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Effect of Certain Biocontrol Agents on The Root-Knot Nematode and Some Enzymatic Activities in Tomato Rhizosphere

Alaa H. A. Abu Habib[1], Hala H. Badry[2], Abeer A. Mohamed[1*], Manal M. Zen El-Dein[3]

[1] *Plant Pathology Research Institute, Agricultural Research Center (ARC), Alexandria 21616, Egypt*.

[2] *Soil and Water Science Department, Faculty of Agriculture, Alexandria University, Aflaton Street, El-Shatby, EG21545 Alexandria, Egypt.*

[3]*Fungicide, Bactericide and Nematicide Research Department, Central Agricultural Pesticide Laboratory (CAPL), Agriculture Research Center (ARC), Alexandria 21616, Egypt*.

[*]Corresponding author: abeera.mohamed81@gmail.com

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ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are a group of the most destructive plant pathogens of tomato plants all over the world. Biological control is considered as a safe and efficient tool to control these pathogens. The present study aimed to evaluate the potency of the marine algae (*Ulva fasciata*) and two bacterial isolates isolated from the seeds and rhizosphere of tomato plants against the root-knot nematode, *M. incognita*, infecting tomato plants. The bacterial isolates were identified using polymerase chain reaction (PCR) through the amplification of 16S rRNA as *Bacillus amyloliquefaciens* (PQ821314) and *Serratia marcescens* (PQ012666). *Bacillus amyloliquefaciens* and *S. marcescens* were used at 10^8 CFU/ml, while *U. fasciata* was used at 5000, 2500, 1250 and 625 mg/l concentrations *in vitro*. A comparable treatments included; Nemacross® 2% and oxamyl 24% (SL) beside the nontreated check. Results showed that *B. amyloliquefaciens*, *S. marcescens*, *U. fasciata* and oxamyl significantly reduced ($P \leq 0.05$) egg hatching of *M. incognita* and increased ($P \leq 0.05$) the mortality of *M. incognita* J_2s, compared to the control checks. However, Nemacross® caused 28.84% larval mortality and 23.72% egg hatchability. Under greenhouse conditions, the disease severity (root galling) and the nematode reproduction (no. egg mass/plant) of *M. incognita* were greatly suppressed by all treatments. Maximum reduction of root galling (98.77%) and nematode reproduction (98.10%) was obtained by *B. amyloliquefaciens*. Fresh weights of shoot and root systems were generally increased by the application of the nematicide oxamyl and all the tested bioagents, compared to the control treatment (*M. incognita*). The soil enzyme activities were also evaluated under greenhouse conditions. The application of the biological products in nematode-infested soils significantly ($P \leq 0.05$) enhanced the soil enzyme activities, particularly urease, dehydrogenase, and alkaline phosphatase. However, oxamyl showed detrimental effects on soil enzyme activities.

INTRODUCTION

Tomato, *Solanum lycopersicum* L., is one of the most important vegetable crops all over the world (Mohamed *et al*., 2021). According to Sahu *et al*. (2013), it is susceptible to various diseases caused by different pathogens, including nematodes, bacteria, viruses, and fungi. Controlling soil-borne plant pathogens, including nematodes, is one of the greatest challenges in contemporary agriculture globally. Root-knot nematode, *Meloidogyne incognita*, is one of the most prevalent nematode species all over the world, where they infect nearly most of the cultivated plants (Khalil and Darwesh, 2018; Lian *et al*., 2022; Kantor *et al*., 2022). Globally, the root-knot nematodes caused tomato yield losses of 27% (Sharma and Sharma, 2015).

Chemical nematicides have been known as one of the main methods for controlling root-knot nematodes for several decades. However, farmers have become less interested in using chemical nematicides to safeguard their crops from nematode infections because of their negative effects and environmental hazards (Baidoo *et al*., 2017). Biological control promises to be such an option (Radwan and Farrag, 2012; Saad *et al*., 2019; Lian *et al*., 2022). Lately, one of the biological control procedures that proved to have suppression effects against nematodes are bacteria and aqueous extracts of marine algae. Seaweeds have also received an attractive attention because they are a source of many active compounds against plant nematodes (Shimizu, 2003; Bahadur *et* al., 2021; Aioub *et al*., 2022; Gowda *et al*., 2022). The use of marine algae as a control agent against plant parasitic nematodes has been studied by many researchers (Nour El-Deen and Issa, 2016). Nour El-Deen *et al*., (2013) stated that different marine algae exhibited very significant nematicidal activities against the root-knot nematodes, *M. incognita* and *M. javanica* and suppressed the fecundity of these nematodes. Also, treating *M. incognita*-infected sunflower plants with marine algae increased the growth parameters of the plants and reduced the numbers of root galls and egg masses produced by the nematodes on infected plants (Ibrahim *et al*., 2007).

