The integration efficacy of formulated abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* for managing *Meloidogyne incognita* (Kofoid and White) Chitwood on tomatoes

Mohamed S. Khalil[1] and Shimaa S. I. Abd El-Naby[1]

ABSTRACT

A pot trial was conducted to evaluate the impact of formulated abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* alone or in combinations against the root-knot nematode (*Meloidogyne incognita*) development in tomato plants. Abamectin was the superior treatment which reduced galls and soil populations by 85.87 and 85.13%, respectively. The single treatments showed that the high rate of *B. thuringiensis* and / or *B. subtilis* recorded the highest reductions than the low rate. The single treatments of *B. thuringiensis* and *B. subtilis* gave reductions in galls ranged from 66.22 to 78.88%, and in soil populations ranged from 70.63 to 80.45%. Moreover, the binary mixtures of abamectin + *B. thuringiensis* recorded the highest reductions among mixtures in galls (85.20%) and soil populations (76.56%).On the other side, all the applied single treatments increased the tomato plant growth criteria over control. The binary blend of abamectin + *B. thuringiensis* recorded the highest augmentation in root length, root fresh weight and shoot fresh weight by 41.84, 49.58 and 56.72%, respectively.

Keywords: *Meloidogyne* sp., Abamectin, *Bacillus thuringiensis*, *Bacillus subtilis*, Integrated Nematodes Management.

MS History: 03.09.2018 (Received)-20.11.2018 (Revised)-23.11.2018 (Accepted).

Citation: Mohamed S. Khalil[1] and Shimaa S. I. Abd El-Naby[1]. 2018. The integration efficacy of formulated abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* for managing *Meloidogyne incognita* (Kofoid and White) Chitwood on tomatoes *Journal of Biopesticides*, **11**(2):146-153.

INTRODUCTION

Tomato is a crop affected by certain types of pests and/or diseases which decreased the quality and quantity of production. In Egypt, tomato is considered one of the most important vegetable crops for fresh consumption and processing (Abd El-Ghany, 2011). Tomatoes are mainly used as rich source of carbohydrates, minerals and vitamins (Ibrahim *et al*., 2010). One of the main pests in tomato crop is the root-knot nematodes, *Meloidogyne* spp., which are responsible for more than 27% yield losses in tomato globally (Sharma and Sharma, 2015).

Research papers and reports for many organizations have established he hazards of pesticides on the human life and his surrounding environment. It has become necessary to find alternative approaches to synthetic nematicides. Abamectin which is a mixture of macrocyclic lactone metabolites produced by the bacterium *Streptomyces avermectinius* is an effective alternative against plant parasitic nematodes according to other investigations of Huang *et al*., (2014), El-Nagdi *et al*., (2015) and Saad *et al*., (2017). Also, the use of bio-management agents was very effective against the phytonematodes. *Bacillus thuringiensis* Berliner is one of the untraditional microbes used against different genera of plant nematodes especially root-knot nematodes. *B. thuringiensis* produce parasporal crystalline proteinaceous inclusions or δ- endotoxins which are toxic to parasitic nematodes (Kotze *et al*., 2005; Iatsenko *et al*., 2014).

Furthermore, *Bacillus subtilis*, is one of the famous plant growth promoting rhizobacteria

(PGPR), which attracted global attention as a bio-control agent in the management of plant pathogenic nematodes (Prakob *et al*., 2009 and Xiang *et al.*, 2017). It was found that *B. subtilis* improved the plant growth indices significantly (Khalil *et al.*, 2012). Therefore, this study aimed to evaluate the efficacy of abamectin, *B. thuringiensis* and *B. subtilis* alone and / or in combinations against the root-knot nematode (*Meloidogyne incognita*) as alternative solutions to chemical control problem on tomato.

METRIALS AND METHODS

Identification of root-knot nematode

The eggs of the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, were isolated from infested roots of eggplant (*Solanum melongena*) with sodium hypochlorite (NaOCl) according to the method of Hussey and Barker (1973). The eggs suspension was passed through a 200 mesh sieve and collected on a 400 mesh sieve to obtain free eggs before carrying out the experiment. The species of the root-knot nematode (*Meloidogyne incognita*) was identified by using the perineal patterns method according to Taylor and Nelscher (1974).

