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Original article (Orijinal araştırma)

Integrated management of *Mi-1* virulent *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) in greenhouse tomatoes[1]

Örtüaltı domates yetiştiriciliğinde *Mi-1* virülent *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)'ya karşı entegre mücadele

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Abstract

The *Mi-1* gene in tomato provides a safe and economical strategy for managing root-knot nematodes (RKN). However, the resistance conferred by the *Mi-1* gene is not effective against virulent populations of RKNs. In this study, the efficacy of combining the dose effect of the *Mi-1* gene with *Bacillus firmus* Bredemann & Wermer, 1933 (Bacillales: Bacillaceae) + fluopyram against *Mi-1*-virulent *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) after soil solarization was assessed for tomatoes grown in a greenhouse. The study was conducted in a greenhouse in Kepez, Antalya between 2015 and 2016. The second-stage juveniles *M. incognita* were not detected in the soil for 2 months after solarization completed. The reactions of homozygous or heterozygous resistant tomato plants against *Mi-1*-virulent populations were not statistically different under greenhouse conditions. Furthermore, *B. firmus* + fluopyram controlled the RKN population when applied to the soil both during the planting period and when J2 count in the soil started to increase. This study suggests that the soil solarization should be combined with other management methods, and the dose effect of the *Mi-1* gene is not important against virulent RKN populations. Additionally, combined *B. firmus* + fluopyram have the potential to be used as a suitable management tool for RKN control in tomato production. These findings will help improve integrated management practices for controlling *Mi-1*-virulent RKN populations.

Keywords: Management, *Mi-1* gene, root-knot nematode, solarization, tomato, virulent

Öz

Domatesteki *Mi-1* geni, kök-ur nematodları kontrol için güvenli ve ekonomik mücadele sağlamaktadır. Bununla birlikte, *Mi-1* geninin sağladığı dayanıklılık virülent kök-ur nematodlarına karşı etkili değildir. Bu çalışmada, solarizasyon uygulamasından sonra serada yetiştirilen domatesler için *Mi-1* virülent *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) popülasyonuna karşı *Bacillus firmus* Bredemann & Wermer, 1933 (Bacillales: Bacillaceae) + fluopyram kombinasyonları ile *Mi-1* geninin doz etkisinin kombinasyonunun etkinliği değerlendirilmiştir. Çalışma 2015-2016 yılları arasında Antalya, Kepez bölgesindeki serada yürütülmüştür. Solarizasyon uygulamasından sonra toprakta iki ay boyunca *M. incognita* ikinci dönem larva tespit edilmemiştir. Sera koşullarında *Mi-1* virülent popülasyona karşı, homozigot-heterezigot dayanıklı domates bitkilerinin reaksiyonlarında istatistiksel farklılık olmamıştır. Ayrıca, *B. firmus* + fluopyram kombinasyonu, hem bitki dikim döneminde uygulandığında hem de toprakta J2 sayısı artmaya başladığı anda uygulandığında kök-ur nematod popülasyonunu kontrol altına almıştır. Bu çalışma, toprak solarizasyonunun diğer mücadele yöntemleriyle kombine edilebilir ve *Mi-1* geninin doz etkisinin virülent kök-ur nematodlarına karşı önemli olmadığını göstermiştir. Ayrıca, *B. firmus* + fluopyram kombinasyonu, domates üretimlerinde kök-ur nematolarının kontrolünde uygun bir mücadele aracı olarak kullanılabilme potansiyeline sahiptir. Bu bulgular, *Mi-1* virülent kök-ur nematod popülasyonlarının kontrolü için entegre mücadele yöntemlerinin gelişmesine yardım edebilecektir.

Anahtar sözcükler: Mücadele, *Mi-1* geni, kök-ur nematodları, solarizasyon, domates, virülent

[1] This study represents first author's master thesis.

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Introduction

Tomato is one of the most important vegetables in Turkey, grown over about 170 kha with an annual yield of 12 Mt in 2015 (TUİK, 2018). Tomatoes are produced in many parts of Turkey and the western Mediterranean Region of Turkey is the most significant production area.

Root-knot nematodes (RKNs) are widespread in many parts of the world. So far, 98 RKN species have been described worldwide (Jones et al., 2013). In previous studies, the presence of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae), *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 was determined in greenhouses of the western Mediterranean Region of Turkey (Devran & Söğüt, 2009; Devran et al., 2017).

