EFFECTIVENESS OF BACILLUS THURINGIENSIS AS A BIOCONTROL AGENT AGAINST ROOT-KNOT NEMATODES ON TOMATO PLANT

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ABSTRACT

This study aimed to evaluate the nematicidal effect of three local *Bacillus thuringiensis* isolates in controlling the root-knot nematodes Meloidogyne spp. under laboratory and shade house conditions. We amplified 16S rRNA and performed polymerase chain reaction on the isolates to confirm them as *B. thuringiensis*. PCR was carried out with a universal primer for the 16S rRNA amplification for confirmation, where all three isolates yielded the predicted amplicon. The isolates were identified as *B. thuringiensis* and documented using sequencing based on their 16S rRNA genes. Sequencing results were deposited at NCBI. The laboratory experiments revealed that Sa.Sa.Kh.3 isolate outperformed the other two isolates, Sa.Sa.Kh.1 and Sa.Sa.Kh.2, in terms of egg hatching inhibition and second-stage juvenile mortality, reached 94.33% and 90% after 72 hours of exposure, respectively. Pot trial showed that the Sa.Sa.Kh.3 isolate reduced root gall index and improved plant growth parameters shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, plant height, and root length of tomato plants by 1.33g, 77.00 g, 16.63 g, 12.15 g, 2.77 g, 88.00 cm, and 34.33, respectively, when compared to the control groups. Results showed that all isolates, both pre-nematode inoculation and post-nematode inoculation, revealed significant effects on root gall index and enhanced plant growth parameters compared to control groups.

Keywords: 16s ribosomal RNA, *meloidogyne* spp., phylogenetic tree.

فعالية بكتريا Bacillus thuringiensis كعامل حيوي للسيطرة على ديدان العقد الجذرية لنبات الطماطة

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المستخلص

تم تقييم التأثير القاتل للنيماتودا لثلاث عزلات محلية من بكتيريا Bacillus thuringiensis في السيطرة على نيماتودا عقد الجذور *Meloidogyne spp*. تحت ظروف المختبر والظل. قمنا بتضخيم 16S rRNA وأجرينا تفاعل البوليميراز المتسلسل على العزلات لتأكيد أنها Bacillus thuringiensis. تم إجراء تفاعل البوليميراز المتسلسل باستخدام برايمر عالمي لتضخيم S rRNA16 للتكوين، حيث أعطت العزلات الثلاث أمبليكون متوقع. تم التعرف على العزلات على أنها Bacillus thuringiensis وتم توثيقها باستخدام تقنيات التسلسل بناءً على جينات 16S rRNA الخاصة بها. تم إيداع نتائج التسلسل في NCBI. وأظهرت التجارب المختبرية أن عزلة Sa.Sa.Kh.3 ثبطت أكثر من العزلتين Sa.Sa.Kh.1 و Sa.Sa.Kh.2 فقس البيض ومعدل وفيات الصغار في المرحلة الثانية حيث بلغت 94.33% و90% بعد 72 ساعة من التعرض على التوالي. وأظهرت تجربة الأصيص أن عزلة Sa.Sa.Kh.3 خفضت مؤشر التصاق الجذور وحسنت معايير نمو النبات وزن الساق الطازج ووزن الساق الجاف ووزن الجذر الجاف وارتفاع النبات وطول الجذر لنباتات الطماطم بمقدار 1.33 جرام و77.00 جرام و16.63 جرام و12.15 غرام و2.77 جرام و88.00 سم و34.33 سم على التوالي عند مقارنتها بمجموعات المقارنة. وأظهرت النتائج أن جميع العزلات سواء قبل التلقيح بالنيماتودا أو بعد التلقيح بالنيماتودا أظهرت تأثيرات كبيرة على مؤشر التصاق الجذور وتحسين معايير نمو النبات مقارنة بمجموعات الضبط.

الكلمات المفتاحية: 16S ribosomal RNA، شجرة التطور، *meloidogyne* spp.

