DOI: 10.22034/ijpp.2025.2021825.468
بیماری‌های گیاهی / جلد ۶۰ / شماره ۱/ سال ۱۴۰۳: ۵۹-۴۸

**مقاله پژوهشی**

# نوسانات جمعیت نماتد مرکبات *Tylenchulus semipenetrans* در یک باغ آلوده در استان فارس، جنوب ایران*

**محمد رومیانی، اکبر کارگربیده** و حبیب‌اله حمزه‌زرقانی**

(تاریخ دریافت: ۱۴۰۳/۰۸/۲۶ ، تاریخ پذیرش: ۱۴۰۳/۱۲/۲۴)

## چکیده

نماتد مرکبات (*Tylenchulus semipenetrans*) یکی از مهم‌ترین عوامل تهدیدکننده تولید مرکبات در دنیا محسوب می‌شود. مدیریت مؤثر این بیمارگر مستلزم درک پویایی جمعیت آن است. در این پژوهش، نوسانات فصلی و اوج‌های جمعیتی نماتد مرکبات در شهرستان فسا، یکی از مناطق اصلی تولید مرکبات استان فارس در جنوب ایران، طی سال‌های ۱۳۹۷-۱۳۹۸ بررسی شد. به منظور پایش تراکم و پویایی جمعیت نماتد، نمونه‌های خاک و ریشه به صورت ماهیانه جمع‌آوری شدند. نتایج این مطالعه نشان داد که در باغ‌های شهرستان فسا دو اوج جمعیتی سالانه وجود دارد. بیشترین جمعیت نماتد در خاک در اواخر ماه‌های اردیبهشت و مهر-آبان مشاهده شد، درحالی‌که جمعیت ریشه در اواخر اردیبهشت و آذر به اوج خود رسید. کم‌ترین سطح جمعیت در خاک (لاروهای سن دوم و نرها) و ریشه (تخم‌ها، لاروها و ماده‌ها) در تیرماه مشاهده شد. بررسی‌ها نشان داد که نوسانات جمعیت نماتد در ریشه، به‌ویژه ماده‌ها، کم‌تر از نوسانات جمعیت در خاک است. علاوه بر این در بیشتر نمونه‌ها، با افزایش فاصله از تنه درخت، تراکم نماتد کاهش یافت. این یافته‌ها نشان‌دهنده اهمیت شناخت پویایی جمعیت نماتد برای اجرای راه‌کارهای مدیریتی در دوره‌های اوج جمعیت است.

**کلید واژه‌ها: اوج جمعیت، درختان مرکبات، نماتد مرکبات، شهرستان فسا**

---

* قسمتی از رساله دکتری نویسنده اول ارائه شده به دانشکده کشاورزی دانشگاه شیراز

**مسئول مکاتبات، پست الکترونیکی: karegar@shirazu.ac.ir
گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه شیراز

Iran. J. Plant Path., Vol. 60, No .1, 2024: 48-59

DOI: 10.22034/ijpp.2025.2021825.468

**Research Article**

# Population dynamics of the citrus nematode *Tylenchulus semipenetrans* in an infested orchard in Fars province, southern Iran*

**Mohammad Rumiani, Akbar Karegar **and Habiballah Hamzehzarghani**

(Received: 16.11.2024; Accepted: 14.03.2025)

## Abstract

The citrus nematode *Tylenchulus semipenetrans* is considered one of the most significant threats to citrus production worldwide. Effective management of this pathogen requires an understanding of its population dynamics. In this study, seasonal fluctuations and population peaks of the citrus nematode were examined in Fasa, one of the main citrus-producing regions in Fars Province, Southern Iran, during the years 2018-2019. To monitor the nematode population density and dynamics, soil and root samples were collected on a monthly basis. The results of this study showed that there are two annual population peaks in the orchards of Fasa. The highest nematode population in the soil was observed at the end of May and in December, while the highest root population was observed at the end of May and October-November. The lowest population levels in the soil (second-stage juveniles and males) and in the roots (eggs, juveniles, and females) were recorded in July. The study revealed that population fluctuations in the roots, especially females, were less than in the soil. Additionally, in most samples, nematode density decreased as the distance from the tree trunk increased. These findings highlight the importance of understanding the nematode population dynamics to implement effective management strategies during population peak periods.

Keywords**:** Population peak, Citrus trees, Citrus nematode, Fasa city

* A Part of Ph.D. Dissertation of the First Author Submitted to School of Agric., Shiraz Univ., Shiraz, Iran.

**Corresponding author's E-mail address: karegar@shirazu.ac.ir
Department of plant protection, faculty of agriculture, Shiraz university, Iran

## Introduction

Citrus is one of the most economically important crops in the world. With a total production of 3,865,098 tons of citrus, Iran is among the top 10 citrus producing countries in the world (Food and Agriculture Organization of the United Nations 2023). Fars province produces about 24.47% of the total citrus in Iran and is the second largest citrus producer after Mazandaran province in the north (Ahmadi *et al.* 2021). More than 40 species of plant parasitic nematodes (PPN) were reported from the citrus orchards around the world. However, only a few species can cause economically serious losses to citrus trees (Timmer *et al.* 2000). The slow decline of citrus plants is caused by infection with *Tylenchulus semipenetrans* Cobb, 1913 and *Radopholus citrophilus* (Duncan 2005). The citrus nematode is the most common PPN of citrus, affecting about 80 species or cultivars of the genus *Citrus* (Timmer *et al.* 2000, Ahmad *et al.* 2004). To date, three biotypes of the citrus nematode are known worldwide, which differ in their host range (Inserra *et al*. 1980, Inserra *et al*. 1994, Verdejo-Lucas *et al*. 2003). The life span of the citrus nematode varies depending on environmental and edaphic conditions and ranges from four to eight weeks (Van Gundy *et al*. 1964, Pretorius 2017). *Tylenchulus semipenetrans* has spread across both northern and southern provinces (especially Fars) of the country at various population levels. Studies are available reporting the frequent occurrence of this pathogen in citrus orchards of Fars, Hormozgan and Mazandaran provinces (Izadpanah & Saffarian 1968, Abiverdi *et al*. 1970, Minassian & Moadab 1970, Maafi & Kheiri 1991, Ayazpour & Ghanaatian 2004, Tanha Maafi & Damadzadeh 2008). Nematode-infected seedlings are the most important factor for the spread of the nematode in the world (Timmer *et al.* 2002).

