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Original article (Orijinal araştırma)

Interaction of *Meloidogyne incognita* (Kofoid & White, 1919) (Nemata: Meloidogynidae) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker in tomato F1 hybrids with differing levels of resistance to these pathogens

Meloidogyne incognita (Kofoid & White, 1919) (Nemata: Meloidogynidae) ve *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker'ya karşı farklı seviyelerde dayanıklılık sağlayan domates hibritlerinde bu patojenlerin etkileşimi

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Abstract

The interaction of *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker (FORL) on nematode reproduction and wilt severity was investigated in tomato hybrids in this study. The study included with five combinations of individual, simultaneous and sequential inoculations of *M. incognita* and FORL to tomato F1 hybrids Adel, Alberty, Armstrong, Body, Gülizar and Kaplan in January-May 2021 under controlled conditions. The experiment was completed after 60 days. Simultaneous inoculation increased *M. incognita* galls and egg masses in Adel, Armstrong, Body, and Gülizar. The highest gall and egg mass numbers occurred with FORL inoculation 10 days after *M. incognita* inoculation (N+10 FORL) in Alberty and Kaplan. The highest disease incidence occurred in all tomato hybrids at the application of N+10 FORL and was followed by Gülizar, Kaplan, Body, Alberty, Armstrong and Adel. *Meloidogyne incognita* showed high reproductive rates in Alberty and Body, and FORL resistance was overcome with treatment N+10 FORL. *Meloidogyne incognita* was unable to reproduce in Adel and Armstrong and thus no disease was seen. The results indicated that the development of root-knot nematodes is a significant factor affecting the durability of FORL resistance.

Keywords: FORL, interaction, resistance, root-knot nematode, tomato

Öz

Bu çalışmada, *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) ve *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker (FORL) etkileşiminin, nematod üremesi ve solgunluk şiddeti üzerine etkisi hibrit domates çeşitlerinde araştırılmıştır. Çalışma, kontrollü koşullar altında 2021 yılı Ocak-Mayıs ayları arasında Adel, Alberty, Armstrong, Body, Gülizar ve Kaplan F1 domates hibrit çeşitlerinde, *M. incognita* ve FORL'nin bireysel, eş zamanlı ve sıralı inokulasyonlarından oluşan beş kombinasyonu içermektedir. Deneme 60 gün sonra sonlandırılmıştır. Eş zamanlı inokulasyon Adel, Armstrong, Body ve Gülizar domates çeşitlerinde *M. incognita* gal ve yumurta paketi sayısını artırmıştır. Alberty ve Kaplan domates çeşitlerinde ise en yüksek gal ve yumurta paketi sayısı *M. incognita* inokulasyonundan 10 gün sonra FORL inokulasyonunda (N+10FORL) tespit edilmiştir. En yüksek hastalık şiddeti tüm domates hibritlerinde N+10FORL uygulamasında meydana gelmiş ve Gülizar, Kaplan, Body, Alberty, Armstrong ve Adel şeklinde izlemiştir. *Meloidogyne incognita* Alberty ve Body çeşitlerinde yüksek üreme göstermiş ve N+10FORL uygulaması ile FORL dayanımı kırılmıştır. *Meloidogyne incognita* Adel ve Armstrong domates hibritlerinde üreyememiş ve bu nedenle hastalık görülmemiştir. Sonuçlar, kök-ur nematodlarının gelişiminin FORL dayanımının sürekliliğini etkileyen önemli bir faktör olduğunu göstermiştir.

