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CONTROL OF THE ROOT KNOT NEMATODE, *Meloidogyne javanica* INFECTED TOMATO PLANTS

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ABSTRACT

The effects of one isolate of *Bacillus subtilis* and two isolates of *B. thuringiensis* subsp. *aegypti* (*Bt*), the bioproducts Agarin® and Vertemic® and the chemical nematicide Vydate L 24% on *Meloidogyne javanica* infection on tomato plants were tested in the greenhouse. Treatment with Vydate L 24% caused the great reduction (90-93.7%) in nematode infection and reproduction (numbers of nematode root galls, egg-masses/plant and number of 2nd stage juveniles (J_2) /250 cc soil). Treatments with sporulated bacterial cells suspension of the tested *Bacillus* isolates resulted in 59.1-80.1% reduction in nematode infection whereas treatment with the cell free-filtrates gave low reduction (16-57.7%) in nematode infection and reproduction. In another test, treatments with Vydate L 24%, Agarin® and Vertemic® induced great reduction (66.3-91.8%) in nematode infection and reproduction on infected tomato plants. Also, treatments with Vydate L 24% and the tested bioproducts showed significant increase in the dry weight of the shoot and root systems in comparison with control treatment.

Keywords: Biological control – Root-knot nematode - *Meloidogyne javanica* - *Bacillus* spp. – Agarin® Vertemic® - Vydate L 24%-Tomato.

INTRODUCTION

Over the past twenty years a large number of studies have been undertaken to investigate the effects of using different microorganisms (biocontrol agents) and bioproducts compounds in comparison with the chemical nematicides against nematode pests (Jansson and Rabatin, 1998; Cochran *et al*, 2007 and El-Bagory, 2008). Plant-parasitic nematodes cause serious crop losses worldwide and are among the most important agricultural pests (Meyer *et al.*, 2002 and Mitkowski *et al.*, 2002). Chemical nematicides are effective, easy to apply, and show rapid effects, but they have begun to be withdrawn from the market owing to concerns about public health and environmental safety (Badawi and Abu-Gharbieh, 2000 and Schneider *et al.*, 2003). The search for novel, environmentally friendly alternatives to manage plant-parasitic nematode populations has therefore become increasingly important. Nematodes in soil are subject to infection by bacteria and other micro-organisms. This creates the possibility of using soil microorganisms to control plant-parasitic nematodes (Gugino *et al.*, 2006). Bacteria are numerically the most abundant organisms in soil, and some of them, for example members of the genus *Bacillus*, has a wide range of suppressive activities on plant-parasitic nematodes (Jonathan *et al.*, 2000, and El-Moflehi, 2005). The most thoroughly studied is probably *B. subtilis* (Siddiqui and Mahmood, 1995; Cannayane and Rajendran, 2001; and Siddiqui, 2002).

Number of studies have reported direct antagonism by *B. thuringiensis* towards plant-parasitic nematode species belonging to *Meloidogyne* spp. (Tohamy *et al.*, 1995; Hammad, 2005 and Basyony, 2008). Previous and recent studies have shown significant reductions in nematode infestation by using certain bioproducts (Wright *et al.*, 1984; Osman *et al.*, 2000; El-saedy *et al.*, 2001; El-Nagdi and Youssef, 2004; Cochran *et al.*, 2007; Monfort *et al.*, 2006 and El-Bagory, 2008).

The objective of this study was to evaluate the effects of the sporulated bacterial cells suspension and cell free-filtrates of one isolate of *Bacillus subtilis* and two isolates of *B. thuringiensis* subsp. *aegypti* (*Bt*); the bioproducts Agarin® and Vertemic®, and the chemical nematicide Vydate L 24% on controlling *M. javanica* infected tomato plants.

MATERILAS AND METHODS

Used bacteria isolates and bioproducts:-

The Egyptian isolate of *B. subtilis* used in this study was obtained from the collection of the Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Also, the bioproduct Agarin® (*Bt*.C18) and two Egyptian isolates of *B. thuringiensis* subsp. *aegypti* (7N and Soto), were obtained from the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Centre, Giza, Egypt.

