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Effects of bacterial isolate, *Priestia megaterium* HB8 and its filtrate on Controlling the root-knot nematode, *Meloidogyne incognita*

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ABSTRACT

This study was conducted to isolate biological control agents from soil samples surrounding the roots of uninfected cucumber, watermelon, eggplant, tomato, and okra plants in fields infested with root-knot nematodes and to evaluate their effectiveness. Therefore, fifteen bacterial isolates were selected to study their effects on the vitality of the second stage juveniles (J2) of the root-knot nematode, *Meloidogyne incognita*. Nine bacterial isolates showed a mortality rate of juveniles ranged between 80 and 100%, while isolate HB8 was unique in having a high ability to influence the vitality of the juveniles, as the mortality rate reached 100%. This isolate was chosen to test its effectiveness in inhibiting egg hatching. The results showed that the inhibition rate after 72 h of incubation reached 94.33%. The effect of the bacterial filtrate of isolate *Priestia megaterium* HB8 was tested using three concentrations (10, 20, and 30%) on the mortality rate of J2 and the inhibition rate of egg hatching. Moreover, the 30% concentration exhibited a high ability to increase the mortality rate of a juvenile to 97.66% after 48 h of incubation, while the inhibition rate of egg hatching was 96.66% after 72 h.

Keywords: *Priestia megaterium*, Root-knot nematode, *Meloidogyne incognita*.
Article type: Research Article.

INTRODUCTION

The root-knot nematodes, *Meloidogyne* spp. is among the most important plant pathogens and the first among the ten most important genera of plant-parasitic nematodes in the world (Mukhtar *et al.* 2013). This genus includes over 100 species (Hunt & Handoo 2009) which infect more than 3000 hosts (Jones *et al.* 2013). The most common species are *M. javanica, M. incognita, M. arenaria*, and *M. hapla*, where this pest causes economic losses to crops of up to 100% (Al-Hazmi 2009). Besides, it is considered as one of the most important determinants of plant and crop growth, since it causes economic losses estimated at $77 billion annually (Ralmi *et al.* 2016, Yadav 2017). A total of 111 hosts were recorded in Iraq (Stephan 1987). Five species have also been identified: *M. javanica, M. incognita, M. arenaria, M. hapla*, and *M. thamisi* (Stephan 1988) as well as *M. cruciani* (Hasan *et al.* 2020). However, the long-term use of these pesticides has led to the ban or restriction of many of them all over the world. The harms of these pesticides lie in their wide spectrum as well as being non-selective and thus indirect effect on non-target organisms and their severe toxicity to the environment (Kepenkci *et al.* 2017). Biological control agents are one of the most promising agents for the natural control of plant-parasitic nematodes (Vagelas 2015). Biological control by fungi and bacteria plays an important role in controlling root-knot nematodes, since the roots of plants are surrounded by many organisms, as they constitute one of the most complex ecosystems on Earth (Jones & Hinsnger 2008; Raaijmakers *et al.* 2009). *B. megaterium*, currently known as *Priestia megaterium*, is considered as an economical and effective bio-inoculator not only in the process of fertilization and preparation of pathogens for plants, but also in controlling bacterial, fungal, and nematode pathogens through its production

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of antibiotics, ammonia, enzymes, and others (Abhilash *et al.* 2010). Furthermore, *Bacillus megaterium* is one of the fifteen species belonging to the genus *Bacillus* sp. identified as an effective biological agent in the management of the root-knot nematode, *M. incognita* (Mendoza *et al.* 2008). Youessf *et al.* (2017) reported that *B. megaterium* exhibits an effective impact on controlling *M. incognita*, as well as improving growth parameters of sugar beet plants. Similarly, El-Hadad *et al.* (2010) reported the ability of *B. megaterium* PSB2 to produce high levels of the enzymes, i.e., protease, chitinase and gelatinase, which is reflected in its ability to affect the vitality of juvenile *in vitro*, since this isolate caused the mortality of second-stage juveniles by 100%. The present study aimed to find biological control agents isolated from the Iraqi environment due to the scarcity of local studies on the effects of this species, or its filtrate on root-knot nematode *M. incognita*

MATERIALS AND METHODS

Thirty six soil samples were collected from six sites, five of them in Baghdad City, Iraq, distributed over the following areas: Abu Ghraib - Al Zaidan Village, Abu Ghraib - Hamid Shaaban Village, Al Radwaniyah, Al Yusufiyah, and Salman Pak. Conversely, the sixth site from different fields was located in Al Saqlawiyah district, west of Baghdad. Soil samples surrounding the roots of cucumber, melon, eggplant, tomato, and okra plants uninfected or lightly infected with root-knot nematode were collected from fields infested with this pest. The samples were placed in polyethylene bags and transferred to the nematode laboratory for isolation. Several bacterial species were isolated, 15 bacterial isolates were selected to test their efficiency in killing second-stage juveniles.

Bacterial identification

The Vitek2 Compact System was used to identify the selected bacterial isolate, cultured on the NA, Overnight media. The BCL card or identification kit of the Bacillaceae family, which was supplied by the French company Biomeriux, was selected.

