Effect of some Biocides and Entomopathogenic Nematodes on Suppressing Root-Knot Nematode, *Meloidogyne Incognita* Infecting Fig Plants under Green House

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ABSTRACT

Anti-nematodes properties of some bio-products viz., BioNematon (a.i. *Paecilomyces lilacinus*) BioZeid® (a.i. *Trichoderma album*), BioArc® (a.i. *Bacillus megaterium*), NemaStop® (a.i. *Streptomyces avermitilis*), Anti-Nema (a.i. *Serratia marcescens*) besides two native entomopathogenic nematode (EPNs) identified as *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. Results showed that post RKN- inoculation application of Biocides and EPNs were better than pre addition. The three selected bio-products were significantly inhibited nematode indices; BioNematon was the best as it achieved 68.2% reduction in final nematode population followed by Anti-Nema 63.5% while the lowest effect (59.7%) was achieved by NemaStop. Also the fig growth was improved after addition of various bio-products and EPNs. In this respect further studies, including various conditions of soil, climate and different agrochemicals already used in crop production, are important before expanding the application of these bio-nematicides. also the optimizing the use of EPNs, besides economic view must be considered. Additionally searching for novo species of EPNs in Egyptian soils are recommended for obtained effective biocontrol agents against phytonematodes.

Keywords: *Bio-nematicides; Entomopathogenic nematodes, Biological control; Nematicidal activity.*

INTRODUCTION

Plant parasitic nematodes (PPNs) are serious pests that cause considerable crop losses estimated by more than one hundred or about 173 billion dollars universally **(**Gamalero & Glick, 2020 and Kantor *et al.,* 2022). Root-knot nematode (RKN) consider one of the most important genus of PPNs, this due to many reasons like; its wide host range as it can attack more than 5000 plant hosts, live inside the roots as it endoparasitic and have a great adverse impacts on their hosts health, besides interaction with other soil pathogen especially wilt pathogens or some viruses (Jones *et al.,* 2013 and Ntalli, 2020).

Managing phytonematodes are essential process to keep their damage at the lowest level or completely avoiding their impact. The easiest way for combating phytophages nematodes is synthetic nematicides. The use of conventional chemical pesticides is known to be accompanied by various hazardous effects as these compounds easily vaporize and accumulate in the ecosystem and effect on all environment components including non-target organisms and subsequently the man health. Accordingly, they are being progressively restricted, currently many effort devoted for using more safe alternatives that became an urgent need. Generally, control strategies including physical measures such as solarization, cultural practices like as crop rotation and biological control using various agents like fungi, bacteria, actinomycetes and predaceous nematodes. Recently, biological control approach had attained importance in agriculture production to minimize the hazards of pesticides. Interaction between various organisms and parasitic nematodes are beneficial when nematode population was reducing to non-harmful level. Fungi like Paecilomyces and Trichoderma have been reported to suppress plant parasitic nematodes (Isaac *et al.,* 2021; Massoud *et al.,* 2021; Ibrahim *et al.,* 2019; Abo-Korah *et al.,* 2022 and Khalil, *et al.,* 2022). Bacteria can effect on PPNs via some mode of actions; siderophores production, antagonistic products or induced plant resistance (El-Nuby, 2014, Metwaly & Zawam, 2015; Mostafa *el al.,* 2018; Ibrahim *et al.,* 2019; Ramalakshmi *et al.,*2020 Abo-Korah *et al.,* 2022). Actinomycetes like *Streptomyces avermitilis* and others were used to suppress nematodes (Ruanpanun & Chiradej, 2015; Liu *et al.,* 2019 and Metwally *et al.,* 2019).

Entomopathogenic nematodes (EPNs) such as *Heterorhabditis* and *Steinernema* spp. were found to interact with phytonematodes and inhibit their population (Caccia *et al,* 2012; El-Aatif *et al.,* 2015; Ashry *et al.,* 2018; El Aimani *et al.,* 2022: Li *et al.,* 2023; Srivastava *et al.,* 2022 and Yang *et al.,* 2022). The symbiotic bacteria *Xenorhabdus* spp. (motile, gram-negative bacteria) live in symbiosis with the genus *Steinernema* and *Photorhabdus* spp.

(bioluminescent, gram-negative bacilli) is endosymbionts for *Heterorhabditis* nematodes possessed harmful effects on PPNs and showed considerable nematicidal activity (Danilov and Kaplin, 2020; Li *et al.,* 2023).

Biopesticides**,** also known as biological pesticides, are certain type of pesticides that have detrimental effect on specific pests. They obtained from various natural materials like animals, microorganisms (Fungi & Bacteria), plants and certain minerals. They can categorize to three major items namely; Biochemical pesticides, Microbial pesticides and Plant-Incorporated-Protectants (Anonyms, 2022). Some of using biopesticides' advantages over synthetic chemicals are; safe to environment, affect only the specific pest, inhibit or prevent pesticide resistance development, and low production cost, less harm to beneficial species and are biodegradable (Pratibha, 2017). Biopesticides are a valuable component of pest control strategies for various pests.

Fig production represent an economic importance in Egypt and particularly in western north coast when the small holder farmers (Bedouins), as they profit from selling fig fruits. One of the major pathogens of Fig is parasitic nematodes called the root-knot nematode (RKN), which considering the most damaging nematodes to fig trees, *Ficus carica* (Abrantes *et al.,* 2008). Additional current reports stated that RKN is a devastating pest affecting fig production worldwide (Rodriguesa *et al.,* 2022). Accordingly, chemical control are the most common method for managing RKN, that mean more pollution for ecosystem, so adopting of biological control as an ecofriendly strategy for controlling PPns are considerable mean. The aims of this research are to evaluate the antinematodal efficacy of five biopesticides namely; BioNematon (a.i. *Paecilomyces lilacinus*) BioZeid® (a.i. *Trichoderma album*), BioArc® (a.i. *Bacillus megaterium*), NemaStop® (a.i. *Streptomyces avermitilis*) and Anti-Nema (a.i. *Serratia marcescens*) besides some Egyptian isolates of entomopathogenic nematodes under lab and greenhouse conditions. Also the impact of these treatments on Fig seedlings growth was studied.