RKNs feed on plant roots, resulting in the root gall formation that causes poor uptake of water and nutrients (Abad et al., 2003). In addition, infested plants can be more susceptible to soilborne plant pathogens (Karssen & Moens, 2006). Therefore, RKNs cause yield losses in conjunction with other factors in crops (Schomaker & Been, 2006). Several management tactics such as soil solarization, organic amendments, biological agents, chemicals and resistant cultivars are used to control RKNs (Collange et al., 2011). Soil solarization is also commonly used alone or in combination with other methods to manage soilborne pathogens (Katan, 1996). Biological control has no residual effects and is eco-friendly. Fungi, such as *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson, 2011 (Hypocreales: Ophiocordycipitaceae) and *Hirsutella rhossiliensis* Minter & Brady 1980 (Hypocreales: Ophiocordycipitaceae), and bacteria, such as *Pasteuria penetrans* Thorne, 1940 (Bacillales: Pasteuriaceae) and *Bacillus* spp., are parasites of nematodes (Keren-Zur et al., 2000; Walia et al., 2000; Walia & Vats, 2000; Anastasiadis et al., 2008). Moreover, biological agents, including *P. lilacinus, P. penetrans* and *Bacillus firmus* Bredemann & Wermer, 1933 (Bacillales: Bacillaceae), are commercially available (Hallman et al., 2009). However, the efficacy of these agents can decrease under field conditions. Nematicides are commonly used to control RKN, although the use of many nematicide are banned or restricted because of health concerns (Devran & Söğüt, 2010; Wesemael et al., 2011). Recently, fluopyram was developed initially as a fungicide by Bayer Crop Science. It is a succinate dehydrogenase inhibitor (SDHI) in the phenyl-benzamide chemical group that is used to manage fungal diseases in plants. Additionally, fluopyram has been used against plant-parasitic nematodes including RKNs (Faske & Hurd, 2015).

Plant resistance is considered an alternative, economical and environment-friendly method to manage RKNs (Devran & Söğüt, 2010). In tomatoes, resistance to RKN is controlled by the *Mi-1* gene. The *Mi-1* gene was introgressed into cultivated tomato from *Solanum peruvianum* L. (Solanaceae) in the 1940s (Smith, 1944). This gene confers resistance against *M. incognita*, *M. javanica* and *M. arenaria* (Roberts & Thomason, 1986). The *Mi-1* gene has been successfully incorporated into many commercially-available tomato cultivars. However, virulent RKN populations overcome the *Mi-1* gene in tomato (Kaloshian et al., 1996; Ornat et al., 2001; Tzortzakakis et al., 2005). In nature, virulent populations occur spontaneously without selection (Castagnone-Sereno et al., 1994). Besides, Jarquin-Barberena et al. (1991) reported that populations of *M. incognita* virulent to *Mi-1* have developed with repeated exposure to this resistance gene under laboratory and field conditions.

The existence of virulent populations has also been documented in many countries (Roberts, 1995; Ornat et al., 2001; Tzortzakakis et al., 2005; Devran & Söğüt 2010; Iberkleid et al., 2014). Therefore, the development of new management methods is needed in the presence *Mi-1* virulent population of RKNs. The objectives of this study were a) to determine the performance of homozygous and heterozygous resistant tomato cultivars grown in a greenhouse infested with *Mi-1*-virulent *M. incognita,* b) to identify the population density of second-stage juveniles (J2s) in the soil planted tomato plants, during the growing season after soil solarization, c) to determine the effect of two different applications of commercial formulations including the combination of chemical and biological components on *Mi-1*-virulent *M. incognita* population and tomato yields in the greenhouse.

Material and Methods

Plant materials

Susceptible tomato cv. Tueza F_1 (*mimi*), heterozygous resistant cv. Seval F_1 (*Mimi*) and homozygous resistant cv. Browny F_1 (*MiMi*) were used in this experiment. All tomato seedlings were provided by Multi Tohum Tar. San. Tic. A.Ş. (Antalya, Turkey).

Commercial formulations

Velum Prime SC 400 and Flocter WP 5, registered by Bayer CropScience LP (Monheim, Germany) were used to control the RKNs. Velum Prime SC 400 is a nematicide containing the active ingredients 400 g/l fluopyram, which inhibits mitochondrial respiration by blocking the electron transport in the respiratory chain of succinate dehydrogenase (Garris, 2017; Heiken, 2017). Flocter WP 5 includes the active biological agent 50 g/kg *B. firmus* I-1582, a gram-positive bacterium. The studies reported that *B. firmus* paralyzes *M. incognita* J2s and inhibits hatching of eggs (Giannakouet et al., 2004; Mendoza et al., 2008; Terefe et al., 2009).

Glasshouse location

This study was conducted in a glasshouse in Kepez District, Antalya, Turkey (36°54'46.83" N, 30°45'13.11" E). The soil was 55% sand, 30% silt, 15% clay, and heavily infested with *M. incognita.* Initial nematode population density in the greenhouse was 3,328 J2s/100 g of soil.

Nematode culture

Soil samples were collected from different parts of the greenhouse used for the experiment in a zigzag pattern. Initially, seven populations of *Meloidogyne* spp. were collected from the root systems of a commercial candidate tomato cultivar with *Mi-1* gene grown in a previous season in the glasshouse. Afterwards, pure cultures were multiplied on susceptible tomato according to Mıstanoğlu et al. (2016).

