JOURNAL OF PLANT PROTECTION RESEARCH Vol. 51, No. 2 (2011)

CONTROLLING ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* INFECTING SUGAR BEET USING SOME PLANT RESIDUES, A BIOFERTILIZER, COMPOST AND BIOCIDES

Wafaa Mohamed Abd Elhameed El-Nagdi[1]*, *Ahmed Ibrahim Abd El Fattah*[2]*

[1] Plant Pathology Department, Nematology Lab., National Research Center, P.O. Box 12622, Dokki, Egypt
[2] Sugar Crops Research Institute, Plant Protection Department, Agricultural Research Centre (ARC) P.O. Box 12619 Giza, Egypt

Received: December 11, 2009
Accepted: December 12, 2010

Abstract: Sugar beet (*Beta vulgaris* L.) is considered an important sugar crop in Egypt and the world and it is highly infested by *Meloidogyne incognita.* This nematode causes damage to epiderm, cortex and stele regions including giant cells in these regions that then reflect on the water and nutrient absorption. As a result, sugar beet produces a poor yield. The obtained results can be summarized as follows: 1 – in general, all plant residues, biofertilizer and organic compost alone or in combination with biocides significantly reduced the number of nematode juveniles (J_2) in soil, number of galls and eggmasses in roots. All plant residues, biofertilizer and organic compost alone or in combination with biocides also significantly increased the studied sugar beet growth and technological characteristics as percentage sucrose, total soluble solids and juice purity. 2 – adding plant residues, organic compost (OC), nile fertile (NF) and biocides alone in the soil gave significant reduction in the number of juveniles in the soil, the galls and the eggmasses on sugar beet roots. In the greenhouse, bionema (B) proved to be the most effective material causing significant reduction (91.0, 81.3 and 83.2%, for respective nematode criteria). Also, organic compost caused a reduction of 86.3, 75.0 and 80.0% for the respective nematode criteria followed by dry leaves of fleabane (F), nemaless (N), mud sugar beet (M), nile fertile (NF) and dry leaves of sugar beet (S), respectively. The best results (92.3, 82.5 and 84.6) were given by 3 – combination of B+NF in reducing the nematode parameter followed by B+M, B+OC, B+S and B+F, respectively. A significant reduction of nematode parameters in this study was provided by 4 – combination of N+F, followed by N+M, N+NF, N+OC and N+S. Under field conditions, after three months, bionema proved to be the most effective material causing significant reduction (55.6, 67.9, 78.5 and 57%) in number of: juveniles in the soil, females, galls on sugar beet roots and rate of nematode build-up, respectively. After six months, a combination of B+M gave the best results (82.3, 70.8, 78.3, 84.1 and 81.1%) in reducing the nematode parameter. These results show how improved plant growth and technological characteristics help reduce the nematode *Meloidogyne incognita*.

Key words: control, root-knot nematode, plant residues, mineral fertilizer, compost, biocides, sugar beet

INTRODUCTION

Plant parasitic nematodes cause severe damage to a wide range of economic crops. These nematodes produce an annual loss of over US$ 100 billion to world agriculture and an estimated US$ 500 million are usually spent on nematode control (Keren-Zur *et al*. 2000). In Egypt, *Meloidogyne incognita*, due to its frequency of occurrence, high level of infestation and possible interactions with other pathogens, is considered the predominant species attacking sugar beet (*Beta vulgaris* L.) crop (Ibrahim 1982; Oteifa and El-Gindi 1982; Abd El-Massih *et al*. 1986; Maareg *et al*. 1998; El-Nagdi *et al*. 2004, Korayem 2006). Reduction of crop losses due to nematodes is one way of increasing crop yields. Therefore, some additives such as soil amendments (Maareg *et al.* 1999, Maareg *et al*. 2008) and certain biocontrol agents (Maareg and Badr 2000, Youssef *et al*. 2008) were tested against root-knot nematode on sugar beet, to minimize environmental pollution and keep management processes more economical. The aim of this research was to evaluate two biocides (bioagents as antagonistic bacteria) singly or in combination with some residues plant, organic compost, and a biofertilizer, for controlling the root-knot nematode infecting sugar beet under green house and field conditions.

