Impact of Some Antagonistic Organisms in Controlling Meloidogyne Arenaria Infesting Tomato Plants

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Abstract—Effect of three isolates of Bacillus subtilis, two isolates of B. thuringiensis subsp. aegypti, three species of Trichoderma and the nematicide Furadan®10G, were evaluated on Meloidogyne arenaria infected tomato plants. Treatments with Furadan®10G, washed pellet of B. thuringiensis, sporulated bacterial cells of B. subtilis isolates and mixed Trichoderma filtrates caused (63.9-96.8%) inhibition on M. arenaria egg-hatching and J; activity in the laboratory experiment and showed 66.2-98.4% reduction in nematode reproduction in the pot experiment. In the field experiment, the greatest reductions (70.0-99.8%) in the number of nematode root galls, egg-masses/plant and number of J2/250 cc soil and greatest increase (40.2-62.2%) in the dry weight of shoot and root systems were recorded with the treatments of Furadan®10G, mixed filtrates of the tested Trichoderma species and washed pellet of B. thuringiensis isolate 7N compared to check treatment.

Index Terms—Bacillus spp, Root-knot nematodes, meloidogyne arenaria, tomato, trichoderma spp.

I INTRODUCTION

Root-knot nematodes Meloidogyne spp. are one of the major pathogens of tomato world wide and limit fruit production, estimated yield losses ranging from 28-68% [18]. Root-knot nematode infestations on tomato (Lycopersicon esculentum Mill) are common in Egypt and cause severe crop damage especially in light soils [15]. These nematodes can be managed effectively by chemical treatments but many of the nematicides are expensive, pose human and environmental risk or have been withdrawn from use [15]. Management of root-knot nematodes with biological control agents has been receiving growing consideration [14]. A wide range of bacteria and fungi create the possibility for control plant-parasitic nematodes [24]. Numerous Bacillus species and strains especially B. subtilis and B. thuringiensis can suppress root-knot nematodes [1], [9], [10]. Also, Trichoderma spp. can produce a variety of fungal metabolites, termed mycotoxins, which affect adult nematode activity and inhibit egg-hatching and juvenile development and enhance plant growth [2], [8], [12], [24]. Hence, the present study designed to showed the efficacy of three B. subtilis isolates and two B. thuringiensis isolates, three species of Trichoderma (T. hamatum, T. harzianum and T. viride) compared with the chemical nematicide, Furadan®10G, on egg-hatching and J2 stage juvenile (J2) activity of M. arenaria under laboratory as well as its infection on tomato plants under greenhouse and filed conditions.

II MATERIALS AND METHODS

A. Bacillus & Trichoderma Cultures

Three isolates of B. subtilis & T. hamatum (Bon.) Bain., T. harzianum Rifai and T. viride Per. Ex Gray, used in this study, were obtained from the cultures collection of the Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. The two isolates of B. thuringiensis subsp. aegypti (7N and Soto) were obtained from the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Centre, Giza, Egypt.

B. Root-knot Nematode, M. arenaria Inoculum Preparation

Cultures of M. arenaria (Neal) Chitwood were established from single egg-masses of adult females previously identified by the morphological characteristics of the female perineal patterns [4] [11] and reared on tomato plants (Lycopersicon esculentum Mill) cv. Super Strain B in a greenhouse. The root-knot nematode eggs were extracted from infected tomato roots using sodium hypochlorite (NaOCl) solution as described by [21]. M. arenaria eggs and the hatched 2nd stage juveniles (J2) were placed in sterile distilled water and used in all tests.

C. Doses of Bacillus Isolates, Trichoderma and Furadan®10G

One dose of sporulated bacterial cells for each of B. subtilis isolates (5×10⁶ cfu/ml distilled water), five ml/pot or plant was used, and (10 ml of each of bacterial cell free-filtrates of B. subtilis isolate/pot or plant) was used for pot and field experiments. Treatments were applied at the same time of nematode inoculation and 21
days later [19] [20]. One dose of washed pellet of *B. thuringiensis* subsp. *aegypti* (4mg/pot or plant) and (10 ml of supernatant/pot or plant) were used for pot and field experiments, for each isolate and applied at the same time with *M. arenaria* inoculation and 21 days later. The granular nematicide carbofuran, Furadan® 10G, 2,3-dihydro-2,2-dimethylbenzofuran-7-yl Methylcarbamate, FMC Chemical, FPRL. Agrochemical Products Group, Philadelphia-PA 19103 (USA) was used at the rate of (0.25 g nematicide/100 ml distilled water) for egg-hatching and *J₂* activity experiments and (2.5 g nematicide/pot or plant) for pot and field experiments, one week before nematode inoculation, and 21 days later.