MATERIALS AND METHODS

Preparation of root-knot nematode culture

Meloidogyne incognita pure population was maintained on tomato plants in the greenhouse as source of nematode for experimental studies. Second stage Juveniles of RKN were extracted from tomato roots by allowing the egg to hatch in distilled water supplemented with air pump. Newly hatched J_2s were used through two days in the nematicidal assay.

Source of entomopathogenic nematodes

Nine EPNs, isolates were selected according their nematicidal properties against RKN from 148 isolates (which recovered from samples collected during the survey conducted in 2021 and 2022 in northern Egypt). According to preliminary screening (Data not shown), the nine EPNs strains were chosen and reared on *Galleria mellonella* at 25 °C, according to van Zyl C (2012). Dead larvae of *G. mellonella* were placed on white trap 48 hours and infective juveniles (IJs) were harvested then stored at 8 °C in a 500 ml container filed with distilled water. the viability of nematodes was checked by observing the movement of IJs before use. After screening against RKN the most effective two EPNs were selected and morphologically identified as *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* then used in pot experiments.

Source of commercial biocides

Five commercial biocides (Table1) were tested for their antinematodal activity towards RKN *M. incognita* in pot experiments which available in Egyptian market. All compounds were purchased from the Soil, Water and Environment Research Institute, Agricultural Research Center, Giza.

Greenhouse pot experiments

Evaluation of certain biocides and EPNs against M. incognita applied pre or post inoculation

Fig, *Ficus carica*, seedlings (variety Sultani 1) were planted individually in 2.5 liter (20 cm diameter) plastic pots filled with approximately 2.5 kg of autoclaved soil mixture (2 sand :1 clay). After 4 weeks, Fig seedlings were inoculated with 3500 J2s of *M. incognita* in 6.0 mL. Nematode juveniles were delivered to plants by pipetting the suspension above the roots after removing the surface layer of soil particles. Five commercial bio-products viz., BioNematon, Anti-Nema, NemaStop, BioArc and BioZeid were used for pot experiment. The commercial products were drenched to Fig seedlings one week after and one week before from *M. incognita* inoculation. Two genera of EPNs; *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*

were used also in greenhouse investigation by rate of 2500 infective juveniles stage per pot after one day before and one day after inoculation. Nematicide Vydate® 240/liter Oxamyl, was applied to soil at the rate of 0.3 ml/plant served as a positive check. Three plants were nematized only to represent control plants, besides unnematized three (only received water) served as healthy check. All treatments were arranged in a completely randomized design and replicated 3 times and kept in a greenhouse bench in DRC. Two months after nematode inoculation, experiment was terminated and fig plants were uprooted then gently washed. Nematological parameters included; gall number, egg masses numbers, developmental stages, eggs per egg mass and final population (summation of DS+MF+EM+ total eggs) besides the reduction percentage (R%) were calculated via equation; (R%) = [(control - treatment)/ control]*100

Also plant growth parameters were recorded including weight and length of each roots and shoots. The increment percentage (I%) due to application of treatments was calculated using the following equation; (I%) = [(treatment - control)/ treatment]*100

According the results of this experiment the best three biocides were selected besides the previously selected two EPNs for revaluation and validation their efficacy in final experiment in the proper application time (per or post inoculation).

Evaluation of three selected Biocides and two EPNs on suppression M. incognita

This experiment was conducted to re-valuate and validate the efficacy of the best 3 biocides, Bionematon, Anti-nema and Nemastop, beside the EPNs on Fig, seedlings infected with *M. incognita*. The same conditions were offered as the previous experiments (as mentioned above) except the treatments were added one week after RKN-inoculation. All nematode criteria were recorded as above after two months form inoculation also plant growth parameters were recorded similar to the prior experiment.

Statistical analysis

Meloidogyne javanica and plant parameters were subjected to ANOVA procedure using the SPSS software, ver. 17. A two-way ANOVA test was performed to examine sources of variation in the observed variables. Significant differences among variables were tested using Duncan at $P < 0.05$.

RESULTS

The application of the five biocides and two EPNs either pre inoculation or post inoculation were carried out to show the proper time for gained maximum nematicidal activity. Data in table 2 revealed that all tested nematode criteria were diminished by application EPNs when add before or after RKN-inoculation. BioNematon decreased the percentage of galls formed in fig roots by 40.7% when applied after inoculation vs 37.1% before inoculation, followed by Anti-Nema then NemaStop. The dissenting trend was observed in EPNs as they hinder the penetration on RKN and subsequently reduced the formation of galls when applied before inoculation of RKN; *S. carpocapsae* achieved reduction percent in gall numbers 31.5% and 27.4% in pre and post inoculation adding, respectively. *H. bacteriophora* behaved like *Steinernema* where it recorded 44% and 41.9% reduction in gall numbers wen applied pre and post, successively. The final population were reduced after applying various biocides and EPNs to fig plants infecting with *M. incognita*. The highest reduction in final population was recorded by *H. bacteriophora* when applied post-inoculation (83.4%) followed by *S. carpocapsae* (75.4%). Concerning biocides; BioNematon treatment was surpass other treatments achieving 74.5% reduction in final population, the Anti-Nema was occupied the second rank after BioNematon in reducing final population by 70.8%m while the third place went to NemaStop as it reduced the final population by 68.9%. The rest biocides BioArc and BioZeid were less effective in suppressing nematode.

The antinematodal activity of selected three biocides were examined again to validate and confirm their efficacy under greenhouse conditions with considering the proper time of application (pre or post RKN- inoculation). Data in Table 3 showed that all treatments able to diminish nematode parameters under investigation. EPNs were the most effective treatments compared to biocides tested. *H. bacteriophora* was the best treatment caused reduction in nematode criteria soil population, galls, developmental stages, immature females, mature females, egg masses, eggs per egg mass as well as final population. The reduction in final population achieved by *H. bacteriophora* (82.8%) while *S. carpocapsae* recorded 74.2%. The three biocides examined showed antinematodal activity varied according the biocides type; the highest effect was obtained from application of BioNematon as it

decreased galls by 59.8% and egg masses by 41.3% as well as final population by 68.2%. Anti-Nema occupy the second category in reducing nematode parameters, it caused reduction in egg masses (36.4%) and final population (63.5%). The bio-compound NemaStop was reduced the total population by 59.7% compared to control which received nematodes only. The nematicide Oxamyl was recorded the highest reduction (89.3%) in nematode final population and this achievement was near to *H. bacteriophora* effect.

