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Selection of *Bacillus thuringiensis* for control of root-knot nematodes on tomato

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Abstract. Root-knot nematodes (*Meloidogyne* spp.) are among the most serious pests, reducing the yield and quality of tomatoes (*Solanum lycopersicum*) in Vietnam. Prolonged use of chemical pesticides has led to numerous negative impacts on the environment and human health. This study was conducted to select native *Bacillus thuringiensis* strains capable of controlling *Meloidogyne* spp. From soil samples collected from tomato fields in Danang, we isolated seven *Bacillus* strains, of which two strains, M2 and M5, were capable of producing toxic crystals. Strain M5 exhibited the highest inhibitory activity against *Meloidogyne* spp. J2 larvae in *in vitro* tests, achieving a mortality rate of 86.67% after 12 hours. Strain M5 was capable of producing key extracellular enzymes, including chitinase, protease, and cellulase. Sequencing of the 16S rRNA gene confirmed that strain M5 belongs to the species *Bacillus thuringiensis* (99.86% similarity with *Bacillus thuringiensis* MK743981.1). At a concentration of 10^9 CFU/ml, strain M5 caused 100% mortality of J2 larvae after 10 hours and inhibited 88.9% of egg hatching. In greenhouse conditions, treatment with strain M5 significantly reduced the number of root galls on tomato plants (14.93 galls/plant) compared to the infected control (35.84 galls/plant). These results indicate that *B. thuringiensis* strain M5 is a promising biological control agent for managing root-knot nematodes on tomatoes.

Keywords: *Bacillus thuringiensis*, *Meloidogyne* spp., biological control, tomato, chitinase

1 Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops globally and in Vietnam, with a cultivation area of approximately 23,000–25,000 hectares in recent years. It serves as a key component of both domestic consumption and export due to its nutritional and economic value. In order to improve yield and fruit quality, various biotechnological approaches have been applied, including the development of high-yielding, pest- and disease-resistant hybrid cultivars adapted to different ecological regions.

However, tomato production is significantly challenged by a wide range of pathogens, among which root-knot nematodes (Meloidogyne spp.) are particularly destructive. These obligate plant parasites are globally distributed and capable of infecting a wide spectrum of cultivated crops. Their infection induces gall formation on roots, disrupts water and nutrient uptake, and leads to severe reductions in plant vigor and yield. Reported tomato yield losses vary depending on nematode species, initial population density, host cultivar, and environmental conditions, ranging from 28% to complete crop failure [1],[2];[3]. More than sixty species of plant-parasitic nematodes have been recorded to attack tomato, but root-knot

nematodes remain the most destructive group, responsible for substantial economic losses [4]

In Vietnam, root-knot nematodes are considered a major constraint on tomato production, causing serious yield reductions. Studies have reported that heavy infestations of *Meloidogyne incognita* can significantly reduce plant height, leaf number, and fruit yield under greenhouse and field conditions [5]. In severely infested fields, tomato yield losses ranging from 30% to 70% have been observed [6, 7].

Current methods for controlling nematodes primarily rely on specific chemical pesticides such as Furadan, Marshal, Oncol, Nokap, and Vimoca. While these chemicals provide immediate effects, they are costly, have inconsistent efficacy in reducing nematode populations, and lead to negative consequences such as pesticide resistance, disruption of soil ecosystems, increased secondary pest outbreaks, environmental pollution, and chemical residues in agricultural products [8]. In light of these challenges, research into biological control methods, particularly the use of native microorganisms with high parasitic and antagonistic activities against nematodes, is a new, sustainable, and safe approach as a promising alternative. This approach not only aims to effectively manage nematode populations but also enhances soil health and promotes biodiversity. Thus, farmers can achieve sustainable pest management while minimizing the reliance on chemical inputs by harnessing the natural abilities of microorganisms.

