Original article (Orijinal araştırma)

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

Örtüaltı domates yetiştiriciliğinde Mi-1 virulent Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogyниdeae)’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: Meloidogyниdeae) 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 virulent kök-ur nematodlara karşı etkili değildir. Bu çalışmada, solarizasyon uygulaması sonrası serada yetiştirilen domatesler için Mi-1 virulent Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogyниdeae) popülasyonunu karşı Bacillus firmus Bredemann & Wermer, 1933 (Bacillales: Bacillaceae) + fluopyram kombinasyonları ile Mi-1 geninin doz etkinliği kombinasyonunun etkinliği değerlendirilmiştir. Çalışma 2015-2016 yılları arasında Antalya, Kepez bölgesindeki serada yürütülmüştür. Solarizasyon uygulaması sonrası toprakta iki ay boyunca M. incognita ikinci dönem larva tespit edilmemişştir. Çalışma 2015-2016 yılları arasında Antalya, Kepez bölgesindeki serada yürütülmüştür. Solarizasyon uygulaması sonrası toprakta iki ay boyunca M. incognita ikinci dönem larva tespit edilmemişştir. Sera koşullarında Mi-1 virulent 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 altında almıştır. Bu çalışma, toprak solarizasyonunun diğer mücadelde yöntemleriyle kombine edilebilir ve Mi-1 geninin doz etkinliğin virulent kök-ur nematodlara karşı önemli olmadığını göstermiştir. Ayrıca, B. firmus + fluopyram kombinasyonu, domates üretiminde kök-ur nematodların kontrolünde uygun bir mücadele aracı olarak kullanılabilme potansiyeline sahiptir. Bu bulgular, Mi-1 virulent kök-ur nematod popülasyonlarının kontrolü için entegre mücadele yöntemlerinin gelişmesine yardımcı edebilecektr.

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

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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: Pasteuriacaeae) 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 F1, Seval F1, and Browny F1 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       | Section 1|
| Band 2 | Tueza F1       |          |
| Band 3 | Browny F1      |          |
| Band 4 | Seval F1       |          |
| Band 5 | Tueza F1       | Section 2|
| Band 6 | Browny F1      |          |
| Band 7 | Seval F1       |          |
| Band 8 | Tueza F1       |          |
| Band 9 | Seval F1       |          |
| Band 10| Tueza F1       | Section 3|
| Band 11| Browny F1      |          |
| Band 12| Seval 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 F₁ | Seval F₁ | Tueza F₁ | Seval F₁ |
| 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).

![Figure 2. Population density curves of Mi-1 virulent Meloidogyne incognita J2s in Section 3.](image)

**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 collected 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* |
|-----------------|-----------|----------------|--------------------|--------------------|---------------------|
| 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               |                     |                     |
|                 | Application 2 | 339            | 669                | F = 4.55 p = 0.01  | 3 > 1 and 2         |
|                 | Control      | 4170           | 8532               |                     |                     |
| Browny F1       | Application 1 | 814            | 1442               |                     |                     |
|                 | Application 2 | 319            | 579                | F = 5.49 p = 0.01  | 3 > 1 and 2         |
|                 | 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 soil when the J2 population started to increase)
Control (Section 3): No application.

Figure 3. Population density curves of Mi-1 virulent M. incognita J2s in Section 2.
Integrated management of Mi-1 virulent Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) in greenhouse tomatoes

