Survey of Citrus Nematode *Tylenchulus semipenetrans* Causing Citrus Slow Decline in Karbala Province of Iraq

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Abstract

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The study aimed to determine the rate and severity of infection with citrus nematodes associated with the citrus slow decline in orchards and nurseries in different regions of Karbala governorate, including Aoun, Hay Al-Amel, Al-Haidariat, Al-Hurr Al-Saghir, Al-Bubiyat, Al-Hussainiya, and Al-Hindiya. The nematodes associated with citrus slow decline were isolated and phenotypically diagnosed based on microscopic examination of the infected stained roots, and juveniles in the soil based on the phenotypic characteristics of the citrus nematodes. Infestation rate in citrus orchards was assessed, and the severity of infestation was determined based on the number of females in 2 gm roots and the number of juveniles in 100 gm of the rhizospheric soil surrounding the roots. The causes of infection dissemination and the factors that contribute to the increase of infection in the severely affected orchards were investigated. The results of the field survey showed the presence of citrus nematodes infestation in all orchards and in all regions. The incidence and severity of citrus nematode infection differed between the different sites. It was found that the severity of infection was significantly higher with older trees compared to newly established orchards with young plants. The results of the questionnaire accompanying the field survey showed that most citrus orchards were not subject to nematode control practices. In addition, most farmers were not aware of the importance of plant parasitic nematodes and the damage they can cause, especially citrus nematodes.

Keywords: Citrus nematode, *Tylenchulus semipenetrans*, survey, Iraq.

Introduction

Citrus production in the tropics and subtropics is located between latitudes 40 north and 40 south of the equator. Citrus fruits production is ranked third after grapes and apples, and sweet oranges occupy the first place in the world among citrus species (Gamal & Susu, 2009). Iraq's production of oranges in 2020 amounted to 142,717 tons, with an average productivity of 22.4 kg per tree for the winter season. The highest production rate was in the governorates of Salah al-Din, Baghdad and Diyala, respectively (Central Statistical Organization, 2020).

Citrus trees are attacked by viral, fungal, bacterial pathogens, and parasitic plant nematodes, including citrus slow decline caused by the citrus nematode *Tylenchulus semipenetrans* (Cobb, 1913). Citrus nematodes are one of the most important nematodes specialized on citrus, infecting more than 80 citrus species and varieties. Yet, no citrus cultivar is reported to be immune or non-host for the citrus nematodes. However, the susceptibility of different citrus cultivars to infection is variable *T. semipenetrans* was reported to infects 19 species and 21 hybrids of citrus, in addition to persimmon, olive, grape and lilac (Sharif, 2012). In the last two decades, citrus slow decline was widely spread in Iraq's orchards and nurseries by more than 95% and the severity of infection reached 250,000 juveniles/kg of soil, with a root size reduction of 80% compared to healthy roots (Sharif, 2012). The slow decline of citrus trees in Iraq was first recorded during the period 1966-1969, and a recent survey conducted by the Abu Ghraib Department of Plant Diseases showed that these nematodes suggest that they are widespread on citrus in all regions of Iraq (Luqaa, 2021)

Trees infected with citrus nematodes at high density are weak and small in size, with yellowish leaves that fall off early, and the terminal branches usually showing die-back symptoms (Luqaa, 2021). Lesions appear on the roots in a dark brown color due to the adhesion of the soil granules to the gelatinous egg masses. The adhesion of the clay to the roots increases with increase of infections and egg masses, and washing with a slight stream of water may lead to the separation of root cortex from the vascular cylinder. The nematode infection may be associated with some pathogenic fungi, and bacteria causing root rot. On stained infected roots, the nematode female appears clear with their posterior part prominent on the surface of the roots. Usually, symptoms of slow decline appear on the affected trees, 3-5 years post infection. The branches may become completely bare of leaves, which greatly affects the quantity and size of fruits (Hofmann *et al.*, 2020). Because Karbala is famous for its citrus cultivation, and for the lack of studies on the nematodes causing citrus slow decline in Karbala, this survey was carried out to investigate the causes of citrus trees decline, assess the rate and severity of infection in the affected orchards, and to determine the factors that leads to wide spread of infection.

Materials and Methods

Field survey

The field survey was conducted during the fall season of 2021 and included seven regions in the Karbala governorate,

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namely Imam Aoun district, Al-Amel neighborhood (Manzel gardens), Al-Haidriat orchards, Al-Hur Al-Sagheer nurseries, Al-Boubiat orchards, Al-Hussainiya orchards, and Al-Hidyah district orchards. The samples were collected randomly, five samples for each region. Soil samples were collected together with a quantity of citrus nutritious roots (five trees or seedlings for each type of citrus). The survey included gathering data on citrus varieties, methods and source of irrigation water, type of fertilization, control method used, farmer's awareness of the plant nematode problem, and the level of production in the sampled orchards (Table 1). Soil and roots samples were collected from under plant canopy, especially from plants that showed symptoms of yellowing and weak growth, dead and stiff branches, and small size of fruits (Figure 1-A). Samples were placed in polyethylene bags, tagged and brought to the laboratory. The samples were stored at 5 C, then processed and examined within three days (Van Bezooijen, 2006).