Anahtar sözcükler: FORL, interaksiyon, dayanıklılık, kök ur nematodu, domates

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Introduction

Root-knot nematodes (RKNs), one of the most important plant parasitic nematode groups, cause heavy economic losses worldwide (Bird et al., 2009). The galls formed by RKNs limit the intake of water and nutrients from the soil. In high population densities, it causes the plants to dry out completely. Also, wounds inflicted on the roots by RKNs facilitate the entry of soilborne fungal and bacterial pathogens (Back et al., 2002). *Meloidogyne arenaria* (Neal, 1889), *Meloidogyne incognita* (Kofoid & White, 1919), and *Meloidogyne javanica* (Treub, 1885) (Rhabditida: Meloidogynidae) have been identified as most common and economically important RKN species in vegetable growing areas of Turkey (Elekcioglu et al., 1994; Kaşkavalcı & Öncüer, 1999; Devran & Söğüt, 2009; Özarslandan & Elekcioğlu, 2010; Cetintaş & Cakmak, 2016; Özarslandan, 2016; Uysal et al., 2017; Gürkan et al., 2019). *Meloidogyne incognita* is the most common RKN species and can infest almost all plants and causes significant economic damage (Sasser & Freckman, 1987). The most common method used for controlling RKNs is genetic resistance (Gilbert & McGuire, 1956; Jacquet et al., 2005; Lobna et al., 2016). *Mi* gene provides high resistance to *M. arenaria, M. incognita* and *M. javanica* (Roberts & Thomason, 1986; Verdejo-Lucas et al., 2009; Devran & Söğüt, 2011). However, *Mi* virulent *M. arenaria*, *M. incognita* and *M. javanica* populations that overcome this resistance have also been reported in many countries (Ornat et al., 2001; Tzortzakakis et al., 2005; Devran & Söğüt, 2010; Aydınlı & Mennan, 2019).

Fusarium oxysporum Schltdl. is one of the most widespread soilborne pathogens of tomato plants and has two forms: *Fusarium oxysporum* f. sp. *lycopersici* W.C. Snyder & H.N. Hansen (FOL) and *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker (FORL). While FOL causes Fusarium wilt, FORL causes Fusarium root and root rot (Attitalla et al., 2004). FORL, which causes tomato root rot, is an important pathogen species and causes more than 60% yield loss in open field and greenhouse tomato cultivation (Ozbay & Newman, 2004; Hibar et al., 2007; Manzo et al., 2016). FORL was first identified by Can et al. (2004) in 2004 in Turkey and since then it has started to spread in tomato growing areas (Erol & Tunalı, 2007; Yücel et al., 2008). Although various methods have been used to control this pathogen, the use of resistant hybrids is the most preferred and economic control method (Szczechura et al., 2013). The single genetic locus *Frl*, the gene expressing resistance to FORL in the tomato plant, was integrated into *Solanum lycopersicum* L. (Solanaecae) cultured from the wild *Solanum peruvianum* L. (Laterrot & Moretti, 1991; Fazio et al., 1999) and has been used in commercial production (Devran et al., 2018).

In previous studies on nematode and fungal pathogen interactions, early infestion of RKN was associated with increased severity of the disease observed (Lobna et al., 2016, 2017). The interaction between the RKN and *F. oxysporum* has been observed in plants such as bananas, beans, cotton, gerbera and grapes (Harris & Ferris, 1991; France & Abawi, 1994; Jonathan & Gajendran, 1998; Jeffers & Roberts, 2003; Meena et al., 2015). Simultaneous infection of both RKN and FORL causes greater and enhanced damage to the host plant compared to the pathogens alone (El-Sherif & Elwakil, 1991; McGawely, 2001; Hajji-Hedfi et al., 2018). It has been reported that the *M. javanica* and FORL interaction in FORL sensitive and resistant species can affect tomato growth and disease severity in different ways (Hajji-Hedfi et al., 2018). Since RKN can form disease complexes with *Fusarium* spp., the use of resistant hybrids is not suitable for the Fusarium wilt disease control. In general, *Meloidogyne* spp. overcome wilt resistance in the host plant (Morrell & Bloom, 1981; Fattah & Webster, 1983; Lobna et al., 2016). Çolak-Ates et al. (2018) found that AL-4, AL-9 and AL-21 tomato genotypes with FORL resistance, lost their resistance to FORL in simultaneous and sequential inoculations with *M. incognita*.

There are only a few studies on the interaction of RKNs and FORL in tomato plants and most of these studies have been conducted with *M. javanica*. A few studies have investigated the *M. incognita* interaction, which is the most widespread species in the world and in Turkey. In this study, the aim was to evaluate the interaction of *M. incognita* and FORL on reproduction of *M. incognita* and severity of Fusarium wilt diseases using resistant and susceptible tomato hybrids.

