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# Investigation of the resistance of some citrus rootstocks against citrus nematode [*Tylenchulus semipenetrans* (Cobb, 1913) (Tylenchida: Tylenchulidae)] under field conditions in the Eastern Mediterranean Region

Doğu Akdeniz Bölgesi'nde doğa koşullarında bazı turunçgil anaçlarının Turunçgil nematodu'na [*Tylenchulus semipenetrans* (Cobb, 1913) (Tylenchida: Tylenchulidae)] karşı dayanıklılığının araştırılması

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ABSTRACT

Rootstocks play a significant role in citriculture in terms of fruit yield, quality, and tolerance/resistance to abiotic and biotic stress conditions. One of the limiting factors to citrus production is *Tylenchulus semipenetrans* (Cobb, 1913) (Tylenchida, Tylenchulidae). Resistant rootstocks had been one of the best options of choice in the management of *T. semipenetrans*. In this study, soil and root samples were taken from 54 selected rootstocks from the citrus collection at Cukurova University, which are naturally infested with citrus nematode *T. semipenetrans* in Adana province between 2012 and 2016. Nematodes were extracted from soil samples using the modified 'The Baermann Funnel Method', and root samples were stained by acid fuchsin. When the root and soil samples were examined, citrus nematode was found a few in the roots and rhizosphere soil of 17 rootstock varieties, while it was very intense in rootstocks of *Citrus aurantium* L. (Rutaceae) except Tuzcu 31-25T, Tuzcu 31-30T, and Tuzcu 891. According to the population density of citrus nematode in rootstock and rhizosphere, Tuzcu 31-25T moderately susceptible; Tuzcu 31-30T, Nasnaran, Poncirus trifoliata, Cleopatra ant, Local trifoliate Cloex swingle, Citrumelo 4475, C-35, Gou tou, Sunki, Tuzcu 891, Carrizo citrange resistant; all other rootstocks were identified as susceptible. All rootstocks supported nematode reproduction but showed different levels of susceptibility.

## INTRODUCTION

Citriculture is widespread throughout the world and significant in economic terms in tropical and subtropical regions where climate and soil conditions are amenable. According to 2018 statistics, global citrus production was 152.448.800 tons, including 75.413.374 tons of oranges [*Citrus sinensis* (L.) Osb.], 34.393.430 tons of mandarins (*Citrus reticulata* Blanco), 19.368.838 tons of lemons (*Citrus limon* Burm. F.) and limes [*Citrus latifolia* Tan. and

*Citrus aurantifolia* (Christm.) Swingle], 9.374.739 tons of grapefruits (*Citrus paradisi* Macf.) and pummelos [*Citrus maxima* (Burm.) Merr.], and 13.898.418 tons of other citrus varieties (FAO 2018).

A vegetatively propagated citrus tree is normally composed of the rootstock and scion. Rootstocks play an important role in the rapid development of citrus and the breeding new cultivars of rootstocks. The necessity of using rootstocks for citrus is to have a profitable production against some limiting factors, such as climate, inappropriate soil conditions, and diseases. Rootstocks enhance tolerance to salinity, iron chlorosis, flooding, drought, compatibility with commercial species/cultivars, high yields of good fruit quality, reduced tree size, resistance to Citrus Tristeza Virus (CTV) (Martellivirales, Closteroviridae), resistance to citrus blight, resistance to fungal diseases affecting citrus [*Phytophthora* spp. (Peronosporales, Peronosporaceae), *Armillaria mellea* (Agaricales, Physalacriaceae), etc.], and resistance to nematodes.