The plant growth was positively responded to application of biocides and EPNs. Results in Table 4 showed increasing in growth parameters; root weight was enhanced by 17.2% and 15.8% in Steinernema and Heterorhabditis, respectively. The shoots mass of fig also enhanced by application of various treatments; EPNs recorded 13-14% increment in shoot weight, the Biocide showed similar effects and BioNematon was the best promotor. Root length was increased in EPNs treatments more than other biocides and Steinernema was better than Heterorhabditis without significant differences. The biocides achieved increase in root length and the BioNematon was more effective achieving 35.6% than the rest biocides. Shoot length was enhanced as a result of application EPNs and biocides; the highest increment in length of shoot was achieved by Steinernema and Heterorhabditis, consequently.

The bio-product also caused increase shoot length of fig plants; BioNematon was the best promoter biocide which improved plant length followed by Anti-Nema and NemaStop without significant differences. Chlorophyll content (CC) in fig leaves was slightly increased by application all treatments. *S. carpocapsae* caused the maximum increment in total chlorophyll content (7.9%). The biocide BioNematon was best bio-product as it increase CC by 7.8% over infected control, followed by Anti-Nema (7.3%) and NemaStop (6.3%).

DISCUSSION

Evaluation of tested commercial biocides against PPNs *in vivo* was carried out to select the most nematode suppressor. Results showed that all tested bio-nematicides possessed various antinematodal effects. These findings are in harmony with (Metwally *et al.,* 2019; Isaac *et al.,* 2021 and Massoud *et al.,* 2021). BioNematon (*P. lilacinus*) was the best biocide; it significantly reduced nematode parameters compared to control, these results are in accordance with previous results of Kiewnick and Sikora, (2006). Recently, other researchers proved the nematode suppression potential of Bio- Nematon and Paecilomyces isolates (Ibrahim *et al.,* 2019; Almohithef *et al.,* 2021; Ahmed *et al.,* 2022; Abo-Korah 2022 and El-Marzoky *et al.,* 2023).

The antinematodal activity of BioNematon® which contains *Paecilomyces lilacinus* fungus as a bioagent may due to increasing frequency in the treated rhizosphere of saprophytic fungi such as (El-Nagdi *et al*., 2011). *P. lilacinum* possessed a highly egg parasitizing ability (80%) as well as egg masses and cysts (Goswami and Mittal, 2002 and Sharf *et al.,* 2011). It can also infect different stages of the genus *Meloidogyne* spp. (Yang *et al.,* 2015). Collectively, the bioactivity of *P. lilacinus* towards nematodes including suppressing egg hatching via direct parasitism and colonization, production of toxic metabolites as well as lytic enzymes also attacking various nematode stages (Ahmad *et al.,* 2019) besides induced plant resistance. It should consider that the reduction in nematode parameters such as formed gall and reproduction factor is dependent on plant, fungus, and nematode species as well as prevailing environmental conditions (Campos, 2020).

The antinematodal effect showed by Anti-Nema® which contain bacterium *Serratia marcescens*, was documented by other researchers; result of Mokbel and Alharbi (2014) showed that *S. marcescens* achieved 62% mortality of nematode juveniles. exposure time. *S. marcescens* significantly reduced number of egg masses (83.7%) as well as soil population (80.7%), respectively. Zaghloul et al. (2015) found that *S. marcescens* was the best antagonist to RKN as it kill about 99.1% of second stage juveniles of *M. incognita.* They also mentioned that this bacterium possessed a chitinolytic, protelytic and gelatinolytic activity according to their secreted enzymes viz., Chitinase, Protease and Gelatinase. Mohamd et al. (2020) showed reduction in rate of build-up of RKN as a result of using *S. marcescens* on infected tomato plants. The growth parameters were improved after treating with *S. marcescens*. The Serratia nematicidal properties is perhaps not only attributed to the chitinolytic activity of the strain, but also to the activity of various enzymes (Méndez-Santiago *et al.,* 2020).

The *Streptomyces avermitilis* represent an active ingredient of NemaStop® showed nematicidal activity against *M. incognita* in current study. Various researchers exploited

actinomycetes for selected the nematode antagonistic isolates; Jonathan et al. (2000) found that tested actinomycetes isolates decreased gall numbers of *M. incognita* on tomato in compared with control and enhanced the crops growth. *Streptomyces avermitilis* is a soil bacterium that has the ability to produce secondary metabolites like abamectin, which comes from mixing of two types of avermectins, which showed nematicidal activity against RKN in a many crops under different conditions (Jayakumar *et al.,* 2005 and Khalil, 2013). Avermectins block the transmittance of electrical activity in nerves and muscle cells by stimulating the GABA (y-aminobutyric acid) release and binding it at nerve endings which leads to subsequent paralysis of the neuromuscular systems and then death (Burkart, 2000 and Martin *et al.,* 2002).

BioZeid®, *Trichoderma album,* showed antinematodal properties because it suppress nematode development and multiplication, this finding are in harmony with the finding of (El-Nagdi *et al.,* 2011; Metwally *et al.,* 2019; Ahmed *et al.,* 2022 and Khalil *et al.,* 2022). Moreover, the antagonist potential of Trichoderma genus toward RKN was proved by other researchers (Al-Hazmi and Javeed, 2016). Also other PPNs genera like citrus nematode was suppressed by application *T. harzianum* (Ibrahim *et al.,* 2019). Trichoderma fungi have different proposed mechanisms of action such as competition on space and nutrients with the pathogen, suppressing reproduction of PPNs by secreting toxic metabolites either volatile or nonvolatile, antibiosis, enhancing plant growth and induce the plant resistance toward specific pathogen and production of lytic enzymes that degrade nematode cuticle like chitinases and proteases (Harman, 2006 and Vey *et al.,* 2001). Other researchers demonstrated that two actions of Trichoderma spp. against phytonematodes the first is direct parasitism of eggs, juveniles and females (Suárez *et al.,* 2004; Yang *et al.,* 2010) and the second is enhancing plant defense through the increase in enzymatic activities (Sahebani and Hadavi, 2008). Additionally, plant growth enhancement due to application of Trichoderma may be a results of Root colonization by this fungus and subsequently enhances root growth and development, crop productivity, resistance to abiotic stresses and uptake nutrients (Sharon *et al.,* 2001).