Examine case studies where native microorganisms have successfully minimized chemical use in farming. *Bacillus thuringiensis* has been reported as a potential control agent for several nematodes [9]. A notable study by Zuckerman and colleagues, which patented the *Bacillus thuringiensis* strain CR-371, demonstrated its ability to protect tomato plants from root-knot nematode attacks [10]. Treatment with strain CR-371 in the root zone significantly reduced gall indices and nematode densities in the soil compared to the control [11]. In 2008, Mohammed and colleagues evaluated the biological activity of native *Bacillus thuringiensis* strains against *Meloidogyne incognita* in tomatoes. The results showed that several isolated strains exhibited strong nematicidal activity. Notably, strains *Bacillus thuringiensis* 7N, Den, 18, K73, Soto, and 7 achieved mortality rates of J2 larvae ranging from 86% to 100% under laboratory conditions [12].

In Vietnam, there is limited research on *B. thuringiensis* for nematode control. Nematode management primarily relies on chemical agents. The use of biological agents such as *B. thuringiensis* for nematode control is a promising new direction for the future. To contribute to exploiting the potential of native microbial resources, which are highly effective in preventing pathogens on tomatoes, we conducted the study "Selection of *Bacillus thuringiensis* for Control of Root-Knot Nematodes on tomato."

2 Materials and Methods

2.1 Research Subjects

Bacillus thuringiensis strains isolated from soil samples collected from various tomato fields in La Huong, Tuy Loan, Danang City.

Root-knot nematodes (*Meloidogyne* spp.) causing galls on tomato plants.

Tomato plants (*Solanum lycopersicum* L).

2.2 Research Methods

Soil Sample Collection Method

Soil samples were collected from the rhizosphere of healthy tomato plants in both diseased and non-diseased fields and brought to the laboratory

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for isolation. Each sample consisted of 100 g of soil, placed in separate bags labeled with the date and location of collection, and allowed to dry naturally in the laboratory [13].

Isolation of *Bacillus* spp.

Soil samples were isolated on LB (Luria-Bertani) agar medium. Samples were diluted with sterile distilled water in a series ranging from 10^{-4} to 10^{-6}. A 100 µl aliquot of each diluted sample was spread evenly on LB agar plates. The plates were incubated at 30–32°C for 18–20 hours to obtain single colonies, which were then purified. The isolated *Bacillus* strains were identified based on colony morphology, cell shape, Gram staining, and biochemical characteristics using Bergey's classification key [14].

Biochemical characterization and extracellular enzyme production

Selected strains were tested for basic biochemical characteristics (oxidase, catalase, Voges-Proskauer (VP), indole, citrate utilization, urease and gelatin hydrolysis). The ability to produce extracellular enzymes was evaluated on specific media: 1% chitin for chitinase, 1% casein for protease, and 1% carboxymethyl cellulose (CMC) for cellulase [15].

Molecular identification of bacteria

Antagonistic bacteria were identified by PCR with primers 27F and 1492R to amplify the entire 16S rRNA gene region [16]. PCR products were directly sequenced using PCR primers. Nucleotide sequences were edited and assembled using Seqman software and searched against the GenBank database using the online BLAST tool at NCBI.

Isolation and propagation of *Meloidogyne* spp. in the laboratory

Meloidogyne spp. nematodes were collected from galled roots of tomato plants, identified, and propagated in the laboratory at the Microbiology Technology Laboratory, University of Science and Education, Danang. Nematodes were propagated on tomato plants as described [17] with modifications: Tomato seeds (using a variety susceptible to *Meloidogyne* spp.) were surface-sterilized with 70% ethanol for 5 minutes, rinsed, and soaked in 1% sodium hypochlorite (NaOCl) for 15 minutes to eliminate external pathogens. The seeds were then washed multiple times with distilled water to remove residual sodium hypochlorite. The seeds were sown in sterilized soil trays, and when the plants developed four true leaves, they were transplanted into pots (250 cm^3 soil). After the plants established roots, they were inoculated with 200 nematode larvae per pot. Nematodes were harvested after 30–60 days.

Roots of tomato plants infected with *Meloidogyne* spp. were washed to remove soil, cut into 1–2 cm segments, and placed in 1.5% NaOCl solution. The mixture was shaken for 5 minutes to disrupt the gelatinous egg sac structure. J2 larvae were collected using a 40 µm sieve, and eggs were collected using a 25 µm sieve [18].