MATERIALS AND METHODS

Greenhouse conditions

This experiment was carried out under greenhouse conditions at the Sugar Crops Research Institute (SCRI), Agricultural Research Center (ARC), Egypt. Sugar beet (*B. vulgaris*) cv. Top was used as the host plant for *M. incognita*. Plastic pots, 30 cm. diam. containing 6 kg solarized sandy loam (1:1) soil were put on a bench in a completely randomized block design in a greenhouse at 20±5°C. The

*Corresponding address:
ahmedscri@yahoo.com, wafaaelnagdi@yahoo.com

tested materials were incorporated into the soil two weeks before planting. These materials were plant residues viz., dry leaves of fleabane [(*Conyza dioscoridis*) (F), sugar beet (S), mud sugar beet (M)], organic compost (OC) as sugar cane residues at a rate of 12 g/pot, Nile fertile (NF) as a biofertilizer at a rate of 2 g/pot and two biocides [bionema (B) containing *Bacillus penetrans* and nemaless (N) containing *Serratia marcescens*] at a concentration of 20% (200 ml/pot) alone or incombination with plant residues. After germination, 21 days from sowing, plants in each pot were thinned to one plant. One week later, the pots were inoculated with 1,000 of the newly hatched second stage juveniles (J_2) of *M. incognita* per pot obtained from pure culture tomato (*Lycopersicum esculentum* cv. Peto UC 82). The untreated six pots were left as the control. Observations were recorded 6 months after nematode inoculation, by extracting nematode from the soil using sieving and decanting methods (Barker 1985). The number of galls and eggmasses in roots were also counted. Plant growth criteria as indicated by the number of leaves, and the weight of shoots and roots were recorded, and technological characters of roots, including percentage of sucrose (S%), was determined according to Le-Docte (1927). Percentage of Total soluble solids (TSS) was measured in the fresh weight of roots by using refractometer. Percentage of juice purity was determined as the ratio between S% and TSS% according to Carruthers and Oldfield (1961). Chemical analysis and C/N ratios of the tested organic amendments were illustrated in table 1.

Field conditions

This experiment was carried out in clay loam soil infested with *M. incognita* root-knot nematode. The selected site was at Nubaryia County; Beheira Governorate, Egypt. The experimental field in the 2008/2009 season was 294 m^2, divided into four blocks and twenty eight plots, seven for each block (3 m x 3.5 m = 10.5 m^2 *i.e.* 1/400 Fadden) with six rows. Seeds of sugar beet, *B. vulgaris*. Top were sown in the last week of October 2008. Seeds were planted to provide the normal density of plants/Fadden (40 000 plants) (1 ha = 2.4 Fadden). The tested materials were plant residue viz., mud sugar beet (M), Nile fertile (NF) as a biofertilizer at a rate of Ton/Fadden (Fed.) and a biocide bionema (B) containing *Bacillus penetrans* at a concentration of 20% (10 l/Fed.) alone or in combination with mud sugar beet or Nile fertile. Oxamyl 24% L was applied at rate of 3 l/Fed. These materials were put into the soil two weeks before planting. The seven treatments were allotted randomly in each block. All treatments were managed throughout the growing season by standard agricultural practices and were irrigated as needed. The treatments were allotted in plots with average Pi equal to 180 juveniles/200 gm soil. Four replicates were maintained for each treatment. Observations were recorded, 3 and 6 months after sowing by extracting nematode from the soil using sieving and decanting methods (Barker 1985). The number of females, galls and eggmasses in roots were counted and the rate of nematode build up was calculated, as follows:

$$\text{Rate of build-up} = \frac{\text{No. of juveniles + No.of females + No. of eggmasses}}{\text{Initial nematode populations}}$$

The same criteria except for the number of leaves were recorded as described above.

Statistical analysis

Data were analyzed using Fisher's Least Significant Difference (LSD). The means were compared by LSD at the 0.01 and 0.05 levels of significance (Steel and Torrie 1980).