The results of current study appeared the antagonistic activity of commercial biocide named BioArc®, *Bacillus megaterium* towards *M. incognita* also showed antinematodal properties because it suppress nematode development and multiplication, this result are in the same track with the result –(El-Nagdi *et al.,* 2011; Radwan *et al.,* 2011 and Mostafa *et al.,* 2018). Improving of Fig growth due to adding bio-arc was supported by other finding (El Deriny, 2009 and El-Zawahry *et al.,* 2015). Similarly, results of indicate that bio-arc enhanced greatest improvement in total plant mass (Mostafa *et al.,* 2018 and Metwally, *et al.,* 2019). Furthermore, Huang et al. (2009) stated that of *B. megaterium* possessed nematicidal activity against *M. incognita* through the production of nematicidal volatiles. Increasing of plant growth parameters as a result of application of *B. megaterium*, may be attributed some factors such as it can help in availability of phosphorus to plant, producing growth promotes, improving uptake of water and nutrients, production antagonistic metabolites and kinds of vitamin B that induce rooting process and adversely impacted on soil microbiome (Rai, 2006).

The utilization of Entomopathogenic nematodes (EPNs) for suppressing PPNs and antagonistic effects of them towards phytonematodes had been documented previously; by some researchers (Pérez and Lewis, 2004; Caccia *et al.,* 2012; Sayedain *et al.,* 2021 and El Aimani *et al.,* 2022). In current study, the antagonistic activity of two native EPNs (*S. carpocapsae* and *H. bacteriophora*) were assessed against the RKN, *M. incognita* infecting fig plants under greenhouse conditions, all *M. incognita* criteria were significantly suppressed by different EPNs treatments applied to the soil. the negative effects of the direct application EPNs in this study are compatible with previous investigation; Caccia et al. (2012) mentioned that PPNs populations was reduced in the presence of EPNs. Similarly, Sayedain et al. (2021) stated that *H. bacteriophora* and *S. carpocapsae* were diminished all the RKN-pathogenicity parameters under greenhouse conditions. It was reported that EPNs species are varied in its efficacy against PPNs species (Pérez and Lewis, 2004), they found that the tested EPNs were able to suppress the penetration of *M. incognita* and minimize the number of eggs/egg mass on tomato plants, but these EPNs were not efficient against *M. javanica*. Also it was observed that *S. carpocapsae* was significantly more effective in reducing nematode impact when compared to *H. bacteriophora* these results were confirmed by many researchers (El Aimani *et al.,* 2022). They explained the superiority of *S. feltiae* over

Heterorhabditis in reducing nematode population by ease of entry to roots then releasing its bacteria better than *H. bacteriophora*, causing a more consistent effect. The antagonistic effects of EPNs toward root-knot nematode species are closely associated with the application time, inoculum density, host plant, and the species of both the PPNs and EPNs (El Aimani *et al.*, 2022). Additionally, interaction between EPNs strains and the host plant in reducing the invasion of RKN is affected with infection behavior of RKN towards the root system. It was observed that the movement of RKN toward the roots was inhibited when EPNs were placed between the position of the RKN and the roots (Li *et al.*, 2023). However, EPNs may not be active against all PPNs and depends on the species and host aspects (Lewis and Grewal, 2005). Additionally, antinematodal activity of EPNs against *M. incognita* in this study might be highly related to allelochemicals, ammonium and other metabolites production by the associated symbiotic bacteria (Grewal & Georgis, 1999 and El Aimani *et al.*, 2022).

CONCLUSION

Our investigation proofs the antagonistic activity of tested biocides and EPNs towards RKN, also our finding pushing towards more studies in this area of employing biocontrol agents for managing nematodes and expanding their application vs. pesticides. Additionally, it is beneficial to employ the interaction between PPNs and EPNs. The multiple relations between two types of nematodes including various mechanisms, which resulted in distinct antagonistic patterns that could have implications for implementation in crop production and attempts to reduce the use of chemical methods. These promising elements can insert in integrated nematode management scheme as well as reduce the dependence on chemical nematicides. This finding indicated that searching and adopting new *Steinernema* or *Heterorhabditis* strains may induce stronger antagonistic effects against PPNs. More studies are necessary to optimized application of EPNs under field conditions in various climatic zones. Also growers should know that biological control as nematode-combating strategy need suitable time for increasing the percent of sharing in integrated nematode management program, till completely avoided using pesticides in control strategy.

REFERENCES:

Aatif, H.M., Javed, N., Khan, S.A., Ahmed, S., Raheel, M. 2015: Virulence of entomopathogenic nematodes against *Meloidogyne incognita* for invasion, development and reproduction at different application times in brinjal roots. Int. J. Agric. Biol., (17): 995-1000

Abo-Korah, M.S. 2022: Integrated management of root-knot nematodes, *Meloidogyne incognita,* infecting cowpea plants. Menoufia J. Plant Protection, (7): 89- 95

Abo-Korah, M.S., Sweelam, M.E., Yassin, A.M. 2022: Effect of different bioagents on the population density of *Meloidogyne incognita* infected tTomato plants. Egypt. J. Agronematol., 21(1): 14-22.

Abrantes, I.M.O., dos Santos, M.C.V., da Conceição, I.L.P.M., Santos, M.S.N., Vovlas, N. 2008: Root-knot and other plant-parasitic nematodes associated with fig trees in Portugal. Nema. Mediterr, (36): 131-136

Ahmed, H.M., Gad, S.B., El-Sherif, A.G., El-Hadidy, E.M. 2022: Efficacy of five biopesticides for the management of root-knot nematode, *Meloidogyne incognita* Infecting Pepper (*Capsicum annum* L.) Plants. Egyptian Journal of Agronematol., 21 (1): 23-33.

