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Disease complex of *Rhizoctonia solani* and *Meloidogyne hapla* Chitwood, 1949 (Nemata: Meloidogynidae) on tomato

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ABSTRACT

The root rot disease complex of *Meloidogyne hapla* Chitwood, 1949 (Nemata: Meloidogynidae) and *Rhizoctonia solani* Kühn was investigated in 2021 under controlled conditions with different applications on tomato. Three week-old seedlings (cv. Alberty F1) were inoculated with *M. hapla* and/or *R. solani* according to priority of the applications. After sixty days, the parameters of plant and root height, plant and root wet weight, numbers of gall and egg masses, disease severity, *M. hapla* second stage juvenile density and *R. solani* density in the soil were recorded. The plant growth parameters were more adversely affected in plants inoculated with *R. solani* 2 weeks after the *M. hapla* application, whereas the number of galls and egg masses in the roots were negatively affected in only *M. hapla* inoculation 2 weeks after the inoculation of *R. solani* application. The disease severity of *R. solani* inoculation 2 weeks after *M. hapla* application (44.7%) and simultaneous *M. hapla* and *R. solani* application (33.6%) were found to be higher than only *R. solani* application (21.6%) and *M. hapla* inoculation 2 weeks after *R. solani* application (22.9%). In this study, it was found that *M. hapla* infestation of tomato increased root rot disease caused by *R. solani*.

1. Introduction

Tomato is one of the most commonly produced, consumed and traded agricultural products in the world. Turkey is third in the world in tomato production with 12.8 million tons (Anonymous 2021). Plant parasitic nematodes are one of the main pests that cause the damage losses in tomato production and quality. This damage caused by plant parasitic nematodes is estimated to be US$173 billion per year (Elling et al. 2013). Karajeh et al. (2008) stated that, about 5% of the world crop production is destroyed annually by *Meloidogyne* species. Similar research indicated that *Meloidogyne* species cause yield losses of up to 80% in tomato growing areas in the Western Anatolia region of Turkey (Kaşkavalcı 2007). *Meloidogyne hapla* Chitwood, 1949 (Nemata: Meloidogynidae) called "Northern Root-Knot Nematode", is one of the four most common root-knot nematode species worldwide and causes significant economic losses in various vegetables, fruit trees and pasture crops, including tomatoes (Moens et al. 2009). *Meloidogyne hapla* is seen more widely in temperate climates. Although it grows north of 39°N, it can be encountered in high altitudes of tropical regions as well (Whitehead 1969; Taylor and Buhrer 1958; Moens et al. 2009). It is possible to find it in northern Europe, northern Asia, southern Canada, and North America (Al Abadiyah Ralmi et al. 2016), and is detected on pepino, kiwi, tomato, pepper, and eggplant in Turkey (Özarslandan et al. 2005; Akyazı et al. 2012; 2017; Kepenekçi et al. 2014; Uysal et al. 2017). *Meloidogyne hapla* infection initially causes the development of small galls in the secondary roots (Gugino et al. 2006), and is more widespread in cool and temperate regions than tropical (Seid et al. 2015). In a study conducted in the vegetable producing areas of Isparta and Burdur Provinces in Turkey, 83 (51.8%) of 160 samples were found to be infected with root-knot nematodes, whereas 68 samples of root-knot nematodes were identified, and 22 of them were *M. hapla* (Uysal et al. 2017).

There are many soil-borne plant pathogens that affect the yield and quality of tomatoes (Bruehl 1987). *Rhizoctonia solani* Kühn. (Teleomorph: *Thanatephorus cucumeris*), one of these disease factors, is an important fungal disease that causes tomato wilt and root rot. *R. solani*, which is very difficult to control and causes serious damage both on seedlings and in the later stages of the plant (Solanki et al. 2011). The pathogen has infected at least 200 plant species with a wide host range (Lehtonen et al. 2008). This pathogen causes post emergence damping-off of seedlings and a weakening of seeds or seedlings before or after they germinate. Infected plants have cankers with red-brown spots on the stems and roots (Parmeter 1970). The fungus can survive for a long time in plant material or in soil as sclerotia (Anderson 1982). In many studies conducted throughout the world, including Turkey, it has been reported that *R. solani* is intensely isolated from tomato plants. These isolates belong to many anastomosis groups, but AG-4 isolate is encountered more frequently (Demirci and Döken 1995; Yıldız and Döken 2002; Kuramae et al. 2003; Taheri and Tarighi 2010; Bayar 2018; Demirer Durak and Ok 2019).

