Citrus Nematode in Fruit Crops and Their Management by Biological and Biotechnological Interventions

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Abstract

One of the most common fruit crops cultivated worldwide is citrus, which is also a significantly traded horticulture product. In places of the world where citrus is grown, *Tylenchulus semipenetrans*, one of the main nematode pest that parasitizes plants, significantly reduces yields in the world where citrus is grown. The management of plant parasitic nematodes in citrus can be done alternatively by using biological control because of its lower toxicity to the environment, specificity of the target, and safety for nontarget organisms. Even though various bacteria, mites, and fungi have been employed to reduce *T. semipenetrans* population in citrus, a dedication to the creation of high-quality products, extension programs, and industrial partnerships will help to promote the widespread use of biological control agents.

Keywords

Nematode · Biological control · Citrus · Management

20.1 Introduction

One of the most popular fruit crops and a significant horticulture traded commodity in the globe is citrus (Matheyambath et al. 2016). Oranges account for 55% of all citrus production worldwide, followed by 25% mandarins, 13% lemons, and 7%

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grapefruits (Global Citrus Outlook 2019). One of the main reasons restricting citrus production globally is plant-parasitic nematode infection. Since citrus crops become perennial, they nourish and encourage nematode population growth throughout the year around (Reddy 2018). There are many plant nematodes associated to the citrus rhizosphere; however, only a small number of species affect the trees (Khan 2023). *Tylenchulus semipenetrans, Pratylenchus coffeae, Radopholus citrophilus*, and *Meloidogyne indica* are among the groups of plant-parasitic nematodes that significantly reduce citrus crop yields worldwide (Kumar and Das 2019; Duncan 2009; Verdejo-Lucas and McKenry 2004).

The main pathogenic species almost every region where citrus is grown in the world is the citrus nematode, *T. semipenetrans*. The young adult females enter the cortical cortex more deeply, settle down, and create nurse cells, which serve as a permanent feeding site and food sink for the nematode (Khan 2008). Depending on the level of infestation, yield losses brought on by this nematode are predicted to range from 10% to 30% globally (Verdejo-Lucas and McKenry 2004). However, researchers estimate those orchard infestations in many regions of the world range from 50% to 90% due to insufficient regulatory exclusion measures (Sorribas et al. 2008, 2000; Maafi and Damadzadeh 2008; Iqbal et al. 2006; de Campos et al. 2002). Citrus is slowly declining as a result of it, and it is also responsible for other complexities like citrus dieback. The age and health of the tree, the nematode population density, and the rootstock's vulnerability all have a role in how much damage a nematode infection causes (Ravichandra 2014). Some of the symptoms that are noticeable include chlorosis, leaf defoliation, smaller fruit, fruit loss before maturity, and twig dieback from above branches. In contrast to healthy roots, the branch rootlets on the infected feeder roots are shorter, darker, and covered in soil (Abd-Elgawad 2020; Duncan 2009).

The most effective preplant nematicides employed in citrus nurseries and orchards against *T. semipenetrans* were fumigants such as 1,3-dichloropropene, metham sodium, and methyl bromide (Shokoohi and Duncan 2018). Due to their toxicity to the environment and negative effects on human health, many pesticides have been taken off the market. In some cases, using resistant rootstocks to control *T. semipenetrans* has been effective (Verdejo-Lucas and McKenry 2004; Verdejo-Lucas et al. 2000; Gottlieb et al. 1987; Kaplan and O'Bannon 1981), but these hybrids perform poorly in alkaline soils, and over time, resistance-breaking biotypes were developed (Abd-Elgawad 2020). Citrus plant-parasitic nematodes can be managed through biological control since these are less hazardous to the environment, more particular in their target species, and safe for nontarget creatures. The world's citrus-growing regions have been subject to biological control methods based on fungi, bacteria, and mites or their bioactive components.

The current chapter examines the potential for biological control agents in citrus to evolve in the future and provides an overview of the biological control agents now being used in citrus to combat *T. semipenetrans*.