ALmohithef, A.H., AL-Yahya, F.A., AL-Hazmi, A.S., Dawabah, A.A.M., Lafi, H.A. 2021: Relative Efficacy of Some Products Against *Meloidogyne Javanica* (Treub) Chitwood on Tomato Under Greenhouse Conditions. Bangladesh J. Bot. 50(3): 709-712, DOI:https://doi.org/10.3329/bjb.v50i3.55852

Anonyms, 2022: What are Biopesticides?; available online: https://www.epa.gov/ingredients-used-pesticide-products/what-are-biopesticides

Danilov, L.G., Kaplin, V.G. 2020: Nematicidal activity of Nematode-Symbiotic Bacteria, *Xenorhabdus Bovienii* and *X. nematophila* against root-knot nematode *Meloidogyne incognita*. ПАРАЗИТОЛОГИЯ, том 54, № 5, с. 413-422.

El Aimani, A., Houari, A., Laasli, S., Mentag, R., Iraqi, D., Diria, G., Khayi, S., Lahlali, R., Dababat, A.A., Mokrini, F. 2022: Antagonistic potential of Moroccan entomopathogenic nematodes against root-knot nematodes, *Meloidogyne javanica* on tomato under greenhouse conditions. Scientific Reports, 12:2915 https://doi.org/10.1038/s41598-022-07039-0

El-Marzoky, A.M., Elnahal, A.S.M., Jghef, M.M., Abourehab, M.A.S., El-Tarabily, K.A., Mohamed, A.M.S. 2023: *Purpureocillium lilacinum* strain AUMC 10620 as a biocontrol agent against the citrus nematode *Tylenchulus semipenetrans* under laboratory and field

conditions European Journal of Plant Pathology, (167): 59-76.

El-Nagdi, W.M.A., Haggag, K.H.E., Abd-El-Fattah, A.I., Abd-El-Khair, H. 2011: Biological control of *Meloidogyne incognita* and *Fusarium solani* in sugar beet. Nematol. medit. , (39): 59-71.

El-Nuby, A.S.M. 2014: Evaluation of some Resistance Inducers Against the root-knot nematode. PhD Thesis, Faculty of Agriculture, Cairo University, pp 297.

Elsherbiny, E.A., Taher, M.A., Elsebai, M.F. 2019: Activity of *Purpureocillium lilacinum* fltrates on biochemical characteristics of *Sclerotinia sclerotiorum* and induction of defense responses in common bean. European Journal of Plant Pathology,(155): 39-52.

El-Zawahry, A.M., Khalil, A.E.M., Allam, A.D.A., Mostafa, R.G. 2015: Effect of the Bio-agents (*Bacillus megaterium* and *Trichoderma album*) on Citrus Nematode (*Tylenchulus semipenetrans*) Infecting Baladi orange and Lime Seedlings. J Phytopathology and Pest Management 2(2): 1-8.

Gamalero, E., Glick, B.R. 2020: The use of plant growth-promoting bacteria to prevent nematode damage to plants. Biology, 9, 381.

Goswami, B.K., Mittal, A. 2002: Effect of some fungal bioagents on root-knot nematode, *Meloidogyne incognita* infecting brinjal. Pakistan Journal of Nematology, (20): 55-59.

Harman, G.E. 2006: Overview of mechanisms and uses of *Trichoderma* spp., Phytopathol. (96): 190-194

Hegazy, M.I., Salama, A.S.A., El-Ashry, R.M., Othman, A.I. 2019: *Serratia marcescens* and *Pseudomonas aeruginosa* are promising candidates as biocontrol agents against root-knot nematodes (*Meloidogyne* spp.), Middle East Journal of Agriculture Research, 8(2):pp 11

Hu, K., Jianxiong, L., Webster, J.M. 1999: Nematicidal metabolites produced by *Photorhabdus luminescens* (Enterobacteriaceae), bacterial symbiont of entomopathogenic nematodes. Nematology (1): 457-469.

Ibrahim, D.S.S., Ali, A.M., Metwally, H.A. 2019: Bio-management of Citrus Nematode, *Tylenchulus semipenetrans* and Dry Root Rot Fungi, *Fusarium solani* under Laboratory and Field Conditions. Egypt. J. Agronematol., 18 (2):118-128

Isaac, G.S., El-Deriny, M.M., Taha, R.G. 2021: Efficacy of *Purpureocillium lilacinum* AUMC 10149 as biocontrol agent against root-knot nematode *Meloidogyne incognita* infecting tomato plant. Brazilian Journal of Biology, vol. 84, e253451| https://doi.org/10.1590/1519-6984.253451

Jonathan, E.I., Arulmozhiyan, R., Muthusamy, S., Manuel, W.W. 2000: Field application of *Paecilomyces lilacinus* for the control of *Meloidogyne incognita* on betelvine, *Pipper betel*. Nematol. Med., (28):131-33.

Jones, J.T., Haegeman, A., Danchin, E.G., Gaur, H.S., Helder, J., Jones, M.G., Kikuchi, T., anzanilla-López, R.M. Palomares-Rius, J.E., Wesemael, W.M., Perry, R.N. 2013: Top 10 plant-parasitic nematodes in molecular plant pathology, Mol. Plant Pathol., (14): pp. 946-961

Kantor, M., Handoo, Z., Kantor, C., Lynn, C. 2022: Top Ten Most Important U.S.-Regulated and Emerging Plant-Parasitic Nematodes Horticulturae 8 (3): 208. https://doi.org/10.3390/horticulturae8030208

Khalil, M.S.H., Gaber, A.F., Allam, Barakat, A.S.T. 2012: Nematicidal activity of some biopesticides agents and Microorganisms against root-knot nematode on tomato plants under greenhouse conditions. Journal of Plant Protection Research 52 (1), 2012

Khalil, M.S., Abd El-Aziz, M.H., El-khouly, A.M. 2022: Optimization the Impact of Fluopyram and Abamectin against the Root-Knot Nematode (*Meloidogyne incognita*) on Tomato Plants by Using *Trichoderma album*. *Egypt. J. Agronematol*., 21(1):79 -90

Khan, A., Williams, K.L., Nevalainen, H.K. 2006: Infection of plant parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum*. Biol. Cont. 51: 659-678.

