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RESEARCH ARTICLE

Biological control of *Meloidogyne javanica* on tomato with Dazitol® and soil solarization

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Abstract

Pot and greenhouse trials were conducted for the management of root-knot nematode, *Meloidogyne javanica*, infestation in tomato. Growth parameters, gall index, soil, and root nematode populations were measured to assess the effect of a novel bio-pesticide (Dazitol®), made from mustard oil and oleoresin of *Capsicum*, on plant growth and nematode reproduction. Data generated within the pot experiment showed that the tested bio-pesticide did not improve plant growth, but it reduced significantly root-knot nematode damage resulting in a decrease in gall index and root (91%) and soil (62%) population of *M. javanica* compared with untreated plants. The greenhouse experiment showed that Mocap® and Dazitol® decreased nematode incidence significantly ($P < 0.05$) on tomato. The result of this study suggested that the best nematode control was obtained by combining soil solarization with chemical or botanical nematicides as an integrated pest management approach.

Keywords Biopesticides · Tomato · Root-knot nematode · Solarization · Pest management

Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp., are the most economically important plant-pathogenic nematodes affecting vegetable crops in all regions of the world and cause both considerable losses in crop production and quality (Sikora and Fernandez 2005; Moens et al. 2009). The yield losses caused by the nematode to tomato in Tunisia ranged from 12 to 60% (Horrigue-Raouani 2003). Although tomato yield suppression caused by root-knot nematodes could reach up to 100% (Seid et al. 2015).

Effective management practices of root-knot nematodes have been and still are the objective of several studies and chemical nematicides are widely used for the control of plant-parasitic nematodes (Talavera et al. 2012; Mekete et al. 2015). Additionally, the efficiency of chemical nematicides is for a limited period only besides being expensive and environmentally hazardous (Abd-Elgawad 2008).

Biofumigation, a sustainable management method considered as an alternative to methyl bromide, was reported efficient in controlling several pests including nematodes (Salem 2014). Biofumigation through the toxic volatile isothiocyanates (ITCs), the released biocidal compounds from brassicaceous plants, suppresses various soil-borne pathogens and plant diseases (Thoden et al. 2011).

Botanicals and plant extracts are perceived by the public as natural products safer than synthetic chemicals and thus have been proposed as tools for integrated RKN management because they provide nematode control as well as reduced risks of pesticide residues, thereby boosting production in sustainable agriculture (Caboni and Ntalli 2014).

Soil solarization, a non-chemical and soil thermal disinfection method for controlling diseases, nematodes, and weeds, was developed as thermic disinfection through mulching soil in hot seasons (Katan et al. 1976). This management tool was also successful in controlling plant-parasitic nematodes (Stapleton and Heald 1991).

Currently, the trend is to use control measures alternative to chemical pesticides. The global focus on sustainability in the agricultural environment is increasing in order to produce healthy, safe and good-quality food. This focus includes the implementation of integrated pest management (IPM). The

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Table 1 Effects of the biological nematicide Dazitol and the synthetic nematicide Vydate on top part and roots of tomato plants under pot conditions, 60 days after inoculation with the root-knot nematode, *Meloidogyne javanica*

Treatment	Plant height (cm)	Top plant weight (g)		Root length (cm)	Fresh root weight (g)
		Fresh (g)	Dried (g)		
Vydate	55.75 ± 3,.75 a	22.15 ± 1.50b	3.17 ± 0.08c	19.56 ± 2.38 ab	24.37 ± 8.52 bc
Dazitol	53.25 ± 3.73a	18.58 ± 2.59a	2.07 ± 0.32 a	22.25 ± 2.76 bc	18.46 ± 7.06 b
Control inoculated	54.5 ± 4.30a	22.64 ± 1.47b	2.81 ± 0.40 b	24.56 ± 2.87 c	27.35 ± 7.89c
Control not inoculated	68.5 ± 14.28b	18.81 ± 2.86a	2.32 ± 0.32 a	17 ± 6.35 a	5675 ± 3.41 a

Means within each column followed by the same letter are not significantly different according to Tukey's multiple range test at $P = 0.05$ ($n = 9$; control inoculated = plant inoculated with root-knot nematode and not treated with nematicides; control not inoculated: plant not inoculated with nematode and not treated with nematicides)

most sustainable approach to root-knot nematodes control will integrate several tools and strategies, including reasonable use of nematicides, crop rotation, resistant cultivars, and soil solarization (Stoddard et al. 2010).