Table 1. Chemical analysis and C/N ratio of the tested amendments

Treatments	Organic carbon [%]	Organic matter [%]	Nitrogen [%]	C/N
Fleabane (F)	33.6	57.9	1.27	26.5:1
Mud sugar beet (M)	18.6	32.0	1.35	13.8:1
Organic complementary (OC)	20.5	40.3	1.30	15.8:1
Sugar beet (S)	19.8	29.o	1.38	14.3:1

C/N – carbon/nitrogen

RESULTS

Greenhouse conditions

In general, all plant residues, biofertilizer, and organic compost alone (Table 2) or in combination with biocides (Table 3), significantly reduced the number of nematode juveniles (J_2) in the soil, and the number of galls and eggmasses in roots. All plant residues, biofertilizer and OC alone or in combination with biocides significantly increased the studied sugar beet growth and technological characteristics as percentage sucrose, total soluble solids and juice purity (Tables 4, 5). Adding plant residues, OC, NF and biocides alone to the soil gave significant reduction in number of juveniles in the soil, the number of galls and the eggmasses on sugar beet roots. Bionema proved to be the most effective material causing significant reduction (91.0, 81.3 and 83.2%, respectively). Also, OC caused 86.3, 75.0 and 80.0% for the respective nematode criteria followed by F, N, M, NF and S, respectively.

The combination of B+NF gave the best results (92.3, 82.5 and 84.6%) in reducing the nematode parameter followed by B+M, B+OC, B+S and B+F, respectively. The combination of N+F significantly caused reduction (86.3, 72.5 and 75.4%) for number of juveniles in soil, number of galls and eggmasses on sugar beet roots, respectively, followed by N+M, N+NF, N+OC and N+S which significantly reduced nematode parameters in this study. Fleabean has the highest C/N ratio (26.5:1) followed by the organic complementary (15.8:1), sugar beet (14.3:1) and mud sugar beet (13.8:1).

Table 2. Effect of bionematicide, organic soil amendments and a biofertilizer as single treatments on *M. incognita* infecting sugarbeet cv. Top

Treatments	No. of juveniles in soil	Reduction [%]	No. of galls	Reduction [%]	No. of eggmass	Reduction [%]
Fleabane (F)	380	83.8	22	72.5	16	75.4
Mud sugar beet (M)	500	78.6	32	60	20	69.2
Organic complementary (OC)	320	86.3	20	75.0	13	80.0
Sugar beet (S)	540	76.9	40	50.0	28	56.9
Nile fertile (NF)	480	79.5	35	56.3	25	61.5
Bionema (B) (10 l)	210	91.0	15	81.3	11	83.2
Nemaless (N) (10 l)	380	83.8	28	65	22	66.2
Untreated (control)	2340		80		65	
LSD at 0.05	20.99		10.60		15.90	
LSD at 0.01	40.12		14.61		21.91	

Values are averages of six replicates

Table 3. Effect of bionematicide, organic soil amendments, and a biofertilize as combined treatments on *M. incognita* infecting sugar beet cv. Top

Treatments	No. of juveniles in soil	Reduction [%]	No. of galls	Reduction [%]	No. of eggmass	Reduction [%]
B+F	380	83.8	24	70.0	18	72.3
B+M	200	91.5	26	67.5	16	75.4
B+NF	180	92.3	14	82.5	15	84.6
B+OC	240	87.7	25	68.8	22	66.2
B+S	300	87.2	20	75.0	14	78.5
N+F	320	86.3	22	72.5	16	75.4
N+M	500	78.6	40	50.0	25	61.5
N+NF	380	83.8	28	65.0	20	69.2
N+OC	480	79.5	32	60.0	22	66.2
N+S	540	76.9	35	56.3	28	56.9
Untreated (control)	2340		80		65	
LSD at 0.05	40.76		2.90		4.76	
LSD at 0.01	60.49		3.95		6.49	

Values are averages of six replicates; B – bionema; F – fleabane; M – mud sugar beet; NF – nile fertile; OC – organic complementary; N – nemaless; S – sugar beet

As for sugar beet plant growth, it was noticed that all plant residues, a biofertilizer and organic compost alone (Table 4) or in combination (5) with biocides achieved the highest increases for studied plant growth criteria.