Preparation of *Bacillus* isolates:-

One isolate of *B. subtilis* (Egyptian isolate) was transferred from the stock culture to 100ml nutrient broth medium (Hanson *et al.*, 1964). The inoculated flasks were incubated overnight at 35±2°C, and then centrifuged at 10,000 rpm for 10 min. After centrifugation the sporulated bacterial cells were washed and re-suspended in 10ml of sterile distilled water, for making a suspension. The sporulated bacterial cells suspension was adjusted to 10^5 colony forming unit (cfu)/ml. The cell free-filtrates of *B. subtilis* isolate was transferred into another sterilized tube.

Two Egyptian isolates 7N and Soto of *B. thuringiensis* subsp. *aegypti* (*Bt*) were grown in liquid T3 medium (Travers *et al.*, 1987), on a rotary shaker (200 rpm) at 28°C for 72 hours then the sporulated bacterial cells were harvested by precipitation using the ultra speed centrifugation at 13.000 rpm at 4°C for 12 min. in sterilized Eppendorf tubes. The sporulated bacterial cells were washed 3 times using sterilized distilled water, then re-centrifuged and resolved in the same volume of sterilized distilled water and adjusted to 10^5 cfu/ml. The cell free-filtrates of each *Bt* isolate was transferred into another sterilized tube.

Doses of the used *Bacillus* isolates, bioproducts and Vydate L 24%

Two doses (10^5 and 2×10^5 cfu/pot) were used of sporulated bacterial cells, and (10ml/pot) of bacterial cell free-filtrates were used undiluted (S) and fifty percent dilution S/2, which was made by adding equal volume of distilled water to 5 ml of S solution for each tested bacteria; two doses (5 and 10g/pot) of the bioproducts Agarin® and (900 and 1800µl/ pot) of Vertemic®(1.8% Ec Abamectin), (Syngenta Crop Protection, Greensboro,

NC) is one member of the macrocyclic lactones, isolated as an aqueous solution of a fermentation product from a strain of bacterium, *Streptomyces avermitilis*, were applied twice, at the same time of nematode inoculation and 21 days later. One dose (50ml/pot) of the nematicide Vydate L 24% (Oxamyl, DuPont Crop Protection, Wil-mington, DE; containing 0.24 Kg a.i/Liter) was used at the rate of (1% per pot), in addition to a single foliar spray three weeks after transplanting.

Root-knot nematode inoculum preparation

Cultures of *M. javanica* were established from single egg-masses of adult females previously identified by the morphological characteristics of the female perineal patterns (Taylor and Sasser, 1978) and reared on Rutgers tomato plants in a greenhouse. The root-knot nematode eggs were extracted from infected tomato roots using sodium hypochlorite (NaOCl) solution as described by Hussey and Barker (1973).

Effect of *B. subtilis*, *B. thuringiensis* isolates and Vydate L 24% on *M. javanica* on tomato plants

Four-wk-old tomato seedlings (*Lycopersicon esculentum* Mill) of cv. Marmand L. were transplanted in eighty clay pots, 15 cm diameter as one seedling/pot. Pots were filled with 1Kg autoclaved sandy clay soil/pot, (2:2, v:v). Pots were inoculated with *M. javanica* suspension of (1000 eggs and larvae/pot), two days prior treatments with two doses (10^5 and 2×10^5 cfu/pot) of sporulated bacterial cells, and (10ml S and S/2/pot) of bacterial cell free-filtrates of each *Bacillus* isolate, and (50ml/pot) of Vydate L 24% was used at the rate of (1% /pot). Each dose considered a treatment.