20.2 Biological Control of *T. semipenetrans* in Citrus

Biocontrol fungi/bacteria alone (Stirling 1991; Khan 2007; Khan and Anwer 2011) or along with oil, neem cakes (Sikora and Roberts 2018; Khan et al. 2021) or pesticides (Mohiddin and Khan 2013) are getting popularity in achieving sustainable nematode management in agricultural crops (Khan 2023; Khan et al. 2023). The microbial antagonists, *Aspergilus niger, Pochonia chlamydosporia, Purpureocellium lilacinum, Pasturia penetrans* etc. (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016), and phosphate solubilizing microorganisms such as *Aspergillus, Bacillus, Penicillium, Pseudomonas* etc. (Khan et al. 2009, 2016a, b; Sikora and Roberts 2018) may significantly contribute in the sustainable management of plant nematodes.

20.2.1 Fungi

20.2.1.1 *Trichoderma* spp.

As a biocontrol agent, *Trichoderma* spp. has been utilized extensively against plant pathogens like bacteria, plant and soil nematodes, and fungus. The chitinases, glucanases, and proteases generated by fungi are crucial in the fight against diseases (Sharon et al. 2001). In recent years, *Trichoderma* has also been found effective in suppressing plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018). Various species of *Trichoderma* were employed to combat citrus nematodes. The effectiveness of *Trichoderma* spp. has been established in numerous experiments carried out under various circumstances. According to Narendra et al. (2008), when *T. harzianum* (4 kg/soil) was applied to *C. jambhiri* under pot conditions, the juvenile and female populations of *T. semipenetrans* were reduced by 30.58% and 64.85%, respectively, in comparison to the untreated control. The commercial formulations of *Trichoderma* spp. are available in market (Khan et al. 2011), which are quite effective against soil nematodes and other pathogens (Mohammed and Khan 2021; Sikora and Roberts 2018; Shahid and Khan 2019).

T. hamatum, however, significantly reduced the amount of delicious orange under greenhouse conditions (86.68% and 61% at 3×10^8 spore/mL, respectively) (Hanawi 2016). While applying *T. harzianum* (3×10^8 spore/mL) to citrus *cv.* volkameriana resulted in the highest control (91.1%) in J_2 population compared to other treatments, according to Montasser et al. (2012). The same findings were reported by Shawky and Al-Ghonaimy (2015) who found an 86.3% decrease in *T. semipenetrans* J_2 on volkameriana seedlings when *T. harzianum* was administered at the highest rate (5×10^8 cfu/pot). According to recent studies, combining *T. harzianum* with Nemastop (natural oils) boosted the mortality rate of *T. semipenetrans* juveniles from 46% to 80% in vitro experiments (Ibrahim et al. 2019).

Based on field trials on sweet orange, *T. viride* (3×10^8 spores/mL) decreased J_2 and female *T. semipenetrans* populations by 64.9% and 44.8%, respectively (Hanawi 2016). While a month after, *T. harzianum* (5×10^8 cfu) was applied to volkameriana. According to Shawky and Al-Ghonaimy (2015), citrus nematode in the soil as well as roots had decreased by 55%. However, 4 months after application in the field, a striking suppression (72%) in the nematode population was seen in comparison to untreated control plots. Ibrahim et al. (2019) investigated the effectiveness of *T. harzianum* alone or combined with nemastop (natural oils), other biocontrol agents, and chemical pesticide to maintaining citrus nematode under control on Washington navel orange trees in Menia EL-kamh, Sharkia governorate, Egypt (Nemaphos). After 12 months of treatments in the field, *T. harzianum* mixed with Nemastop and when applied as a soil drench (500 mL/tree), compared to 33.1% for Nemaphos and 35.85% for *T. harzianum* alone, it caused a 51.7% reduction in the number of nematodes.