Selection of *Bacillus thuringiensis* strains inhibiting nematodes

Twenty nematodes were added to 300 µl of culture broth of isolated *Bacillus thuringiensis* strains at a concentration of 10^7 CFU/ml in 1.5 ml Eppendorf tubes containing 50 µg/ml ampicillin. After 12 hours, the number of dead J2 larvae (immobile nematodes) was counted under an optical microscope. Experiments were performed in triplicate [19].

Assessment of hydrogen cyanide (HCN) synthesis

The ability of selected *Bacillus thuringiensis* strains to synthesize HCN was tested using the method

of Castric (1980): Selected strains were grown on LB medium containing 4.5 g/L glycine. Sterile filter paper saturated with 2% sodium carbonate and 1% picric acid was placed in the lid of petri dishes. The dishes were sealed with parafilm and incubated at 30°C for four days. A color change of the filter paper from yellow to reddish-brown was used as an indicator of anaerobic activity [15].

Assessment of Inhibitory Concentrations of *Bacillus thuringiensis* against *Meloidogyne* spp.

Inhibition of Egg Hatching: The experiment was conducted as described by Chahal: 30 eggs were placed in vials containing 4 ml of culture broth of the selected *Bacillus thuringiensis* strain at concentrations of 10^5, 10^7, and 10^9 CFU/ml. The vials were incubated at 30°C, and the number of hatched J2 larvae was counted every 24 hours for 10 days. Distilled water was used as the control. The experiment was repeated three times [20].

Inhibition of J2 Larvae: The experiment was conducted as described by Yap Chin Ann with modifications: 20 nematodes were added to 300 µl of culture broth of isolated *Bacillus thuringiensis* strains at concentrations of 10^5, 10^7, and 10^9 CFU/ml in 1.5 ml Eppendorf tubes containing 50 µg/ml ampicillin (ampicillin in tubes to limit bacterial interference. After incubation at 30°C for 2, 6, and 10 hours, the number of dead J2 larvae (immobile nematodes) was counted under a light microscope. Controls were incubated with water and LB medium. Experiments were performed in triplicate and repeated at least three times [14].

Evaluation of *Bacillus thuringiensis* efficacy against *Meloidogyne* spp. in greenhouse conditions

The efficacy of selected *Bacillus thuringiensis* strains in controlling *Meloidogyne* spp. on tomato was evaluated under greenhouse conditions. Soil used for planting was autoclaved to eliminate some microorganisms. Tomato seeds were germinated, and seedlings with four true leaves were transplanted into pots. The pots were maintained in a greenhouse environment under controlled temperature and humidity conditions (Figure 1).

Fig. 1. Experimental tomato plants
((A). Germinated tomato plants; (B). Tomato plants transplanted into pots).

The experiment consisted of four treatments (CT), each with 30 tomato plants:

CT1: No nematode egg inoculation' CT2 (Negative control): Inoculated with *Meloidogyne* spp. Eggs; CT3: 30 ml of selected *Bacillus thuringiensis* + *Meloidogyne* spp. Eggs; CT4 (Positive control): 30 ml of commercial *Bacillus thuringiensis* formulation + *Meloidogyne* spp. eggs.

Each pot was inoculated with 1,000 eggs. The number of root galls was recorded after 4 weeks.

Data Processing

Data were statistically analyzed using procedures of the Statistical Package for Social Sciences (SPSS, version 27 for Windows. All data were analyzed by analysis of variance (ANOVA) and the treatment means were separated by using Duncan's multiple range test at $P \leq 0.05$.

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3 Results and Discussions

3.1 Isolation and selection of *Bacillus* spp. from soil samples in La Huong, Tuy Loan

From soil samples collected from tomato fields in La Huong, Tuy Loan, seven *Bacillus* strains capable of growing at 42°C were isolated, designated M1 to M7. The colony morphology and biological characteristics of the strains are presented in Table 1.