Tested materials and their rates of applications

Dipel® 54% DF containing 32,000 iu/mg of bacterium *Bacillus thuringiensis* var. *kurstaki*. It was applied at 5 and 10 g / kg soil

Sting® containing 1×10^9 cell/ ml of bacterium *Bacillus subtilis***.** It was applied at 5 and 10 ml / kg soil**.**

Tervigo® 2% SC (Abamectin).It was applied in the recommended dose 2.5 L / feddan.

The experimental design

Pots experiment was carried out using tomato plants cv. YARA, each pot were filled with 1kg of loamy sand soil. Five weeks old tomato seedlings were transplanted and infected with the eggs suspension of *Meloidogyne incognita* which applied at the rate of 5000 eggs / pot by making holes around the root system at uniform distance. Nine treatments were applied and each treatment was replicated five times, and each replicate contains one plantlet. Sixty days after planting, the seedlings were uprooted and assessed for the shoot and root lengths and fresh weights. Meanwhile, the numbers of J_2 populations' were evaluated in the soil (250 g) using the sieving and Baermann plates' technique (Ayoub, 1980), in addition to galls number on plant roots.

The applied treatments were; *Meloidogyne incognita* only (Untreated check), *M. incognita* + Abamectin (Recommended dose), *M. incognita* + *Bacillus thuringiensis* (at 5g / kg soil), *M. incognita* + *Bacillus thuringiensis* (at 10g / kg soil), *M. incognita* + *Bacillus subtilis* (at 5ml / kg soil), *M. incognita* + *Bacillus subtilis* (at 10ml / kg soil), *M. incognita* + Abamectin(at half dose) + *B. thuringiensis* (at 5g/ kg soil), *M. incognita* + Abamectin(at half dose) + *B. subtilis* (at 5ml/ kg soil) and *M. incognita* + *B. subtilis* (at 5ml/ kg soil) + *B. thuringiensis* (at 5g/ kg soil).

Statistical analysis

The pots were arranged in complete randomized design (CRD). The least significant differences (LSD) at the 5% level of probability were determined using a computer program Costat Version: 6.303 (2005).

RESULTS AND DISCUSSION

The biological performance of formulated abamectin, *Bacillus thuringiensis* and *Bacillus subtilis*, alone and in combinations was evaluated against galls formation and soil population density of *Meloidogyne incognita* infecting tomato plants (Table 1). The single treatments showed that abamectin, *B. thuringiensis* and *B. subtilis* both at high rates recorded the reductions in galls by 85, 78.88 and 71.02%, respectively. However, the low rate of *B. thuringiensis* and *B. subtilis* recorded the least reductions in galls formation by 68.57 and 66.22%, consecutively. The combined treatments showed that the binary mixture between abamectin + *B. thuringiensis,* abamectin + *B. subtilis* and *B. thuringiensis* + *B. subtilis* recorded decrement in galls by 85.20, 75.92 and 68.16%, respectively.

On the other hand, all the applied treatments exhibited decreasing in soil populations' density significantly over control. The results in Table 1 indicated that abamectin alone

Table 1. The effect of abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* alone and in combinations against *Meloidogyne incognita* in tomato plants.

Treatments	Rate (ml or g / kg soil)	Galls / root system		Soil populations / 250g soil	
		Mean ± SE	% decrease in galls over control	Mean ± SE	% decrease in J2 over control
Abamectin	0.0025 ml	27.70±2.72	85.87	212.60±11.49	85.13
Ba. thuringiensis	5g	61.60±6.17	68.57	420.00±28.85	70.63
	10g	41.40±5.77	78.88	308.60±15.20	78.42
Ba. subtilis	5 ml	66.20±3.18	66.22	306.20±13.30	78.59
	10 ml	56.80±4.88	71.02	279.60±12.05	80.45
Abamectin + *B. thuringiensis*	0.0013 ml + 5g	29.00±3.81	85.20	335.20±23.49	76.56
Abamectin + *B.subtilis*	0.0013 ml + 5 ml	47.20±4.53	75.92	343.50±27.43	75.98
B. thuringiensis + *B.subtilis*	5g + 5 ml	62.40±4.46	68.16	390.90±30.64	72.66
Untreated Check	----	196.00±6.14	----	1430.00±54.42	----
LSD 0.05	------	13.48	------	78.39	------

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05

suppressed J_2 in the soil by 85.13%, followed by the single treatments of *B. thuringiensis* at high rate, *B. thuringiensis* at low rate, *B. subtilis* at high rate and *B. subtilis* at low rate with reductions by 80.45, 78.59, 78.42 and 70.63%, respectively. Meanwhile, the combined treatments revealed that abamectin + *B. thuringiensis*, abamectin + *B. subtilis* and *B. thuringiensis* + *B. subtilis* recorded suppression in J_2 by 76.56, 75.98 and 72.66%, consecutively.

