Plant Bulletin
ISSN: 2959-3379 (Print), 2959-3387 (Online)

Research Article

Evaluation of Nematicidal Potential of Plant Growth Promoting Rhizobacteria against *Meloidogyne incognita*

[a]Muhammad Anwar ul Haq, [a]Muhammad Shahid, [a]Muhammad Zeeshan Niaz, [a]Kamra Mahmood, [a]Ussama Yaseen, [a]Muhammad Tariq Adnan Khan

[a] *Plant Pathology Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.*

[a] *Department of Plant Pathology, PMAS Arid Agriculture University, Rawalpindi, Pakistan.*

**Correspondence:* tariqadnan8123@gmail.com, anwaruaf@gmail.com

Article History

Received: July 10, 2022

Accepted: September 18, 2022

Published: December 30, 2022

ABSTRACT

Root-knot nematodes have a wide host range, causing damage to many annual and perennial crops. More than 100 species of *Meloidogyne* are known in which *M. incognita* (Kofoid and White) Chitwood is one of the most common and important plant parasitic nematode in tropical and subtropical regions of the world including Pakistan. Therefore, the present study was planned to check the effect of PGPRs on juvenile mortality and egg hatching inhibition *of M. incognita* under lab conditions. Eggs were isolated from egg masses by using NaOCl solution. Rhizobacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Azospirillum* sp., *Rhizobium leguminosarum*) were multiplied on nutrient broth and kept overnight at 28 ± 2 °C in shaking incubator. Cell free culture filtrates were obtained in falcon tubes by centrifuging PGPRs @ 4000 RPM twice for 30 minutes. Two ml of toxins of each PGPR was poured into each 5cm-dia. petri plate and 100 microliters of egg suspension containing approximately 100 eggs were put in each petri plate. Similar experimental conditions and protocols were used for juveniles' mortality. In this experiment, instead of eggs, 100 J2s contained in 100 microliters of juvenile suspension were put in each petri plate. The petri plates were placed in completely randomized design in incubator at 28 ± 2 °C with ten replicates. Petri dishes containing distilled water were kept under control. All the PGPRs caused larval mortality and inhibition in egg hatching with varying degrees. The maximum egg hatching was inhibited by *Bacillus subtilis* while *Rhizobium leguminosarum* was found the least effective. The other PGPRs i.e. *Pseudomonas fluorescens*, *Azotobacter chroococcum*, and *Azospirillum* sp. showed intermediate results.

Keywords: PGPR, Root knot nematode, Mortality, Hatching, *Meloidogyne incognita*, nematicides.

Introduction

Meloidogyne spp. obligate sedentary endoparasites of host plants which attack plant roots. Five root-knot species viz. *M. arenaria, M. graminicola*, *M. hapla, M. incognita,* and *M. javanica* out of more than 100 known *Meloidogyne* spp. are found more frequently in Pakistan as well as all over the world as major pests of vegetables, fruit plants and field crops (Anwar, 1989; Anwar et al., 1991; Anwar & Khan, 1992; Anwar and McKenry, 2012;

Eisenback et al., 1981; Fourie and McDonald, 2000; Hunt and Handoo, 2009; Mateille et al., 2000; Menjivar et al., 2011; Moens et al., 2009). Root knot nematodes are polyphagous and more than 3000 plant species have been reported as hosts of these nematodes (Abad et al., 2003; Agrios, 2005). Due to such wide host arrange, root knot nematodes cause major economic damage to vegetables, fruit plants and field crops and an estimated loss of 125 billion $ occurs annually worldwide (Chitwood, 2003; Collange et al., 2011; Dodzia et al., 2012; Koenning et al., 1999). In Pakistan as well as worldwide, 10-100% yield losses on vegetables were reported by many scientists (Anwar and McKenry, 2012; Kamran et al., 2010; Shahid et al, 2007).

Many management strategies i.e., host plant resistance; cultural practices, physical, biological and chemical methods are used for the management of root knot nematodes, but chemicals have given relatively quick and better results to farmers. Therefore, some microbial antagonists were potentially used in the replacement of chemical nematicides against root knot nematodes (Barker and Koenning, 1998; Brand et al., 2010; Hussain et al., 2014; Jairajpuri et al., 1990; Mukhtar et al., 2017; Nico et al., 2004; Siddiqui and Shaukat, 2003; Sikora and Fernandez, 2005; Veremis and Roberts, 1996; Whitehead, 1998). Plant growth promoting rhizobacteria (PGPR) have the potential as bio-control agents to substitute chemicals because they are ecofriendly and significantly reduce the disease.