Table 1. Colony morphology and biological characteristics of *Bacillus* strains isolated at 42°C

Strain	Colony Morphology	Cell Morphology	Spore	Crystal
M1	Milky white colony, sunken center, wide-spreading wrinkled edge	Rod-shaped, single, central elliptical spore	+	-
M2	Milky white colony, spreading edge, central knob	Rod-shaped, chained, central spore, spherical crystal	+	+
M3	Milky white colony, round, mucoid	Rod-shaped, single, eccentric spore	+	-
M4	Creamy white center, translucent periphery, wavy edge, tightly adhered to agar, 3–4 mm diameter	Rod-shaped, chained, central egg-shaped spore	+	-
M5	Similar to M4 but with radiating flower-like pattern, central knob	Rod-shaped, single, central elliptical spore, diamond-shaped crystal	+	+
M6	Milky white colony, spreading wavy edge, tightly adhered to agar, 3–4 mm diameter	Rod-shaped, single, eccentric spore	+	-
M7	Large creamy white colony, radiating flower-like pattern, wrinkled surface	Rod-shaped, single, central elliptical spore	+	-

The results showed that two strains, M2 and M5, produced toxic crystals.

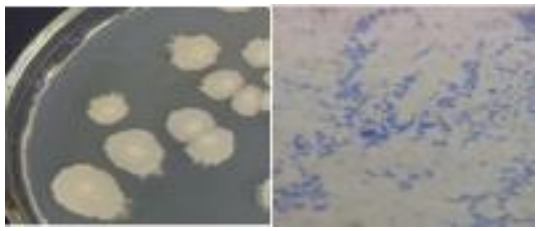

Fig. 2. Colony morphology, cell morphology, and crystal of strain M2 (100X)

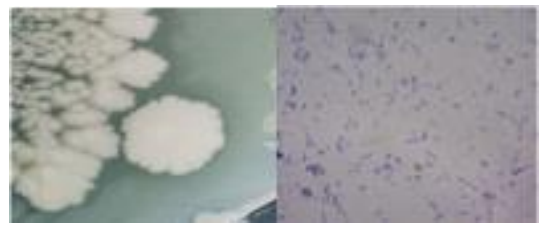

Fig. 3. Colony morphology, cell morphology, and crystal of strain M5 (100X)

Both presumed *Bacillus thuringiensis* strains shared common characteristics: colonies ranging from white to creamy, with a central knob and spreading edges. All isolated strains were Gram-positive, with central elliptical spores. Both strains

produced protein crystals. These characteristics align with published descriptions of *Bacillus* [21,22], which include white, wrinkled, or smooth colonies with spreading edges; rod-shaped cells with slightly blunt ends; central egg-shaped spores; and bipyramidal, cubic, or spherical crystals [21, 22].

3.2 Physiological and biochemical characterization of isolated *Bacillus thuringiensis*

Bacillus thuringiensis is characterized by the presence of protein crystals. To distinguish *Bacillus thuringiensis* from *Bacillus cereus*, physiological and biochemical tests were conducted on the two presumed *Bacillus thuringiensis* strains following the method described by Claus and Berkeley (1986) [23]. The results are presented in Table 2.

Table 2. Biochemical characteristics of Two presumed *Bacillus thuringiensis* strains

Test	M2	M5
Gram staining	+	+
Oxidase	+	+
Catalase	+	+
Indole	-	-
Citrate utilization	+	+
Gelatin hydrolysis	+	+
Urease	+	+
Motility	+	+
Growth at 50°C	-	-
Growth at 42°C	+	+
Growth in 10% NaCl	-	-
Fermentation: Glucose	+	+
Fermentation: Sucrose	+	+
Fermentation: Galactose	-	+

The presumed *Bacillus thuringiensis* strains showed positive reactions for catalase, urease, gelatin hydrolysis, nitrate reduction, and glucose and sucrose utilization. Both strains were negative for indole production, grew at 42°C but not at 5°C, and could not grow in 10% NaCl. Most results were consistent with Claus and Berkeley (1986), though the strains differed in their ability to ferment galactose. These physiological and biochemical characteristics further confirmed that both strains belong to *Bacillus thuringiensis*.

3.3 Selection of *Bacillus thuringiensis* Strains Inhibiting *Meloidogyne* spp. J2 Larvae

The effect of culture broth from the two isolated *Bacillus thuringiensis* strains on J2 larvae of *Meloidogyne* spp. from infected tomato plants was studied. The results are presented in Table 3 and Figure 4.

