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Infestation of *Tylenchulus semipenetrans* on citrus plantations and their management

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Abstract
Many plant parasitic nematodes can harm citrus, but *Tylenchulus semipenetrans* is one of the most significant ones globally. This nematode causes a higher degree of damage as compared to other nematodes. It feeds on the feeder roots of the plant and damages them. Therefore, the plant cannot take up water or nutrients from the soil essential for its growth and development, causing the slow decline of citrus. There have been reports of the *Tylenchulus* from many countries, including India. At the global level, it is majorly reported from the countries like the USA, Egypt, Pakistan, Iraq and many European countries. In India, it is reported from the states like Assam, Tamil Nadu, Himachal Pradesh etc. In this article, we will discuss the major affected areas by *Tylenchulus semipenetrans* in the world including India, and go through various research at national and international levels in which the *Tylenchulus semipenetrans* were reporting and also discuss their management which involves Pre-Plant Management Techniques, Botanical control, Bioagents, Chemical Control, and Genetic Control (Hybrid Root Stock).

Keywords: *Tylenchulus semipenetrans*, parasitic nematodes, hybrid root stock

Introduction
Nematodes that parasitize plants are the main issue with citrus crop cultivation. *Tylenchulus semipenetrans*, *Xiphinema index*, *Meloidogyne spp.*, *Radopholus similis*, and *Ratylenchus coffee* are the main nematode pests affecting citrus crops (Kumar and Das). About Rs. 242.1 billion is lost annually in crop production resulting from plant-parasitic nematode infestation (Jain *et al.*). Plant-parasitic nematodes (PPNs) feed on the majority of the plant's tissues, such as the roots, stems, and leaves, to infect plants all over the world, specially cultivated crops. The influence of the soil's organic matter has also led to a huge effect on the abundance of nematodes in the root rhizosphere (Bernard *et al.*).
Citrus plantations are infested by various plant parasitic nematodes but one of the most significant citrus plant nematodes is *T. semipenetrans* which parasitized the citrus plantations (Ciancio and Mukerji). The yield loss due to *T. semipenetrans* ranges from 10 to 30% based on the degree of the infestation (Duncan and Cohn). The first time it was reported in California (USA) was in 1912 and after this, it was reported in every region of the world where citrus is produced (Duncan). *T. semipenetrans*, is economically relevant in all nations that grow citrus (Ciancio and Mukerji). At the global level, The *T. semipenetrans* is distributed in various parts of the US including California, Texas, Arizona and Florida (Baines *et al.*) (Gottlieb *et al.*). Their infestation is also reported in the Mediterranean region which includes the Mediterranean Basin and South African areas (R. N. Inserra *et al.*) (Gottlieb *et al.*). The *T. semipenetrans* population is also reported in the countries like Brazil, Argentina, Australia, Venezuela and India (Crozzoli P and Funes).
One of India's most significant fruit crops is citrus and stands third after Banana and Mango (Priyadarshini and Abhilash). In India, *T. semipenetrans* was initially recorded by Siddiqui in 1961 from Aligarh (UP). Citrus production is slowly declining due to the infestation of *T. semipenetrans* (Kumar and Das). It damages the citrus plantations when new seedlings of plants are introduced in the heavily infested older orchard, and this is called the citrus replant problem. It leads to slow growth, yellowing of leaves, and delayed fruiting in the young citrus plants (Khan).

Biology and Life cycle of *Tylenchulus semipenetrans*
The body length of the adult female *Tylenchulus* is 328 to 477µm long with a thick and cylindrical tail that ends bluntly at a rounded terminal portion (Maafi *et al.*). The body of the mature female is white and translucent (CABI). Its stylet contains rounded and small knobs (Maafi *et al.*). The length of the stylet is 11.9 µm in mature females and while in J2 females the length of the stylet is 12.3 µm (R. Inserra *et al.*). The body's midsection contains excretory pores (Maafi *et al.*). In adult females, the length of distance between the anterior head region and excretory pore is 84% of the total body length while 54.4% is in the case of J2 females (R. Inserra *et al.*). A hemispherical lip region is present and distinct annuli are absent. *Tylenchulus semipenetrans* also contain a delicate stylet with small, rounded knobs. Procorpus and meta corpus are not intertwined (Maafi *et al.*) and the length of the meta corpus in adult females is 21.2 µm, while in J2 females the length is 15.3 µm (R. Inserra *et al.*). The distinct isthmus and post corpus are present in the bulb. *Tylenchulus semipenetrans* infect seedlings in nurseries, therefore it is one of the most significant hazards to citrus plants, trees as well as orchards (Abd-Elgawad *et al.*).
They show sexual dimorphism males have mature to long thread-like structures without feeding (Duncan). Without males, females can produce male and female offspring and are amphimictic in nature. The second stage juvenile female infests the epidermis of the root and after 6 to 7 days they moult into the 3rd, 4th and adult stages. The young adult female penetrates the cortical cells of the root with their