Samples from infected roots (Figure 1-A) were washed, cut into small pieces of about 1 cm and stained by the acid Fuchsin method (Bird & Wallace, 1965). The roots were examined using a stereo microscope (Figure 1-B). The infection rate was calculated in the presence and absence of citrus nematodes, and the severity of infection was calculated according to the equation of McKinney (1923) based on the number of females per 1 g roots (Figure1-C and D).

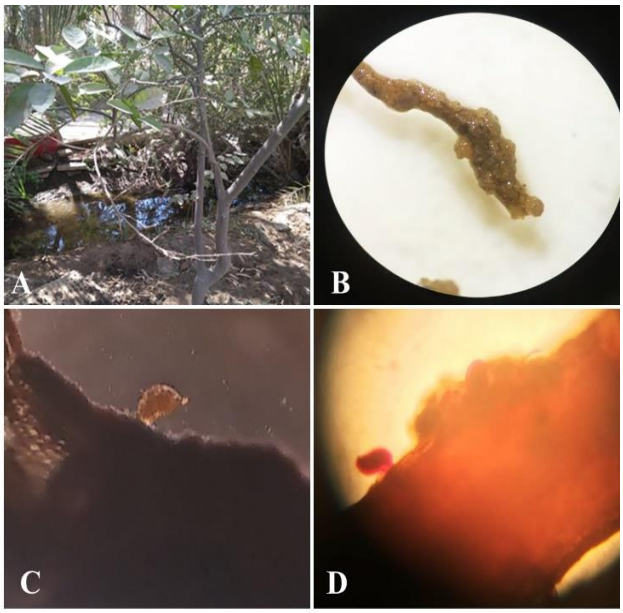

Figure 1. (A) a citrus tree showing yellowing of leaves and die-back, (B) adhesion of clay to egg masses attached to roots infected with *T. semipenetrans*, (C & D) *T. semipenetrans* adult female feeding on citrus nutrient roots.

Results and Discussion

Field survey results showed the presence of nematodes associated slow citrus deterioration and the prevalence of soil infestation with nematodes in all surveyed regions at different rates of infestation. The results obtained (Table 2) showed that the highest infection rate was recorded in the home gardens of Al-Amel neighborhood and the orchards of Al-Hindia district, with an infection rate of 83.3%, followed by Aoun 66.6%, Al-Hussainiya 60%, then the orchards of Al-Boubayat 51.5% and Al-Haidriat 40%, compared to the lowest infection rate of 10% in Al-Hurr Al-Sagheer region.

Findings of this survey were in agreement with previous studies and confirmed infection with *T. semipenetrans* which led to various symptoms ranged from the branches die back to small size fruits and bark cracking and dryness (Jaiman *et al.*, 2022). The reason for the variation in infection rates from one site to another is due to several factors, the most important of which is the variation in the age of the plants. In the houses, the age of the trees was large compared to what is found in the Aoun, Amel and Al-Hurr Al-Sagheer regions nurseries, where seedlings age did not exceed one to three years. This led to the nematodes being able to produce many generations on the roots of large trees due to the availability of the feeder roots, on which the nematodes feed more abundantly than in young seedlings (Al-Hakim & Abdel-Rasoul, 2009). Abu-Gharbieh & Al-Azzeh (2004) indicated that the number of nematodes varied, and they have a positive intrinsic relationship to the density of the nutrient roots and their starch content.

The results of the field survey in the nurseries of the Al-Hurr Al-Sagheer region showed that the workers deliberately expose the seedling soil to drying and solarization before the soil is used for cultivation, an approach used to reduce the number of nematodes in the soil. It was also found that workers also use insecticides, fungicides, nematicides and herbicides as preventive and curative sprays, and this is another reason for the decrease or absence of nematodes at this site (Al-Hazmi, 2009). Such measures in combating non-nematode causes lead to a reduction in opportunistic fungi and bacteria that attack the plant after infection with nematodes including root-knot, lesion and citrus nematodes.

