

SHORT NOTES

Efficacy of *Bacillus thuringiensis jordanica* against *Meloidogyne javanica* infecting tomato

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Summary. A local strain of *Bacillus thuringiensis jordanica* (Btj), serotype H71, was evaluated for its efficacy in controlling *Meloidogyne javanica* attacking tomato. Glasshouse experiments were conducted using a soil drench of the bacterium applied one week before transplanting the tomato seedlings to the soil. Results showed a reduction in tomato root galling by 51–59% when *M. javanica* eggs or second stage juveniles (J2) were used as inoculum respectively. Single (14 days) and double (14 and 7 days) applications of Btj before plantation, both achieved a significant reduction in root galling. A field trial also showed that Btj, alone or in combination with peptone, significantly reduced root galling.

Key words: *Bacillus*, biological control, root galling, root knot nematodes.

Introduction

Tomatoes are the most widely cultivated vegetable crop in Jordan, with a total area of 20,000 acres, comprising 25% under the total area of vegetable cultivation, and a total annual yield of 360,000 tons (Annual agricultural statistics, AA.VV., 2003). Tomato production is threatened by several pests and parasites, including the root knot nematodes (RKN), *Meloidogyne* spp. (Abu Gharbieh, 1994, Karajeh, 2004). Root knot appears as stunted growth coupled with severe deficiency

symptoms of some nutritional elements, loss of yield, and a reduction in product quality. RKN are managed by chemical control and soil solarization (Abu Gharbieh, 1994; Badawi, 1999). Soil solarization has proved effective in areas where it was applied, but fumigants and non-fumigant nematicides have a negative impact on the environment. Alternative and safe control methods such as biological control therefore have to be considered. *Bacillus thuringiensis* (Bt) was reported as a potential control agent to some nematodes (Borgonie *et al.*, 1996, Marroquin *et al.*, 2000, Griffiths *et al.*, 2001).

Several strains of Bt are pathogenic to insects and nematodes. Both *in vitro* and *in vivo* experiments have been conducted to test the effectiveness of Bt strains against the hatching, motility,

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penetration, development and reproduction of RKN (Ignoffo and Dropkin, 1977; Osman *et al.*, 1988; Rehberger, 1992; Zuckerman *et al.*, 1993; Sharma, 1994; Carneiro *et al.*, 1998).

In Jordan, local strains of Bt were isolated from various locations. They showed lethality to eggs and second-stage juveniles (J2) of RKN under laboratory conditions (Abu-Dhaim, 2002; Khyami-Horani *et al.*, 2003; Al Banna and Khyami-Horani, 2004). The objective of the present work was to evaluate the efficacy of *Bt. jordanica* (Btj) strain H71, against *M. javanica* in glasshouse experiments and under field conditions.

Materials and methods

Nematode culture

The two populations of *M. javanica* used in this study, were obtained from cucumber grown in plastic houses in Baqa area, and from infested roots of eggplant grown in the Jordan Valley. The populations were multiplied and maintained by culturing on tomato plants (*Lycopersicon esculentum* Mill cv. GS 12) in a greenhouse at the University of Jordan campus at 25±5°C. Eggs were collected by treating the egg masses with 0.5% solution of sodium hypochlorite (Barker *et al.*, 1985). Second-stage juveniles were obtained by incubating hand-picked egg masses in water at 25°C.

Bacillus culture

Cultures of *Bt jordanica* (strain H71) were maintained on nutrient broth supplemented with a mineral salt solution (1 ml l⁻¹) to allow adequate sporulation (Johnson *et al.*, 1998). Cultures were incubated on a rotary shaker (200 rpm) at 32°C for two days to ensure sporulation and cell lysis (Sela *et al.*, 1998). Spores and crystals were harvested by centrifugation (10,000 rpm) for 10 min at 4°C. The pellets were washed in deionized water, and serial dilutions of the spore-crystal suspensions were prepared.

Effect of Btj on *Meloidogyne javanica* and its damage to tomato

Application as a soil drench under glasshouse conditions

Meloidogyne javanica inoculum consisting of 300 J2 or 2800 eggs, obtained from infested eggplant roots, was added to 100 cm³ pot containing

oven-dried sterilized sandy clay loam soil. Concurrently, 30 ml of an aqueous Btj suspension of 10⁷ viable spores ml⁻¹ was added to each pot. Pots containing J2, untreated or treated with the nematocide Vydate[®], served as control treatments. Six days later, one tomato seedling (cv. GS 12) was transplanted to each pot. The pots were then placed in the glasshouse. Root systems were harvested after one month and assessed for galling (No. of galls/root system). A randomized complete design was used with either 3 or 4 replicates per treatment and J2 or eggs as inoculum respectively.

Effect of the number of applications of Btj under glasshouse condition

To evaluate the effect of the number of applications of the Btj suspension on RKN, 1000 eggs of *M. javanica*, were added to each pot (100 cm³) containing sterilized sandy clay loam soil. Two sets of experiments were carried out. In the first set, pots received one dose of Btj 2 weeks before transplanting and in the second, pots received an additional dose of Btj one week before transplanting. Pots containing J2 untreated or treated with Vydate[®] served as control treatments. Four replications were used. The pots were maintained for one month in the glasshouse at 25°C. The root systems were then harvested and assessed for galling.