Molecular identification of the selected bacterial isolate

DNA extraction

In a tube of 10 mL, 3 mL Brain Heart Infusion (BHI) liquid medium was added, which was sterilized by an autoclave device at a temperature of 121 °C and a pressure of 1 bar for 20 min. The liquid medium was inoculated with a pure colony of the selected isolate and incubated at 30 °C for 24 h. Afterward, 1.5 mL of the bacterial inoculum was withdrawn using a pipette and placed in a sterile Eppendorf tube to be placed in the centrifuge at a speed of 13,000-16,000 g × r/min for 1 min. The filtrate was removed and the bacterial precipitate was retained for genetic material isolation using the Wizard Genomic DNA Purification Kit from Promega Company.

PCR polymerase chain reaction

A pair of primers for the S rRNA16 gene prepared from the Korean company Macrogen used the forward primer Forward: 27-5`-AGAGTTTGATCCTGGCTCAG-3` and the reverse primer: 1494TACGGTTACCTTGTTA CGACTT-3`-5`. The Polymerase Chain Reaction (PCR) was carried out with 25 µL reaction components. Components involved in gene amplification 16S rRNA by PCR technique, Go Taq Green Master Mix 12.5 µL, forward primer 1 µL, reverse primer 1 µL, DNA template 2 µL deionized distilled water 8.5 µL. Amplification was carried out using a thermocycler PCR from Thermo Fisher Scientific, USA, pre-programmed with the amplification program for 32 cycles: Initial Denaturation 95 °C for 5 min per one cycle (Denaturation 95 °C for 30 seconds, Annealing 60 °C for 30 seconds, Extension 72 °C for 30 seconds). For 30 cycles, Final Extension 72°C for 7 min, Hold 10°C for 10 min for 1 cycle.

Electrophoresis of PCR products

Agarose gel was prepared at a concentration of 1.5% by adding 100 mL TBE 1x solution to 1.5 g Agarose in a 250 mL glass bottle. Nozzle was closed and placed in the microwave until the mixture melted to form a transparent liquid. It was left to cool at laboratory temperature and when the temperature was reduced to 50 °C, 1 µL ethidium bromide dye was added, with stirring, to ensure that it was mixed with the mixture, taking care not to form any bubble. The mixture was poured into the casting mold after fixing the electrophoresis combs in place inside the mold and allowed the mixture to solidify. After solidification was completed, the combs were carefully withdrawn

and the gel was transferred to the electrophoresis bath. TBE 1x solution was added to it, ensuring that the gel surface was covered to a height of 3-5 mL.

Preparation of the bacterial inoculum for the selected isolate

100 mL sterile NB liquid media was inoculated by taking one colony from the pure culture of the selected isolate and incubating at 28 ± 2 °C for 48 h (Mahesha *et al.* 2017).

Preparation of the bacterial filtrate for the selected isolate

100 mL NB liquid media was sterilized and inoculated with a pure colony of the selected isolate using a loop and incubated at 28 ± 2 °C for 48 h. Then the inoculum was distributed into 10 sterile test tubes of 10 mL and placed in a refrigerated centrifuge 4500 g x cycle/ minute for 20 min at a temperature of 4 °C. This process was repeated for another cycle to ensure the complete precipitation of bacteria. The filtrate was withdrawn using a sterile 10 mL syringe and passed through a sterile 0.20 µm syringe filter prepared by the Spanish company CHMLAB to be collected in a sterile glass tube (Mahesha *et al.* 2017) with some modifications.

Preparation of root-knot nematode inoculum for laboratory experiments

The roots of infected cucumber plants were collected and washed with a light stream of water to remove the dirt suspended in them. Thereafter, the ripe egg masses were picked and placed in a 30 mL beaker, to which 1% commercial solution containing 6% NaOCl sodium hypochlorite was added for 2 min, then the solution containing the eggs was poured into a 0.01 mL sieve and washed with distilled water several times to get rid of remains of the sterilization solution. Afterward, the eggs were collected in a beaker and the volume was completed to 100 mL. To obtain the second-stage juveniles, the eggs were placed in a glass dish and incubated at 25 ± 2° C for 72 h.

Testing the effect of bacterial isolates on the mortality of juvenile root-knot nematodes

The efficiency of 15 bacterial isolates was tested on the vitality of newly-hatched second-stage juveniles by preparing 100 mL of each bacterial isolate. Thus, 5 mL of each isolate was taken and added to a sterile petri dish of 6 cm in diameter containing 0.2 mL of the root-knot nematode inoculum at a concentration of 100 ± 3 juveniles of the newly-hatched second-stage, with 3 replicates for each bacterial isolate, in addition to the two comparison treatments with sterile distilled water and the comparison with the sterile NB liquid nutrient medium. The dishes were incubated at 28 ± 2 °C and after 24 h, the number of juveniles killed were taken and the mortality rate of juvenile was calculated according to the following equation.

$$\text{Mortality rate (\%)} = \frac{\text{Number of dead J2}}{\text{Total number of J2}} \times 100$$