The combination of two or more management methods are encouraged in an IPM context. The use of biofumigation, botanicals, and solarization are particularly interesting for organic agriculture. The current investigation focused on determining the effect of a mixture of botanicals, Dazitol®, made from mustard and pepper extract plus soil solarization to control root-knot nematodes on tomato under pot and greenhouse conditions.

Materials and methods

Nematode inoculum

Egg masses of a monoxenic population of *Meloidogyne javanica* extracted from tomato galled roots were shaken with diluted 1% sodium hypochlorite for 4 min following the Hussey and Barker (1973) method. The eggs were washed three times with distilled water and filtered through 100 and 20 μm sieves. Eggs retained by the 20 μm sieve were collected by washing the sieve with distilled water over a container. The distilled water-egg suspension was incubated at 25 ± 3 °C during 3 days in a Baermann funnel to allow egg-hatching. The number of freshly emerged juveniles per ml of suspension was determined three times using a counting chamber under light microscope. The solution was adjusted to1500 J2 per 1 ml.

Pot experiment

Tomato seeds (*Lycopersicum esculentum* Mill. cv. Riogrande, susceptible to *Meloidogyne*) were sterilized by immersion in 5% hypochlorite sodium for 10 min soon followed by three washes by distilled water and sown in alveolus plates filled with peat. Germinated, four leaves and uniform seedlings were transplanted into plastic pots (12-cm diameter) filled with an autoclaved (during 1 h at 120 °C) mix of soil (1 l), sand, and peat at rates of 1:1:1, *v*/v. One day after transplanting, plants were inoculated with 1500 freshly emerged juveniles (J2) by pouring 1 ml of root-knot nematode suspension into two holes around the plant root. The tested nematicide (Dazitol®, Champon Millennium Chemicals Inc., Northvale, NJ, USA) (0.42% *w*/*v* capsaicin + 3.7% *w*/*v* allyl-isothiocyanate) was applied at recommended label rates of 10 l/ha, corresponding to 0.75 ml per pot, 5 days before seedlings transplantation by pipette at top of soil. The commercial nematicide Vydate®, with active chemical substance oxamyl (DuPont de Nemours South Africa (Pty) Ltd.; 10% oxamyl) was applied, 2 days after nematode inoculation, at rates of 20 kg/ha, corresponding to 0.15 g per pot by soil application. Controls (positive and negative) were non-inoculated pots and inoculated but non-treated pots. Nine replicated pots per treatment were arranged in a completely randomized design and the whole experiment was repeated twice. All pots were maintained under greenhouse conditions with controlled temperature at 25–30 °C and humidity at 60–75%. Pots were watered regularly and fertilized with nutrient

Table 2 Effects of the biological nematicide Dazitol and the synthetic nematicide Vydate on root galling of tomato plants, 60 days post-inoculation with *Meloidogyne javanica*

Treatment	Gall index	Galls/g of root	Egg masses/g of root
Vydate	1 ± 00 a	21.11 ± 12.46 a	10.56 ± 7.19 a
Dazitol	1.22 ± 0.66 a	22.78 ± 12.30 a	8.89 ± 7.70 a
Control inoculated	3.33 ± 0.70 b	124 ± 48.29 b	46 ± 13.75 b

Means within each column followed by the same letter are not significantly different according to Tukey's multiple range test at $P = 0.05$ ($n = 9$; control inoculated = plant inoculated with root-knot nematode and not treated with nematicides)