Crop losses caused by the citrus nematode reach 90% in some heavily infested areas (Baines *et al*. 1962, Duncan 2005). The economic threshold for nematode infestation depends on the nematode reproduction rate, host and environmental factors, rootstock susceptibility (Duncan 1999), the cost of nematode management (Sorribas *et al*. 2008), climatic conditions (Bello *et al*. 1986, Navas *et al*. 1992, Timmer *et al*. 2000), and soil physicochemical properties such as moisture (Duncan & Eissenstat 1993), density, texture (Baines *et al*. 1962, Martin *et al*. 1963, Duncan 2005, Sorribas *et al*. 2008, Rashidifard *et al*. 2015b), organic matter, salinity and soil acidity (Timmer *et al*. 2000, Sorribas *et al*. 2008, Salahi Ardakani *et al*. 2014). In addition, seasonal fluctuation of the citrus nematode populations varies with environmental and geographic conditions.

The use of nematode-resistant or -tolerant citrus rootstocks is considered the most promising and sustainable measure to prevent damage from this pathogen (Kaplan 1981, Lee *et al*. 1999, Ling *et al*. 2000, Galeano *et al*. 2003). However, replacing susceptible rootstocks with resistant cultivars is an expensive and time-consuming process. Therefore, monitoring nematode population density in the rhizosphere and roots of infested citrus trees can lead to obtaining information about the peak of the population and consequently implementing IPM strategies at the right time. In northern Iran, the citrus nematode population peaks in summer and declines to lower levels in autumn and winter (Tanha Maafi & Damadzadeh 2008). A preliminary study conducted in Khafr region of Fars province revealed that the citrus nematode has two generations per year. It was demonstrated that the life cycle of *T. semipenetrans* begins with egg laying in the soil in mid-May (Sharafeh 1972). Moreover, the peak of the citrus nematode population in Kerman province was observed in autumn, while the minimum was recorded in spring (Rashidifard *et al*. 2015b). Despite the remarkable importance of *T. semipenetrans* in the country, its life cycle and biology have hardly been studied in Fars province. Also, population dynamics of this pathogen in the province have not been extensively studied. The aims of this study were to determine the: i) biology and monthly fluctuations of citrus nematode in a citrus orchard in Fasa region, Fars province, ii) correlation between nematode population density and temperature.

## Materials and Methods

### Survey area

A 2-ha nematode-infested citrus orchard was selected as part of a preliminary sampling of several areas in Fasa region, Fars province. The orchard was drip irrigated with a drip spacing of 60-80 cm and planted approximately 5 m × 6 m apart. Irrigation applied at 5 to 7 days' interval during summer and every 10 to 14 days during winter. The flow rate of water was around 6 liters per hour (L/h). Three 10 to 12-year-old orange trees (*Citrus sinensis* L. var. Valencia), which were grafted onto sour orange (*C. aurantium* L.) rootstocks, were sampled to ensure an approximately even distribution of the selected trees in the orchard (828: 28°95′54.5″N & 53°60′14.2″E, 829: 28°95′60.3″N & 53°60′19.3″E and 830: 28°95′58.1″N & 53°60′16.8″E). Monthly samples were collected from the roots and rhizosphere for 14 months, at a depth of 20-30 cm below each of the selected trees. To minimize sampling errors and allow simultaneous sampling, trees were selected close to each other in an orchard. The experiment was conducted randomly in three replicates (tree). The physicochemical properties of the soil were measured using a composite soil sample taken from three trees studied.

### Species identification

After extraction, the nematodes were fixed with hot FAA (4:1), transferred to anhydrous glycerol, and microscopic preparations were made. Identification was based on morphological and morphometric characteristics and using nematode identification keys (Inserra *et al*. 1988, Tanha Maafi *et al*. 2012), Dino Capture software, and a light microscope. To visualize root populations, nematode-infested roots were stained with lactophenol-acid fuchsin. To confirm the results of the morphological and morphometric studies, genomic DNA was extracted and the ITS fragment and D2–D3 expansion segments of 28S were sequenced.

### Sampling

Due to the rocky nature of the soils, it was not possible to use an auger as a conventional soil sampling device, so a hand trowel was used for sampling. Two composite samples were randomly taken from the moist margin created on both sides of the irrigation drip line as hypothetical inner (A: four subsamples) and outer (B: six subsamples) circles under each tree canopy (Rumiani *et al.* 2021). The subsamples were 10-15 cm in diameter and 20-30 cm deep. Composite samples A and B were taken at a radius of 60 and 120 cm around the tree trunk, respectively. The subsamples in each circle were mixed and gently homogenized, then approximately 2 kg of soil (1550 $cm^3$) was collected, placed in separate labeled bags, and transported to the laboratory.

### Nematode extraction and counting

The soil inhabiting stages of citrus nematode, including second-stage juveniles and males, were extracted from 100 $cm^3$ of soil using the tray method (Whitehead & Hemming 1965), after 48 hours, the nematode suspension was collected, transferred to a counting dish. The second-stage juveniles and males were counted under a stereomicroscope in three replications. To estimate the root population, including eggs, juveniles and females, the roots of each composite sample were taken from soil, washed thoroughly, dried with tissue paper to remove the excess moisture and then chopped to one-centimeter pieces. To extract the nematodes 5 g of the roots were randomly obtained and subjected to a blender containing 0.05% NaOCl, at high speed for 30 seconds (in two consecutive 15-second intervals). The blended roots were then placed on a nested 200 mesh sieve over a 500-mesh sieve, sprayed with a tap water then collected from the lower sieve. The suspension was mixed thoroughly and one ml used for counting which repeated three times and expressed per 5 g of fresh root (McSorley *et al*. 1984, Sorribas *et al*. 2008). The mean number of nematodes extracted from soil and root of two composite samples (A & B) were considered as the final population per 100 $cm^3$ of soil and 5 g of roots of the citrus trees.