Materials and Method

Materials

The FORL isolate used in this study was obtained from a tomato plant in the Serik District of Antalya Province, and its morphologically identification was made in accordance with Gerlach et al. (1982) and Davis & Raid (2002). *Meloidogyne incognita* DR17 isolate, obtained in a previous study (N: 37°47'44" N, 30°30'47" E), was mass produced on tomato F1 hybrid Tueza under controlled climate conditions (24 ± 1°C, 60 ± 5% RH) (Uysal et al., 2017). In the study, six tomato F1 hybrids (Adel, Alberty, Armstrong, Body, Gülizar and Kaplan) that have resistance to RKNs and FORL were used (Table 1).

Table 1. Resistance of tomato hybrids to *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*

Tomato F1 hybrids	Nematode	Fungal resistance*
Adel	HR	HR
Alberty	X	HR
Armstrong	IR	HR
Body	IR	IR
Gülizar	X	X
Kaplan	IR	X

*HR, high/standard resistance; IR, moderate/intermediate resistance; and X, unknown

Method

Preparation of fungal inoculum

FORL isolate was incubated in sterile Petri dishes at 25°C for 7 days on potato dextrose agar medium. Then, 1 cm^2 pieces of the fungus colonies were taken and five pieces were added to 250 ml sterile flasks with 50 ml potato dextrose broth. The flasks were incubated in the dark at 25°C for 7 days in the laboratory and shaken manually on a daily basis. Seven days later, the culture filtrate was passed through two layers of filter paper (Whatman No. 1) to remove fungal spores and mycelium. Using a hemacytometer in the light microscope, each plant was adjusted to 3×10^6 ml suspension and kept at 4°C until the experiment was set up (Lobna et al., 2016).

Preparation of nematode inoculum

Mass production of *M. incognita* was done on Tueza with 20 replicates under climate room conditions (24 ± 1°C, 60 ± 5% RH). Tomato seedlings were transplanted into pots containing sterilized soil (68% sand, 21% silt and 11% clay) and about 1000 second-stage juveniles (J2s) inoculated into the soil. The tomato roots were removed 8 weeks after inoculation and were washed in tap water. Then the egg masses were removed from the roots under a stereomicroscope. Collected egg masses were incubated in water at 25 ± 2ºC for 3 days in a Petri dish containing a sterile sieve (3 cm diameter). The J2s hatched after 3 days were counted under the light microscope and placed in 1 ml tubes, adjusted to 1000 J2s to be used in the experiment (Lobna et al., 2017).

Fusarium oxysporum f. sp. *radicis-lycopersici* and *Meloidogyne incognita* interaction in tomato hybrids with different levels of resistance to these pathogens

The study was conducted between January and May 2021. There were five treatment combinations consisting of individual, simultaneous and sequential inoculations of *M. incognita* and FORL on tomato hybrids with different resistance levels to RKNs and FORL. Treatments were consisted of (1) *M. incognita* only (N); (2) FORL only; (3) Simultaneous inoculation of *M. incognita* and FORL (N+FORL); (4) first inoculation of FORL and 10 days later inoculation of *M. incognita* (FORL+10N); and (5) first inoculation of *M. incognita* and 10 days later FORL (N+10FORL). The study was conducted in a climate room under controlled conditions (24 ± 1°C, 60 ± 5% RH) in plastic pots and in a randomized block design with five

replicates. Three-week-old tomato seedlings were transplanted into 14-cm plastic pots containing approximately 1500 g of sterile soil (68% sand, 21% silt and 11% clay). As initial inoculum density, 1000 J2s/1 ml *M. incognita* and 3×10^6/10 ml FORL/seedling were used. Inoculations were made according to the treatment priority. The nematode inoculum was evenly distributed by a pipette into three small 2-3-cm holes drilled in the soil around the seedling stem and deep enough to contact the roots. Fungi inoculation was poured into these holes opened on the soil surface of the pots using a graduated cylinder (Lobna et al., 2016, 2017).