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 homoyzgous 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               |               |                 |
|          | Seval F1       | 1.15                | 1.89               | 0.09          | 0.91            |
|          | Browny F1      | 1.18                | 1.61               |               |                 |
| Harvest 2 | Tueza F1       | 1.88                | 2.39               |               |                 |
|          | Seval F1       | 1.86                | 2.43               | 0.05          | 0.95            |
|          | 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 fluopyram on various fungi has been assessed, including its ability to control Neocosmospora virguliformis (O’Donnell & T. Aoki) L. Lombard & Crous, 2015 (Hypocreales: Nectriaeae) 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 trials, 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.
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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 | | | |
| | Application 2 | 0.28 | 0.73 | 88.4 | 0.01 | 3 > 1 and 2 |
| | Control | 2.65 | 2.24 | | | |
| Harvest 2 | Application 1 | 1.33 | 1.87 | | | |
| | Application 2 | 0.59 | 1.02 | 82.2 | 0.01 | 3 > 1 > 2 |
| | 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 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 |
|-----------------|-------------|-----------------|--------------------|---------------|-----------------|---------------------|
| Tuezza F1       | Application 1 | 25.28 | 11.5 | | | |
| | Application 2 | 27.65 | 11.7 | 4.12 | 0.01 | 2 > 1 > 3 |
| | Control | 21.85 | 10.4 | | | |
| Seval F1        | Application 1 | 24.73 | 12.3 | | | |
| | Application 2 | 26.53 | 11.2 | 3.86 | 0.01 | 2 > 1 > 3 |
| | Control | 21.44 | 12.4 | | | |
| Browny F1       | Application 1 | 15.92 | 10.1 | | | |
| | Application 2 | 17.52 | 11.2 | 5.22 | 0.01 | 1 and 2 > 3 |
| | Control | 13.58 | 10.2 | | | |

Tuezza 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 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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References

Abad, P., B. Favery, M. N. Rosso & P. Castagnone-Sereno, 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Molecular Plant Pathology, 4 (4): 217-224.

Anastasiadis, I. A., I. O. Giannakou, D. A. Prophetou-Athanasiadou & S. R. Gowen, 2008. The combined effect of the application of a biocontrol agent Paecilomyces lilacinus, with various practices for the control of root-knot nematodes. Crop Protection, 27: 352-361.

Castagnone-Sereno, P., M. Bongiovanni & A. Dalmaso, 1994. Reproduction of virulent isolates of Meloidogyne incognita on susceptible and Mi-resistant tomato. Journal of Nematology, 26 (3): 324-328.

Collange, B., M. Navarrete, G. Peyre, T. Mateille & M. Tchamitchian, 2011. Root-knot nematode (Meloidogyne) management in vegetable crop production: the challenge of an agronomic system analysis. Crop Protection, 30 (10): 1251-1262.

Devran, Z., I. Mıstanoğlu & T. Özalp, 2017. Occurrence of mixed populations of root-knot nematodes in vegetable greenhouses in Turkey, as determined by PCR Screening. Journal of Plant Diseases Protection, 124: 617-630.

Devran, Z. & M. A. Söğüt, 2009. Distribution and identification of root-knot nematodes from Turkey. Journal of Nematology, 41 (2): 128-133.

Devran, Z. & M. A. Söğüt, 2010. Occurrence of virulent root-knot nematode populations on tomatoes bearing the Mi gene in protected vegetable-growing areas of Turkey. Phytoparasitica, 38: 245-251.

Faske, T. R. & K. Hurd, 2015. Sensitivity of Meloidogyne incognita and Rothylenchulus reniformis to Fluopyram. Journal of Nematology, 47 (4): 316-321.

Ferris, H. & J. W. Noling, 1987. “Analysis and Prediction as a Basis for Management Decisions, 49-81”. In: Principles and Practice of Nematode Control in Crops (Eds. R. H. Brown & B. R. Kerry). Academic Press, Sydney, New York, London, Montreal, Tokyo, 447 pp.

Garris, S., 2017. Velum prime technical presentation. (Web page: http://www.laca1.org/Presentations/2017/GeneralAndET/Emerging%20Technologies%20-%20Bayer.pdf) (Date accessed: December 2018).

Giannakou, I. O., I. A. Anastasiadis, S. R. Gowen & D. A. Prophetou-Athanasiadou, 2007. Effects of a non-chemical nematicide combined with soil solarization for the control of root-knot nematodes. Crop Protection, 26: 1644-1654.

Giannakou, I. O., D. G. Karpouzas & D. Prophetou-Athanasiadou, 2004. A novel non-chemical nematicide for the control of root-knot nematodes. Applied Soil Ecology, 26: 69-79.