Evaluating the effect of a selected bacterial isolate, HB8 on the inhibition of egg hatching

0.5 mL of root-knotted nematode inoculum containing 100 ± 5 eggs were added to sterile petri dishes of 6 cm in diameter. The dishes were treated by adding 5 mL bacterial inoculum to the selected isolate containing 4×10^8 CFU mL^{-1} with three replicates/treatments, in addition to the two comparison treatments with sterile distilled water and sterile NB liquid nutrient medium. The dishes were incubated at 28 ± 2 °C, then after 72 h, the hatched eggs were counted, and the inhibition rate (%) of hatching was calculated according to the following equation:

$$\text{Hatching inhibition rate (\%)} = \frac{\text{Inhibitory eggs in treatment} - \text{inhibitory eggs in control}}{100 - \text{inhibitory eggs in control}} \times 100$$

Evaluation the effect of three concentrations of bacterial filtrate on the mortality of juveniles

Three concentrations (10, 20, and 30%) of the bacterial filtrate of the selected isolate were prepared. Then, 0.5 mL of root-knot nematode inoculum containing 100 ± 5 newly-hatched juveniles was added to petri dishes of 6 cm in diameter and three replicates/treatment, so that the final solution volume was 5 mL in each dish. The dishes were then incubated at 28 ± 2 °C, and after 48 h, the number of dead juveniles were counted. The mortality of juveniles were calculated according to the following equation:

$$\text{Mortality rate (\%)} = \frac{\text{Number of dead J2}}{\text{Total number of J2}} \times 100$$

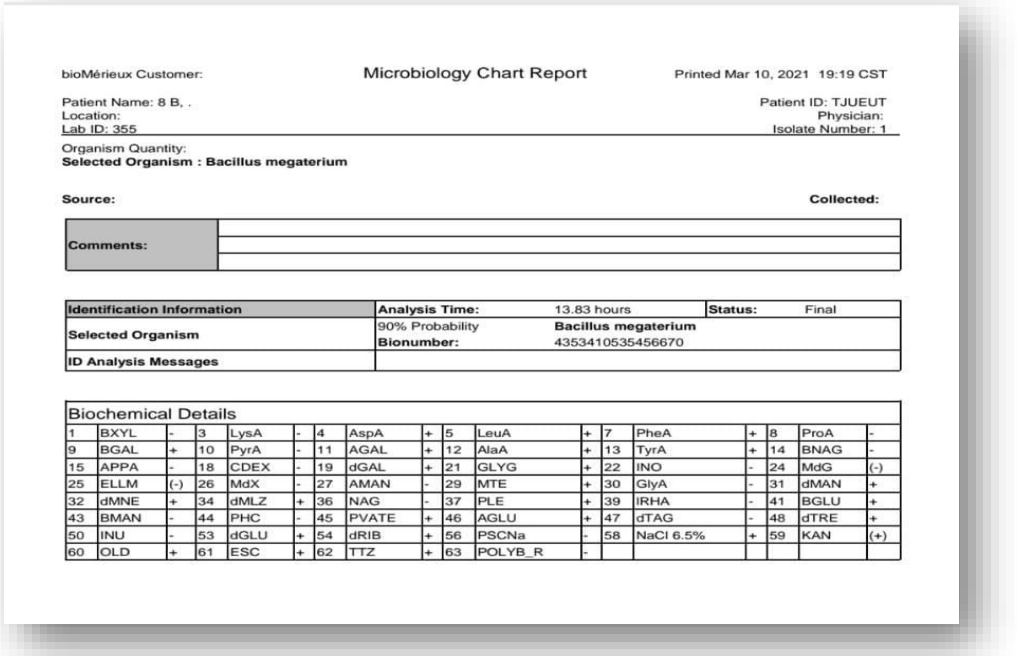
Evaluating the effect of three concentrations of bacterial filtrate on the inhibition of egg hatching

Three concentrations (10, 20, and 30%) of the bacterial filtrate of the selected isolate were prepared. Further, 0.5 mL of root-knot nematode inoculum containing 100 ± 5 eggs were added to petri dishes of 6 cm in diameter and in three replicates/treatment, so that, the final volume of the solution was 5 mL/dish. The dishes were incubated at 28 ± 2 °C, and after 72 h the hatched eggs were counted, and the rate of hatching inhibition was calculated according to the following equation:

$$\text{Hatching inhibition rate (\%)} = \frac{\text{Inhibitory eggs in treatment} - \text{inhibitory eggs in control}}{100 - \text{inhibitory eggs in control}} \times 100$$

RESULTS AND DISCUSSION

The results of 63 tests contained in the BCL diagnostic kit of the Bacillaceae family with Vitek 2 technology, of those 46 biochemical tests (Fig. 1) exhibited that this isolate belongs to the species *Bacillus megaterium* with a probability of 90%.



bioMérieux Customer: Microbiology Chart Report Printed Mar 10, 2021 19:19 CST

Patient Name: 8 B, . Patient ID: TJUEUT
Location: Physician:
Lab ID: 355 Isolate Number: 1