DNA isolation

DNA was isolated from J2s with the DNAeasy Tissue and Blood Kit (Qiagen, Hilden, Germany), based on the manufacturer's protocol.

Molecular identification

The RKN populations were identified according to previous studies using species-specific primers Inc14F/Inc14R (Randig et al., 2002), Fjav/Rjav (Zijlstra et al., 2000) and Far/Rar (Zijlstra et al., 2000).

Virulence test

Seedlings of susceptible tomato cv. Tueza F_1 and resistant cv. Seval F_1 were planted singly in 250ml plastic pots containing steam-sterilized sandy soil. The establishment of the experiment and nematode inoculation were conducted as previously described (Mıstanoğlu et al., 2016).

Egg masses on roots of plants were examined under a stereomicroscope with. the number of egg masses on each plant root scored on a 0-5 scale according to Hartman & Sasser (1985) as follows: 0, no galls (resistant); 1, 1-2 galls or egg masses (resistant); 2, 3-10 galls or egg masses (resistant); 3, 11-30 galls or egg masses (susceptible); 4, 31-100 galls or egg masses (susceptible); and 5, more than 100 galls or egg masses per root system.

The J2s from 100 g of soil from each pot were extracted by modified Baermann funnel (Hooper, 1986). Reproduction factor (RF; i.e., final J2 population density/initial nematode population, 1000 J2s) was calculated (Ferris & Noling, 1987).

Soil preparation

The tomato plants from the previous season were removed from the glasshouse in 1 June 2015. About 15-20 cm depth of soil was processed with rotavators. The soil was then arranged using a shovel and 12 bands (double rows) were formed for the planting of seedlings. Drip irrigation pipes were placed in the soil.

Soil solarization

Soil solarization in the greenhouse was performed from 11 July to 22 August 2015 (6 weeks). No chemical was applied to the soil during the solarization period. The soil was covered with plastic of 20µ thickness and watered in the early morning at 5-d intervals for heat transmission to the deep layers of the soil. To detect the effectiveness of solarization, soil samples were taken from the greenhouse using a soil drill after soil solarization was completed. Three samples were taken from each subsection and then combined for analysis. The J2s were extracted from the soil (100 g) by modified Baermann funnel and counted under a microscope.

Planting of tomato seedlings

The glasshouse was divided into three sections, each formed from four bands. In addition, each band was split into three subsections (Figure 1). Seedlings of tomato cvs Tueza F_1, Seval F_1 and Browny F_1 were arranged in a randomized block design, with four replicates in each section (Figure 1). Forty tomato seedlings were planted for each replicate. The plants were planted 20 cm apart from each other in glasshouse. In total, 1440 seedlings were planted on 4 September 2015. Fertilization, irrigation and pests and pathogen management were done as needed.

Bands	Sub-Sections			Sections
Band 1	Seval F1	Tueza F1	Browny F1	**Section 1**
Band 2	Tueza F1	Browny F1	Seval F1	
Band 3	Browny F1	Seval F1	Tueza F1	
Band 4	Seval F1	Tueza F1	Browny F1	
Band 5	Tueza F1	Browny F1	Seval F1	**Section 2**
Band 6	Browny F1	Seval F1	Tueza F1	
Band 7	Seval F1	Tueza F1	Browny F1	
Band 8	Tueza F1	Browny F1	Seval F1	
Band 9	Seval F1	Tueza F1	Browny F1	**Section 3**
Band 10	Tueza F1	Browny F1	Seval F1	
Band 11	Browny F1	Seval F1	Tueza F1	
Band 12	Seval F1	Tueza F1	Browny F1	

Figure 1. Experimental layout.

Application of commercial formulations

As mentioned above, the glasshouse was divided into three sections corresponding to the three treatments (Figure 1). Commercial formulations were applied in Sections 1 and 2 via drip tubes into the soil according to the recommended doses (40 kg/ha for Flocter WP 5 and 600 ml/ha for Velum Prime SC 400). Treatments of the sections were:

Section 1: Flocter WP 5 was applied 1 week before (28.08.2015) planting and 1 week after (11.09.2015) planting. Velum Prime SC 400 was applied 1 d (05.09.2015) after planting and 15 d after the first application (20.09.2015) (Figure 1).

Section 2: Flocter WP 5 and Velum Prime SC 400 were applied twice at 15 d apart (01.12.2015 and 16.12.2015) because the number of J2s in the soil increased (Figure 1).

Section 3: Control, no commercial formulations.

Monitoring of J2s

The soil samples were collected 27 times at 10-d intervals, after the soil solarization. Three samples were obtained from each subsection and were combined to detect the number of J2s in the soil by modified Baermann funnel. The number of J2s was monitored during the growing season.