anterior head portion while the posterior portion becomes swollen and exposed out from the root surface. The head of the female surrounds by the various nurse cells on which it feeds. The eggs are produced by the by 6 weeks old female and an egg mass is composed of gelatine which is released from their excretory pore (Cohn).

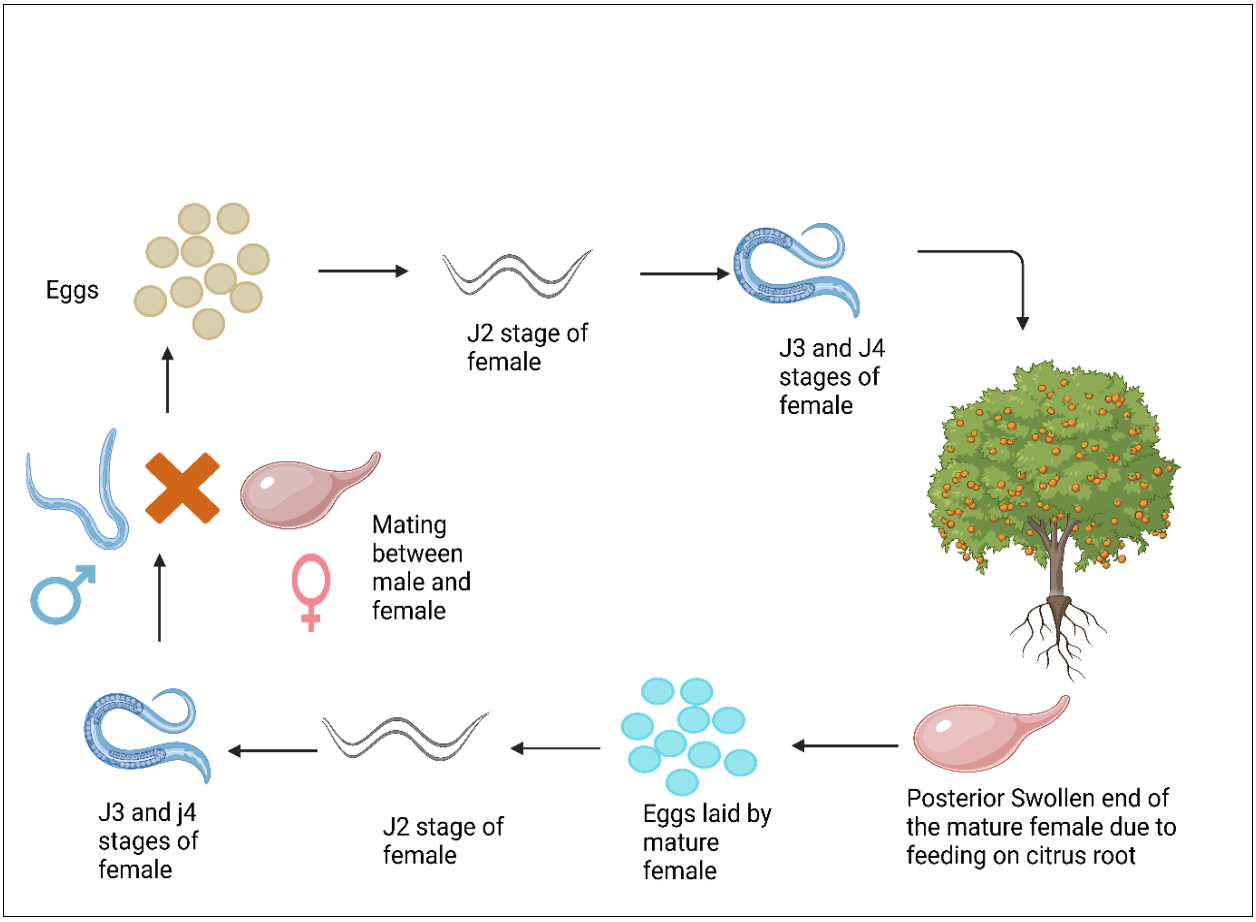

Fig 1: Life cycle of *T. semipenetrans*

Occurrence of *Tylenchulus semipenetrans* in India and the World

This "citrus parasite," *T. semipenetrans*, seems appropriately named since it may be found in all regions of the world where citrus is planted and inhibits citrus fruit output in a variety of environmental and edaphic circumstances. Various surveys were conducted in the primary citrus-producing areas in the United States. According to estimates, the worm infests the
commercial citrus orchards of California, Florida, Texas and Arizona and cause 50 to 90 % of damage to citrus yield (Van Gundy and Meagher) (Heald and O'Bannon).

There are 80% of orchards in Spain suffered from *T. semipenetrans* infestations. In *T. semipenetrans*-infested soil, older citrus fruit trees can function well, while newly planted trees suffer significant damage that causes uneconomical growth and development with low harvests (Verdejo-Lucas and McKenry). *T. Semipenetrans* is also reported from the Mediterranean region where 73 samples were collected out of which 8.2% of samples are at the Economic Threshold Level (ETC) and 21.9% were crossing Economic Injury Level (EIL) (Bozbuga *et al*.). In China, 24 soil samples were collected from various citrus orchards out of which *T. semipenetrans* is detected in 14 samples