Organism Quantity:
Selected Organism : Bacillus megaterium

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	13.83 hours	**Status:**	Final
Selected Organism	90% Probability	**Bacillus megaterium**		
	Bionumber:	4353410535456670		
ID Analysis Messages				

Biochemical Details

1	BXYL	-	3	LysA	-	4	AspA	+	5	LeuA	+	7	PheA	+	8	ProA	-
9	BGAL	+	10	PyrA	-	11	AGAL	+	12	AlaA	+	13	TyrA	+	14	BNAG	-
15	APPA	-	18	CDEX	-	19	dGAL	+	21	GLYG	+	22	INO	-	24	MdG	(-)
25	ELLM	(-)	26	MdX	-	27	AMAN	-	29	MTE	+	30	GlyA	-	31	dMAN	+
32	dMNE	+	34	dMLZ	+	36	NAG	-	37	PLE	+	39	IRHA	-	41	BGLU	+
43	BMAN	-	44	PHC	-	45	PVATE	+	46	AGLU	+	47	dTAG	-	48	dTRE	+
50	INU	-	53	dGLU	+	54	dRIB	+	56	PSCNa	-	58	NaCl 6.5%	+	59	KAN	(+)
60	OLD	+	61	ESC	+	62	TTZ	+	63	POLYB_R	-						

Fig. 1. Bacterial identification form and results of biochemical tests for the selected bacterial isolate HB8 using the Vitek2 compact system.

Molecular identification of selected bacterial isolate

The results of agarose gel electrophoresis for 16S rRNA gene (Fig. 2) showed the presence of a band resulting from the polymerase chain reaction (PCR) whose size is estimated at 1500 bp based on the DNA-marker ranged between 100 and 1500 bp. Accordingly, this confirms the success of the amplification process for the target gene.

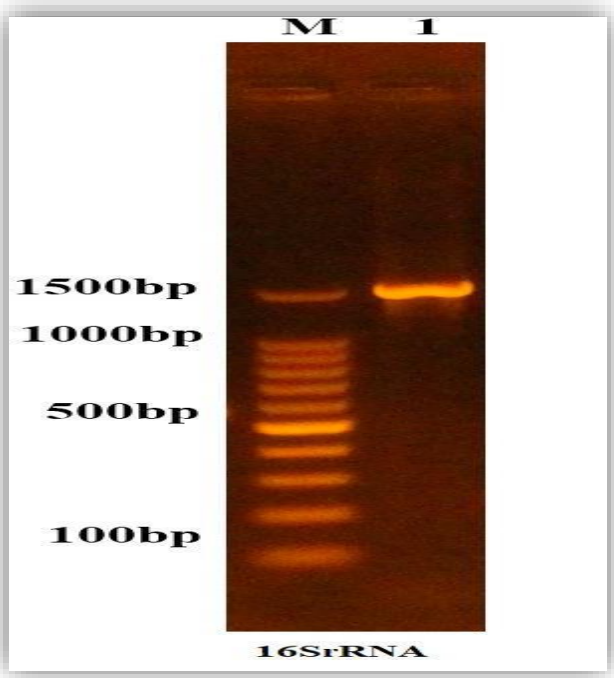


Fig. 2. 16S rRNA amplification product by PCR technique and electrophoresis on 1.5% agarose gel, 1 represents 16S rRNA amplification product for the selected bacterial isolate, M represents the DNA-Marker 100-1500 bp.

Determination of the nitrogenous base sequences of the S rRNA16 gene and their comparison with similar genes

The 16S rRNA amplified gene sequences were determined by the Korean company Macrogen, where their concordance was studied with the sequences of nitrogenous bases of bacterial strains on the NCBI GenBank. The results of the concordance study showed that the 16S rRNA amplified gene from the selected bacterial isolate HB8 was 100% identical to more than one strain of bacteria with its previous name, i.e., *Bacillus megaterium* and registered under the new name, i.e., *Priestia megaterium*. This concordance covered 100% of the 1411 nitrogenous base sequences of the gene found in the GenBank. These results were confirmed by the results of biochemical identification using phytic technology. Besides, this isolate belongs to the bacterium *P. megaterium*, and it was registered in the GenBank under the name *P. megaterium* HB8 and with the identification number OM189443.1.

Testing the effect of bacterial isolates on the vitality of juvenile root-knot nematodes

The results of the efficiency test of 15 bacterial isolates recovered from soil samples surrounding the roots of cucumber, watermelon, eggplant, tomato, and okra uninfected or lightly infected with nematode root knots from fields infested with this pest on the vitality of *M. incognita* juveniles illustrated in Fig. 3. These results showed that almost all bacterial isolates caused mortality after 24 h in J2, so the highest mortality of J2 was in the isolated HB8 with 100% mortality rate, followed by isolates G1, B7, and G18 with a mortality rates of 97.67% 95.67%, and 91.33%, respectively. Moreover, the mortality rates in nine isolates ranged between 57.66-88.33 %, while the lowest mortality was in isolates D9 and C4, amounted to 43.33% and 37.67%, respectively (Fig. 3). In an experiment Tran *et al.* (2019) tested the ability of 34 bacterial isolates recovered from the roots of healthy black pepper plants from root-knot nematode infection in fields infested with this pest on the vitality of second-stage juveniles of *Meloidogyne* spp. A total of 16 isolates exhibited a high ability to elevate the mortality rate of juveniles between 85.56% and 100%.