**D. Laboratory Experiments:**

Effects of *Trichoderma* filtrates and Furadan® 10G on egg-hatching and *J₂* activity of *M. arenaria*

The collected culture filtrates of each of the three tested *Trichoderma* spp. were used alone or as a mixed filtrate of the three *Trichoderma* species on egg-hatching and *J₂* activity. Treatments were applied in 24- well tissue culture plates; each well received 3 ml of each treatment. A total of 270 *M. arenaria* eggs or 95 fresh hatched *J₂*/well were added in 50 µl of water. Each treatment was replicated eight times. For check treatments, *M. arenaria* eggs or *J₂* were incubated in sterile distilled water and sterilized CDL medium. Treatments were maintained at 25±2°C in an incubator.

Effects of *B. subtilis*, *B. thuringiensis* isolates and Furadan® 10G on egg-hatching and *J₂* activity of *M. arenaria* under laboratory conditions

Sporulated bacterial cells (5×10⁵ cfu/ml distilled water) or bacterial cell free-filtrates for each *B. subtilis* isolate, washed pellet (4 mg/ml distilled water) and supernatant for each *B. thuringiensis* isolate and (0.25 g/100 ml distilled water) of Furadan® 10G were tested to study their effect on egg-hatching and *J₂* activity. Treatments were done in 24-well tissue culture plates; each well received 3 ml of each treatment. A total of 200 *M. arenaria* eggs/well or 125 fresh hatched *J₂* was added in 50 µl of water. Each treatment was replicated eight times. For check treatments, *M. arenaria* eggs or *J₂* were placed in sterile distilled water, sterilized NB medium and sterilized T3 medium. Treatments were maintained at 25±2°C in an incubator.

Observation of the on the effects of different treatments on egg-hatching and *J₂* activity was taken 24 hours after adding nematode eggs or *J₂* in *Trichoderma* filtrates. The nematode *J₂* were considered to be inactive when they did not move.

**E. Pot experiment**

Effects of *B. subtilis*, *B. thuringiensis* isolates and Furadan® 10G on *M. arenaria* on tomato under greenhouse conditions

Four-wk-old tomato seedlings cv. Super Strain B were transplanted in 120 plastic pots, 30 cm diam., one seedling/pot. Pots were filled with sandy clay soil (2:1, v:v) and inoculated with *M. arenaria* (600 eggs or *J₂*/pot), Pots were treated with 5 ml/pot of the sporulated bacterial cells suspension (5×10⁵ cfu/ml distilled water) and 10 ml of bacterial cell free-filtrates/pot of each *B. subtilis* isolate. Four mg washed pellet/pot and 10ml supernatant/pot of each tested *B. thuringiensis* isolate were used. Furadan®10G was used at the rate of 2.5 g nematicide/pot. Treatments were applied at the same time of *M. arenaria* inoculation and 7 days later.

Fifteen pots, 5 pots each, received sterile distilled water, sterilized NB medium and liquid T3 medium were served as check treatments. Each treatment was replicated five times. Pots were arranged in randomized complete block maintained at 27±2 °C and irrigated daily. The experiments were terminated 35 days after nematode inoculation. Numbers of nematode root galls and egg-masses/plant were determined.

**F. Field Experiment:**

Effects of two isolates of *B. subtilis*, isolate 7N of *B. thuringiensis, Trichoderma* filtrates and Furadan® 10G on *M. arenaria* on tomato under field conditions