### Soil temperature

Due to the geographic distance of the sampling site, the soil temperature was estimated using mean monthly air temperature data and empirical models (Islam *et al*. 2015). The equations $y = 0.9x + 3.83$, $y = 0.842x + 6.224$ and $y = 0.708x + 9.871$ were used to estimate soil temperature at three depths of 5, 10 and 20 cm, respectively (Islam *et al*. 2015) where x and y are the monthly mean air temperature (Farsmet 2020) and the monthly mean ground temperature of Fasa, respectively. Microsoft® Excel® 2013 (15.0.4420.1017) software was used to draw the graphs.

## Results

The studied population was identified as *T. semipenetrans* based on morphological and morphometric characteristics and identification keys (Inserra *et al*. 1988, Tanha Maafi *et al*. 2012). In addition, the sequencing results (ITS fragment and D2– D3 expansion segments of 28S rRNA) confirmed the identification. NCBI blast showed that the sequences of the present study differed from corresponding sequences of the *T. semipenetrans* populations deposited in the database by only a few nucleotides and had an identity of more than 99%.

Based on the physicochemical analysis, the soil under the canopy of the trees was classified into the group of silt-loam soils with 25.08% sand, 57.64% silt and 17.28% clay. The organic matter contents and pH of the soil were 1.96% and 7.56, respectively, and the electrical conductivity (EC) was 1900 µmhos/cm. The soil contained 71.6 mg/kg phosphorus, 15.26 mg/kg potassium, 55.00 mg/kg calcium carbonate, 4.3 mg/kg calcium, 7.3 mg/kg magnesium, 125.41 milliequivalents (meq)/liter sodium, 1.05 mg/kg zinc, 3.5 mg/kg iron, 1.26 mg/kg copper and 2.20 mg/kg manganese.

Monitoring the citrus nematode population showed fluctuation of root and rhizosphere nematodes under the canopy of citrus trees. The lowest number of nematodes in the soil was recorded in July (931 J2s and males / 100 ml soil), while the highest numbers were recorded in two separate peaks in May (3900/ 100 ml soil) and December (5403/ 100 ml soil) (Fig. 1 middle). Root nematode populations declined in June, July, and August. The peak of the female population was recorded in late May and October (1561 and 3582 females per 5 g of root, respectively). Also, the highest number of eggs and juveniles in the root was observed in late May (3178 and 3013, respectively) and November (19044 and 15486, respectively) (Fig. 1). The nematode data showed that in most cases, the mean number of nematodes in the roots and soil was higher in the inner part of the irrigation drip line (A circle) than in the outer part (B circle) (Fig. 2).

The mean monthly air temperature in the warmest (July) and coldest months (December and January) during 2019-2020 in Fasa was found 31.9 and 8°C, respectively (Farsmet 2020). Mean soil temperature at a depth of 5 to 20 cm under a tree canopy was also estimated 32.7 and 13.2°C in the warmest and coldest months, respectively (Islam *et al*. 2015). Soil temperature was determined 29°C for the first peak of the nematode population in the soil and roots (May). For the second peak of the soil and the root populations (December & November), the above values were 13.2°C and 14.8°C, respectively (Fig. 1).

## Discussion

The citrus root nematode (*Tylenchulus semipenetrans*) is found in most citrus growing regions worldwide and significantly affects the citrus production under different environmental conditions and soil physico-chemical properties (Baines *et al*. 1962, Bello *et al*. 1986, Duncan 2005, Sorribas *et al*. 2008, Rashidifard *et al*. 2015a).

The results of the present study showed that the population of root-dwelling stages of the citrus nematode decreased when soil temperature exceeded 30°C. This is consistent with research showing that the citrus nematode, which invade citrus roots, has its populations decline as soil temperature rises above 30°C (O'Bannon *et al*. 1966).

The higher temperatures during the summer months, especially in June and July, were not conducive to the penetration of J2s into the roots, causing the nematode population to decrease drastically in the present study. However, soil moisture content did not significantly affect *T. semipenetrans*, especially the female population in the roots.

Because the citrus nematode co-evolved with deep-rooted woody plants, these plants can support the nematodes during the dry season by hydraulic upwelling through the root xylem from deeper soil layers to the drier surface soils (Duncan & El-Morshedy 1996). In this study, the nematode did not suffer from water deficit along the rhizoplane, even when the soil was very dry overall. On the other hand, juveniles and males cannot survive in soil under drought conditions. Duncan and El-Morshedy (1996) found that almost no eggs of *T. semipenetrans* hatched after 23 and 37 days of uniform drought. Other researchers have also demonstrated that adequate soil moisture is required for nematode eggs to hatch (Timmer *et al*. 2000). Therefore, along with high soil temperature,