Table 3. Inhibitory effect of Two *Bacillus thuringiensis* strains on J2 Larvae

Strain	Live J2 Larvae	Dead J2 Larvae	Mortality Rate (%)
M2	4.67 ± 0.52^{b}	15.33 ± 0.58^{b}	76.67 ± 0.03^{b}
M5	2.67 ± 0.52^{c}	17.3 ± 0.58^{a}	86.67 ± 0.03^{a}
Distilled water	20.0 ± 0.0^{a}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
LB medium	20.0 ± 0.0^{a}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}

Note: Different letters in the same column indicate statistically significant differences at $p < 0.05$.

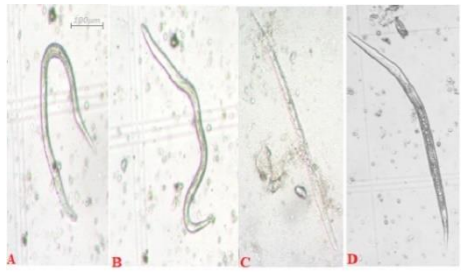

Fig. 4. Effect of ***Bacillus thuringiensis*** culture broth on J2 larvae

From the results in Table 3, both presumed *Bacillus thuringiensis* strains inhibited the tested

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nematodes. However, strain M5 exhibited stronger activity against *Meloidogyne* spp., with a mortality rate of 86.67 ± 0.03% within 12 hours. Based on these screening results, strain M5, with its high inhibitory activity against *Meloidogyne* spp., was selected for further experiments.

3.4 Assessment of extracellular enzyme production and hydrogen cyanide synthesis by Strain M5

The enzymatic activity of bacteria was determined by their capacity to degrade substrates and cause a color change in specific media around the bacterial colonies. Hydrogen cyanide (HCN) production was assessed using the method of Castric (1980) [19], with a color change of filter paper from yellow to reddish-brown indicating anaerobic activity. The results of enzyme production and HCN synthesis by strain M5 are presented in Table 4 and Figure 5.

Table 4. Extracellular enzyme and HCN synthesis by Strain M5

Characteristic of Strain M5	Chitinase	Protease	Cellulase	HCN Synthesis
Synthesis Ability	+	+	+	-
Degradation Zone Diameter	2.27 ± 0.21^{ab}	1.57 ± 0.12^{b}	2.53 ± 0.06^{a}	-

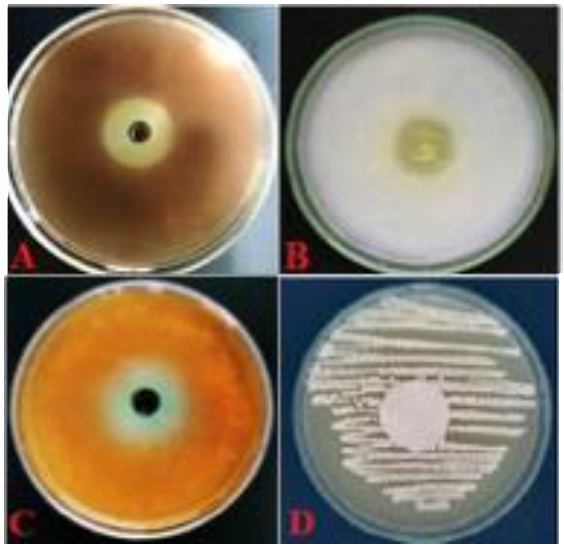

Fig. 5. Degradation zone diameters for chitin, protein, and cellulose by strain M5
(A). Chitinase; (B). Protease; (C). Cellulase; (D). Hydrogen cyanide (HCN) production

The results from Table 4 and Figure 5 show that strain M5 can degrade chitin, cellulose, and protein. The chitinase activity was the highest, with a degradation zone diameter of 2.27 ± 0.21 cm. However, strain M5 did not produce HCN, consistent with the findings of Yap Chin Ann (2013), who reported that three strains (MPB04, MPB115, and MPB93) showed no significant color change from yellow to reddish-brown, indicating negative cyanogenic activity [18].

3.5 Molecular Identification of Strain M5

Strain M5 was identified by amplifying the 16S rRNA gene region using PCR. The PCR product was purified and sequenced at Phu Sa Biochemistry Co., Ltd. (Can Tho). The results are shown in Figure 6.