The study was completed 60 days after the fungi and nematode inoculation of the plants. At the end of the treatment, the tomato roots were washed carefully under tap water and then exposed to 0.25% triptan blue for 3 min (Sharma & Ashokkumar, 1991). Then, the gall formations and egg masses were counted under a stereomicroscope. The J2 density in soil (using a 100 g sample) was calculated using the Baermann funnel technique (Hooper, 1986). The severity of disease caused by FORL was scored on the 0 to 4 scale of Chandler & Santelman (1968): 0, no damage to the seedling (resistant); 1, discoloration and small lesions at the junction of the seedling with the soil surface; 2, larger lesions bending the stem (sensitive); 3, large lesions surrounding the stem, resulting in a concave appearance (vulnerable); and 4, dead plant due to fungal damage (very vulnerable) (Erol & Tunalı, 2007).

Statistical analysis

SPSS (version 20.0) program was used for statistical analysis of the data and one-way analysis of variance was performed to test the differences between the means. Tukey's test was used to determine the means of different groups when variances were homogeneous ($P \leq 0.05$).

Results and Discussion

The results showed that differences in treatment time with *M. incognita* and FORL in tomato hybrids were affected the number of galls and egg masses. The lowest gall formation was detected in the Armstrong with treatment FORL+10N (5 per pot) and 22 per pot with treatment N+10 FORL. Although the number of galls was higher with treatment N+FORL in Armstrong than with treatment N, there was no significant difference between groups (Table 2). There was no significant difference between treatments N (117 per pot), N+FORL (126 per pot) and N+10FORL (143 per pot) in terms of gall number in Alberty. The lowest gall number in Alberty was found with treatment FORL+10N (69 per pot). Although the number of galls with treatment N+FORL (42 per pot) in Body, which is resistant to RKNs and FORL, was higher than with treatment N (36 per pot), there was no significant difference. However, gall number with treatments FORL+10N (18 per pot) and N+10FORL (21 per pot) in Body were lower than these treatments. Although the number of galls with treatment N+10FORL (41 per pot) in Kaplan, which is tolerant to RKN, was higher with treatment N (34 per pot), there was no significant difference between them. The lowest gall number was in Kaplan with treatment FORL+10N (18 per pot). Adel, which is resistant to RKNs and FORL, had the highest gall with treatments N (26 per pot) and N+FORL (32 per pot) and the lowest with treatments FORL+10N (7 per pot) and N+10FORL (10 per pot). The highest gall number in the Gülizar, which is susceptible to RKNs and FORL, was found with treatment N+FORL (166 per pot) and the lowest with treatment FORL+10N (86 per pot). Also, the lowest gall number was determined with treatment FORL+10N in all tomato hybrids. With *M. incognita* infection most gall formation occurred in susceptible Gülizar and Alberty. The gall numbers of *M. incognita* were similar in Adel, Armstrong, Body and Kaplan with RKN resistance.

Table 2. Effect of sequentially and concomitantly inoculation of *Meloidogyne incognita* (N) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) on number of galls per pot (mean ± SE[1]) of resistant and susceptible tomato hybrids

Treatment[2]	Adel		Alberty		Armstrong		Body		Gülizar		Kaplan	
N	26 ± 1.4	a B	117 ± 10.5	a A	29 ± 3.0	ab B	36 ± 5.1	ab B	137 ± 4.9	b A	34 ± 2.6	ab B
N+FORL	32 ± 2.4	a C	126 ± 6.7	a B	35 ± 2.8	a C	42 ± 3.1	a C	166 ± 4.5	a A	27 ± 3.5	b C
FORL+10N	7 ± 1.2	b C	68 ± 7.7	b A	5 ± 1.2	c BC	18 ± 3.2	c BC	86 ± 3.7	c A	18± 3.2	c B
N+10FORL	10 ± 1.0	b C	143 ± 13.0	a A	22 ± 4.6	b BC	21 ± 4.6	bc BC	140 ± 6.0	b A	41 ± 4.1	a B

[1] Means followed by the same lowercase letter within columns or uppercase letter within rows are not significantly different ($p \leq 0.05$).
[2] Treatments: N, *M. incognita* only; N+FORL, simultaneous inoculation of *M. incognita* and FORL; FORL+10N, first inoculation with FORL and 10 days later inoculation with *M. incognita;* and N+10FORL, first inoculation with *M. incognita* and 10 days later with FORL.