The irrigation method adopted in the low-infested areas was drip and sprinkler irrigation, compared to the high infestation areas in home gardens where they apply flooding. Workers in the same low infested area were using diversified fertilization methods with mixtures of animal manure and chemical fertilizers. Bakker *et al*. (2006) indicated that the incidence of nematodes was lower under drip irrigation, and that proper management of irrigation and fertilization can give positive results in controlling the damage resulting from nematode infection. This is consistent with the low incidence of infection observed in the two regions of Al-Amel and Al-Hindiya, compared to Al-Haidriat region, which suffers from high humidity due to poor irrigation and absence of control. Dry soils restrict the movement of nematodes, and the disease symptoms are exacerbated by rising ground water level and increasing salinity and organic matter content (Sharif, 2012). In general, the study indicated that high ground water level and increased humidity did not seem to encourage nematode reproduction, but rather negatively affect plant growth and thus contribute to reduced plant resistance to nematodes. Nematodes are also negatively affected by increased humidity, and the host-range was limited to perennial plants with shallow roots. Abu-Gharbieh *et al*. (2010) indicated that adding organic matter to the soil affects the density and survival of nematodes. This is either directly, by the action of its intermediate decomposition

products that are toxic to nematodes, especially the resulting gases and fatty acids such as butyric acid, or indirectly by encouraging the growth and reproduction of the natural enemies of nematodes, especially fungi. The addition of organic matter leads to increased plant growth and thus increases its tolerance to infection. Organic matter works to bring about natural and chemical changes in the soil and changes the physiology of the host plant, making it more resistant to nematodes. Furthermore, organic matter provides nutrients to the plant and increases its growth, which reduces the negative effects of nematodes (Al-Hazmi, 2009).

Table 1. Data collected from the field survey of citrus nematodes *T. semipenetrans* in different regions of Karbala province in Iraq.

Sample region (Region)	Type of citrus	Fertilizer used	Irrigation method	Water source	Awareness of infection presence	Level of production	Control practices
Aoun area	Sweet, sour orange, lemon, mandarin	Manure	Flood	River	No	Low	Yes
Al-Amel neighborhood	sour orange	Chemical and manure	Hose	Tap	No	Average	No
Haidriyat	Grafted, regular, sour orange	Multiple	Flooding	Drainage	No	Very good	Yes
Al-Hurr Al-Sagheer	Grafted, regular, sour orange, mandarin	Multiple	Regular	River	No	Good	No
Al-Bobiatt	Orange grafted cuts saplings, sour orange	Mix	regular	Well	yes	low	Yes
Al-Husayniyah	Sour, regular orange	Mix	Flooding	Well	No	Average	No
Hindia	Sour, regular orange	Mix	Spray	Well	No	Average	Yes

Table 2. Infection rate and severity of citrus nematodes *T. semipenetrans* in different regions of Karbala province in Iraq.

Sampling region (location)	No. of tree samples	No. of infected trees	Infection rate %	Infection severity (No. of female/1g root)	Infection severity (%)
Aoun	15	10	66.6	22.0	53.30
Al-Amel neighborhood	6	5	83.3	27.6	83.33
Haidriyat	250	100	40.0	13.3	19.20
Al-Hurr Al-Sagheer	50	5	10.0	3.3	1.20
Al-Bobiatt	37	17	51.5	16.6	28.40
Al-Husayniyah	10	6	60.0	20.0	43.20
Hindia	6	5	83.3	27.6	83.30

الملخص

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أجريت هذه الدراسة بهدف تحديد نوع النيماتودا التي أدّت الى تدهور الحمضيات/الموالح في بساتين ومناطق محافظة كربلاء، والتي شملت المناطق التالية: الحسينية، البوبيات، الحيدريات، عون، الهندية، الحر الصغير وحيّ العامل. تمّ عزل النيماتودا المسببة لتدهور الحمضيات وتشخيصها من خلال الفحص المجهري ومطابقتها للصفات المظهرية/المورفولوجية لنيماتودا الحمضيات، وتقييم نسبة الإصابة في البساتين المصابة، بالإضافة للبحث في أسباب انتشار الإصابة والعوامل المساعدة على انتشارها. أظهرت نتائج المسح، بالاعتماد على التشخيص المظهري، أن جميع العينات المأخوذة من جذور نباتات الحمضيات/الموالح كانت مصابة، وفي جميع المناطق، ولكن بنسب متباينة من موقع لآخر. بيّنت نتائج الاستبيان المرافقة للمسح الحقلي أن اغلب بساتين الحمضيات/الموالح لاتخضع لعمليات مكافحة، فضلاً عن عدم معرفة المزارعين بأهمية نيماتودا النبات المتطفلة، وخصوصاً نيماتودا الحمضيات/الموالح (نيماتودا التدهور البطيء).

كلمات مفتاحية: نيماتودا الحمضيات، *Tylenchulus semipenetrans* ، مسح حقلي، العراق.

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