Mostafa et al. Egyptian Journal of Biological Pest Control (2018) 28:66
https://doi.org/10.1186/s41938-018-0068-6

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

The role of *Bacillus megaterium* and other bio-agents in controlling root-knot nematodes infecting sugar beet under field conditions

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Abstract

A micro-plot field experiment was conducted in loamy soil naturally infested with *Meloidogyne* spp. to assess the potential of bio-agents namely bio-arc (*Bacillus megaterium*), nemastrol (a mixture of active ingredients), humisun (humic acid), and dried sweet basil callus to suppress nematodes' population and induce resistance in sugar beet. Results indicated that integration of two or more components of such bio-agents gave better results in sugar beet growth parameters than did single ones. Hence, nemastrol and humisun in concomitant with bio-arc, sweet basil callus, and oxamyl (half recommended dose) induced significant ($P \leq 0.05$) and maximum improvement in total plant fresh weight and shoot dry weight. Similar trend was also noticed in root diameter and number of leaves of sugar beet infected with *Meloidogyne* spp. Additionally, the greatest suppression in nematodes' population (95.7%), root galling (83.0%), and number of egg masses (100%) was also sustained at the soil amended with nemastrol + humisun + bio-arc + sweet basil callus + oxamyl since incorporation of such organic materials into soil might enhance *B. megaterium* activity that initiates antibiotics towards nematode population. However, single application of dried sweet basil callus showed better performance than did the dried leaves in terms of female fecundity and total nematodes' population. Thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR) indicated the presence of higher content of triterpenoides that belong to three groups, i.e., lupane, ursane, and oleanane, in dried sweet basil callus compared to native dried leaves powder. Concomitant treatment with nemastrol + humisun + bio-arc + sweet basil callus + oxamyl exhibited significantly increased in sucrose (17.1%), total sugar solids (21.8%), and purity (79.0%). The activities of both peroxidase (PO) and polyphenol oxidase (PPO) showed detectable fluctuations at the end of the experiment compared to untreated plants.

Keywords: Bio-agents, *Bacillus megaterium*, Root-knot nematodes, Control, Sugar beet

Background

Root-knot nematodes (RKNs) *Meloidogyne* spp. are worldwide plant pathogens playing a detectable role in limiting the productivity of economic agriculture crops in temperate regions. RKN, *Meloidogyne incognita* (Kofoid & White) Chitwood, is among the most important nematode species in sugar beet fields (Korayem 2006) causing damage to the epidermis, cortex, and stele regions which leads to giant cells and galls formation on fibrous and lateral roots that affect water and nutrient absorption (El-Nagdi and Abd El Fattah 2011). Biological control and other eco-friendly disease control measures have gained increasing interest among researchers after the environmental restrictions on nematicidal use for controlling plant parasitic nematodes. Plant growth rhizobacterium (PGPR) that belongs to *Bacillus* spp. is being exploited commercially for plant protection to induce systemic resistance against various pests and pathogens (Mostafa et al. 2014). However, several plant species have been demonstrated to have nematicidal activity against plant parasitic nematodes (Salim et al. 2016; El-Deriny 2016; Khairy 2016). Callus tissues are among plant parts that have been evaluated for their nematicidal

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effects and introduced to pharmacological research tests (Rateb et al. 2007). Many researchers have investigated the antifungal and antimicrobial activities of certain plant callus extracts (Shariff et al. 2006), but little attention has been given to their use in nematode management (Rocha et al. 2004; Osman et al. 2008; Nour El Deen 2008; Nour El-Deen and Darwish 2011). Several studies demonstrated that callus cultures of sweet basil accumulate various phenolic compounds with antioxidant activity (Makri and Kintzios 2008).

The present work was carried out in order to study the impact of certain bio-agents as resistance inducers singly or concomitantly on *Meloidogyne* spp. infecting sugar beet under field conditions.