The use of beneficial bacteria might be arising as one of the safest measures to control the root-knot nematodes as well. *Serratia marcescens* has emerged as a promising agent for the biological control of nematodes, particularly in organic farming systems (Attia and Nofel, 2023). Its potential lies in its production of bioactive compounds, including proteases, chitinases, and other hydrolytic enzymes that directly affect nematode larvae and eggs. These enzymatic activities disrupt nematode development and reduce populations in the soil, mitigating their impact on crops (Kaur and Kaur, 2021). *Bacillus amyloliquefaciens* plays a pivotal role as a biocontrol agent in organic farming systems due to its multifaceted mechanisms of action and environmental compatibility. Its importance lies in its ability to enhance plant health and yield while minimizing reliance on synthetic chemicals (Kloepper *et al*., 2004; Hegazy *et al*., 2019). Application of *B. amyloliquefaciens* also promoted plant growth. Thus, *B. amyloliquefaciens* are considered environmentally safe for application as biocontrol and are abundant in soil (Abd-Elgawad and Askary, 2018).

Soil enzymes are critical biological catalysts that mediate essential nutrient cycling processes within terrestrial ecosystems. Among these enzymes, urease, dehydrogenase, and alkaline phosphatase play important roles in nitrogen and phosphorus dynamics and serve as indicators of microbial activity and soil health. Urease catalyzes the hydrolysis of urea into ammonia and carbon dioxide, which influences nitrogen availability for plants and microorganisms (Zhang et al., 2021). These enzymatic activities are vital for promoting soil fertility. Dehydrogenase enzymes are involved in the oxidation of organic substrates, acting as indicators of microbial metabolic activity. Their activity reflects overall soil microbial biomass and functional diversity, which are crucial for ecosystem stability and nutrient cycling. Elevated dehydrogenase activity has been associated with enhanced microbial diversity and resilience, emphasizing the importance of these enzymes in maintaining soil health (Yang *et al*., 2023). Alkaline phosphatase is essential for phosphorus availability, catalyzing the hydrolysis of phosphate esters and releasing inorganic phosphate for plant uptake (Chen *et al*., 2021). This enzyme's activity is particularly important in phosphorus-deficient soils, where it facilitates nutrient mobilization, thereby supporting plant growth and productivity (Azene *et al*., 2023). Monitoring these enzymatic activities allows researchers and land managers to assess soil quality and microbial function effectively, leading to informed decisions in sustainable agricultural practices (Chen *et al*., 2021).

The present study aimed to: 1) isolation and molecular identification of two local bacterial isolates to be evaluated along with the marine alga *U. fasciata* for controlling the root-knot nematode, *M. incognita in vitro* and *in vivo*, 2) determination of urease, dehydrogenase, and alkaline phosphatase activities in the *M. incognita*-infected and non-infected tomato plants.

MATERIALS AND METHODS

The effectiveness of *Bacillus amyloliquefaciens*, *Serratia marcescens*, and *Ulva fasciata* on *Meloidogyne incognita* was evaluated *in vitro* and under greenhouse conditions. Comparable treatments included: non-treated control, a bio-nematicide (Nemacross® 2%), and a chemical nematicide (oxamyl 24% SL).

Algal extracts preparation

Samples of the green alga, *Ulva fasciata*, were obtained from the National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. Aqueous extracts of the alga were prepared and washed thoroughly with tap water to get rid of salts. The water was drained off, and the algal material was spread on blotting paper to remove the excess water. After completely drying, the different seaweed materials were ground to a fine powder using an electrical grinder, and 40 g of powdered seaweeds were extracted in 200 ml of distilled water using a shaker for 3h. The extract was filtered through a Whatman No. 1 filter paper and stored at 4°C until further dilution as per dose requirements (Guiry and Guiry, 2013). Concentrations of *U. fasciata* were prepared at 5000, 2500, 1250, and 625 mg/l, and their effects on hatching and mortality of *Meloidogyne incognita* second-stage juveniles were evaluated *in vitro*. The concentration of 5000 mg/l was chosen for the experiment under greenhouse conditions.