The lowest number of egg masses in Armstrong was with treatment FORL+10N (6 per pot) and the highest with treatments N+FORL (40 per pot) and N (32 per pot) (Table 3). The number of egg masses with treatment FORL+10N (76 per pot) in Alberty was lower than with treatments N (129 per pot), N+FORL (136 per pot) and N+10FORL (152 per pot) (Table 3). There was no significant difference between treatments N (38 per pot), FORL+10N (21 per pot) and N+10FORL (25 per pot) in egg mass numbers in Body. In Kaplan, significant difference was found only between treatments N+10FORL (43 per pot) and FORL+10N (28 per pot) in egg mass numbers. The highest number of egg masses in Adel was with treatments N (30 per pot) and N+FORL (34 per pot) and the lowest with treatments FORL+10N (8 per pot) and N+10FORL (12 per pot). The highest number of egg mass in Gülizar was with treatment N+FORL (182 per pot) and the lowest with treatment FORL+10N (91 per pot). The number of egg masses parallel to the gall numbers in Gülizar and Alberty, which were most susceptible to RKN. The number of egg masses in Adel, Armstrong, Body and Kaplan, which have resistance and tolerance to RKN, were similar with treatment N.

Table 3. Effect of sequentially and concomitantly inoculation by *Meloidogyne incognita* (N) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) on number of egg masses per pot (mean ± SE[1]) of resistant and susceptible tomato hybrids

Treatment[2]	Adel		Alberty		Armstrong		Body		Gülizar		Kaplan	
N	30 ± 1.6	a B	129 ± 10.6	a A	32 ± 2.8	ab B	38 ± 5.7	ab B	150 ± 4.5	b A	37 ± 2.8	ab B
N+FORL	34 ± 3.0	a C	136 ± 6.4	a B	40 ± 2.2	a C	49 ± 4.5	a C	182 ± 4.8	a A	36 ± 3.5	ab C
FORL+10N	8 ± 1.4	b C	76 ± 8.4	b A	6 ± 1.6	c C	21 ± 3.1	b BC	91 ± 3.3	c A	28 ± 2.3	b B
N+10FORL	12 ± 1.8	b C	152 ± 11.7	a A	25 ± 5.7	b BC	25 ± 5.7	b BC	144 ± 5.9	b A	43 ± 3.5	a B

[1] Means followed by the same lowercase letter within columns or uppercase letter within rows are not significantly different ($p \leq 0.05$).
[2] Treatments: N, *M. incognita* only; N+FORL, simultaneous inoculation of *M. incognita* and FORL; FORL+10N, first inoculation with FORL and 10 days later inoculation with *M. incognita;* and N+10FORL, first inoculation with *M. incognita* and 10 days later with FORL.

The lowest J2 density with Armstrong was with treatment FORL+10N (31 per kg soil) and the highest with treatment N+FORL (95 per kg soil) (Table 4). The highest J2 density with Alberty was with treatment N+10FORL (210 per kg soil) and the lowest with treatment FORL+10N (130 per kg soil). The J2 density with Body was 23 per kg soil with treatment FORL+10N, lower than with treatment N+10FORL (48 per kg soil). There was no significant difference in J2 density treatments N (70 per kg soil) and N+FORL (71 per kg soil) with Body. The J2 density was highest with treatments N+10FORL (116 per kg soil) and N (100 per kg soil), and lowest with treatment FORL+10N (51 per kg soil) in the Kaplan. The J2 density with Adel with treatment FORL+10N was 20 per kg soil, lower than with treatment N+10FORL (50 per kg soil). There was no significant difference between treatments N (68 per kg soil) and N+FORL (76 per kg soil) in J2 density in Adel. The highest J2 density in the soil was with Gülizar, which is the most susceptible to RKN, followed by the RKN susceptible Alberty. In Gülizar, there was no significant difference between J2 densities with

treatments N (368 per kg soil), N+FORL (383 per kg soil) and N+10FORL (354 per kg soil) but these treatments gave densities than treatment FORL+10N (304 per kg soil). In the Adel, Armstrong, Body and Kaplan, with RKN resistance, the J2 densities were similar with treatment N.