Materials and methods

Tested bio-agents

A) Bio-arc
   A native commercial formulation of phosphorus soluble bacterium, *Bacillus megaterium* (25×10^6 cfu/g) at 2.5 g/L of distilled water
B) Nemastrol
   A native commercial formulation of active ingredients containing glycosynolates (12%), chitinase (12×10^5 IU), cytokinins (200 ppm), flavonoids (5%) and β 1–3, Glucanase (2×10^5 IU) at the rate of 5 L/feddan
C) Humisun
   A native commercial formulation of humic acid
D) Sweet basil *Ocimum basilicum* callus
   In vitro propagation was carried out in biotechnology laboratory at Nematological Research Unit (NERU), Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt, to produce sterilized multiple shoots, the main source of callus (Murashige and Skoog 1962).
E) Sweet basil *Ocimum basilicum* leaf
   Native fresh leaves of *O. basilicum* were sun dried and powdered.

Experimental site

A micro-plot field experiment was carried out in a loamy soil (a mixture of coarse sand (2.9%), fine sand (20.7%), silt (39.8%), and clay (36.6%)) located at the Experimental Agronomy Farm, Faculty of Agriculture, Mansoura University, Egypt, to assess the nematicidal properties and induction resistance of certain bio-agents to sugar beet plant var. Negma. The plots were naturally infested with *Meloidogyne* spp. ($308J_2$/250 g), *Rotylenchulus reniformis* (453 immature females/250 g), and other nematode genera, i.e., *Tylenchorhynchus*, *Helicotylenchus*, *Aphelenchus*, and *Xiphinema*.

Agricultural practices

Inorganic fertilizers, i.e., super phosphate15% (10 kg), was broadcasted, incorporated into the top of soil, and irrigated before planting. Urea as nitrogen fertilizer (30 kg) and potassium sulfate (10 kg) were introduced 1 month after planting. Urea was reapplied after 2 weeks.

Design and micro-plot field layout

A field experiment, an area of 140 m^2, with a randomized complete block design (RCBD) and replicated three times was practiced. Each block included untreated control and 12 treated plots. A plot consisted of two rows, 60 cm wide and 5 m long. Plots were then planted with seeds of sugar beet var. Negma (three to four seeds/hill). Plants were thinned to one seedling/hill after 30 days of germination. One week after urea fertilization, all treatments were introduced. Oxamyl was applied at a rate of 0.3 g/plant in a single application and at half dose (0.15 g/plant) in concomitant applications. Plots were treated by the bio-control agents, i.e., bio-arc (a commercial product of *Bacillus megaterium* (20 ml/plant)); nemastrol (a mixture of active ingredients at 0.25 ml/plant) was applied two times at 1-week interval; humisun (a commercial product of humic acid) was applied at the rate of 100 ml/plant and powdered dried sweet basil callus (*O. basilicum*) as well as native leaves were applied three times at 1-week interval at the rate of 0.1 g/plant and incorporated into soil. Plants were harvested 6 months after planting, and roots were washed free from adhering soil. Data dealing with number of leaves, fresh shoot and root weight, dry shoot weight, shoot and root length, and root diameter were recorded. From each plot, a composite soil (250 g) was processed for nematode extraction by sieving and modified Baermann technique (Goodey 1957). At each treatment, root hairs (1 g) were stained in 0.01 acid fuchsine lactic acid (Bybd et al. 1983) and examined for the developmental stages, females, galls, and egg masses under a stereomicroscope.

Chemical analysis

One gram of dry weight of roots from each treatment was subjected to chemical analysis in order to evaluate total sugar solids (TSS), sucrose, and sugar purity.

Enzyme activity

Peroxidase (PO) and polyphenol oxidase (PPO) activities were determined in dried root tissues (0.5 g), according to the methods of Amako et al. (1994) and Coseteng and Lee (1987), respectively.

Determination of rosmarinic acid (RA) and terpenoid compounds

The presence of rosmarinic acid and terpenoids in methanolic extract of dried callus and powdered leaves of sweet basil was evaluated, using thin-layer chromatography (TLC)

and nuclear magnetic resonance (NMR) (Kintzios et al. 2003). Authentic rosmarinic acid was used as standard. Extractions and measurements of both rosmarinic acid and terpenoides were carried out at the Faculty of Pharmacy, Mansoura University, Egypt.

Data analysis

Statistically, the obtained data were subjected to analysis of variance (ANOVA) (Gomez and Gomez 1984), followed by Duncan's multiple range tests to compare means (Duncan 1955).