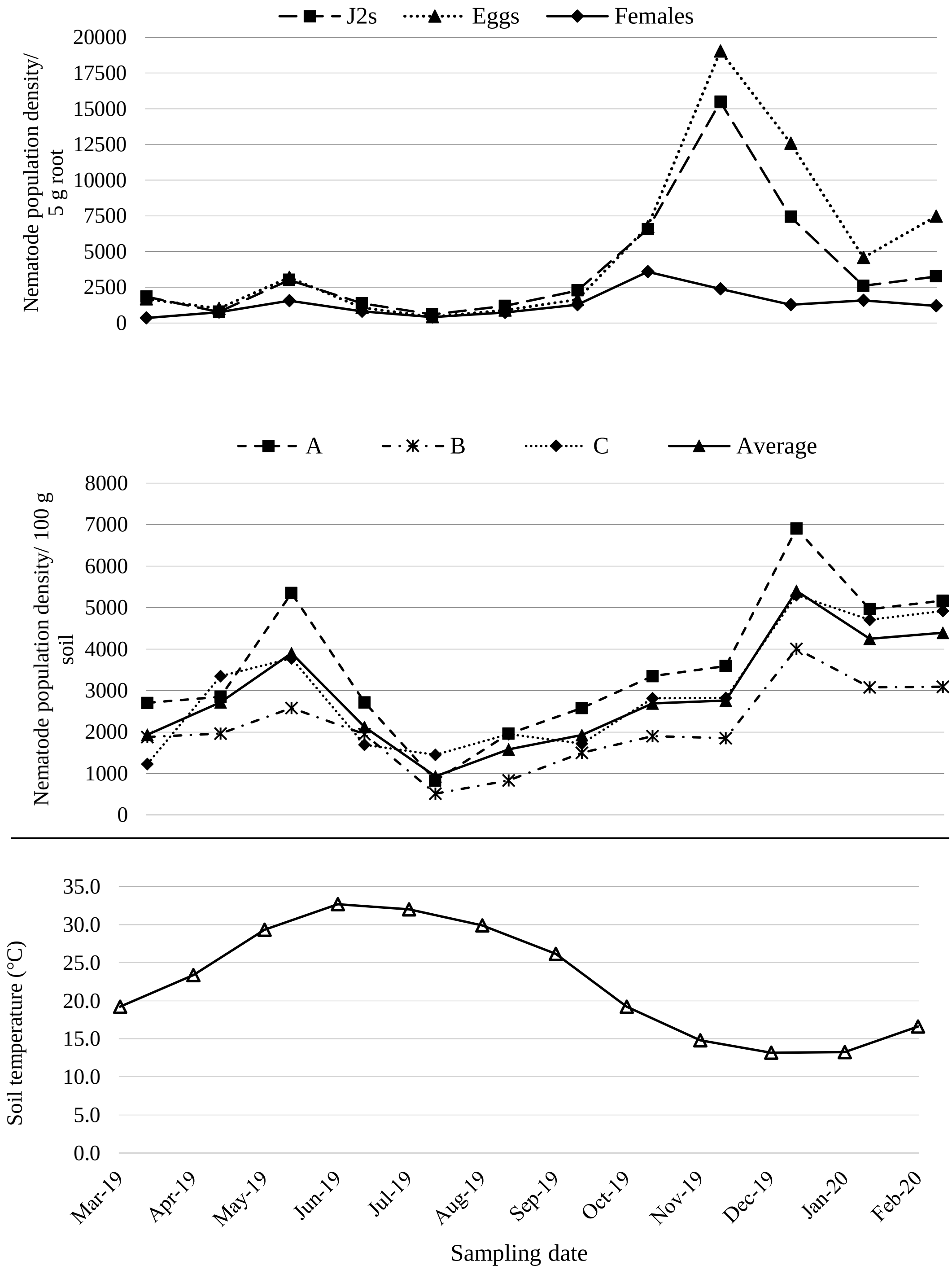
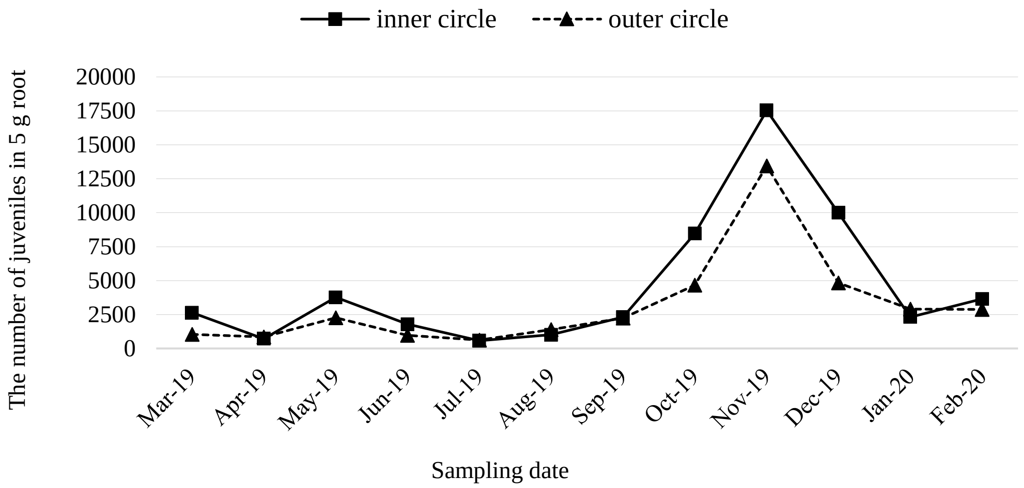
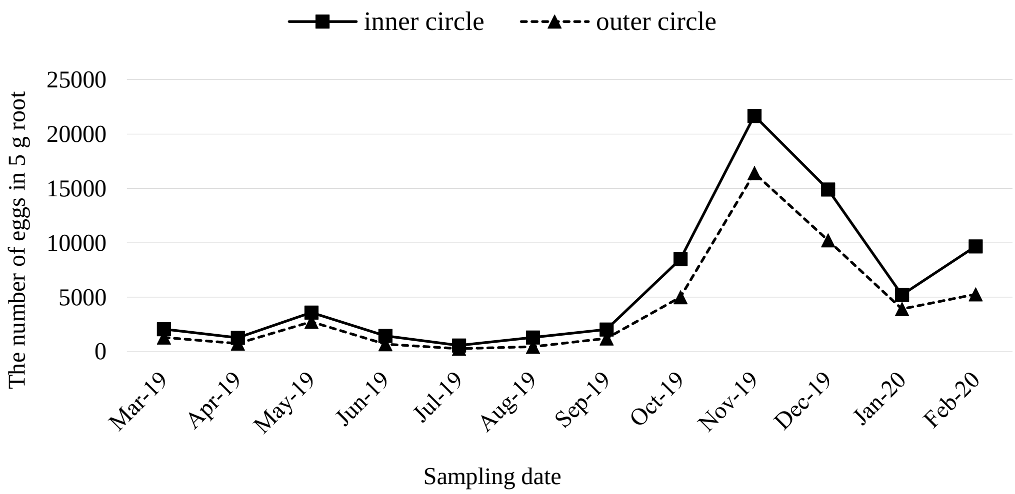