Culturing and identification of bacterial isolates

One bacterial isolate of *Bacillus* sp. was isolated from the seeds of tomato plants collected from a field in Behera Governorate, Egypt. After washing the tomato seeds with running tap water, they were surface sterilized for two minutes in 1% sodium hypochlorite and then rinsed in sterile water. After drying on a sterile filter paper, the surface-sterilized seeds were plated on nutrient agar medium (NA), and the plates were incubated at 25°C for 24 h (Mohamed *et al*., 2019). The second bacterial isolate, *Serratia* sp., was isolated from the rhizosphere of tomato plants. Initially, soil samples were collected from the tomato rhizosphere, which were then processed to obtain a soil suspension. This suspension was prepared by blending a one g soil with a sterile phosphate-buffered saline to release the bacterial cells into the solution. The next step involves serial dilution of the soil suspension to reduce microbial load and facilitate the isolation of

individual colonies. Diluted samples were then plated onto nutrient agar supplemented with appropriate antibiotics (Rahayu *et al*., 2023).

DNA extraction from the bacterial isolates

DNA was extracted according to Behiry *et al*. (2018) with some modifications. After sedimentation of *Serratia* sp. and *Bacillus* sp., cells were grown in nutrient broth medium for 48 h using a microcentrifuge set to 6000 g for 5 minutes. TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was used to wash the cells in each culture. Cells were then suspended in a solution of 30 µL of 10% sodium dodecyl sulphate (SDS), 567 µL Tris-EDTA, and 3 µL of Proteinase K (20 mg/ml). Following 60 minutes of incubation at 37°C, 80 µL of CTAB/NaCl solution and 100 µl of 5M NaCl were added, and the tubes were thoroughly inverted before being incubated for 10 minutes at 65°C in a water bath. After adding and properly mixing 0.8 ml of phenol/chloroform /isoamyl alcohol (1:24:1), the tubes were centrifuged at 11,000 *g* for five minutes. Then, using the aqueous supernatant, the phenol/chloroform procedure was repeated. To precipitate the DNA, an equivalent volume of isopropanol was added, then washing was done with 70% ethanol. The DNA pellets were suspended in either sterilized distilled water or 100 µL of TE buffer.

Amplification and sequencing of the 16S rRNA gene

PCR was performed with the universal 16S primers designed. 16S rRNA gene was amplified using two universal primers namely: P0 (5′-GAAGAGTTTGATCCTGGCTCAG-3′) and P6 (5′-CTACGGCTACCTTGTTACGA-3′). The PCR amplification was carried out in a total volume of 25 µL containing 3 µL of template DNA, 12.5 µL PCR Green Master Mix (Thermo Scientific™), 8.5 µL molecular grade water and 0.5 µL of each primer (10 pmol) (Poussier *et al*., 2000b). The reactions were subjected to the following temperature cycling profile: one cycle at 95°C for 5 min, followed by 35 cycles at 92°C for 45 sec denaturation, 50°C for 30 sec annealing and 72°C for 2 min extension and final extension at 72°C for 10 min.

Bacillus sp. and *Serratia* sp. were sent for sequencing (Macrogen, Scientific Services Company, Korea) (Kumar *et al*., 2016). The National Centre for Biotechnology Information (NCBI) BLAST search was used to match the sequences to those in GenBank (http://www.ncbi.nlm.nih.gov).

Nematode culture and morphological characteristics

Single egg mass cultures of the root-knot nematode *Meloidogyne* sp. (Kofoid and White, 1919), Chitwood, 1949, were established and maintained on eggplant (*Solanum melongena* L.) cv. Black Beauty in the greenhouse (24 ±2 ºC) (Taylor and Netschert, 1974; Singh and Chahar (2021). Nematode species was identified as to be *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, based on the morphological characteristics of the female perineal pattern (Taylor and Sasser, 1978; Mukesh *et al*., 2024). Whenever needed, the root-knot nematode eggs were extracted from the infected eggplant roots using sodium hypochlorite (NaOCl) solution as described by Hussey and Barker (1973) and Van (2006).