by Loop-Mediated Isothermal Amplification (LAMP) technique (Lin *et al*.). Its high prevalence is also reported in Saudi Arabia (Al-Yahya). In South Africa, various *T. semipenetrans* biotypes were isolated in the soil of *Citrus jambhiri* and *Poncirus trifoliata* (Mathabatha *et al*.). In Southern Iran, the *T. semipenetrans* is widely distributed which is responsible for citrus decline (Abivardi *et al*.). In the Punjab province of Pakistan, *T. semipenetrans* is considered a major pest which is detected in the soil samples of *Citrus reticulata*, *Citrus sinensis*, *Citrus paradisi*, *Citrus limettioides* and *Citrus limon* (Iqbal *et al*.) (Khanzada *et al*.).

In India, Kumaon district of Uttarakhand, *T. semipenetrans* were reported in the composite soil samples of sweet oranges (Rathour and Sudarshan, 2015) [55]. The nematode species that were detected were *Tylenchorhynchus spp*., *Helicotylenchus dihystera*, *Hoplolaimus indicus*, and *T. semipenetrans*. In Assam, the citrus nematode *T. semipenetrans* was described as the major plant parasitic nematode of several citrus species such as Sweet Orange, Khasi Mandarin, Trifoliate Orange, Grapefruit, Pummelo, Rough Lemon, Cleopatra Mandarin, Assam Lemon, Troyer Citrange and Citrange. Out of 10 different citrus species, it was found that *T. semipenetrans* was most prevalent in rough lemon, next is in the grapefruit and Assam lemon, while least prevalent in trifoliate orange and Troyer citrus. (Kumar and Das). *T. semipenetrans* is highly prevalent in the south zone of Andhra Pradesh and affect many species of citrus and also infests other crops such as grapes and castor in Telangana (Sunanda and Sathish).