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INTRODUCTION

Nematode root-knot disease, caused by root-knot nematodes, is one of the most common of these diseases (15, 23). *Meloidogyne* spp. is the most destructive plant-parasitic nematodes (PPNs), causing economic harm to agricultural and horticultural crops (16). The genus *Meloidogyne* has 98 species, which can cause damage to more than 3000 host plants by forming root knots in their roots (19, 29), leading to decreased absorption of water and nutrients, resulting in a weak and stunted plant of smaller stature, wizened at specific times of the day, and low yield (24). *Meloidogyne* spp. is so difficult to control due to their short reproduction time, high reproduction rate, endoparasite, and sedentary nature; therefore, non-host crop rotation was used in its management. In addition to *Meloidogyne* spp. developing strains resistant to the effects of nematicides, increased cost and severe impact on climate (9). Recent studies focus on finding alternatives to chemical to control *Meloidogyne* spp. (20, 22). Biological control has a larger role and impact in combating and controlling plant-parasitic nematodes (1), due to the positive results when being applied via different means and has different modes of action (18). *Bacillus* genus is used in the biological control of agricultural pests, due to its rapid and massive reproduction efficiency and its no harmful effect to humans, plants, animals, or beneficial insects (2). *B. thuringiensis* produced different cry proteins identified according to their amino acid sequence. Among these known Cry toxin families, Cry5, Cry6, Cry12, Cry13, Cry14, and Cry21 showed nematicidal activity (10). Researchers indicated the effectiveness of *B. thuringiensis* CR-371 in protecting tomatoes from root-knot nematodes, *Meloidogyne incognita*, and bacterial wilt diseases under glasshouse conditions (8). The current study was conducted as an alternative to chemical nematicides to protect tomato plants against root-knot nematodes by using three local *B. thuringiensis* isolates on reducing root-knot nematodes under laboratory and shade conditions.

MATERIALS AND METHODS

Isolation and identification: *B. thuringiensis*was isolated from three different soil samples collected from Abu Ghraib, Baghdad governorate. Each sample underwent serial dilution individually; one ml from each dilution was disseminated over a petri dish containing a Nutrient Glucose 1% Agar medium and incubated at 28 °C for 48 hours. Smears were taken carefully in sterile disposable transport media swaps, then kept in an incubator at 37ºC (24–48 hr.). Such swabs were cultured in a brain-heart infusion (B.H.I. broth) medium and incubated at 37ºC for 24 hours to promote bacterial growth. Then, they were streaked on the general and differential culture media and also incubated for 18–24 hours at 37ºC. The growth was diagnosed depending on shape, size, margin, consistency, and color of colonies, microscopic features, molecular diagnosis, and biochemical tests by using the Vitek II system. The microscopic identification was prepared from a single colony of young bacterial isolate grown on nutrient agar (NA) medium for 18–24 hours and fixed on a clean and dry slide, then dyed with Gram stain (Fluka/Switzerland) and examined under a light microscope to see the shape, arrangement, and colors of the cells according to their interaction with the gram stain as shown in Figure (1). Vitek II system used, according to the manufacturing company (BioMérieux/ France), by inoculation of gram-positive bacterial isolates on a blood agar plate (BAP) and then incubation at 37 ºC for 24 hours. All bacterial isolates were identified at the species level by using the VITEK-2 compact system for the identification of gram-positive bacteria. A colorimetric reagent card (BCL) is used to detect the spore-forming Gram-positive bacilli (e.g., *Bacillus*) provided by the BIOMERIEUX/French company.

Table 1. The sequence of primer used to identify *B. thuringiensis*

Primer	Sequence	Primer sequence	Tm (°C)	GC%	Size of Product (bp)
16S rRNA	F	AGAGTTTGATCCTGGCTCAG	54.3	50.0	1150
	R	GGTTACCTTGTTACGACTT	49.4	42.1	

Molecular diagnosis

The DNA of *Bt* isolates was extracted by transferring the appropriate number of cells (up to 5 x 10^9) to a 1.5 ml microcentrifuge tube, according to the protocol of FavorPrep Total DNA Mini Kit Extraction

(FAVORGEN/Korea). The NanoDrop UV spectrophotometer was used to determine the concentration and purity of DNA isolated at 260 and 280 nm. The extracted DNA was stored at 4ºC or -20ºC until used for PCR reactions. Molecular diagnosis was performed using the primer 16SrRNA shown in Table (1), which was prepared by Macrogen Company (Korea), and gene amplification by PCR was performed to diagnose the *Bt* isolates. Agarose gel electrophoresis was adopted to confirm the presence of amplification. 5µl of PCR products were loaded directly into the well, and a 100bp DNA marker ladder (TransGen/China) was used to detect the size of the PCR product. The isolates of *B. thuringiensis* sequencing were analyzed using MEGA6 software and deposited at the NCBI under the accession numbers PQ069228.1, PQ069229.1, and PQ069230.1. Phylogenetic tree relationship was deciphered out by MEGA6 software and compared with seventeen isolates that were deposited in the NCBI gene bank.