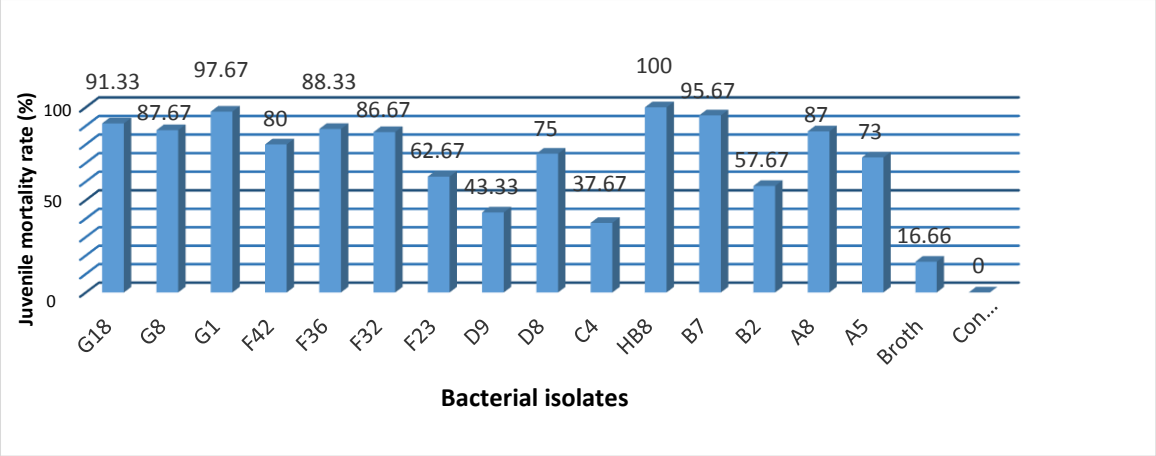


Fig. 3. Efficiency test of bacterial isolates on the vitality of juvenile root-knot nematodes. Least significant difference (LSD) at p = 0.05 between the treatments was 3.79 **

Evaluating the effect of selected HB8 bacterial isolate on egg hatching inhibition

The bacterial isolate exhibited efficiency in the hatching of root-knot nematodes with a significant difference compared to the two control groups. In addition, the inhibition rate on hatching eggs after 72 h of contamination was 94.33% (Fig. 4). Borrajo *et al.* (2021) reported the efficiency of local Argentine isolates of *Bacillus* sp. and *Pseudomonas fluorescens* in reducing the hatching egg of root-knot nematodes, *M. javanica* and a high mortality rate of J2 juveniles. In the same role, the bacterial isolate *Bacillus* sp. B9T displayed the highest rates of inhibition on the egg hatching and mortality rate of juveniles compared to isolates of *Pseudomonas* sp.

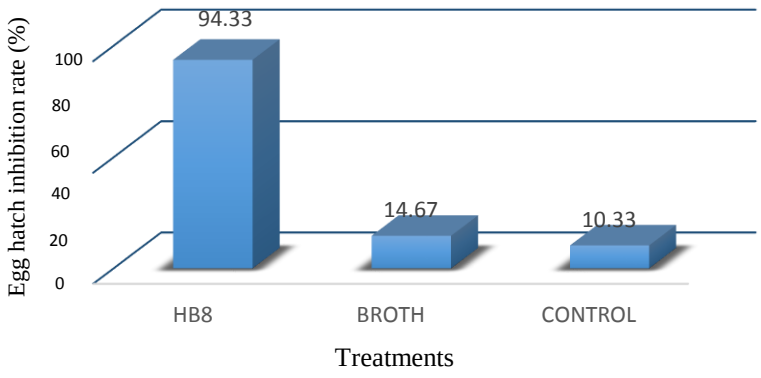


Fig. 4. Efficiency of the selected bacterial isolate to inhibit egg hatching; LSD value at p =0.05 was ** 4.159; Bacterial strain containing 4×10^8 CFU mL^{-1}.

Evaluation the effect of three concentrations of the bacterial filtrate of HB8 on the vitality of J2 juveniles.

The effectiveness of the three concentrations of bacterial filtrate in killing J2 juveniles with significant differences compared to the two comparison treatments is illustrated in Fig. 5. The concentration of 30% exceeded in influencing the vitality of J2 juveniles, so that, the juvenile mortality rate (%) after 48 h was 97.66%. However, the concentration of 10% recorded the lowest mortality rate in juveniles, reaching 51.66% after 48 h of contamination. Gastaňeda-Alvaez *et al.* (2016) conducted an *in vitro* study to investigate the secretion of lipase enzyme by bacterial strains belonging to *B. thuringinsis, B. megaterium, and B. amyloliquefaciens* reporting that strains belonging to these species capable of secreting this enzyme displayed a high ability to kill the nematode, *Xiphinema index*. In addition, it was found that the strain belonging to the type *B. megaterium* FB133M, which cannot secrete lipase enzyme, exhibited the least killing effect.