Results and discussion

Application of bio-arc (*B. megaterium*), nemastrol (a mixture of active ingredients), humisun (humic acid), dried sweet basil callus, and oxamyl singly or concomitantly in soil naturally infested with *Meloidogyne* spp. revealed that integrated of two or more components gave better results in sugar beet growth parameters than did single ones (Table 1). Single application of humisun (98%) and *B. megaterium* (58%) performed the best in ameliorating total plant fresh weight. Whilst growth parameters were significantly promoted ($P \leq 0.05$) by the application of nemastrol and humisun in concomitant with bio-arc, sweet basil callus, and oxamyl (half recommended dose), such application induced significant ($P \leq 0.05$) and maximum improvement in shoot (47.7%) and root (18.1%) lengths. Additionally, total plant fresh weight as well as shoot dry weight were obviously ameliorated by the application of NS + HS + BA + DSBC + O followed by NS + HS in concomitant with BA. Similar trend was also noticed with root diameter and number of leaves of sugar-beet infected with *Meloidogyne* spp. These findings supported the reports that humic acid at 0.04% not only offers significant nematode control but also improves growth of banana infected with *M. incognita* (Seenivasan and Senthilnathan 2017). Also humic acid treatments improved the yield of grape infected with *M. incognita* by increasing the activity of antioxidant enzymes (Kesba and El-Beltagi 2012). The rhizobacteria that belong to *Bacillus* viz. *B. subtilis*, *B. megaterium*, and *B. pumilus* have shown nematicidal activity against *M. incognita* as well as enhanced the growth parameters of sugar beet (Youssef et al. 2017). *B. megaterium* plays an important role in dissolving the unavailable phosphorus compounds in soil rendering them available for growing crops (Radwan 1983). However, oxamyl (standard nematicide) exhibited moderate increment in shoot weight (68.4%), total plant fresh weight (40.0%), root diameter (11.8%), and number of leaves (35.3%) of sugar beet (Table 1).

All tested materials showed antagonistic potential against *Meloidogyne* spp. infecting sugar beet. The integration of *B. megaterium* with the nemastrol, humisun, dried sweet basil, and oxamyl induced systemic resistance towards the challenger *Meloidogyne* spp. in sugar beet. Nematode population densities within 250 g soil and number of females (1 g /root) were significantly suppressed with single and

Table 1 Impact of certain bio-agents singly and concomitantly on plant growth parameters of sugar beet var. Negma infected with *Meloidogyne* spp. under field conditions

Treatments	*Plant growth response										
	Length (cm)				Plant fresh wt. (g)		Inc. % Total plant fresh wt.	Shoot Dry wt. (g)	Inc.%	Root Diam.	No. of leaves
	Shoot	Inc.%	Root	Inc.%	Shoot	Root					
NS	44.0^{f}	10.6	40.3^{f}	0.0	340.0^{i}	1050.0^{i}	39.0	150.0^{f}	200.0	13.75^{h}	17.0^{j}
HS	45.7^{cd}	14.8	42.0^{e}	4.2	480.0^{f}	1500.0^{d}	98.0	200.0^{d}	300.0	15.3^{e}	27.0^{f}
BA	40.4^{gh}	1.5	40.8^{f}	1.2	380.0^{h}	1200.0^{g}	58.0	90.0^{h}	80.0	14.63^{fg}	25.0^{g}
DSBC	40.6^{gh}	2.0	40.5^{f}	0.5	320.0^{k}	990.0^{k}	31.0	80.0^{i}	60.0	11.5^{j}	20.0^{i}
O	41.0^{g}	3.0	42.0^{f}	4.2	400.0^{g}	1000.0^{j}	40.0	100.0^{g}	100.0	14.25^{g}	23.0^{h}
NS + HS	46.4^{c}	16.6	44.5^{c}	10.4	500.0^{c}	2500.0^{c}	200.0	250.0^{c}	400.0	16.75^{c}	35.0^{c}
NS + BA	45.2^{de}	13.6	42.5^{de}	5.5	493.0^{d}	1350.0^{e}	84.3	150.0^{f}	200.0	15.88^{d}	32.0^{d}
NS + DSBC	44.5^{ef}	11.8	41.8^{e}	3.7	480.0^{f}	1100.0^{h}	58.0	100.0^{g}	100.0	15.0^{ef}	29.0^{e}
NS + O	45.0^{de}	13.1	43.3^{d}	7.4	490.0^{e}	1250.0^{f}	74.0	170.0^{e}	240.0	15.25^{e}	30.0^{e}
NS + HS + BA	47.6^{b}	19.6	45.8^{b}	13.6	520.0^{b}	3600.0^{b}	312.0	300.0^{b}	500.0	18.88^{b}	37.0^{b}
NS + HS + BA + DSBC + O	58.8^{a}	47.7	47.6^{a}	18.1	660.0^{a}	4200.0^{a}	386.0	350.0^{a}	600.0	19.5 ^{a}	46.0 ^{a}
DSBL	40.0^{h}	0.5	40.5^{f}	0.5	338.0^{j}	1000.0^{j}	33.8	100.0^{g}	100.0	13.0^{j}	25.0^{g}
Untreated plants	39.8^{h}	0.0	40.3^{f}	0.0	237.5^{l}	762.5^{l}	0.0	50.0^{j}	0.0	12.75^{i}	17.0^{j}

Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan's multiple range test
NS nemastrol, HS humisun, BA bio-arc (*Bacillus megaterim*), DSBC dried sweet basil callus, DSBL dried sweet basil leaf, O oxamyl
*Each value presented the mean of three replicates

concomitant applications by a reduction percentage in final nematode population ranged from 48.2 to 95.7% (Table 2). The greatest reduction in nematode population was sustained by the application of NS + HS + BA + DSBC + O (95.7%). Root galling (83.0%) and egg masses number (100.0%) were significantly suppressed for such treatment. Chitinase plays an important role in hydrolyzing chitin, the structure component in egg shell, and thus reducing nematode multiplication. Therefore, nemastrol (chitinase12 × 10^5 IU) could be speculated to have a defense role during infection causing severe adverse effects on crucial biological processes of *Meloidogyne* spp. Results of this study support the findings of Mostafa et al. (2014) and El Deriny (2016) in respect of microbial activity, i.e., *B. megaterium* in the soil is enhanced on incorporation of organic matter that initiated antibiosis towards the nematode activity. Padgham and Sikora (2007) reported that *B. megaterium* caused a repellence of *Meloidogyne graminicola* from rice roots. Production of repellent substances or modification of the plant's exudates by the antagonistic bacteria were suggested as mechanisms for this effect (Sikora et al. 2007).

Sweet basil, *O. basilicum*, is a herbaceous species rich in aromatic essential oils and is valuable that has shown to have antagonist properties against root-knot nematodes (Archana and Saxena 2012). Herein, sweet basil callus (53.5%) and dried leaf powder (48.2%) exhibited the least reduction in nematodes' population. However, dried sweet basil callus showed better performance than did dried leaves in terms of female fecundity. Thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR) indicated the presence of high content of triterpenoides that belong to the three groups, i.e., lupane, ursane, and oleanane, in dried *O. basilicum* callus compared to native dried leaves powder. Meanwhile, *O. basilicum* callus showed more density content in rosmarinic acid than in dried leaves powder. Rosmarinic acid has a number of interesting biological activities, i.e., antiviral, antibacterial, anti-inflammatory, antinematode (Caboni et al. 2013), and antioxidant. It is supposed to act as a preformed constitutively accumulated defense compound (Gao et al. 2005).

On the other hand, untreated sugar beet plants showed a decrease in size of storage roots and malformation appearance (forked roots) with percentage value amounted to 26.7 (Table 2). Less branched roots were noticed with the introduction of sweet basil callus, sweet basil dried leaf powder, and oxamyl. However, sugar beet plants showed healthy storage root with NS + HS + BA + DSBC +O and NS + HS + BA as well. Technological characters in terms of sucrose (17.1%), TSS (21.8%), and purity (79.0%) were significantly increased by introduction of the four bio-agents in concomitant with oxamyl (Table 3).