Evaluation of treatments

Plants were harvested at two different periods, 16 April and 31 May 2016. Ten tomato plants were harvested from each subsection. A total of 720 plants were harvested and evaluated at the end of the experiment. The gall indices of the roots were rated on a scale of 0 to 10 (Zeck, 1971).

Applications effects on yield

Tomato fruit of 20 plants from each subsection were harvested from 15 November 2015 to 20 March 2016 and the cumulative yield calculated.

Data analysis

Raw data were used in all analyses. ANOVA was used to compare triplet groups, and the Sidak test for binary comparisons. Two-way repeated measures ANOVA was used for the number of J2 and yield. One-way ANOVA was used for egg masses. Statistical analyses were performed using the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) at $p < 0.05$.

Results and Discussion

Molecular confirmation of *M. incognita*

Pure cultures were confirmed by PCR, using species-specific primers. Only PCR primers Inc14F/Inc14R, which are specific primers for *M. incognita*, produced an ~400 bp amplicon in all nematode samples, while the other primers did not yield any PCR products (data not shown). The results indicated that all populations were *M. incognita* and concur with previous studies (Randig et al., 2002; Devran & Söğüt, 2009).

Virulence test

Meloidogyne incognita pure cultures were tested for virulence. All *M. incognita* populations multiplied well in both susceptible cv. Tueza F_1 and resistant cv. Seval F_1. The RF values of populations were >1 in both susceptible and resistant cultivars. Similarly, the number of egg masses per root system was more than 100 (Table 1). All *M. incognita* populations were virulent for the *Mi-1* gene. The *Mi-1* gene confers resistance against three RKNs including *M. incognita*, *M. javanica* and *M. arenaria* (Roberts & Thomason, 1986). However, the *Mi-1* gene has been overcome by virulent populations that occur naturally or by a selection pressure in field and laboratory experiments (Jarquin-Barberena et al., 1991; Devran & Söğüt, 2010; Verdejo-Lucas et al., 2012). Results indicated that all *M. incognita* populations collected from the glasshouse were *Mi-1* virulent.

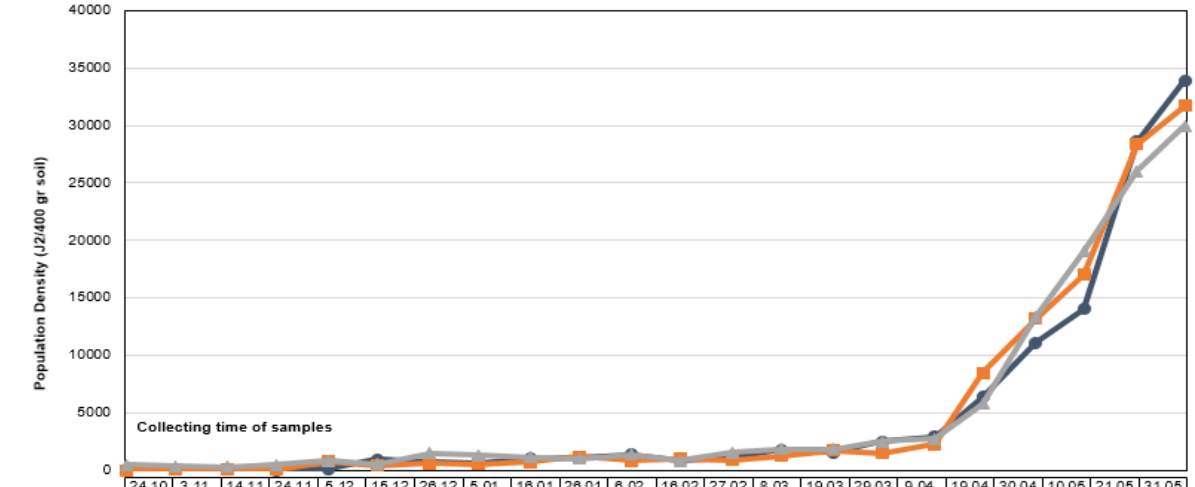
Table 1. The determination of the virulence of *Meloidogyne incognita* populations under laboratory conditions

Sample No	Egg mass index*		Reproduction factor	
	Tueza F_1	Seval F_1	Tueza F_1	Seval F_1
1	5	5	26.5	27.5
2	5	5	53.1	46.5
3	5	5	9.6	8.0
4	5	5	11.8	13.3
5	5	5	27.6	28.0
6	5	5	31.7	25.3
7	5	5	31.1	34.7

* Hartman and Sasser (1985) scale. Tueza F1, susceptible (*mimi*); and Seval F1, heterozygous resistant (*Mimi*).