More successful nematode control was achieved by combining hostile bacteria with agricultural waste, such as compost, than by employing only one microbial strain or compost. It was reported that *T. semipenetrans* population density was less in the soil and roots by the application of *T. harzianum* mixed with neem, karanj, and castor oil cakes, and acid lime seedling growth was found to be boosted (Reddy et al. 1996). El-Mohamedy et al. (2016) reported that the population of citrus nematode that developed on sour oranges under greenhouse conditions decreased from 0.73 to 0.80 after the application of compost containing either *T. harzianum* or *T. viride* (1×10^6 cfu/mL) to 0.38 and 0.41, respectively. Similar results were achieved by combining compost with *T. viride* or *T. harzianum* at the same rate, which led to a lower nematode population (0.40, 0.42) as a comparison to the fungal cultrate alone (1.1, 0.76) in volkameriana (Hammam et al. 2016).

20.2.1.2 *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*)

This fungus can parasitize citrus nematode eggs, egg masses, and females (Kumar 2020). Seven different *P. lilacinus*-based treatments are utilized globally to control citrus nematodes at different phases of their life cycles. According to Maznoor et al. (2002), the application of *P. lilacinus* (8 g/kg soil) made with rice bran-reduced nematode populations on khasi mandarin in India by 64.4% compared to nematode populations reduced by formulations with mustard oil cake (63.9%). However, in terms of nematode population decrease, the bioefficacy of the fungi developed in both environments was comparable. While Narendra et al. (2008) reported that when *P. lilacinus* (4 kg/soil) was applied to *C. jambhiri* plant, J_2 and the female population of *T. semipenetrans* were significantly reduced (64.7% and 75.7%, respectively), compared to the control under pot conditions. When *P. lilacinus, T. harzianum*, and *G. fasciculatum* were all applied together, the population of *T. semipenetrans* was decreased by 73.04% and 89.08%, respectively. This helped *C. jambhiri* plants grow more quickly. Similar to this, applying 10 g of *P. lilacinus*, 10 g of *Pseudomonas fluorescens*, and 250 g of neem seed cake per tree once every 6 months for 2 years decreased the plant nematodes and increased the yield (30.24 kg/tree) in comparison to control (17.20 kg/tree) (Rao 2008). In future, market growth for *P. lilacinus*-based commercialization products manages the domestic citrus nematode strains. Verdejo discovered 20 fungal strains from citrus rhizosphere in Spain, among them

P. lilacinus and *Talaromyces cyanescens* showed promising against citrus nematode infesting Carrizo citrange and Cleopatra mandarin in greenhouse conditions.

20.2.1.3 *Pochonia chlamydosporia*

P. chlamydosporia-based talc formulation was applied to the soil, Kumar and Prabhu (2009) claim that this resulted in a considerable reduce citrus nematode after 30 days over control (52.5, 9.5, respectively), under nursery conditions. As *P. chlamydosporia* (20 g/tree) was applied in orchard, Deepa et al. (2011) reported that the population of citrus nematode decreased by 42.76% when compared to untreated controls. Successful reduction of the *M. javanica* infects root gall in nursery by adding *P. chlamydosporia* and *P. lilacinus* to the soil @ 5 and 10 g/kg, respectively (Rao 2005).

20.2.1.4 *Mycorrhizae* (*Glomus* spp.)