Materials and methods

Single egg mass culture of *M. incognita*

Eggplants showing characteristic root knot symptoms (poor and stunted growth, and galled roots) were selected during surveys of vegetable fields. Plants were uprooted carefully along with rhizosperic soil, put in polythene bags, labeled properly and brought to the laboratory. Soil was detached from the roots and roots were washed under tap water carefully. After washing, the roots were cut into small pieces. Healthy light brown egg masses were picked singly with the help of needle and inoculated individually the five-week-old seedlings of tomato in the root zone. After 50 days of inoculation, tomato plants were harvested carefully, and root systems were cut into pieces. From each root system, fifteen mature whitish females were randomly isolated under stereoscope, put in 40% lactic acid and perineal patterns were made (Taylor, 1967). The perineal patterns were compared with standard diagrams and females were confirmed to be *M. incognita* (Eisenback et al., 1981).

Mass culturing of *M. incognita*

For mass culturing, three-week-old tomato seedlings (Money Maker) were transplanted singly in 15-cm-diam. pots having formalin sterilized soil. Two weeks after transplantation, two thousand eggs of *M. incognita* contained in 10 ml of water were inoculated in each plant in the root zone by making three holes around the stems of plants grown in pots containing 2 kg of sterilized soil. The earthen pots were watered according to their requirement. For the continuous supply of culture throughout the experiments, the same procedure was repeated regularly.

Collection of Bio-control Agents

Bio-control agents, plant growth promoting rhizobacteria (PGPRs) were collected from Plant Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad. Five PGPRs (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Azospirillum* sp., *Rhizobium leguminosarum*) were used for experimental purpose. These PGPRs were multiplied on Nutrient Broth for their culture maintenance.

Evaluation of inhibitory influence of PGPRs on egg hatching of *M. incognita*

The effect of cell free cultural filtrates of PGPRs was assessed on inhibition of eggs hatching of *M. incognita*. Eggs were isolated from egg masses by using NaOCl solution (Hussey and Barker, 1973). Rhizobacteria were multiplied on nutrient broth and kept overnight at 28 ± 2 °C in shaking incubator. Cell free culture filtrates were obtained in falcon tubes by centrifuging PGPRs @ 4000 RPM twice for 30 minutes. Two ml of toxins of each PGPR was poured into each 5cm-dia. petri plate and 100 microliters of egg suspension containing approximately 100 eggs were put in each petri plate. The petri plates were placed in completely randomized design in incubator at 28 ± 2 °C with ten replicates. Petri dishes having distilled water were kept as control. Data were recorded after 24, 48, 72, 96 and 120 hours and percentage egg inhibition were calculated using.

$$\text{Percentage Egg Inhibition} = \frac{\text{Number of Eggs Hatched}}{\text{Total number of Eggs}} \text{X100}$$

Evaluation of nematicidal potential of PGPRs on the mortality of *M. incognita* juveniles

The effect of cell free cultural filtrates of PGPRs was investigated against mortality of *M. incognita* juveniles. Similar experimental conditions and protocols were used as described in pervious experiments. In this experiment, instead of eggs, 100 J2s contained in 100 microliters of juvenile suspension were put in each petri plate. Data were recorded after 24, 48, 72, 96 and 120 hours and percentage mortality were determined using.

$$\text{Mortality Percentage} = \frac{\text{Number of Juveniles killed}}{\text{Total number of Juveniles}} \text{X100}$$

Straight, unmoved J2s were considered as dead if they did not move after pinching with fine bamboo needle. Dead J2s were also transferred in distill water to confirm their death.

Results and Discussion

Efficacy of PGPR cell free cultural filtrates on egg hatching of *M. incognita*

Cell free cultural filtrates of five PGPRs were evaluated for *in vitro* percent egg hatching inhibition against *M. incognita* at different time intervals at 30 ± 2 °C. All the five PGPRs showed great potential to inhibit the percent egg hatching over control. After 24 hours, the maximum % egg hatching inhibition was examined in *Bs* (0.400) fallowed by *Pf* (1.4), *Azto* (2.50), *Azo* (3.60) while the minimum was in *Rhiz* (6.60) as compared to control (40.30) (Table 4.2). Similar trend was noted in all the treatments after 48, 72, and 96 hours after exposure to filtrates. Egg hatching was increased after exposure to filtrates but after 120 hours, the maximum % egg hatching inhibition was examined in *Bs* (21.400), while *Pf*, *Azto* and *Azo* (25.20, 27.30, and 29.90) showed intermediary results. The minimum percent egg inhibition was found in *Rhiz* (35.30) over control (98.70) (Table 1).