Field experiment was conducted at the farm of Faculty of Agriculture, Alexandria University, Alexandria, Egypt. The soil was sandy loam with 2.5% organic matters. Field plot experiments were 3 m × 5.0 m for each treatment (4 rows of 3 meter long and 85-cm wide with 50-cm gap between rows). All rows were irrigated to full water holding capacity and three weeks old tomato seedlings, cv. Super Strain B, were transplanted at 50-cm distance three days later, (5 plants per row). Each plant received 2000 eggs&*J₂* of *M. arenaria* at the same time with the following treatments; 5×10⁵ cfu/ml distilled water of sporulated bacterial cell for each *B. subtilis* isolate (isolates 2&3) at 5 ml of the suspension/plant, washed pellet (4mg/plant) for *B. thuringiensis* isolate 7N, *T. harzianum* or *T. viride* filtrates (10ml/plant) either alone or as a mixture filtrates of *Trichoderma* species and Furadan®10G at the rate of 2.5 g/plant were applied. Rows of tomato plants received only sterilized distilled water without nematode served as control treatment. Separate rows of tomato plants treated with sterilized CDL, NB and T3 mediums plus *M. arenaria* were included as check treatments. All treatments were replicated five times and were laid out in a completely randomized block design for two months duration. Data were collected 60 days after final treatments. Twelve plants and 10 soil samples from the rhizosphere of tomato plants up to a depth of 20 cm (250 cc soil/sample) from each plot were taken for analysis. Dry weight of shoot and root systems, number of nematode root galls and egg-masses/plant and number of *J₂*/250cc soil were determined.

**G. Statistical Analysis:**

Data obtained were statistically analyzed according to SAS software program [25]. Data of the numbers of nematode root galls, egg-masses and juveniles were transformed to √(x+1) before statistical analysis. Comparison among means was made via the least
significant difference (LSD) at the 5% level of probability.

III RESULTS

A. Effects of Trichoderma Filtrates on Egg-hatching and J2 Activity of M. arenaria under Laboratory Conditions:

The effects of Trichoderma filtrates and Furadan® 10G on M. arenaria egg-hatching and J2 activity were presented in Table I. The greatest reduction in egg-hatching and J2 activity (96.1–96.8%) was achieved with Furadan® 10G. Also, treatments with Trichoderma mixture filtrates caused (79.5–89.6%) inhibition in egg-hatching and J2 activity, respectively, whereas treatments with T. hamatum, T. harzianum and T. viride filtrates showed 47.6-74.6% inhibition in egg-hatching and J2 activity of M. arenaria compared with the check treatment (Table I).

![Table I](image)

B. Effects of B. subtilis, B. thuringiensis Isolates and Furadan® 10G on egg-hatching and J2 activity of M. arenaria under Laboratory Conditions:

Data presented in Table II indicated that Furadan® 10G caused the greatest reduction (94.6-96.8%) in egg-hatching and J2 activity, followed by treatments with isolate 7N (4mg washed pellet /ml) of B. thuringiensis with (76.7-81.8%) reductions in egg-hatching and J2 activity. Treatments with (5x10^3 cfu/ml) of sporulated bacterial cells of B. subtilis isolate (2&3) and (4mg of washed pellet/ml Dist. water) of Soto isolate caused 63.9-73.4% reduction in egg-hatching and J2 activity, while treatments with (5x10^3 cfu/ml Dist. water) of sporulated bacterial cells of B. subtilis isolate 1, bacterial cell free-filtrates and supernatant of all Bacillus spp.

![Table II](image)

![Table III](image)

C. Effects of B. subtilis, B. thuringiensis Isolates and Furadan® 10G on M. arenaria on Tomato Plants under Greenhouse Conditions:

Data presented in Table III indicated that treatments with Furadan® 10G (2.5g/pot) caused the greatest reduction 97.2% and 98.4% in number of nematode root galls and egg masses/plant, respectively. Treatments with sporulated bacterial cells and washed pellet showed the highest reduction than bacterial cell free-filtrates and supernatant treatments on nematode root galls and egg masses /plant for all B. subtilis and B. thuringiensis isolates. Treatments with (5x10^3 cfu/pot) of sporulated bacterial cells of B. subtilis isolates (2&3) and (4mg...
washed pellet/pot) of *B. thuringiensis* isolate 7N showed great reductions ranging from (76.7-82.6%) in the number of nematode root galls and egg masses/plant. Also, treatments with washed pellet (4mg/pot) of *B. thuringiensis* isolate Soto showed significant reductions ranged from (66.2-67.4%) nematode root galls and egg masses/plant. Meanwhile, treatments with (5× 10^5 cfu/plant) of sporulated bacterial cells of *B. subtilis* isolate 1, bacterial cell free-filtrates and supernatant of all tested isolates caused considerable reductions (43.2-55%) of nematode root galls and egg masses/plant compared to check treatments.