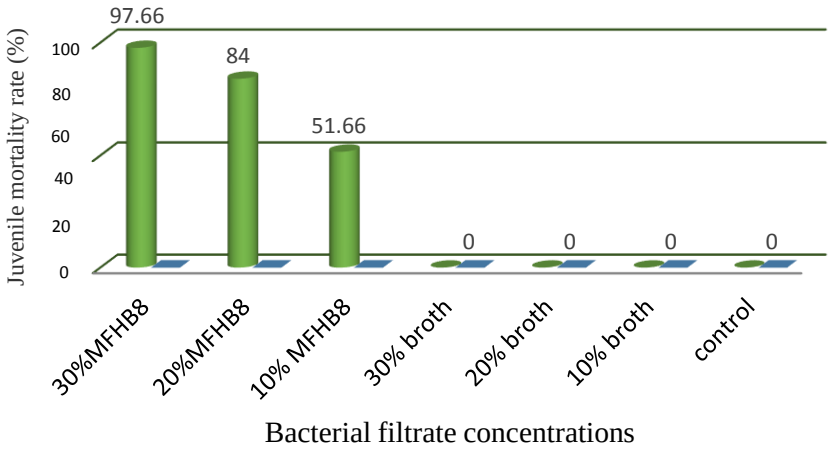


Fig. 5. Effect of three concentrations of bacterial filtrate on the vitality of J2 juveniles. Bacterial strain containing 4×10^8 CFU mL^{-1}; The value of LSD at p = 0.05 was **2.818.

Evaluation the effect of three concentrations of bacterial filtrate on inhibiting egg hatching

The results illustrated in Fig. 6 exhibits the effectiveness of the three concentrations of bacterial filtrate in inhibiting the hatching of root-knot nematode eggs with significant differences compared to the two comparison treatments. The concentration was 30% exceeded in the effect on egg hatching, and the inhibition rate (%) on hatching eggs after 72 h of contamination was 96.66%. However, the two concentrations (20% and 10%) recorded inhibition rate of 88.31% and 70.34%, respectively. This was in line with the results of Ying *et al.* (2010), on the efficiency of the bacterial filtrate of some types of bacteria belonging to the genus *Bacillus* sp. in reducing the number of hatched eggs of root-knot nematodes, along with its ability to elevate the mortality rate of juveniles. On the other hand, Mehasha *et al.* (2017) estimated that the number of bacteria of the same genus upraise the inhibition rate on the egg hatching. Moreover, the same study reported the efficiency of bacterial filtrate *Bacillus megaterium* IIHR to increase inhibition rate to 92.93% at 100% concentration and after 120 h of contamination.

