6. Fruit quality of tomatoes

The effect of nitrogen treatments (250 N/ha and 500 N/ha) and bacterial treatments (F7, Bt, Bc, *A.* Ax, Ox, and the control) on tomato fruit quality characteristics (average fruit weight (g), fruit diameter (cm), and TSS%) are presented in Table 5. Tomato plants treated with 500 N/ha and F7 as a soil drench produced the highest average fruit weight (138.80 g), followed by 500 N/ha with Bc as a soil drench. The highest fruit diameter values were achieved with 500 N/ha treatments using F7 and Bc as soil drench, with no significant differences between them (6.10 cm and 5.95 cm, respectively). Conversely, the lowest values for both average fruit weight (64.83 g) and fruit diameter (4.42 cm) were observed in plants treated with 250 N/ha and the control. Regarding the effect of the interaction between nitrogen and bacteria treatments on TSS%, no clear trend was observed.

Table 5. Effect of the interaction of nitrogen deficiency and bacterial treatments on average fruit weight, fruit diameter and TSS % of tomato plants

Nitrogen dose	Treatments	Application	Average fruit weight (g)	Fruit diameter (cm)	TSS %
250 N/ha	F7	Foliar spraying	103.50^{gh}	5.50^{efg}	4.93^{abc}
	Bt		102.10^{h}	5.43^{fgh}	4.72^{abc}
	Bc		90.77$_{i}$	5.27^{ghi}	4.50^{cd}
	Ax		102.00^{h}	5.43^{fgh}	4.78^{abc}
	Oxamyl		89.03^{j}	5.23^{hi}	4.17^{de}
	Control		64.83^{l}	4.42^{k}	3.55^{f}
	F7	Soil drench	125.40^{c}	5.70^{b-e}	5.00^{ab}
	Bt		106.60^{f}	5.53^{ef}	5.00^{ab}
	Bc		119.30^{d}	5.63^{c-f}	5.00^{ab}
	Ax		106.90^{f}	5.50^{efg}	5.17^{a}
	Oxamyl		91.77^{i}	5.26^{ghi}	4.50^{cd}
	Control		65.22^{l}	4.83^{j}	3.65^{f}
500 N/ha	F7	Foliar spraying	116.70^{e}	5.81^{bc}	5.05^{ab}
	Bt		115.20^{e}	5.73^{b-e}	4.85^{abc}
	Bc		104.00^{g}	5.56^{c-f}	4.58^{bcd}
	Ax		115.50^{e}	5.71^{b-e}	4.88^{abc}
	Oxamyl		102.10^{h}	5.55^{def}	4.23^{d}
	Control		78.07^{k}	4.86^{j}	3.67^{f}
	F7	Soil drench	138.80^{a}	6.10^{a}	5.10^{a}
	Bt		119.90^{d}	5.80^{bcd}	5.03^{ab}
	Bc		132.50^{b}	5.95^{ab}	5.03^{ab}
	Ax		120.00^{d}	5.82^{bc}	5.08^{a}
	Oxamyl		105.00^{g}	5.52^{efg}	4.60^{bcd}
	Control		78.45^{k}	5.15^{i}	3.77^{ef}

Means with the same letter(s) are not statistically ($P < 0.05$) different from each other, F7: fusant, Bt: *B. thuringiensis*, Bc: *B. cereus*,Ax: *A. xylosoxidans*

The data presented in Figures 10 A-C show the impact of nitrogen treatments (250 N/ha and 500 N/ha) on various fruit quality characteristics of tomatoes. Plants treated with 250 N/ha produced the highest values for fruit weight (g), fruit diameter (cm), and average fruit weight (g), with no significant differences found between the 250 N/ha and 500 N/ha treatments for total soluble solids (TSS%).

The effects of bacteria treatments including F7, Bt, Bc, Ax, Oxamyl, and the control applied as either foliar sprays or soil drenches on tomato fruit quality characteristics are shown in Figures 11 A-C. The results indicate that plants treated with F7 as a soil drench produced the highest values for fruit weight (g), fruit diameter (cm), and average fruit weight (g), followed by Bc as a soil drench treatment. However, no distinct trend in TSS% was observed across the bacteria treatments.