Recently, nematode-fungi interactions and their damage to many economically important crops have attracted the attention of scientists (Back et al. 2002). The interaction between root-knot nematodes and the root rot caused by *R. solani* has been studied on different hosts, and most of these studies have reported a synergistic interaction between these two important pathogens (Powell 1971; Mai and Abawi 1987; Shahzad and Ghaffar 1992; Evans and Haydock 1993; Bhagawati et al. 2007; Mokbel et al. 2007; Al Hazmi and Al Nadary 2015). *Meloidogyne incognita* (Kofoid and White 1919) Chitwood, 1949 (Nemata: Meloidogynidae) and *Rhizoctonia solani* interaction in tomato was reported in previous studies (Golden and Van Gundy 1974; Goswami et al. 1975; Mehta et al. 1995; Kumar and Haseeb 2009; Sagar et al. 2012). However, there are not many studies on the interaction of *M. hapla* with *R. solani* although it is the most widespread root knot nematode species, especially in temperate regions. Only Irvine (1964) reported that the highest death rates in alfalfa plants were in the treatment of *M. hapla* and *R. solani* together, followed by *M. hapla* treatment alone.

The root knot nematodes and *R. solani* interaction can cause serious damage to tomatoes, especially in greenhouse conditions. Developing a successful strategy to manage this nematode / fungus disease complex primarily depends on identifying the interaction between these two pathogens. The aim of this study was to investigate the interaction of local *M. hapla* and *R. solani* isolates in tomato root rot disease complex under controlled conditions.

2. Materials and Methods

2.1. Material

This study was carried out on the Alberty F1 tomato variety, which is known to be susceptible to both pathogens. *Rhizoctonia solani* isolate was obtained from tomato roots collected in the Deregümü region of Isparta province and was identified according to Barnett and Hunter (1998). Root knot nematode, DR15 isolate, was previously taken from a tomato greenhouse in the Deregümü region of Isparta province, identified morphologically and molecularly (Uysal et al. 2017) and mass production continued under controlled conditions (24±1°C, 60±5% humidity).

2.2. Nematode inoculum

In this study, an inoculum density of 1200 eggs per seedling was used. Eggs were obtained by soaking approximately 1 cm of diced tomato roots in 1% sodium hypochlorite for 5 minutes (Coolen and D'Herde 1972). First of all, the eggs were poured on a 75 µm sieve, then collected on a 5 µm sieve and washed with tap water to remove sodium hypochlorite (Nico et al. 2004; Liu et al. 2008). Finally, they were washed with sterilised distilled water and adjusted to a suspension of 1200 eggs in tubes containing 10 ml of distilled water (Al Hazmi and Al Nadary 2015).

2.3. Preparation of Inoculum of Rhizoctonia solani

Approximately 10 g of barley seeds were placed in 250 ml flasks and autoclaved with sterilised water for 30 minutes for two consecutive days. After the barley flasks had cooled, they were inoculated with one disc (5 mm in diameter) of 7 day old cultures of *R. solani* on Potato Dextrose Agar (PDA). The flasks were then incubated at 27±20°C for two weeks. During incubation, the flasks were shaken twice a day to ensure proper growth of the fungal mycelium on the barley seeds. Two weeks later, the fungi-colonised barley seeds were mixed in a bowl to ensure homogeneity and the inoculum amount was used at 15 g seedling (Al Hazmi and Al Nadary 2015).