Kiewnick, S., Sikora, R. 2006: Evaluation of *Paecilomyces lilacinus* strain 251 for the biological control of the northern root-knot nematode *Meloidogyne hapla* Chitwood. Nematol. 8: 69-78

Lewis, E.E., Grewal, P.S. 2005: Interactions with plant-parasitic nematodes. In Nematodes as Biocontrol Agents (eds. Grewal, P. S., Elhers, R. U., Shapiro-Ilan, D. I) 349-362 (CABI)

Li, J., Li, Y., Wei, X. 2023: Direct antagonistic effect of entomopathogenic nematodes and their symbiotic bacteria on root-knot nematodes migration toward tomato roots. Plant Soil (484**):** 441-455.

Lima-Rivera, D.L., Lopez-Lima, D., Desgarennes, D., Velazquez-Rodriguez, A.S., Carrion, G. 2016: Phosphate solubilization by fungi with nematicidal potential. J. Soil Sci. Plant Nutr., (16): 507-524.

Liu, M.J., Hwang, B.S., Jin, C.Z., Li, W.J., Park, D.J., Seo, S.T., Kim, C.J. 2019: Screening, isolation and evaluation of a nematicidal compound from actinomycetes against the pine wood nematode, *Bursaphelenchus xylophilus*. Pest Manag Sci. 2019 Jun; 75(6):1585-1593.

Massoud, M.A., Khalil, M.S., Shawer, R., El-bialy, M.M., Abdel-Fattah, S.A.S. 2021: Biological Performance of Certain Bio-Agents, Fluopyram and Fosthiazate against *Meloidogyne* spp. on Guava Trees (*Pisidum guava*). Alexandria Science Exchange Journal, 42(4): 789-797

Méndez-Santiago, E.W., Sánchez-Cruz, R., Folch Mallol, J.L., Aguilar-Marcelino, L., Hernández-Velázquez, V.M., Gómez-Rodríguez, O., Villar-Luna, E., Wong-Villarreal, A. 2020: *Serratia* sp., an endophyte of *Mimosa pudica* nodules with nematicidal, antifungal activity and growth promoting characteristics. Arch. Microbiol. (203): 549-559.

Metwally, W.E., Khalil, A.E., Mostafa, F.A.M. 2019: Biopesticides as Eco-friendly Alternatives for the Management of Root-Knot Nematode, *Meloidogyne incognita* on Cowpea (*Vigna unguiculata* L.). Egypt. J. Agronematol., 8 (2):129-145

Metwaly, H.A., Zawam, H.S. 2015: efficacy of some bioagents and nemaStop compound in controlling root-knot disease on Peanut. J. Plant Prot. and Path., Mansoura Univ., 6 (3):535-547.

Mohamd, O.M., Hussein, R.A.A., Ibrahim, D.S.S., Badawi, M.H., Makboul, H.E. 2020: Effects of Serratia marcescens and Prodigiosin Pigment on the Root-Knot Nematode *Meloidogyne incognita*, Middle East Journal of Agriculture Research, 9(2): 243-252

Mokbel, A.A., Alharbi, A.A. 2014: Suppressive effect of some microbial agents on root-knot nematode, *Meloidogyne javanica* infected eggplant. AJCS 8(10):1428-1434.

Mostafa, F.A.M., Khalil, A.E., Nour El-Deen, A.H., Ibrahim, D.S. 2018: The role of *Bacillus megaterium* and other bio-agents in controlling root-knot nematodes infecting sugar beet under field conditions. Egyptian Journal of Biological Pest Control (28):66, doi.org/10.1186/s41938-018-0068-6

Park, J.O., Hargreaves, J.R., McConville, E.J., Stirling, G.R., Ghisalberti, E.L., Sivasithamparam, K. 2004: Production of leucinostatins and nematicidal activity of Australian isolates of *Paecilomyces lilacinus* (Thom) Samson. Lett. Appl. Microbiol. , 38, 271-276.

Pérez, E., Lewis, E. 2004: Suppression of *Meloidogyne incognita* and *Meloidogyne hapla* with entomopathogenic nematodes on greenhouse peanuts and tomatoes. Biol. Control.; (30): 336-341.

Pratibha S.S.K.C. 2017: Bio pesticides: A Safe and Ecofriendly Alternative. Indo Global Journal of Pharmaceutical Sciences, 7(1): 3

Radwan, M.A., Abu-ELamayem, M.M., Farrag, S.A.A., Ahmed, N.S. 2011: Integrated management of *Meloidogyne incognita* infecting tomato using bio-agents mixed with either oxamyl or Organic amendments. Nematol. medit. (39): 151-156.

Ramalakshmi, A., Sharmila, R., Iniyakumar, M., Gomathi, V. 2020: Nematicidal activity of native *Bacillus thuringiensis* against the root knot nematode, *Meloidogyne incognita* (Kofoid and White). Egyptian Journal of Biological Pest Control (30):90

Rodriguesa, M.G.F., Firminoa, A.C., Valentima, J.J., Pavanb, B.E., Ferreirab, A.F.A., Monteirob, L.N.H., Ramosc, E.S., Soutelloa, R.V.G. 2022: Correlation of genome methylation of fig tree accessions with natural nematode and rust incidence. https://doi.org/10.1590/1519-6984.263041

Ruanpanun, P., Chiradej, C. 2015: Potential of actinomycetes isolated from earthworm castings in controlling root-knot nematode *Meloidogyne incognita*. Journal of General Plant Pathology 82(1), DOI:10.1007/s10327-015-0637-4

Sahebani, N., Hadavi, N. 2008: Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harziannum*. Soil Biol. Biochem. 40 (8): 2016- 2020.

Sayedain, F.S., Ahmadzadeh, M., Fattah-Hosseini, S., Bode, H.B. 2021: Soil application of entomopathogenic nematodes suppresses the root-knot nematode *Meloidogyne javanica* in cucumber. J. Plant Dis. Prot. 128(1), 215-223.

Sharf, H.R., Abbasi, R.M., Ambreen, A. 2011: Study of efficacy of leaf extracts of some plants on germination and sporulation of fungi *Paecilomyces lilacinus.* Journal of Natural Product and Plant Resources, 1(4): 86-89.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O., Spiegel, Y. 2001: Biological control of root knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology (91): 687-693.