Effect of Agarin®, Vertemic® and Vydate L 24% on *M. javanica* infected tomato plants

Three-wk-old tomato seedlings of cv. Marmand L. were transplanted in thirty clay pots filled with 1Kg autoclaved sandy-clay soil/pot (2:1, v:v). Pots inoculated with *M. javanica* suspension, (1000 eggs and larvae/pot) and treated twice, at the same time of nematode inoculation and 21 days later with two different doses of the tested bioproducts, Agarin® (5 and 10g/pot) and Vertemic® (900 and 1800µl/pot) and (50ml/pot) of Vydate L 24% was used at the rate of (1%/pot). Each dose considered a treatment.

Five pots in each experiment received no bacterial treatment served as control. In each experiment treatments were replicated five times. Pots were arranged in randomized complete block design and irrigated daily. The experiments were terminated 60 days after nematode inoculation. Dry weight of shoot and root systems, number of nematode root galls and egg-masses/plant and number of juveniles (J_2)/250 cc soil were determined.

Statistical Analysis

Data obtained were statistically analyzed according to SAS software program (SAS Institute, 1997). Data of the numbers of nematode root galls, egg-masses and juveniles were transformed to $\sqrt{x+1}$ before statistical analysis. Comparison among means was made via the least significant difference (LSD) at the 5% level of probability.

RESULTS

Effect of *B. subtilis*, *B. thuringiensis* isolates and Vydate L 24% on *M. javanica* on tomato plants

Data presented in Table 1 indicated that treatments with the nematicide Vydate L 24% (1%/pot) caused the greatest reduction (90-93.7%) in number of nematode root galls, egg-masses/plant and number of J_2/250 cc soil. Treatments with sporulated bacterial cells showed more significant reduction than bacterial cell free-filtrates in nematode parameters for all *Bacillus* isolates. Treatments with two doses (10^5 and 2×10^5 cfu/pot) of sporulated bacterial cells of the tested *Bacillus* isolates showed great reduction ranged from (59.1-80.1%) in number of nematode root galls, egg-masses/plant and number of J_2/250 cc soil. Meanwhile, treatments with bacterial cell free-filtrates dilutions S and S/2 of all tested isolates caused a considerable reduction (43-57.7%) in number of nematode root galls, egg masses/plant, and low reduction (16-37.1%) in number of J_2/250 cc soil compared to control treatment (nematode alone).

Table 1. Effect of *B. subtilis*, *B. thuringiensis* isolates and Vydate L 24% on *M. javanica* on tomato plants

Treatment	No. of galls/ plant	Reduction%	No. of egg masses/ plant	Reduction%	No. of juveniles/250 cc soil	Reduction%	Shoot dry weight (g)	Increase %	Root dry weight (g)	Increase %
M. javanica (control)	401.0 a	---	395.0 a	---	350.0 a	-	9.1 g	--	7.6 f	--
Nutrient broth medium alone	386.5 a	*3.6*	379.6 a	*3.9*	340 a	*2.9*	9.4 g	*3.2*	8.1 f	*9.2*
B. subtilis										
Sporulated bacterial cells / pot										
10^5cfu/pot	106.8 g	*73.4*	101.0 f	*74.4*	82.0 g	*76.6*	12.1 e	*24.8*	10.9 d	*30.3*
2×10^5 cfu/pot	85.9 h	*78.6*	78.5 h	*80.1*	80.0 g	*77.1*	21.6 b	*57.9*	16.0 b	*52.5*
Bacterial cell free-filtrates/pot										
S/2	193.2 c	*51.8*	215.4 b	*45.5*	248.0 c	*29.1*	10.9 f	*16.5*	8.9 ef	*14.6*
S	169.6 f	*57.7*	184.0 c	*53.4*	220.0 d	*37.1*	14.7 d	*38.1*	11.4 cd	*33.3*
T3 Medium alone	385.9 a	*3.8*	381.5 a	*3.4*	336.0 a	*4.0*	9.8 g	*7.7*	7.8 f	*2.6*
B. thuringiensis isolate 7N										
Sporulated bacterial cells / pot										
10^5cfu/pot	130.4 e	*67.6*	124.8 d	*68.4*	143.0 e	*59.1*	12.4 e	*26.6*	10.1 de	*24.8*
2×10^5 cfu/pot	101.6 g	*74.7*	100.0 f	*70.8*	116.0 f	*66.9*	21.8 a	*58.2*	16.2 b	*53.1*
Bacterial cell free-filtrates/pot										
S/2	213.2 b	*46.8*	224.8 b	*43.0*	280.0 b	*20.0*	11.0 f	*17.3*	8.7 ef	*12.6*
S	178.7 d	*55.4*	209.0 b	*47.1*	264.0 c	*24.6*	14.1 d	*35.5*	11.1 d	*31.5*
Soto										
Sporulated bacterial cells/ pot										
10^5cfu/pot	133.2 e	*66.8*	126.0 d	*68.1*	112.0 f	*68.0*	14.2 d	*35.9*	11.2 d	*32.1*
2×10^5 cfu/pot	114.0 g	*71.6*	100.0 f	*74.4*	110.0 f	*68. 6*	18.2 c	*50.0*	12.7 c	*40.2*
Bacterial cell free-filtrates/pot										
S/2	214.6 b	*46.5*	210.9 b	*46.6*	294.0 b	*16.0*	10.8 f	*15.7*	8.5 f	*10.6*
S	195.4 c	*51.3*	188.7 c	*52.2*	258.0 c	*26.3*	12.1 e	*24.8*	10.0 e	*24.0*
Vydate L 24% 1%per pot	40.0 i	*90.0*	25.0 h	*93.7*	69.0 h	*90.0*	22.4 b	*59.4*	18.5 a	*58.9*