Laboratory experiments

Bacterial culture filtrates were prepared by inoculating each *Bt* isolate in a conical flask (500 ml) including 400 ml of nutrient glucose (2%) broth (NGB) medium prepared according to the following formula (glucose 10 g, beef extract 3 g, peptone 5 g, distilled water 1 L). Then, the pH was adjusted to 7.2. The inoculated flasks were incubated at 28 ± 2 °C for four days (7). A McFarland standard solution tube with a concentration of 1 $\times 10^9$ CFU/ml (BIOMERIEUX) was used as a reference to adjust the turbidity of the *Bt* inoculum of each isolate.

***Meloidogyne* spp. inoculum**

Root samples infected with root-knot nematodes were collected from naturally infested okra crops in the field to obtain the nematode inoculum according to the (11). The roots were chopped into small pieces (2–3 cm) after being washed with water to remove the soil and placed in a 1000-ml measuring cylinder containing 250 ml of sodium hypochlorite solution with 0.5% concentration (229 ml of water and 21 ml of commercial Clorox) to dissolve the gelatinous matrix. Then the roots were placed in an electric blender and spun for 10 seconds to ensure that the eggs did not rupture, and then they were left for three minutes to precipitate the root pieces. The supernatant is taken and passed through different-sized sieves (75, 50, and 25 microliters), respectively. The eggs were collected from a 25-microliter sieve and placed in a 1000-ml measuring cylinder after sieving through a mesh. The eggs were counted per 1 ml using a special nematode counting slide.

Egg hatching impact

The experiment conducted in a laboratory to determine the effect of *B. thuringiensis* isolates on the hatching rate of eggs of the root-knot nematode *Meloidogyne* spp. Three ml of *Bt* strain suspension at a concentration of 1×10^9 CFU/ml were added to 10 ml test tube containing 300 µL of nematode inoculum at a concentration of 100 ± 5 eggs. Three ml of NGB medium and sterilized distilled water were separately added to 300 µL of nematode suspension containing 100 ± 5 eggs, which served as standard checks. All treatment included three replications to test the inhibition of egg hatching in a completely randomized design (CRD). The test tubes were incubated at a temperature of 28 ± 2 °C, while the numbers of unhatched eggs were counted after 24, 48, and 72 hours of exposure by a Sedgewick rafter cell counting slide under a compound light microscope with 4x magnification. The corrected percentage of egg inhibition was calculated according to the following equation:

$$\text{Corrected percentage\%} = \frac{\text{\% Unhatched eggs in treatment} - \text{\% Unhatched eggs in control}}{100 - \text{\% Unhatched eggs in control}} \times 100$$

Second-stage juvenile mortality impact

The experiment conducted in the laboratory to investigate the nematicidal effectiveness of *B. thuringiensis* (*Bt*) isolates on second-stage juvenile mortality of the root-knot nematodes (RKN). A concentration of 1×10^9 CFU/ml of each *Bt* strain inoculum was prepared. Furthermore, 300 µL of root-knot nematode inoculum containing 100 ± 5 juveniles (J2) was added to the test tube capacity of 10 ml. Then three ml of each *Bt* strain inoculum was added to each test tube, in addition to the two

comparison treatments of NGB medium and sterilized distilled water. Each treatment consisted of three replications to assess the mortality of juveniles in a completely randomized design (CRD). The test tubes were incubated at 28 ± 2 °C. Observation on the number of dead juveniles (J2) was counted after 24, 48, and 72 hours of exposure by a Sedgewick rafter cell counting slide under a compound light microscope with 40x magnification. When probed with a small needle, the juveniles did not move, indicating that they had died. The mortality percentage of juveniles was estimated using the following equation:

$$\text{Mortality percentage\%} = \frac{\text{Number of dead juveniles}}{\text{Total number of juveniles}} \times 100$$