2.4. Determination of the interaction of M. hapla and R. solani on tomato

In the present study, the experiments consisted of 5 different applications involving individual, simultaneous and sequential inoculations of *M. hapla* and *R. solani* on tomato. As a control, plants without nematode and fungi were used. Applications; 1) *M. hapla* only (N); 2) *R. solani* only (F); 3) Simultaneous inoculation of *M. hapla* and *R. solani* (N + F); 4) Fungus inoculation 2 weeks after nematode application (N+2F); 5) Nematode inoculation 2 weeks after fungus application (F + 2N). This study was carried out under controlled conditions (24±1°C, 60±5% humidity) and was designed in a randomised plot design with 10 replications. Three-week-old tomato seedlings were transplanted into 14 cm plastic pots containing approximately 1500 g of soil (68% sand, 21% silt and 11% clay). Inoculations were made 3 days after the seedling transplantation. One thousand two hundred *M. hapla* eggs 10 ml^{-1} and 15 g *R. solani* inoculum per seedling were used as the initial inoculum density, and inoculations were carried out according to the application priority. The nematode inoculum was equally distributed through three small holes made in the soil around the seedling stem and deep enough to contact the roots. Fungus inoculation was made by dispersing the seeds on the soil surface and mixing them well. Then, a small amount of soil was added and irrigated (Al Hazmi and Al Nadary 2015).

The study was completed 60 days after inoculation. After determining the plant height and fresh weight, the plants were uprooted and carefully washed thoroughly with tap water. Root lengths and root fresh weights were measured. Roots were exposed to 0.25% trypan blue for 3 minutes, then the gall and egg masses were counted under a stereomicroscope (Sharma and Ashokkumar 1991). The Baermann funnel technique was used to obtain the second stage juvenile density in the soil. The diseased plant rate at the end of the experiment (number of plants with root rot/number of healthy plants x 100) was calculated and then the *R. solani* density in the soil (cfu) was determined (Sagar et al. 2012).

2.5. Statistical analysis

Statistical analysis of the findings was calculated by using the SPSS (version 20.0) programme and analysis of variance (ANOVA) was used to test the differences between the means. In order to compare the means of different groups, "Tukey" was used when the variances were homogeneous ($P \leq 0.05$).

3. Results and Discussion

In the present study, the interaction effects of *R. solani* and *M. hapla* on plant growth and nematode and fungus density were investigated on the Alberty F1 tomato variety, by seperate and combined applications. Plant and root length and fresh weight values of all applications were found to be lower than the control application. The highest plant height values were found in seperate *M. hapla* (37.6 cm) and *R. solani* applications (39.8 cm) while the lowest values were in simultaneous *M. hapla* and *R. solani* applications (23.9 cm). There was no statistical difference between plant heights of simultaneous inoculations (30.1 cm) and *M. hapla* inoculation (31.0 cm) found 2 weeks after *R. solani*

inoculation (*P≤0.05)*. While plant fresh weight values were found to be close to each other in N+F (simultaneous *M. hapla* and *R. solani* applications), F+2N (*M. hapla* inoculation 2 weeks after *R. solani* application) and N+2F (*R. solani* inoculation 2 weeks after *M. hapla* application) applications, it was observed that it was lower than seperate nematode and fungus applications. While there was a statistical difference between N (*M. hapla* only) and F (*R. solani* only) applications in root length, no statistical difference was found between plant height, plant and root fresh weight values. It was determined that the plant growth parameters in simultaneous nematode and fungus applications were lower than seperate *M. hapla* and *R. solani* applications ($P \leq 0.05$). However, the lowest root fresh weight value was in N+2F (2.2 g) application and there was a statistical difference between N+F (4.0 g) and F+2N (3.3 g) applications ($P \leq 0.05$). It was determined that plant growth was more adversely affected when *R. solani* was inoculated 2 weeks after *M. hapla* inoculation (Table 1).

The lowest gall (83.3/root) and number of egg masses (105.0/root) in roots were determined in F+2N application. There was no statistical difference between the number of gall and egg masses of N, N+F and N+2F applications ($P \leq 0.05$). The second stage juvenile density of N (2949.8/250 cc soil) and N+F (2853.6/250 cc soil) applications in the soil was found to be higher than F+2N and N+2F applications. It was observed that the density of the second stage juvenile in the soil was adversely affected by inoculation with *M. hapla* 2 weeks after *R. solani* inoculation and *R. solani* application 2 weeks after inoculation with *M. hapla*. However, when the number of galls in the roots and the number of egg masses were analysed, the application in which nematode density was most negatively affected was F+2N application (Table 2).