D. Effects of Two Isolates of *B. subtilis*, Isolate 7N of *B. thuringiensis*, Trichoderma Filtrates and Furadan® 10G on *M. arenaria* on Tomato Under Field Conditions

Data presented in Table IV showed that the greatest reductions (99.5-99.8%) in the number of nematode root galls, egg-masses/plant and numbers of *J. J* 250 cc soil were achieved with Furadan® 10G (2.5g/plant). Treatment with mixture filtrates of all *Trichoderma* species and washed pellet (4mg/kg soil) of *B. thuringiensis* isolate 7N showed significant reductions ranged from (81.1-87.6%) in the number of nematode root galls, egg-masses/plant and number of *J. J* 250 cc soil. Meanwhile, treatment with sporulated bacterial cells (5× 10^5 cfu/plant) of each *B. subtilis* isolates (2 & 3) and treatment with filtrates of *T. harzianum* and *T. viride* each alone showed (68.6-73.2%) reductions in the number of nematode root galls, egg-masses/plant and number of *J. J* 250 cc soil compared to check treatments.

Data presented in Table V showed that treatment with the nematicide Furadan®10G (2.5g/plant) caused the greatest increase (60.2-61.4%) in the dry weight of shoot and root systems. Also, treatments with the mixture filtrates of *Trichoderma* species, washed pellet (4mg/kg soil) of *B. thuringiensis* isolate 7N and filtrates of *T. harzianum* or *T. viride* alone showed great increases ranged from 47.8-59.3%, followed by treatments with sporulated bacterial cells (5× 10^5 cfu/plant) of *B. subtilis* isolates 2 & 3, which caused 39.2-42.5% increase in the dry weight of shoot and root systems compared to check treatment.

**TABLE IV. EFFECTS OF TWO ISOLATES OF B. SUBTILIS, ISOLATE 7N OF B. THURINGIENSIS, TRICHODERMA FILTRATES AND FURADAN® 10G ON M. ARENARIA (MA) ON TOMATO UNDER FIELD CONDITIONS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Galls/plant</th>
<th>EM/plant</th>
<th>J/250 cc soil</th>
<th>Red. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check treatment*</td>
<td>1901.5 a</td>
<td>0.0</td>
<td>1884.2 a</td>
<td>0.0</td>
</tr>
<tr>
<td>CDL medium+MA</td>
<td>1893.6 a</td>
<td>0.2</td>
<td>1879.4 a</td>
<td>0.3</td>
</tr>
<tr>
<td>NB Medium + MA</td>
<td>1890.0 a</td>
<td>0.4</td>
<td>1880.8 a</td>
<td>0.5</td>
</tr>
<tr>
<td>T3 medium+MA</td>
<td>1895.1 a</td>
<td>0.2</td>
<td>1875.1 a</td>
<td>0.1</td>
</tr>
<tr>
<td><em>B. subtilis</em> isolates (Sporulated cells)+ MA</td>
<td>568.2 b</td>
<td>70.1</td>
<td>554.2c</td>
<td>70.6</td>
</tr>
<tr>
<td><em>B. thuringiensis</em> isolate 7N + MA</td>
<td>576.1 b</td>
<td>69.7</td>
<td>581.2b</td>
<td>69.2</td>
</tr>
<tr>
<td>Washed pellet</td>
<td>307.2 d</td>
<td>83.8</td>
<td>265.2 e</td>
<td>85.9</td>
</tr>
<tr>
<td><em>Trichoderma</em> filtrates + MA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>512.2 c</td>
<td>73.1</td>
<td>500.0 d</td>
<td>73.2</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>534.5 bc</td>
<td>71.9</td>
<td>513.1d</td>
<td>72.7</td>
</tr>
<tr>
<td>Mixture filtrates</td>
<td>278.5 d</td>
<td>85.4</td>
<td>251.0 f</td>
<td>86.7</td>
</tr>
<tr>
<td>Furadan® 10G + MA</td>
<td>9.5 e</td>
<td>99.5</td>
<td>7.0 g</td>
<td>99.6</td>
</tr>
</tbody>
</table>


Data are averages of 5 replicates. EM= Egg-masses. Values of each column, followed by the same letter are not significantly different at P = 0.05 of LSD test.