Increased activity of defense-related enzymes, i.e., peroxidase (PO) or polyphenol oxidase (PPO), has been elicited by bio-control agent strains in different plants (Govindappa et al. 2010). PO and PPO are thought to reinforce cell walls (lignification and suberization) at the border of infection and further limit spread of pathogens (Passardi et al. 2004). Previous studies reported that application of bio-arc + nemastrol under greenhouse conditions increased the activities of related enzymes, i.e., PO and PPO, in roots of sugar beet infected with *M. incognita*, as they reached their peaks at day 9 from

Table 2 Impact of certain bio-agents singly and concomitantly on reproduction of *Meloidogyne* spp. infecting sugar beet var. Negma under field conditions

Treatments	*Population/ 250 g soil	Female/ 1 g root	Final population	Red. %	No. of galls/ 1 g root	Red. %	No. of egg masses/ 1 g root	Red. %	Root malformation %
NS	1150.0^{f}	35.0^{f}	1185.0	79.9	33.0^{f}	29.8	8.0^{i}	77.1	0.0
HS	2150.0^{e}	40.0^{c}	2190.0	62.9	38.0^{e}	19.1	14.0^{f}	60.0	0.0
BA	2585.0^{d}	42.0^{b}	2627.0	55.5	40.0^{d}	14.9	16.0^{de}	54.3	0.0
DSBC	2700.0^{c}	43.0^{b}	2743.0	53.5	43.0^{c}	8.5	20.0^{c}	42.9	20.0
O	1100.0^{g}	33.0^{g}	1133.0	80.8	19.0^{c}	59.5	10.0^{h}	71.4	6.7
NS + HS	800.0^{j}	33.0^{g}	833.0	85.9	28.0^{b}	40.4	15.0^{ef}	57.1	0.0
NS + BA	850.0^{i}	37.0^{e}	887.0	85.0	30.0^{h}	36.2	21.0^{c}	40.0	0.0
NS + DSBC	950.0^{h}	39.0^{d}	989.0	83.2	33.0^{g}	29.8	17.0^{d}	51.4	0.0
NS + O	700.0^{k}	32.0^{g}	732.0	87.6	27.0^{i}	42.6	12.0^{g}	65.7	0.0
NS + HS + BA	630.0^{l}	27.0^{h}	657.0	88.9	25.0^{j}	46.8	5.0^{j}	85.7	0.0
NS + HS + BA + DSBC + O	240.0^{m}	12.0^{i}	252.0	95.7	8.0^{k}	83.0	0.0^{k}	100.0	0.0
DSBL	3010.0^{b}	47.0^{ab}	3057.0	48.2	45.0^{b}	4.3	25.0^{b}	28.6	20.0
Untreated plants	5850.0^{a}	49.0^{a}	5899.0	0.0	47.0^{a}	0.0	35.0^{a}	0.0	26.7

Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan's multiple range test
NS nemastrol, HS humisun, BA bio-arc (*Bacillus megaterium)*, DSBC dried sweet basil callus, DSBL dried sweet basil leaf, O oxamyl
*Each value presented the mean of three replicates

Table 3 Impact of certain bio-agents on technological characters as well as peroxidase (PO) and polyphenol oxidase (PPO) activities in roots of sugar beet var. Negma infected with *Meloidogyne* spp. under field conditions

Treatments	Sucrose %	TSS %	Purity %	Δ Absorbance units/mg protein	
				PO activity	PPO activity
NS	15.9^{g}	20.5^{g}	77.5^{f}	0.298^{f}	0.428^{f}
HS	15.7^{h}	20.3^{h}	77.5^{f}	0.307^{e}	0.436^{e}
BA	15.5^{i}	20.1^{i}	77.0^{g}	0.315^{c}	0.447^{c}
DSBC	15.3^{j}	19.9^{j}	77.0^{g}	0.311^{d}	0.442^{d}
O	16.1^{f}	20.7^{f}	77.9^{e}	0.295^{g}	0.425^{g}
NS + HS	16.9^{b}	21.6^{b}	78.0^{d}	0.275^{j}	0.406^{j}
NS + BA	16.7^{c}	21.3^{d}	78.6^{b}	0.284^{h}	0.419^{h}
NS + DSBC	16.4^{e}	20.9^{e}	78.4^{c}	0.281^{i}	0.413^{i}
NS + O	16.5^{d}	21.0^{e}	78.4^{c}	0.266^{k}	0.403^{k}
NS + HS + BA	17.0^{a}	21.5^{c}	78.4^{c}	0.259^{l}	0.395^{l}
NS + HS + BA + DSBC + O	17.1^{a}	21.8^{a}	79.0^{a}	0.253^{m}	0.391^{m}
DSBL	15.2^{k}	19.7^{k}	77.0^{g}	0.321^{b}	0.453^{b}
Untreated plants	15.0^{l}	19.5^{l}	76.9^{h}	0.329^{a}	0.458^{a}