Root-knot nematodes impact

The experiment in a pot conducted under shade conditions to determine the nematicidal activity of *B. thuringiensis* isolates against *Meloidogyne* spp. Seeds of tomato (a Feton variety obtained from the local market) were sown on February 15, 2024, in a Styrofoam plug tray, with one seed per cell. One month after sowing, tomato seedlings were transferred into plastic pots of 8 kg size, filled with sterilized sandy soil and Patmos (2:1). Each plastic pot contained one seedling, and when 2-3 true leaves appeared, the seedlings were inoculated by making three holes in the plastic pot's soil near the roots at the same distance in a manner of 1.5 cm deep so that the roots did not get damaged (5) at a rate of 5 ml of the suspension, containing 1000 j2 per plant, and then covered with the sterilized soil. Forty milliliters per plant of each *Bt* isolate suspension at a concentration of 1.2×10^9 CFU/ml were applied in the pot near the root zone (rhizosphere) at two different intervals. The first interval was three days before root-knot nematode inoculation, and the second interval was three days after root-knot nematode inoculation. Standard checks included pots treated with carbofuran 10% GR at a rate of three g per plant + RKN, nematodes alone (positive control), NGB medium + RKN, and healthy plants (negative control). The trial included ten treatments with three replications in a randomized complete block design (RCBD).

Gall index and plant growth parameters: After two months of inoculation, the tomato plants were uprooted from the soil pots, and the roots were carefully washed free of soil. The following observations were taken for each interval: plant height, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, root length, and the number of root knots. Gall index (GI) was expressed according to scale (28) shown in Table (2).

Table 2. Gall index of root-knot nematodes, according to the number of root knots per root system

Scale	Number of root- knots
0	No knots
1	1-2 knots
2	3-10 knots
3	11-30 knots
4	31-100 knots
5	greater than 100

Statistical Analysis====The data on egg hatching and second-stage juvenile mortality were analyzed statistically using a complete randomized design (CRD) by the statistical program GenStat 12, and the data were arranged using Excel. The data of the Pot experiment were analyzed using a randomized complete block design (RCBD). All trials included three replications, with one plant per replication, and significant differences between means were compared with the least significant difference (LSD) test at a significance level of 0.05.

RESULTS AND DISCUSSION

Identification of *B. thuringiensis:* The microscopic and morphological features of the three *Bt* isolates revealed the presence of gram-positive rods that produced oval spores and illustrated hemolytic activity in the blood agar plate (BAP), indicated by β-hemolysis zones (Figure 1). The results of the 46 biochemical tests of three isolates using the VITEK-2 compact system with identification (BCL) cards exhibited that all the isolates belong to the species *B. thuringiensis* with a probability of 99%.

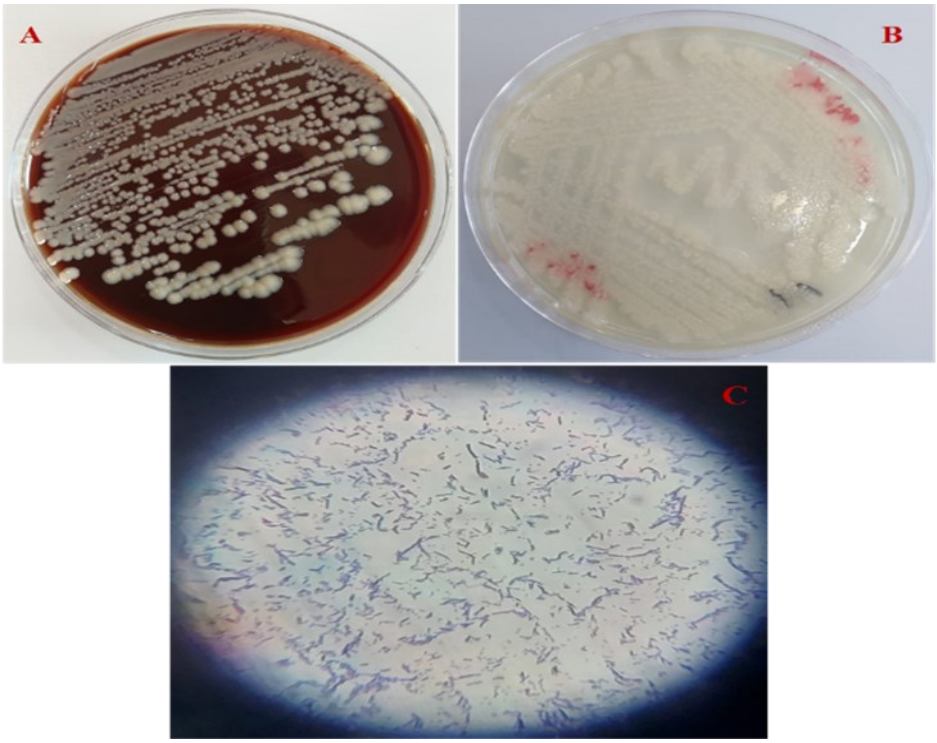

Figure 1. (A): *Bacillus thuringiensis* isolate were grown on a blood agar plate. (B): Bacterial on nutrient agar. (C): *B. thuringiensis* spores with Gram stain were illustrated as gram-positive with a purple color observed under a light microscope at 40x magnification