Data are averages of 5 replicates.
Values within a column followed by the same letter(s) are not significantly different at $P = 0.05$.
cfu = colony forming unit/ml.

Data in Table 1 indicated that infected tomato plants which treated with Vydate L 24% (1% /pot) and (2×10^5 cfu/pot) of all *Bacillus* isolates gave the highest increase (40.2-59.4%) in the dry weight of shoot and root systems. In addition, treatment with (10^5 cfu/pot) and S/pot of the tested isolates, showed 24-38.1% increase in the dry weight of shoot and root systems. Also, there were no significant differences between treatment with nutrient broth medium alone and T3 medium alone in nematode parameters and dry weight of shoot and root systems compared with control treatment (nematode alone).

Effect of Agarin®, Vertemic® and Vydate L 24% on *M. javanica* infected tomato plants

Data presented in Table 2 showed that treatment with the nematicide, Vydate L 24% (1%/pot) caused great reduction (86.7-91.8%) in nematode infection and reproduction (numbers of nematode root galls, egg-masses/plant and number of J_2 /250 cc soil). Treatments with high dose of the bioproducts, (10g/pot) of Agarin® and (1800µl/pot) of Vertemic® showed 66.3-82.1%. Treatments with low dose of the bioproducts, (5g/pot) of Agarin® and (900µl/pot) of Vertemic® which showed 48.8-57.7% reduction in number of nematode root galls, egg-masses/plant and number of J_2/250 cc soil. Also, treatments with Vydate L 24% (1%/pot) and high dose of the previous bioproducts, resulted in great increase (60.1-83.5%), followed by treatments with low dose of the bioproducts, (5g/pot) of Agarin® and (900µl/pot) of Vertemic® which showed 22.1-43% increase in dry weight of shoot and root systems in comparison with control treatment.

Table 2. Effect of Agarin ®, Vertemic® and Vydate L 24% on *M. javanica* infected tomato plants