Srivastava, S., Chaubey, A.K. 2022: *In vitro* study on the nematicidal activity of entomopathogenic bacteria against the root knot nematode *Meloidogyne incognita*. Journal of Applied and Natural Science, 14(1): 1-8.

Suárez, B., Rey, M., Castillo, P., Monte, E., Llobell, A. 2004: Isolation and characterization of PRA1, a trypsin-like protease from the biocontrol agent *Trichoderma harzianum* CECT 2413 displaying nematicidal activity.Appl. Microbiol. Biotechnol. (65): 46-55.

van Zyl, C. 2012: The *in vivo* production of *Heterorhabditis zealandica* and *Heterorhabditis bacteriophora*. Master of Agricultural Sciences Thesis, Stellenbosch University, Stellenbosch.

Vey, A., Hoagland, R.E., Butt, T.M. 2001: Toxic metabolites of fungal biocontrol agents. In: Fungi as biocontrol agents: Progress, problems and potential, (Butt, T. M., C. Jackson and N. Magan eds.). CAB International Bristol, pp. 311-346.

Yang, L.i., Xianqin, W., Yonghe, C., Xinhu, G., Xingyue, L., Toyoshi, Y., Abd-Elgawad, M.M., Shapiro-Ilan, D., Ruan, W., Sergio, R. 2022: Direct antagonistic effect of entomopathogenic nematodes and their symbiotic bacteria on root knot nematodes migration toward tomato roots. Jingjing Li. Nankai University. https://orcid.org/0000-0003-2140-4840

Yang, F., Abdelnabby, H., Xiao, Y. 2015: The role of a phospholipase (PLD) in virulence of *Purpureocillium lilacinum* (*Paecilomyces lilacinum*). Microbial Pathogenesis, vol. 85, pp. 11

Zaghloul, R.A., Neweigy, N.A., Abou-Aly, H.E., El-Sayed, S.A., Bahloul, A.M. 2015: Nematicidal activity of some biocontrol aAgents against root-knot nematodes *In-Vitro*. RJPBCS 6(1): 429- 438

Table 1: List of commercial compounds tested in this investigation.

Trade name	Bio-agents and its concentration	Concentration used
Anti-Nema®	*Serratia marcescens* 25×10^9 CFU/g of bacterium	2.5g/100 ml distilled water
BioArc® 6% Powder	*Bacillus megaterium* 25×10^6 CFU/g of bacterium	2.5g/100 ml distilled water
BioNematon® 1.75% WP	*Paecilomyces lilacinus* 1×10^8 CFU/g of fungus	0.25g/100 ml distilled water
BioZeid® 2.5% Powder	*Trichoderma album* 25×10^6 CFU/g of fungus	2.5g/100 ml distilled water
NemaStop® 5% CS	*Streptomyces avermitilis* Abamectin (5% CS)	2.5ml/100 ml distilled water

Table 4: Effect of selected Biocides and EPNs on fig seedlings growth infected *M. incognita* under greenhouse conditions

Treatments	Shoot weight (g.)	%I	Root weight (g.)	%I	Shoot length (cm.)	%I	Root length (cm.)	%I	Chlorophyll content	%I
S. carpocapsae	44.0a	14.3	19.3a	17.2	48.5a	29.9	12.3a	44.2	41.67ab	7.9
H. bactriophora	43.4a	13.1	19.0a	15.8	44.6ab	23.9	11.0a	37.2	38.97a	1.5
BioNematon	43.3a	12.9	18.8a	15.1	42.8ab	20.6	10.7ab	35.6	41.60ab	7.8
Anti-Nema	42.5a	11.4	18.7a	14.3	41.5ab	18.1	9.2ab	25.3	41.40a	7.3
NemaStop	41.7ab	9.5	18.0a	11.1	40.5ab	16.1	8.9ab	22.5	40.93ab	6.3
Control infected	37.70b	0.0	16.0a	0.0	34.0b	0.0	6.9b	0.0	38.37a	0.0

S= Steinernema, H= Heterorhabditis

Within the same column numbers followed by the same letter are significantly equal at P=5.0 according to Duncan Multiple Range Test

Table 2: Effect of some biocides and two EPNs applied pre and post nematode inoculation on suppression *M. incognita* population infecting fig seedlings under greenhouse conditions**:**

Treatments	AT	Gall	%R	DS	%R	MF	%R	EM	%R	Eggs/ EM	%R	Total eggs	%R	Pf	%R
S. carpocapsae	Pre	56.7 bcd	31.5	53.3 bcd	26.3	49.7 bc	24.9	48.0 bcd	27.7	113.3 e	33.9	5440.0 cd	52.2	5591.0 cd	51.8
	Post	60.0 b	27.4	36.7 cd	49.3	32.7 bcd	50.6	32.3 c	51.3	85.0 d	50.4	2748.3 c	75.9	2850.0 c	75.4
H. bactriophora	Pre	46.3 d	44.0	43.3 e	40.1	41.7 c	37.0	41.7 cd	37.3	105.0 f	38.7	4375.0 d	61.6	4501.7 d	61.2
	Post	48.0 c	41.9	28.7 e	60.4	28.7 cd	56.7	26.3 d	60.4	70.0 e	59.1	1843.3 d	83.8	1927.0 d	83.4
BioNematon	Pre	52.0 d	37.1	46.7 de	35.5	43.0 c	35.0	42.3 cd	36.3	125.0 d	27.0	5291.7 d	53.5	5423.7 d	53.2
	Post	49.0 c	40.7	30.0 de	58.5	26.0 d	60.7	29.3 cd	55.8	98.0 c	42.8	2874.7 c	74.7	2960.0 c	74.5
Anti-Nema	Pre	55.0 cd	33.5	50.0 cde	30.9	46.3 bc	30.0	39.3 d	40.8	127.7 cd	25.5	5113.3 d	55.1	5249.0 d	54.7
	Post	52.0 c	37.1	35.0 de	51.6	35.0 bc	47.1	31.7 cd	52.3	103.7 bc	39.5	3282.8 c	71.2	3384.4 c	70.8
NemaStop	Pre	65.0 bc	21.4	60.0 b	17.0	55.0 b	16.9	43.3 cd	34.8	130.0 cd	24.1	5633.3 cd	50.5	5791.7 cd	50.0
	Post	60.0 b	27.4	36.7 cd	49.3	32.7 bcd	50.6	33.3 c	49.8	105.0 bc	38.7	3500.0 c	69.2	3602.7 c	68.9
BioArc	Pre	66.3 b	19.8	60.0 b	17.0	57.0 b	13.9	50.0 bc	24.7	135.0 bc	21.2	6750.0 bc	40.7	6917.0 bc	40.3
	Post	62.0 b	25.0	45.3 b	37.3	40.0 b	39.5	42.7 b	35.8	102.0 bc	40.5	4352.0 b	61.8	4480.0 b	61.4
BioZeid	Pre	65.0 bc	21.4	59.0 bc	18.4	56.3 b	14.9	56.7 b	14.7	140.0 b	18.3	7933.3 b	30.3	8105.3 b	30.1
	Post	48.3 c	41.5	43.0 bc	40.6	38.3 b	42.1	40.0 b	39.8	108.3 b	36.8	4333.3 b	61.9	4454.7 b	61.6
Control infested		82.7 a	0.0	72.3 a	0.0	66.2 a	0.0	66.4 a	0.0	171.3 a	0.0	11381.7 a	0.0	11592.8 a	0.0