3.7. Tomato plant chemical composition and soil available nitrogen

Data in Table 6 reveal the effect of nitrogen treatments (250 N/ha and 500 N/ha) and bacterial treatments (F7, Bt, Bc, Ax, Ox, and the control) on the chemical composition of tomato plants and soil available nitrogen characteristics (leaf N%, P%, K%, and soil available nitrogen in mg/kg). The results show that tomato plants treated with 250 N/ha and F7 as a soil drench produced the highest significant values for leaf N% and leaf K% (4.27% and 5.20%, respectively), followed by 250 N/ha with F7 as a soil drench (4.14% and 4.98%). The lowest values for leaf N% and leaf K% were observed in the 250 N/ha control treatment (2.43% and 1.88%, respectively). For leaf P% and soil available nitrogen (mg/kg), tomato plants treated with 250 and 500 N/ha with F7 as a soil drench had the highest significant values (0.654% and 0.639% for P%; 254.40 mg/kg and 252.00 mg/kg for soil nitrogen), followed by 500 N/ha with F7 applied as a foliar spray. The lowest values for P% and soil available nitrogen were found with the 250 N/ha control treatment (0.0707% for P% and 145.10 mg/kg for soil nitrogen).

Table 6. Effect of the interaction of nitrogen deficiency and bacterial treatments on leaf N, P and K percentage and soil available nitrogen of tomato plants

Nitrogen dose	Treatments	Application	N %	P %	K %	Soil available nitrogen mg kg-1
250 N/ha	F7	Foliar spraying	3.80^{cd}	0.539^{bc}	4.68^{d}	242.30^{cd}
	Bt		3.65^{fg}	0.467^{de}	4.14^{f}	226.60^{gh}
	Bc		3.13^{j}	0.252^{f}	3.06^{i}	183.80^{k}
	Ax		3.54^{h}	0.467^{de}	4.11^{f}	224.00^{h}
	Oxamyl		2.64^{l}	0.115^{gh}	2.52^{k}	164.30^{l}
	Control		2.43^{n}	0.0707^{h}	1.88^{m}	145.10^{n}
	F7	Soil drench	4.14^{b}	0.639^{a}	4.98^{b}	252.00^{ab}
	Bt		3.57^{gh}	0.436^{e}	3.84^{g}	226.40^{gh}
	Bc		3.74^{def}	0.517^{bcd}	4.38^{e}	234.80ef
	Ax		3.57^{gh}	0.432^{e}	3.85^{g}	227.10^{fgh}
	Oxamyl		3.22^{j}	0.285^{f}	3.12^{i}	192.40^{ij}
	Control		2.48^{mn}	0.113^{gh}	1.90^{m}	152.50^{m}
500 N/ha	F7	Foliar spraying	3.82^{cd}	0.551^{b}	4.84^{c}	246.00^{bc}
	Bt		3.78^{de}	0.484^{cde}	4.35^{e}	230.30^{fgh}
	Bc		3.19^{j}	0.266^{f}	3.29^{h}	187.40^{jk}
	Ax		3.76^{de}	0.489^{cde}	4.368^{e}	232.20^{efg}
	Oxamyl		2.76^{k}	0.130^{g}	2.74^{j}	168.00^{l}
	Control		2.57^{lm}	0.0817^{gh}	2.13^{l}	149.70^{mn}
	F7	Soil drench	4.27^{a}	0.654^{a}	5.20^{a}	254.40^{a}
	Bt		3.69^{ef}	0.450^{e}	4.05^{f}	229.50^{fgh}
	Bc		3.88^{c}	0.533^{bc}	4.62^{d}	238.10^{de}
	Ax		3.70^{ef}	0.450^{e}	4.07^{f}	230.40^{fgh}
	Oxamyl		3.35^{i}	0.298^{f}	3.35^{h}	194.60^{i}
	Control		2.64^{l}	0.128^{gh}	2.16^{l}	152.80^{m}

Means with the same letter(s) are not statistically ($P < 0.05$) different from each other. F7: fusant, Bt: *B. thuringiensis*, Bc: *B. cereus,* Ax: *A. xylosoxidans*

Regarding the effect of nitrogen treatments on the chemical composition of tomato plants and soil available nitrogen characteristics, data presented in Figures 12 A-D show that leaf N%, leaf K%, and soil available nitrogen (mg/kg) were negatively affected by increasing nitrogen fertilizer levels. The 250 N/ha treatment produced the highest significant values compared to the 500 N/ha treatment. There were no significant differences between 250 N/ha and 500 N/ha for leaf P%.

For the effect of bacteria treatments on the chemical composition of tomato plants and soil available nitrogen, results showed that all bacteria treatments positively influenced both the chemical composition of the plants and the soil available nitrogen characteristics, regardless of the application method (foliar or soil drench), when compared to Oxamyl and the control treatments (Figures 13 A-D). The highest significant values for leaf N%, leaf P%, leaf K%, and soil available nitrogen (mg/kg) were achieved with F7 applied as a soil drench, followed by F7 applied as a foliar spray.