Many plant-parasitic nematodes infect the citrus rootstocks, but a few nematodes cause damage to the trees. Citrus nematode (*Tylenchulus semipenetrans*) is one of the important plant parasitic nematodes that affect citrus growth and yield citrus (Duncan 2005). The nematode may damage young roots depending on soil structure and water condition (Duncan and Noling 1987). Heavily infected feeder roots are thicker than healthy roots and have a dirty appearance on the root surface. The infection of rootstocks by the citrus nematode, resulting in the reduction of the yield in the root system, decreasing the nutrient and water intake from the soil, and the production of unqualified fruit on trees. Citrus trees may show signs of decline symptoms (Duncan 2005). This situation may be correlated with higher daily temperature and lower relative humidity. Physical damage to roots can cause by feeding *T. semipenetrans,* and insects can also break resistance mechanisms and significantly increase *Phytophthora* disease. In many cases, the infection of *T. semipenetrans* biotypes has reduced the efficacy of resistant rootstocks worldwide. To date, it was determined four biotypes as Poncirus, citrus, Mediterranean, and grass (Gottlieb et al. 1986, Inserra et al. 1980, Kwaye et al. 2008, Mashela et al. 2010). Moreover, it is reported that the nematode causes 10-20% annual production loss (Bilgrami and Gaugler 2004, Bongers and Ferris 1999, Philis 1989, Sasser and Freckman 1987). The citrus nematode has been recorded in every commercial citrus-producing country, and the infection percent is 24-60% in California and Florida states; 70-90% in Texas, Arizona, Brazil, and Spain (Cobb 1913, 1914, de Campos et al. 2002, Esser et al. 1993, Heald and O'Bannon 1987, Sorribas et al. 2000). Also, *T. semipenetrans* were found firstly on pomegranate in Iran and worldwide (Rashidifard et al. 2015). In the Aegean region of Turkey, 89.33% and 88.88% of citrus nematodes were detected in Izmir and Aydın, respectively (Emre and Kaşkavalcı 2015, Kesici 2016). Moreover, in the Eastern Mediterranean region of Turkey, it is known that 90% of orchards are infested with the Mediterranean race at above the economic loss threshold (Elekcioğlu 1992, Elekcioğlu1995, Elekcioğlu et al. 2013). Because of high pH soils in the Eastern Mediterranean Region, *Citrus aurantium* L. is the most widely grown and is the most tolerant plant variety against diseases and pests. As the use of chemical preparation is expensive and harmful to human health, resistant rootstock and biological control are considered for appropriate control methods (Duncan and Cohn 1990, Jones 2017). It is known that different rootstocks show different resistance to *T. semipenetrans* (Duncan et al. 1994, Edwards 1988). Some selections of *Poncirus trifoliata* and Cleopatra mandarin have moderately susceptible to populations of *T. semipenetrans*, while other selections can be a high level of resistance (Baines et al. 1969, Galeano et al. 2003).

There are many studies about resistant rootstocks (Alian et al. 2018), but there is no study about rootstock resistance to the Mediterranean race of citrus nematode. This study was deemed necessary due to the lack of literature about the different resistant rootstocks against the Mediterranean race of the nematode. The Mediterranean race is the most common in Adana province (Toktay et al. 2005). In this study, the resistance of different citrus rootstocks and varieties to *T. semipenetrans* was investigated. Since *C. aurantium* is the most commonly used rootstock in this region, the resistance of 54 different rootstocks in field conditions was studied.

## MATERIALS AND METHODS

*Field studies*

Field studies were carried out between March and April months in 2012-2016 on 54 different rootstocks at Çukurova University Faculty of Agriculture in the Citrus Collection plots. Rootstocks were planted at the orchard in 1976, 1980, and 1981. Since the highest population occurred between 23-25 °C, this time interval was considered, and the samples were collected during this period (Tanha Maafi and Damadzadeh 2008, Toktay and Elekçioğlu 2001). The roots and soil samples were taken from 3 different trees of the same rootstock to investigate the development of this nematode from different citrus rootstock varieties and different rootstocks in that time.

Southey's proposal (1986) was performed taking samples from at least 50-60 different points per 0.4 ha. The samples were taken from a depth of 0-30 cm in a zigzag type, considering the crown roots of the trees in the direction of the drip irrigation hose by using a soil probe. Plant root samples and soils were placed into polyethylene bags after cleaning. The label information of the bags includes as follows: the date of receipt, the place where was taken, and the name of the rootstock. These samples were brought to the laboratory for examination. After the necessary labelling, the root/soil samples were stored at +4 °C.

*Laboratory studies*

The modified Baermann Funnel Method was followed to obtain the 2$^{nd}$ juveniles and male individual nematodes in the soil (Barker 1985, Hooper 1986, Southey 1986). The number of nematodes in 100 g of soil was counted under a light microscope.

Citrus root samples brought to the laboratory were carefully washed and the soil was cleaned to see the female individuals clearly. After that, weighed as 1 gram on a precision scale and stained in an acid-fuchsin solution (10 ml of 1% acid-fuchsin, 17.5 ml of lactic acid, 12.6 ml of glycerine, 12.4 ml of pure water) (Moltmann 1988). In this method, the nematodes absorb the dye and get a dark red color. Stained root hairs were placed between two slides and the number of adult female individuals in the roots was counted in the binocular stereomicroscope and their numbers were determined. Results were evaluated based on citrus nematode resistance and susceptibility scale of Javed et al. (2008) (Table 1). Based on the results, the nematode population density counted in the root and soil was determined according to the resistance scale from 1 to 9.