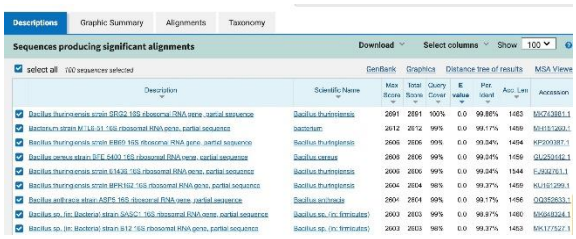

Fig. 6. Sequence similarity search results for strain M5

Based on the sequence similarity search, a phylogenetic tree was constructed for strain M5, as shown in Figure 7.

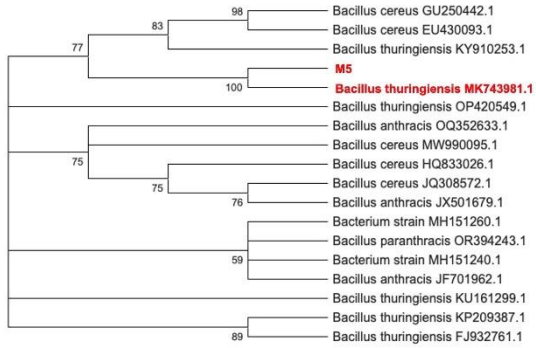

Fig. 7. Phylogenetic tree based on 16S rDNA sequences of strain M5 and related ***Bacillus*** strains

The phylogenetic tree indicates that strain M5 belongs to the *Bacillus thuringiensis* branch, with a sequence similarity of 99.86% to *Bacillus thuringiensis* MK743981.1 (Figure 7). Therefore, we conclude that strain M5 belongs to the species *Bacillus thuringiensis*. This provides a foundation for further studies on this species.

3.6 Effect of *Bacillus thuringiensis* (M5) concentration on *Meloidogyne* spp. inhibition

Inhibition of egg hatching

Compared to egg masses, individual eggs are known to be more susceptible to exotoxins and enzymes produced by *Bacillus thuringiensis* [20]. The inhibitory effect of *Bacillus thuringiensis* (M5) on egg hatching varied with concentration. The detailed results are summarized in Table 5 and illustrated in Figure 8.

Table 5. Effect of *Bacillus thuringiensis* (M5) concentration on egg hatching inhibition

Cell Concentration (*Bacillus thuringiensis*/ml)	J2 Larvae Emergence/30 Eggs	Egg Hatching Inhibition (%)
10^5	8.33 ± 1.53^b	72.22 ± 5.09^c
10^7	5.33 ± 0.58^c	82.22 ± 1.9^b
10^9	3.33 ± 0.58^d	88.9 ± 1.9^a
Distilled water	30.0 ± 0.0^a	0.0 ± 0.0^d

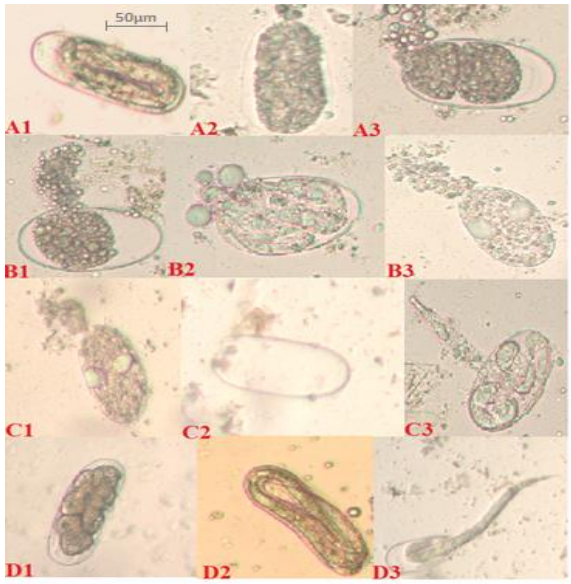

Fig. 8. Effect of *Bacillus thuringiensis* (M5) concentration on egg hatching inhibition
(A1–A3. Eggs treated with Bacillus thuringiensis *(M5) at 10^5; B1–B3. Eggs treated with* Bacillus thuringiensis *(M5) at 10^7; C1–C3. Eggs treated with* Bacillus thuringiensis *(M5) at 10^9; D1–D3. Eggs and J1, J2 larvae in distilled water.)*