Data in table 4 indicated that adding plant residues, OC, NF and biocides alone in soil gave a significant increase in the number of leaves (fleabane, 44.4%), weight of shoots (bionema, 58.3%), and weight of roots (mud sugar beet, 38.5%), Sucrose and TSS (bionema, 23.3 and 20%); respectively. Also, data in table 5 indicated that the combination of (B+NF) showed the best results in the number of leaves, sucrose, and TSS (55.6, 18.4 and 20%), respectively. A combination of B+M caused an increase in the weight of shoots and roots (158.3 and 100.3%), respectively.

Table 4. Effect of bionematicide, organic soil amendments and a biofertilizer as single treatments, on sugar beet growth, yield and technological characters infected by *M. incognita*

Treatments	No. of leaves	Weight of shoots [g]	Weight of roots [g]	Sucrose [%]	TSS [%]	Juice purity [%]
Fleabane (F)	26	160	174	11.8	17.7	74.4
Mud sugar beet (M)	25	175	180	11.7	16.7	76.0
Organic complementary (OC)	20	190	160	12.1	18.0	75.0
Sugar beet (S)	22	165	150	11.3	16.7	75.5
Nile fertile (NF)	24	180	145	11.4	17.3	76.1
Bionema (B) (10 l)	25	190	175	12.7	18.0	75.0
Nemaless (N) (10 l)	24	160	160	11.8	17.7	74.4
Untreated (control)	18	120	130	10.3	15.0	76.1
LSD at 0.05	4.9	37.6	25.9			
LSD at 0.01	ns	60.8	52.7			

Values are averages of six replicates; ns – not significant
TSS – Total soluble solids

Table 5. Effect of bionematicide, organic soil amendments and a biofertilizer as combined treatments on sugar beet growth, yield and technological characters infected by *M. incognita*

Treatments	No. of leaves	Weight of shoots [g]	Weight of roots [g]	Sucrose [%]	TSS [%]	Juice purity [%]
B+F	25	180	194	11.8	16.7	74.4
B+M	26	310	251	11.7	17.8	76.0
B+NF	28	243	190	12.2	18.0	75.0
B+OC	27	184	178	11.6	16.7	75.5
B+S	24	176	170	12.1	17.3	80.6
N+F	25	157	169	12.4	16.0	76.1
N+M	24	168	165	12.1	16.8	75.0
N+NF	22	170	190	11.7	17.0	74.4
N+OC	26	185	180	11.4	16.9	76.0
N+S	24	155	160	12.0	15.8	75.0
Untreated (control)	18	120	130	10.3	15.0	75.5
LSD at 0.05	5.8	40.7	27.6			
LSD at 0.01	ns	70.5	50.7			

Values are averages of six replicates; ns – not significant; B – bionema; F – fleabane; M – mud sugar beet; NF – nile fertile; OC – organic complementary; N – nemaless; S – sugar beet

Field conditions

Data reported in table 6 showed that there were a significantly reduced number of nematode juveniles (J_2) in soil, number of females, number of galls, eggmasses in roots, rate of build-up. Table 6 also shows the significantly increased studied sugar beet growth and technological characteristics as percentage increase in sucrose, total soluble solids and juice purity (Table 7). After three months, bionema proved to be the most effective material causing significant reduction (55.6, 67.9, 78.5 and 57%) in number of juveniles in soil, females, galls on sugar beet roots and rate of build-up, respectively. Adding plant residue viz., mud sugar beet (M) alone in soil gave significant reduction followed by Nile fertile (NF) as a biofertilizer (Table 6). The combination of B+M gave the best results (50, 64.3, 67.6 and 52.2%) in reducing the nematode parameter followed by B+NF, respectively. After six months, the combination of B+M gave the best results (82.3, 70.8, 78.3, 84.1 and 81.1%) in reducing parameter of nematode followed by B alone, B+NF, M, oxamyl and NF alone, respectively.