4. Discussion

In Egypt, root-knot nematodes (RKNs) are among the most significant agricultural pests, causing substantial crop losses. The use of synthetic nematicides poses risks to both human health and the environment, emphasizing the need for sustainable agricultural practices such as biological control. However, controlling RKNs is challenging since they are obligatory root parasites that spend most of their life cycle inside host roots (Tian *et al*., 2007). One of the most effective biological control methods is breaking the nematode life cycle. The eggshell, which is the most resilient component of nematode eggs, plays a key role in their resistance to chemical and biological pesticides (Wharton, 1980). The inner protein layer is the most common eggshell structure (Bird and McClure, 1976). Lytic bacterial activity has been associated with biocontrol mechanisms for years (Kassab *et al*., 2017).

Many plant growth-promoting rhizobacteria (PGPRs), including *Bacillus* species, contribute significantly to agroecosystem sustainability by promoting plant growth and productivity while reducing the need for chemical fertilizers through nitrogen fixation (Albdaiwi *et al*., 2019, Sivasakthi *et al*., 2014). Biological control methods, biofertilization, and biostimulation techniques using Bacillus spp. can decrease disease prevalence and enhance agricultural productivity (Borriss, 2011; Yao *et al*., 2006).

Lytic enzymes, particularly proteases, play a crucial role in breaking down structural components of nematodes. There is a positive correlation between bacterial nematode-killing efficiency and alkaline protease production, supporting findings by Kassab *et al.* (2017). The amplification of the alkaline protease gene ASP16 from Bc) and Bt) strains, as well as their F7, successfully generated 1100-base pair fragments, confirming the presence of the targeted gene. Visualization on an agarose gel (Figure. 2) validated this amplification process.

Analysis of the amino acid sequence of ASP16 showed a strong relationship with the S8 family peptidases of *B. cereus* (Figure. 3), classifying ASP16 as a serine protease, in line with findings by Mahmoud *et al.* (2021) and Ariyaei *et al*. (2019). Identifying ASP16 as a serine protease is significant as it provides insights into its enzymatic activity and functional properties. Further studies can focus on characterizing ASP16 and its role in biological processes.

The serine protease from *Paecilomyces lilacinus* strain 251 was found to significantly alter the eggshell structure of *M. javanica*. Protease-treated eggs exhibited a thinner chitin layer and loss of the lipid layer compared to the control (Khan *et al*., 2004). In this study, the protease gene was detected in F7 and its parental strains (Bt, and Bc).

Soil drenching was the most effective treatment, showing the highest number of protein bands in soluble protein electrophoresis compared to foliar spraying and control. The highest number of bands (18) appeared in plants exposed to F7 via foliar spraying and soil drenching, while the control exhibited only 8 bands. This finding aligns with Ramadan and Soliman (2020), who reported that biotic stress from nematode infection induced increased protein bands in plants treated with bio-agents such as *A. xylosoxidans*. Protein synthesis and enzymatic alterations occur due to biotic stress, as evidenced by SDS-PAGE. Soil drenching with *A. xylosoxidans* at the time of planting was more effective in protecting against *M. incognita* and promoting tomato growth than foliar spraying.

Induction of resistance genes is associated with an increase in protein bands, correlating with improved tomato growth (Vyomesh *et al*., 2018). Similar findings were reported by Sharaf *et al*. (2016), who suggested that these proteins inhibit nematode infections. Resistance genes influence plant growth, with changes reflected in shoot length, fresh weight, and dry weight (Ramadan and Soliman, 2020). An increase in protein band density was observed when *B. subtilis* strains were applied against *M. incognita* in tomatoes (Sharaf *et al*., 2016).