The results show that *Meloidogyne* spp. eggs were highly sensitive to exotoxins produced by the selected *Bacillus thuringiensis* (M5) strain after 10 days. The hatching rate of J2 larvae varied with cell concentration, with the highest inhibition ($88.9 \pm 1.9\%$) observed at 10^9 cells/ml. Compared to the findings of Chahal (1991), strain M5 exhibited lower egg inhibition activity. Chahal (1991) reported that no J2 larvae of *Meloidogyne incognita* emerged from eggs in the entire *Bacillus thuringiensis* culture medium [20]. This inhibitory

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effect may be explained by the ability of *B. thuringiensis* to kill eggs through bacterial enzymes or toxins that dehydrate the gelatinous matrix surrounding the eggs [24]. Exotoxin production by *B. thuringiensis* has been reported [25]. Chigaleichik (1976) noted that chitinase produced by *B. thuringiensis* hydrolyzes chitin (a polymer of N-acetyl-glucosamine) found in the protein-chitin layer of eggshells and the gelatinous matrix of egg masses, with chitinase and exotoxin activity contributing to egg mortality [26].

Inhibition of J2 Larvae

The inhibitory effect of *Bacillus thuringiensis* (M5) on J2 larvae of *Meloidogyne* spp. at different concentrations is presented in Table 6 and Figure 9.

Table 6. Effect of *Bacillus thuringiensis* (M5) concentration on J2 larvae mortality

***Bacillus thuringiensis* Concentration/ml**	**J2 Larvae Mortality**		
	2 hours	**6** hours	**10** hours
10^5	38.33 ± 0.08^c	58.33 ± 0.03^c	80.0 ± 0.05^c
10^7	56.67 ± 0.06^b	76.67 ± 0.06^b	91.67 ± 0.03^b
10^9	80.0 ± 0.05^a	91.67 ± 0.03^a	100.0 ± 0.0^a
Distilled water	0.0 ± 0.0^d	0.0 ± 0.0^d	0.0 ±0.0^d

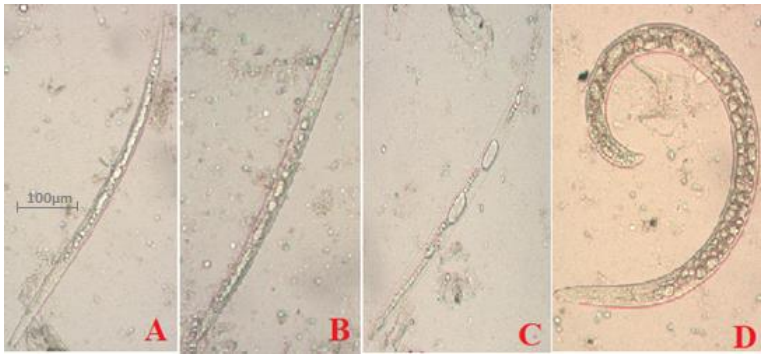

Fig. 9. Effect of *Bacillus thuringiensis* (M5) concentration on J2 larvae mortality
(A. J2 larvae treated with Bacillus thuringiensis (M5) at 10^5; B. J2 larvae treated with Bacillus thuringiensis (M5) at 10^7; C. J2 larvae treated with Bacillus thuringiensis (M5) at 10^9; D. J2 larvae in distilled water)

The results in Table 6 and Figure 9 show that the selected *Bacillus thuringiensis* (M5) strain killed J2 larvae at all tested concentrations (10^5, 10^7, 10^9 CFU/ml). The highest mortality (100%) was observed at 10^9 CFU/ml after 10 hours. At this concentration, significant changes in the gut morphology of J2 larvae were observed, including the formation of vacuole-like structures in gut cells, gut contraction away from the body wall, constrictions along the gut, and gut degeneration. These findings align with the report by Marroquin et al. (2000) [27].