**Fig 1. Monthly fluctuations of the population densities of the citrus nematode *Tylenchulus semipenetrans* in roots (top) and soil (middle) under the canopy of citrus trees in Fasa region, Fars province and its relationship to soil temperature (bottom) in 2019 to 2020. A, B and C are replicates of the experiment, and the nematode population curve data in roots (top) are the mean of replicates A, B and C.**



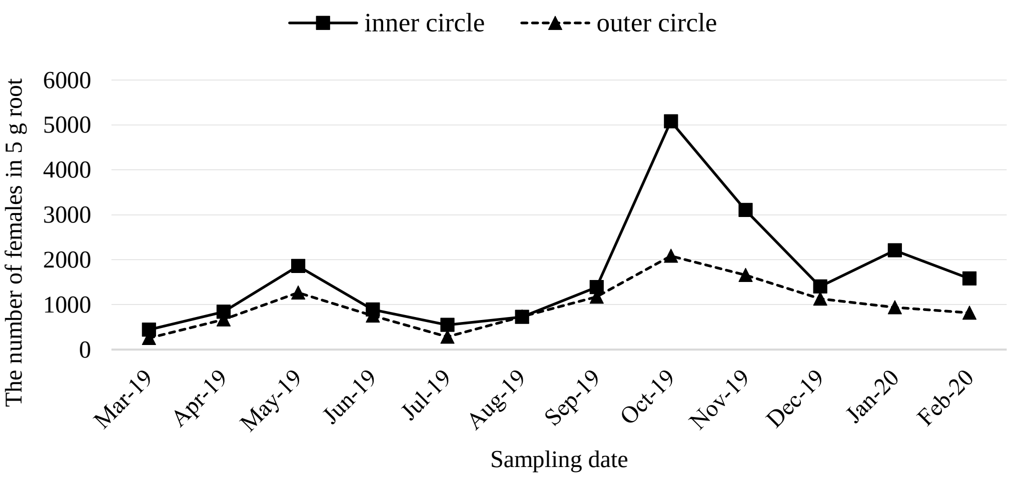




**Fig 2. Monthly fluctuations of the population densities of the citrus nematode *Tylenchulus semipenetrans* in citrus roots in Fasa region, Fars province, from 2019 to 2020. The top, middle, and bottom figures show the populations of juveniles, eggs and females, respectively, in citrus roots.**

inadequate moisture is considered one of the factors contributing to the sharp decline in nematode population (especially eggs, J2s, and males) during the summer in the present study. The marked decrease in the nematode population in June suggests that the roots of citrus plants were affected to a much lesser extent by the nematodes infesting them (July & August) (Fig. 1).

Studies have shown that the phenology of citrus growth also influences nematode population densities. During the fruiting season (summer), the carbohydrate supply in citrus trees is relatively low (Sanz *et al*. 1987), and *T. semipenetrans* may not be able to take up excess carbohydrates until the plant's demands are met. Therefore, carbohydrate competition between developing citrus fruit and the citrus nematode may be an important factor in the population dynamics of the nematode during the fruit production season (Duncan & Eissenstat 1993). Thus, the summer decline in nematode population in the present study could be linked to the high carbohydrate demand of fruit and the reduced carbohydrate reserves in the roots of the plant. However, drought and high soil temperatures are considered key factors for the summer decline in nematode density (both in the soil and in the roots) in our study. The results indicate that soil temperature was above 30°C in summer (Table 1). Furthermore, the temperature tolerance of most plant-parasitic nematodes (PPNs) is low, and they become inactivated at high temperatures (Dwinell 1990). Therefore, the over-summering of PPNs in warm climates, such as the Fasa region, seems more important than their overwintering, as shown in the present study, where a low population of nematodes occurred during the summer (Fig. 1).

The citrus nematode population peaked twice during this study, which can be attributed to nematode generations. The first peak occurred in spring, and the second, which was higher in population, occurred in late autumn and early winter (Fig. 1). During the first peak, soil temperature was 23°C, a temperature favorable for the penetration and development of the nematode (Fig. 1) (O'Bannon *et al*. 1966). In this study, soil temperature was estimated based on air temperature to investigate the relationship between fluctuations in the population of the citrus nematode and soil temperature. The estimated temperatures may slightly differ from actual values; however, this discrepancy does not affect the number or timing of nematode population peaks. The applicability of this approach depends on the objective of the experiment. In certain studies, such as examining the effect of soil solarization in nematode management, this method is not recommended due to the crucial role of both air and soil temperatures in nematode populations. In such cases, directly measuring the temperature of the soil surface and at various depths is essential.

**Table 1. Estimation of mean monthly soil temperature at a depth of 5, 10 and 20 cm, using mean monthly air temperature in Fasa region, Fars province, from 2019 to 2020.**

| | Time (month) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature | Jan-19 | Mar-19 | Apr-19 | May-19 | Jun-19 | Jul-19 | Aug-19 | Sep-19 | Oct-19 | Nov-19 | Dec-19 | Jan-20 | Feb-20 |
| AT | 9.8 | 15.4 | 20.5 | 27.8 | 31.9 | 31.1 | 28.5 | 23.9 | 15.4 | 10.0 | 8.0 | 8.1 | 12.2 |
| ST 5 cm | 12.7 | 17.7 | 22.3 | 28.9 | 32.5 | 31.8 | 29.5 | 25.3 | 17.7 | 12.8 | 11.0 | 11.1 | 14.8 |
| ST 10 cm | 14.5 | 19.2 | 23.5 | 29.6 | 33.1 | 32.4 | 30.2 | 26.3 | 19.2 | 14.6 | 13.0 | 13.0 | 16.5 |
| ST 20 cm | 16.8 | 20.8 | 24.4 | 29.6 | 32.5 | 31.9 | 30.0 | 26.8 | 20.8 | 17.0 | 15.5 | 15.6 | 18.5 |
| Mean ST | 14.6 | 19.2 | 23.4 | 29.3 | 32.7 | 32.0 | 29.9 | 26.2 | 19.2 | 14.8 | 13.2 | 13.3 | 16.6 |

AT: Monthly air temperature (mean) in Fasa region retrieved from Fars Meteorological Office (2022).