Our findings demonstrate that all treatments reduced RKN proliferation compared to the control. F7 exhibited the highest nematicidal efficiency, reducing J2 populations in soil, gall numbers, and egg masses more effectively than its parental strains or the control. Several factors contribute to nematode suppression, including:

1. Biocontrol agents produce lytic enzymes, bioactive compounds, iron siderophores, antioxidant enzymes, antibiotics, and compete for nutrients (Beneduzi et al., 2012).
2. PGPRs activate plant defense mechanisms, inducing systemic resistance (ISR) against phytopathogens via signaling pathways such as *B. cereus* AR156 (Chowdappa *et al.,* 2013). ISR is frequently induced in the rhizosphere by Pseudomonas and Bacillus species (Choudhary and Johri, 2009).
3. PGPRs employ eco-friendly biocontrol strategies, including exopolysaccharides (EPSs), salicylic acid, biosurfactants, N-acyl-homoserine lactones (AHLs), and antibiotics (Wang *et al*., 2015; Zou *et al*., 2018).
4. PGPRs produce plant hormones such as indole-3-acetic acid (IAA), gibberellins (GAs), and cytokinins, promoting root and shoot growth, chlorophyll content, hydration, and nutrient uptake (Arora *et al*., 2013; Bhutani *et al*., 2018).

Soil drenching proved to be the most effective application method, corroborating Ramadan and Soliman's (2020) findings that soil drenching enhances bacterial efficiency as a bio-agent in plant nematode biocontrol, plant growth induction, and protein band augmentation.

In our previous study, F7 and its parental strains (Bc and Bt) were assessed for nitrogen fixation ability using glucose nitrogen-free mineral media. Parental strains exhibited a blue color, while F7 displayed a dark blue color (Mohammed *et al*., 2021). Recent data indicate that fertilizers lead to significant losses of nitrogen (40–70%), phosphorus (80–90%), and potassium (50–90%) (Verma and Sandeep, 2023). Consequently, biofertilizers are being

explored as alternatives to chemical fertilizers to improve plant growth and yield.

Tomato plants receiving minimal nitrogen (250 N/ha) exhibited the highest plant length, branch numbers, leaf numbers, and leaf fresh and dry weights. F7 soil drenching at 500 or 250 N/ha yielded the tallest plants, while 500 N/ha F7 treatment produced the highest branch numbers. These findings align with Rascio and La Rocca (2013), who highlighted bacteria's role in sustainable agriculture through biological nitrogen fixation (BNF). Bacteria facilitate BNF by converting atmospheric nitrogen into plant-accessible compounds using nitrogenase (Udvardi and Poole, 2013).

Higher nitrogen levels negatively impacted cluster numbers, fruit numbers, and yield, with 250 N/ha treatments yielding the best results. F7 soil drenching significantly improved fruit yield over foliar application. Increased biological nitrogen fixation during flowering correlates with plant nitrate demands and yield enhancement (Kapulnik *et al*., 1985). PGPRs improve plant growth through hormone production, phosphate solubilization, and antimicrobial activity (Günsu *et al*., 2015; Bhutani *et al*., 2018; Liu *et al*., 2019; Reis *et al*., 2022). These phytohormones stimulate root growth, enhancing water and nutrient uptake (Arora *et al*., 2013). Our results confirm that nitrogen application, F7 treatment, and soil drenching influence tomato chemical composition and soil nitrogen availability.

5. Conclusion

Fusant F7 enhances biocontrol activity against *Meloidogyne incognita*, significantly reducing J2 nematode populations in soil, as well as the number of galls and egg masses in tomato roots. The reduction in nematode infestation, coupled with its bio-fertilization effects, positively influences tomato growth parameters and protein band expression in leaves. Overall, F7 exhibited the most significant impact when applied as a soil drench, achieving the greatest reduction in nematode-related parameters, enhancing nitrogen fixation, and improving plant growth and yield. Notably, soil drenching proved to be more effective than foliar spraying in all measured outcomes.