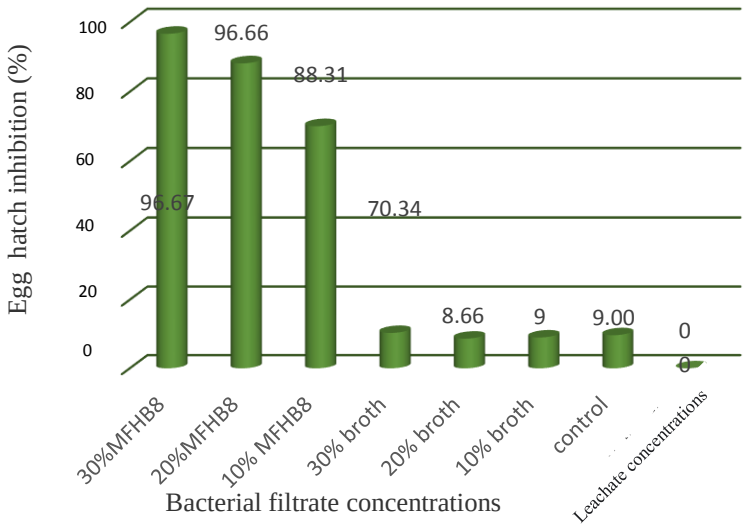
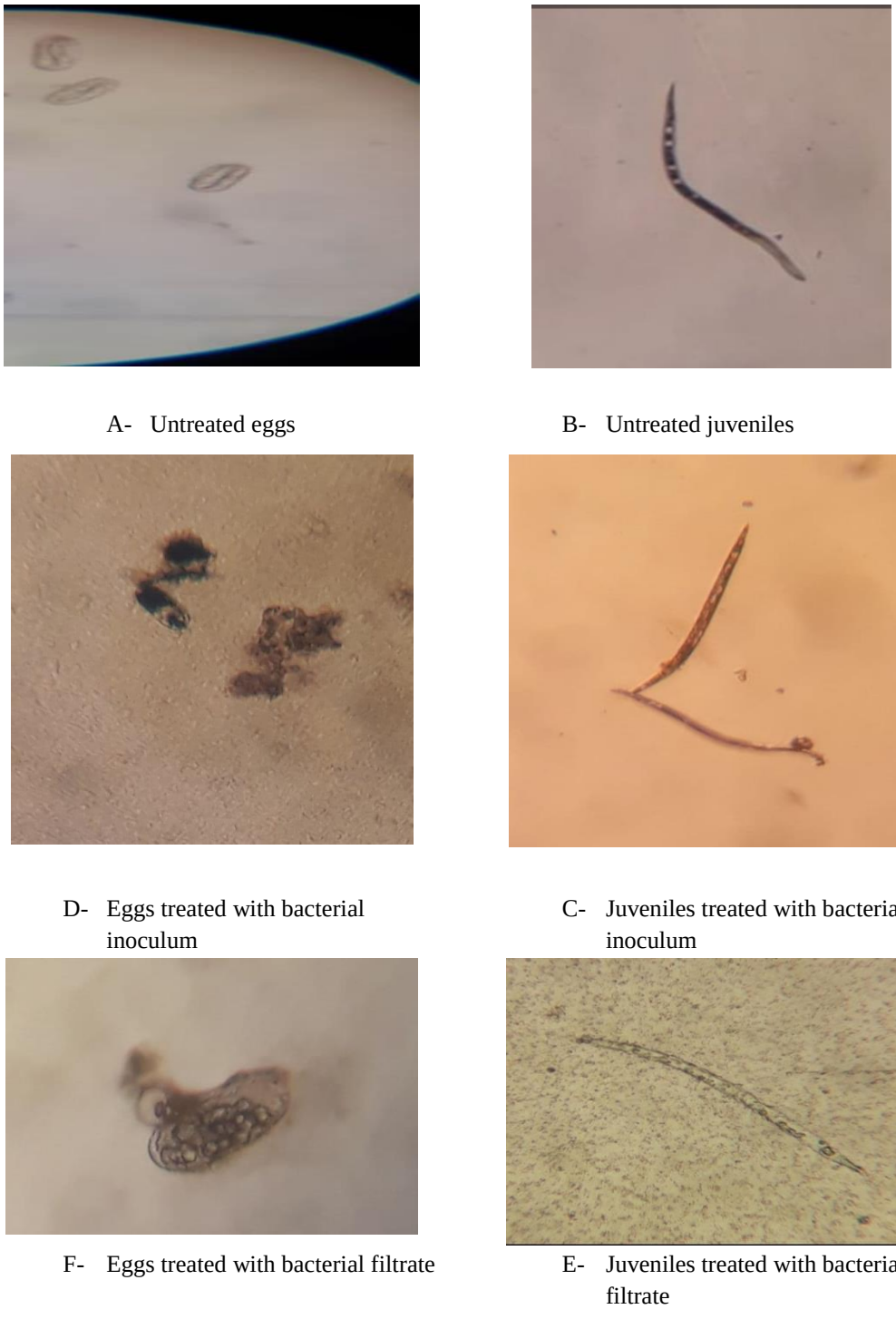


Fig. 6. Effects of the three bacterial filtrate concentrations on the inhibition rate of the egg hatching. Bacterial strain containing 4×10^8 CFU mL^{-1}; LSD value at p = 0.05 was 3.35**

The addition of bacterial inoculum of the selected isolate to the root-knot nematode juveniles *in vitro* after 24 h of contamination resulted in the death of all tested individuals. It was observed that the juvenile's dormancy was straight, horizontally or vertically, with the presence of a bubble on some of the juveniles on one side of the body. Their colour was changed to dark with the contents of the body coming out from the front side of the body mostly in the form of Fig. 7C. Anyhow, the effect of bacterial filtrate appeared at a concentration of 30% on the juveniles after 48 h of treatment. Juvenile dormancy was straight, with their body mostly free of internal viscera (Fig. 7E). As for the effect of bacterial inoculum on the eggs after 72 h of treatment, the eggs turned to dark colour at first,

and the symptoms developed to leave the egg empty of its contents (7D). In addition, as for the effect of the bacterial filtrate at a concentration of 30% after 72 h, it was distorting the contents of the egg and its exit from one of the egg sides (Fig. 7F).



A- Untreated eggs

B- Untreated juveniles

D- Eggs treated with bacterial inoculum

C- Juveniles treated with bacterial inoculum

F- Eggs treated with bacterial filtrate

E- Juveniles treated with bacterial filtrate

Fig. 7. Effect of the selected local isolate *Priestia megaterium* BH8 and its filtrate on the vitality of second-stage juveniles and egg hatching of root-knot nematodes with a magnification of 40 X.

REFERENCES

Abhilash, M, Shiva Reddy, DM, Mohan, BK, Nataraja, S & Krishnappa, M 2010, Isolation and molecular characterization of Bacillus megaterium isolated from different agro climatic zones of Karnataka and its

effect on seed germination and plant growth of *Sesamum indicum*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 1: 3.