ST: Estimated soil temperature (monthly mean) at a depth of 5, 10 and 20 cm using the equations $y = 0.9x + 3.83$, $y = 0.842x + 6.224$, and $y = 0.708x + 9.871$, respectively (Islam *et al*. 2015).

In addition to favorable temperatures during the first peak, the frequent initiation of new fibrous root growth (every 4 to 6 weeks) and high carbohydrate concentration during spring may have played roles as potential triggers for increasing nematode density on the young roots. This situation might be considered the most suitable for citrus nematode penetration and reproduction (Cohn 1964, Hamid *et al*. 1989). The carbohydrate content in citrus fibrous roots begins to increase in autumn and peaks in late autumn and early winter, which coincides with the second peak in the present study (Hamid *et al*. 1985, Duncan & Eissenstat 1993).

Population changes of the citrus nematode were studied in the Khafr region of Fars province, where the highest population density of the nematode was found in October (Sharafeh 1972). Maafi and Kheiri (1991) observed the highest number of J2s of the citrus nematode as two peak populations in spring (May-July) and late summer (September) in Hormozgan province. These results are somewhat consistent with the results of the present study. The peak of *T. semipenetrans* population in soil was recorded in July and August in northern Iran and the lowest in autumn and winter (Tanha Maafi & Damadzadeh 2008). The timing of the peak of nematode populations varies. However, the soil temperature at the time of the first peak in our study and the peak of the citrus nematode population in the north were almost the same (23°C) (Fig. 1 and Table 1). Another difference between the present survey and the northern study (Tanha Maafi & Damadzadeh 2008) was the number of peaks, which was two and one, respectively. The main reason for this discrepancy is probably related to environmental conditions, especially temperature. The average soil temperature in winter was higher in the Fasa region (14°C) than in the study carried out in the north (6.5°C) (Tanha Maafi & Damadzadeh 2008, Farsmet 2020). This provides suitable conditions for the nematode to reproduce, allowing the nematode to reach its second peak in the present study.

It was also reported that the population of citrus nematode increases in winter. A study conducted in South Africa showed that in areas with summer rains, the J2 population of the citrus nematode peaked after each rooting period in spring, summer, and autumn. However, in areas with winter precipitation (e.g., Fasa city), seasonal fluctuations in carbohydrate flux and temperature, seem to be more effective than the timing of rooting. In such regions, the J2 population begins to increase with the onset of precipitation and then peaks in winter (Le Roux 1995).

The results of the present study showed that the slope of the citrus nematode population line was positive (Fig. 1). This means that the nematode population may increase annually followed by a slow decline, the well-known nematode symptom, occurs over time. Citrus root proved to be a safe harborage for nematodes during environmental changes, especially extreme moisture (Le Roux 1995). The lower fluctuation of the root nematode population compared to soil nematodes (Fig. 1) suggests that root nematodes are less affected by environmental conditions such as drought (Duncan & El-Morshedy 1996).

Our results showed that the citrus nematode population decreased with increasing distance from the tree trunk, which is consistent with other studies (Duncan 1986). A direct relationship between nematode counts and root density (Duncan *et al*. 1993) was confirmed by our results as well.

As claimed by other researchers, hatching of the citrus nematode eggs in vitro takes 12-24 days. After that, the hatched J2s were attracted to the root exudates (Timmer *et al*. 2000). After completion of the juvenile stages and formation of immature females, invasion of the root interior and formation of nurse cells occurs. The egg-to-egg life cycle of *T. semipenetrans* on sweet orange seedlings (*C. sinensis*), sour orange (*C. aurantium*) and Troyer citrange (*C. sinensis* × *Poncirus trifoliata*) lasts 6-8 weeks (Van Gundy 1958), 8 weeks, and 9 weeks, respectively (Tanah Maafi & Damadzadeh 2008). However, *T. semipenetrans* can survive as eggs in the absence of citrus trees; in the absence of a host, citrus nematode has been detected in the soil for up to nine years (Van Gundy *et al*. 1967).

It appears that the life cycle of the citrus nematode in Fasa region begins when soil temperatures fall in late summer. Then, surviving eggs from the summer hatch, and in September, favorable conditions such as root exudates attract the J2s to the roots of citrus trees. As mentioned earlier, the life cycle of this nematode lasts between one and two months (Van Gundy *et al*. 1964, Tanah Maafi & Damadzadeh 2008). Thus, invading nematodes complete their life cycle in the roots of citrus plants after September. Consequently, the peak population of root nematodes was detected in the following months (October-November) (Fig. 1). In November, however, not only the soil population did not increase significantly, but in some cases, it even decreased slightly (two out of three replicates; B & C), indicating that the nematode population had invaded the roots at this time. After the females matured, the root nematodes laid their eggs in the soil, so most of the soil nematodes found in December. The newborn individuals then re-invade the citrus roots in December and January. It takes about two months for this peak to complete. Finally, another peak of the total nematode population might be reached in May (Fig. 1).

In summary, the citrus nematode *T. semipenetrans* is capable of completing its life cycle within two months and possibly producing multiple peaks (or generations) per year. However, the phenology of citrus trees and the environmental conditions including soil temperature and moisture, as well as seasonal carbohydrate flux in the roots of citrus trees in Fasa, Fars province were not favorable for the completion of all cycles. Therefore, the nematode completes only two peaks per year. The first peak population occurs in late May and the second in November-December. It is strongly recommended that management measures against this pathogen be implemented during these two periods. The results of this study can be applied in citrus orchards in other areas with similar citrus varieties and environmental conditions.

**Acknowledgement:** The authors greatly acknowledge Shiraz University for providing financial support to carry out this work.

**Conflict of Interest:** The authors declare no potential conflicts of interest pertaining to this submission to Iranian Journal of Plant Pathology..