It was observed that the high rate of *B. thuringiensis* and/or *B. subtilis* was more effective than the low rate. The binary mixture between abamectin + *B. thuringiensis* and abamectin + *B. subtilis* recorded the same significance in galls and soil population reductions. Also, the binary mixture of *B. thuringiensis* + *B. subtilis* did not significantly differ from using each alone in the case of galls reduction.

The present investigation was confirmed by previous studies which proved that abamectin is an effective nematicide against root-knot nematodes (*Meloidogyne* spp.), in different crops (Huang *et al*., 2014; El-Nagdi *et al*., 2015 and Saad *et al*., 2017). It was reported that abamectin diminished tomato root galling and the soil population significantly (Khalil *et al*., 2012). While Saad *et al*. (2017) found that abamectin relatively suppressed the galls, egg masses, eggs / mass and soil population of *Meloidogyne incognita* in tomato plants by 66.69, 66.31, 16.34 and 75.34%, respectively.

Abamectin is a new generation of nematicides that considered a new tool in the field of plant parasitic nematodes management. Abamectin have a unique mode of action in comparison with traditional nematicides. Abamectin is targeted the δ- amino-butyric acid (GABA) receptors causing increments in the permeability of chloride ions which finally causing death (Martin *et al.*, 2002). Also, the effect of abamectin as a nematicide is attributed to the strong adsorption to soil particles and the immobility in the soil (Lopez-Perez *et al.*, 2011; Muzhandu *et al.*, 2014). The stability of abamectin in soils leads to more exposure periods of the plant nematodes to the compound. The commercial products of *Bacillus thuringiensis* have proved the ability for managing the root-knot nematode (*Meloidogyne* spp.) in different investigations (Radwan, 2007; Khalil *et al.*, 2012; Khalil, 2013). Both Dipel® (6.4% WG) and Dipel 2X®were reported to decrease galls, egg masses and soil population in roots of tomato plants significantly (Khalil *et al.*, 2012; Khalil, 2013). Also, it was proved that *B. subtilis*, *B. thuringiensis and B. cereus* were effective against *M. javanica* (Dawar *et al.*, 2008), and *Heterodera avenae* (Ahmed *et al*., 2018). Different isolates of *B. thuringiensis* were reduced galls of *M. incognita* ranging from 81.8 to 94.6%, and egg masses ranged from 87.7 to 93.9% (Ashoub and Amara, 2010)

In the same context, Radwan (2007) evaluated the impact of different commercial formulations and concentrations of *B. thuringiensis* namely; Dipel 2x®, Delfin®, Ecotech Bio®, Turex® and Xentari® against *M. incognita*. All tested treatments of *B. thuringiensis* suppressed galls ranging from 49.3 to 78.2% and the second stage juveniles in soil ranged from 63.7 to 76.7%. All treatments induced the root and shoot systems growth. Khan *et a.*, (2010) documented that certain isolates of B. *thuringiensis* recorded nematicidal potential against the root-knot nematodes infection in okra and mung beans under greenhouse conditions. The suspension of BT isolates reduced number of galls, egg masses, eggs/egg mass, nematode population /g roots and nematode population /200g soil as compared to untreated check.

The suggested actions of *B. thuringiensis* against plant parasitic nematodes are attributed to the production of Bt parasporal crystal (s) which are known to be toxic to a wide range of insect species (Feitelson *et al.,* 1992). Some Cry proteins are also toxic to nematodes (Feitelson *et al.*, 1992). There are five Cry proteins (Cry5B, Cry6A, Cry13, Cry14A, Cry21A) known to be toxic to larvae of a number of free-living and parasitic nematodes (Crickmore *et al.*, 1998; Marroquin*et al*., 2000; Kotze *et al*., 2005). Also, it was described that there are certain Cry proteins which produced by *B. thuringiensis* such as Cry5, Cry6, Cry12, Cry13, Cry14, Cry21, Cry55 have nematicidal activity (Wei *et al*., 2003; Iatsenko *et al*., 2014). Additionally, a number of studies have reported direct antagonistic effects of other bacteria to pathogenic nematodes belonging to the genera *Heterodera* and *Meloidogyne*, included *B. amyloliquefaciens*, *B. cereus*, *B. licheniformis*, *B. megaterium* and *B. thuringiensis*. Bacteria are suggested to produce metabolites which reduce hatch and attraction and/or degradation of specific root exudates which control nematode behavior (Sikora and Hoffmann-Hergarten, 1993).

