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Biological Management of the Root-knot Nematode on Strawberry in Egypt

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ABSTRACT

The effect of commercial biocontrol products viz. Nemakill, Nemaless, Micronema as well as an Egyptian entomopathogenic nematode (EPN) strain of *Heterorhabditis bacteriophora*-infective juveniles (IJs) within either cadavers of *Galleria mellonella* last instar larvae or EPN-IJs in water suspension, compared to chemical nematicides of Nema Plus Zero and Oxamyl on *Meloidogyne incognita* developmental parameters, strawberry growth and yield and rhizosphere microbial community were determined in two separated field experiments. Six months after strawberry transplanting, 30-88% reduction in nematode juveniles (J_2) in soil was achieved by Nemakill, Nemaless and Micronema, while reduction range was 37-64% by EPNs compared to 64-73%, by chemicals. The commercial products followed by EPNs significantly reduced the numbers of *M. incognita*-J_2, galls, and egg masses in strawberry roots. All treatments enhanced strawberry growth and yield parameters. Nemakill followed by EPNs in water and within insect gave best fruit yield of strawberry cv. Festival. Nemaless followed by Nemakill and Micronema gave best fruit yield of strawberry cv. Fertona. Effect of tested treatments on rhizosphere microbial community and mycoflora frequency in treated strawberry plants was presented and discussed.

Key words: Biological control, entomopathogenic nematode, *Meloidogyne*, rhizosphere microbial community.

INTRODUCTION

Strawberry (*Fragaria ananassa* Duch.) is one of the most economically important crops worldwide. It is grown under a wide range of climatic conditions, where cultivated for producing small delicious fruits (Kurze et al., 2001). Strawberry is important as a source of macronutrients and beneficial dietary compounds (Bianco et al., 2009) with benefits on neurodegenerative and cardiovascular diseases (Bombarely et al., 2010). The root-knot nematodes, RKNs (*Meloidogyne* spp.) especially *M. incognita* in Egypt (Ibrahim, 1985), cause considerable losses in many economically important crops. It is highly pathogenic to staple crops such as maize, potato, soybean, banana, tomato, sweet potato and yam; and industrial crops such as tobacco, coffee, sugar cane, sugar beet, cotton and pepper. Often this species also causes economic damage to fruit crops such as guava, pineapple, papaya, grapes, and strawberry (e.g. Pinkerton and Finn, 2005; Perry et al., 2009). *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are among the most economically important species of root-knot nematodes. *Meloidogyne hapla* has been observed in four major strawberry

producing areas in Québec, Canada (Bélair and Khanizadeh, 1994). Dickstein and Krusberg (1978) classified 33 strawberry cultivars as susceptible and least susceptible to *M. hapla* infection under greenhouse conditions on the basis of a galling index on the root system. Edwards et al. (1985) and Szczygiel (1981) suggested such a rating to be misleading, because cultivars with moderate root galling showed no significant growth reduction and thus were identified as tolerant. Eleven strawberry varieties were screened for their resistance against *M. incognita* by Neelam and khan (2013) in micro plots infested fields; out of which Tiago and Torrey were found resistant but Chandler was highly resistant against root knot nematode.

Growing dissatisfaction with chemical nematicides due to their environmental and health hazards has created markets for bio-nematicide products worldwide. In this respect, *Bacillus megaterium* greatly reduced the numbers of galls, females and egg-masses of *M. incognita* in the roots of sugar beet, followed by *Bacillus subtilis*, *Paecilomyces lilacinus*, *P. fumosoroseus* and *Trichoderma album* in greenhouse and field experiments (El-Nagdi et al., 2011). The commercial products Stanes sting (*B. subtilis*), Bio-Nematon (*P. lilacinus*), Stanes symbion vam plus (*Glomus fasciculatum* and *Gigaspora* sp.) and Rhizo-N (*B. subtilis*) reduced nematode parameters of *M.arenaria* on potato plants in the field. These treatments also increased the averages of growth and yield parameters (Abd-El-Khair and El-Nagdi, 2014). *Bacillus methylotrophicus* and *Lysobacter antibioticus*, when applied as soil drenches or seed treatments in greenhouse experiments, reduced root-knot severity and incidence of *M. incognita* on tomato compared to no-bacterium control. The treatments reduced root-knot disease levels and increased yields, compared to the untreated check in field trials (Zhou et al., 2016). Hammam et al. (2016) found that the two formulations of entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora*-infective juveniles (IJs) within either cadavers of *Galleria mellonella* last instar larvae or IJs in water suspensions, Bio-Nematon, Stanes sting, Micronema, Nema Plus Zero and Carbofuran could control the citrus nematode, *Tylenchulus semipenetrans*, population levels in Mandarin orchard.

The present work aimed at studying the effect of commercial products of Nemakill, Nemaless, and Micronema, in addition to the above-mentioned two formulations of EPNs, compared to the chemical nematicides Nema Plus Zero and Oxamyl, on *M. incognita* developmental parameters, growth & yield of strawberry and rhizosphere microbial community.

MATERIALS AND METHODS

1. Experimental field:

Two separate field experiments were conducted at Badr County, El Bahera Governorate, Egypt, during 2017-2018 season to test the antagonistic effects of selected biocontrol agents on *M. incognita*, rhizosphere microbial community, frequency of mycofolra and strawberry growth parameters and yield. Strawberry cultivars Festival and Fertona were separately transplanted on 20 and 11 September, 2017 in the two *M. incognita*-infected fields, respectively. Each field experiment, with loam sandy soil, consisted of 40 plots; each plot (3 × 1 m) consisted of four plant rows. All tested treatments were applied as soil treatments. Five plots were used as replicates for each treatment as well as the untreated check. All agricultural practices were carried out as recommended (El-Shemy et al., 2013).

2. Treatments:

On 30 October, 2017, eight treatments applied as soil drench in a randomized complete block design were as follows:

1. Nemakill® (natural oils) was applied at 85 ml/10 L/plot

2. Nemaless® (containing10^9 colony forming unit (CFU)/ml of *Serratia marcescens* was applied at 85 ml/10 L/plot

3. Micronema® (containing10^9 CFU/ml of *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *Bacillus thuringiensis*) was applied at 170 ml/10 L/plot

4. An Egyptian entomopathogenic nematode strain of *Heterorhabditis bacteriophora* at rate of 100 ml (125 of infective juveniles/cm^2) on the soil surface under strawberry seedling.

5. *H. bacteriophora*-infected cadavers of *Galleria mellonella* last instar larvae was applied at a rate of 5 insect (*Galleria mellonella*) beneath soil surface under strawberry seedling.

6. Nema Plus Zero® (a.i. 2.4-dinydroxybenzene 12.5%, 2.4-dichloropropene and inert ingredients 81.5%) was applied at 25 g/10 L/plot

7. Vydate® 24% L (Oxamyl) [N, N-dimethyl-2-methylcarbamoyloxyimino-2-(methylthio) acetamide] was applied at 5 ml /1 L/plot.

8. Untreated control.

3. Effect on *M. incognita* developmental parameters:

Initial soil samples were taken from each field experiment before transplanting. Then, soil and root samples were taken two, four and six months after transplanting. Five 200-g-soil samples were taken from each plot at a depth of 15-30 cm. Nematodes were extracted from soil by sieving and decanting method described by Barker (1985). The numbers of juveniles (J_2) of *M. incognita* in soil were counted using Hawksly slide under light microscope. Root samples were gently washed free of soil and an aliquot of 5 g per plot (5 plants) was cut into 2-cm-long pieces. At each sampling time, the root pieces were placed in Petri dishes with distilled water incubated under laboratory conditions (25 ± 5 ºC) for a week to extract and count *M. incognita*-J_2. The numbers of nematode galls and egg masses were also counted in another 5 g root. The percentage reductions of the root-knot nematode populations in soil (J_2) induced by the treatments were determined according to the formula of Handerson and Tilton (Puntener, 1981):

$$\text{Nematode reduction (\%)} = [1- (PTA/PTB \times PCB/PCA)] \times 100$$

Where PTA = Population in the treated strawberry plant after application, PTB = Population in the treated strawberry plant before application, PCB = Population in the check strawberry plant before application and PCA = Population in the check strawberry plant after application.

4. Effect on strawberry growth and yield parameters:

Effect of tested treatments on plant growth and yield parameters was recorded. A random sample of ten strawberry plants was separately taken from each treatment as well as control six months after transplanting. The averages of shoot lengths (cm), fresh & dry weights of shoot, and root fresh weight (g) were determined. So, the yield parameters was recorded as only one random harvest time of fruit yield (kg) per plot, then divided by plant numbers/plot to get one harvest fruit yield (g) per plant, and multiplied by number of collection times to obtain harvest fruit yield (kg) per plot-season. Strawberry fruits were collected almost weekly for 16 weeks (16 harvest times).

5. Effect on rhizosphere microbial counts:

Effect of the tested treatments on total counts of aerobic bacteria; spore-forming bacteria and fungi at the rhizosphere soil of strawberry plants were determined by the plate count technique using dilution method on suitable media (Ghini et al., 2007). Ten grams of each soil sample were separately shaken in 90 ml of sterilized distilled water in a 250 ml flask, 20 minutes on a shaker, to give a dilution of 10^{-1}. Then, serial dilutions up to 10^{-7} of fresh suspension was prepared, for each sample, by transferring 1 ml of sample suspension to 9 ml sterilized distilled water in a test tube under sterile conditions. Five replicated plates were prepared for each dilution per soil sample. To determine the aerobic bacterial count, Aliquots 1.0 ml of 10^{-5} - 10^{-7} dilution were transferred onto separated sterilized Petri plates filled with nutrient agar (NA) medium; Peptone 5 g, Beef extract 3 g, Agar 15 g, Distilled water 1L, pH 7 (Bridson , 1995). After 2 days of incubation at 30^{o}C ± 2, the resulted aerobic bacteria are presented as a number of colony-forming units (CFU) per 10 gram of soil.

After pasteurization of dilution of 10^{-1} at 80 °C for 20 min., the count of spore-forming bacteria was determined. Aliquots 1.0 ml of 10^{-3} - 10^{-5} dilutions were transferred onto separated sterilized Petri plates filled with NA for 2 days of incubation at 30 ±2 ^{o}C (Bridson, 1995). The resulted spore-forming bacteria were presented as CFU/10 g of dry soil. To count the fungi, aliquots 1.0 ml of each 10^{-3} and 10-^{4} dilution were transferred onto separated sterilized Petri plates filled with Martin medium; Glucose 10g, Peptone 5g, KH_2PO_4 1g, $MgSO_4$ 0.5g, Rose Bengal 30µg, streptomycine 0.03g,Agar 15g, Distilled water 1L (Bridson, 1995). The inoculated plates were incubated at 30 ±2 ^{o}C for 7 days before count of the fungi.

6. Effect on Mycoflora frequency:

Effects of tested treatments on the percentages of fungi occurrence in the rhizosphere of strawberry was determined as CFU per 10 gram of soil on Martin medium by the pour plate method and dilution technique (Ghini et al., 2007). One gram of soil was suspended in 90 ml sterile water to obtain a 1/100 dilution. Then, serial dilutions were prepared up to 10^{-5}. Five replicated plates were prepared for each dilution per soil sample. The plates were incubated at 30^{o}C±2 for 7 days. Fungi that grew out were count as CFU /plate. The resulted fungi were identified to genus and species level according to the key of morphological and cultural characters described by Ellis (1971) and Barnett & Hunter (1972). Each isolated fungus was counted and its frequency percentage was calculated according to the following formula:

Frequency of common mycoflora (%) = (fungus no. / total fungi no.) X 100

7. Statistical analysis:

Statistical analyses of experimental data were carried out according to ANOVA procedures (Snedecor and Cochran, 1999). Treatment means were compared at 5% level of probability by Duncan's New Multiple Range Test using Computer Statistical Package (CO-STAT) User Manual Version 3.03, Barkley Co.

RESULTS

1. Effect on nematode developmental parameters:

Effect of the tested treatments on *M. incognita* reproductive parameters 2, 4 and 6 months after treatments in two separate field experiments are listed in Tables (1 and 2). In Experiment I, Nemakill reduced the J_2 number in soil after two months by 59%, followed by Nema Plus Zero (57%), *H. bacteriophora* (IJs) in water suspensions treatment (46%), Micronema (42%), Oxamyl (40%), Nemaless (26%) and *H. bacteriophora* as insect treatment (22%), respectively. After four months, the highest percentage reduction was recorded with Nemaless (99%), followed by *H. bacteriophora* (IJs) in water suspensions (83%) and Micronema (80%). After six months of treatment, the greatest reduction of J_2 in soil, was obtained with Nemakill (88%), followed by Micronema (87%), Nemaless (85%) and Oxamyl (80%), respectively. All treatments significantly reduced numbers of each J_2 in roots, galls and egg masses, compared to untreated control 2, 4 and 6 months after treatment applications (Table 1).

In experiment II, Nemakill reduced the J_2 number in soil after two months by 46%, followed by Oxamyl (29%), Micronema (25%), each of Nemaless or Nema Plus Zero (17%), and each of liquid IJs treatment or *Galleria mellonella* infected with *H. bacteriophora* (6%), respectively. After four months, the highest percentage reduction was recorded with Oxamyl (46%), followed by Micronema (44%) and Nemakill (40%). After six months of treatment, the greatest reduction of J_2 in soil, was obtained with Nemakill (82%), followed by Oxamyl (73%), Nema Plus Zero (72%), and Micronema (66%), respectively. All treatments significantly reduced numbers of each J_2 in roots, galls and egg masses, compared to untreated plots 2, 4 and 6 months after applications (Table 2).

2. Effect on strawberry growth and yield parameters:

Effect of the applied treatments on growth parameters of strawberry *viz*. shoot length, fresh & dry shoot weights and root fresh weight as well as yield parameters as one harvest per plant, one harvest per plot and yield per plot-season of the two field experiments are shown in Tables (3 and 4). In experiment I, the shoot length ranged from 21.0 to 24.7 cm in treated plots, compared to 19 cm in the untreated control. The fresh and dry weights of shoots and fresh root weights were in the ranges of 36.9-75.6 g, 13.70-18.33 g and 10.0-14.6 g, compared to 40.3, 9.88 and 9.1 g in the untreated check, respectively. The yield weight of one harvest per plant, the fruit yield of one harvest per plot, and the fruit yield per plot-season of treated plants

Table 1. Effect of bio-control agents and chemical nematicides on developmental parameters of *Meloidogyne incognita* infecting strawberry cv. Festival under field conditions (Experiment I).

Treatments	Nematode parameters at months after transplanting												
	Initial	Two		Four					Six				
	J_2 in soil	J_2 in soil	Red. %	J_2 in soil	Red. %	J_2 in roots	Galls No.	Egg masses	J_2 in soil	Red. %	J_2 in roots	Galls No.	Egg masses
Nemakill[@]	110b[*]	35d	59	160c	58	125b	2bc	1b	120e	88	60g	6d	3d
Nemaless[@]	70ef	40c	26	150d	99	50d	1c	1b	100f	85	70f	4e	2d
Micronema[@]	100c	45b	42	70g	80	50d	2bc	2b	120e	87	70f	3e	2d
Heterorhabditis bacteriophora in water	120a	50a	46	70g	83	40e	1c	1b	400b	64	130e	8c	6bc
Heterorhabditis bacteriophora in insect	75e	45b	22	133e	49	50d	2bc	2b	320c	54	200b	10b	7b
Nema Plus Zero[@]	90d	30e	57	180b	42	60c	3b	2b	300d	64	150d	7cd	5c
Oxamyl	65fg	30e	40	100f	55	50d	2bc	2b	120e	80	180c	4e	3d
Untreated	60g	45b	-	210	-	130a	10a	7a	560a	-	900a	23a	14a

*Means, averages of five plots followed by same letter(s) in a column are not significant ($P \leq 0.05$) according to Duncan's Multiple Range Test

Table 2. Effect of bio-control agents and chemical nematicides on developmental parameters of *Meloidogyne incognita* infecting strawberry cv. Fertona under field conditions (Experiment II).

Treatments	Nematode parameters at months after transplanting												
	Initial	Two		Four					Six				
	J_2 in soil	J_2 in soil	Red. %	J_2 in soil	Red. %	J_2 in roots	Galls No.	Egg masses	J_2 in soil	Red. %	J_2 in roots	Galls No.	Egg masses
Nemakill[@]	70b[2]	30c*	46	100c	40	40b	1b	1b	60f	82	180d	3d	2c
Nemaless[@]	30f	20d	17	60f	16	20d	1b	1b	100c	30	210c	4cd	2c
Micronema[@]	50d	30c	25	60f	44	20d	2b	1b	80e	66	130g	3d	3c
Heterorhabditis bacteriophora in water	40e	30c	6	133b	- 37	20d	2b	1b	90d	53	180g	5c	4bc
Heterorhabditis bacteriophora in insect	40e	30c	6	100c	-56	30c	2b	1b	120b	37	220b	7b	5b
Nema Plus Zero[@]	60c	40b	17	90d	37	30c	1b	0c	80e	72	140f	4cd	2c
Oxamyl	70b	40b	29	80e	46	20d	1b	0c	90d	73	150e	4cd	3c
Untreated	75a	60a	-	160a	-	80a	7a	5a	350a	-	700a	18a	13a

*Means, averages of five plots, followed by same letter(s) in a column are not significant (P≤ 0.05) according to Duncan's Multiple Range Test.

Table 3. Effect of bio-control agents and chemical nematicides on growth and yield of strawberry cv. Festival infected by *Meloidogyne incognita* under field conditions (Experiment I).

Treatments	Growth and Yield parameters						
	Growth				Yield		
	Shoot Length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Plant/ one harvest (g)	Plot /one harvest (kg)	Plot /season (kg)
Nemakill[@]	24.7a*	75.6a	18,33a	11.9ab	68.7a	3.02a	48.37a
Nemaless[@]	23.7ab	36.9c	17.24b	10.0ab	48.5d	2.13d	34.15d
Micronema[@]	23.3ab	67.1abc	16.66b	13.0ab	47.0d	2.07d	33.09d
Heterorhabditis bacteriophora in water	24.7a	74.8ab	18.19a	12.9ab	61.3b	2.70b	43.16b
Heterorhabditis bacteriophora in insect	21.7ab	51.6abc	13.70d	14.3a	53.1c	2.34c	37.38c
Nema Plus Zero[@]	22.0ab	53.1abc	15.50c	14.6a	37.7e	1.66e	26.52e
Oxamyl	21.0ab	51.7abc	15.38c	11.0ab	32.0f	1.41f	22.53f
Untreated	19.0b	40.3bc	9.88d	9.1b	26.8g	1.18g	18.87g

*Means, averages of five plots, followed by same letter(s) in a column are not significant ($P \leq 0.05$) according to Duncan's Multiple Range Test.

Table 4. Effect of bio-control agents and chemical nematicides on growth and yield of strawberry cv. Fertona infected by *Meloidogyne incognita* under field conditions (Experiment II).

Treatments	Growth and Yield parameters						
	Growth				Yield		
	Shoot Length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Plant/one harvest (g)	Plot /one harvest (kg)	Plot /season (kg)
Nemakill[@]	20.0abc[*]	58.3abc	15.20f	14.8ab	86.6b	3.81b	60.97b
Nemaless[@]	23.0a	66.9ab	17.27e	16.0ab	94.8a	4.17a	66.76a
Micronema[@]	20.0abc	64.2ab	18.94c	14.6ab	83.6b	3.68b	58.85b
Heterorhabditis bacteriophora in water	20.7abc	61.6abc	18.40d	19.5a	62.0c	2.73c	43.65c
Heterorhabditis bacteriophora in insect	20.7abc	83.8ab	25.04a	17.5ab	63.2c	2.78c	44.49c
Nema Plus Zero[@]	22.3ab	88.0a	24.30b	15.6ab	57.2c	2.52c	40.27c
Oxamyl	18.0bc	55.1bc	14.46g	16.3ab	57.3c	2.52c	40.34c
Untreated	16.7c	31.4c	10.98h	11.1b	32.2d	1.42d	22.67d

*Means, averages of five plots, followed by same letter(s) in a column are not significant ($P \leq 0.05$) according to Duncan's Multiple Range Test.

ranged 32.0-68.7 g, 1.41-3.02 kg, and 22.53-48.37kg, compared to 26.8 g, 1.18 kg, and 18.87 kg in the untreated plots, respectively (Table 3). Nemakill followed by EPNs in water and within insect gave best fruit yield of strawberry cv. Festival. In experiment II, the shoot length in treated plots ranged 18.0-23.0 cm compared to length of 16.7 cm in the untreated control. The fresh and dry weight of shoot and fresh weight of roots were in the ranges of 55.1- 88.0 g, 14.46-25.04 g and 14.6-17.5 g, compared to 40.30, 9.88 and 9.10 g in the untreated plants, respectively. Yield weight of one harvest per plant, fruit yield of one harvest per plot, and fruit yield per plot-season ranged 57.2-94.8 g, 2.52-4.17 kg, and 40.27-66.76 kg, compared to 32.2 g, 1.42 kg and 22.67 kg in untreated control, respectively (Table 4). Nemaless followed by Nemakill and Micronema gave best fruit yield of strawberry cv. Fertona.

3. Effect on rhizosphere microbial counts:

Effect of the applied treatments on total microbial counts of aerobic bacteria, spore-forming bacteria and total fungi in the two field experiments are listed in Table (5). In experiment I, the total aerobic bacteria counts in the rhizosphere of strawberry ranged from 7.06 to 7.57 $\log_{10}$ CFU/10g soil, the spore-forming bacteria counts ranged 4.98-5.26 $\log_{10}$ CFU/10g soil, while the total fungi counts ranged from 4.27 to 4.53 $\log_{10}$ CFU/10g soil, compared to counts of 7.55, 5.28 and 4.47 $\log_{10}$CFU/10g soil, in untreated plots, respectively.

In experiment II, the total aerobic bacteria counts in the rhizosphere of strawberry ranged from 7.11 to 7.57 $\log_{10}$ CFU/10g soil, the spore-forming bacteria counts ranged from 4.97 – 5.27 $\log_{10}$ CFU/10g soil, while the total fungi counts ranged from 4.28 to 4.52$\log_{10}$ CFU/10g soil, compared to counts of 7.58, 5.29 and 4.44 $\log_{10}$CFU/10g soil in the untreated plants, respectively (Table 5). Yet, *Aspergillus* spp. and *Penicillium* spp had higher frequency than others. Details of frequency of the common fungi in two field experiments are listed in Tables (6 and 7).

DISCUSSION

Environmental and health concerns over the use of chemical pesticides have increased the need for alternative measures to control plant-parasitic nematodes. Biological control is considered ecologically friendly and a potential alternative in pest and disease management. Therefore, we examined the effect of a few biocontrol agents, in the form of commercial and EPN products compared herein to the chemical nematicides Nema Plus Zero and Oxamyl, on *M. incognita* population densities, rhizosphere microbial community, and strawberry growth and yield in two field experiments. Nemakill, Nemaless and Micronema could highly reduce *M. incognita*-J_2 in soil than EPNs especially after four and six months of transplanting strawberry cv. Festival, while Nemakill, Nemaless and Micronema were the best for strawberry cv. Fertona. Yet, all tested treatments enhanced strawberry growth parameters viz. shoot length, fresh & dry shoot weights and fresh root weights as well as yield parameters of both cultivars. These results partially agreed with those recorded by El-Nagdi and Abd-El-Khair (2014) where Micronema and other biocontrol agents significantly reduced *M. incognita* population levels and increased common bean yield. Also, the highest percentages reduction of juveniles (J_2) in soil and each of J_2, J_3, females and eggs in roots were achieved by Fornem x5®, followed by Micronema® after six months of application (El-Nagdi et al., 2015). Combining apple fruit fermentation plus *Bacillus licheniformis* in one treatment resulted the largest leaf area,

plant height, root length and plant weight (Zhang et al., 2016). Such a combination could increase antioxidant enzymes activities in strawberry seedlings, optimize the status of rhizosphere microbial, and promote plant growth.

Table 5. Effect of bio-control agents and chemical nematicides on total microbial counts in the rhizosphere of strawberry cvs Festival (Exp. I) and Fertona (Exp. II) six months after transplanting under field conditions.

Treatments	Total microbial counts (Log_{10} CFU/10g soil)					
	Aerobic bacteria (10^6)		Spore-forming bacteria (10^4)		Total fungi (10^3)	
	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II
Nemakill[@]	7.46b[*]	7.23d	5.17bc	4.97d	4.27b	4.33ab
Nemaless[@]	7.57a	7.57a	5.26ab	5.27a	4.32b	4.38ab
Micronema[@]	7.31c	7.40b	5.03de	5.10b	4.28b	4.28b
H.bacteriophora in water	7.55a	7.56a	5.15c	5.00cd	4.51a	4.28b
H.bacteriophora in insect	7.06d	7.11e	5.03de	5.01cd	4.46a	4.45ab
Nema Plus Zero[@]	7.27c	7.30c	4.98e	5.02c	4.53a	4.46ab
Oxamyl	7.25c	7.27cd	5.10cd	5.00cd	4.51a	4.52a
Untreated	7.55a	7.58a	5.28a	5.29a	4.47a	4.44ab

[*]Means are averages of five plots followed by same small letter (s) in a column are not significant according to Duncan's Multiple Range Test at P≤ 0.05.

Our results showed that some tested treatments affected the rhizosphere microbial levels as well as the frequency of common fungi, compared to the untreated control. These results are in agreement with those recorded by Abd-El-Khair and El-Nagdi (2014) found also that other biocontrol agents; *i.e. Trichoderma album*, *T. viride*, Rhizo-N® (*Bacillus subtilis*), Bio-Arc®, (*Bacillus megaterium*) and Bio-Zeid®, (*T. album*), induced significant differences in counts of bacteria and fungi in the rhizosphere of potato plants under field conditions.They also reported that *Aspergillus* spp., *A. niger*, *Fusarium solani*, *Penicillium* spp., *Rhizopus* spp., *Rhizoctonia solani*, and *Trichoderma* spp. were the common fungi in the rhizosphere of treated potato. El-Nagdi and Abd-El-Khair (2014) showed that *Aspergillus* spp., *A. niger*, *Fusarium* spp., *F. solani*, *Penicillium* spp., *R. solani*, *R. nigricans* and *Trichoderma* spp.were the common fungi in the rhizospheres of common bean plants treated with Stanes sting® (*Bacillus subtilis*), Bio-Nematon® (*Paecilomyces lilacinus*), *T. hamatum*, *T. album*, Stanes symbion vam plus® (*Glomus fasciculatum* and *Gigaspora* sp.) and Rhizo-N®

Table 6. Effect of bio-control agents and chemical nematicides on the frequency (%) of fungi in the rhizosphere of strawberry cv.Festival six months after transplanting under field conditions (Experiment I).

Fungi	Frequency % of fungi in treatments							
	Nemakill[@]	Nemaless[@]	Micronema[@]	*H. bacteriophora* in water	*H. bacteriophora* in insect	Nema Plus Zero[@]	Oxamyl	Untreated
Aspergillus spp.	7.7	9.7	18.6	16.7	15.6	15.9	11.8	8.9
Aspergillus niger	7.7	6.5	2.3	13.9	11.8	9.1	8.8	5.9
A.terreus	3.9	0	9.3	2.8	0	6.8	-	2.9
Alternaria spp.	3.9	3.2	2.3	0	3.1	2.3	-	5.9
Botrytis spp.	3.9	0	0	0	0	2.3	2.9	5.9
Penicillium spp.	23.1	25.9	27.9	16.7	15.6	20.5	20.6	14.7
P. chrysogenum	15.4	16.1	16.3	8.3	9.4	11.4	20.6	8.9
P. citrinum	11.5	16.1	9.3	5.6	11.8	13.6	17.7	8.9
Rhizopus nigricans	7.7	9.7	0	13.9	11.8	2.3	5.9	11.8
Rhizoctonia solani	3.9	3.2	4.7	5.6	3.1	2.3	2.9	8.9
Fusarium spp.	3.9	3.2	4.7	8.3	6.3	6.8	2.9	11.8
Trichoderma spp.	3.9	3.2	2.3	5.6	3.1	4.6	-	-
Others	3.5	3.2	2.3	2.6	8.4	2.1	5.2	5.5

Table 7. Effect of bio-control agents and chemical nematicides on the frequency (%) of fungi in the rhizosphere of strawberry cv. Fertona six months after transplanting under field application (Experiment II).

Fungi	Frequency % of fungi in treatments							
	Nemakill[@]	Nemaless[@]	Micronema[@]	*H. bacteriophora* in water	*H. bacteriophora* in insect	Nema Plus Zero[@]	Oxamyl	Untreated
Aspergillus spp.	20.6	19.2	14.3	17.1	18.2	16.2	17.7	12.8
Aspergillus niger	8.8	7.7	2.9	5.7	9.1	8.1	5.9	5.1
A. terreus	11.8	7.7	-	5.7	3.0	5.4	5.9	5.1
Alternaria spp.	2.9	3.9	5.7	2.9	6.1	2.7	5.9	7.7
Botrytis spp.	2.9	3.9	-	2.9	3.0	2.7	2.9	5.1
Penicillium spp.	11.8	15.4	25.7	22.9	21.2	10.2	20.6	12.8
P. chrysogenum	2.9	11.5	20.0	14.3	15.2	16.2	11.7	7.7
P. citrinum	5.9	7.7	14.3	11.4	12.1	13.5	8.8	7.7
Rhizopus nigricans	11.8	7.7	2.9	5.7	3.0	5.4	5.9	5.1
Rhizoctonia solani	5.9	3.9	2.9	2.9	3.0	5.4	2.9	12.8
Fusarium spp.	2.9	3.9	5.7	5.7	3.0	8.1	5.9	10.3
Trichoderma spp.	8.8	3.9	-	-	-	2.7	2.9	2.6
Others	3.0	3.6	5.7	2.8	3.1	2.8	3.0	5.2