Each value presented the mean of three replicates. Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan's multiple range test

NS nemastrol, HS humisun, BA bio-arc *(Bacillus megaterium)*, DSBC dried sweet basil callus, DSBL dried sweet basil leaf, O oxamyl, TSS total soluble solids

nematode inoculation (Ibrahim 2013; Mostafa et al. 2014). In current investigation, the activities of PO and PPO were evaluated at the end of the experiment and showed detectable fluctuations among all treatments. The greatest activities of PPO and PO were recorded in control plants. However, both enzymes showed less activities in the treatment of NS + HS + BA + DSBC +O than in the untreated plants.

Conclusions

The use of screened organic materials, i.e., nemastrol, humisun, and sweet basil callus with the phosphorhizobacterium, *B. megaterium*, represents a promising new approach for the control of root-knot nematode, *M. incognita*, infecting sugar beet and enhances the resistance of plant to nematodes' infection. Moreover, the importance of using natural resources instead of synthetic antioxidants has risen globally. Therefore, attempts to increase active compounds in ornamental plants, i.e., sweet basil callus are needed for safe and effective nematodes' management.

Acknowledgements
The authors would like to thank to Dr. Hadeer Y. Darwish, Plant Res. Institute, Giza, Egypt, for her guidance in the preparation and propagation of sweet basil callus. Sincere thanks are also given to Dr. Fatma Abdel Rahman, Faculty of Pharmacy, Mansoura University, Egypt, for the evaluation of rosmarinic acids and terpenoids in dried leaf callus of sweet basil using the thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR).

Availability of data and materials
The data sets supporting the conclusions of this article are included within the article.

Authors' contributions
All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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Received: 12 April 2018 Accepted: 23 July 2018
Published online: 09 August 2018

References

Amako A, Ghen GX, Asala K (1994) Separate assays specific for the ascorpate peroxides and guaiacol peroxidase and for the chloroplastic and cytosolic isozyme of ascorbate peroxidase in plants. Plant Cell Physiol 53:497–507

Archana B, Saxena R (2012) Nematicidal effect of root extract of certain medicinal plants in control of J_2 of *Meloidogyne incognita* in vitro and in vivo conditions. Pak J Nematol 30(2):179–187

Bybd DW, Kirapatrick T, Barker KR (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. J Nematol 15(1):142–143

Caboni P, Saba M, Tocco G, Casu L, Murgia A, Maxia A, Menkissoglu-Spiroudi U, Ntalli N (2013) Nematicidal activity of mint aqueous extracts against the root-knot nematode *Meloidogyne incognita*. J Agric Food Chem 61(41):9784–9788

Coseteng MY, Lee CY (1987) Change in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. J Food Sci 52:985–989