**IV DISCUSSION**

Over the past twenty years a numerous studies have been undertaken to investigate the effects of using microorganisms (as biocontrol agents) and bioproducts in comparison with the nematicides against nematode pests

**TABLE V. EFFECTS OF B. SUBTILIS AND B. THURINGIENSIS ISOLATES, TRICHODERMA SPP. FILTRATES AND FURADAN® 10G ON DRY WEIGHTS OF TOMATO INFECTED WITH M. ARENARIA (MA) UNDER FIELD CONDITIONS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot DW (g)</th>
<th>Inc. %</th>
<th>Root DW (g)</th>
<th>Inc. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check treatment*</td>
<td>14.6 c</td>
<td>0.0</td>
<td>11.9 c</td>
<td>0.0</td>
</tr>
<tr>
<td>CDL Medium+MA</td>
<td>14.8 c</td>
<td>1.4</td>
<td>12.4 c</td>
<td>4.0</td>
</tr>
<tr>
<td>NB Medium + MA</td>
<td>15.1 c</td>
<td>3.3</td>
<td>12.0 c</td>
<td>0.8</td>
</tr>
<tr>
<td>T3 Medium+MA</td>
<td>14.9 c</td>
<td>2.0</td>
<td>12.1 c</td>
<td>1.7</td>
</tr>
<tr>
<td><em>B. subtilis</em>+ MA</td>
<td>25.4 b</td>
<td>42.5</td>
<td>19.9 b</td>
<td>40.2</td>
</tr>
<tr>
<td>Isolate 2 Sporulated cells</td>
<td>24.0 b</td>
<td>39.2</td>
<td>20.7 b</td>
<td>42.5</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>34.6 a</td>
<td>57.8</td>
<td>25.7 a</td>
<td>53.7</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>30.0 a</td>
<td>51.3</td>
<td>22.8 b</td>
<td>47.8</td>
</tr>
<tr>
<td>Mixture filtrates</td>
<td>35.9 a</td>
<td>59.3</td>
<td>26.0 a</td>
<td>54.2</td>
</tr>
<tr>
<td>Furadan® 10G + MA</td>
<td>37.8 a</td>
<td>61.4</td>
<td>29.9 a</td>
<td>60.2</td>
</tr>
</tbody>
</table>

Legend, as in Table IV. [13], [15]. Microorganisms which grown in soil rhizosphere are ideal for use as biocontrol agents since rhizosphere of many plants provides front line defense for roots against attack by soil-borne pathogens [3], [5], [13], [23].
The present efforts of laboratory and field experiments showed that treatments with mixture filtrates of *Trichoderma* species caused great inhibition in egg-hatching, J2 activity and great reduction in the incidence and overall severity of *M. arenaria* infections on tomato plants. These findings are in agreement with those of other workers [6] [16] [17]. *Trichoderma* spp. has been reported to produce chitinases into the culture, which might help in the inhibition of eggs hatching. *Trichoderma* also have been shown to have activity towards root-knot nematode [7]. Some *Trichoderma* isolates were reported to do both enhance plant growth and reduce root-knot nematode damage [24]. Many reports suggested that the main anti-nematode activity caused by *T. harzianum* takes place in the soil and not within the roots and the proteolytic activity of *Trichoderma* strains are important for the nematode biocontrol [7].

Treatments with *Bacillus* isolates have the abilities to suppress root knot nematodes infection on tomato and showed a significant increase of shoot and root dry weights. These findings are in agreement with those of other workers [3], [22], [23]. Treatments with sporulated bacterial cells suspension of the tested *B. subtilis* isolates was more effective than treatments with bacterial cell free-filtrates in reducing *M. arenaria* multiplication on infected tomato plants and showed a significant increase in the dry weight of shoot and root systems. These findings were similar to those of other workers [1]. Many reporters indicated that using culture filtrates of *B. subtilis* were less effective in controlling nematode multiplications due to *M. javanica* than using bacterial spores [9]. Also, treatments with washed pellet of *B. thuringiensis* isolates more effective than treatments with bacterial supernatant in reducing numbers of nematode root galls, egg-masses/plant and number of J3/J250 cc soil of compared with untreated control and resulted in a significant increase of shoot and root systems dry weights. These findings are in agreement with those of other workers [1] [9].

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 are used properly.

**REFERENCES**


Dr. Asmaa Abd El-Hamid Mokbel, was born in Alexandria, Egypt - 20/April/1971. Her Educational background: B.Sc. in Plant Pathology, 1992, M.Sc. in Plant Pathology (Nematology), 1996 and Ph.D. in Plant Pathology (Nematology), 2002, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt. The scientific degrees: Demonstrator, Assistant Lecturer, Assistant Professor and Associate Professor of Plant Pathology, Faculty of Agriculture, University of Alexandria, Egypt. She works now as an Associate Professor of Botany- Faculty of Science- Biology Department- Jazan University- Kingdom of Saudi Arabia. She published more than 15 paper in the filed of plant nematology.

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