Nematode mortality and egg hatching tests *in vitro*

The nematicidal activities of the bacterial isolates; *Bacillus amyloliquefaciens*, *Serratia marcescens* at 10^8 CFU/ml and the alga *Ulva fasciata* were evaluated against *M. incognita* juveniles and eggs. The bacterial isolates were prepared by adding the appropriate volumes of sterilized distilled water to the standard solution (S). One ml of each dilution containing 10^8 CFU/ml of both *B. amyloliquefaciens* and/or *S. marcescens*, as well as different dilutions of *U. fasciata* (5000, 2500, 1250 and 625 mg/l), the bio-nematicide (Nemacross®) at 10^9 CFU/ml and oxamyl 24% (SL) at 3000 mg/l were used. The treatments were transferred to glass vials each one separately, then 300 *M. incognita* eggs at a final volume of 2.5 ml were added. Eggs were added to each glass vial, and all vials were incubated at room temperature (24 ± 2°C). Glass vials containing distilled water plus nematode eggs served as controls. All treatments were replicated three times. Numbers of the hatched juveniles were recorded in each glass vial, 7 days after incubation. The percentages of hatched J_2s were calculated where, egg hatchability (%) = the number of hatched juveniles/total number of eggs × 100. The same treatments were used for nematode juveniles (J_2s), using 300 J_2s. Distilled water was used as control and the treatments were also replicated three times. Juvenile mortality (%) = (Number of dead J_2s /total number of incubated juveniles) × 100 was estimated, 24 hr. after incubation (Ismail and Fadel, 1997; Younis *et al*., 2016). Juvenile mortality % was corrected using Abbotts formula before statistical analysis (Abbotts. 1925).

Greenhouse experiment

Seedlings of the susceptible tomato cultivar (Dosera) were transplanted in 30 cm diameter clean plastic pots, each containing 5 kg of steam-sterilized sandy clay soil (1:1). Two weeks after transplanting, tomato seedlings were inoculated with *M. incognita* @15000 eggs/pot. All treatments and the control (non-infected plants) were replicated 3 times. Pots were arranged in a complete randomized design on a greenhouse bench. Treatments included; *Bacillus amyloliquefaciens*, *Serratia marcescens*, and Nemacross® at 10^8 CFU/ml (each), *U. fasciata* extracts at 5000 mg/ml and oxamyl 24% SL at the

recommended rate (5 ml/L) were applied as soil drench in 150 ml water per pot after 7 days of inoculation. Pots were kept on a greenhouse bench in a complete randomized design where, they were irrigated and fertilized as needed. Sixty days after nematode inoculation, plants were harvested and roots were washed free of soil by the running tap water. Roots were stained with phloxin B solution (0.15 g / liter tap water) for 15 min and the numbers of galls and egg masses/plant were determined (Taylor and Sasser, 1978; Ibrahim and Ibrahim 2000; Mukesh *et al*., 2024). Also, the fresh weights of shoot and root systems of the harvested plants were determined.

Determination of Urease activity

Urease activity was measured as described by Tabatabai and Bremner (1969). A 1 g soil sample was incubated with 0.1 M urea solution (10 ml) and 0.05 M phosphate buffer (pH 7.0) in a sealed container at 37 °C for 2 hours. After incubation, the reaction was stopped by adding 10 mL of 1 N HCl. The ammonia released was determined using the phenate method (Fiore and O'Brien, 1962), where the ammonium concentration was measured colorimetrically at 625 nm. Urease activity was expressed as µg NH_4^+ released per g of soil per hour.

Determination of Dehydrogenase activity

Dehydrogenase activity was determined using the method adapted from Casida *et al*. (1964). A 1 g soil sample was incubated with 1 ml of 3% triphenyltetrazolium chloride (TTC) solution and 0.5 ml of distilled water in an airtight container at 30 °C for 24 hours. The reaction was terminated by adding 10 ml of methanol. The resulting triphenylformazan (TPF) was extracted by shaking and filtered. The absorbance of the supernatant was measured at 485 nm. Dehydrogenase activity was reported as mg TPF formed per g of soil per day.

Determination of Alkaline Phosphatase activity

Alkaline phosphatase activity was measured using the method outlined by Tabatabai and Bremner (1969). A 1 g soil sample was incubated with 1 ml of 0.1 M p-nitrophenyl phosphate (pNPP) solution and 10 ml of 0.5 M bicarbonate buffer (pH 8.5) at 37 °C for 1 hour. The reaction was stopped with 10 ml of 0.5 M NaOH. The p-nitrophenol released was quantified colorimetrically at 405 nm. Alkaline phosphatase activity was expressed as µg p-nitrophenol released per g of soil per hour.

Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) (Anon, 1989), and means were separated using the Fisher's protected LSD ($P \leq 0.05$).