Effect of soil solarization on J2s

J2s were first detected in the soil 2 months after the soil solarization completed and were also found in the soil for 51 d after planting of seedlings (Figure 2). Greco (1999) demonstrated that soil solarization for 45-60 d during July-August can eradicate nematodes from top soil, even in heavily infested plastic houses. However, the effectiveness of solarization depends on some parameters, such as the soil color, structure and moisture, the air temperature, length of day and sunlight intensity (Souza, 1994).



	24.10.2015	3.11.2015	14.11.2015	24.11.2015	5.12.2015	15.12.2015	26.12.2015	5.01.2016	16.01.2016	26.01.2016	6.02.2016	16.02.2016	27.02.2016	8.03.2016	19.03.2016	29.03.2016	9.04.2016	19.04.2016	30.04.2016	10.05.2016	21.05.2016	31.05.2016
Tueza F1	0	80	60	20	140	920	700	660	980	1060	1340	900	1070	1780	1540	2500	2930	6400	11120	14050	28570	33920
Seval F1	0	100	40	60	760	420	580	520	720	1200	840	1020	880	1260	1700	1510	2240	8500	13160	17000	28350	31720
Browny F1	470	360	280	460	840	540	1460	1300	1100	1000	1370	850	1560	1860	1830	2520	2720	5780	13380	19040	26020	29960

Figure. 2. Population density curves of *Mi*-1 virulent *Meloidogyne incognita* J2s in Section 3.

Effect of commercial formulations and cultivars on J2 numbers

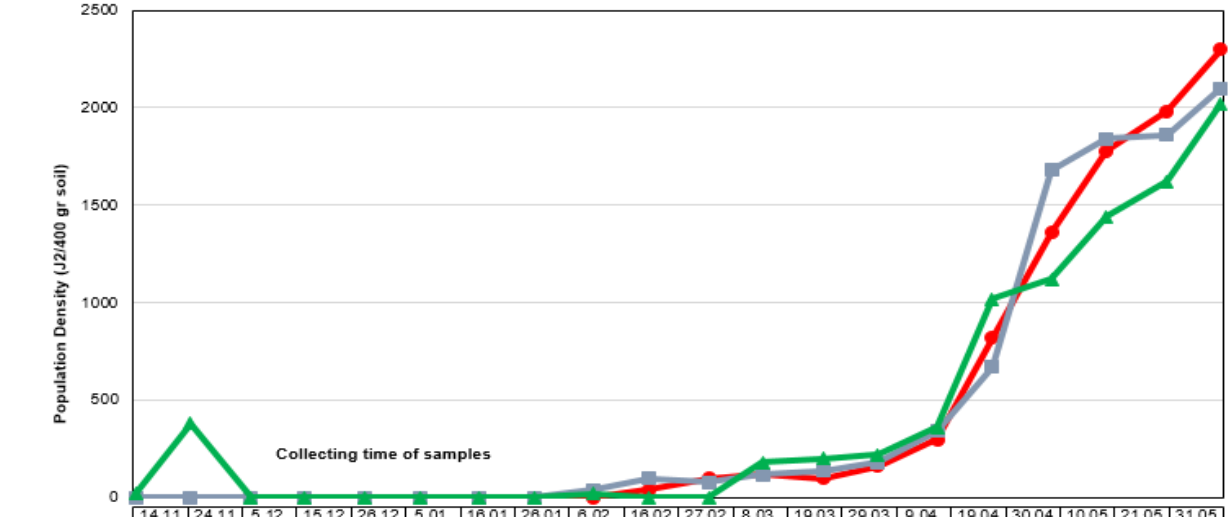
The number of J2s in the soil of the three tomato cultivars within each section were not statistically different ($F = 1.06$, $p > 0.05$) (Table 2). Nonetheless, when comparing the sections, the number of J2, in Section 3 was significantly high than 1 and 2 sections ($F = 4.56$, $F = 4.55$, $F = 5.49$, $p < 0.05$) (Table 2). Curves of J2s in the soil shown in Figures 2-4. The lowest J2 population density curve was detected in

Section 2, followed by Section 1, and the highest J2 density curve corresponded to Section 3, during the growing season (Figures 2-4). In Section 2, the combination of Flocter WP 5 + Velum SC 400 was applied to the soil when the soil population of J2 started to increase. No J2s were detected in the samples colleced between 5 December 2015 and 6 February 2016 (Figure 3).

