Effect of Different Management Practices for Controlling Root-Knot Nematode Meloidogyne incognita on Squash E.M.A. Noweer and Susan A.A. Hasabo Plant Pathol. Dept., Nat. Res. Centre, Giza, Egypt.

Under field conditions, farmyard manure, urea, ammonium sulphate (20.5 N %), ammonium nitrate (33.5 N%), Agerin (commercial formulation of the bacterium, Bacillus thuringiensis), Nemaless (a product of the bacterium, Serratia marcescens), Nile fertile (a product contains a sulphur bacterium) and Yeast (a product contains cells of Saccharomyces cerevisiae) were applied either singly or in different combinations for controlling Meloidogyne incognita infecting squash, Cucurbita pepo var. Melopepo. The combined treatments were more effective in reducing numbers of the second stage juveniles in soil and root gall formation more than the single treatments. Also the tested materials improved fruit yield as compared to the untreated check.

Key words: Bioagents, management, *Meloidogyne incognita* and squash.

Squash, Cucurbita pepo var. Melopepo., represents one of the most important cucurbitaceous vegetable crops in Egypt. However, its production remains limited due to many diseases caused by fungi, bacteria, virus and nematodes especially the root knot nematode, Meloidogyne spp. of which M. incognita being the most frequent (Moussa et al., 1981 and Montasser, 1982). The use of chemicals to control phytoparasitic nematodes has always been an expensive remedy and may also reduce populations of beneficial antagonistic microorganisms in soil. Thus, there is a need to develop alternative strategies to manage plant parasitic nematode. In cooperation of organic amendments into soil as nutrient sources for crop production have been found to be effective in controlling nematodes. Different hypotheses which may explain their effectiveness were discussed by Alam et al. (1977 and 1980). Mineral fertilizers, which release ammonia in soil, were very effective against nematodes (Huebner et al., 1983). Suppressive effects of some microbial agents on nematodes have recently been considered. Ali (1996) and El-Sherif et al. (1999) found that liquid cultures of some bacterial species including Serratia marcescens or its filtrates could inhibit egg hatching and juvenile survival of there plant parasitic nematodes in soil. Sulphur bacterium (Thiobacillus sp.) that oxidizes sulphur to sulphate which in turn can be reduced by sulphate reducing bacteria to hydrogen sulphide toxic to nematodes, (Jaco and Fortuner, 1979). Bacillus thuringiensis is one of the biocontrol agents used as a microbial pathogen to control plant parasitic nematodes (Osman et al., 1988; Mena et al., 1996; El-Nagdi and Youssef, 2004 and Radwan et al., 2004). Nemaless, a commercial biofertilizer contains a strain of the bacterium S. marcescens, was used as soil treatment by Ismail and Hasabo (2000) and El-Nagdi and Youssef (2004). Several researches on

fungal biocontrol agents affecting root-knot nematodes have been reported by Godoy et al. (1983) and Dube and Smart (1987), but a little was found on the effect of yeast fungus (Youssef and Soliman, 1997). The present work aimed to asses the potentials of four different bioagents in commercial biofertilizer products against M. incognita infecting squash when applied separately or in combination with farmyard manure and different nitrogen sources under field conditions.

Materials and Methods

The present study was conducted during November, 2003 to March, 2004, in a salty sand soil naturally infested with root-knot nematodes *M. incognita* due to successive cultivation of peanut crop, located at Abd-Elsamad village, Giza governorate. A field experiment perpendicularly ploughed twice, then comprised as 10 rows, 70 cm in between. Two cubic meters of farmyard manure were mixed with soil except that of the untreated check. Squash seeds (*Cucurbita pepo* var. Melopepo) were sown in 84 rows; each was 4 m in length and 1 m in width.

Before sowing, one composite soil sample was collected from each row on November 25/2003 and the nematode 2nd stage juveniles were extracted by sieving and decanting methods to estimate the initial population density (Goodey, 1963).