These are the obligate root symbionts, which increase nutrient intake to promote plant growth and reduce plant stress brought on by nematodes that parasitize plants (Schouteden et al. 2015; Vos et al. 2012). Nematode and mycorrhizal fungal interactions depend on the association of plant cultivars, fungi, and nematode species and appear to be highly particular (Ingham 1988). In a preliminary greenhouse investigation, rough lemon seedlings grew more quickly than nonmycorrhizal seedlings after being transplanted into soil contaminated with *Glomus mosseae* and infected with *T. semipenet*rans (O'Bannon et al. 1979). *Radopholus citrophilus*, a citrus-burrowing nematode, was later found to have lower population densities in mycorrhizal-infested or nonmycorrhizal, high-phosphorus plants than in nonmycorrhizal, low-P plants of rough lemon. However, there was no discernible difference in the seedlings' growth. According to Reddy et al. (1995), citrus nematode was successfully controlled after *G. fasciculatum* was treated with neem cake in nursery. The soil treatment of *G. fasciculatum* (@ 500 spores/kg soil) reduced the citrus nematode infesting *C. jambhiri* by 66.77–82.22% (Narendra et al. 2008). While Ravichandra (2014) reported that, *T. semipenetrans*-infesting citrus might be controlled by applying *G. fasciculatum* or *G. mossae* @ 50–100 g/plant. Despite having a biocontrol effect on PPN, arbuscular mycorrhizal fungi's usage in citrus is fairly limited because of variable results.

20.2.1.5 Nematophagous Fungi

Nematophagous fungus and citrus nematodes coexist in the rhizosphere of the soil. They are successful in controlling these nematode species. According to Martinelli et al. (2012), the abundance of the *Pratylenchus jaehni* in pera orange under natural conditions in Spain was successfully reduced by applying formulations of *Arthrobotrys robusta, A. musiformis, A. oligospora, Monacrosporium eudermatum,* and *Dactylella leptospora*, enriched with sugarcane bagasse and rice bran mixture separately at doses of 1 and 2 L/plant. However, Noweer (2018) reported that the use of a combination of egg-parasitizing fungus *Verticillium chlamydosporium* and nematode-trapping fungus *Dactylaria brochopaga* (0.5 kg/tree) for 2 seasons caused a significant decline in the population of *T. semipenetrans* (97% and 70%, respectively) compared to control in mandarin trees.

20.2.2 Bacteria

Among the most effective and extensively used bacteria against several plant nematodes infecting citrus over the world include *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. *Pasteuria* species have also been utilized as biocontrol agents in addition to these *Serratia marcescens.*

20.2.2.1 *Bacillus* spp.

This genus has successfully controlled plant nematodes at an amazing level on a variety of horticulture crops in multiple instances. *B. thuringiensis*, *B. firmus, B. subtilis*, and *B. megaterium* species have all been investigated in citrus under various circumstances. According to Montasser et al. (2012), of the seven isolates of fungi and bacteria used as biocontrol agents that were tested in vitro*, B. subtilis* had the highest level of success against *T. semipenetrans* (J_2) (100% mortality at 3×10^8 cfu/mL), followed by *S. marcescens* (99.9%) after 72 h of exposure. When compared to untreated plots, field tests in Egypt using the commercial formulation of *Bacillus thuringiensis*—Agerin® (3 kg/4200 m^2) grafted onto 15-year-old baladi mandarin (*Citrus reticulata*) trees on sour orange (*Citrus aurantium*) trees boosted yields by 52.9–69.2% over two seasons (El-Nagdi et al. 2010). On 16-year-old Valencia sweet orange trees, Abd-Elgawad et al. (2010) showed a sharp decrease in *T. semipenetrans* juveniles and the maximum fruit output (85.6–90.2 kg/tree) following the application of *B. subtilis* (107 cells/mL).

Hammam et al. (2016) reported that, the effectiveness of *T. semipenetrans* population was higher when *B. subtilis* combined with compost (10^{16} cfu/mL) was administered to the soil of volkameriana seedlings in Egypt after 3 months of treatment under greenhouse conditions. *B. subtilis* had similar results in sour oranges (1×10^6 cfu/mL) mixed with compost, which caused 73.7% more *T. semipenetrans* death across all life stages than *B. subtilis* alone (66.8%) (El-Mohamedy et al. 2016). El-Tanany et al. (2018) found that soil treatment of a combination of commercial formulations including *B. megaterium* and *T. album* (Bio Arc + Bio Zeid) over two seasons boosted fruit yield and significantly decreased (66.20–78.79%) *T. semipenetrans* populations in Washington navel orange trees over two seasons under field conditions in Egypt.