Table 2. Analysis of mean number of J2 between treatments within tomato cultivars and between cultivars (n = 108)

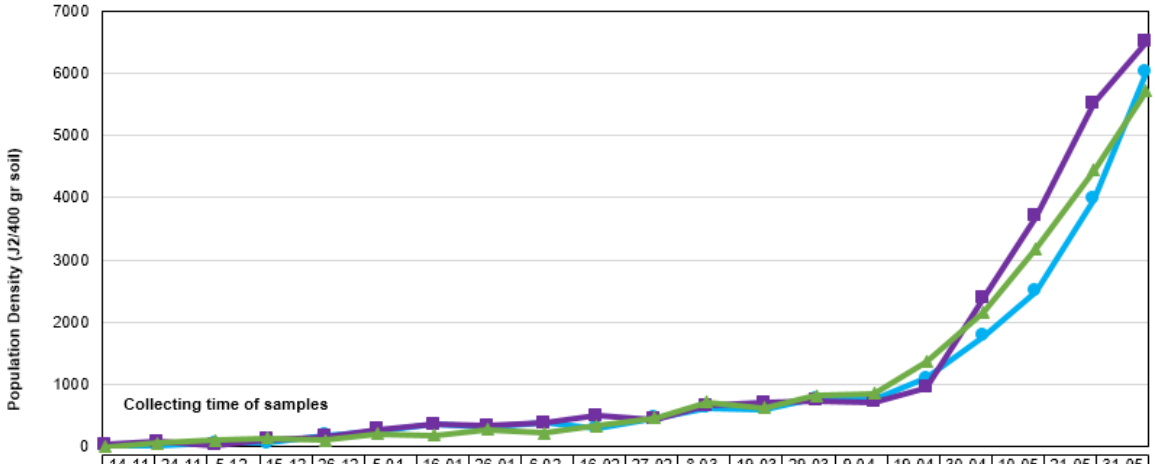
Tomato cultivar	Treatment	Mean J2 number	Standard deviation	Between treatments	Pairwise comparison*	Between cultivars
Tueza F1	Application 1	761	1384	F = 4.56 p = 0.01	3 > 1 and 2	
	Application 2	336	680			
	Control	4101	8554			
Seval F1	Application 1	911	1682	F = 4.55 p = 0.01	3 > 1 and 2	F = 1.06 p = 0.22
	Application 2	339	669			
	Control	4170	8532			
Browny F1	Application 1	814	1442	F = 5.49 p = 0.01	3 > 1 and 2	
	Application 2	319	579			
	Control	4248	8076			

*3 = Control.
Tueza F1: Susceptible (*mimi*), Seval F1: Heterozygous resistant (*Mimi*), Browny F1: Homozygous resistant (*MiMi*)
Application 1 (Section 1): Flocter WP 5 + Velum Prime SC 400 (applied in the soil when plants were planted)
Application 2 (Section 2): Flocter WP 5 + Velum Prime SC 400 (applied in the the soil when the J2 population started to increase)
Control (Section 3): No application.



	14.11.2015	24.11.2015	5.12.2015	15.12.2015	26.12.2015	5.01.2016	16.01.2016	26.01.2016	6.02.2016	16.02.2016	27.02.2016	8.03.2016	19.03.2016	29.03.2016	9.04.2016	19.04.2016	30.04.2016	10.05.2016	21.05.2016	31.05.2016
Tueza F1	0	0	0	0	0	0	0	0	0	40	100	120	100	160	300	820	1360	1780	1980	2300
Seval F1	0	0	0	0	0	0	0	0	40	100	80	120	140	180	340	670	1680	1840	1860	2100
Browny F1	20	380	0	0	0	0	0	0	20	0	0	180	200	220	360	1020	1120	1440	1620	2020

Figure 3. Population density curves of *Mi*-1 virulent *M. incognita* J2s in Section 2.



	14.11.2015	24.11.2015	5.12.2015	15.12.2015	26.12.2015	5.01.2016	16.01.2016	26.01.2016	6.02.2016	16.02.2016	27.02.2016	8.03.2016	19.03.2016	29.03.2016	9.04.2016	19.04.2016	30.04.2016	10.05.2016	21.05.2016	31.05.2016
Tueza F1	20	20	60	60	180	260	360	320	380	300	460	620	600	780	760	1100	1780	2500	3980	6020
Seval F1	40	80	20	120	160	280	360	340	380	500	440	660	700	740	720	950	2400	3700	5520	6500
Browny F1	0	60	100	140	100	210	180	280	220	340	460	720	620	820	860	1360	2160	3180	4440	5720

Figure 4. Population density curves of *Mi*-1 virulent *M. incognita* J2s in Section 1.