Data in table 7 indicated that adding bionema alone in soil gave a significant increase in shoot weight (35.8%), root diameter (80.7%), weight of roots (55.6%), roots yield (84.7%), sucrose and % juice purity (28.6 and 14.9%); respectively followed by mud sugar beet and Nile fertile. Data in table7 also indicated that the combination of (B+M) showed the best results for shoot weight, root diameter, weight of roots; roots yield, sucrose, and TSS (38.9, 88.6, 61.1, 75.9, 35.7 and 15.3%), respectively, followed by B+NF.

Table 6. Effect of bionema, organic soil amendment, a biofertilizer as single or combined treatments on *M. incognita* infesting sugar beet cv. Top

Treatments	Nematode criteria after three months				Nematode criteria after six months				
	No. of J_2 [200 g soil]	No. of female	No. of galls	Rate of build-up	No. of J_2 [200 g soil]	No. of female	No. of galls	No. of eggmasses	Rate of build-up
Bionema (B)	160 (55.6)*	18 (67.9)	16 (78.5)	0.99 (57.0)	130 (77.0)	30 (68.8)	34 (71.7)	28 (79.7)	1.0 (75.0)
Mud sugar beet (M)	200 (44.4)	30 (46.4)	38 (44.1)	1.3 (43.5)	180 (68.1)	38 (60.4)	40 (66.7)	36 (73.9)	1.4 (68.2)
Nile fertile (NF)	240 (33.3)	24 (57.1)	32 (52.9)	1.5 (34.8)	210 (62.8)	50 (47.9)	44 (63.3)	40 (71.0)	1.7 (61.4)
B+M	180 (50.0)	20 (64.3)	22 (67.6)	1.1 (52.2)	100 (82.3)	28 (70.8)	26 (78.3)	22 (84.1)	0.83 (81.1)
B+NF	200 (44.4)	22 (60.7)	28 (58.8)	1.2 (47.8)	160 (71.6)	34 (64.6)	32 (73.3)	26 (81.2)	1.2 (72.7)
Oxamyl 24% l	180 (50.0)	20 (64.3)	18 (73.5)	1.1 (52.2)	180 (68.1)	40 (58.3)	36 (70.0)	30 (78.3)	1.3 (70.5)
Untreated (control)	360	56	68	2.3	564	96	120	138	4.4
LSD at 0.05	25.9	5.8	4.7		20.4	8.6	6.5	6.9	

*figures in parenthesis indicate percentage nematode reduction

Table 7. Effect of single or combined treatments of bionema, organic soil mendments and a biofertilizer on sugar beet growth, yield and technological characters infested by *M. incognita*

Treatments	Shoot weight [gm]	Root diameter [cm]	Root weight [kg]	No. of survival plants/fed.	Roots yield Ton/ Fadden	Sucrose [%]	TSS [%]	Juice purity [%]
Bionema (B)	978	15.9	2.800	13400	37.5	18	19	94.7
Mud sugar beet (M)	870	13.2	2.300	11600	26.7	16	18	88.9
Nile fertile (NF)	860	11.8	2.000	12800	25.6	17	19	89.5
B+M	1000	16.6	2.900	12300	35.7	19	20	95.0
B+NF	940	14.4	2.500	14100	35.3	20	22	90.9
Oxamyl 24% l	890	13.2	2.200	13800	30.4	18	20	90.0
Untreated	720	8.8	1.800	11300	20.5	14	17	82.4
LSD at 0.05	56.9	3.8		120.9	5.8			

TSS – Total soluble solids

DISCUSSION

According to the obtained results, the effect of the tested materials is more pronounced when mixed together than as single treatments. This could be attributed to those plant residues having a synergistic effect. The addition of organic compost and Nile fertile to biocides increased the activity and reproduction of the tested microorganisms. Similar results were obtained by Aboa-Elamayem *et al*. (1989), Radwan (1999), Keren-Zur *et al*. (2000), Amer and Zaki (2002) and Radwan *et al*. (2004). Biological control agents of soil borne pathogens when applied to soils in combination with organic materials reduced nematode occurrence (Rodriguez-Kabana *et al*. 1987; Mittal *et al*. 1995; Chen *et al*. 2000; Youssef *et al*. 2008). In addition, the organic materials act as carriers to these microorganisms and decomposition increased in the soil. Toxic gasses and compounds against nematodes formed, which in turn increased soil fertility and plant growth criteria (Chen *et al*. 2000). Breakdown of organic materials may release toxic and nematicidal substances that contribute to nematode control. In this present study, it is possible that nematicidal activity, at least nitrogenous by-products, should be the most evident when the C/N ratio of the amendment is less than 20:1 (Stirling 1991). As a result, the less the C/N ratio, the more the effect on the nematodes.