As for the bio-based product of the bacterium, *B. subtilis*, the present results complement those of Khalil *et al.*, (2012) and Khalil (2013) who found that *B. subtilis* was effective against the galls and egg masses of *M. incognita* in tomato plants and population density in the soil. In micro-plots trial, the *B. subtilis* strains significantly reduced the number of eggs/g roots with values ranged from 73.63 to 80.72 % after 48 days of application on cotton and increased cotton yield **(**Xiang *et al.*, 2017). Also, Mohamedova and Samaliev (2011) tested the nematicidal activity of two local strains of *B. subtilis* applied to the soil at different time intervals. The application of *B. subtilis* significantly reduced the developmental stages, females, egg masses and eggs/ mass of *M. arenaria* on roots of potato plants.

The reduction of plant parasitic nematodes associated with *B. subtilis* as a plant growth promoting rhizobacteria (PGPR) may be attributed to diverse mechanisms which involve phytohormones production, mineral solubilization, reduction of the activity of egg hatching factors, alteration of root exudates and inhibition of nematode penetration into the roots as well as reducing galling (Karanja *et al.*, 2007). Furthermore, several reports clarified that the basic mechanisms of *B. subtilis* included direct parasitism, production of extracellular antibiotics or other substances, enhance plant growth, induce systemic resistance in plants, managing the plant diseases and secreting volatile nematicidal products (Ji *et al.*, 2006 and Huang *et al*., 2009). On the other hand, the effect of applied treatments on the tomato growth indices was recorded in Table 2.The root system length was recorded and the best single treatment was

Table 2. The effectiveness of abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* alone and in combinations on growth criteria of tomato plants infected with *Meloidogyne incognita*

Treatments	Rate (ml or g / kg soil)	Root Length		Root Fresh Weight		Shoot Length		Shoot Fresh Weight	
		Mean ± SE	Increase (%)	Mean ± SE	Increase (%)	Mean ± SE	Increase (%)	Mean ± SE	Increase (%)
Abamectin	0.0025 ml	21.10±0.78	7.65	2.05±0.17	8.11	26.90±1.82	16.96	5.85±0.39	33.17
Bacillus thuringiensis	5g	21.70±0.89	10.71	2.37±0.12	24.95	25.60±2.05	11.30	5.58±0.21	27.20
	10g	27.60±0.81	40.82	2.47±0.18	29.89	27.60±2.68	20.00	6.14±0.39	39.95
Bacillussubtilis	5 ml	21.20±0.86	8.16	2.19±0.07	15.26	23.50±1.18	2.17	5.19±0.13	18.27
	10 ml	22.80±1.33	16.33	2.24±0.07	18.11	27.80±1.56	20.78	5.39±0.37	22.73
Abamectin + *B. thuringiensis*	0.0013 ml + 5g	27.80±1.11	41.84	2.84±0.21	49.58	25.20±1.07	9.57	6.88±0.32	56.72
Abamectin + *B.subtilis*	0.0013 ml + 5 ml	23.20±1.69	18.37	2.23±0.04	17.26	28.40±1.28	23.48	6.87±0.46	56.40
B. thuringiensis + *B.subtilis*	5g + 5 ml	21.40±0.76	9.18	2.34±0.04	23.26	26.20±1.46	13.91	6.00±0.37	36.58
Untreated Check	-----	19.60 ±1.02	-----	1.90±0.09	-----	23.00±1.41	-----	4.39±0.30	-----
LSD 0.05	-----	3.06	-----	0.36	-----	4.82	-----	0.98	-----

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05

B. thuringiensis at the high rate with 40.82% increasing, followed by *B. subtilis* at high rate (16.33%), *B. thuringiensis* at low rate (10.71%), *B. subtilis* at low rate and abamectin (7.65%). The combined treatments which were abamectin + *B. thuringiensis*, abamectin + *B. subtilis* and *B. subtilis* + *B. thuringiensis* increased the root length significantly by 41.84, 18.37 and 9.18%, respectively.