Pre= Pre inoculation, Post= post inoculation, AT= Application Time
DS= Developmental stages, MF= Mature females, EM= Egg masses, pf= Final population
Within the same column numbers followed by the same letter are significantly equal at P=5.0 according to Duncan Multiple Range Test

Table 3: Suppressive effect of selected Biocides and EPNs towards *M. incognita* infecting fig plants under greenhouse conditions

Treatments	Galls	%R	SP	%R	DS	%R	IF	%R	MF	%R	EM	%R	Eggs/ EM	%R	Total eggs	%R	Pf	%R
S. carpocapsae	100.0b	50.0	255.0b	51.4	44.3c	62.5	57.3c	63.0	86.7d	56.7	105.7cd	44.5	256.c7	53.3	27121.1cd	74.1	27564.4a	74.2
H. bactriophora	70.0de	65.0	215.0b	59.0	35.0cd	70.4	38.7d	75.1	80.7de	59.7	90.3d	52.5	200.0d	63.6	18066.7d	82.7	18436.0bc	82.8
BioNematon	80.3cd	59.8	223.7b	57.4	36.7cd	69.0	43.7	71.8	110.0c	45.0	111.7c	41.3	301.0b	45.3	33611.7cd	67.9	34025.7c	68.2
Anti-Nema	86.0bcd	57.0	244.0b	53.5	57.7b	51.3	77.0b	50.3	118.7bc	40.7	121.0bc	36.4	318.0b	42.2	38478.0bc	63.2	38975.3bc	63.5
NemaStop	91.0bc	54.5	259.0b	50.6	61.0b	48.4	79.0b	49.0	127.3b	36.3	128.7b	32.4	330.3b	39.9	42502.9c	59.4	43029.2bc	59.7
Nematicide (Oxamyl)	54.7e	72.7	194.3b	63.0	30.3d	74.4	32.7d	78.9	71.3e	64.3	69.3e	63.6	160.3e	70.8	11116.4b	89.4	11445.1bc	89.3
Control infected	200.0a	0.0	1733.3a	0.0	118.3a	0.0	155.0a	0.0	200.0a	0.0	190.3a	0.0	550.0a	0.0	104683.3a	0.0	106890.0b	0.0

S= Steinernema, H= Heterorhabditis

SP= Soil population, DS= Developmental stages, IF= immature females, MF= Mature female, EM= Egg masses, Pf= Final population

Within the same column numbers followed by the same letter are significantly equal at P=5.0 according to Duncan Multiple Range Test

تأثير بعض المبيدات الحيوية والنيماتودا الممرضة للحشرات في مكافحة نيماتودا تعقد الجذور المرتبطة بجذور نبات التين في الصوبة

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الملخص العربي

تم تقييم الخصائص المضادة للنيماتودا لبعض المركبات الحيوية BioNematon (*Paecilomyces lilacinus*)، ®BioZeid (*Trichoderma album*)، ®BioArc (*Bacillus megaterium*)، ®NemaStop (*Streptomyces avermitilis*)، وAnti-Nema (*Serratia marcescens*) بالإضافة إلى عزلتان محليتان من النيماتودا الممرضة للحشرات (*Heterorhabditis bacteriophora* & *Steinernema carpocapsae*) تحت ظروف الصوبة قبل العدوي وبعدها بأسبوع. ثم تقييم أفضل المبيدات الحيوية (BioNematon، NemaStop وAnti-Nema) بالإضافة إلى نيماتودا الممرضة الحشرات للحصول علي افضل النتائج في التجربة السابقة بعد إختيار ميعاد التطبيق الأفضل، بعد العدوي، تحت ظروف الصوبة علي نبات التين المعدي بنيماتودا تعقد الجذور. وأظهرت نتائج تجارب الصوبة، حدوث تثبيط لمؤشرات النيماتودا بشكل ملحوظ في المنتجات الحيوية الثلاثة المختارة؛ كان BioNematon هو الأفضل حيث حقق إنخفاضًا بنسبة 68.2% في العدد النهائي للنيماتودا يليه Anti-Nema بنسبة 63.5% بينما حقق NemaStop أقل تأثير (59.7%). كما تم تحسين نمو التين بعد إضافة المنتجات الحيوية المختلفة ونيماتودا الحشرات. وفي هذا الصدد، من المهم إجراء المزيد من الدراسات، مع مراعاة الظروف المختلفة للتربة والمناخ والأسمدة والمبيدات الكيميائية الزراعية المختلفة المستخدمة فعليًا في الإنتاج النباتي قبل التوسع في إستخدام هذه المبيدات الحيوية. ويجب أيضًا النظر في الإستخدام الأمثل لنيماتودا الحشرات، إلى جانب مراعاة البعد الإقتصادي للتطبيق.

الكلمات الاسترشادية: