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An ecofriendly Root- Knot Nematode Pest Management Strategy on Sugarbeet 1- Utilizing Microbial Agents

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Abstract

Filtrates of several fungi *(Arthrobotry oligospora, Dactylella brochopage, Nematochomus concurrence, Fusarium exsporium, Trichoderma harzianum* and *Varticillium chlamydosporium*) and bacteria (*Bacills cereus, B. thuringiensis, Psendornonas fluorescens and Serratia odorifera*) as biocontrol agents were tested for their nematicidal against the root-knot nematode, *M. javanica* infecting sugarbeet plants, as well as for their effects on yield and quality parameters. These microbial filtrates were applied as soil drench at the three concentrates (50, 75 and 1000%).

Results showed that all fungi and bacteria filtrates significantly reduced the numbers of nematode population and reproduction factor comparing to the check treatment. The reduction percentage of nematode counts and reproduction factor was affected by microbial filtrate type and concentration used. Enhanced reduction followed increased concentration in each microbial filtrate. Moreover, root, leaves and sugar yields, as well as quality characters (i.e. T.S.S., sucrose and purity %) were also significantly increased.

Among the fungi filtrates, *V. chamydosporiusm* filtrate at the highest concentration recorded the maximum effect in reducing number of nematode population and reproduction factor. Also, the highest increase percentages of leaves, root, sugar yields and sucrose, T.S.S and purity% were obtained at the highest concentrations of *V. chlamydosporium*. In case bacteria filtrates, the highest reduction of nematode population and reproduction factor as well as, the greatest root and sugar yields increases were achieved at the highest concentration of *B. cereus and S. odorifera*, filtrates.

Generally, the plants treated with *V. chlamydosporium, B. cereus and S. odorifera* filtrates had less nematode population and higher productivity of sugar-beet than those plants treated with the other tested microbial agents. In addition, these filtrates had nearly the same effect of the nematicide, Oxamyl on root-knot nematode, *M. javanica*. Also, these biocontrol agents are ecological sound, economical viable and partial substitutes for costly and pollution causing chemical nematicides and have been a successful instead of these chemical nematicides management strategy when used alone or in combination with other strategies.

Key words: *Arthrobotry oligospora, Dactylella brochopage, Nematochomus concurrence, Fusarium exsporium, Trichoderma harzianum, Varticillium chlamydosporium*, *Bacills cereus, B. thuringiensis, Psendornonas fluorescens, Serratia odorifera,* root- knot nematode, bioagent, biocontrol.

Introduction

Nematodes of the genus, *Meloidogyne* are also know as RKN, because they develop knots in the roots of infected plants during their parasitic life-cycle. Rootknots are giant cells of plants and, once developed nematodes use them as a source for their nutrition. *Meloidogyne* species have great economic importance as they can cause severe damage to several important crop plants.

Among the important species of the genus, *M. incognita and M. javanica* are widely distributed around the world and attack several crops, which belong to Solanaceae, Cucurbitaceae, Leguminaceae, Chenopodiaceae and other families. They also cause severe damages to some other staple crops such as cereals (rice, maize, soybean etc…..) as well as to industrial crops such as (sugarbeet, sugarcane, cotton, tobacco etc……). Economic losses have also been reported in vegetable fruit crops.

Since 1950, the control of phytoparasitic nematodes has been based on chemical pesticides, although several of them are being withdrawn from the market due to issues related to the environment and public health. However, in recent decades the utilization of chemical pesticide is being discouraged due to severe environmental problems, including ground water contamination, avian and mammalian toxicity, and accumulation of pesticides in food materials **(Bird and Kaloshian, 2003).** Nematodes also developed resistance against most of the known pesticides, and this triggered worldwide research for new alternative agents and methods for nematode control **(Fernandez *et al*., 2001, Gohar, 2003, Ibrahim *et al*., 2007; Maareg *et al.,* 1999, a& b; Maareg *et al*., 2005, a, b & c; Maareg *et al.,* 2008; Mostafa, 1998 and Youssef *et al*., 2008).**

Biological control, an ecofriendly pest management strategy that utilizes deliberate introduction of living natural enemies to lower the population level of a target pest **(Delfosse, 2005; Gohar, 2003; Maareg *et al*., 2003 and Maareg *et al*., 2005 a, b & c).** These enemies are commonly referred to as biological control agents (BCAs), which must demonstrate some characteristics for success in field, including ability for rapid colonization of the soil, persistence, virulence, predictable control blow economic threshold, easy production and application, good viability under storage, low cost of production, compatibility with agrochemicals, and safety **(Kerry, 1987).** In natural, it is observed that many natural enemies, such as viruses, bacteria, rickettsia, fungi, nematode, acari and others can attack plant parasitic nematodes, but in the search for suitable BCAs more attention has been given to fungi and bacteria. Biological control can be either natural (i.e., when a natural population of a particular organism inhibits the growth and development of nematodes), or induced (i.e., when BCAs have been introduced artificially). There are two approaches for introduction: microbial pesticide application for rapid control of pest, and the introduction or mass release of a biocontrol agent to provide long

lasting control. The suppression can be specific or non specific, when only one or two organisms are involved **(Abdel-Rahman, 1999 a & b; Akhtar and Malik, 2000; Davies *et al*., 1991; El- Sherief *et al.,* 1994; Gohar, 2003; Jatala, 1986; Kerry, 1987; Maareg *et al.,* 2003; Maareg *et al.,* 2005 b & c and Mostafa, 1998).**

Researchers have made several attempts to utilize bacteria and fungi for nematode control. Nematicidal bacteria are of two types: nematode parasites and rhizobacteria. However, nematophagous fungi are organisms that control the development of plant parasitic nematodes by way of attacking nematodes or their eggs, and they utilize them as a source of nutrients. BCAs should be safe to humans and other non- target species. The aim of the present study was evaluate the nematicidal of some soil bacteria and fungi as BCAs against *M. Javanica* infection on sugarbeet as well as their effects on crop yield and quality.

Material and Methods

Propagation of *Meloidogyne javanica* nematode in stock culture

Heavily galled sugarbeet roots var. Helios were collected and carefully washed from the adhering soil particles with tap water. The eggmasses from the egglaying females which were previously identified as *M. javanica* were picked up from the infested roots and singly inoculated into soil planted with 45-days-old tomato seedlings (*Lycopersicon esculentum* L.) in 1 m^2 lysameters filed with stemsterilized sandy loam soil, and watered as needed regularly. The infested tomato roots which contained females with their eggmasses were used to renew the stock culture. The pure stock culture in this respect was prepared from infested tomato roots through extraction by the Baermann-pan technique according to Southey (1970).

Inoculation procedures

In greenhouse experiments, the inoculation was achieved by pouring the second stage juveniles (J_2) water suspension into four holes (3-5 cm) depth around the sugarbeet root system which were immediately covered and mixed with soil. Each pot was inoculated with 2000 fresh J_2 at the fourth leaf stage seedlings.

Microbial agents

Six different antagonistic fungi (*Arthobotrys oligospora*, *Dectytella brochopage*, *Nematoclomus concurrence*, *Fusariun exsporium*, *Trichoderma horizianum* and *Verticillum chlamudosporium*) and four bacteria (*Bacillus cereus*, *B. thuringiensis*, *Pseudomonas fluorscens* and *Serratia odorifera*) isolates were used to test their effectiveness against *Meloidogyne javanica* nematode infecting sugarbeet plants as well as their effects on plant yield and quality parameters.

Preparing the microbial filtrates

Liquid culture was established for each pure fungal isolate used to inoculate 150 ml of potato dextrose broth, and each pure bacterial isolate was used to inoculate 150 ml of nutrient broth, All inoculations were carried out in 500 ml glass flasks. Flasks were incubated on 28°C under complete darkness conditions. After 10 days, the culture was blended for three minutes and the mixture was filtered first by filter paper, afterwards the filtrate was centrifuged for 15 minutes at 3000 rpm to separate the fungal spores. Filtrates were steem sterilized. Three different concentrates (100, 75 and 50%) of the sterilized microbial filtrates were applied to the plant root at a rate of 150 ml pot^{-1}. Applications of microbial agents even as fungi or bacteria were carried out twice, at the fourth leaf seedling stage and one month later.

Nematicide, Oxamyl 10%G.

Inoculated plants were treated by the systemic nematicide, Vydate® as a comparable treatment. Mixed with soil inoculated at levels of 1,2 and 4 g pot^{-1}, which were applied one time at the fourth leaf seedling stage.

Chemical name: N-N-dimethyl – 2 – methylcarbamoyloxyimino – 2 - (methylthio) acetamide structural formula:

$$(CH_3)_2NCOC(=NOCONHCH_3)SCH_3$$

Experimental design

The investigation was carried out using loamy sand soil from Banger El-Soukar district, Burg El-Arab Sector. The soil was air-dried and sieved to pass through a 2mm. sieve. The chemical characteristic of this soil is shown in Table (1).

Table (1). Chemical properties of the soil at the experimental site.

Soil depth cm	pH 1:25	EC m.m	OM %	$CaCO_3$ %	Soluble cations (mg/l)				Soluble anions (mg/l)			
					Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	Co_3^{-2}	HCO_3^{-}	SO_4^{-2}	Cl^{-}
0–30	**8.4**	**1.17**	**1.05**	**28.31**	**7.49**	**1.68**	**9.55**	**4.50**	**–**	**8.80**	**6.90**	**8.0**

The pot experiment was conducted in the greenhouse under controlled conditions (23 ± 5°C and 65 ± 5 HR). Seeds of sugarbeet var. Helios were sown in 40 cm diameter clay pots. Each pot was filed with about 7 kg of the sterilized experimental soil in October. After germination and at fourth leaf stage, seedlings were thinned to one vigorous plant pot^{-1}. After one week, pots were inoculated with 2000 freshly hatched juveniles (J_2) of root-knot nematode, *Meloidogyne javanica.* Several treatments were applied to control the population of *M. javanica.*

Each treatment level was replicated eight times. Each experiment included 16 pots (8 pots inoculated only with nematodes and the other 8 free of nematodes or any treatment) as control, 144 pots of fungi, 96 pots of bacteria and 24 pots of nematicide. Pots were watered daily with tap water. The experiment lasted six months.

At the end of each experimental period, the soil of each pot was well irrigated before removing the plant. Roots were washed in a gentle flow tap water. Fresh weights of leaves and roots were recorded (as growth parameters). However, the quality parameters in sugarbeet roots included sucrose percentage determined according to **Le-Docte (1927),** total soluble solids (TSS) percentage was measured using hand refractometer and juice purity percentage was determined as a ratio between sucrose and TSS % according to **Carruther and Olfield (1961).** However sugar weight plant^{-1} was calculated (root weight X sucrose %). Number of nematode in soil was determined by extracting through sieve and modified Baermann- pan technique **(Goodey, 1975)** and recorded. Roots were examined for developmental stages after staining by acid – fuchsin **(Byrd, *et al.,* 1983)** and recorded. Also, the rates of nematode reproduction factor and reduction% were calculated. Data analyzed statistically using the least significant differences **(Steel and Torrie, 1981).**

Results

Filtrates of some fungi (*Arthrobotry oligospora, Dactylella brochopage, Nematochomus concurrence, Fusarium exsporium, Trichoderma harzianum* and *Varticillium chlamydosporium*) and bacteria (*Bacillus cereus, B. thuringiensis, Pseudomonas fluorescens* and *Serratia odorifera*) were tested for their nematicidal against the root- knot nematode, *Meloidogyne javanica* infecting sugarbeet plants and their effects on plant growth and quality as shown in Tables (2, 3, 4, 5, 6 and 7).

Effect of selected microbial filtrates on development and reproduction of root-knot nematode, *Meloidogyne javanica* infecting to sugarbeet

The results in Tables (2 & 3) indicated that all microbial filtrates applied impaired nematode reproduction. All treatments significantly reduced the numbers of J_2 in soil, immature stages, mature females in root system and consequently the nematode reproduction factor (RF) comparing to the check treatment. The reductions percentages of nematode counts and reproduction factor was affected by microbial filtrate and concentration used. Significant ($P < 0.05$) differences in nematode suppression were noticeable among the microbial filtrates treatments and/or concentrations used. The reduction of nematode counts and fecundity increased by increasing the concentration in each microbial filtrate treatment. The highest reduction in nematode parameters was achieved when microbial filtrates were applied at their highest concentrations.

Table (2). Effect of selected fungi filtrates on the development and reproduction of root-knot nematode, *Meloidogyne javanica* infecting sugarbeet

Treatments	Level	J_2 in soil pot^{-1}		In root system				Final Population (P_f)		RF
		No.	R%	Immature stages	Mature females	Total	R%	No.	R%	
***Arthrobotry oligospora* (%)**	50	4996	52.6	1100	332	1432	64.8	6428	56.0	3.21
	75	4548	56.9	972	276	1248	69.3	5796	60.3	2.90
	100	3420	67.6	800	208	1008	75.2	4428	69.7	2.21
Mean		4321.3	59.0	957.3	272	1229.3	69.8	5550.7	62.0	2.78
***Dactylella brochopage* (%)**	50	5976	43.3	1328	412	1740	57.2	7712	47.2	3.86
	75	4628	56.1	1100	332	1432	64.8	6060	58.5	3.03
	100	3160	70.0	900	244	1144	71.9	4304	70.5	2.15
mean		4588	56.5	110.3	329.3	1438.7	64.6	6025.3	58.7	3.01
***Nematochomus concurrence* (%)**	50	5356	49.2	1320	388	1708	58.0	7064	51.6	3.53
	75	4352	58.7	1172	276	1448	64.4	5800	60.3	2.90
	100	3440	67.4	1012	172	1184	70.9	4624	68.3	2.31
Mean		4382.7	58.4	1168	278.7	1446.7	64.4	5829.3	60.1	2.91
***Fusarium exsporim* (%)**	50	5396	48.8	1440	504	1944	52.7	7320	49.9	3.66
	75	4848	54.0	1140	444	1584	61.1	6432	56.0	3.22
	100	3244	69.2	1040	200	1240	69.5	4484	69.3	2.24
Mean		4496	57.3	1206.7	382.7	1589.3	61.1	6078.7	58.4	3.04
***Trichoderma harzianum* (%)**	50	5772	45.2	1608	440	2048	49.7	7820	46.5	3.90
	75	5028	52.3	1300	328	1628	60.0	6656	54.4	3.33
	100	4156	60.6	932	268	1200	65.6	5556	62.0	2.78
Mean		4985.3	52.7	1280	345.3	1625.3	58.4	6677.3	54.3	3.34
***Varticillium chlamydosporium* (%)**	50	4552	56.8	1516	284	1800	55.8	6352	56.2	3.18
	75	3524	66.6	1040	220	1260	69.0	4784	67.3	2.39
	100	2908	72.5	832	160	992	75.6	3900	73.3	1.95
Mean		3661.3	65.3	1129.3	221.3	1350.7	66.8	5012	65.6	2.51
Oxamyl (g pot^{-1})	1	5864	44.4	1508	300	1808	55.6	7672	47.5	3.84
	2	4328	58.9	1236	252	1488	63.4	5816	60.2	2.91
	4	2784	73.6	760	164	924	77.3	3708	74.6	1.85
Mean		4325.3	59.0	1168	238.7	1406.7	65.4	5732	60.8	2.87
Check				3320	748	4068		14608		7.30
$LSD_{0.05}$ between treatments (A)		51.1				15.9		343.2		0.03
$LSD_{0.05}$ between levels (B)		33.4				10.4		224.7		0.02
A × B		62.6				19.5		420.4		0.04

J= Juveniles R= reduction RF = reproduction factor

Table (3). Effect of selected bacteria filtrates on the development and reproduction of root-knot nematode, *Meloidogyne javanica*. infecting sugarbeet.

Treatments	Level	J_2 in soil pot^{-1}		In root system				Final Population (P_f)		RF
		No.	R%	Immature stages	Mature females	Total	R%	No.	R%	
Bacillus Cereus **(%)**	50	6303	40.2	2070	395	2465	39.4	8768	40.0	4.38
	75	4427	58.0	1382	367	1749	57.0	6176	57.7	3.09
	100	3320	68.5	900	273	1139	72.0	4459	69.5	2.23
Mean		4683	55.6	1450.7	345	1784	56.1	6467	55.7	3.23
Bacillus thuringiensis **(%)**	50	6714	36.3	2129	409	2538	37.6	9252	36.7	4.63
	75	5049	52.1	1405	373	1778	56.3	6835	53.2	3.42
	100	3773	64.2	1087	300	1387	65.9	5160	64.7	2.58
Mean		5178	50.9	1540.3	360.7	1901	53.3	7082	51.5	3.54
Pseudomonas fluorescens **(%)**	50	7199	31.7	2162	421	2583	36.5	9783	33.0	4.89
	75	5460	48.2	1404	382	1786	56.1	7238	50.5	3.62
	100	4163	60.5	1101	331	1432	64.8	5595	61.7	2.80
Mean		5607	46.8	1555.7	378	1933	52.5	7538	48.4	3.77
Serratia odorifera **(%)**	50	5997	43.1	2055	370	2425	40.4	8422	42.3	4.21
	75	4342	58.8	1101	331	1432	64.8	5774	60.5	2.89
	100	3130	70.3	856	250	1106	72.8	4236	71.0	2.12
Mean		4489	57.4	1337.3	317	1654	59.3	6144	57.9	3.07
Oxamyl (g pot^{-1})	1	5864	44.4	1508	300	1808	55.6	7672	47.5	3.84
	2	4328	58.9	1236	252	1488	63.4	5816	60.2	2.91
	4	2784	73.6	760	164	92.4	77.3	3708	74.6	1.85
Mean		4325	59.0	1168	238.7	1406.7	65.4	573.2	60.8	2.87
Check		10540	0.0	3320	748	4068	0.0	14608	0.0	7.30
$LSD_{0.05}$ between treatments (A)		107.0		146.3	63.3	151		204.6		0.10
$LSD_{0.05}$ between levels (B)		75.7		103.4	44.8	107		144.7		0.07
A × B		131.1		179.2	77.5	185		250.6		0.12

R= Reduction RF = Reproduction factor

Table (4). Effect of selected fungi filtrates on leaves, root and sugar weights of sugarbeet infecting by root-knot nematode, *Meloidogyne javanica*.