After seed germination, the following materials were applied either separately or in combination as shown in (Table 1), each in four replicates (rows):

- 1- Agerin, Agriculture research centre commercial bioproduct, as a bioagent containing an Egyptian isolate of *Bacillus thuringiensis*, was added to the soil at the rate of 5g/plant.
- 2- Nemaless, Agriculture Research Centre, commercial bioproduct, as a bioagent containing an Egyptian isolate of Serratia marcescens, at the rate of 5 ml/plant.
- 3- Yeast, imported commercial product, containing cells of Saccharomyces cerevisiae, at the rate of 5 g/plant.
- 4- Nile fertile, a commercial bioproduct from Giza Com. for biofertilizers industry, as a biofertilizer contains sulphur bacterium (*Thiobacillus* sp.) and some nutrient elements (S, 38%; N, 2.7%; P₂O₂, 3.5%; K₂O, 1.2%; CaO, 5%; MgO, 2.7% and Fe, 1%) at the rate of 3g/plant.
- 5- The mineral fertilizers used were urea (NH₃)₂Co, 48%, ammonium nitrate NH4 NO₃, 33.5% and ammonium sulphate (NH₄)₂ SO₄, 20.5% at the rate of 3g/plant.

The materials were added biweekly starting December 15, 2003 to end of the experiment (March, 2004). Traditional agricultural practices were carried out according to technical recommendations in squash cultivation.

Nematode count in soil samples was recorded during February and March, 2004 using sieving and decanting procedure. Number of galls/1g root was counted for each particular treatment. Accumulated yield of squash for each treatment was recorded through 4 harvest times.

Data were statistically analyzed following Duncan's multiple range test (Duncan, 1955) and percentage of reduction in the second juveniles (J2) counts was estimated according to Anderson and Tilton formula (Puntener, 1981) as follows:

Whereas: J2 PTA= Population of the treated plots after application, J2 PCB= Population of the check plots before application, J2 PTB= Population of the treated plots before application and J2 PCA= Population of the check plots after application.

Results

Data in Table (1) reveal the percentages of reduction in *M. incognita* second stage larvae in soil as affected by the individual and combined treatments of the tested materials. Two months after materials application on November, 2003, the individual treatment of farmyard manure gave the least reduction percentage in count of second stage juveniles (33.7%). Statistically significant differences among treatments of the mixtures were noticed in reduction percentage in counts of the second stage juveniles when compared with that of the untreated check. The mixture

Table 1. Effect of different biomaterials and their combinations on soil

juveniles of M. incognita infecting squash roots

Sampling time and nematode population							
		2/2/2004		28/3/2004			
Treatment	25/11/2003	No. of	Red. (%)	No. of	Red. (%)		
	[Initial	Juveniles/		Juveniles/			
	population	kg soil		kg soil			
F m+ urea + Agerin	2180	642	76.5 ab	215	95.5 a		
F m+ urea + Nemaless	1940	616	74.6 ab	316	92.6 ab		
F m+ urea + yeast	1385	420	75.8 ab	185	94.0 ab		
F m+ urea	1745	865	60.4 c	1046	72.9 c		
Fm+ ammonium nitrate + Agerin	980	384	68.7 bc	335	84.5 ab		
F m + ammonium nitrate + Nemaless	1120	714	49.1 cd	464	81.3 bc		
F m + ammonium nitrate + yeast	2205	975	64.7 bc	433	91.1 ab		
F m+ ammonium nitrate	1620	1035	51.0 cd	1174	67.2 cd		
F m+ ammonium sulphate+ Agerin	785	224	77.2 ab	118	93.2 ab		
Fm+ ammonium sulphate+ Nemaless	974	276	77.4 ab	109	94.9 ab		
F m+ ammonium sulphate + yeast	1086	375	72.4 b	114	95.3 a		
F m+ ammonium sulphate.	840	425	59.6 cd	885	52.4 e		
F m+ Nile fertile + Agerin.	2165	614	77.3 ab	224	95.3 a		
F m+ Nile fertile+ Nemaless.	2065	412	84.1 a	188	95.9 a		
F m+ Nile fertile+ yeast	1630	504	75.3 ab	165	95.4 a		
F m+ Nile fertile	965	720	40.4 de	936	56.2 de		
F m+ Agerin	745	615	34.1 e	520	68.5 cd		
F m+ Nemaless	1050	584	55.6 cd	318	86.3 ab		
F m+ yeast.	875	316	71.2 b	123	93.6 ab		
F m alone	1108	920	3307 e	1209	50.7 e		
Control	965	1208		2135			

- Values are averages of 4 replicates. F m= Farmyard manure.