RESULTS AND DISCUSSION

Molecular Identification of Bacterial isolates

Basic local alignment search tool (BLAST) results revealed that molecular Characterization of the two bacterial isolates by polymerase chain reaction (PCR) through the amplification of 16S rRNA region was 99% similar to the type strain of *Bacillus amyloliquefaciens* (accession no. PQ821314) and *Serratia marcescens* (accession no. PQ012666). The complete 16S rRNA sequence has been deposited in GenBank. The findings showed that the two isolates had different genetic makeups. The two isolates were not closely related, as evidenced by the significant level of genetic variability shown by the differences in their DNA sequences (Mohamed *et al*., 2019; Jamal *et al*., 2017; Ashour *et al*., 2022).

Nematode mortality and egg hatching tests *in vitro*

Data in Table (1) showed that treatments of the aqueous extracts of *U. fasciata*, *B. amyloliquefaciens*, *S. marcescens*, oxamyl and Nemacross® suppressed ($P \leq 0.05$) *M. incognita* egg hatching and

Table 1: Effect of *Bacillus amyloliquefaciens, Serratia marcescens* and *Ulva fasciata* as compared with Nemacross® and oxamyl on *Meloidogyne incognita* juvenile mortality and egg hatchability.

Treatments	Juvenile mortality (%)	Egg hatchability (%)
M. incognita (Control)	69.60c	79a
M. incognita + *B. amyloliquefaciens* (10^8 CFU/ml)	100a	0.00f
M. incognita + *S. marcescens* (10^8 CFU/ml)	100a	0.00f
M. incognita + *U. fasciata* 5000 mg/l	100a	7.42e
M. incognita + *U. fasciata* 2500 mg/l	91.34b	17.58d
M. incognita + *U. fasciata* 1250 mg/l	49.68d	24.14c
M. incognita + *U. fasciata* 625 mg/l	25.30e	35.82b
M. incognita + Nemacross® (10^9 CFU/ml)	28.84e	23.72c
M. incognita + oxamyl (5000 mg/l)	100a	0.00f

Data are average of three replicates each.

Means in a column followed by the same letter (s) are not significantly different at $P \leq 0.05$ according to Fisher's protected LSD.

B.= Bacillus, S.= *Serratia*, *U.= Ulva.*

increased nematode second-stage mortality percentages with the superiority of *B. amyloliquefaciens*, *S. marcescens* at 10^8 CFU/ml, *U. fasciata* at 5000 mg/l and the nematicide oxamyl which almost gave the highest percentages of nematode juvenile mortality (almost 100%) and inhibition of egg hatching, compared to the control treatments. However, Nemacross® gave percentages of larval mortality (28.84 %) and percentages of egg hatching (23.72 %). Previous studies showed that the most efficient bacteria for controlling *Meloidogyne* species are those belonging to the genus *Bacillus*, which includes species such as *B. cereus*, *B. amyloliquefaciens*, *B. circulans* and *B. megaterium*. Actually, *B. amyloliquefaciens* was one of the most effective species against *M. incognita*, just as it was in this investigation. According to Jamal *et al*. (2017) and Hegazy *et al*. (2019), *Serratia liquefaciens* has anti-nematode properties against *Meloidogyne* spp. The present study proved that *S. marcescens*, as an endophytic bacterium, was one of the most effective species against *M. incognita*. As well, many previous studies also showed that algae have a good potential to control root-knot and other nematode species in general (Rizvi and Shameel, 2006; Manilal *et al*., 2011; Nour El-Deen *et al*., 2013; Kumar, 2014; Khan *et al*., 2015).

Greenhouse experiment

Table 2 showed that the disease index (root galling) and reproduction (egg mass production) of *M. incognita* on tomato plants were greatly suppressed ($P \leq \leq 0.05$) by all the tested treatments. Reductions ranged from 37.13 to 98.77% in the number of galls and 16.42 to 99.10% in the number of egg masses per plant. Maximum reduction in root galling (98.77%) and nematode reproduction (99.10%) was found where *B. amyloliquefaciens* was used. However, *B. amyloliquefaciens* gave approximately 9.75 and 30.67 folds reductions in root galling and egg mass production, respectively, compared to oxamyl. Generally, all biocontrol agents used in this study and the nematicide oxamyl provided a good control of *M. incognita* on tomato plants. It has been previously suggested that biocontrol agents release certain nematotoxic compounds which kill nematodes and/or increase the plant resistance (Ibrahim and Ibrahim 2000). Based on egg mass production by *M. incognita* on tomato plants, *B. amyloliquefaciens* gave the best control followed by oxamyl, *S. marcescens*, Nemacross®, and *U. fasciata*. Previous studies showed that the most efficient bacteria for controlling *Meloidogyne* species are those belonging to the genus *Bacillus* and *Serratia*, such as *B. amyloliquefaciens* and *S. marcescens*, which were among the most effective species against *M. incognita* (Jamal *et al*., 2017, Hegazy *et al*., 2019). In a previous work also, *U. fasciata* gave approximately similar results in controlling *M. incognita* on common bean (Ibrahim and Ibrahim, 2000).