The data established that this application was effective against *M. incognita* during this period. In addition, the J2 population densities in the soil fluctuated though winter because of the low soil temperature. However, J2 population densities steadily increased with every sampling from March to June. These findings show that the combination of *B. firmus* + fluopyram was effective against RKNs and the individual components were not antagonist against each other. Faske & Hurd (2015) revealed that fluopyram could be used successfully to control *M. incognita* and *Rotylenchulus reniformis* Linford & Oliveira, 1940 (Tylenchida: Hoplolaimidae). Giannakou et al. (2004) investigated the efficacy of bionematicide including *B. firmus* in a field naturally infected with *Meloidogyne* spp. and under laboratory conditions. The treatments were compared with fumigant nematicides and the biocontrol agent *P. penetrans*. Under field conditions, 1,3-dichloropropene and dazomet + sodium tetrathiocarbonate were typically superior to bionematicide application. However, the recommended dose of the bionematicide significantly suppressed the numbers of J2s at the end of the cropping season in comparison with 1,3-dichloropropene. In addition, in pot experiments, findings indicated that the bio-nematicide was typically more efficient in controlling RKNs than the biocontrol agent *P. penetrans*. In another study, Giannakou et al. (2007) evaluated the effectiveness of a formulated bionematicide product containing lyophilized *B. firmus* spores against RKNs under greenhouse and field conditions. In the laboratory, a decrease in J2s hatching was recorded with bionematicide at a dose of 0.9 g/kg of soil, while a further decline was noted by doubling the dose. In a field experiment, the combination of soil solarization with bionematicide improved nematode control and gave results similar to the chemical treatment.

Dose effect of *Mi-1* gene

Gall indices did not differ between susceptible, and homozygous resistant and heterozygous resistant tomatoes in two separate harvest periods, against *Mi-1*-virulent *M. incognita* populations ($F = 0.09$, $F = 0.05$, $p > 0.05$) (Table 3). Therefore, no significant difference was evident in the reactions of homozygous and heterozygous resistant tomato plants against *Mi-1*-virulent *M. incognita* population, under greenhouse conditions. Tzortzakakis et al. (1998) stated that virulent isolate 1 was unaffected by the *Mi* gene copy number. In another study, Iberkleid et al. (2014) found that the virulent nematodes reproduced well on all susceptible and resistant tomatoes and showed both high reproduction on the susceptible, and

heterozygous and homozygous *Mi-1* genotypes. These findings indicate that reproduction of the *Mi-1*-virulent population was not affected by the allelic variation. Our findings agreed with these studies. In contrast, for an avirulent RKN population, the dosage of the *Mi-1* gene negatively impacted the reproduction of RKNs. Tzortzakakis et al. (1998) observed that expression of the *Mi-1* gene could be affected by gene zygosity, depending on whether the *Mi-1* gene is homozygous or heterozygous, and temperature. Many factors, including the background genotype, the structure of the nematode population and the use of resistant genotypes, can affect virulence (Castagnone-Sereno et al., 1994; Jacquet et al., 2005; Verdejo-Lucas et al., 2009).

Table 3. Comparison of gall index ratings in tomato cultivars (n = 120)

Harvest	Tomato cultivar	Average gall index*	Standard deviation	ANOVA F value	Probability (p)
Harvest 1	Tueza F1	1.25	1.93	0.09	0.91
	Seval F1	1.15	1.89		
	Browny F1	1.18	1.61		
Harvest 2	Tueza F1	1.88	2.39	0.05	0.95
	Seval F1	1.86	2.43		
	Browny F1	1.95	2.46		

* Samples were evaluated according to Zeck (1971). Harvest 1, 16 April 2016; and Harvest 2, 31 May 2016.
Tueza F1, susceptible (*mimi*); Seval F1, heterozygous resistant (*Mimi*); and Browny F1, homozygous resistant (*MiMi*).

Effect of applications on gall formation

Gall indices of the roots of susceptible, and heterozygous and homozygous resistant tomato cultivars were evaluated in two separate harvest periods. Gall indices of the roots did not differ between susceptible, and homozygous and heterozygous resistant tomatoes planted in all sections (Table 3). Nevertheless, Flocter WP 5 + Velum SC 400, which were applied in different periods, decreased galls on roots compared to plants in the control section. At the first harvest, there was no difference between Sections 1 and 2. Whereas, at the second harvest, galls numbers on the root of plants in Section 2 were lower than in Section 1 ($F = 88.36$, $F = 82.17$, $p < 0.05$) (Table 4). As mentioned above, fluopyram is an SDHI fungicide that is being evaluated for management of soilborne fungi and plant-parasitic nematodes in agronomic crops. The effect of fluopyramy on various fungi has been assessed, including its ability to control *Neocosmospora virguliformis* (O'Donnell & T. Aoki) L. Lombard & Crous, 2015 (Hypocreales: Nectriaceae) and many isolates of *F. virguliforme* showed sensitivity to fluopyram (Wang et al., 2017). Recently, a formulation that consists of fluopyram + imidacloprid has been considered as an in-furrow treatment for suppression of *M. incognita* and *R. reniformis* in cotton. In these field trials, fluopyram was found to suppress nematode densities at levels that were numerically more effective than those achieved by thiodicarb applied as a seed treatment (Lawrence et al., 2015). Faske & Hurd (2015) reported that in tomatoes nematode infestation of roots was reduced, and root galling lowered by 31 to 84% at concentrations of 1.3 to 5.3 mg/ml fluopyram. In another study, Giannakou et al. (2004) showed that band application of the bionematicide at all dose levels failed to decrease the midseason nematode population in soil compared to the control. However, there were significantly fewer nematodes at the end of the season in plots treated with the bionematicide at all dose levels than in the control plots. Terefe et al. (2009) examined the influence of BioNem on *M. incognita* in the greenhouse and field. They reported that BioNem applied at 8 g/pot planted with tomato seedlings reduced gall formation by 91%, final nematode populations by 76% and the number of eggs by 45% in the greenhouse trials. In addition, in the field trails, BioNem applied at 200 and 400 kg/ha was effective in reducing the number of galls (75-84%), and increased shoot height (29-31%) and weight (20-24%) over the untreated control, at 45 d after treatment.