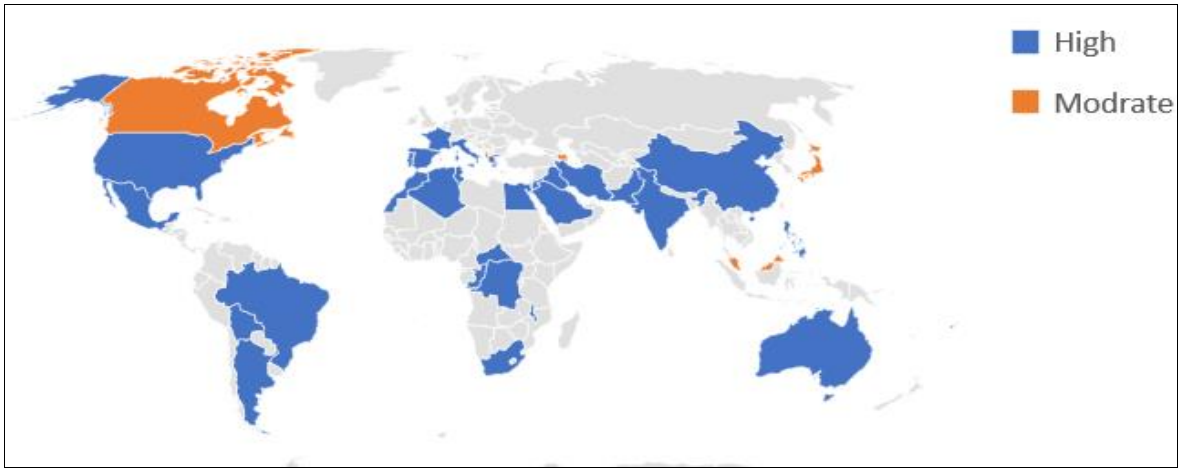

Fig 2: Infestation of *T. semipenetrans* at the Global Level (CABI).

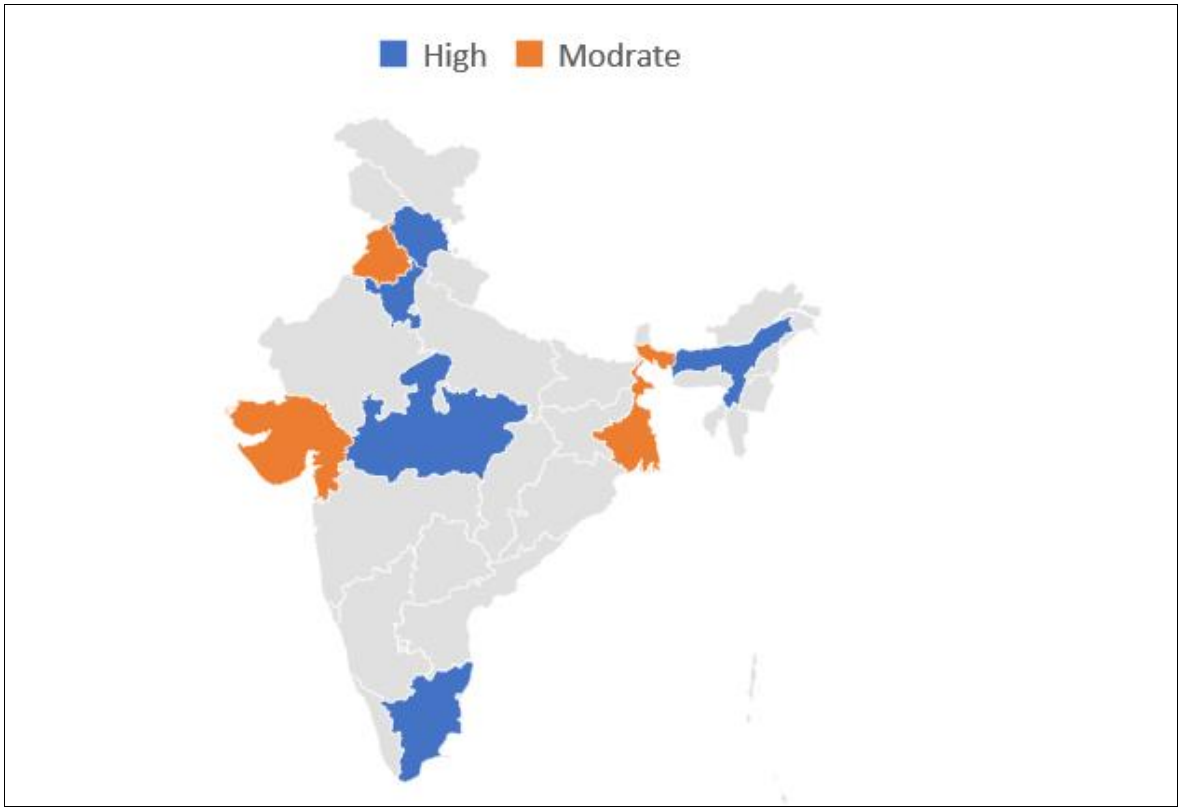

Fig 3: Infestation of *T. semipenetrans* at the National Level (M. Khan *et al*.)