Table 2: Effect of *B. amyloliquefaciens* and *Serratia marcescens* and *Ulva fasciata* as compared with Nemacross® and oxamyl on the development of *Meloidogyne incognita* on tomato cv. Dosera, 60 days after inoculation.

Treatment	No. of galls per plant	No. of Egg masses per plant	Relative efficacy	
			Galls	Egg masses
M. incognita (Control)	1300.67a	111.67a	--	--
M. incognita + *B. amyloliquefaciens*	16.00d (98.77)*	1.00cd (99.10)	9.75	30.67
M. incognita + *S. marcescens*	67.67d (94.79)	65.33b (41.50)	2.31	0.47
M. incognita + *U. fasciata*	817.33b (37.13)	93.33ab (16.42)	0.19	0.33
M. incognita + Nemacross®	307.67c (76.33)	82.67ab (25.97)	0.51	0.37
M. incognita + oxamyl	156.00cd (88.00)	30.67c (72.54)		

Data are average of three replicates each.

Means in a column followed by the same letter (s) are not significantly different at $P \leq 0.05$ according to Fisher's protected LSD.

*Values in parenthesis are percent reduction over the control.

Relative efficacy= No. galls or egg mass in the nematode-infected treatment (nematode control) ÷ No. galls or egg mass in a certain treatment.

B.= Bacillus, S.= *Serratia*, *U.= Ulva.*

Efficacy of *U. fasciata* in the control of *M. incognita* could be attributed to the production of ammonical nitrogen and organic acids during the microbial decomposition. It also increases the soil pH, which consequently stimulates the production of nitrates that are toxic to nematodes (Khan *et al*., 1995).

Fresh weights of shoots and roots of tomato plants infected with *M. incognita* were greatly decreased ($P \leq 0.05$) due to nematode infection (Table 3). However, all the soil treatments, gave general increase ($P \leq 0.05$) in the fresh weights of shoots and roots of nematode-infected plants, especially the treatments with *U. fasciata*, *B. amyloliquefaciens* and oxamyl. Several studies showed that the treated plants with *U. fasciata* might increase the plant growth parameters. This might be due to the action of some growth-promoting substances (Zaki *et al*., 2005). The improvement in plant growth following the use of bioagents may be due also to the nematode suppression (Hegazy *et al*., 2019).

The enzymatic activities

Urease Activity

Results in Table(4) showed significant variations in urease activity by the different treatments. The nematode non-infected plants exhibited the lowest urease activity at 0.11 µmol NH_4/g soil/h, while the treatments involving nematode presence showed markedly higher activities. The highest urease activities were recorded in treatments with nematodes and biological products (*M. incognita*, *S. marcescens*, *B. amyloliquefaciens* and Nemacross®). All those treatments exceeding 1.1 µmol NH_4/g soil/h. The chemical treatment (oxamyl) had a moderate effect on urease activity to 0.60 µmol NH_4/g soil/h. This increase in urease activity in nematode-infested soils treated with biological products may be attributed to the enhanced microbial activity facilitated by these amendments, as suggested by Singh *et al*. (2022a) who noted that microbial diversity plays a critical role in nutrient cycling in nematode-infested soils. This finding aligns with recent studies indicating that biological treatments can significantly influence soil enzyme activities by stimulating microbial communities (Zhang *et al*., 2023; Li *et al*., 2023). Furthermore, microbial agents such as *Bacillus amyloliquefaciens* have been shown to increase urease activity by promoting beneficial soil microorganisms (Wang *et al*., 2021a).