Application as soil drench under field conditions

A field experiment was carried out at the Agricultural Station of the University of Jordan in the Jordan Valley. The soil type was sandy loam and it was divided into blocks (3×1 m each). Selected plots were covered with black plastic mulch containing holes 50 cm apart that were to be planted with tomato seedlings. All plots except the controls, were artificially infested by incorporating segments of tomato roots infected with *M. javanica* originally obtained from infested cucumber roots. Research plots comprised the following five treatments: infested soil treated with 10 ml of Btj (10⁶ viable spores ml⁻¹); infested soil treated with Btj (10⁶ viable spores ml⁻¹) plus peptone (0.02% w:w); infested soil treated with peptone (0.02% w:w); infested untreated soil; and uninfested untreated soil. One tomato seedling (cv. GS 12) was planted in each hole one week after the addition of the bacteria. Plants were harvested three and a half months after planting, examined and scored for root gall-

ing severity on a scale of 1 to 5, with 1, no galls; 2, 1–25%; 3, 26–50%; 4, 51–75%; and 5, 76–100% of roots with galls.

A completely randomized design was used with three replicates (5 plants/replicate) for each treatment.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) test was performed to separate the means (Steele and Torrie, 1980).

Results and discussion

In the glasshouse pot experiments the Btj strain reduced root galling severity both when J2s and when eggs were used as inoculum (59 and 51% respectively) (Table 1). Additional pre-applications of Btj did not further reduce root galling (Table 2).

In the field experiment, Btj alone reduced root

galling severity but the results did not differ significantly from those of the other treatments (Btj plus peptone and peptone, alone) (Table 3).

Previous studies have already shown that Bt strain CR-371 applied as a soil drench lead to a 53% reduction of tomato root galling caused by *M. incognita* (Rehberger, 1992; Zuckermann *et al.*, 1993).

Abu-Dhaim (2002) found that exposure of RKN J2 to Btj (10^6 spores ml⁻¹) for 4 days caused 100% mortality, and that exposure of eggs to the same concentration of Btj significantly reduced of hatching. In our glasshouse and field experiments root galling still occurred, even when higher concentrations of Btj were applied (Table 1–3). This was due to the soil type and to other abiotic and biotic factors in the soil. Bt applied in unfumigated soil decreased root galling caused by *M. hapla* on roots of lettuce, more than when the bacterium was applied in fumigated soil (Chen *et al.*, 2000). The difference was thought to be due to the fact that unfu-

Table 1. Effect of *Bacillus thuringiensis jordanica* (H71) on *Meloidogyne javanica* and its damage to tomato in a glasshouse pot experiment^a.

Treatment	No. of galls/root system (J2 as inoculum)	No. of galls/root system (eggs as inoculum)
Btj 10 ⁷ viable spores ml ⁻¹	9 b	22 b
Untreated	23 a	46 a
Vydate 2 ml l ⁻¹	0 c	0 c
LSD	8.7	20.7

^a Data for each treatment are the mean of four (J2 as inoculum) and three (eggs as inoculum) replicates. Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P=0.05$).

Table 2. Effect of the number of applications of *Bacillus thuringiensis jordanica* (H71) on *Meloidogyne javanica* and its damage to tomato in a glasshouse pot experiment^a

Treatment	No. of galls/root system
Btj 10 ⁸ viable spores ml ⁻¹ (one application)	56 b
Btj 10 ⁸ viable spores ml ⁻¹ (two applications)	62 b
Untreated	124 a
Vydate 2 ml l ⁻¹	0 c
LSD	22.9

^a Data for each treatment are the mean of four replicates. Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P=0.05$).

Table 3. Effect of *Bacillus thuringiensis jordanica* (H71) (Btj) on *Meloidogyne javanica* and its damage to tomato in a field experiment^a

Treatment	Root galling severity ^b
Btj 10 ⁶ viable spores ml ⁻¹	1.93 b
Btj 10 ⁶ viable spores ml ⁻¹ and Peptone 0.02% w:w	1.98 b
Peptone 0.02% w:w	2.5 b
Nematode	3.5 a
Untreated	1.0 c
LSD	0.74

^a Data for each treatment are the means of three replicates (5 plants/replicate). Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P=0.05$).

^b Rating scale: 1, no galls; 2, 1–25%; 3, 26–50%; 4, 51–75%; and 5, 76–100% of roots with galls.

migated soil contained proteic compounds that accelerated the effect of Bt.

Although the combination of Btj and peptone did not improve control effectiveness, peptone reduced root galling. Oka *et al.* (1993) found that peptone inhibited root galling by reducing the percentage of J2 penetration. These workers also reported that *B. cereus* applied with peptone had greater negative effect on J2 penetration than *B. cereus* alone. This effect can be due to the release of ammonia and nitrate, both of which are lethal to nematodes.

It is concluded that Btj has the potential to be a biocontrol agent of RKN. However, further research, including a more quantitative approach, is needed to explore the interaction between the control organisms with biotic and abiotic soil factors.

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