Management of *Tylenchulus semipenetrans*

Pre-Plant Management Techniques

The best nematode management methods for perennial citrus are pre-plant techniques because they lower starting populations before developing an orchard. They will encourage the establishment of young trees, enhance yields, and lessen the requirement for frequent post-plant treatments in an orchard (Verdejo-Lucas). *T. semipenetrans* was managed by pre-plant fumigation under control (Le Roux *et al*.) (Sorribas *et al*.). Weed treatment has little direct effect on *T. semipenetrans*, but it will boost tree development by lowering the conflict for nutrients and water between weeds and plants. Lowering weeds and mulching new tree plantings in the row helps in the establishment of a tree. Anti-weed nets mulched around trees in mature orchards to inhibit weed growth while having no impact on nematode populations (Verdejo-Lucas). Subsoiling and ploughing the orchard land after a specific interval of time will expose the nematode to direct sunlight which decreases the population of *T. semipenetrans* up to 90% (Sorribas *et al*.). Sometimes young orchards are also affected by the infestation of *T. semipenetrans* by the introduction of new plantings from contaminated nursery soils (Verdejo-Lucas). Major factors must be taken into account when managing nematodes in citrus. Which include effective collection and proper nematode sampling, enhanced PPN quarantine, the development of certification procedures, and the development of PPN limiting factor evaluation concepts. Nematicidal applications should consider practical knowledge gathered from prior research on the citrus nematode conducted globally. In this regard, a thorough understanding of nematode biology, ecology, and their considerations of the worm's economic significance should be analyzed for improved pest control (Abd-Elgawad).

Chemical Control

Rugby and Cartap are considered the most important nematicides against the *T. semipenetrans* (W. A. Khan *et al*.). Rugby and Cartap are organophosphates and responsible for the deactivation of the Acetylcholinesterase enzyme and hampers the nervous system of nematode. (Ali *et al*.). Lufenuron also known as Match also used as a chemical nematicide which suppresses the production of chitin. So, the larva of *T. semipenetrans* is not able to develop a hard outer covering. Therefore, their internal organs are exposed during the process of hatching and moulting and are more prone to damage (Meola *et al*.). When these above-listed chemicals are used in combination with *T. harzianum* (a fungus) will increase plant growth and fruit yield by reducing the population of nematode (W. A. Khan *et al*.). Oxamyl is another chemical which is used to suppress the population of *T. semipenetrans*. When the infected soil samples are treated with Oxamyl, the female population of *T. semipenetrans* reduces to 63.8% (Swelam *et al*.) and also increases the percentage of fruit weight to 166.84% (Hammad *et al*.). Nemaphos is another chemical nematicide of the organophosphate category which is used against a wide spectrum of plant parasitic nematodes including *T. semipenetrans* and causes a 33.1 to 39.9% reduction in the population of nematode (Ibrahim *et al*.). The *T. semipenetrans* population can also be controlled by the use of Ozone in combination with copper sulphate and calcium sulphate. Both the combinations i.e., Ozone + $CuSO_4$ and Ozone + $CaSO_4$ will reduce the population of *T. semipenetrans* and also increases the yield and nutritional value of the lemon tree and increases the diameter and weight of the fruit (Abdel-Sattar and Hammad). Carbofuran is also used as a chemical nematicide to control the population of *T. semipenetrans* and decreases its population by up to 55.60% and increases the fruit yield by up to 33.27% (Bhagawati *et al*.). Azadirachtin and Humic Acid are also used to kill the population of *T. semipenetrans* in an acid lime tree by delivering it through a drip irrigation method into the root and soil of the plant (Nagachandrabose *et al*., "Effect of Application of Organic Bio-Products through Drip Irrigation against Tylenchulus Semipenetrans in Citrus").