The diseased plant rate was highest in N+2F (44.7%) application, followed by N+F (33.6%). The lowest disease rate was determined in F (21.6%) and F+2N (22.9%) treatments, and no statistical difference was found between them ($P \leq 0.05$). The disease rate in simultaneous nematode and fungus inoculations was found to be higher than the application of *R. solani* only. The highest concentration of *R. solani* in the soil was found in N+2F (2111.4 cfu g^{-1} soil) application, while the lowest was determined in F (1109.4 cfu g^{-1} soil) application. No statistical difference was found between the *R. solani* concentrations in the soil of N+F and F+2N applications ($P \leq 0.05$). It was determined that *R. solani* was more intense on plants inoculated with *R. solani* 2 weeks after *M. hapla* inoculation. It was found that the infection of roots with *M. hapla* contributed to the increase of the disease (Table 3).

Table 1. Effect of the interaction of *Meloidogyne hapla* and *Rhizoctonia solani* on plant growth parameters of Alberty F1 tomato variety

Applications*	Plant length (cm)	Plant wet weight (g)	Root length (cm)	Root wet weight (g)
	Average ± Standard Error			
C	47.5 ± 1.1a**	8.7 ± 0.4a	28.6 ± 1.2a	6.1 ± 0.1a
N	37.6 ± 1.5b	5.4 ± 0.3b	17.0 ± 0.6c	4.3 ± 0.1b
F	39.8 ± 1.5b	5.6 ± 0.3b	24.3 ± 0.7b	4.4 ± 0.2b
N+F	30.1 ± 1.1c	3.8 ± 0.1c	16.3 ± 0.5cd	4.0 ± 0.1bc
F+2N	31.0 ± 0.6c	3.3 ± 0.1c	14.7 ± 0.6cd	3.3 ± 0.2c
N+2F	23.9 ± 0.7d	2.7 ± 0.1c	13.4 ± 1.6d	2.2 ± 0.1d

*N: Nematode inoculation, F: Fungus inoculation, N+F: Simultaneous nematode and fungus inoculation, N+2F: Fungus inoculation 2 weeks after nematode application, F+2N: Nematode inoculation 2 weeks after fungus application, C: Control. ** Lowercase letters indicate statistical differences between applications in the same column (*P≤0.05*).

Table 2. Effect of *Meloidogyne hapla* and *Rhizoctonia solani* interaction on nematode density in Alberty F1 tomato variety

Applications*	Number of galls / root	Number of egg masses / root	2nd stage juvenile density in 100 g soil
	Average ± Standard Error		
C	-	-	-
N	168.6 ± 5.0a**	190.9 ± 3.8a	2949.8 ± 96.3a
F	-	-	-
N+F	176.4 ± 6.0a	196.8 ± 7.3a	2853.6 ± 99.0a
F+2N	83.3 ± 4.3b	105.0 ± 5.0b	2242.0 ± 204.5b
N+2F	160.3 ± 5.5a	178.8 ± 7.4a	1848.4 ± 49.0b

*N: Nematode inoculation, F: Fungus inoculation, N+F: Simultaneous nematode and fungus inoculation, N+2F: Fungus inoculation 2 weeks after nematode application, F+2N: Nematode inoculation 2 weeks after fungus application, C: Control. ** Lowercase letters indicate statistical differences between applications in the same column (*P≤0.05*).

Table 3. Effect of *Meloidogyne hapla* and *Rhizoctonia solani* interaction on disease rate on Alberty F1 tomato variety

Applications*	Disease rate (%)	*Rhizoctonia solani* concentration (cfu g^{-1} soil)
	Average ± Standard Error	
C	-	-
N	-	-
F	21.6 ± 1.7c**	1109.4 ± 34.7c
N+F	33.6 ± 1.8b	1731.6 ± 35.2b
F+2N	22.9 ± 1.5c	1535.9 ± 82.4b
N+2F	44.7 ± 1.7a	2111.4 ± 93.0a

*N: Nematode inoculation, F: Fungus inoculation, N+F: Simultaneous nematode and fungus inoculation, N+2F: Fungus inoculation 2 weeks after nematode application, F+2N: Nematode inoculation 2 weeks after fungus application, C: Control. ** Lowercase letters indicate statistical differences between applications in the same column (*P≤0.05*).