Dehydrogenase Activity

Data in Table (4) also showed that dehydrogenase activity, a key indicator of microbial activity and overall soil health, was found to be highest in the *M. incognita*-infested soil, measuring 0.13 µg TPF/g soil/h, which is consistent with the urease results. All treatments with nematodes (*S. marcescens*, *B. amyloliquefaciens*, and Nemacross®) maintained similar levels of dehydrogenase activity, highlighting the role of these biological amendments in sustaining microbial metabolic processes even under nematode stress. Conversely, the oxamyl treatment resulted in the lowest dehydrogenase activity (0.06 µg TPF/g soil/h), suggesting that chemical control may negatively impact soil microbial communities (Wang *et al*., 2021b). These findings align with previous research indicating that chemical nematicides can have detrimental effects on beneficial soil microorganisms, thus impairing soil health (Li *et al*., 2023; Zhang *et al*., 2022). Biological treatments, in contrast, support microbial activity and can enhance soil enzyme activity, further supporting sustainable agricultural practices (Singh *et al*., 2022b).

Table 3: Effect of *B. amyloliquefaciens*, *Serratia marcescens* and *Ulva fasciata* as the compared with nematicides Nemacross® and oxamyl on the growth of tomato plants cv. Dosera, 60 days after inoculation.

Treatment	Shoot weight (g)	Root weight (g)
Seedling only	37.653 b	13.507 b
M. incognita (Control)	17.040 c	09.520 b
M. incognita + *B. amyloliquefaciens*	37.237 b	24.343 a
M. incognita + *S. marcescens*	26.250 bc	26.800 a
M. incognita + *U. Fasciata*	62.513 a	23.487 a
M. incognita + Nemacross®	30.167 bc	11.563 b
M. incognita + oxamyl	37.387 b	14.443 b

Data are average of three replicates each.

Means in a column followed by the same letter (s) are not significantly different at $P \leq 0.05$ according to Fisher's protected LSD.

B.= Bacillus, S.= *Serratia*, *U.= Ulva.*

Table 4: Enzymatic activity profiles in soil samples collected from the rhizosphere of tomato plants infected with *Meloidogyne incognita*.

Treatments	Urease µ mol NH/g Soil/h	Dehydrogenase µg TPF/g Soil/h	Alkaline phosphatase µg PNP/g Soil/h
Untreated	0.11 c	0.05 b	1.67 b
M. incognita	1.24 a	0.13 a	3.33 a
S. marcescens	1.15 a	0.11 a	2.67 a
B. amyloliquefaciens	1.12 a	0.11 a	2.67 a
U. fasciata	1.20 a	0.13 a	3.33 a
Nemacross®	1.20 a	0.13 a	3.23 a
Oxamyl	0.60 b	0.06 b	1.33 b

Data are average of three replicates each.
Means in a column followed by the same letter (s) are not significantly different at $P \leq 0.05$ according to Fisher's protected LSD.
B.= Bacillus, S.= *Serratia*, *U.= Ulva.*

Alkaline Phosphatase Activity

Alkaline phosphatase activity (Table 4) which reflects phosphorus availability in soil, showed a marked increase in treatments with nematodes, particularly in the control with nematodes (*M. incognita*-infected plants), registering 3.33 µg PNP/g soil/h. This trend was similarly observed in the *S. marcescens* and Nemacross® treatments, suggesting that these biological agents might enhance phosphorus solubilization, thus benefiting plant growth under nematode pressure. In contrast, the lowest alkaline phosphatase activity was recorded in the oxamyl treatment (1.33 µg PNP/g soil/h). Further corroborating the negative impact of chemical nematicides on soil enzyme activity and nutrient availability (Jiang *et al*., 2020; Zhang *et al*., 2021). These results align with previous studies indicating that the use of biological amendments can significantly enhance soil enzyme activity that improving nutrient availability in nematode-infested soils (Singh *et al*., 2022b; Li *et al*., 2023).

CONCLUSION

Bacillus amyloliquefaciens, *Serratia marcescens* and *Ulva fasciata* extracts proved to be effective bio-control agents against the root-knot nematode, *Meloidogyne incognita*. As a safe bio-control agent for the root-knot disease, local Egyptian inoculums containing both bacterial species could be produced. The application of such biological products in nematode-infested soils might offer significant enhancements in soil enzyme activities, particularly urease, dehydrogenase, and alkaline phosphatase. These results underscore the potential of biological treatments not only for the nematode management but also for improving soil health and fertility. Conversely, chemical nematicides like oxamyl showed detrimental effects on soil enzyme activities, emphasizing the need for sustainable practices in nematode management.

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الملخص العربي

تأثير بعض عوامل المكافحة الأحيائية على نيماتودا تعقد الجذور وبعض النشاطات الإنزيمية الحادثة في المنطقة المحيطة بجذور نباتات الطماطم

علاء حبيب عبد السلام[١]، هالة حسن بدري[٢]، عبير أبو زيد محمد[١]، منال محمد زين الدين[٣]

[١]معهد بحوث أمراض النباتات– مركز البحوث الزراعية.