Reduction (%)= (efficiency %) according to Anderson and Tilton formula (Puntener, 1981).

Data with the same letters within a column are not significantly different according to Duncan's a new multiple range test.

of farmyard manure + Nile fertile + Nemaless gave the highest significant reduction percentage (84.1%) in counts of the 2nd stage juveniles in soil followed by farmyard manure + ammonium sulphate + Nemaless (77.4%), farmyard manure + Nile fertile + Agerin (77.3%), farmyard manure + urea + Agerin (76.5%) and then farmyard manure + urea + yeast (75.8%). Four months after materials treating on March, 2004, the mixture of farmyard manure + Nile fertile + Nemaless caused also the highest significant nematode reduction (95.9%) followed by farmyard manure + urea + Agerin (95.5%), farmyard manure + Nile fertile + yeast (95.4%), farmyard manure + Nile fertile + Agerin and farmyard manure + ammonium sulphate + yeast (95.3%).

With regard to gall formation in squash roots under stress of different treatment, it is evident (Table 2) that no galls were observed with farmyard manure+ ammonium sulphate+ Nemaless on February. Relatively a few gall counts was obtained by farmyard manure+ urea+ yeast or farmyard manure + ammonium sulphate+ yeast, as they were reduced gall formation by 88.9%. The highest gall number was detected with the farmyard manure when applied alone as it was 15galls/Ig roots (Table 2).

Table 2. Effect of different biomaterials on gall formation of Meloidogyne

incognita infecting squash roots

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	Sampling time and nematode population					
Treatment	2/2/200)4	28/3/2004			
i reathern	No. of galls/g	Red. (%)	No. of galls/g	Dad (9/)		
	roots		roots	Red. (%)		
F m+ urea + Agerin	6	66.7 cd	8	63.6 d		
F m+ urea + Nemaless	5	72.2 c	5	77.3 bc		
F m+ urea + yeast	2	88.9 ab	_ 6	72.7 cd		
F m+ urea	11	38.9 f	14	36.4 e		
Fm+ ammonium nitrate + Agerin	8	55.6 de		72.7 cd		
F m + ammonium nitrate + Nemaless	6	66.7 cd	6	72.7 cd		
F m + ammonium nitrate + yeast	3	83.3 Ъ	5	77.3 bc		
F m+ ammonium nitrate	14	22.2 g	18	18.1 f		
F m+ ammonium sulphate+ Agerin	4	77.8 bc	6_	72.7 cd		
Fm+ ammonium sulphate+ Nemaless	-	100 a	4	81.8 bc		
F m+ ammonium sulphate + yeast	2	88.9 ab		100 a		
F m+ ammonium sulphate.	9	50.0 ef	14	36.4 e		
F m+ Nile fertile + Agerin.	6	66.7 cd	8	63.6 d		
F m+ Nile fertile+ Nemaless.	4	77.8 bc	5	77.3 bc		
F m+ Nile fertile+ yeast	4	77.8 bc	4	81.8 b		
F m+ Nile fertile	6	66.7 cd	16	27.3 ef		
F m+ Agerin	8	55.6 de	8 -	63.6 d		
F m+ Nemaless	4	77.8 bc	6	72.7 cd		
F m+ yeast.	3	83.3 b	4	81.8 bc		
F m alone	1.5	16.7 g	19	13.6 fg		
Control	18	•	22			

- Values are averages of 4 replicates. F m = Farmyard manure.

⁻ Data with the same letters within a column are not significantly different according to Duncan's new multiple range test.

⁻ Reduction % = (efficiency %) according to Anderson & Tilton formula (Puntener,1981).

Four months, after materials treatment on March 2004, the highest percentage of gall reduction (100%) was achieved by farmyard manure+ ammonium sulphate + yeast followed by farmyard manure+ Nile fertile + yeast or farmyard manure + ammonium sulphate + Nemaless (81.8%). Also, the least reduction of galls (13.6%) was obtained by farmyard manure when applied alone.