The maximum percent egg hatching was inhibited (99.00, 89.76, 83.42, 80.80, and 78.31) by Bs while Rhiz showed the minimum percent egg inhibition (83.62, 73.24, 69.40, 71.09, and 64.23) while all other treatments revealed intermediary results after 24, 48, 72, 96 and 120 hours respectively (Figure 1).

Table 1. Efficacy of PGPRs cell free culture filtrates on egg hatching of *M. incognita.*

*Treatments	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
Bs	0.40 e	5.70 e	11.70 e	17.20 e	21.40 f
Pf	1.40 de	7.80 d	14.30 d	19.70 d	25.20 e
Azto	2.50 cd	9.70 c	15.80 cd	21.10 d	27.30 d
Azo	3.60 c	11.40 c	17.20 c	23.70 c	29.90 c
Rhiz	6.60 b	14.90 b	21.60 b	25.90 b	35.30 b
Control	40.30 a	55.70 a	70.60 a	89.60 a	98.70 a

Means within a column sharing the same letters are not significantly different from each other at P =0.05 according to Tukey HSD Test

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp., Rhiz = Rhizobium leguminosarum.*

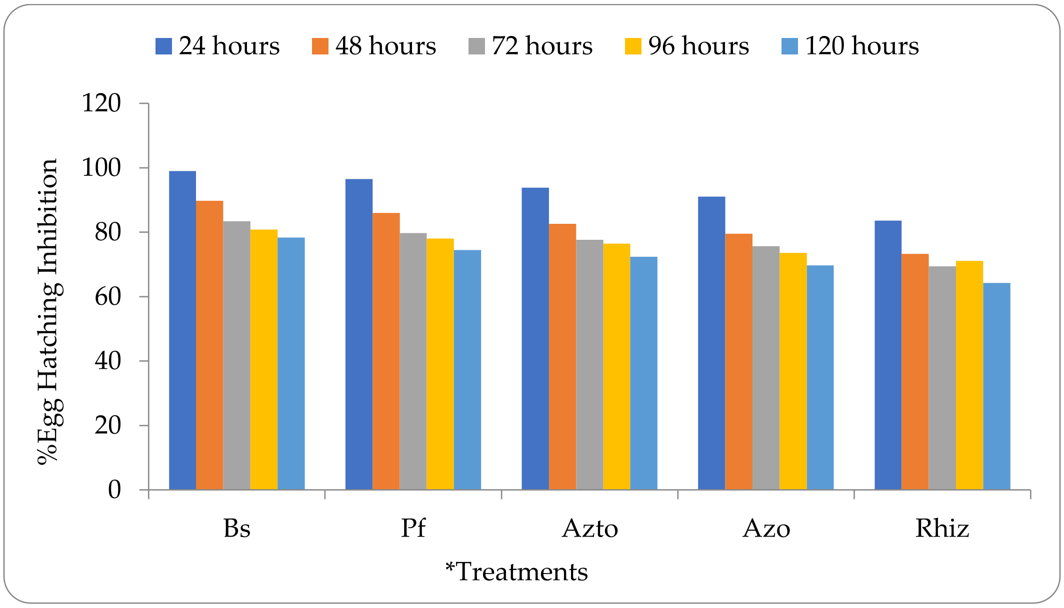

Figure 1. Efficacy of PGPRs cell free cultural Filtrates on % egg hatching inhibition of *M. incognita*

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp., Rhiz = Rhizobium leguminosarum.*

Efficacy of PGPR Cell Free Cultural Filtrates on Juvenile Morality of *M. Incognita*

Cell free cultural filtrates of five PGPRs were also evaluated *in vitro* against juvenile mortality of *M. incognita* at different time intervals at 30 ± 2 °C. All the PGPRs caused the mortality of J2s with varying degrees. After 24 hours, the maximum mortality of J2s was examined in *Bs* (44.7) followed by *Pf* (42.3), *Azoto* (41.2), and *Azo* (39.8) while the minimum mortality was observed in *Rhiz* (32.7) as compared to control (00) (Table 4.3). A similar tendency was recorded in all the treatments after 48, 72, and 96 hours after exposure to filtrates. J2s mortality increased after exposure to filtrates and after 120 hours, the maximum J2s mortality was examined in *Bs* (94.5), while *Pf*, *Azoto* and *Azo* (90.1, 86.2, and 79.5 respectively) showed intermediate results. The minimum J2s mortality was found in *Rhiz* (75.5) as compared to control (00) (Table 2).