Table 4. Effect of sequentially and concomitantly inoculation of *Meloidogyne incognita* (N) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) on second-stage juvenile density in soil (per kg, mean ± SE[1]) of resistant and susceptible tomato hybrids

Treatment[2]	Adel		Alberty		Armstrong		Body		Gülizar		Kaplan	
N	68 ± 6.6	a C	162 ± 9.0	bc B	74 ± 7.5	ab C	70 ± 6.9	a C	368 ± 5.9	ab A	100 ± 6.9	ab C
N+FORL	76 ± 4.3	a C	180 ± 7.2	ab B	95 ± 3.8	a C	71 ± 5.5	a C	383 ± 10.9	a A	81 ± 6.2	bc C
FORL+10N	20 ± 3.8	c D	130 ± 9.7	c B	31 ± 5.4	c CD	23 ± 4.2	c C	304 ± 22.9	b A	51 ± 9.0	c BC
N+10FORL	50 ± 3.7	b C	210 ± 13.9	a B	52 ± 6.1	bc C	48 ± 4.9	b C	354 ± 20.4	ab A	116 ± 12.0	a BC

[1] Means followed by the same lowercase letter within columns or uppercase letter within rows are not significantly different ($p \leq 0.05$).
[2] Treatments: N, *M. incognita* only; N+FORL, simultaneous inoculation of *M. incognita* and FORL; FORL+10N, first inoculation with FORL and 10 days later inoculation with *M. incognita;* and N+10FORL, first inoculation with *M. incognita* and 10 days later with FORL.

The disease severity in Armstrong, which is resistant to RKNs and FORL, was scored a 1.0 with treatments N+FORL and N+10FORL, which was higher than with treatments FORL (score 0.4) and FORL+10N (score 0.2) (Table 5). This shows that *M. incognita* contributed to the increase in disease severity in the Armstrong, however, the FORL resistance was not overcome. With Alberty which is only resistant to FORL only, Body, which is resistant to RKN and FORL, the disease severity was lower with treatments FORL and FORL+10N than with treatments N+FORL and N+10FORL. As disease severity was high with treatment FORL+10N, this shows that *M. incognita* enhances the fungal disease severity. In these two hybrids, especially with treatment N+10FORL, it was observed that the plant was not resistant to FORL and becomes susceptible. In Kaplan, which is susceptible to FORL but resistant to RKN, the lowest disease severity was with treatment FORL+10N (score 1.8) and the highest with treatment N+10FORL (score 4.0). The disease severity in the Adel, with RKN and FORL resistance, was low, and no significant difference was found between these treatments. It was found that *M. incognita* did not enhance the disease severity and FORL resistance was not overcome in Adel. There was no significant difference between disease severity in Gülizar, which is susceptible to RKNs and FORL. With treatment FORL, the highest disease severity among tomato hybrids was in Gülizar (score 3.4) without FORL resistance, followed by FORL-resistant Body (score 1.2) and FORL-susceptible Kaplan (score 2.0). With treatment N+10FORL, the disease severity was ranked highest to lowest as Gülizar > Kaplan > Body > Alberty > Armstrong > Adel.