As for squash yield, Table (3) shows that the highest increase in yield/plant (88.2%) was achieved by the combined treatment of farmyard manure + ammonium nitrate + Agerin followed by farmyard manure + urea+ Nemaless (56.9%), farmyard manure+ ammonium sulphate + yeast (54.8%), farmyard manure + ammonium sulphate + Agerin (50.0%).

Table 3. Effect of application with different biomaterials on some growth parameters of squash plants grown in soil naturally infested with *M. incognita*

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Treatment	No. of plants/treatment	No. of fruits/plant	Yield/ plant (kg)		Increase (%)
F m + urea + Agerin	50	19 bc	1.85 b	92.5	48.2
F m + urea + Nemaless	48	20 bc	2.04 bc	97.9	56.9
F m + urea + yeast	42	18 abc	1.9 b	79.8	27.9
F m + urea	50	19 bc	1.6 b	80.0	88.2
F m + ammonium nitrate + Agerin	46	17 ab	1.9 b	87.4	40.1
F m + ammonium nitrate + Nemaless	48	18 abc	1.8 b	86.4	38.5
F m + ammonium nitrate + yeast	46	20 bc	1.95 b	89.5	43.4
F m + ammonium nitrate	42	16 a	1.4 a	58.8	-
F m + ammonium sulphate+ Agerin	52	18 abc	1.8 b	93.6	50.0
Fm + ammonium sulphate + Nemaless	49	17 ab	1.96 b	96.04	54.5
F m + ammonium sulphate + yeast	46	15 a	2.ĭ c	96.6	54.8
F m + ammonium sulphate	44	14 a	1.5 a	66.0	5.8
F m + Nile fertile + Agerin.	48	18 abc	1.78 b	85.4	36.9
F m + Nile fertile+ Nemaless.	43	20 bc	1.82 b	78.3	25.5
F m + Nile fertile+ yeast	44	21 c	1.94 b	85.4	36.9
F m + Nile fertile	46	18 ab	1.35 a	62.1	-
F m + Agerin	52	l6 ab	1.6 ab	83.2	33.3
F m + Nemaless	49	17 ab	1.73 ab	84.8	35.9
F m + yeast.	50	15 a	1.8 b	90.0	44.2
F m alone.	49	14 a	1.4 a	68.6	9.9
Control.	52	16 ab	1.2 a	62.4	

- Values are averages of 4 replicates. - F m = Farmyard manure.

Discussion

Results of the present investigation revealed that all the tested materials either applied singly or in combination have significantly affected J2 of M. incognita in soil and root galling in squash and increased yield of the plant under field conditions. Results obtained for the effect of farmyard manure on nematode population density were similar to those reported for other organic amendments.

Data with the same letters within a column are not significantly different according to Duncan's new multiple range test.

Such effect may be attributed to the decomposition processes with the liberal water resulted in releasing nematotoxic substances (Alam et al., 1977 and Badra et al., 1979). It is also possible that the amendments change the physical and chemical properties of the soil to be unfavourable to nematodes (Ahmed et al., 1972) or have induced resistance in the plant (Alam et al., 1977 and Sitaramaiah and Singh, 1978).

The reduction in nematode population obtained by mineral fertilizers may be due to the liberation of ammonia which may inhibit of giant cell formation and suppress nematode development in infected roots (Mojtahedi and Lownsbery, 1976; Alam, 1991; Orion and Chitwood, 1995; Youssef and Soliman, 1997 and Shah et al., 2003). Also, urea suppressed nematode and gall formation. These findings are in agreement with those of Huebner et al. (1983) who reported that compound is readily converted to ammonia by urea present in the soil; thus urea acts as a fertilizer and nematicide. The commercial product (Agerin) had also adverse effect as it reduced number of the second stage juveniles of M. incognita in soil and galls on roots. These results confirm those obtained by Osman et al. (1988), Mena et al. (1996), Radwan et al. (2004) and El-Nagdi and Youssef (2004). The mode of action of B. thuringiensis toxins is mainly inhibition of protein and nucleic acid synthesis (Sebesta et al., 1969).

