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Efficacy of *Bacillus thuringiensis* alone and in combination with oxamyl against *Meloidogyne arenaria* infecting greenhouse tomato

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Abstract. *A local strain of Bacillus thuringiensis (B44) was evaluated for its efficacy in controlling Meloidogyne arenaria attacking tomato. Greenhouse experiments were conducted using a soil drench of the bacterium applied alone and in combination only with Vidate 10 G. Results showed reduction in invasion of M. arenaria on the tomato roots by 86-94% when the bacterium was applied threefold alone and twice in combination with Vidate 10 G during vegetation. Results also showed that at the end of the vegetation of tomatoes, the threefold application of an aquatic suspension of Bt (strain B44) on 7th, 25th and 40th days after planting, significantly decreased a final nematode density (Pf) of eggs and J2/g soil and of eggs and J2/g root.*

Keywords: *Bacillus thuringiensis*, biological control, root-knot nematode, tomato, greenhouse

Introduction

Crop production in greenhouses is a method for economically maintaining a warm environment during cool season and to protect crops, especially vegetables, from unfavourable weather condition. Tomatoes are the second most widely cultivated vegetable crop in greenhouses in Bulgaria, with a total annual yield of 35 668 tons (Annual agricultural statistics, MZP, 2009). However, soil borne fungi and root knot nematodes (RKN) greatly affect plant growth and yield of tomatoes in greenhouses. RKN of genus Meloidogyne (Goeldi) are the most pathogenic nematodes which parasitize on tomato and cucumber plants growing in greenhouses (Stoyanov, 1980; Samaliev and Stoyanov, 2008). Traditionally RKN are managed by chemical control. Negative effects of fumigant and non-fumigant nematicides on the environment and increasing concern about the effects of nematicides on user and consumer has increased the amount of research into biological control of plant parasitic nematodes (Johnson et al. 1998). *Bacillus thuringiensis* (Bt) was reported as a potential control agent to some nematodes, including RKN (Borgonie et al., 1996; Marroquin et al., 2000; Griffits et al., 2001; Khyami-Horani and Al-Banna, 2006). 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, penetration and development of RKN (Osman et al., 1988; Rehberger, 1992; Zuckerman et al., 1993; Sharma et al., 1994; Carniero et al., 1998).

In Bulgaria, local strains of *Bt* were isolated from various locations. Some of them showed lethality to eggs and second-stage juveniles (J2) of *Meloidogyne arenaria* under laboratory conditions (Mohamedova and Samaliev, 2006). The aim of present experiments was to evaluate the efficacy of *Bt* (strain B44) alone and in combination with oxamyl against *M. arenaria* in greenhouse conditions.

Materials and methods

Culture of nematode

The root-knot nematode *M. arenaria* was obtained from cultures derived from single egg masses maintained on tomato (*Lycopersicon esculentum*, cv. Rudgers) in a greenhouse at 24-26єC. Eggs of *M. arenaria* were extracted from roots by the sodium hypochlorite method (Hussey and Barker, 1973), second-stage juveniles (J_2) were obtained from these eggs by a modified Baermann funnel (Baermann, 1917) and used within 3 days.

Isolation of bacterium

B44 is a local strain, which was isolated for the first time from roots of cucumber plants growing in Plovdiv region. Subsequently the strain B44 was determined as *Bacillus thuringiensis*.

Culture of bacterium

Cultures of *Bt* (strain B44) 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 32єC 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 4єC. The pellets were washed in deionized water, and serial dilutions of the spore-crystal suspensions were prepared.

Design of experiments

Our tests were conducted in 2007 in plastic-covered greenhouse in the experimental field of the Department of Entomology - Agriculture University, Plovdiv. For the purpose microplots were prepared (pots 30 cm high and 30 cm in diameter disposed so as to stick out 5 cm from the soil) with 10 l loam-sand soil (65% sand, 25% loam and 10% clay), preliminarily sterilized. The distance between the microplots was 60/30 cm.

Aqueous suspension of *Bt* (B44) was applied as a soil drench

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with a watering can in a dosage 2.5 ml^{-1} (containing 10^7 cells/ml) per plant preliminarily diluted in 200/350 ml of water. Vidate 10 G (oxamyl) was applied with incorporation. The soil was lightly irrigated before and after the bacteria and nematicide treatment. Plants in untreated soil with and without *M. arenaria* served as control treatments. The following recordings and observations were made: 1) During the period of vegetation - development of plants and number of galls and egg sacs of *M. arenaria* (on the 50th day of planting the tomatoes); 2) At the end of vegetation - degree of invasion of the root system, number of eggs and J2 in soil, number of eggs and J2 in a root, and yield of fruit. The samples on the 50th day were taken by means of a drill (2 cm/d) at a distance 10 cm from the plant stem, upon which the roots were separated, washed and the number of galls with and without egg sacs was determined per 1 g of randomly chosen roots. At the end of the vegetation, the nematodes were extracted from soil samples by Coolen's method (modified by Di Vito et al., 1985). Eggs were extracted from 10 g of root sub-samples by the sodium hypochlorite method (Hussey and Barker, 1973) and then processed by Coolen's method to extract the remaining eggs and swollen stages of the nematode. A root gall index of tomato was determined on a 0-10 scale (Bridge and Page, 1980).