Duncan DB (1955) Multiple range and multiple, F-test. Biometrics 11:1–42
El-Deriny MM (2016) Integrated control of certain plant parasitic nematodes infecting cucurbitaceae plants, PhD Thesis. Egypt: Faculty of Agriculture, Mansoura University Egypt; p 156
El-Nagdi WMA, Abd El Fattah AI (2011) Controlling root-knot nematode, *Meloidogyne incognita* infecting sugar beet using some plant residues, a biofertilizer, compost and biocides. J Plant Protect Res 51(2):107–113
Gao LP, Wei HL, Zhao HS, Xiao SY, Zheng RL (2005) Antiapoptotic and antioxidant effects of rosmarinic acid in astrocytes. Pharmazie 60(1):62–65
Gomez KA, Gomez AA (1984) Statistical procedures for agriculture research, 2nd edn. Wiley, New York
Goodey JB (1957) Laboratory methods for work with plant and soil nematodes. Tech Bull No.2 Min Agric. England: Fish Ed London; p 47
Govindappa M, Lokesh S, Ravishankar VR, Rudranaik V, Raju SC (2010) Induction of systemic resistance and management of safflower *Macrophomina phaseolina* root rot disease by biocontrol agents. Arch Phytopathol Plant Protect 43:26–40
Ibrahim DS (2013) Induction of resistance to root-knot nematodes in sugar-beet plants, Ph D Thesis. Egypt: Facuty of Agriculture, Mansoura University; p 157
Kesba HH, El-Beltagi HS (2012) Biochemical changes in grape rootstocks resulted from humic acid treatments in relation to nematode infection. Asian Pac J Trop Biomed 2(4):287–293. https://doi.org/10.1016/S2221-1691(12)60024-0
Khairy D (2016) Management of root-knot nematode *Meloidogyne incognita* by the use of certain bioagents, M ScThesis. Egypt: Faculty of Agriculture, Mansoura University; p 119
Korayem AM (2006) Relationship between *Meloidogyne incognita* density and damage to sugar beet in sandy clay soil. Egypt J Phytopathol 34(1):61–68
Makri O, Kintzios S (2008) *Ocimum* sp. (Basil) botany, cultivation, pharmaceulical properties and biotechnology. J Herbs Spices Med Plants 13(3):123–150
Mostafa FAM, Khalil AE, Nour El Deen AH, Ibrahim DS (2014) Induction of systemic resistance in sugar-beet against root-knot nematode with commercial products. J Plant Pathol Microbiol 5:236. https://doi.org/10.4172/2157-7471.1000236
Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497
Nour El Deen AH (2008) Potential use of tissue culture technology in controlling plant parasitic nematodes, PhD Thesis. Egypt: Faculty of Agriculture, Mansoura University; p 152
Nour El-Deen AH, Darwish HY (2011) Nematicidal activity of certain Egyptian weeds and bald cypress callus extracts against *Meloidogyne incognita* infecting eggplant under greenhouse conditions. Egypt J Agronematol 10(2):242–254
Osman HA, El-Gindi AY, Taha HS, El-Kazzaz AA, Youssef MMA, Ameen HH, Lashein AM (2008) Biological control of root-knot nematode *Meloidogyne incognita*: 2- evaluation of the nematicidal effects of *Tagetes erecta* tissue culture under laboratory and greenhouse conditions. Egypt J Phytopathol 36(1–2):33–44
Padgham JL, Sikora RA (2007) Biological control potential and modes of action of *Bacillus megaterium* against *Meloidogyne graminicola* on rice. Crop Prot 26:971–977
Passardi F, Penel C, Dunand C (2004) Performing the paradoxal: how plant peroxidases modify the cell wall. Trends Plant Sci 9:534–540
Radwan SM (1983) Effect of inoculation with phosphate dissolving bacteria on some nutrients uptake from newly cultivated soil, M.Sc Thesis. Egypt: Faculty of Agriculture, Ain Shams University; p 184
Rateb MEM, El-Hawary SS, El-Shamy AM, Yousef EMA (2007) Production of parthenolide in organ and callus cultures of *Tanacetum parthenium* (L.). Afr J Biotechnol 6(11):1306–1316
Rocha FS, Campos VP, Silva RC (2004) Culture of cells extract of several plants on second stage juveniles of *Meloidogyne incognita*. Nematol Bras 28(2):191–198
Salim HA, Salman SI, Majeed II, Hussein HH (2016) Evaluation of some plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne* sp. J Genet Environ Resour Conserv 4(3):241–244
Seenivasan N, Senthilnathan S (2017) Effect of humic acid on *Meloidogyne incognita* (Kofoid & White) Chitwood infecting banana (*Musa* spp.). Int J Pest Manag 64(2):110–118. https://doi.org/10.1080/09670874.2017.1344743
Shariff N, Sudarshana MS, Umesha S, Hariprasad P (2006) Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. Afr J Biotechnol 5(10):946–950
Sikora RA, Schafer K, Dababat AA (2007) Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. Australas Plant Pathol 36:124–134
Youssef MMA, Abd-El-Khair H, El-Nagdi WMA (2017) Management of root knot nematode, *Meloidogyne incognita* infecting sugar beet as affected by certain bacterial and fungal suspensions. vol 19. Agric Eng Int: special issue 293–special issue 301