Greco, N., 1999. Alternatives to methyl bromide to control plant parasitic nematodes in greenhouses. (Web page: http://www.minagric.gr/ greek/data/files2251/greco1.doc) (Date accessed: 10 February 2018).

Hallman, J., K. G. Davies & R. Sikora, 2009. “Biological Control Using Microbial Pathogens, Endophytes and Antagonists, 380-411”. In: Root-Knot Nematodes (Eds. R. N. Perry, M. Moens & J. L. Starr). CABl, London, 488 pp.
Integrated management of Mi-1 virulent *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) in greenhouse tomatoes

Hartman, K. M., J. N. Sasser, 1985. “Identification of *Meloidogyne* species on the Basis of Different Host Test and Perineal Pattern Morphology, 69-77”. In: An Advanced Treatise on *Meloidogyne*, Vol. 2: Methodology (Eds. K. R. Barker, C. C. Carter & J. N. Sasser). North Carolina State University Graphics, Raleigh, 223 pp.

Heiken, J. A., 2017. The Effects of Fluopyram on Nematodes. North Carolina State University, (Unpublished) MSc Thesis, Raleigh, North Carolina, USA, 75 pp.

Hooper, D. J., 1986. “Handling, Fixing, Staining and Mounting Nematodes, 58-80”. In: Laboratory Methods for Work with Plant and Soil Nematodes (Ed. J. F. Southey). Her Majesty's Stationery Office, London, 202 pp.

Iberkleid, I., R. Ozalvo, L. Feldman, M. Elbaz, B. Patricia & S. B. Horowitz, 2014. Responses of tomato genotypes to avirulent and Mi-virulent *Meloidogyne javanica* isolates occurring in Israel. Phytopathology, 104: 484-496.

Jacquet, M., M. Bongiovanni, M. Martinez, P. Verschave, E. Wajnberg & P. Castagnone, 2005. Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the Mi gene. Plant Pathology, 54: 93-99.

Karssen, G. & M. Moens, 2006. “Root-Knot Nematodes, 59-90”. In: Plant Nematology (Eds. R. N. Perry & M. Moens). CABI, London, 568 pp.

Katan, J., 1996. “Soil Solarization: Integrated Control Aspect, 250-278”. In: Principles and Practice of Managing Soilborne Plant Pathogens (Eds. R. Hall). APS Press, St. Paul, 330 pp.

Keren-Zur, M., J. Antonov, A. Bercovitz, K. Feldman, A. Husid, G. Kenan, N. Markov & M. Rehun, 2000. *Bacillus fiacil* formulations for the safe control of root-knot nematodes. (Web page: http://www.cabdirect.org/cabdirect/abstract/200003031309) (Date accessed: 10 February 2019).

Lawrence, K., P. Huang, G. Lawrence, T. Faske, C. Overstreet, T. Wheeler, H. Young, R. Kemarait & H. Mehl, 2015. Beltwide nematode research and education committee 2014 nematode research report cotton varietal and nematicide responses in nematode soils. (Web page: http://www.cotton.org/beltwide/proceedings/2005-2018/data/conferences/2015/papers/15955.pdf) (Date accessed: 10 February 2019).

Mendoza, A. R., S. Kiewnick & R. A. Sikora, 2008. In vitro activity of *Bacillus firmus* against the burrowing nematode *Radopholus similis*, the root-knot nematode *Meloidogyne incognita* and the stem nematode *Ditylenchus dipsaci*. Biocontrol Science and Technology, 18: 377-389.

Mistanoğlu, I., T. Özalp & Z. Devran, 2016. Response of tomato seedlings with different number of true leaves to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949. Turkish Journal of Entomology, 40 (4): 377-383.

Ornat, C., S. Verdejo-Lucas & F. J. Sorribas, 2001. A population of *Meloidogyne javanica* from Spain virulent to the Mi resistance gene in tomato. Plant Disease, 85: 271-276.

Randig, O., M. Bongiovanni, R. M. Carneiro & P. Castagnone-Sereno, 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR marker specific for the coffee damaging species. Genome, 45 (5): 862-870.