Effect of Bt (strain B44) alone and in combination with Vidate 10 G on M. arenaria infecting greenhouse tomato

Tomato seedlings (F1 hybrid Caruso) were transplanted in the greenhouse on 10 March and grew until 29 July 2007. The soil had been infested with *M. arenaria* at a rate of 28 eggs and J2/g soil. The experiments included the following variants: A) Control (without *M. arenaria*); B) Control (with *M. arenaria*); C) Application of *Bt* (B44) in a dosage 2.5 ml/plant with 350 ml water on the 7th, 25th and 40th day after planting; D) Combined introduction of 1 g/plant Vidate 10 G and *Bt* (B44) in a dosage 2.5 ml/plant with 350 ml water on the 7th and 25th day after planting and application of *Bt* (B44) on 40th day after planting; E) Treatment of the seedlings with Vidate 10 G in a dosage 1 g/plant and introduction of 2 g/plant on the 7th and 25th day after planting. Each variant was in 8 replications. Records of fruit yield started on 30 May.

Statistical analysis

Data were analyzed by analysis of variance, using procedures of the SPSS programme. Least significant differences (LSD) were calculated at P=0.05 and Duncan`s multiple range test was employed to test for significant differences between treatments.

Results

It was found out that on 50th day after planting of tomato in the variant with combined application of *Bt* (B44) plus Vidate 10 G (variant "D") and variant "E" with Vidate 10 G applied alone, 20 and 26 galls/g roots, respectively, were established on the roots of tomato and all galls were without egg sacs. (Table 1a). *Bt* (B44) applied alone (variant "C") showed slightly lower efficacy for this period - 34 galls/g roots, but only 7 out of them were with egg sacs (Table 1a). In the control there was a high invasion of *M. arenaria* as the whole number of recorded 301 galls/g roots were with egg sacs (Table 1a). Negative influence of the *Bt* (B44) applied alone and in combination with Vidate 10 G on the development of tomato plants was not established (Table 1a). The plant height in the control with *M. arenaria* on the 50th day after planting of tomatoes was 30% lower than plants in the control without nematode. These positive results of the treatment with *Bt* (B44) and Vidate 10 G in tomatoes are preserved by the end of the vegetation (Table 1a).

Tomato yield varied between the different variants (Table 1b). The recorded overall weight of tomato/plant in the control (without nematodes) and the variant *Bt* (B44) plus Vidate 10 G (variant "D") is highest – 5.55 kg/plant and 5.6 kg/plant, respectively. Then follows the one in the variant with individual application of Vidate 10 G (5.4 kg/plant, variant "E") and *Bt* (B44) (6.2 kg/plant, variant "C"). The yield at the control (with *M. arenaria*) is almost 1.5 lower (3.5 kg/plant) than in the control without *M. arenaria*.

Table 1. Influence of Bt (strain B44) alone and in combination with Vidate 10 G on Meloidogyne arenaria and its damage on tomato in a greenhouse pot experiment

a) Number of galls and height of tomato plans - 50 days after planting

Variants	No. of galls in 1 g root on the 50th day			Height
	♀ without egg sac	♀ with egg sacs	Total	on 50th day (cm)
A	-	-	-	118.5 a
B	0	301	301 b	83.0 b
C	34	7	41 a	113.5 a
D	20	0	20 a	110.0 a
E	26	0	26 a	107.0a

b) Weight, levels of invasion and number of eggs on the root system, number of J2 in the soil at the end of vegetation

Variants	Weight (kg/plant)	Gall index	Eggs and J2/g root	Eggs and J2/g soil
A	5.55 a	-	-	-
B	3.5 c	6.3 c	5665 c	89 c
C	5.2 b	3.9	3410 b	51 b
D	5.6 a	2.0 a	2010 a	34 a
E	5.4 ab	2.7 ab	2240 ab	40 ab

* The mean values with different letters have significant difference (P0.05) according to Duncan's Multiple