**Table 1.** Resistance or susceptibility scale to citrus nematode (Javed et al. 2008).

| Scale | | Population Density | |
|---|---|---|---|
| | | J2/100 cm$^3$ soil | Female individual/ g root |
| 1 | Resistance | <250 | <100 |
| 3 | Medium-Resistance | 250-500 | 100-200 |
| 5 | Medium-Susceptible | 500-1000 | 200-300 |
| 7 | Susceptible | 1000-1600 | 300-500 |
| 9 | Very Susceptible | >1600 | >500 |

## RESULTS AND DISCUSSIONS

In this study, all 37 *Citrus aurantium* and 17 other rootstock varieties at the Cukurova Citrus Collection plot were evaluated and found to be moderately and highly susceptible to the citrus nematode. Only Tuzcu 31-30T and Tuzcu 891 were found to be resistant to 141 individuals/100 g, 162 individuals/100 g in the soil in Tuzcu's collection (Table 2). As the resistance level of other Tuzcu's rootstocks were 5 and above, it was determined as susceptible. Nasnaran, *Poncirus trifoliata*, Cleopatra ant, Local trifoliate, Cloex swingle, Citru melo 4475, C-35, Gou tou, Sunki, Tuzcu 891, Carrizo citrange resistance level was determined as 1. Carrizo and Troyer citranges, which were found to be resistant in the first studies, were later evaluated as sensitive to *T. semipenetrans* (Lo Giudice and Inserra 1980). Based on Javed et al. (2008) (Table 1), Carrizo citrange was resistant (Table 2) 42 individuals/100 g in soil. This rootstock Troyer citrange originated from Riverside-California. However, the difference in their resistance level to nematodes should be related due to different rootstock varieties and biotypes of *T. semipenetrans* (Kwaye et al. 2008, Verdejo-Lucas et al. 2003). Although there is a dense population in the soil, Swingle citrumelo 4475 rootstock was also resistant, and this resistance was reported in previous studies by Verdejo-Lucas et al. 1997a.

The damaging threshold of *T. semipenetrans* (number of larvae per 100 g of soil) can be affected by several factors (Duncan and Cohn 1990). *T. semipenetrans* leads to penetration of second microorganisms (Broadbent 2000, Gams 2000). Trees infected by *Fusarium solani*, *Phytophthora* spp. can be severely stressed and weak. Then, this complex disease is injured with infection of citrus trees, which causes nondevelopment of the root system. Besides, the reproduction factor of the nematode differs between species of Citrus, and their hybrids are affected by tree age (Bello et al. 1986, Cohn 1965).

*T. semipenetrans* has different races that are known to infect different host plants (Inserra et al. 1980). In this study, the Mediterranean race had a low reproduction in *Poncirus trifoliata*. It is known that the rootstock has resistant genes and resistance against the different races of *T. semipenetrans* (Baines et al. 1969, Inserra et al. 1994, Ling et al. 2000). As a matter of fact, Kallel et al. (2006) reported that *Citrus aurantium* is sensitive to citrus nematode. However, by grafting with *Poncirus trifoliate*, the resistance was observed cellular necrosis and allelochemicals are produced in roots against to Mediterranean biotype of the Citrus nematode. Moreover, rootstocks which limited nematode reproduction also have fewer nematodes due to hypersensitive reaction (Kaplan 1981). Therefore, it was concluded that, in the future citrus rootstock breeding studies, *Poncirus trifoliata* could be hybridized with Tuzcu due to resistance. While Troyer citrange is known to be moderately susceptible to the citrus race of *T. semipenetrans*, also it was determined susceptible to the Mediterranean race in this study (Baines et al. 1969, Verdejo-Lucas et al. 1997a). It is thought to be the constant exposure to the high nematode population, and due to the age of the rootstocks which may cause the susceptibility. Resistance to citrus nematode infection was reported when Troyer citrange exposure over time and with high nematode population (Verdejo-Lucas et al. 1997a, Verdejo-Lucas et al. 2003, Verdejo-Lucas and McKenry