Table 5. Effect of sequentially and concomitantly inoculation by *Meloidogyne incognita* (N) and *F*usarium *oxysporum* f. sp. *radicis-lycopersici* (FORL) on disease severity scores (mean ± SE[1]) of resistant and susceptible tomato hybrids

Treatment[2]	Adel		Alberty		Armstrong		Body		Gülizar		Kaplan	
FORL	0.4 ± 0.2	a C	0.6 ± 0.2	b C	0.4 ± 0.2	b C	1.2 ± 0.2	b BC	3.4 ± 0.2	a A	2.0 ± 0.3	b B
N+FORL	0.6 ± 0.2	a D	1.4 ± 0.2	ab CD	1.0 ± 0.0	a CD	1.6 ± 0.2	ab BC	3.6 ± 0.2	a A	2.4 ± 0.2	b B
FORL+10N	0.4 ± 0.2	a C	0.8 ± 0.2	b BC	0.2 ± 0.2	b C	1.2 ± 0.2	b BC	3.6 ± 0.2	a A	1.8 ± 0.3	c B
N+10FORL	0.6 ± 0.2	a C	2.2 ± 0.2	a B	1.0 ± 0.0	a C	2.4 ± 0.2	a B	4.0 ± 0.0	a A	4.0 ± 0.0	a A

[1] Means followed by the same lowercase letter within columns or uppercase letter within rows are not significantly different ($p \leq 0.05$).
[2] Treatments: N, *M. incognita* only; N+FORL, simultaneous inoculation of *M. incognita* and FORL; FORL+10N, first inoculation with FORL and 10 days later inoculation with *M. incognita;* and N+10FORL, first inoculation with *M. incognita* and 10 days later with FORL.

In the present study, tomato hybrids were found to be affected by a *M. incognita* and FORL interaction. Although the nematode density changed with treatment in Adel, Armstrong, Body, and Kaplan, resistance to RKN remained evident. Treatment time differences for *M. incognita* affected the response of

the tomato hybrids in the number of galls, egg masses and J2 density. Simultaneous inoculation enhanced the number of galls and egg masses in Adel, Armstrong, Body and Gülizar. The highest number of galls and egg masses in Alberty and Kaplan were with treatment N+10FORL. Treatments N+FORL and N+10FORL increased the number of galls, egg masses and J2 density. These data show that FORL has a positive influence on nematode reproduction. In some studies, it has been reported that nematode penetration was enhanced by formation of fungal pathogen enzymes in the roots (Edmunds & Mai, 1966a, 1966b, 1967; Nordmeyer & Sikora, 1983). Although root lesion nematodes have a different trophic behavior to RKNs, there are studies that show interaction between these nematodes and plant pathogenic fungi increases the nematode density (Vrain, 1987; Hasan, 1988). Also, the lowest number of galls and egg masses in all tomato hybrids in the present study was with treatment FORL+10N because of the root rot caused by FORL affected the nematode feeding process in the root tissues and then negatively affected nematode reproduction. Either the existence of a fungal hyphae prevents nematode penetration or invasion sites the nematode chooses to feed may cause a decrease in nematode density (Davide & Triantaphyllou, 1967; Mokbel et al., 2007). Additionally, *Fusarium* species secrete toxic compounds against plant parasitic nematodes and these compounds affect hatching, viability and juvenile movement (Nitao et al., 1999; 2001). In order to develop and deposit eggs of RKNs, they must provide their nutritional needs from giant cells. If *Fusarium spp*. colonizes these feeding cells and depletes their nutrient content, the female nematode can die without depositing eggs (Nordmeyer & Sikora, 1983). It has been reported that the reproduction of *M. incognita* and galling in the roots of blackgram plants (*Vigna mungo* L.) are significantly reduced in the presence of *F. oxysporum* (Mahapatra & Swain, 2001). Akram & Khan (2006) found that gall formation, egg mass production and soil population of *M. incognita* were negatively affected by FOL in greenhouse tomato plants. In this study, the change in the interaction of nematode and fungus according to the treatment time shows that the pathogen enters the plant first is important in the development of other pathogens. Lobna et al. (2016), in their study with *M. javanica* and FOL in tomato, found that disease severity depends on nematode population and inoculation time. Ramalingam (2019) found that when *M. incognita* is applied to tomato plants before FOL inoculation, wilt intensity is highest, followed by simultaneous inoculation, and the lowest wilt was with FOL application before nematode inoculation. Göze Özdemir (2020), in her research on *Pratylenchus thornei* Sher & Allen, 1953, *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans-Stekhoven, 1941 and *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941, and with *F. culmorum* (WG Smith) Sacc. under controlled conditions, determined that the inoculation time of *F. culmorum* is important and that the pathogen that enters the wheat first negatively affects the development of the other pathogen. These results also confirm the findings of the present study.