Treatments	Level	Leaves plant^{-1}		Root plant^{-1}		Sugar plant^{-1}	
		g.	Inc. %	g.	Inc. %	g.	Inc. %
***Arthrobotry oligospora* (%)**	25	251.19	60.73	621.86	43.56	110.44	81.59
	50	313.38	100.52	792.45	82.94	149.77	146.26
	100	376.84	141.13	978.79	125.95	197.23	224.29
Mean		313.80	100.79	797.70	84.15	152.48	150.71
***Dactylella brochopage* (%)**	25	219.74	40.61	489.00	13.02	79.36	30.49
	50	263.27	68.46	646.83	49.32	112.74	85.38
	100	315.18	101.68	831.22	91.89	153.44	152.30
Mean		266.06	70.25	655.68	51.41	115.18	89.39
***Nematochomus concurrence* (%)**	25	291.60	86.59	484.40	11.78	84.83	39.48
	50	315.93	102.16	803.10	85.40	150.66	147.72
	100	335.43	114.63	862.75	97.86	166.77	174.21
Mean		314.32	101.13	716.68	65.01	134.09	120.47
***Fusarium exsporium* (%)**	25	263.27	68.46	646.83	49.30	112.74	85.38
	50	298.89	91.25	729.52	68.41	137.22	125.63
	100	329.78	111.02	883.88	104.05	184.38	203.16
Mean		297.31	90.24	753.41	73.92	144.78	138.05
***Trichoderma harzianum* (%)**	25	219.74	40.61	490.90	13.23	79.67	31.00
	50	251.19	60.73	621.86	43.56	110.44	81.59
	100	291.80	86.72	754.26	74.12	138.93	128.44
Mean		254.24	62.69	622.34	43.64	109.68	80.35
***Varticillium chlamydosporium* (%)**	25	313.38	100.52	702.45	62.16	129.95	113.67
	50	365.40	133.81	891.83	105.88	179.26	194.74
	100	427.55	173.58	1020.19	135.50	214.24	252.26
Mean		368.78	135.97	871.49	101.18	174.48	186.89
Oxamyl (g pot^{-1})	1	177.27	13.43	508.76	17.45	84.40	38.78
	2	214.41	37.20	555.54	28.25	96.50	58.66
	4	265.14	69.66	728.13	68.09	136.31	124.12
Mean		218.94	40.10	597.48	37.93	105.74	73.85
Check		156.28	00.00	433.18	00.00	60.82	0.00
Healthy		252.69	61.69	746.77	72.39	136.73	124.82
$LSD_{0.05}$ between treatments (A)		15.64		13.97		4.37	
$LSD_{0.05}$ between levels (B)		9.58		8.56		2.68	
A × B		19.15		17.11		5.36	

g. = gram Inc. = Increase

Table (5). Effect of selected fungi filtrates on quality parameters of sugarbeet infecting by root-knot nematode, *Meloidogyne javanica*.

Treatments	Level	Sugar		TSS		Purity	
		%	Inc. %	%	Inc. %	%	Inc. %
Arthrobotry oligospora (%)	25	17.76	26.50	21.39	18.05	83.03	7.16
	50	18.90	34.62	22.60	24.72	83.63	7.93
	100	20.15	43.52	24.04	32.67	83.82	8.18
Mean		18.94	34.88	22.68	25.15	83.49	7.75
***Dactylella brochopage* (%)**	25	16.23	15.60	19.50	7.62	83.23	7.42
	50	17.43	24.15	20.72	17.34	84.12	8.57
	100	18.46	31.48	21.75	20.03	84.87	9.54
mean		17.37	23.74	20.66	15.00	84.08	8.51
***Nematochomus concurrence* (%)**	25	17.52	24.15	21.11	16.50	82.99	7.11
	50	18.76	33.62	22.33	23.23	84.01	8.43
	100	19.33	37.68	23.00	26.93	84.04	8.47
Mean		18.54	31.82	22.15	22.22	83.68	8.00
***Fusarium exsporium*(%)**	25	17.43	24.15	20.68	14.13	84.28	8.78
	50	18.81	33.97	22.10	21.96	85.11	9.85
	100	20.86	48.58	24.43	34.82	85.39	10.20
Mean		19.03	35.57	22.40	23.64	84.93	9.61
***Trichoderma harzianum* (%)**	25	16.23	15.60	19.30	6.51	84.09	8.53
	50	17.76	26.50	21.11	16.50	84.13	8.58
	100	18.42	31.20	21.80	20.31	84.50	9.05
Mean		17.47	24.43	20.74	14.44	84.24	8.72
***Varticillium chlamydosporium* (%)**	25	18.50	31.77	22.21	22.57	83.30	7.50
	50	20.10	43.16	24.00	32.45	83.75	8.09
	100	21.00	49.57	24.50	35.21	85.71	10.62
Mean		19.87	41.50	23.57	30.08	84.25	8.74
Oxamyl (g pot^{-1})	1	16.59	18.16	19.81	9.33	83.75	8.08
	2	17.37	23.72	20.47	12.97	84.86	9.51
	4	18.72	33.33	22.06	21.74	84.86	9.52
Mean		17.56	25.07	20.78	14.68	84.49	9.04
Check		14.04	00.00	18.12	00.00	77.48	0.00
Healthy		18.31	30.41	22.00	21.41	83.23	7.41
$LSD_{0.05}$ between treatments (A)		0.42		0.38		2.05	
$LSD_{0.05}$ between levels (B)		0.26		0.24		1.25	
A × B		0.53		0.46		2.51	

Inc. = Increase

Table (6). Effect of selected bacteria filtrates on leaves, root and sugar weights of sugarbeet infecting by root-knot nematode, *Meloidogyne javanica*.