**Table 2.** Reactions of Citrus rootstock varieties in "Collection parcels" against Tylenchulus semipenetrans.

| Varieties of Citrus Rootstock | FPD* | PD$^x$ | SC** | RL*** | Varieties of Citrus Rootstock | FPD* | PDx | SC** | RL*** |
|---|---|---|---|---|---|---|---|---|---|
| TUZCU 31-22T (*Citrus aurantium L.*) | 357 | 1260 | 7 | S | TUZCU 31-26T (*C. aurantium L.*) | 420 | 1551 | 7 | S |
| TUZCU 31-29T (*C. aurantium L.*) | 312 | 1104 | 7 | S | TUZCU 31-24T (*C. aurantium L.*) | 330 | 1320 | 7 | S |
| TUZCU 33-10T (*C. aurantium L.*) | 402 | 1401 | 7 | S | TUZCU 31-31T (*C. aurantium L.*) | 450 | 1500 | 7 | S |
| TUZCU 01-17T (*C. aurantium L.*) | 315 | 1116 | 7 | S | TUZCU 31-30T (C. aurantium L.) | 27 | 141 | 1 | R |
| TUZCU 01-24T (*C. aurantium L.*) | 330 | 1101 | 7 | S | TUZCU 01-19T (*C. aurantium L.*) | 342 | 1254 | 7 | S |
| TUZCU 33-2T (*C. aurantium L.*) | 363 | 1602 | 7 | S | TUZCU 33-9T (*C. aurantium L.*) | 426 | 1560 | 7 | S |
| TUZCU 33-12T (*C. aurantium L.*) | 372 | 1608 | 7 | S | TUZCU 33-4T (*C. aurantium L.*) | 420 | 1557 | 7 | S |
| TUZCU 01-13T (*C. aurantium L.*) | 360 | 1461 | 7 | S | TUZCU 33-8T (*C. aurantium L.*) | 456 | 1302 | 7 | S |
| TUZCU 31-27T (*C. aurantium L.*) | 306 | 1302 | 7 | S | TUZCU 01-16T (*C. aurantium L.*) | 411 | 1542 | 7 | S |
| TUZCU 01-14T (*C. aurantium L.*) | 453 | 1590 | 7 | S | TUZCU 01-15T (*C. aurantium L.*) | 402 | 1548 | 7 | S |
| TUZCU 01-18T (*C. aurantium L.*) | 309 | 1047 | 7 | S | TUZCU 31-25T (*C. aurantium L.*) | 321 | 960 | 5 | MS |
| TUZCU 33-11T (*C. aurantium L.*) | 321 | 1062 | 7 | S | TUZCU 01-20T (*C. aurantium L.*) | 336 | 302 | 7 | S |
| TUZCU 33-32T (*C. aurantium L.*) | 327 | 302 | 7 | S | TUZCU 01-21T (*C. aurantium L.*) | 345 | 1440 | 7 | S |
| TUZCU 01-23T (*C. aurantium L.*) | 471 | 1596 | 7 | S | TUZCU 11-28T (*C. aurantium L.*) | 342 | 1260 | 7 | S |
| TUZCU 33-5T (*C. aurantium L.*) | 288 | 1362 | 7 | S | TUZCU 33-7T (*C. aurantium L.*) | 480 | 1581 | 7 | S |
| TUZCU 33-1T (*C. aurantium L.*) | 477 | 1560 | 7 | S | TUZCU 33-6T (*C. aurantium L.*) | 291 | 1380 | 7 | S |
| Tuzcu 01-18T (*C. aurantium L.*) | 510 | 2640 | 9 | VS | Tuzcu 891 (*C. aurantium L.*) | 9 | 162 | 1 | R |
| *Citrus taiwanica* | 324 | 1020 | 7 | S | Tuzcu 01-14T (*C. aurantium L.*) | 150 | 1260 | 7 | S |
| *Citrus aurantium* | 345 | 1260 | 7 | S | Troyer citrange (*C.sinensis*P.trifoliata*) | 390 | 1461 | 7 | S |
| *Citrus ampullaceae* | 363 | 1380 | 7 | S | *Citrus volkameriana* | 492 | 1641 | 9 | VS |
| *Citrus obovoidea* | 399 | 1422 | 7 | S | *Poncirus trifoliata* | 6 | 81 | 1 | R |
| Nasnaran (*C. amblycarpa)* Antalya) | 39 | 40 | 1 | R | Local trifoliate (*P. trifoliata*) Sunki | 42 | 135 | 1 | R |
| Cleopatra mandarin (*Citrus reshni* Tan. cv | 1 | 20 | 1 | R | Sunki (*Citrus* sunki (Hayata) hort ex. Tan) aka | 51 | 141 | 1 | r |
| Cloex swingle (*Citrus reshni* Tan X *Citroncirus* spp.) | 1 | 40 | 1 | R | *Citrus sulcata* | 285 | 1302 | 7 | S |
| Citru melo 4475 X *Citroncirus* spp. RUTACEAE | 42 | 20 | 1 | R | Cleopatra mandarin (*Citrus reshni* Tan.) | 303 | 1362 | 7 | s |
| C-35 (X *Citroncirus* spp.) | 5 | 40 | 1 | R | Carrizo citrange (*C. sinensis* X *P. trifoliata*) | 9 | 42 | 1 | R |
| Gou tou (*C. aurantium* L.) | 30 | 40 | 1 | R | Yuzu (*Citrus changensis* X *C.reticulata*) | 474 | 1584 | 7 | S |
| *Citrus olkameriana* | 204 | 980 | 7 | S | | | | | |