Treatment	No. of galls/ plant	Reduction %	No. of egg masses/ plant	Reduction %	No. of juveniles/ 250 cc soil	Reduction %	Shoot dry weight (g)	increase %	Root dry weight (g)	increase %
M. javanica(control)	542.0 a	---	529.0 a	----	602.0 a	---	13.6 e	----	7.9 c	----
Agarin®										
5g/pot	236.6 b	*56.3*	223.6 b	*57.7*	308.0 c	*48.8*	16.6 d	*22.1*	11.3 b	*43.0*
10g/pot	182.8 d	*66.3*	165.8 c	*68.7*	140.0 d	*76.7*	21.8 b	*60.1*	13.4 b	*69.6*
Vertemic®(1.8% Abamectin)										
900µl/pot	241.0 c	*55.5*	231.4 b	*56.3*	292.0 b	*51.5*	18.5 c	*36.0*	10.3 b	*23.3*
1800µl/pot	132.0 e	*75.6*	122.8 d	*76.8*	108.0 e	*82.1*	23.2 a	*70.6*	13.9 a	*75.9*
Vydate L 24% 1%/ pot	61.0 f	*88.7*	43.5 e	*91.8*	80.0 f	*86.7*	24.2 a	*77.9*	14.5 a	*83.5*

Data are averages of 5 replicates.

Values within a column followed by the same letter(s) are not significantly different at $P=0.05$.

Discussion

The present study indicated that in autoclaved and non-autoclaved soil treatments with the nematicide Vydate L 24% resulted in a great reduction in the incidence and overall severity of *M. javanica* infections on the roots and increased the dry weight of both shoot and root systems. These findings are in agreement with those of other workers (Badawi and Abu-Gharbieh, 2000; Schneider *et al.*, 2003; Hasabo and Noweer, 2005 and Gugino *et al.*, 2006).

In the present work, treatments with the three tested *Bacillus* isolates showed significant reduction, and treatments with sporulated bacterial cells

suspension of the tested *Bacillus* isolates was more effective than treatments with bacterial cell free-filtrates in reducing *M. javanica* multiplication on infected tomato plants and showed a significant increase in the dry weight of shoot and root systems. These findings are much similar to those of other workers (Jonathan *et al.*, 2000 and Cannayane and Rajendran, 2001). Siddiqui (2002) reported that using culture filtrates of *B. subtilis* were less effective in controlling nematode multiplications due to *M. javanica* than using bacterial spores. Also, significant reduction in numbers of nematode root galls, egg-masses/plant and number of J_2/250 cc soil were achieved when tomato plants treated with sporulated bacterial cells and bacterial cell free-filtrates of *B. thuringiensis* isolates compared with untreated control (El-Moflehi, 2005; Hammad, 2005 and El-Bagory, 2008).

Tohamy *et al.* (1995) evaluated the efficacy of an isolate of *Bt*, isolated from Egyptian soil, in controlling the root-knot nematode, *M. incognita* on tomato in a greenhouse trial. They found a significant reduction in numbers of nematode root galls and egg-masses developed on tomato roots compared with untreated control.

In the present study treatments with the nematicide Vydate L 24% and the high dose of the bioproducts, Agarin® and Vertemic® caused great reductions in *M. javanica* multiplications on infected tomato and showed a significant increase in the dry weight of shoot and root systems compared to control treatment. These findings are in agreement with those obtained by (El-saedy *et al.*, 2001; Cochran *et al.*, 2007; Monfort *et al.*, 2006 and Faske and Starr, 2007). Osman *et al.* (2000) reported that Agarin® produced a variety of toxin proteins during vegetative and sporulated phases of growth against insect larvae. Moreover, its spore-crystal complex showed nematicidal activity against root-knot and citrus nematodes.

El-saedy *et al.* (2001) evaluated three concentrations of Agarin® in comparison with three commonly used commercial nematicides to improve the status and productivity of Washington navel orange and Williams banana plants grown in soil infested by *Tylenchulus semipenetrans* and *M. javanica*. They found that Temik® and Agarin® 50g treatments gave the highest reduction in numbers of citrus nematode juveniles (92 and 89%) and females (91 and 89%), respectively, followed by Mocap® 10G and Vydate ® L 24% with 83-84% reduction.

Also, Monfort *et al.*, (2006) found that abamectin can be used successfully as a seed treatment against *M. incognita* on cotton plants and appears to have considerable potential as a nematicide.