For additional identification, we amplified 16S rRNA and performed PCR on the isolates to confirm them as *B. thuringiensis*. We used a universal primer for the 16S rRNA amplification, and all three Sa.Sa.Kh.1, Sa.Sa.Kh.2, and Sa.Sa.Kh.3 isolates yielded the predicted 1150 bp amplicon. *B. thuringiensis* isolates were identified and documented using sequencing techniques based on their 16S rRNA genes. The results of the sequencing of the *B. thuringiensis* isolates were deposited at the National Center for Biotechnology Information (NCBI) as shown in table 3. The amplified products of each isolate were sequenced and analyzed using MEGA6 software. The evolutionary history was inferred using the UPGMA method (25). The evolutionary distances were computed using the Maximum Composite Likelihood method (26) and are in units of base substitutions per site. The analysis involved 20 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd, and noncoding region. All positions with gaps or incomplete data were eliminated. There were 389 positions in the final dataset. Evolutionary analyses were conducted utilizing MEGA6 software (27). This finding confirms the correspondence of the biochemical tests and microscopic features with a molecular diagnosis for the three isolates. Researchers investigated the phenotypic characterization and identification of recovered *Bt* isolates using the morphological roperties of colonies, spores, and parasporal crystals in addition to hemolytic activity (6).

Table 3. Molecular identification of the *Bacillus thuringiensis* isolates by 16S ribosomal RNA

No. of sample	Isolates name	Accession no.	Nucleotide alignment dissimilarity	Sequence Identities	Most related isolate	similarity
1	Sa.Sa.Kh.1	PQ069228.1	ATCGGCTACACTGGGA .....C.....	99%	MT534571.1: *B. thuringiensis*: China:Shaanxi	99%
2	Sa.Sa.Kh.2	PQ069229.1	------	100%	MT102737.1: *B. thuringiensis*: Turkey	
3	Sa.Sa.Kh.3	PQ069230.1	AATGGACGCAAGTCTG .....A.....	99%	MN845148.1: *B. thuringiensis*: Poland	

The data in Tables (4) and (5) shows significant differences between *Bt* isolates and control groups when applied as nematicidal against eggs and second-stage juveniles of root-knot nematodes. The result revealed that all isolates effectively inhibited eggs and the mortality of juveniles (J2) when observed under a microscope, as presented in Figure 2. All *Bt* isolates demonstrated a high percentage of egg inhibition. The highest egg inhibition was observed in the Sa.Sa.Kh.3 isolate in the ranges of 99.33%, 97%, and 94.33%, with a corrected percentage of egg inhibition of 98.942%, 95.833%, and 93.814% after 24, 48, and 72 hours of treatment, respectively, followed by Sa.Sa.Kh.2 and Sa.Sa.Kh.1 isolates, compared to the two comparison treatments, NGB medium and control (sterilized distilled water), which gave the lowest inhibition percentage of eggs 38.67%, 30.00%, 9.67%, and 36.67%, 28.00%, and 8.33% after 24, 48, and 72 hours, respectively (Table 4). Comparably, the mortality of J2 was observed under a compound microscope, and the results showed that the cultural filtrate-treated juveniles were dead, as they were straight and did not move even when probed with a small needle. In contrast, untreated juveniles were found to be alive due to their curved and comma-shaped movements (Figure 2). Moreover, the highest mortality percentage of second-stage juveniles (J2) was recorded in Sa.Sa.Kh.3 isolate amounted to 34.67%,68.33%, and 90% with a corrected percentage of juveniles mortality 32.41%, 66.54%, and 89.16% after 24, 48, and 72 hours of exposure respectively. This was followed by Sa.Sa.Kh.2 and Sa.Sa.Kh.1 isolates in the ranges of 30.33%, 61.00%, 85.00%, and 25.00%, 53.33%, 81.00% with a corrected percentage of juveniles mortality 27.93%, 58.80%,83.75%, and 22.41%, 50.70%, 79.42% after 24, 48, and 72 hours of exposure respectively.