Treatments	Level	Leaves plant^{-1}		Root plant^{-1}		Sugar plant^{-1}	
		g.	Inc. %	g.	Inc. %	g.	Inc. %
***Bacillus cereus* (%)**	25	220.21	40.91	666.79	53.93	115.44	89.68
	50	237.70	52.10	734.45	69.55	135.61	122.82
	100	251.19	60.73	797.70	84.15	151.05	148.19
Mean		236.37	51.25	732.98	69.21	134.04	120.23
***Bacillus thuringiensis* (%)**	25	190.26	21.74	606.32	39.97	103.81	70.58
	50	204.51	30.86	626.95	49.35	111.75	83.62
	100	219.74	40.61	729.52	68.41	137.25	125.51
Mean		204.84	31.07	654.26	52.58	117.60	93.23
***Pseudomonas fluorescens* (%)**	25	182.93	17.05	565.78	30.61	87.73	44.15
	50	200.84	28.51	604.59	39.57	97.42	60.07
	100	214.41	37.20	655.68	51.41	113.99	87.29
Mean		199.39	27.59	608.68	40.53	99.71	63.84
***Serratia odorifera* (%)**	25	224.70	43.78	644.23	48.72	109.63	80.14
	50	239.26	53.10	704.52	62.64	126.21	107.37
	100	254.24	62.69	754.26	74.12	138.95	128.31
Mean		239.40	53.06	701.00	61.83	124.93	105.27
Oxamyl (g pot^{-1})	1	177.27	13.43	508.76	17.45	84.46	38.78
	2	214.41	37.20	555.54	28.25	96.55	58.65
	4	265.14	69.66	728.13	68.09	136.29	123.94
Mean		218.94	40.10	597.48	37.93	105.77	73.79
Check		156.28	0.00	433.18	0.00	60.86	0.00
Healthy		252.69	61.69	746.77	72.39	136.79	124.75
$LSD_{0.05}$ between treatments (A)		18.11		37.15		6.80	
$LSD_{0.05}$ between levels (B)		11.86		24.32		4.45	
A × B		22.18		45.50		8.33	

Inc. = Increase

Among, the fungi filtrates, the results indicated that the fungus, *V. chlamydosporium* at its highest concentration achieved the strongest effect on *M. javanica* population and fecundity with 2908 J_2 pot^{-1}, 832 immature stages and 160 mature females root^{-1}. Similar effects were also achieved by filtrates of *D. brochopage*, *A. oligospora* and *N. concurrence* followed by *T. harzianum*.

The highest percentage in nematode population reduction, 74.6% was achieved when using the nematicide, Oxamyl at its highest concentration (4g pot^{1}). Similar result (73.3%) was recorded with V. *chlamydosporium* filtrate at the maximum concentration. Filtrates of *D. brochopage, A. oligospora, N. concurrence, F. exsporium* followed by *T. harzianum* recorded reductions in number of P_f with 70.5, 69.7, 69.3, 68.3 and 62.0 %, respectively at their highest concentrations.

Table (7). Effect of selected bacteria filtrates on quality parameters of sugarbeet infecting by root-knot nematode, *Meloidogyne javanica*.

Treatments	Level	Sucrose		TSS		Purity	
		%	Inc. %	%	Inc. %	%	Inc. %
***Bacillus cereus* (%)**	25	17.31	23.29	21.16	16.78	81.99	5.81
	50	18.46	31.48	22.35	23.34	82.81	6.87
	100	18.94	34.88	22.68	25.15	83.52	7.78
Mean		18.24	29.88	22.06	21.76	82.77	6.82
***Bacillus thuringiensis* (%)**	25	17.10	21.79	20.47	12.98	83.53	7.80
	50	17.84	27.07	21.16	16.78	84.32	8.82
	100	18.81	33.97	22.10	21.96	85.12	9.84
Mean		17.92	27.61	21.24	17.24	84.33	8.82
***Pseudomonas fluorescens* (%)**	25	15.51	10.47	19.85	9.55	78.14	0.84
	50	16.11	14.74	20.39	12.53	79.10	2.08
	100	17.37	23.72	21.66	19.54	80.19	3.49
Mean		16.33	16.31	20.63	13.87	79.14	2.14
***Serratia odorifera* (%)**	25	17.00	21.08	20.72	14.35	82.04	5.87
	50	17.91	27.56	21.39	18.05	83.73	8.05
	100	18.42	31.20	21.80	20.31	84.50	9.05
mean		17.78	26.61	21.30	17.57	83.43	7.66
Oxamyl (g pot^{-1})	25	16.59	18.16	19.81	9.33	83.76	8.10
	50	17.37	23.72	20.47	12.97	84.86	9.51
	100	18.72	33.33	22.06	21.74	84.86	9.52
mean		17.56	25.07	20.78	14.68	84.49	9.04
Check		14.04	0.00	18.12	0.00	77.49	0.00
Healthy		18.31	30.41	22.00	21.41	83.23	7.41
$LSD_{0.05}$ between treatments (A)		0.253		0.626		2.629	
$LSD_{0.05}$ between levels (B)		0.166		0.410		1.721	
A × B		ns		ns		ns	

ns= not significant Inc. = Increase

The rates of nematode reproduction factor (RF) were significantly decreased with treatment by fungal filtrates at all concentrations compared to the check treatment. The highest reduction in nematode RF was achieved using the highest filtrate concentrations. The lowest RF of *M. javanica* was achieved by the nematicide, Oxamyl and filtrate of *V. chlamydosporium* at their highest concentrations, resulting in 1.85 and 1.95 fold, respectively. Generally, effectiveness of *V. chlamydosporium* against *M. javanica* pest was equivalent the effects of the nematicide, Oxamyl (Tables, 2).

In case bacterial filtrates, the bacterium, *S. odorifera* filtrate at its maximum concentration had the highest significant effect on *M. javanica* counts and fecundity with 3130 J_2 in soil, 856 immature stages and 250 mature females in root system compared with the check treatment. This was followed by *B. cereus* (3320, 900 and 273 ind.) *B. thuringiensis* (3773, 1087 and 300 /ind.) then *P. fluorescens* (4163 J_2, 1101, 331/ind), at their highest concentrations.

The highest reduction was achieved using filtrates of *S. odorifera,* (71.0%) and *B. cereus,* (69.5%) followed by *B. thuringiensis, (*64.7%) and *P. fluorescens* (61.7%) at their highest concentrations, respectively.

Significant reduction in nematode reproduction factor (RF) at all concentrations following treatment with bacteria filtrates. The highest reductions in RF were achieved when using the highest treatment concentrations. The minimum value (1.85 fold) of *M. javanica* RF was achieved by the nematicide, Oxamyl at its highest concentration, followed by *S. odorifera*, *B. cereus, B. thuringiensis, P. fluorescens* with 2.12, 2.23, 2.58 and 2.8 fold, respectively. Significant differences were detected between the treatments (Table, 3).

Effect of selected microbial filtrates on growth and quality parameters of sugarbeet infecting by root-knot nematode, Meloidogyne *javanica*

Data of sugarbeet plant growth response and quality parameters as influenced by microbial filtrates are listed in Tables (4, 5, 6& 7). The results revealed that, growth and quality parameters influenced by type of filtrate, as well as their concentrations. Also, the obtained data displayed positive relations between all treatments and both plant yield and quality parameters. It was noticed that all treatment concentrations showed increases in the parameters of plant growth (leaves and root weight plant^{-1}) and sugar weight plant^{-1} as well as quality parameters (sucrose, TSS and purity percentages) as compared to the check treatment.

With regard to fungi filtrates the results indicated that all fungi filtrates achieved significant increases at the all applied concentrations in the leaves and root weights plant^{-1} compared to the check treatment. The maximum increases compared to the check were achieved by using fungi filtrates at their maximum concentrations. According to the leaves weight, the highest value of leaves weight (427.55 g. plant^{-1}) was recorded at the highest concentration of *V. chlamydosporium* filtrate when compared to the check treatment (156.28 g. plant^{-1}).Filtrates of *A. oligospora*, *N. concurrence, F. exsporium, D. brochopage, T. harzianum* and the nematicide, Oxamyl at their highest concentrations showed similar effect but to a lesser degree (376.84, 335.43, 329.87, 315.18, 291.80 and 265.14 g. plant^{-1}, respectively) as shown in Table (4).

Also, data in the same Table revealed that, root weight influenced by fungi

filtrates and their concentrations. It is worthy to mention that, the increase in root weight followed the increase in dosage concentration of all tested filtrates.

In general, the highest recorded root weight (1020.19 g. plant^{-1}) was with *V. chlamydosporium* filtrate followed by *A. oligospora* (978.79 g. plant^{-1}), *F. exsporium* (883.88 g. plant^{-1}), *N. concurrence* (862.75 g. plant^{-1}), *D. brochopage* (831.99 g. plant^{-1}), *T. harzianum* (754.26 g. plant^{-1}) and finally Oxamyl nematicide (728.13 g. plant^{-1}) at their highest concentrations.

The sugar yield increased in all treatments compared to the check treatment, the highest sugar yield (214.62 g plant^{-1}) compared to the check (60.82 g plant^{-1}) was obtained by *V. chlamydosporium* filtrate followed by *A. oligospora* (197.23 g plant^{-1}), *F. exsporium* (184.38 g plant^{-1}), *N. concurrence* (166.77 g plant^{-1}), *D. brochopage* (153.44 g plant^{-1}), *T. harzianum* (138.93 g plant^{-1}) and finally Oxamyl (136.31 g plant^{-1}) as shown in Table (4).

Results of effect of the fungi filtrates on the quality parameters, sucrose, TSS and purity percentages are summarized in Table (5). Generally speeking, all fungi filtrates increased the quality parameters. All treatments exhibited significant increases of sucrose, TSS and purity% compared to the check treatment. Furthermore, Significant differences were also noticed among treatments and/or concentrations. Filtrate of *V. chlamydosporium* fungus achieved a higher increase in all quality parameters at all concentrations compared to filtrates of the other fungi and nematicide, Oxamyl. The standard concentration (100%) was optimal in increasing quality parameters for all tested fungi. At this concentration, *V. chlamydosporium* filtrate yielded the highest increases in all quality criteria compared to the check with 49.57, 35.21 and 10.62 % of sucrose, TSS and purity, respectively. Similar results was obtained with, *F. exsporium* filtrate (48.58, 34.82 and 10.21 %) for sucrose, TSS and purity were observed (Table, 5). the nematicide, Oxamyl achieved increases in percentage of sucrose, TSS and purity at all concentration, but the increases showed some decline at the higher concentration.

In general, the pots treated with *V. chlamydosporium* filtrate had less nematode population and higher plant growth and yields as well as quality of sugarbeet than those treated with other fungi filtrates.

With regarding to the bacteria filtrates, the data on sugarbeet leaves, roots and sugar yields as well as quality parameters as influenced by bacteria filtrates are listed in Tables (6 & 7). The data showed positive relationships among all the treatments and the studied criteria. Application of all concentrations of bacteria filtrates caused increase in leaves, roots and sugar weights as compared to the check treatment. Significant increases were observed especially at higher filtrate concentrations (Table, 6).

According to the leaves weight, the treatment achieving the highest increase in leave weight (254.24 g. plant$^{-1)}$ was achieved with *S. odorifera* at its highest concentration, followed by *B. cereus*, *B. thuringiensis* and *P. fluorescens* filtrates at their highest concentrations with 251.19, 241.41 and 219.74 g. plant^{-1}, respectively.