[٢]قسم علوم التربة والمياه، كلية الزراعة، جامعة الإسكندرية.

[٣]قسم بحوث مبيدات الفطريات والبكتيريا والنيماتودا، المعمل المركزي لمبيدات الآفات الزراعية، مركز البحوث الزراعية.

تعد نيماتودا تعقد الجذور (*Meloidogyne* spp.) واحدة من أهم مجموعات مسببات الأمراض النباتية التي تصيب نباتات الطماطم على مستوى العالم، كما تعد وسائل المكافحة الأحيائية من الوسائل الفعالة والآمنة لمكافحة هذه الكائنات الممرضة. وتهدف الدراسة الحالية إلى تقييم فعالية الطحلب البحري *Ulva fasciata* وعزلتين بكتيريتين تم عزلهما من بذور الطماطم ومنطقة التربة المحيطة بجذورها في مكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* على نباتات الطماطم. تم تعريف العزلتين البكتيريتين المختبرتين باستخدام تفاعل البلمرة المتسلسل PCR من خلال تضخيم منطقة الحامض النووي الريبوسومي 16S rRNA فوجد أنهما تنتميان إلى النوع *Bacillus amyloliquefaciens* (PQ821314)، والنوع *Serratia marcescens* (PQ0126660). تم اختبار تأثير كل من نوعي البكتيريا *B. amyloliquefaciens*، و *S. marcescens* بتركيز 10^{8} مستعمرة بكتيرية/مل، وكذلك الطحلب *U. fasciata* بتركيزات ٥٠٠٠، و٢٥٠٠، و١٢٥٠، و٦٢٥ مجم/لتر على بيض ويرقات النيماتودا سابقة الذكر، وذلك تحت ظروف المختبر. تم أيضا استخدام معاملتي مقارنة تشملان المبيد النيماتودي Nemacross® 2%، ومبيد أوكساميل ٢٤٪ (سائل) بالإضافة إلى معاملة الشاهد غير المعامل.

أوضحت النتائج أن المعاملة بكل من: البكتيريا *B. amyloliquefaciens*، و*S. marcescens* والطحلب *U. fasciata* ومبيد الأوكساميل قد خفضت معنويا ($P \leq 0.05$) من نسبة فقس بيض نيماتودا تعقد الجذور *M. incognita*، كما زادت معنويا ($P \leq 0.05$) من نسب موت يرقات الطور الثاني لتلك النيماتودا، وذلك قياسا بمعاملات المقارنة. ومن ناحية أخرى، أدت المعاملة بمبيد Nemacross® إلى موت يرقات الطور الثاني لنيماتودا تعقد الجذور بنسبة ٢٨.٨٤٪ وثبطت فقس بيض تلك النيماتودا بنسبة ٢٣.٧٣٪. أما تحت ظروف البيت الزجاجي، فقد انخفضت شدة المرض بنيماتودا تعقد الجذور *M. incognita* (عدد العقد الجذرية) وكذلك انخفض عامل تكاثر النيماتودا (عدد كتل البيض/نبات) معنويا بكل المعاملات المختبرة. وقد أحدثت المعاملة بالبكتيريا *B. amyloliquefaciens* أكبر خفض في كل من: عدد العقد الجذرية على النباتات بنسبة ٩٨.٧٧٪، وعامل تكاثر النيماتودا (عدد كتل بيض النيماتودا/نبات) بنسبة ٩٨.٧٧٪. زاد الوزن الرطب للمجموع الخضري لنباتات الطماطم المصابة بنيماتودا تعقد الجذور بشكل عام عند المعاملة بالمبيد النيماتودي "أوكساميل" وجميع عوامل المكافحة الأحيائية المختبرة، وذلك بالمقارنة إلى معاملة الشاهد المعدي بنيماتودا تعقد الجذور *M. incognita* فقط. تم تقييم النشاط الإنزيمي في التربة أيضا تحت ظروف البيت الزجاجي، ووجد أن تطبيق المواد الأحيائية في التربة الملوثة بنيماتودا تعقد الجذور قد شجع معنويا ($P \leq 0.05$) نشاط بعض إنزيمات التربة وخاصة إنزيم اليورييز والديهيدروجينيز والفوسفاتيز القلوي. ومن ناحية أخرى، أظهر مبيد الأوكساميل تأثيرات فاعلة على النشاط الإنزيمي بالتربة.