**References**

Abiverdi C., Izadpanah K. and Sharafeh M. 1970. Plant parasitic nematodes associated with citrus decline in southern Iran. Plant Disease Report 56: 186-188.

Ahmad M. S., Mukhtar T. and Ahmad R. 2004. Some studies on the control of citrus nematode (*Tylenchulus semipenetrans*) by leaf extracts of three plants and their effects on plant growth variables. Asian Journal of Plant Science 3: 544-548. https://doi.org/10.3923/ajps.2004.544.548

Ahmadi K., Ebadzadeh H., Hatami F., Afruzi S., Taleghani R., Yari S. and Kalantari M. 2021. Iranian Agricultural Statistics in 2019-2020 Crop Years, Part III Gardening Products. Agricultural ministry press, Tehran

Ayazpour K. and Ghanaatian A. 2004. Determination of root parasite nematodes of citrus in Jahrom (Fars province, Iran). Master's thesis, Islamic Azad University of Jahrom

Baines R. C., Martin J. P., De Wolf T. A., Boswell S. B. and Garber M. J. 1962. Effects of high doses of D-D on soil organisms and the growth and yield of lemon trees. Phytopathology 52: 723 (abstract).

Bello A., Navas A. and Belart C.1986. Nematodes of citrus groves in the Spanish Levanter Ecological study focused to their control, pp. 217-226. In: R. Cavalloro and E. Di-Martino (Eds). Integrated Pest Control in Citrus groves. Balkema Publishing, South Africa. https://doi.org/10.1201/9781003079279-34

Cohn E. 1964. Penetration of the citrus nematode in relation to root development. Nematologica 10: 594-600. https://doi.org/10.1163/187529264x00240

Duncan L. W. 1986. The spatial distribution of citrus feeder roots and of the citrus nematode, *Tylenchulus semipenetrans*. Revue de Nématologie 9: 233–240.

Duncan L. W. 1999. Nematode diseases of citrus, pp. 136-148. In: L. W. Timmer and L. W. Duncan (Eds). Citrus Health Management. American Phytopathological Society Press, California. https://doi.org/10.1079/9780851997278.0437

Duncan L. W. 2005. Nematode parasites of citrus, pp. 437-466. In: M. Luc, R. A. Sikora and J. Bridge (Eds). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Wallingford, UK. https://doi.org/10.1079/9780851997278.0437

Duncan L. W. and Eissenstat D. M. 1993. Responses of *Tylenchulus semipenetrans* to citrus fruit removal: implications for carbohydrate competition. Journal of Nematology 25: 7–14.

Duncan L. W. and El-Morshedy M. M. 1996. Population changes of *Tylenchulus semipenetrans* under localized versus uniform drought in the citrus root zone. Journal of Nematology 28(3): 360.

Duncan L. W., Graham J. H. and Timmer L. W. 1993. Seasonal patterns associated with *Tylenchulus semipenetrans* and *Phytophthora parasitica* in the citrus rhizosphere. Phytopathology 83(5): 573-581. https://doi.org/10.1094/phyto-83-573

Dwinell I. D. 1990. Heat treatment southern yellow pine lumber to eradicate *Bursaphalenchus xylophilus*. Nematologica 36: 346-347.

Fars Meteorological Bureau (2020) Farsmet: agricultural meteorology. http://www.farsmet.ir/DM/Files/files/9.jpg. Accessed 04 August 2022

Food and Agriculture Organization of the United Nations (2023) FAOSTAT Crops and livestock products. https://www.fao.org/faostat/en/#compare. Accessed 12 April 2023

Galeano M., Verdejo-Lucans S., Sorribas F. J., Ornat C., Forner J. B. and Alcaide A. 2003. New citrus selections from Cleopatra mandarin *Poncirus trifoliata* with resistance to *Tylenchulus semipenetrans* Cobb. Nematology 5: 227-234. https://doi.org/10.1163/156854103767139725

Hamid G. A., Van Gundy S. D. and Lovatt C. J. 1985. Citrus nematode alters carbohydrate partitioning in the 'Washington'navel orange. Journal of the American Society for Horticultural Science 110: 642-646. https://doi.org/10.21273/jashs.110.5.642

Hamid G. A., Van Gundy S. D. and Lovatt C. J. 1989. Phenologies of the citrus nematode and citrus roots treated with oxamyl, pp. 993-1004. In: Proceedings of the sixth international citrus congress Middle-East, March, 1988. Margraf Scientific Publishers, Israel.

Inserra R. N., Duncan L. W., O'Bannon J. H. and Fuller S. A. 1994. Citrus nematode biotypes and resistant citrus rootstocks in Florida. Nematology 205: 1-4.

Inserra R. N., Vovias N. and O'Bannon J. H. 1980. A classification of *Tylenchulus semipenetrans* biotypes. Journal of Nematology 12: 283-287.

Inserra R. N., Vovlas N., O'Bannon J. H. and Esser R. P. 1988. *Tylenchulus graminis* n. sp. and *T. palustris* n. sp. (Tylenchulidae) from native flora of Florida, with notes on *T. semipenetrans* and *T. furcus*. Journal of Nematology 20: 266-287.

Islam K. I., Khan A. and Islam T. 2015. Correlation between atmospheric temperature and soil temperature: A case study for Dhaka, Bangladesh. Atmospheric and Climate Sciences 5(03): 200. https://doi.org/10.4236/acs.2015.53014

Izadpanah K. and Safarian A. 1968. Possible role of citrus nematode (*Tylenchulus semipenetrans* Cobb) in the citrus decline in southern Iran, pp. 40-41. In: Proceeding of the 1st National Congress of plant protection of Iran, September, 1968. Iranian Research Institute of Plant Protection, Iran.

Kabata-Pendias A. 2000. Trace Elements in Soils and Plants. 3rd ed., CRC Press, Florida. 432 p. https://doi.org/10.1201/9781420039900-8

Kaplan D. T. 1981. Characterization of citrus rootstock responses to *Tylenchulus semipenetrans* (Cobb). Journal of Nematology 13: 492-498.