In case of the root weight, the greatest root weight (797.70 g. plant^{-1}) increase as compared to the check treatment (433.18 g. plant^{-1}) was achieved at the highest concentration of *B. cereus* filtrate, followed by *S. odorifera* (754.26 g. plant$^{-1)}$, *B. thuringiensis* (729.52 g. plant^{-1}) and *P. fluorescens* (655.85 g. plant^{-1})at their highest concentrations, In this regard the nematicide, Oxamyl come next B. *thuringiensis*.

The highest increase in sugar yield compared to the check treatment was achieved by *B. cereus* filtrate at its highest concentration with 152.46 g. g. plant^{-1} (150.7%), followed by *S. odorifera*, *B. thuringiensis*, Oxamyl and finally *P. fluorescens* at their highest concentrations with 138.93 g. plant^{-1} (128.43 %), 137.22 g. plant^{-1} (125.61 %), 136.31 g. plant^{-1} (124.10 %) and 69.18 g. plant^{-1} (89.43 %), respectively as shown in Table (6).

In concerning the quality parameters, increases in sucrose, TSS and purity percentages were improved primarily at high concentrations. The maximum increments as compared to the check were achieved when using bacteria filtrates at their maximum concentrations.

The filtrate of *B. cereus* at the highest concentration achieved significant increases in both sucrose and TSS., compared to the check treatment with 34.88 g. plant^{-1} and 25.15 %, respectively. This was followed by *B. thuringiensis* filtrate, nematicide, Oxamyl, *S. odorifera* and finally *P. fluorescens* with (33.97 & 21.96%), (33.33 & 21.74 %), (31.20 & 20.31%) and (23.74 & 15.00%), respectively. However, the maximum increment (9.84%) in TSS was achieved when using *B. thuringiensis* filtrate at its maximum concentration, followed by nematicide, Oxamyl (9.52%), *S. odorifera* (9.05%), *B. cereus* (7.78%) and finally *P. fluorescens* (3.49%) at their highest concentrations. Significant differences were noticed among the treatments (Table, 7).

Discussion

With respect to the tested fungal agents under greenhouse conditions, and the systemic Oxamyl nematicide, the results showed that they negatively reduced development and reproduction of *Meloidogyne javanica* on sugarbeet var. Helios. This was indicated by the lower population density of second stage juveniles of *M. javanica* in soil and roots, other developmental stages and consequently the reproduction factor of nematode. This was reflected on the improvement of plant growth and quality parameters. These findings are in agreement with those of **Noweer (1997); Ali and Barakat (1991); Ali *et al.* (1994); Maareg (1984); Maareg**

and Badr (2000); Badr (2000); Gohar (2003); Maareg *et al.* (2005 b & c) and Youssef *et al.* (2008). Who found that filtrates of some fungi species included *V. chlamydosporium*, *T. harzianum*, *T. viride*, *F. exsporium, F. solani* inhibited juveniles survival of plant parasitic nematode, *M. javanica* and *M. incognita* on sugarbeet and other hosts. The effect of these fungi may be due to potential of such fungi to produce compounds against penetration and development of nematodes on host root and soil **(Sharma and Saxena, 1992; El-Hadidy, 1996; Amin and Mostafa, 2000; Maareg and Badr, 2000; Gohar, 2003; Maareg *et al.*, 2005 b & c and Youssef *et al.*, 2008).**

Also, this study concluded that bacterial filtrate of *Bacillus thuringiensis, B. cereus, P Seudomonas fluorescens* and *Serrata odorifera* reduced population density of the nematode second stage juveniles as well as developmental slagis thus, the reproduction factor of *M. javanica* on sugarbeet was reduced plant growth, yield and quality improved.. These results support the findings reported by **Maareg and Badr (2000), Gohar (2003); Maareg *et al.*(2005 b) and Youssef *et al.* (2008).** They found that filtrates of soil bacteria, *B. cereus*, *P. flucorescens* and *S. odorifera* received attention as potential biocontrol against *M. javanica* and *M. incognita* nematodes on sugarbeet.

The present results also, indicated that the bacterial filtrates, *B. thuringiensis* and *S. odorifera* displayed a high nematicidal activity against post infection development of *M. javanica* nematode on sugarbeet in greenhouse than the other selected bacterial species. Similarly, **Ali and Kamal (1998); El-Sherif *et al.* (1994) and El-Nagdi (2001)** found that soil bacteria isolates, *Bacillus* spp. inhibited hatching of *M. javanica* eggs and highly toxic to juveniles of *M. incognita* and *Rotylenchulus reniformis* in laboratory and in greenhouse trials on other hosts. Also, Abdel Rahman (1999) reported that filtrate of soil bacteria, *S. odorifera* pronounced a high nematicidal activity on *M. incognita* and *M. hapla* juveniles in bio assay test. Also, **Gohar (2003) and Maareg *et al.* (2005 b)** found that the filtrates of *S. odorifera* and *P. fluorescens* showed maximum reduction in galls, eggmasses, females and juveniles of *M. incognita* on sugarbeet roots. The effect of these bacteria may be attributed to the accumulation of nematoxic metabolites of these microbial agents in soil.

Also, the data revealed that *Bacillus thuringiensis* was found to reduce the number of juveniles, other developmental stages and consequently the reproduction factor of *M. javanica*. These results are in agreement with those obtained by **Abd-El-Gawad (1995), Ismail and Fadel (1999) and El-Nagdi (2001).** who reported that the application of *B. thuringiensis* under laboratory or greenhouse conditions significantly suppressed populations of several species of plant parasitic nematodes. The mode of action of B.t.-toxins i.e., inhibition of protein and nucleic acid synthesis **(Sebesta *et al.*, 1969)** was generally sufficient to indicate that this toxin will have a wide spectrum of activity against many living organisms.

Also, the data showed that the effect of bacterial and fungal filtrates on growth and quality parameters as well as sugar yield. Results revealed that the inoculation with *M. javanica* induced a reduction in leaves and root weight, sucrose %, TSS % and purity % as well as sugar yield when compared with the unincoulated treatment. All treatments significantly increased plant growth, sucrose percent and sugar yield as compared to the check.