(*B. subtilis*) in field application. Li et al. (2012) reported that such specific bacterial and fungal genera were common communities in different replant soils of strawberry,where the numbers of both bacterial and fungal communities increased in the replant strawberry soil. Zhang et al. (2016) showed that the greatest numbers of bacterial species were observed in the rhizosphere of control matrix treated with water only, while the lowest diversity appeared in the rhizosphere soil treated with *B. licheniformis* alone. Jang et al. (2016) found that a culture filtrate of *A. niger* F22 was highly active against *M. incognita* with marked mortality of second-stage juveniles and inhibition of egg hatching, where the nematicidal component was identified as oxalic acid. Application of *A. niger* in a field naturally infested with *M. incognita*, significantly reduced gall formation on the roots of watermelon plants (Jang et al., 2016). These results suggest that *A. niger* can be used as a microbial nematicide for the control of root-knot nematode disease. The data presented herein documented the importance of the rhizosphere for plant nutrition, health and quality, where the response of microorganisms to root exudates was shown to shape rhizosphere microbial communities (Berg and Smalla, 2009). This complex plant-associated microbial community is crucial for crop production. Berendsen et al. (2012) reported that different plant species may host specific microbial communities when grown on the same soil. So, plants affect their rhizosphere microbial communities that can contain beneficial effect, where one of the mechanisms of disease control is induced systemic resistance (ISR). Such ISR is effective against a wide range of pathogens and thus offers important potential for practical applications in crop protection (Doornbos et al., 2012).

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الملخص العربى

الإدارة الحيوية لنيماتودا تعقد الجذور ميلودوجين انكوجنيتا على الفراولة في مصر

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تم دراسة تأثير منتجات تجارية للمكافحة البيولوجية وهي نيماكل، ونيمالس، وميكرونيما، وكذلك الطور اليرقي المعدي من سلالة مصرية للنيماتودا الممرضة للحشرات (النوع *هتروريتيدس باكتريوفورا*) موجودة إما في معلق مائي أو داخل جثث يرقات الطور الأخير من دودة الشمع الكبري حيث تمت مقارنة هذه المركبات الحيوية بمبيدين من مبيدات النيماتودا الكيميائية هما نيما بلس زيرو ومركب الأكساميل من حيث تأثيرها علي مؤشرات نمو وتطور نيماتودا تعقد الجذور، ونمو ومحصول الفراولة، وكذلك المجتمع البكتيري الموجود في منطقة جذور الفراولة في تجربتين حقليتين منفصلتين. بعد ستة أشهر من زراعة الفراولة أحدثت مركبات نيماكل، ونيمالس، وميكرونيما انخفاض بنسبة 30–88٪ في تعداد يرقات الطور اليرقي الثاني من نيماتودا تعقد الجذور المتواجدة بالتربة، في حين كان هذا الانخفاض بسبب النيماتودا الممرضة للحشرات 37–64٪، أما الانخفاض المتسبب عن مبيدات النيماتودا فكان 64–73٪. خفضت المنتجات التجارية يليها النيماتودا الممرضة للحشرات بشكل ملحوظ من أعداد اليرقات، وكتل البيض، وعقد النيماتودا علي جذور الفراولة. عززت جميع العلاجات نمو ومحصول الفراولة. أعطي مركب نيماكل يليه نيماتودا الحشرات في الماء أوداخل الحشرة أفضل إنتاج من محصول الفراولة صنف فستيفال في حين أعطت مركبات نيماكل، ونيمالس، وميكرونيما أفضل إنتاج من محصول الفراولة صنف فرتونا. تم عرض ومناقشة تأثير العلاجات التي تم اختبارها على المجتمع البكتيري الموجود في منطقة جذور الفراولة.

الكلمات الدالة: المكافحة الحيوية، النيماتودا الممرضة للحشرات، نيماتودا تعقد الجذور، المجتمع البكتيري الموجود في منطقة الجذور، الفراولة.

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