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Appendices

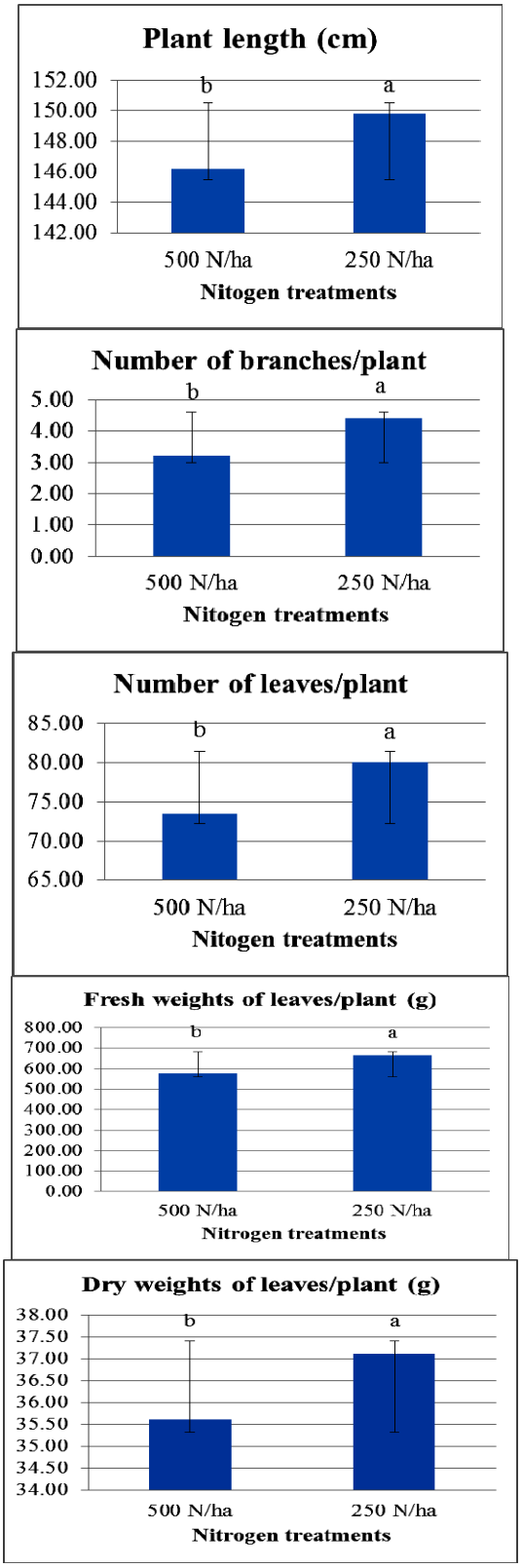

Figure 6A-E. Effect of nitrogen treatments only on vegetative growth of tomato plants

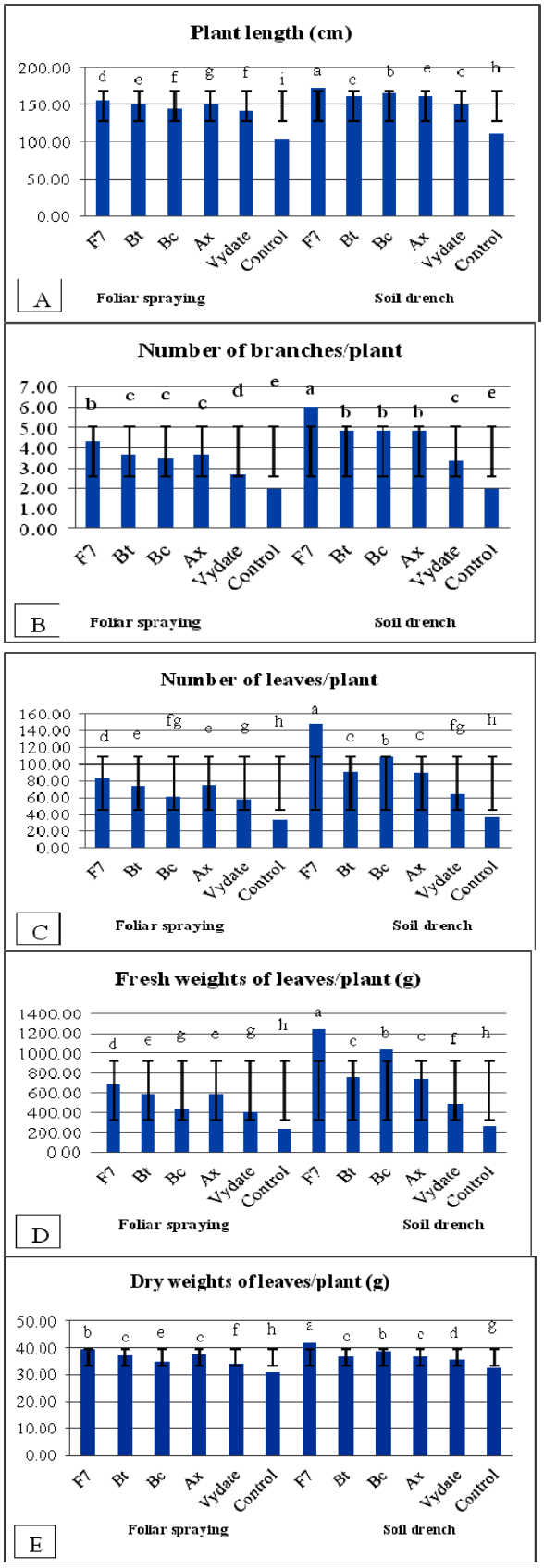

Figure 7A-E. Effect of bacteria treatments only on vegetative growth of tomato plants