Table 4. The Nematicidal impact of *Bacillus thuringiensis* isolates on egg hatching

Treatment	EI 24/hr.	CEI 24/hr.	EI 48/hr.	CEI 48/hr.	EI 72/hr.	CEI 72/hr.	Mean
Control	36.67		28.00		8.33		24.33
NGB	38.67	3.158	30.00	2.333	9.67	1.461	26.11
Bt strain Sa.Sa.Kh.1	96.33	94.204	92.00	88.888	87.00	85.818	91.78
Bt strain Sa.Sa.Kh.2	97.67	96.320	95.00	93.055	90.00	89.091	94.22
Bt strain Sa.Sa.Kh.3	99.33	98.942	97.00	95.833	94.33	93.814	96.89
LSD 5%			3.07**				1.77**
Mean	73.73		68.40		57.87		
LSD 5%			1.37**				

*** Means are averages of three replicates; EI= Egg inhibition %; CEI= Corrected egg inhibition%.**

Figure 2. The effect of culture filtrate of *B. thuringiensis* isolates on egg hatching and second-stage juvenile (J2) mortality. (A): Untreated juveniles. B. An untreated egg matured into a juvenile (J2). (C): A dead second-stage juvenile. (D): Unhatched eggs

The two comparison treatments, NGB medium, and control gave the lowest mortality percentage of J2, exhibited in Table5. The findings in Tables 4 and 5 show that the NGB medium has a minor influence on egg inhibition and juvenile mortality.

Table 5. The Nematicidal impact of *Bacillus thuringiensis* on juvenile mortality

Treatment	JM 24/hr.	CJM 24/hr.	JM 48/hr.	CJM 48/hr.	JM 72/hr.	CJM 72/hr.	Mean
Control	3.33		5.33		7.67		5.44
NGB	4.33	1.03	6.00	0.70	8.67	1.08	6.33
Bt strain Sa.Sa.Kh.1	25.00	22.41	53.33	50.70	81.00	79.42	53.11
Bt strain Sa.Sa.Kh.2	30.33	27.93	61.00	58.80	85.00	83.75	58.78
Bt strain Sa.Sa.Kh.3	34.67	32.41	68.33	66.54	90.00	89.16	64.33
LSD 5%			4.39**				2.53**
Mean	19.53		38.80		54.47		
LSD 5%			1.96**				

***Means are averages of three replicates; JM=Juveniles mortality %; CJM=Corrected juvenile mortality%.**

The study observed the dead second-stage juvenile (J2) was straight or vertical, with the body mostly devoid of internal viscera, and a bubble on one side of its body was noticed. After 72 hours of exposure, the juveniles and eggs turned a dark color, in addition to distorting their contents. Our findings were consistent with a study conducted to find the effectiveness of the fourteen native *B. thuringiensis* isolates against *M. incognita* (20). After 72 hours of treatment, six out of 14 isolates demonstrated 100% inhibition of second-stage juvenile emergence from *M. incognita* egg masses. A study revealed that the nematicidal effects of 10 *B. thuringiensis* isolates in vitro against *M. incognita* exhibited the greatest nematicidal activity (17). A research discovered that the *B. thuringiensis* Berliner strain NBIN-863 has substantial fumigant nematicidal activity and a high attraction impact on the root-knot nematode *M. incognita* (4, 5). Results of a study revealed juvenile death rates ranged from 4- 36% (22). A study used isolates of *B. thuringiensis* with nematode-active cry genes and conducted preliminary bioassays on *M. incognita* (12). According to that, four isolates were selected for further bioassays. At a 2×10^8 CFU/mL concentration, strains YD5 and KON4 exhibited 77% and 81% toxicity against *M. incognita*, respectively. A study found that two strains of *B. thuringiensis* (ToIr65 and ToIr67) exhibited 70% nematicidal activity against juveniles and eggs of *Meloidogyne javanica* (21). After 60 days of nematode inoculation, *B. thuringiensis* Sa.Sa.Kh.3 isolate demonstrated improved plant growth characteristics and a favorably lower gall index as compared to other treatments. According to the data shown in table 6, there were significant differences within growth parameters, these results were in congruent with those of research done to test two forms of strain ToIr65, which significantly reduced the number of galls by 51% compared to the control and promoted tomato plant growth parameters (21). A study found that the use of 3-(methylthio) propionic acid from *B. thuringiensis* reduced gall numbers from 97.58 to 6.97 per gram of root (4). In addition, the plant height and root length increased significantly when compared to the controls. A recent study congruent with our results, applied three dosages of *Bt* suspension (30, 40, and 50 ml/plant) (3). The results showed that a dosage of 40 ml/plant of *Bt* gave the highest reduction of gall number on patchouli root, up to 67% compared to the control. A research evaluated two isolates of Bt under greenhouse circumstances, and the results showed that they had a greater effect on tomato plants by reducing the female population, suppressing the number of egg masses, and decreasing the root gall index compared to control groups (20). A study reported that cuticle-degrading proteases, exhibit nematicidal activity, are likely to be involved in the interactions between bacteria, nematodes, plants, and the environment (13). Although inoculants often have low survival rates and compete with native bacteria for available growth substrates, PGPR strains may temporarily increase the population (14). Researchers indicated the effectiveness of *B. thuringiensis* CR-371 in protecting tomatoes from root-knot nematodes, *M. incognita*, and bacterial wilt diseases under glasshouse conditions (8).