Al Hazmi, ABS 2009, Introduction to plant nematology. 2nd Edition, King Saud University Press, Riyadh, Saudi Arabia, 440 p.

Borrajo, PM, Mondino, EA, Maronich, GA, Fernandez, M & Creus, CM 2021, Potential of rhizobacteria native to Argentina for the control of *Meloidogyne javanica*. *Revista Argentinel de Microbiology*, http: //dol.org/10.1016/j. ram 2021.2.010.

Castaneda Alvarez, C, Prodan, S, Rosales, IM & Aballay, E 2016, Exoenzymes and metabolites related to the nematicidal effect of rhizobacteria on *Xiphinema index*. Thorne and Allen. *Journal of Applied Microbiology*, 120: 413-424.

Dameh, M, Ameen Baqer, A, Zaidi, MK, Hachim, SM, Hameed, NA, Awadh, S, Obaid, AJ, Adhab, ZH 2022, Evaluating the efficacy of flavonoids from *Capparis spinosa* extracts in treatment of *Candida vulvovaginitis*. *Caspian Journal of Environmental Sciences*, 20: 565-570

El Hadad, ME, Mustafa, MI, Selim, ShM, Mahgoob, AEA, El Hasan, ST, Inad, DA & Abd Al Rasoul, AM 2020, Diagnostic of new species of root- knot nematode *Meloidogyne cruciani* associated with eggplant in Babylon governorate, Iraq based on morphological characters and molecular methods. *Equal Employment Opportunity Environmental and Conservation*, 26: 69 -74.

Hunt, DJ & Handoo ZA 2009, Taxonomy, Identification and principal species. In: RN, Perry, M, Moens JL, Starr (Eds): Root-knot Nematodes. Chapter 3. Wallingford, CAB International: pp. 55-97.

Jepson, SB 1983, The use of second stage juvenile tails as an aid in the identification of *Meloidogyne* species. *Nematologica*, 29: 11-28.

Jones, JT, Haegeman, A, Danchin, EGJ, Gaur, HS, Helder, J, Jones, MGK, Kikuchi, T, Manzanilla López, R, Palomares Rius, JE, Wesemael, WM & Perry, RN 2013, Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, 14: 946-961

Kepeneki, L, Dura, O & Dura, S 2017, Determination of nematicidal effects of some bio- pesticides against root- knot nematode *Meloidogyne incognita* on kiwifruit. *Journal of Agricultural Science and Technology*, 7: 546- 551.

Mahesha, HS, Ravichandra, NG, Rao, MS & Narasegowda, NC 2017, Bio-efficacy of different strains of *Bacillus* spp. against *Meloidogyne incognita* under in vitro International. *International Journal of Current Microbiology and Applied Sciences*, 6: 2511-2517.

Mendoza, AR, Kiewnick, S & Sikora, RA 2008, In vitro activity of *Bacillus firmus* against the burrowing nematode *Radopholus similis*, the root-knot nematode *Meloidogyne incognita* and the stem nematode Ditylenchus dipsaci. *Biocontrol Science and Technology*, 18: 377-389.

Mukhtar, T, Kayani, MZ & Hussain, MA 2013, Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. *Industrial Crop Products*, 42: 447-453.

Raaijmakers, JM, Paulitz, TC, Steinberg, C, Alabouvette, C & Moënne Loccoz, Y 2009, The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, 321: 341-361.

Ralmi, NHAA, Khandaker, MM & Mat, N 2016, Occurrence and control of root knot nematode in crops: A review. *Australian Journal of Crop Science*, 10: 1649-1654.

Stephan, ZA 1987, Plant parasitic nematodes on cereal and legumes in Iraq. In: Nematodes parasitic to cereal and legumes in Temperate and Semi arid Regions. Proceeding of a workshop held at Larnaca, Cyprus, pp. 155- 159.

Stephan, ZA 1988, Newly reported hosts of root - knot nematodes in Iraq. *International Nematology Network Newsletter*, 5: 36-43.

Tayeb, TS & Abdel Aziz, NH 2010, In vitro evaluation of some bacterial isolates as biofertilizers and biocontrol agents against the second stage juveniles of *Meloidogyne incognita*. *World Journal of Microbiology and Biotechnology*, 26:2249-2256.

Tran, TH, Wang, S, Nguyen, V, Tran, D, Nguyen, D & Nguyen, A 2019, Study of novel endophytic bacteria for biocontrol of black pepper root- knot nematodes in the central highlands of Vietnam. *Agronomy*, 9: 714.

Vagelas, IK 2015, Novel bacteria species in nematode bio control. In: TH, Askari & PRH Mertinelli (Eds.) Bio control agents of phytonematodes. CABI, Wallingford.

Yadav, U. 2017. Recent trends in nematode management practices: the Indian context. *International Journal of Engineering Research & Technology*, 4: 482-489.

Youssef, MMA, Abd El Khair, H & El Nagdi WMA 2017, Management of root knot nematode, *Meloidogyne incognita* infecting sugar beet as affected by certain bacterial and fungal suspensions. *Agricultural Engineering International*, 9: 293-301.

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