Nemaless, which contains S. marcescens was effective against the nematode and this is in agreement with those obtained by El-Nagdi and Youssef (2004). Also, Ali (1996) and El- Sherif et al., (1999) found that liquid cultures of some bacterial species, including this bacterium, or its filtrates could inhibit egg hatching and juvenile survival of three plant parasitic nematodes in soil. The effect of the biofertilizer "Nile fertile" on nematode population might be due to its contents of sulphur bacterium (Thiobacillus sp.) which oxidises sulphur to sulphate. This can in turn be reduced by sulphate reducing bacteria to hydrogen sulphide toxic to nematodes as suggested by Jaco and Fortuner (1979).

The effect of yeast on M. incognita might be due to the activity of S. cerevisiae to convert carbohydrates to ethyl alcohol and CO_2 toxic to nematodes. This is in agreement with the results obtained by Youssef and Soliman (1997). The increase in number and weight of fruits in all treatments is partially due to the effect of the tested materials on the nematode; besides its role in plant nutrition as suggested by Akhtar and Alam (1990).

In conclusion, effect of the biofertilizers with different sources of nitrogen could suppress population of *M. incognita* and root gall formation and enhance yield production of squash under field conditions.

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(Received 01/06/2005; in revised form 27/11/2005)

تأثير طرق مقاومة مختلفة لمكافحة نيماتودا تعقد الجذور ميلودوجين إنكوجئيتا على الكوسة عزت محمد عبد الباقى نوير - سوزان عبد العظيم حسبو قسم امراض النبات - المركز القومي للبحوث - جيزة - مصر.

تم تحت ظروف الحقل بمحافظة الجيزة إختبار سماد عضوى ومخصبات حيوية وأسمدة معدنية في حقل كوسة مصاب بنيماتودا تعقد الجذور ميلودوجاين إنكوجنيتا وكانت المواد المختبرة هي:-

سماد بلدي بمعدل هجم / للجورة، الأجارين بمعدل هجم/ للجورة ويحتوى على بكتريا باسيلس ثيوريجنسس، محلول نيمالس بمعدل ه مل/ للجورة والمحتوى على بكتريا سيراشيا مارسيسنس، نايل فيرتيل بمعدل ٣ جم/ للجورة، المحتوى على بكتريا الكبريت (ثيوباسيلس)، فطر الخميرة بمعدل هجم/ للجورة، سلفات امونيوم بمعدل ٣جم/ للجورة، نترات امونيوم بمعدل ٣جم/ للجورة، يوريا بمعدل ٣ جم/ للجورة، وقدأستخدمت هذه المواد منفردة أو مجتمعة.

أوضحت نتائج الدراسة قدرة جميع المعاملات المستخدمة على خفض الكثافة العددية لنيماتودا تعقد الجذور في التربة والجذر وذلك في حالة استخدامها منفردة و مجتمعة . وكانت افضل المعاملات هيمعاملة السماد البلدى + نيل فيرتيل + نيمالس وذلك بعد شهرين من المعاملة وفي نهاية التجربة حيث ادت إلى خفض تعداد البرقات بنسبة ١٨٤١/١، ٩٠٩٩ على المتوالى وذلك في التربة أما تأثير المعاملات على النيماتودا في الجذر فكانت افضلها هو استخدام سماد بلدى + سفات أمونيوم + فطر الخميرة بعد شهرين من المعاملة أو في نهاية التجربة وكانت نسبة النقص ٨٨٨٩ ، ١٠٠٠ على التوالى . مع وجود فروق معنوية واضحة .

كما أدت المعاملات الى زيادة معنوية فى محصول نبات الكوسة وكانت الفضل المعاملات معاملة سماد بلدي +نترات امونيوم +الاجارين التى أدى إلى زيادة قدر ها ٢و٨٨ عن الكونترول.

تشير نتائج هذه الدراسة إلى إمكانية استخدام بعض المخصبات الحيوية والسماد البلدى والأسمدة المعدنية منفردة أو مجتمعة كطريقة فعالة لمكافحة نيماتودا تعقد الجذور ميلودوجين الكوجنيتا في نبات الكوسة ـ حيث نتميز هذه المواد بكونها أمنة للبيئة وسهلة الإستخدام مما يؤهلها للإمراج ضمن المكافحة المتكاملة للنيماتودا.