The fungal filtrate of *V. chlamydosporium* was ranked the first in increasing as root weight, leaves weight and sugar yield, followed by *A. oligospora* and *F. exsporium.* However, the other tested fungal filtrates gave lesser increase than those achieved by the prior ones. These results supported the findings reported by **Gohar (2003) and Maareg *et al.* (2005b).** They reported that inoculation of sugarbeet infecting by *M. incognita* with *V. chlamydosporium* and *T. harzianum* showed better enhancement in plant growth, sucrose % purity % and sugar yield. Also, the bacterial filtrate of *S. odorifera* and *B. cereus* recorded the highest increase in growth parameters, sucrose % and sugar yield, followed by *B. thuringiensis* and *P. fluorescens*. Similar results obtained by **Gohar, (2003); Maareg *et al.* (2005 b) and Youssef *et al.* (2008).** They found that the microbial filtrates of *V. chlamydosporium*, *A. oligospora, F. exsporium, B. cereus, S. odorifera, B. thuringiensis* and *P. fluorescens* reduced the ability of juveniles of *M. javanica* to infect and develop on sugarbeet and therefore, improved the root, leave and sugar yields.

In conclusion, IPM program as necessary in a manner that minimizes economic, health and environmental risks, which could be based on the previous findings, consisted of the filtrates of fungi (*Arthrobotry oligospora, Dactylella brochopage, Nematochomus concurrence, Fusarium exsporium, Trichoderma harzianum* and *Varticillium chlamydosporium*) and the filtrates of bacteria (*Bacillus cereus, B. thuringiensis, Pseudomonas fluorescens* and *Serratia odorifera*) for control root- knot nematode, *Meloidogyne javanica* instead of chemical nematicides,.

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الملخص العربي

إستراتيجية إدارة مكافحة نيماتودا تعقد الجذور على بنجر السكر بوسائل صديقة للبيئة

١- استخدام الميكروبات

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نظرًا لافتقار المقاومة في معظم أصناف بنجر السكر لنيماتودا تعقد الجذور التي تسبب أضرار اقتصادية وخسائر فادحة في محصول الجذور والسكر الناتج. ولما كانت المبيدات النيماتودية عالية التكاليف من الناحية الاقتصادية وذات تأثير ضار على البيئة وصحة الإنسان والحيوان فإن الاتجاه الحديث هو البحث عن وسائل بديلة صديقة للبيئة، آمنة، رخيصة التكاليف، وتوجد بوفرة في البيئة لحل المشاكل الناجمة عن هذه الآفة.

ومن هذه الوسائل استخدام الميكروبات الموجودة في التربة الزراعية، ولذا تم تقييم فاعلية رواشح ست عزلات فطرية: *(Arthrobotry oligospora, Dactylella brochopage, Nematochomus concurrence, Fusarium exsporium, Trichoderma harzianum* and *Varticillium chlamydosporium)*

وأربع عزلات بكتيرية:

(Bacills cereus, B. thuringiensis, Psendornonas fluorescens and Serratia odorifera)

بتركيزات ٥٠ و٧٥ و١٠٠% (أضيفت للتربة) لتقييم تأثيرها على درجة تطور وتعداد وتكاثر نيماتودا تعقد الجذور النوع *Meloidogyne javanica* التي تصيب بنجر السكر مع دراسة أثر استخدامها على المحصول والجودة مقارنة بالمبيد النيماتودي أوكساميل.

أظهرت النتائج أن جميع الرواشح الفطرية بتركيزاتها المختلفة قد نجحت معنويًا في خفض تعداد النيماتودا (في التربة وفي جذور النبات)، وكذلك معدل تكاثرها مع تحقيق زيادة معنوية في الصفات المحصولية والجودة مقارنة بمعاملة الكنترول، وتزداد نسبة الخفض في تعداد النيماتودا ومعدل تكاثرها وكذلك نسبة الزيادة في محصول الجذور والأوراق والسكر وصفات الجودة (نسبة السكروز والنقاوة والمواد الصلبة الذائبة الكلية) بزيادة تركيز الراشح الفطري أو البكتيري.

فيما بين الفطريات، وجدَّ أن أعلى خفض معنوي في تعداد النيماتودا ومعدل تكاثرها وأعلى زيادة معنوية في محصول الجذور والأوراق والسكر وجميع صفات الجودة تتحقق من تطبيق راشح الفطر *V. Chlamydosporium* مع أعلى تركيز له، كما وجد أن أفضل الرواشح البكترية تأثيرًا على النيماتودا والمحصول راشحي بكتيريا *B. cereus and S. odorifera* حيث حققت أعلى خفض معنوي في تعداد النيماتودا (في التربة وجذور النبات) ومعدل تكاثرها وأعلى زيادة معنوية في محصولي الجذور والسكر وكذلك صفات الجودة عند أعلى تركيز لهما.

بالمقارنة بتأثير المبيد النيماتودي، وجد أن تأثير راشح الفطر *V. Chlamydosporium* يتساوى مع تأثير المبيد على النيماتودا، كما أن راشحي البكتيريا *B. cereus and S. odorifera* تقترب في تأثيرها على النيماتودا مع تأثير ذلك المبيد النيماتودي فضلاً عن أن هذه الميكروبات حققت زيادة معنوية في المحصول والجودة تفوق المبيد، ونظرًا لأن هذه الميكروبات تمتاز بوجودها في البيئة ويمكن تنميتها اقتصاديًا كما أنها قليلة التكلفة ولم تسبب التلوث البيئي الذي تسببه المبيدات الكيماوية، لذا يمكن استخدامها بنجاح كمبيدات حيوية منفردة بدلاً من المبيدات الكيماوية أو يمكن إدخالها في برامج المكافحة المتكاملة، كما يمكن استخدام رواشح الميكروبات المختبرة الأخرى كعناصر مكافحة حيوية لهذه الآفة.