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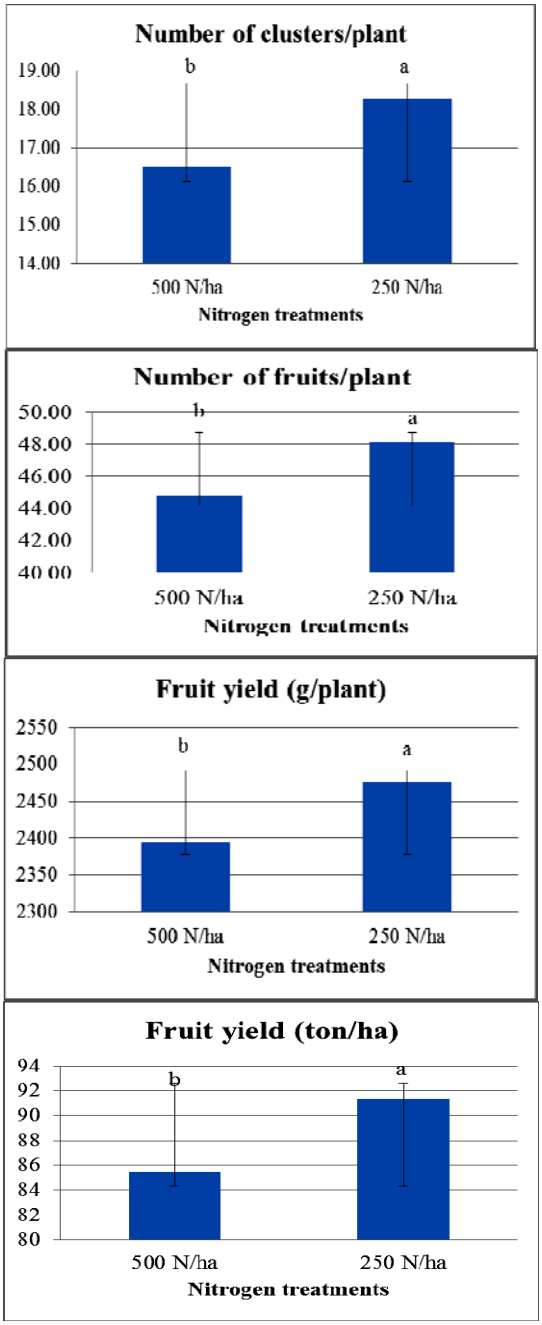
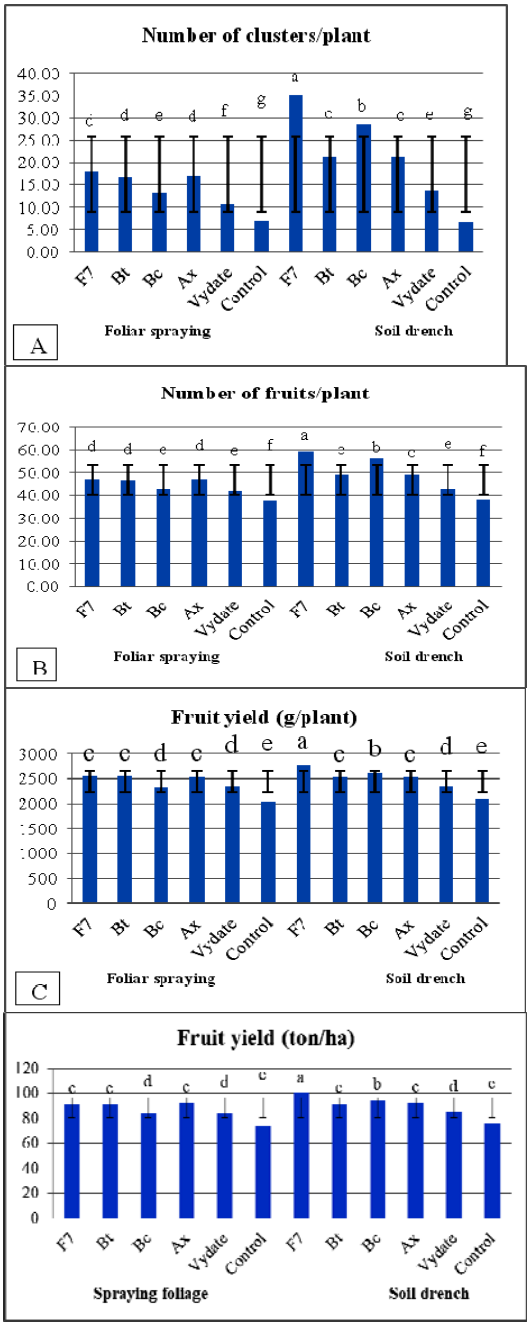
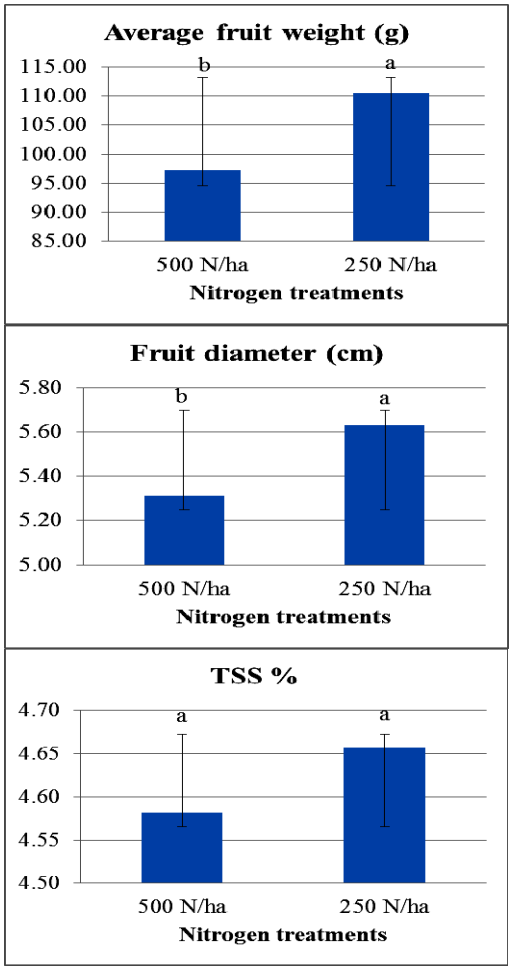