2004). In previous studies, Cleopatra mandarin x *Poncirus trifoliata*, *Citrus volkameriana* x *Poncirus trifoliata*, and Swingle citrumelo 4475 hybrid citrus rootstocks showed resistance to citrus nematodes. Besides, Carrizo and Troyer citranges have different levels of resistance and tolerance (Inserra et al. 1994, Magunacelaya et al. 2004, Verdejo-Lucas et al. 2000). For the first time, Nasnaran, C-35, Gou tou, Trifoliate, Sunki, Tuzcu 31-30T, Tuzcu 891 rootstocks were determined resistant in this study. This study aims to determine rootstocks that are resistant and susceptible to *T. semipenetrans* in field conditions.

As a result, when considering the cost of chemical control and damage to human health, using resistant varieties against citrus nematode is one of the most important solutions in the long term (Emre and Kaşkavalcı 2015). All citrus trees having a high citrus nematode population on the roots are not show aboveground symptoms. Symptom expression may not consider for 5 to 10 years after peak nematode population levels are reached (Heald and O'Bannon 1987). A soil analysis should be performed to assess whether to plant resistant varieties before planting. Also, the economic damage threshold of this pest can be reduced by attaching importance to cultural measures, biological and chemical controls. Determining the various reactions of resistant rootstock varieties under controlled conditions and at different population densities is important for future studies (Verdejo-Lucas et al. 1997b, 2000). The importance and role of biotic and abiotic tensions for penetrating the infection progress need further investigation.

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## ÖZET

Anaçlar, meyve verimi, kalitesi, abiyotik ve biyotik stres koşullarına karşı tolerans/direnç açısından turunçgil üreticiliğinde önemli bir rol oynamaktadır. Turunçgil üretimini sınırlandıran faktörlerden biri de *Tylenchulus semipenetrans* (Cobb, 1913) (Tylenchida, Tylenchulidae)'dır. Dayanıklı anaçlar, *T. semipenetrans*'ın mücadelesinde en iyi seçeneklerden birisi olmuştur. Bu çalışmada Adana ilinde Turunçgil nematodu *T. semipenetrans* ile doğal olarak bulaşık Çukurova Üniversitesi Turunçgil Koleksiyon parsellerinden, seçilen 54 anaçdan 2012-2016 yılları arasında toprak ve kök örnekleri alınmıştır. Toprak örneklerinden nematodlar, geliştirilmiş Baerman Huni yöntemi kullanılarak ekstrakte edilmiştir ve kök örnekleri asit fuksinle boyanmıştır. Kök ve toprak örnekleri incelendiğinde 17 anaç çeşidin köklerinde ve rizosferdeki toprakta Turunçgil nematoduna çok az rastlanırken, *Citrus aurantium* L. (Rutaceae)'ye ait 37 anaçtan Tuzcu 31-25T, Tuzcu 31-30T ve Tuzcu 891 hariç diğerlerinde zararlı yoğun olarak bulunmuştur. Anaç kökleri ve rizosferde bulunan Turunçgil nematodunun popülasyon yoğunluğuna göre, Tuzcu 31-25T orta hassas; Tuzcu 31-30T, Nasnaran, Poncirus trifoliata, Kleopatra ant, Local trifoliate, Cloex swingle, Citrumelo 4475, C-35, Gou tou, Sunki, Tuzcu 891, Carrizo citrange dayanıklı; diğer tüm anaçlar hassas olarak tespit edilmiştir. Tüm anaçlar nematod çoğalmasını desteklemiş ancak farklı duyarlılık seviyeleri göstermiştir.

Anahtar sözcükler: Carrizo sitranjı, *Citrus aurantium* L., *Poncirus trifoliata*, Cloex swingle, Kleopatra mandarini, turunçgil nematodu

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