Regarding the root system fresh weight; it was increased with the solo treatments of *B. thuringiensis* at the high rate, *B. thuringiensis* at low rate, *B. subtilis* at high rate, *B. subtilis* at low rate and abamectin by 29.89, 24.95, 18.11, 15.26 and 8.11%, consecutively. The binary blends of abamectin + *B. thuringiensis*, *B. subtilis* + *B. thuringiensis* and abamectin + *B. subtilis* achieved augmentations reached up to 49.58, 23.26 and 17.26%, respectively.

In the same context, the shoot length of tomato plants was increased significantly with the single treatment of *B. subtilis* at high rate with 20.78%, followed by *B. thuringiensis* at high rate, abamectin, *B. thuringiensis* and *B. subtilis* both at low rate with augmentations by 20.00, 16.96, 11.30 and 2.17%, respectively. While the binary mixture of abamectin + *B. subtilis*, *B. subtilis* + *B. thuringiensis* and abamectin + *B. thuringiensis* were recorded increasing by 23.48, 13.91 and 9.57%, consecutively.

Besides, the shoot system fresh weight was also increased without significant differences by 39.95, 33.17, 27.20, 22.73 and 18.27% in pots received the single treatments of *B. thuringiensis* at high rate, abamectin, *B. thuringiensis* at low rate, *B. subtilis* at high rate and *B. subtilis* at low rate, respectively. The mixed treatments of abamectin + *B. thuringiensis*, abamectin + *B. subtilis* and *B. subtilis* + *B. thuringiensis* were gave the highest augmentations by 56.72, 56.40 and 36.58%, respectively.

The gained results are in conformity with those obtained by Radwan (2007), Khan *et al*. (2010) and Khalil (2013) who found that *B. thuringiensis* increased the shoot system indices significantly. In addition to, abamectin enhanced the shoot and root length and fresh weight of tomato plants (Ibrahim *et al.*, 2010; Khalil *et al.*, 2012; Saad *et al.*, 2012; Muzhandu *et al*., 2014).

In certain investigations it were found that the plant growth characteristics as shoot and root system were increased significantly in the presence of *B. subtilis* (Khalil *et al.*, 2012; Khalil, 2013; Xiang *et al.*, 2017).Also, the using of *P. fluorescens* and *B. subtilis*, singly and in mixture against *M. incognita* ameliorating the plant growth criteria of onion crop (Munshid *et al.*, 2013).

To date, in Egypt, the farmers are still depending on chemical nematicides. But the adverse effects on human health were noticed.

Therefore, in this study we try to study alternative tools such as abamectin that registered newly in Egypt, *B. thuringiensis* and *B. subtilis* alone or in combinations as safe suggestions for the integrated nematodes management programs (INMP).

REFERENCE

Ahmed, S., Liu, Q. and Jian, H. 2018. Bio-control potential of *Bacillus* isolates against cereal cyst nematode (*Heterodera avenae*).Pakistan *Journal of Nematology*, **36** (2):163-176

Ashoub, A. H., Amara, M. T. 2010. Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. *Journal of American Science,* **6** (10): 321- 328.

Abd El-Ghany, N. M. 2011. Molecular evaluation of *Bacillus thuringiensis* isolates from the soil and production of transgenic tomato plants harboring *Bt* gene for controlling lepidopterous insects in Egypt. Dissertation, Ain Shams University, Egypt, P. 270.

Atkinson, H. J., Lilley, C. J., Urwin, P. E. 2012. Strategies for transgenic nematode control in developed and developing world crops. *Current Opinion in Biotechnology*, **23**(2): 251-256.

Ayoub, S. M. 1980. Plant Nematology. An Agricultural Training Aid. Nema. Aid Publications. Sacramento, California, USA, **pp**.195.

CoStat Software. 2005. Microcomputer program analysis, CoHort software, Version 6.303, Monterey, CA, USA.

Crickmore, N., Zeigler, D. R., Feitelson, J., Schnepf, E., Rie, J. V., Lereclus, D., Baum, J., Dean, D. H. 1998. Review of the nomenclature for the *Bacillusthuringiensis* pesticidal crystal proteins. *Microbiology and molecular biology reviews*, **62**(3): 807–813.

Dawar, S., Tariq, M., Zaki, M. J. 2008. Application of *Bacillus* species in control of *Meloidogyne javanica* (Treub) Chitwood on cowpea and mash bean. *Pakistan Journal of Botany*, **40** (1): 439-444.