Table 2. Efficacy of PGPRs cell free cultural filtrates on juveniles' mortality of *M. incognita*

*Treatments	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
Bs	44.7 a	60.1 a	72.2 b	84.5 a	94.5 a
Pf	42.3 b	55.7 b	78.2 a	80.3 b	90.1 b
Azto	41.2 bc	59.9 a	69.7 c	76.5 c	86.2 c
Azo	39.8 c	54.2 b	62.9 d	72.0 d	79.5 d
Rhiz	32.7 d	51.2 c	60.2 e	66.1 e	75.5 e
Control	0.00 e	0.00 d	0.00 f	0.00 f	0.00 f

Means within a column sharing the same letters are not significantly different from each other at P =0.05 according to Tukey HSD Test

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp., Rhiz = Rhizobium leguminosarum*

After 120 hours, the maximum J2s mortality over control was observed in *Bs* (94.5) while *Rhiz* showed the minimum J2s mortality (75.5) while the rest of the treatments showed intermediary results (Figure 2).

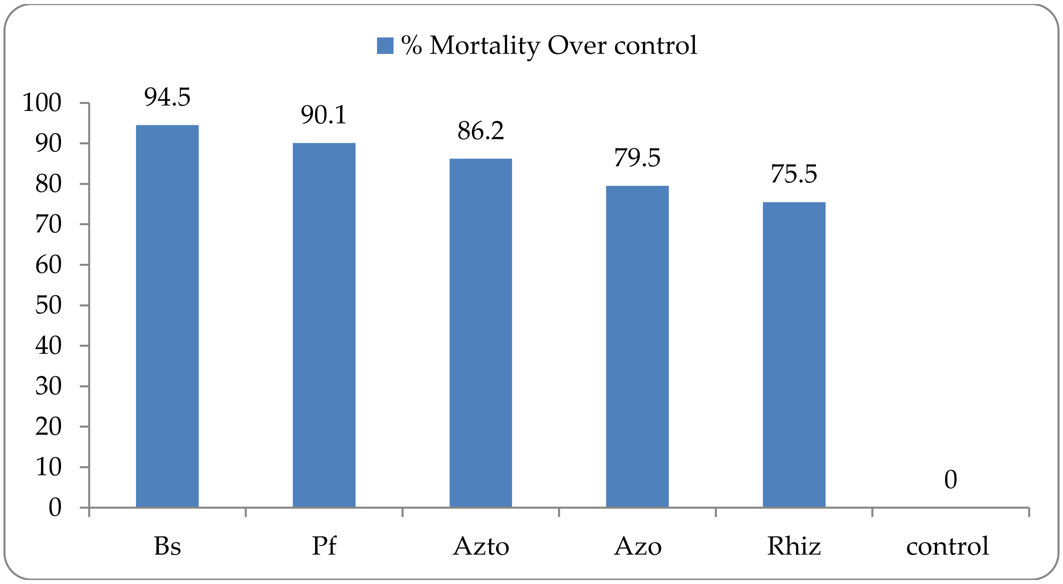

Figure 2. Efficacy of PGPRs cell free cultural filtrates on % juveniles' mortality of *M. incognita* after 120 hours

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp., Rhiz = Rhizobium leguminosarum.*

Many researchers investigated the effects of PGPR metabolites, toxins and suspensions against egg hatching and juvenile mortality of *Meloidogyne* spp. El-Moneim and Massoud (2009) tested the endotoxin of four isolates of *Bacillus thuringiensis* (AI, AII, AIII and AIV) against juveniles of *M. incognita*. AI and AII isolates with crystal toxin (1×10^8 crystal/ml) gave the best results against J2s. Ravari and Moghaddam (2015) tested the nematicidal activity of two *B. thuringensis* strains (ToIr 65 and ToIr 67) against eggs and juveniles of *M. javanica* in the form of bacterial suspension and spore/crystal mixture in lab. Bacterial suspensions of two strains revealed 70% nematicidal efficacy as compared to spore/crystal mixture. Meyer et al. (2009) evaluated 2,4-diacetylphloroglucinol (DAPG) antibiotic produced by various isolates of *P. fluorescens* against eggs and hatched juveniles of many plant parasitic nematodes (*Pratylenchus scribneri, Xiphinema*

https://doi.org/10.55627/pbulletin.001.02.0222

americanum, Heterodera glycines and *M. incognita*) and bacterial feeder nematodes (*Rhabditis rainai, Caenorhabditis elegans* and *Pristionchus pacificus*) in different doses from 0 to 100 mg/ml of DAPG in laboratory. Antibiotic DAPG decreased the egg hatching.

The nematicidal action of culture filtrates of PGPRs against *Meloidogyne* spp. and other plant parasitic nematodes might be ascribed to the production of certain enzymes (Segers et al., 1994; Webb et al., 1972) and toxins (Minato et al., 1973) which help in weakening and dissolving the barriers of their hosts. In the current study, PGPRs strains effectively inhibited egg hatching by changing the eggshell structures due to protease, chitinase and chitosanase activities. Soliman et al. (2019) showed that eggs exposed to chitinase enzyme, more and large vacuoles in the chitin layer of eggshell resulting in an increase in percentage mortality of juveniles. These results agree with those described by Mercer et al. (1992). Soliman et al. (2019) also found that *B. subtilis* and *Paenibacillus polymyxa* produced maximum chitinase enzyme which caused egg hatching inhibition and J2s mortality. From this perspective, Sohrabi et al. (2018) reported that the rhizobacterial strains *P. polymyxa* and *B. subtilis* significantly caused reductions in the juvenile population of *M. javanica*. Batool et al. (2013) revealed that above 90% mortality of *M. javanica* in *in vitro* studies occurred due to high production of chitinase. The bacterial strains tested in the current study may be promising biocontrol agents as PGPR for the future nematode management strategies.

Data availability

The authors declare that the data supporting the findings of this study are available within the article.

Declarations ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

References

Abad, P., Favery, B., Rosso, M. N., & Castagnone-Sereno, P. (2003). Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Molecular Plant Pathology, 4*(4), 217-224.

Agrios, G. N. (2005). *Plant Pathology*: Academic press.

Anwar, S. (1989). Investigations on nematodes associated with field, vegetable and fruit crops. *Technical Bulletin. Barani Agricultural College, Rawalpindi, Pakistan*.

Anwar, S. A., & McKenry, M. (2012). Incidence and population density of plant-parasitic nematodes infecting vegetable crops and associated yield losses in Punjab, Pakistan. *Pakistan Journal of Zoology, 44*(2), 327-333.

Anwar, S., & Khan, M. (1992). Evaluation of four vegetables against *Meloidogyne incognita*. *Journal of Agricultural Research, 30*, 415-421.

Anwar, S., Gorsi, S., Anwar-ul-Haq, M., & Yousuf, P. (1991). Plant parasitic nematodes of some field, vegetable, fruit and ornamental crops. *Journal of Agricultural Research (Pakistan), 29*, 233-249.

Barker, K. R., & Koenning, S. R. (1998). Developing sustainable systems for nematode management. *Annual Review of Phytopathology, 36*(1), 165-205.

Batool, H., Fatima, N., Hira, K., Sultana, V., Ara, J., & Ehteshamul-Haque, S. (2013). Role of *fluorescent Pseudomonas* associated with root nodules of soybean in suppressing the root rotting fungi and root knot nematode of soybean in soil amended with seeds of *Vernonia antihelmenthica*. *International Journal Biology Research*, *1*, 73-79.

Brand, D., Soccol, C., Sabu, A., & Roussos, S. (2010). Production of fungal biological control agents through solid state fermentation: A case study on *Paecilomyces lilacinus* against root-knot nematodes. *Micologia Aplicada International, 22*(1), 31-48.

Chitwood, D. J. (2003). Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture–Agricultural Research Service. *Pest Management Science: Formerly Pesticide Science, 59*(6-7), 748-753.

Collange, B., Navarrete, M., Peyre, G., Mateille, T., & Tchamitchian, M. (2011). Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agronomic system analysis. *Crop Protection, 30*(10), 1251-1262.

Dodzia, B. K., Attia, T., Kodjoa, T. A., Affoha, A., & Mawuenaa, G. (2012). Impact de la fumure organique appliquée seule et en combinaison avec une souche indigène de champignon mycorhizien arbusculaire *Glomus mosseae* sur *Meloidogyne* spp, principal nématode parasitaire de la tomate au Togo. *Journal of Applied Biosciences, 55*, 3973-3986.