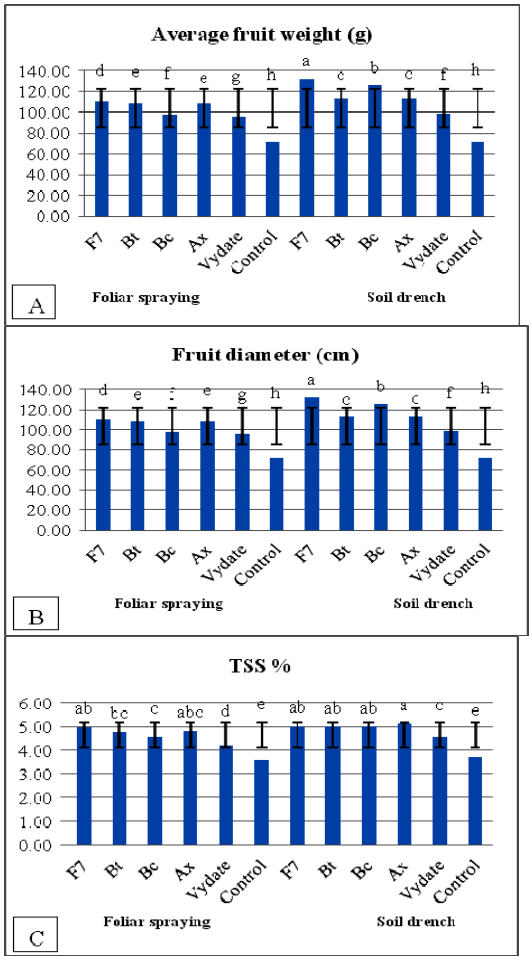
Figures 8A-D. Effect of nitrogen treatments on flowering and fruit yield of tomato plants



Figures 9A-D. Effect of bacteria treatments on flowering and fruit yield of tomato plants



Figures 10A-C. Effect of nitrogen treatments on average fruit weight, fruit diameter and TSS % of tomato plants



Figures 11A-C. Effect of bacteria treatments on average fruit weight, fruit diameter and TSS % of tomato plants