Roberts, P. A., 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. Annual Review Phytopathology, 33: 199-221.

Roberts, P. A. & I. J. Thomason, 1986. Variability in reproduction of isolates of *Meloidogyne incognita* and *M. javanica* on resistant tomato genotypes. Plant Diseases, 70: 547-551.

Schoemaker, C. H. & T. H. Been, 2006. “Plant Growth and Population Dynamics, 275-301”. In: Plant Nematology (Eds. R. N. Perry & M. Moens). CABI, London, 568 pp.

Smith, P. G., 1944. Embryo culture of a tomato species hybrid. Proceeding of American Society for Horticultural Science, 44: 413-416.

Souza, N. L., 1994. Solarizacao do solo. Summa Phytopathologica, 20: 3-15.
Terefe, M., T. Tefera & P. K. Sakhuja, 2009. Effect of a formulation of *Bacillus firmus* on root-knot nematode *Meloidogyne incognita* infestation and the growth of tomato plants in the greenhouse and nursery. Journal of Invertebrate Pathology, 100: 94-99.

Terefe, M., T. Tefera & P. K. Sakhuja, 2012. Biocontrol (formulation of *Bacillus firmus* (BioNem)) of root-knot nematode, *Meloidogyne incognita* on tomato plants in the field. Ethiopian Journal Agricultural Sciences, 22 (1): 102-116.

TUİK, 2015. Production quantities and cultivation of tomatoes and in Turkey. (Web page: http://www.biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul) (Date accessed: 15 November 2018).

Tzortzakakis, E. A., M. A. M. Adam, V. C. Blok, C. Paraskevopoulos & K. Bourtzis, 2005. Occurrence of resistance-breaking populations of root-knot nematodes on tomato in Greece. European Journal Plant Pathology, 113: 101-105.

Tzortzakakis, E. A., D. L. Trudgill & M. S. Phillips, 1998. Evidence for a dosage effect of the *Mi* gene on partially virulent isolates of *Meloidogyne javanica*. Journal of Nematology, 30 (1): 76-80.

Verdejo-Lucas, S., L. Cortada, F. J. Sorribas & C. Ornat, 2009. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of *Mi* resistance gene tomato rootstocks under field conditions. Plant Pathology, 58: 990-998.

Verdejo-Lucas, S., M. Talavera & M. F. Andrés, 2012. Virulence response to the *Mi.1* gene of *Meloidogyne* populations from tomato in greenhouses. Crop Protection, 39: 97-105.

Walia, R. K., S. B. Sharma & R. Vats, 2000. “Bacterial Antagonists of Phytonematodes, 173-186”. In: Biocontrol Potential, and Its Exploitation in Sustainable Agriculture: Crop Diseases, Weeds and, Nematodes, Vol. I. (Eds. R. R. Upadhyay, K. G. Mukerji & B. P. Chamola). Kluwer Academy Plenum, New York, 294 pp.

Walia, R. K. & R. Vats, 2000. “Fungal Antagonists of Phytonematodes, 155-172”. In: Biocontrol Potential, and Its Exploitation in Sustainable Agriculture: Crop Diseases, Weeds and, Nematodes, Vol. I. (Eds. R. R. Upadhyay, K. G. Mukerji & B. P. Chamola). Kluwer Academy Plenum, New York, 294 pp.

Wang, J., C. A. Bradley, O. Stenzel, D. K. Pedersen, U. Reuter-Carlson & M. I. Chilvers, 2017. Baseline sensitivity of *Fusarium virguliforme* to fluopyram fungicide. Plant Diseases, 101 (4): 576-582.

Wesemael, W. M. L., N. Viaene & M. Moens, 2011. Root-knot nematodes (*Meloidogyne* spp.) in Europe. Nematology, 13 (1): 3-16.

Zuck, W. M., 1971. A Rating scheme for field evaluation of root-knot nematode infestation. Pflanzenschutz Nachrichten, 10: 141-144.

Zijlstra, C., D. T. H. M. Donkers-Venne & M. Fargette, 2000. Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. Nematology, 2 (8): 847-853.