In tomato plants the recorded index of root galling at the end of vegetation varied, and was the lowest in the variant with combined application of *Bt* (B44) plus Vidate 10 G - 2.8 and variant with Vidate 10 G applied alone - 2.7. The root gall index on tomatoes in the variants treated with *Bt* (B44) was 3.9. The highest root gall index was found in the control *with M. arenaria* - 6.3 (Table 1b). Similar tendency in tomatoes was observed in the number of eggs and J2/g root, as in the variant with combined application of *Bt* (B44) plus Vidate 10 G it was 2010 eggs/g root and in the one with Vidate 10 G - 2240 eggs and J2/g root. In the variant with *Bt* (B44) the recorded number of eggs and J2/g root of tomato was 3410 eggs and J2/g root (Table 1b). At the control the observed reproduction of *M. arenaria*

into tomato roots was the highest – 5665 eggs and J2/g root.

The established population density of eggs and J2 in the soil, at the end of the vegetation period of tomatoes in the variants with *Bt* (B44) was 51 eggs and J2/g soil (Table 1b). Here also the lowest population density of eggs and J2 in the soil was recorded in the variants with combined application of *Bt* (B44) plus Vidate 10 G - 34 eggs and J2/g soil and with Vidate 10 G - 40 eggs and J2/g soil with tomatoes. In the control with *M. arenaria* the number of recorded eggs and juveniles with tomatoes was 89 eggs and J2/g soil, and is significantly higher than all treated with *Bt* (B44) and Vidate 10 G variants (Table 1b).

Discussion

Results showed that in the first one and a half months after planting the plants, the threefold application of *Bt* (B44) in combination with reduced dosage of Vidate 10 G or alone during the vegetation of tomatoes reduces the invasion of *M. arenaria* on the plant roots by 86-94%, compared with the control (Tables 1a and 1b). Khyami-Horani and Al-Banna (2006) reported that *Bacillus thuringiensis jordanica* (Btj) decreased tomato root galling by 51-59% when *M. javanica* eggs or J2 were used as inoculum, respectively. The effect of *Bt* (B44) and Vidate 10 G applied alone or in combination appeared very soon after the plants were exposed to the nematodes and was longer than 14 days. This quick reaction is crucial for effective root-knot nematode control, since the invasive J2 hatching from eggs, tend to find and penetrate host roots within a few days. Through its prolonged effect the bacterium could protect the plants from the invading juveniles in the most sensitive stage of their development, the formation of the root system.

In all variants with *Bt* (B44) at the 50th day after planting the number of galls on the roots of tomatoes with egg sacs was 97.7-100% less than the control (Tables 1a and 1b), i.e. the bacterium interferes with the development of *M. arenaria* inside roots. The efficacy of *Bt* (B44) was enhanced in combination with reduced dosages of Vidate 10 G. Brown and Nordmeyer (1985) report a significant increase of the efficacy of the bacteruium *Pasteuria penetrans* in combination with reduced dosages of nematicides with systematic action. This synergism of the parasite is recorded by the authors in experiments with aldicarb and carbofuran and is due to the fact that at low concentrations the carbamate nematicides influence the orientation and the faster movement of nematodes to the host roots, rather than killing them (Wright, 1981). Therefore, the higher efficacy in the combined application of *Bt* (B44) plus Vidate 10 G (in reduced dosage) in this experiment probably results from an increase of the number of juveniles around the root system in the first weeks when the action of the bacterium *Bt* (B44) is the highest.

At the end of tomato vegetation the threefold application of a suspension of *Bt* (B44) on 7th, 25th and 40th days after planting resulted in a final nematode density (Pf) of 51 eggs and J2/g soil, which was 1.8 times higher than the initial nematode density (Pi) (Table 1b). In the variant with Vidate 10 G Pf was 1.4 times higher than Pi. When *Bt* (B44) was combined with Vidate 10 (in reduced dosage), the Pf was only 34 eggs and J2/g soil, which is close to the Pi (28 eggs and J2/g soil). These results indicate that *Bt* (B44) is compatible with Vidate 10 G and their combined effect is similar to that of the threefold application of Vidate 10 G alone. However, although final nematode population density was significantly lower compared to the untreated plants, it was higher than the economic threshold which for crop loss from *M. arenaria* with tomato is usually less than 16 eggs and J2/g soil, (Samaliev and Stoyanov, 2008).

Conclusion

The study indicated that *Bt* (B44) has the potential to be a biocontrol agent of RKN. The selected isolate is antagonistic to RKN and could be used alone and in combination with oxamyl to reduce deleterious impact of *M. arenaria* on tomato growth in greenhouse conditions. However, further research, including a more quantitative approach, is needed to explore the interaction between the bacterium with a nematicide and between the bacterium with biotic and abiotic soil factors.

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