In summary, the present data of nematode control are very promising. Further investigations are necessary, especially under field conditions. Since, such studies could help growers to use new biocontrol agents to control root-knot nematodes. In general, information on integrated control of plant-parasitic nematodes is very important and must be applied to suppress nematode populations under field conditions. It is clear that nematode control on economic plant crops can be achieved if resistant plant cultivars, crop rotation, biological control agents or bioproducts are used properly.

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مقاومة نيماتودا تعقد الجذور *M. javanica* التى تصيب نباتات الطماطم

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**** قسم وقاية النبات- كلية ناصرللعلوم الزراعية- جامعة عدن-عدن- اليمن**

تم دراسة تأثيراستخدام عزلة من البكتيرة *Bacillus subtilis* وأثنين من عزلات البكتيرة *B. thuringiensis* (عزلة 7N و عزلة Soto) وكذلك تأثير أثنين من المنتجات الحيوية هما الأجرين والڤيرتيمك مقارنة بأستخدام المبيد النيماتودي الفايدت ل ٢٤% لمقاومة تأثير الأصابة بنيماتودا تعقد الجذور *M. javanica* التي تصيب نباتات الطماطم في أثنين من التجارب المنفصلة.

أظهرت النتائج أن معاملة التربة المعداة بنيماتودا تعقد الجذور *M. javanica* بمعدل (١٠٠٠بيضة و يرقة/أصيص) بالمبيد النيماتودي الفايدت ل ٢٤% بمعدل (١%/أصيص) أدت إلى حدوث خفض بنسبة ٩٠-93.7% في أعداد العقد الجذرية وأكياس البيض لنيماتودا تعقد الجذور/نبات وأعداد الطورالبرقى الثانى لنيماتودا تعقد الجذور/٢٥٠ سم^3 تربة.

نتج عن المعاملة بمعلق الخلايا المتجرثمة بمعدل (١٠^7 و ٢×١٠^{7}خلية متجرثمة) للعزلات البكتيرية السابق ذكرها حدوث خفض بنسبة ٥٩,١-٨٠,١% في أعداد العقد الجذرية وأكياس البيض لنيماتودا تعقد الجذور/نبات وأعداد الطور البرقى الثانى/٢٥٠ سم^3 تربة. فى حين أظهرت المعاملة براشح العزلات البكتيرية السابق ذكرها حدوث خفض بمعدل (١٦-٥٧,٧%) في أعداد العقد الجذرية وأكياس البيض لنيماتودا تعقد الجذور/نبات وفى أعداد الطور اليرقى الثانى /٢٥٠ سم^3 تربة مقارنة بالكنترول. كذلك نتج عن أستخدام المبيد النيماتودي الفايدت ل ٢٤% بمعدل (١%/أصيص) و(٢×١٠^{7}خلية متجرثمة) للعزلات البكتيرية السابق ذكرها حدوث زيادة معنوية بنسبة ٤٠,٢-٥٩,٤% فى الوزن الجاف لكل من المجموع الخضرى والجذرى للنباتات المعاملة مقارنة بالكنترول.

أتضح من النتائج أن أستخدام (٥٠مل/أصيص) من المبيد النيماتودي الفايدت ل ٢٤% بمعدل (١%/أصيص) والجرعة المرتفعة من كلا من المنتج الحيوى الأجرين (١٠ جم/أصيص) و (١٨٠٠ميكروليتر/أصيص) من الڤيرتيمك حدوث خفض بنسبة ٦٦,٣-٩١,٨% في أعداد العقد الجذرية وأكياس البيض لنيماتودا تعقد الجذور/نبات وفى أعداد الطور اليرقى الثانى /٢٥٠ سم^3 تربة. كذلك أظهرت المعاملة بالمبيد النيماتودي أو بالجرعات المرتفعة لكلا من المنتجات الحيوية السابقة حدوث زيادة قدرها (٦٠,١-٨٣,٥%) فى الوزن الجاف لكل من المجموع الخضرى والجذرى للنباتات المعاملة مقارنة بالكنترول.