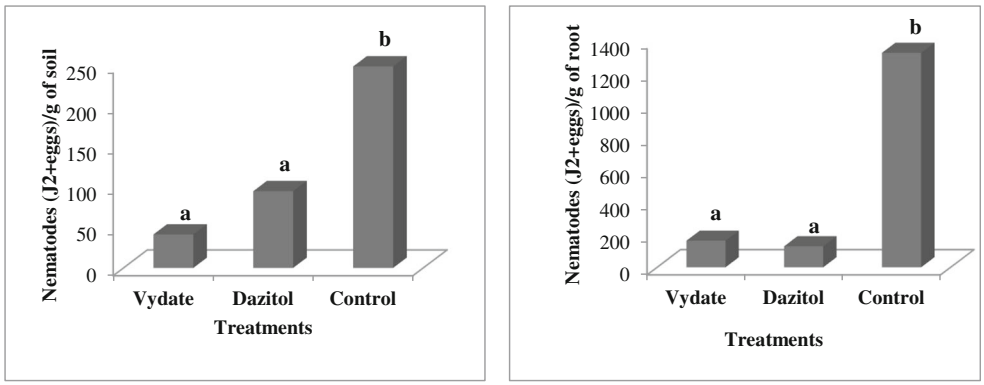

Fig. 1 Effects of the treatments on the numbers of the root-knot nematode, *Meloidogyne javanica*, in soil and roots of tomato in pots, 60 days after nematode inoculation (control = pots inoculated with the nematode and non-treated)

solution according to Pharand et al. (2002). Sixty days after inoculation, tomato plants were uprooted carefully and washed free of soil. Fresh and dried shoot weight and root length and weight were measured for each plant. Nematode damage was rated on a scale of 0 to 5 for gall index according to Hussey and Janssen (2002). Number of galls and egg masses per root system were counted. Root-knot nematodes (second stage juveniles and eggs) were extracted from 5 g roots by blender centrifugal method (Coolen 1979) and soil (500 cm^3) by flotation-centrifugation technique according to the modified method by De Grisse (1969) and counted under a stereo microscope.

Greenhouse experiment

A field experiment was carried out in two adjacent greenhouses in Chatt-Mariem, Sousse, Tunisia from October to June 2015–2017. The greenhouse soil was naturally infested with *Meloidogyne javanica* at the level of 107 J2 s per 100 cm^3 of soil, as assessed by the extraction technique previously described. Soils in both greenhouses had similar soil texture (loamy sandy) and pH 7.4. One greenhouse was soil solarized during the hot season (July–August) while the other was not. Two weeks before solarization, the soil was disked three times. Then, the soil was irrigated up to field capacity and covered with transparent polyethylene film (100 μm thickness) for 6 weeks. The recorded soil temperature reached more than 50 °C (nearly 55 °C at 15–25 cm soil depth). Each plot was planted with four plant rows, 8-m long, with 1-m row spacing. Blocks were separated by a 1.2-m alley. The experiment was arranged in a randomized complete block design in each greenhouse, using three plots per treatment, which were Dazitol, Mocap, and a control (soil naturally infested with *Meloidogyne javanica* and not treated). Each treatment had 20 replicates. At the end of the soil solarization period and 5 days prior to tomato planting, Dazitol® was applied through the drip irrigation system. Mocap (10% ethoprophos; BAYER Cropscience, Rhone-Poulenc, Inc.) was applied at the recommended application rate of 50 kg/ha incorporated into the soil in 30-cm wide bands at planting time. At the end of experiment, plant growth parameters were assessed (plant height, fresh and dried shoot weight, length and weight of root). Evaluation of nematode damage on tomato roots was assessed by visually rating gall indices and counting extracted nematodes from soil and roots, as described before in the pot trial.

Data analysis

Data derived from pot and greenhouse experiments were analyzed for significance by analysis of variance and Duncan's multiple range test ($P < 0.05$), using the software SPSS 20 for windows.

Table 3 Effects of the treatments on tomato growth in greenhouses infested with the root-knot nematode, *Meloidogyne javanica*, and solarized (S) or not (NS)

Treatments		Shoot length (m)	Root length (cm)	Fresh root weight (g)	Collar diameter (cm)
Mocap	S	3.96 ± 0.57 b	43.5 ± 11.26 ab	108.6 ± 35.33 a	2.06 ± 0.30 ab
	NS	2.92 ± 0.59 a	48.7 ± 16.97 b	101.35 ± 36.08 a	2.3 ± 0.50 bc
Dazitol	S	3.87 ± 0.71 b	39.5 ± 9.64 a	105.5 ± 24.12 a	1.99 ± 0.28 a
	NS	2.87 ± 0.67 a	42.75 ± 16.24 ab	198.70 ± 45.02 b	2.29 ± 0.33 bc
Control	S	4.11 ± 0.32 b	41.60 ± 0.58 ab	111.80 ± 36. 03 b	1.99 ± 0.29 a
	NS	3.02 ± 0.51 a	41.25 ± 11.84 ab	211.80 ± 50.40 b	2.36 ± 0.43 c