El-Nagdi, W. M. A., Hafez, O. M., Saleh, M. A. 2015. Impact of a biocide abamectin for controlling of plant parasitic nematodes, productivity and fruit quality of some date palm cultivars. *Scientia Agriculturae,***11** (1): 20-25.

Fettelson, J., Payne, J., Kim, I. 1992. *Bacillus thuringiensis*: insects and beyond. *Biotechnology*, **10**(3): 271-275.

Huang, W. K., Sun, J. H., Cui, J. K., Wang, G. F., Kong, L. A., Peng, H., Chen, S. L., Peng, D. L. 2014. Efficacy evaluation of fungus *Syncephalastrum racemosum* and nematicide avermectin against the root-knot nematode *Meloidogyne incognita* on cucumber. *PLoS one* **9**(2).

Huang, Y., Xu, C., Ma, L., Zhang, K., Duan, C., and Mo, M. 2009. Characterisation of volatiles produced from *Bacillus megaterium* YFM3.25 and their nematicidal activity against *Meloidogyne incognita*. *European Journal of Plant Pathology*, **26**: 417-422.

Hussey, R. S. and Barker, K. R. 1973. A comparison of methods of collecting inocula on *Meloidogyne* spp., including a new technique. *Plant Disease Reporter*, **57** (2): 1025-1028.

Iatsenko, I., Boichenko, I. and Sommer, R. J. 2014. *Bacillus thuringiensis* DB27 produces two novel protoxins, Cry21Fa1 and Cry21Ha1, which act synergistically against nematodes. *Applied and Environmental Microbiology*, **80**(10): 3266–3275.

Ibrahim, H. S., Saad, A. S. A., Massoud, M. A. and Khalil, M. S. H. 2010. Evaluation of certainagrochemicals and biological agents against *Meloidogyne incognita* on tomatoes. *Alexandria Science Exchange Journal*, **31**(1): 10-17.

Ji, P., Campbell, H., Kloepper ,J., Jones, J., Suslow,T. and Wilson, M. 2006. Integrated biological control of bacterial speck and spot of tomato under field conditions using foliar biological control agents and plant growth promoting rhizobacteria. *Biological Control*, **36**: 358–367.

Karanja, N. K., Mutua, G. K. and Kimenju, J. W. 2007. Evaluating the effect of Bacillus and Rhizobium bio-inoculant on nodulation and nematode control in *Phaseolusvulgaris* L. In: Advances in Integrated Soil Fertility Research in SubSaharan Africa: Challenges and Opportunities, (Bationo, A., B. Waswa, J. Kihara and J. Kimetu eds.), Springer, Netherlands, **PP** 863–868.

Khalil, M. S. 2013. The potential of five eco-biorational products on the reproduction of root-knot nematode and plant growth. *eSci journal of plant pathology*, **2**(2): 84-91.

Khalil, M. S. H., Allam, A. F. G. and Barakat, A. T. 2012. Nematicidal activity of some biopesticide agents and microorganisms against root-knot nematode on tomato plants under greenhouse conditions. *Journal of Plant Protection Research*, **52**(1): 47-52.

Khan, M. Q., Abbasi, M. W., Zaki, M. J. and Khan, S. A. 2010. Evaluation of *Bacillus thuringiensis* isolates against root-knot nematodes following seed application in okra and mungbean. *Pakistan Journal of Botany*, **42**(4): 2903-2910

Kotze, A. C., Grady, O., Gough, J., Pearson, J. M., Bagnall, R., Kemp, D. H. and Akhurst, R. J. 2005. Toxicity of *Bacillus thuringiensis* to parasitic and free-living life stages of nematodes parasites of livestock. *International Journal for Parasitology*, **35**: 1013-1022.

Lopez-Perez, J. A., Edwards, S. and Ploeg, A. 2011. Control of root-knot nematodes on tomato in stone wool substrate with biological nematicides. *Journal of Nematology*, **43**(2): 110-117.

Marroquin, L. D., Elyassnia, D., Griffitts, J. S., Feitelson, J. S. and Aroian, R. V. 2000. *Bacillus thuringiensis* (*Bt*) toxin susceptibility and isolation of resistance mutants in the nematode *Caenorhabditis elegans*. *Genetics*, **155**(4): 1693–1699.