3.7 Efficacy of *Bacillus thuringiensis* (M5) in controlling *Meloidogyne* spp. in greenhouse conditions

The ability of *Bacillus thuringiensis* (M5) to inhibit nematodes on tomato plants was evaluated as described in section 2.3.10. The results are presented in Table 7 and Figure 10.

Table 7. Effect of *Bacillus thuringiensis* (M5) on root gall formation in tomato

Treatment	Number of Root Galls
CT1: No nematode egg inoculation	0.0^{c}
CT2 (Negative control): *Meloidogyne* spp. eggs	35.84 ± 4.38^{a}
CT3: 30 ml selected *Bacillus thuringiensis* + *Meloidogyne* spp. eggs	14.93 ± 4.10^{b}
CT4 (Positive control): 30 ml commercial *Bacillus thuringiensis* formulation + *Meloidogyne* spp. eggs	13.33 ± 3.63^{b}

Fig. 10. Effect of *Bacillus thuringiensis* (M5) on root gall formation in tomato
(A. Tomato roots in CT2; B. Tomato roots in CT3; C. Tomato roots in CT4).

The results in Table 6 and Figure 10 show a significant difference in the number of root galls between the untreated control (CT2) and treatments with *Bacillus thuringiensis* (CT3, CT4). In CT2, the total number of galls was the highest, with 35.84 ± 4.38 galls. Compared to the commercial product (CT4), the number of galls in CT3 was higher but not significantly different. These results suggest that *B. thuringiensis* strain M5 is effective in reducing root gall formation in nematode-infected tomato plants. The nematicidal effect of *Bacillus thuringiensis* has been reported in several studies, such as Hala Khyami-Horani et al. (2006), who noted a 51–59% reduction in root gall density on tomato plants treated with *Bacillus thuringiensis* [28]. The mechanism of nematode-killing action may be attributed to the production of chitinase, protease, and endotoxins that hydrolyze eggshells and kill J2 larvae. Reducing J2 larvae density decreases the primary factor causing root infection and gall formation in tomato plants. These findings are consistent with a previous report by Granum et al. (1988) and Ghareeb et al., who noted that *Bacillus thuringiensis* produces lecithinase and endotoxins that contribute to nematode control. The rigid cuticle of nematodes is composed of proteins and chitin [29], so the nematicidal activity of *B. thuringiensis* is closely related to its proteolytic activity, which facilitates the invasion of nematodes [30].

Additionally, the process of intoxication and nematode mortality has been studied by Leyns et al. (1995) and Wei et al. (2003) [31, 32]. The direct effect of *Bacillus thuringiensis* toxins entering the nematode gut through ingestion (inactive insoluble crystal proteins) is activated in the alkaline gut environment, converting protoxins into active toxins (60–65 kDa). These activated toxins bind to specific receptor molecules on the microvilli of the nematode gut cells, altering the electrochemical gradient and forming ion channels. This disrupts the osmotic balance of the cell membrane, leading to cell contraction, thinning of gut cells, detachment of the gut from the body wall, degeneration, cell swelling, and disintegration, resulting in gut damage. The nematode becomes inhibited, paralyzed, and dies [33].

From the above results, *Bacillus thuringiensis* strain M5 is a highly potential biological control agent for nematodes.

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4 Conclusion

From soil samples collected from tomato fields in Danang, we isolated seven *Bacillus* strains, of which two strains, M2 and M5, were capable of producing toxic crystals. Strain M5 exhibited the highest inhibitory activity against *Meloidogyne* spp. J2 larvae in *in vitro* tests, achieving a mortality rate of 86.67% after 12 hours. This strain is also capable of producing important extracellular enzymes, including chitinase, protease, and cellulase. Molecular identification based on 16S rRNA gene sequence confirmed that strain M5 belongs to the species *Bacillus thuringiensis* (99.86% similarity with *Bacillus thuringiensis* MK743981.1). At a concentration of 10^9 cells/ml, strain M5 caused 100% mortality of J2 larvae after 10 hours and inhibited 88.9% of egg hatching. In greenhouse conditions, treatment with strain M5 significantly reduced the number of root galls on tomato plants (14.93 galls/plant) compared to the infected control (35.84 galls/plant). These results indicate that *B. thuringiensis* strain M5 is a promising biological control agent for managing root-knot nematodes on tomato cultivation.

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