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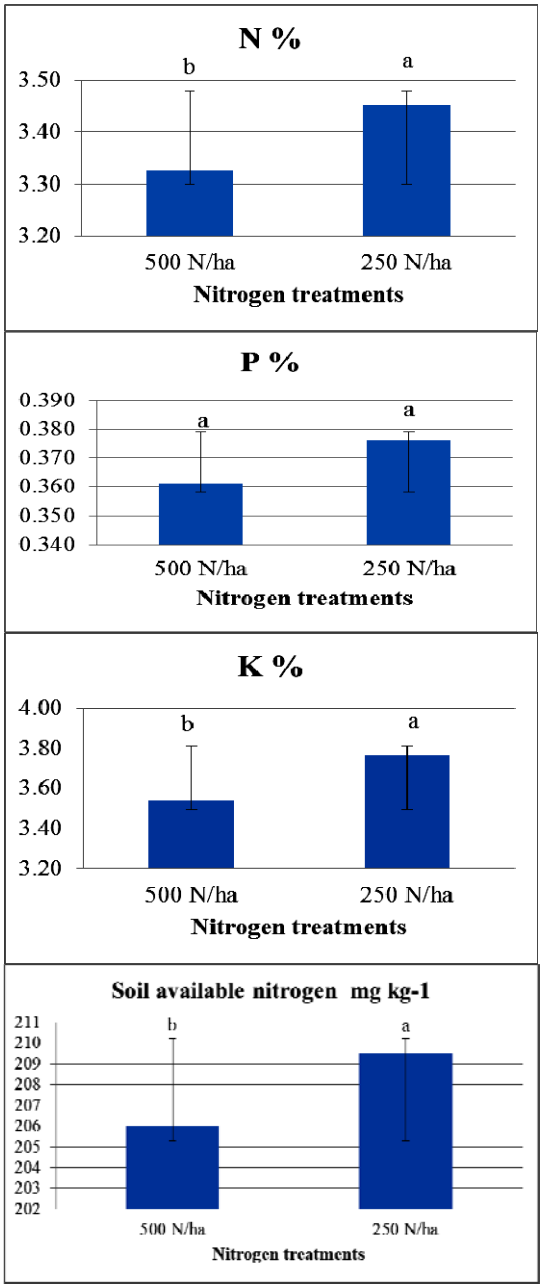
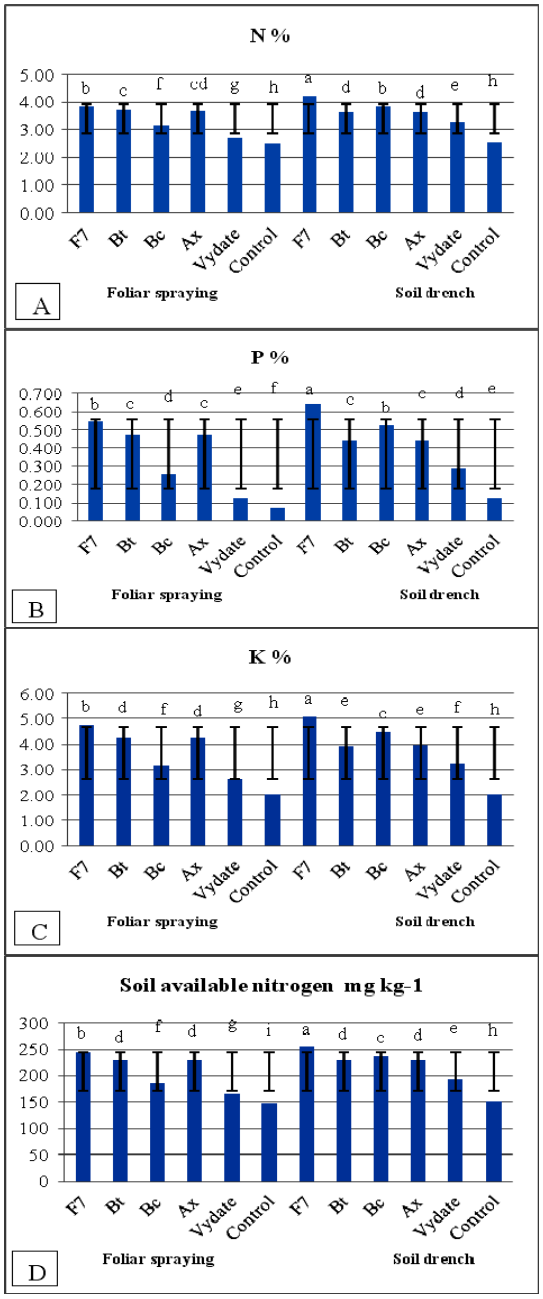


Figures 12A-D. Effect of nitrogen treatments on leaf N, P and K percentage of tomato plants and soil available nitrogen



Figures 13A-D. Effect of bacteria treatments on leaf N, P and K percentage of tomato plants and soil available nitrogen