Martin, R. J., Robertson, A. P. and Wolstenholme, A. J. 2002. Mode of action of the macrocyclic lactones. In: "Macrocyclic lactones in antiparasitic therapy" (J. Vercruysse, R.S. Rew, eds). United Kingdom, Wallingford, **P.** 125.

Mohamedova, M. and Samaliev, H. 2011. Effect of the rhizobacterium *Bacillus subtilis* on the development of the root-knot nematode *Meloidogyne arenaria* at different temperatures. *Agricultural science and technology*, **3** (4): 378-383.

Munshid, H., Simon, S. and Lal, A. A. 2013. Antagonistic potential of *Bacillus subtilis* and *Pseudomonas fluorescens* on *Meloidogyne incognita* of green onion (*Allium fistulosum*). *International Journal of Botany Research*, **3** (3): 15-22.

Muzhandu, R. T., Chinheya, C. C., Dimbi, S. and Manjeru, P. 2014. Efficacy of abamectin for the control of root-knot nematodes in tobacco seedling production in Zimbabwe. *African Journal of Agricultural Research*, **9** (1): 144-147.

Prakob, W., Nguen-Hom, J., Jaimasit, P., Silapapongpri, S., Thanunchai, J. and Chaisuk, P. 2009. Biological control of lettuce root knot disease by use of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Paecilomyces lilacinus*. *Journal of Agricultural Technology*, **5**: 179- 191.

Radwan, M. A. 2007. Bioactivity of commercial products of *Bacillus thuringiensis* on *Meloidogyne incognita* infecting tomato. *Indian Journal of Nematology*, **37** (1): 30-33.

Radwan, M. A., Abu-Elamayem, M. M., Kassem, S. M. I. and El-Maadawy, E. K. 2004. Management of *Meloidogyne incognita*, root-knot nematode by integration of *Bacillus thuringiensis* with either organic amendments or carbofuran. *Pakistan Journal of Nematology*, **22**(2): 135-142.

Saad, A. S. A., Massoud, M. A., Ibrahim, H. S. and Khalil, M. S. 2011. Management study for the root-knot nematodes, *Meloidogyneincognita* on tomatoes using fosthiazate and Arbiscular Mycorrhiza Fungus. *Journal of the Advances in Agriculture Research*, **16** (1): 137-147.

Saad, A. S. A., Massoud, M. A., Ibrahim, H. S. and Khalil, M. S. H. 2012. Activity of nemathorin, natural product and bioproducts against root-knot nematodes on tomatoes. *Archives of Phytopathology and Plant Protection,* **45**(8): 955-962.

Saad, A. S. A., Radwan, M. A., Mesbah, H. A., Ibrahim, H. S. and Khalil, M. S. 2017. Evaluation of some non-fumigant nematicides and the biocide avermactin for managing *Meloidogyne incognita* in tomatoes.*Pakistan Journal of Nematology,* **35** (1): 85-92.

Sharma, I. P. and Sharma, A. K. 2015. Effects of initial inoculum levels of *Meloidogyne incognita* J2 on development and growth of tomato cv.PT-3 under control conditions. *African Journal of Microbiology Research,* **9**(20): 1376-1380.

Sikora, R. A. and Hoffmann-Hergarten, S. 1993. Biological control of plant parasitic nematodes in plant health promoting rhizobacteria. In: "Pest management Biotechnology based technologies" (R.D. Lumsden, J.L. Vaughn, eds) American Chemical Society, Washington. USA. **PP**. 166-172.

Taylor, D. P. and Nelscher, C. 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. *Nematology,* **20**: 268-269.

Wei, J. Z., Hale, K., Carta, L., Platzer, E., Wong, C., Fang, S. C. and Aroian, R. V. 2003. *Bacillus thuringiensis* crystal proteins that target nematodes. In: Proceedings of National Academy of Sciences, USA, **100**(5): 2760– 2765.

Xiang, N., Lawrence, K. S., Kloepper, J. W., Donald, P. A., McInroy, J. A. and Lawrence, G. W. 2017. Biological control of *Meloidogyne incognita* by spore-forming plant growth-promoting rhizobacteria on cotton. *Plant Disease,* **101** (5): 774-784.

Mohamed S. Khalil* and Shimaa S. I. Abd El-Naby

Agricultural Research Center, Central Agricultural Pesticides Laboratory, El-Sabaheya, Alexandria, Egypt

*Corresponding author

Mobile: 002-0100-929-7847

E-mail: melonema@gmail.com