B. megaterium, a similar species, has become a promising citrus biocontrol agent. According to Elzawahry et al. (2015), the use of the commercial formulation Bioarc TM (30 g/L) resulted in 90.5% *T. semipenetrans* J2 mortality following a 72-h exposure period in the laboratory. While a greenhouse study revealed a considerable reduction (89.0%, 89.5%, and 76.6%, 82.9%) in juvenile in the soil and females in the root of baladi orange and lime, respectively.

20.2.2.2 *Pseudomonas fluorescens*

It is possible to control *Meloidogyne* spp. and *T. semipenetrans* in citrus successfully by using *P. fluorescens* as a biocontrol agent (Rajendran et al. 2001). For instance, after 72 h of exposure under in vitro conditions, Montasser et al. (2012) reported the maximum death (99.9%) of *T. semipenetrans* juveniles. Hanawi (2016), however, found that after 48 h of exposure, 94% of juveniles died at a dosage of 3×10^8 cfu/mL. In comparison to untreated control plots under natural conditions in India, the application of commercial talc-based *P. fluorescens* formulation (20 g/tree) to the soil decreased *T. semipenetrans* infesting *C. limon* and increased the yield (Deepa et al. 2011). Despite the fact that the use of experimental culture filtrate led to a sharp decline in *T. semipenetrans* juveniles on sweet orange trees in Egypt when compared to control plots (48.2% at 25 mL/tree—3×10^8 cfu/mL) (Hanawi 2016). Applying neem cake (25 g/plant) and *P. fluorescens* (2×10^9 spores) together considerably decreased the population of *T. semipenetrans* in the soil and on the roots of acid lime seedlings as compared to the control (Reddy et al. 2000).

20.2.2.3 *Streptomyces avermitilis*

The naturally occurring fermentation byproduct of *S. avermitilis*, Abamectin, has enormous promise as a biocontrol agent for a variety of plant nematodes (Saad et al. 2017). El-Nagdi et al. (2010) reported that the application of commercial formulations of *S. avermitilis*—abamectin to mandarin trees grafted on sour orange (*Citrus aurantium*) enhanced yield by 84.6–115.4% over two seasons compared with control plots under field conditions. El-Tanany et al. (2018) evaluated abamectin (Tervigo®), oxamyl (Vydate®), and botanical insecticide to manage *T. semipenetrans* infesting Washington navel orange trees in Egypt. In comparison to oxamyl and azadirachtin (Achook®), the substance used in the soil (2.5 L per feddan) caused a reduction of 78.12–87.06% throughout two growing seasons. However, compared to abamectin (41.45 kg/tree), the average fruit output was much higher with oxamyl treatment (51.87 kg/tree). A similar reduction in *T. semipenetrans* population was found by El-Saedy et al. (2019) following the administration of Tervigo® (15 mL/tree), which also led to an increase in fruit yield (71.1 kg/tree) throughout two seasons among orange trees in Valencia

20.2.2.4 *Pasteuria* spp.

The *Pasteuria* sp. has been associated to *T. semipenetrans* in reports from various citrus-growing regions across the globe (Ciancio et al. 2016; Sorribas et al. 2000, 2008; Gené et al. 2005; Kaplan 1994; Ciancio and Roccuzzo 1992). It could function as an efficient biocontrol agent for *T. semipenetrans* and other plant nematodes due to the density of its endospores and their long-term persistence in soil under challenging conditions (Ciancio 2018). The population of *T. semipenetrans* was effectively reduced by the combined application of *P. penetrans* (2109 spores/plant) and *P. lilacinum* (50 g/plant with 4107 spores/g) (Reddy and Nagesh 2000). The limited host range and obligatory character of this genus have limited the experimental investigations that have been done utilizing it to combat *T. semipenetrans*. The application of these bacteria in the biocontrol of citrus nematode will be further improved by further knowledge of their biology and field ecology as well as artificial culturing of the bacteria employing fermented technology.