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POLISH SUMMARY

ZWALCZANIE GUZAKA *MELOIDOGYNE INCOGNITA* NA BURAKU CUKROWYM PRZY ZASTOSOWANIU RESZTEK ROŚLINNYCH, BIONAWOZU, KOMPOSTU ORAZ BIOCYDÓW

Burak cukrowy jest uważany za jedną z najważniejszych roślin dostarczających surowiec do produkcji cukru, zarówno w Egipcie jak też na świecie. Korzenie buraka cukrowego są w silnym stopniu opanowywane przez nicień *Meloidogyne incognita.* Nicień uszkadza epidermę, tkanki kory pierwotnej i okolice walca osiowego wraz z komórkami olbrzymimi, wpływając destrukcyjnie na proces pobierania wody i składników pokarmowych przez korzeń buraka. W efekcie rośliny buraka wydają niższy plon o gorszej jakości. Prezentowane wyniki badań można podsumować następująco: 1 – resztki roślinne, bionawóz oraz kompost zastosowany samodzielnie lub łącznie z biocydami ograniczyły liczbę larw (stadium J_2) w glebie, liczbę galasów na korzeniach i masę

jaj, a także wpłynęły istotnie na wzrost plonu i cechy technologiczne korzeni buraka, takie jak : procent cukru, zawartość ogólnych rozpuszczalnych substancji stałych oraz czystość soku. 2 – zastosowanie doglebowo resztek roślinnych, kompostu (OC), osadu rzecznego Nilu (NF) i samych biocydów istotnie wpłynęło na obniżenie liczby larw w glebie, galasów na korzeniach oraz masy jaj. W warunkach szklarniowych biocyd Bionema (B) okazał się najskuteczniejszy, a procenty skuteczności wynosiły odpowiednio 91,0, 81,3 i 83,2%, biorąc pod uwagę przyjęte kryteria skuteczności (liczba larw, galasów i masa jaj). Zastosowanie kompostu spowodowało spadek nasilenia występowania larw, liczby galasów oraz masy jaj odpowiednio o 86,3, 75,0 i 80%, a w dalszej kolejności sklasyfikowano działanie następujących kombinacji: suche liście rośliny *Conyza dioscorid* (F), biocyd Nameless (N), resztki roślin buraka cukrowego (M), osad rzeczny Nilu (NF) oraz suche liście buraka cukrowego (S). 3 – biorąc pod uwagę kryteria oceny skuteczności zastosowanych środków, najlepsze wyniki dała kombinacja B + NF (odpowiednio 92,3. 82,5 i 84,6%), a następnie kombinacje B + M, B + OC, B + S oraz B + F. 4 – istotne ograniczenie liczby larw, galasów oraz masy jaj uzyskano przy zabiegach N + F, N + M, N + NF, N + OC i N + S. W warunkach polowych biocyd Bionema po upływie 3 miesięcy dawał najlepsze wyniki pod względem ograniczenia liczby larw w glebie, liczby samic, galasów na korzeniach buraka oraz wskaźnika występowania nicieni, a procentowe wielkości poszczególnych parametrów wynosiły odpowiednio 55,6, 67,9, 78,5 oraz 57.0%. Po upływie 6 miesięcy od zabiegu kombinacja B + M dawała najlepsze wyniki (82,3, 70,8, 78,3, 84,1 oraz 81,1%) biorąc pod uwagę odpowiednie kryteria oceny skuteczności.

Wyniki przeprowadzonych badań dowodzą, że zapewnienie bardziej sprzyjających warunków dla rozwoju roślin buraka cukrowego wpływa pozytywnie na wielkość plonu, jego cechy technologiczne, a także przyczynia się do znacznego ograniczenia występowania *M. incognita*.