Le Roux H. F. 1995. Control of the citrus nematode in South Africa. Dissertation, Pretoria University

Lee R. F., Lehman P. S. and Navarro L. 1999. Nursery practices and certification programs for bud wood and rootstocks. Citrus health management 35-46.

Ling P., Duncan L. W., Deng Z., Dunn D., Hu X., Huang S. and Gmitter F. G. 2000. Inheritance of citrus nematode resistance and its linkage with molecular markers. Theoretical Applied Genetics 100: 1010-1017. https://doi.org/10.1007/s001220051382

Martin J. P. and Van Gundy S. D. 1963. Influence of soil phosphorous level on the growth of sweet orange seedlings and the activity of the citrus nematode (*Tylenchulus semipenetrans*). Soil Science 96: 128-135. https://doi.org/10.1097/00010694-196308000-00009

McSorley R., Parrado J. L. and Dankers W. H. 1984. A quantitative comparison of some methods for the extraction of nematodes from roots. Nematropica 14: 72-84.

Maafi Z. T. and Kheiri A. 1991. Citrus nematode (*Tylenchulus semipenetrans*) in Hormozgan province. Iranian Journal of Plant Pathology 27: 31-42 (in Persian with English Summary)

Minassian V. and Moadab H. 1970. The occurrence and distribution of citrus nematode *Tylenchulus semipenetrans* Cobb in Khuzestan, Iran. Iranian Journal of Plant Pathology 6: 25-28. (In Persian with English summary)

Navas A., Nombela G. and Bello A. 1992. Caracterización de la modalidad de distribución de *Tylenchulus semipenetrans* en elevate Española. Nematropica 22: 205-216.

O'Bannon J. H., Reynolds H. W. and Leathers C. R. 1966. Effects of temperature on penetration, development, and reproduction of *Tylenchulus semipenetrans*. Nematologica 12: 483–487.

Pretorius M. C. and Le Roux H. F. 2017. Nematode pests of citrus, pp. 311-32. In: H. Fourie, V. W. Spaull, R. K. Jones, M. S. Daneel and D. De Waele (Eds). Nematology in South Africa: a view from the 21st century. Springer International Publishing, New York. https://doi.org/10.1007/978-3-319-44210-5_13

Rashidifard M., Shokoohi E., Hoseinipour A. and Jamali S. 2015a. *Tylenchulus semipenetrans* (Nematoda: Tylenchulidae) on pomegranate in Iran. Australasian Plant Disease Notes 10(1): 1-6. https://doi.org/10.1007/s13314-014-0157-7

Rashidifard M., Shokoohi E., Hoseinipour A. and Jamali S. 2015b. Distribution, morphology, seasonal dynamics, and molecular characterization of *Tylenchulus semipenetrans* from citrus orchards in southern Iran. Biologia 70: 771-781. https://doi.org/10.1515/biolog-2015-0098

Rumiani M., Hamhehzarghani H., Karegar A. and Ghaderi R. 2021. Optimization of citrus tree sampling pattern for estimating population of citrus nematode in the soil of infested orchards in Fars province, southern Iran. Crop Protection 142: 105523. https://doi.org/10.1016/j.cropro.2020.105523

Salahi Ardakani A., Tanha Mafi Z., Mokaram Hesar A. and Mohammadi Goltappeh E. 2014. Relationship between soil properties and abundance of *Tylenchulus emipenetrans* in citrus orchards, Kohgilouyeh va Boyerahmad province. Journal of Agricultural Science and Technology 16: 1699-1710.

Sanz A., Monerri C., Gonzlez-Ferrer J. and Guardiola J. E. 1987. Changes in carbohydrates and mineral elements in Citrus leaves during flowering and fruit set. Physiologia Plantarum 69: 93-98. https://doi.org/10.1111/j.1399-3054.1987.tb01950.x

Sharafeh M. 1972. Preliminary study on population dynamics of the citrus nematode, *Tylenchulus semipenetrans* Cobb, in Khafr, an important citrus growing region of Fars. Applied Entomology and Phytopathology 33: 26-30.

Sorribas F. J., Verdejo-Lucas S., Pastor J., Ornat C., Pons J. and Valero J. 2008. Population densities of *Tylenchulus semipenetrans* related to physicochemical properties of soil and yield of clementine mandarin in Spain. Plant Disease 92: 445-450. https://doi.org/10.1094/pdis-92-3-0445

Tanha Maafi Z. and Damadzadeh M. 2008. Incidence and control of citrus nematode *Tylenchulus semipenetrans* Cobb in north of Iran. Nematology 10: 113-122. https://doi.org/10.1163/156854108783360096

Tanha Maafi Z., Amani M., Stanley J. D., Inserra R. N., Van Den Berg E. and Subbotin S. A. 2012. Description of *Tylenchulus musicola* sp. n. (Nematoda: Tylenchulidae) from banana in Iran with molecular phylogeny and characterization of species of Tylenchulus Cobb, 1913. Nematology 14: 353-369. https://doi.org/10.1163/156854111x596242

Timmer L. W., Garnsey S. S. M. and Graham J. H. 2000. Compendium of citrus diseases. 2nd ed., The American Phytopathological Society, USA. 92 p.

Van Gundy S. D. 1958. The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb. Nematologica 3: 283–294.

Van Gundy S. D., Bird A. F. and Wallace H. R. 1967. Ageing and starvation of larvae of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. Phytopathology 57: 571–599.

Van Gundy S. D., Martin J. P. and Tsao P.H. 1964. Some soil factors influencing reproduction of the citrus nematode and growth reduction of sweet orange seedlings. Phytopathology 54: 294-299.

Verdejo-Lucas S., Galeano M., Sorribas F. J., Forner J. B. and Alcaide A. 2003. Effect on resistance to *Tylenchulus semipenetrans* of hybrid citrus rootstocks subjected to continuous exposure to high population densities of the nematode. European Journal of Plant Pathology 109: 427–433

Whitehead A. G. and Hemming J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology 55: 25-38. https://doi.org/10.1111/j.1744-7348.1965.tb07864.x