20.2.3 Mites

It has been determined that mites may be used as plant nematode biocontrol agents. Investigations on various species of mites such as *Macrocheles muscaedomestica, Cosmolaelaps simplex, Macrocheles matrius*, and *Gaeolaelaps acul*e against *T. semipenetrans* have been undertaken on citrus, with the majority of the studies taking place in greenhouses. Al Rehiayani and Fouly (2005) found that the simultaneous application of *C. simplex* (200 individuals/pot) and *T. semipenetrans* juvenile inoculation to citrus seedlings significantly reduced the nematode's reproduction capacity, although mite individuals were less effective than aldicarb (614 juveniles/100 cm^3 soil). Salehi et al. (2014) found that key lime plants that were not treated (398.25 J_2/100 cm^3 soil) produced considerably more juvenile *T. semipenetrans* plants than those that were treated (20 individuals/pot), ranging from 126 to 161. Similar research was conducted by Abo-Korah (2017) on the efficiency of *M. matrius* against citrus nematode and found that it reduced *T. semipenetrans* juvenile population by the highest percentage (77.5%) when compared to carbofuran (76.9%), and that seedling growth was also increased. However, compared to *T. semipenetrans*, *P. penetrans* had a reduced predation efficiency. Despite the possibility of managing *T. semipenetrans*, problems with mass production, soil delivery, and nonspecificity prevent predatory mites from being widely used in the biocontrol of plant nematodes (Cumagun and Moosavi 2015; Viaene et al. 2006). However, the advancement of mass production, delivery, and soil ecological knowledge may boost the use of these agents shortly.

20.3 Biotechnological Interventions in Citrus Nematode Management

There is a dearth of information on biotechnological methods for controlling *T. semipenetrans*. To handle *T. semipenetrans*, methods including gene silencing (RNAi) and the introduction of harmful substances to the invading nematode should be taken into consideration. Natural variation for resistance, extensive germ plasm screening, and genetic markers should all be investigated to find the genes that confer resistance to *T. semipenetrans*. To reduce effective establishment in host cells on a sensitive or tolerant host, transgenic techniques that take advantage of an understanding of nematode-host interactions and direct the infective stage to prevent locating host roots are used (Fosu-Nyarko and Jones 2015). To counteract *T. semipenetrans*, citrus breeding programs are using genomic editing techniques like CRISPR/Cas (Abd-Elgawad 2022). Nanotechnology is a most recent branch of science and offers satisfactory solutions for plant disease management (Khan and Rizvi 2014; Khan et al. 2019a, b, c) and disease detection (Khan and Akram 2020;

Khan and Rizvi 2016; Khan et al. 2020). Nano-sensors are the most important product of nanotechnology, and have great potential for use in plant disease diagnosis (Khan 2023). Sellappan et al. (2022) developed nanobiosensor to early detection and prevention of agricultural crops from harmful microorganisms. Using specific nanoparticles as nano-sensors to detect the plant pathogen early can reduce the plant disease damage and help in proper management of the disease (Khan and Rizvi 2018).

20.4 Conclusion and Future Perspectives

In addition to acting as a safer alternative to toxic chemical pesticides in citrus, biological control is crucial in the management of nematode infections. Although several fungi, bacteria, and mites have been used in citrus, a dedication to the development of high-quality products, extension programs, and collaboration between researchers, farmers, and industry will more strongly advocate the use of biological control agents against plant nematodes in citrus. To create cultivars with long-lasting resistance, major resistance genes or quantitative trait locus (QTLs) must be introgressed alongside low-impact QTLs. The next goal is to precisely identify these low-effect QTLs. This suggests that to acquire a high heritability trait, all resistance testing must be taken into account. Due to the availability of complete genome sequences for the major crops, nematode resistance genes can be found, localized, diagnosed, and cloned, a goal that is likely to be accomplished shortly. This will give breeders a flexible tool for precise resistance breeding.

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