Eisenback, J., Hirschmann, H., Sasser, J., & Triantaphyllou, A. (1981). A guide to the four most common species of root-knot nematodes Meloidogyne species with a pictorial key. *State University, Department. of Plant Pathology*. 48 p

El-Moneim, T. S. A., & Massoud, S. I. (2009). The effect of endotoxin produced by *Bacillus thuringiensis* (Bt.) against *Meloidogyne incognita*. *Egyptian Journal of Natural Toxins, 6*(1), 83-93.

Fourie, H., & McDonald, A. (2000). Nematodes. ARCLNR leaflet. *Crop Protection, 18*(4).

Hussain, M. A., Mukhtar, T., & Kayani, M. Z. (2014). Characterization of susceptibility and resistance responses to root-knot nematode (*Meloidogyne incognita*) infection in okra germplasm. *Pakistan Journal of Agricultural Sciences, 51*(2), 319-324.

Hussey, R. S., & Barker, K. R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter*, *57*, 1025-1028.

Kamran, M., Anwar, S., Javed, M., Khan, S., & Sahi, G. (2010). Incidence of root-knot nematodes on tomato in Sargodha, Punjab, Pakistan. *Pakistan Journal of Nematology, 28*(2), 253-262.

Koenning, S., Overstreet, C., Noling, J., Donald, P., Becker, J., & Fortnum, B. (1999). Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. *Journal of Nematology, 31*(4S), 587-618.

Mercer, C., Greenwood, R., & Grant, J. (1992). Effect of plant and microbial chitinase on the eggs and juveniles of *Meloidogyne hapla* Chitwood (Nematoda: Tylenchida). *Nematologica, 38*, 227-236.

Meyer, S. L., Halbrendt, J. M., Carta, L. K., Skantar, A. M., Liu, T., Abdelnabby, H. M., & Vinyard, B. T. (2009). Toxicity of 2, 4-diacetylphloroglucinol (DAPG) to plant-parasitic and bacterial-feeding nematodes. *Journal of Nematology, 41*(4), 274-280.

Minato, H., Matsumoto, M., & Katayama, T. (1973). Studies on the metabolites of *Verticillium* sp. structure of verticillins A, B and C. *Chemistry Communication,* 1819-

1825.

Moens, M., Perry, R. N., & Starr, J. L. (2009). *Meloidogyne* species A diverse group of novel and important plant parasites. *Root-Knot Nematodes, 1*, 483.

Segers, R., Butt, T. M., Kerry, B. R., & Peberdy, J. F. (1994). The nematophagous fungus *Verticillium chlamydosporium* produces a chymoelastase-like protease that hydrolyses host nematode proteins *in situ*. *Microbiology*, *140*, 2715-2723.

Shahid, M., Rehman, A., Khan, A., & Mahmood, A. (2007). Geographical distribution and infestation of plant parasitic nematodes on vegetables and fruits in the Punjab province of Pakistan. *Pakistan Journal of Nematology, 25*(1), 59-67.

Sikora, R., & Fernandez, E. (2005). Nematode parasites of vegetables, Luc M., Sikora, R. A., and Bridge J.(Eds.), Plantpar asitic Nematodes in Subtropical and Tropical Agriculture. *Wallingford, UK: CABI Publishing*, 39-319.

Sohrabi, F., Sheikholeslami, M., Heydari, R., Rezaee, S., & Sharifi, R. (2018). Evaluation of four rhizobacteria on tomato growth and suppression of root-knot nematode, *Meloidogyne javanica* under greenhouse conditions, a pilot study. *Egyptian Journal of Biology Pest Control*, *28*, 56-60.

Soliman, G. M., Hoda Hussein Ameen, H. H., Abdel-Aziz, S. M., & Ghada M. El-Sayed, G.M. (2019). In vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. *Bulletin of the National Research Centre*, *43*, 171.

Taylor, A. L. (1967). Introduction to research on plant Nematology. An FAQ guide to study of plant parasitic nematodes. *Food and Agriculture Organization, United Nation, Rome*, 133p.

Veremis, J., & Roberts, P. (1996). Relationships between *Meloidogyne incognita* resistance genes in *Lycopersicon peruvianum* differentiated by heat sensitivity and nematode virulence. *Theoretical and Applied Genetics, 93*(5-6), 950-959.

Webb, H. M., Ghafoor, A., & Heale, J. B. (1972). Protein and enzyme patterns in strains of *Verticillium. Transaction of the British Mycology Society, Elsevier, 59*(3), 393-402.

Whitehead, A. (1998). Plant nematode control. *CAB International, 31*(07), 2009.