Table 4. Effect of applications on gall index ratings (n = 120)

Harvest	Application	Average gall index*	Standard deviation	ANOVA F value	Possibility (p)	Pair-wise comparison
Harvest 1	Application 1	0.66	1.04	88.4	0.01	3 > 1 and 2
	Application 2	0.28	0.73			
	Control	2.65	2.24			
Harvest 2	Application 1	1.33	1.87	82.2	0.01	3 > 1 > 2
	Application 2	0.59	1.02			
	Control	3.77	2.75			

* Samples were evaluated according to Zeck (1971). Harvest 1: 16 April 2016, Harvest 2: 31 May 2016.
Tueza F1: Susceptible (*mimi*), Seval F1: Heterozygous resistant (*Mimi*), Browny F1: Homozygous resistant (*MiMi*)
Application 1 (Section 1): Flocter WP 5 + Velum Prime SC 400 (applied in the soil when plants were planted)
Application 2 (Section 2): Flocter WP 5 + Velum Prime SC 400 (applied in the the soil when the J2 population started to increase)
Control (Section 3): No application.

Effect of applications on yield

Given the differences in the genetic backgrounds of the tomato cultivars used in the experiment, the yields were separately evaluated for each cultivar. The yields of tomato cultivars planted in the Sections 1 and 2 were higher than Section 3. Also, yields of susceptible and heterozygous resistant tomato cultivars planted in Section 2 were higher relative to Section 1 ($F = 4.12$, $F = 3.86$, $F = 5.22$, $p < 0.05$) (Table 5). Whereas, the yields of homozygous resistant cultivars grown in Sections 1 and 2 were not statistically different from each other ($F = 4.12$, $F = 3.86$, $F = 5.22$, $p < 0.05$) (Table 3). The results showed that Flocter WP 5 + Velum Prime SC 400 decreased yield losses caused by *M. incognita*. Moreover, application with increasing of *M. incognita* J2 population the soil was more successful than application before planting. Terefe et al. (2012) investigated the effect of BioNem on nematode infestation and plant growth and yield of tomato, and compared two methods of BioNem application for the control of root-knot under field conditions. A significant increase in crop growth and yield relative to the untreated control was reported.

Table 5. Effect of different applications on yield (n = 10)

Tomato cultivar	Application	Mean yield (kg)	Standard deviation	ANOVA F value	Probability (p)	Pair-wise comparison
Tueza F1	Application 1	25.28	11.5	4.12	0.01	2 > 1 > 3
	Application 2	27.65	11.7			
	Control	21.85	10.4			
Seval F1	Application 1	24.73	12.3	3.86	0.01	2 > 1 > 3
	Application 2	26.53	11.2			
	Control	21.44	12.4			
Browny F1	Application 1	15.92	10.1	5.22	0.01	1 and 2 > 3
	Application 2	17.52	11.2			
	Control	13.58	10.2			

Tueza F1, susceptible (*mimi*); Seval F1, heterozygous resistant (*Mimi*); and Browny F1, Homozygous resistant (*MiMi*).
Application 1 (Section 1), Flocter WP 5 + Velum Prime SC 400 (applied in the soil when plants were planted).
Application 2 (Section 2), Flocter WP 5 + Velum Prime SC 400 (applied in the the soil when the J2 population started to increase).
Control (Section 3), No application.

Conclusion

Importantly, no J2s were detected in the soil for 2 months after solarization completed. Furthermore, to control RKNs, soil solarization should be performed in the summer, providing a great opportunity to transplant seedlings into the soil confidently. The solarization effect can be further improved when combined with other management practices. Under these greenhouse conditions, the homozygous or heterozygous resistant tomato plants were not effective against virulent RKN. However, *B. firmus* + fluopyram applied both at planting and when the J2 population in the soil was increasing provided some control of *M. incognita*, with the latter more effective at reducing the *M. incognita* population at the end of the experiment. In future research on integrated management practices, there should be a focus on controlling *Mi-1*-virulent RKNs.

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