Table 6. Evaluation of the impact of *Bt* isolates on gall index and plant growth parameters three days before nematode inoculation.

Treatment	Gall index	Shoot fresh weight/g	Shoot dry weight/g	Root fresh weight/g	Root dry weight/g	Plant height/ cm	Root length/cm
Negative control	0.00	34.00	7.03	2.88	0.84	52.67	17.67
Positive control	5.00	20.00	3.31	6.32	0.76	40.33	12.67
Carbofuran	2.67	27.67	5.37	2.43	0.69	48.33	15.33
NGB	5.00	25.00	4.91	5.76	0.82	45.67	13.67
Bt strain Sa.Sa.Kh.1	2.00	62.33	10.93	6.82	1.61	75.33	26.33
Bt strain Sa.Sa.Kh.2	1.67	69.33	13.23	9.18	2.03	80.33	30.00
Bt strain Sa.Sa.Kh.3	1.33	77.00	16.63	12.15	2.77	88.00	34.33
LSD 5%	0.65**	6.34**	1.86**	1.55**	0.29**	6.31**	2.99**

*** Means are averages of three replicates**

The results reported in Table (7) revealed significant differences between *Bt* isolates and control groups for gall index and plant growth parameters. The Sa.Sa.Kh.3 isolate showed a significant impact on tomato growth parameters compared with control groups. These results correspond with earlier research (15), that suggests the efficacy of *B. thuringiensis* in being a novel biocontrol agent to control root-knot nematodes.

Table 7. Evaluation of the impact of *Bt* isolates on gall index and plant growth parameters during the period 3 days after nematode inoculation.

Treatment	Gall index	Shoot fresh weight/g	Shoot dry weight/g	Root fresh weight/g	Root dry weight/g	Plant height/ cm	Root length/cm
Negative control	0.000	34.000	7.033	2.883	0.840	52.667	17.667
Positive control	5.000	20.000	3.310	6.317	0.760	40.333	12.667
Carbofuran	2.667	27.667	5.367	2.433	0.690	48.333	15.333
NGBcontrol	5.000	25.000	4.907	5.760	0.817	45.667	13.667
strain Sa.Sa.Kh.1	3.000	50.667	8.950	4.637	1.193	61.000	20.667
strain Sa.Sa.Kh.2	2.667	57.333	10.003	5.627	1.317	69.000	23.667
strain Sa.Sa.Kh.3	2.333	61.000	10.570	6.120	1.453	73.000	25.333
LSD 5%	0.593**	6.839**	1.477**	1.128**	0.219**	6.837**	2.804**

*** Means are averages of three replicates**

Reports evaluated six endophytic bacteria (13). Among these, *B. thuringiensis* AK08 provided secondary metabolite substances such as tannins, terpenoids, saponins, steroids, alkaloids, flavonoids, and phenols. *B. thuringiensis* demonstrated the greatest results for the average incubation duration, number of galls and nematodes in the root of tomato plants, and nematode number in the soil.

CONCLUSIONS

It is important to study the interaction among organisms to find alternatives to reduce climate change, the current investigation showed that three native *B. thuringiensis* isolates were effective in controlling root-knot nematodes. The study found that local *Bt* isolates were efficient in egg inhibition and juvenile mortality (J2).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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