Means within each column followed by the same letter are not significantly different according to Tukey's multiple range test at $P = 0.05$ ($n = 20$; control = plant inoculated with root-knot nematode and not treated with nematicides)

Table 4 Effects of the treatments on root galling and reproduction of *Meloidogyne javanica* (RKN) on tomato grown in solarized (S) or non-solarized (NS) greenhouses

Treatment		Gall index	RKN per root system	RKN per g of soil
Mocap	S	1.25 ± 0.27 a	8532.75 ± 17.08 a	0.81 ± 0.31 a
	NS	2.10 ± 0.19 b	15,068.25 ± 45.03 a	0.96 ± 0.19 a
Dazitol	S	1.1 ± 0.19 a	8407.25 ± 16.99 a	0.92 ± 0.30 a
	NS	3.30 ± 0.25 c	21,624.5 ± 39.43 ab	1.33 ± 0.30 b
Control	S	2.15 ± 0.26 b	36,940.75 ± 25.70 b	1.03 ± 0.09 a
	NS	3.95 ± 0.20 c	38,150.25 ± 12.85 b	1.39 ± 0.36 b

Means within each column followed by the same letter are not significantly different according to Tukey's multiple range test at $P = 0.05$ ($n = 20$; control = plant inoculated with root-knot nematode and not treated with nematicides; RKN: root knot nematode and the stages of the nematodes are both juveniles and eggs)

Results

Pot experiment

Nematode infection caused a significant decrease in plant growth (Table 1). All growth parameters were reduced significantly in the inoculated control compared with non-inoculated control. The aerial part of tomato plants showed no significant differences in dried and fresh shoot weight between Dazitol treatment and non-inoculated plants. Dazitol did not improve plant growth compared to inoculated control, while Vydate treatment increased fresh and dry weight of the plants when compared to Dazitol treatment.

Both Vydate and Dazitol decreased significantly *Meloidogyne javanica* development. All treatments significantly reduced root galling index, galls, and egg masses numbers per g of root compared to the untreated inoculated control (Table 2). Moreover, both treatments significantly reduced RKN populations in soil and roots (Table 2, Fig. 1).

Greenhouse experiment

Soil treatment with Mocap and Dazitol had no clear significant effect on tomato growth with or without soil solarization, when compared to the non-treated controls. Although, all solarized treatments (S) showed greater shoot length than their respective non-solarized treatments (NS) (Table 3).

Mocap and Dazitol reduced root-knot nematode reproduction (Table 4) and the greatest reductions in gall index, and number of nematodes per root system or g of soil were obtained by combining soil solarization and a nematicide treatment.

Discussion

The results of the present study prove that Dazitol has the potential to contribute as a successful eco-friendly component in the integrated nematode management package for the tomato. Our results complement those of Ploeg (2008) and Reddy (2011) reporting that isothiocyanates, the active ingredient of brassica crop, had a broad pesticidal activity against weeds, bacteria, fungi, and nematodes. Dazitol was as effective as the synthetic nematicides ethoprophos and oxamyl. This agrees with the results by Fleming et al. (2006) and Martin et al. (2007) who found that isothiocyanates from brassicas are as effective as fenamiphos and fosthiazate (active ingredient of Nemathorin). Additionally, Dazitol, in combination with soil solarization, can be used for root-knot nematode control in organic farms or conventional farming systems (Reddy 2011). For conventional farming systems, soil solarization, alone or in combination with organic amendments, was found effective towards *Meloidogyne javanica* and *Globodera pallida* infecting potato crop as it reduced nematodes multiplication and improved plant growth and yield (Elhajji and Horrigue-Raouani 2012). Moreover, combination of solarization and biofumigation was reported to be efficient in reducing pathogenic fungus severity (Gamliel and Stapleton 1993). Finally, in organic farms, the combination of soil solarization with organic amendments has notable potential for controlling *M. javanica* and *M. incognita* (Oka et al. 2007).

Compliance with ethical standards

This study does not involve any human and/or animal participant.

Conflict of interest The authors declare that they have no conflict of interest.

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