Nematode development affecting FORL resistance of tomatoes is one of the important findings of the present study. Although there were differences between the treatments on Adel and Armstrong with RKN and FORL resistance, J2 density, galls and egg masses, and disease severity were found to be low. The nematode in Alberty caused increased gall formation, egg masses and J2 density, and the disease severity increased. With treatment N+10FORL, the highest number of galls and egg masses, and J2 density was with Alberty. In parallel, the highest disease severity was with treatment N+10FORL and FORL resistance of the plant was overcome. Although the RKN and FORL-tolerant Body developed less *M. incognita* than Alberty, disease severity was found to be similar. Although *M. incognita* developed well in Body, the highest disease severity with treatment N+10FORL and FORL resistance was found to be overcome. In Kaplan, which is sensitive to FORL but tolerant to RKN, disease severity was higher than with treatmentsFORL in N+FORL and N+10FORL. In Kaplan, nematode development was higher than with other treatments, especially with N+10FORL, and the disease severity was the highest with this treatment. Compared to Gülizar, Kaplan appeared to remain nematode resistant, and this resulted in lower disease severity in other treatments, except for N+10FORL. Gülizar, which is sensitive to RKN and FORL, had the highest number of galls and egg massed, and J2 density, and disease severity among all tomato hybrids. Also, the disease severity was found to be similarly high in all treatments in Gülizar. Co-infection of the two

pathogens was determined to increase the severity of Fusarium wilt susceptibly. RKN resistance was found to be important for ensuring the durability of FORL resistance. Resistance of the fungus was overcome with treatment N+10FORL, with the highest disease. This may be due to the wounds caused by RKNs in the roots, or by physiological and biochemical changes in the host cells (Moussa & Hague, 1988; Khan & Hosseini-Nejad, 1991; Marley & Hillocks, 1996). Porter & Powell (1967), in their study with RKNs in tobacco, found severe wilting in plants that were treated with nematodes before the fungus. Bowman & Bloom (1996) reported that the resistance to Fusarium wilt in tomato plants was overcome in the treatments with *M. incognita* inoculation before fungal inoculation. Vargas et al. (1996) found that *Phytophthora capsici* Leon. resistance was overcome when *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 (Rhabditida: Pratylenchidae) was applied to chili pepper. Colak-Ates et al. (2018) found that AL-4, AL-9 and AL-21 tomato genotypes with FORL resistance lost their resistance to FORL disease in simultaneous and sequential inoculations with *M. incognita*. In the present study, it was determined that although nematode resistance was not lost in tomato hybrids with RKN resistance, there was a change in application-based nematode development and this change was due to the synergistic or antagonistic interaction between *M. incognita* and FORL. It was found that these interactions are dependent on the pathogen that was first inoculated onto the plant.

When FORL was first inoculated on tomato plants under controlled conditions, gall formation, egg mass production and soil population of *M. incognita* were found to be negatively affected. It was determined that tomato FORL resistance was overcome with increasing nematode density with treatment N+10FORL in some tomato hybrids. This result shows that the density of *M. incognita* is important in the durability of FORL resistance, and as the population density increases, there may be a risk in the durability of resistance. It was found that the maintenance of FORL resistance is possible with RKN resistance. The disease was not observed in hybrids resistant to both pathogens. Farmers can prevent disease development by choosing tomato hybrids that are resistant to both RKNs and FORL.

It was concluded that RKN or fungal resistance can contribute to the prevention of yield losses caused by co-infection of *M. incognita* and FORL. When cost of resistant hybrids is taken into consideration, it is essential that resistance is not lost under field conditions. Nematode control should be successfully applied whenever FORL resistant hybrids are used. Integrated control methods should be considered to manage these disease complexes.

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