In this study, it was found that the disease rate and soil density of *R. solani* increased in the presence of *M. hapla* on tomato. However, it was determined that the highest increase in disease rate was determined when *R. solani* was inoculated 2 weeks after *M. hapla* inoculation and was followed by simultaneous inoculation. This increase in root rot indicates a synergistic interaction between the two pathogens and greater damage to the plant. When the plant growth parameters were examined, it was determined that the most damage occurred in *R. solani* inoculated 2 weeks after *M. hapla* inoculation. Due to *M. hapla* being a sedentary endoparasite and the physiological and anatomical changes it causes in the root tissues in giant cell formation may be a reason for this damage. The feeding cells of fixed endoparasite nematodes, "syncytia" or "giant cell", contain many golgi apparatus, mitochondria, a dense cytoplasm, and many ribosomes, and have high metabolic activity (Melendez and Powell 1970; McLean and Lawrence 1993; Abdel-Momen and Starr 1998). These nutrient-rich cells are appropriate substrates for fungal colonisation (Porter and Powell 1967; Powell 1968; Batten and Powell 1971; Carter 1981). Many other researchers recorded a synergistic interaction between root knot nematodes and *R. solani* on different hosts (Powell 1971; Mai and Abawi 1987; Evans and Haydock 1993; Bhagawati et al. 2007; Mokbel et al. 2007; Al Hazmi and Al Nadary 2015). Bhattarai et al. (2009) found that *R. solani* damage increased in the combination of *Globodera pallida* with *R. solani* or *G. rostochiensis* with *R. solani* and stem canker index increased significantly in co-inoculation with *G. pallida* and *R. solani* compared with *R. solani* only.

It was determined that *R. solani* inoculations did not increase the number of gall and egg masses in the roots 2 weeks after simultaneous inoculation and *M. hapla* inoculation, and it was in the same statistical group with *M. hapla* application only. However, it was determined that the number of gall and egg masses considerably decreased in the application of *M. hapla* inoculation 2 weeks after *R. solani* inoculation. Root rot caused by *R. solani* may have been affected by the nematode feeding process in root tissues and subsequently negatively affected nematode growth. The existence of a fungal mass that prevents nematode penetration or invading the places that the nematode chooses to feed may cause a decrease in nematode density (Triantaphyllou 1960; Nord-Meyer and Sikora 1983; Mokbel et al. 2007). The decrease in dry and wet weight of the plant as a result of the increase in fungal pathogenicity can reduce the nematode population (Mauza and Webster 1992). In most studies, it has been reported that root gall nematode-induced galling and nematode population decrease in the presence of *R. solani* (Choo et al. 1990; Mehta et al. 1995; Roy and Mukhopadhyay 2004; Kumar and Haseeb 2009; Sagar et al. 2012). Irwine (1964) reported that the highest death in alfalfa plant was in the simultaneous *M. hapla* and *R. solani* applications, followed by *M. hapla* application only. In a study of Göze Özdemir and Arıcı (2021) *in vitro* conditions, they determined that *R. solani* culture filtrates showed toxic effects on *M. hapla* eggs and juveniles which the 2nd stage juvenile death by *M. hapla* in the pure culture filtrate concentration of *R. solani*, live egg and hatching from the egg masses percentages, 83.2%, 76.7% and 54.2%, respectively.

4. Conclusion

In the present study, the results have shown that *M. hapla* infestation of tomato increased root rot disease caused by *R. solani*. With the increase in the disease rate, it was observed that plant growth was negatively affected in simultaneous nematodes and fungi applications. The lowest plant growth and the highest disease severity were determined in the inoculation of *R. solani* 2 weeks after the *M. hapla* inoculation. It was also observed that the density of *R. solani* in the soil was higher in simultaneous nematode and fungus applications than fungus inoculation only. These results indicate that co-infection of *M. hapla* and *R. solani* caused significant losses in yield. To manage this disease complex, the development of a successful strategy must depend on integrated disease management that includes appropriate methods to suppress the populations of both pathogens.

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