Botanical Control

The primary strategy to manage the density of plant parasitic nematodes is the use of chemical nematicides (Maleita *et al*.). But these chemicals have unnecessary effects on the environment and human health. Therefore, these harmful chemicals are removed from the market. So, the options to manage the PPNs including *T. semipenetrans* to an economically accepted level are limited. So, there is a need to use environmentally friendly and sustainable methods to control the infestation of PPNs (Tucuch-Pérez *et al*.). The phytochemical compound is one of the best options for sustainable control of PPNs including *T. semipenetrans* and the desert plant contains effective phytochemical compounds such as tannins, flavonoids, alkaloids and terpenoids that have nematicidal properties against *T. semipenetrans* (Al-Saleem *et al*.). Desert plants are living in extreme conditions and develop various physiological processes to survive extremely hot and dry conditions and release above listed secondary metabolites as an end product (A. Khan *et al*.). Various plants which have medicinal importance produce essential oils which inhibit the growth and development of *T. semipenetrans.* Some medicinal aromatic plants such as *Rosmarinus officinalis, Thymus vulgaris, Laurus nobilis, Verbena officinalis, Foeniculum vulgare, Moringa oleifera*, and *Chamaemelum nobile* produce essential oils which show nematicidal properties against *T. semipenetrans*. The concentration of essential oils in the soil is directly proportional to the J2 mortality and egg hatch imbibition and this would go to increase with time. Out of these *R. officinalis* and *T. vulgaris* are known to be more toxic than *T. semipenetrans* (Zoubi *et al*.). The plant Extract of *Eucalyptus camaldulensis* and *Zataria multiflora* lead to the mortality of J2 juvenile of nematode (PALASHI). Some plant extracts such as Marigold leaf, Marigold root, Datura, Acacia and Eucalyptus also show nematicidal properties against *T. semipenetrans* and decline the population of nematode from 13 to 76 % which depends upon the concentration of the plant extract (Wajid *et al*.). Neem Seed Kernal Extract (NSKE) is considered an effective agent against *T. semipenetrans* and leads to a significant reduction in its population but, when it uses with *P. lilacinum* then it declines the nematode population up to 78 to 88.8% in soil and 73.5 to 79.2% in roots and increases the fruit yield from 26.3% to 33.6% (Seenivasan *et al*.). *M. canadensis* oil and Menthol also show nematicidal activity against J2 juveniles of *T. semipenetrans*. Menthol shows a moderate impact on the nematode (Sangwan *et al*.). Various vegetable extracts such as garlic extract, Ziziphus extract and cabbage extract are also known to be effective against *T. semipenetrans* and increase the yield of the sour orange plant by declining the nematode population in soil and roots (Saadoon *et al*.).

Bioagents

Fungal control

Purpureocillium lilacinum and *Trichoderma viride* are considered excellent fungal bioagents used to control the *T. semipenetrans* population. But, the efficiency of *P. lilacinum* is higher than that of *T. viride*. *P. lilacinum* reduces the population of *T. semipenetrans* in soil and root of the acid lime ranges from 53.4 to 74.4% and also increases the fruit yield from 35.4 to 40.3% (Nagachandrabose *et al*., "Application of Liquid Bio-Inoculants through a Drip Irrigation System to Manage Slow Decline Disease Caused by Tylenchulus Semipenetrans in Acid Lime Trees"). Although other fungi such *as Paecilomyces lilacinus, Fusarium solani, Cylindrocarpon cylindroides, Sepedomium chrysospermum, Veronea botryosa* and *Exophiala pisciphila* are also used as bioagents against *T. semipenetrans*. All these fungal species coexist in citrus roots and soil and damage the eggs, J2 juveniles and mature females of the nematode (Gené *et al*.). In Spain, a bio-nematicide with the active ingredient *Paecilomyces lilacinus* strain 251 (now *Purpureocillium lilacinus*) is used on citrus. *P. lilacinus* parasitizes the juvenile, female, and egg stages of *T. semipenetrans*. Culture filtrates of this fungus immobilize juveniles and decrease the population of the nematode to 45% (Verdejo-Lucas). Mycorrhizae *(Arbuscular mycorrhiza) is* another fungus used as a bioagent against *T. semipenetrans.* It decreases the population of nematodes to 39. 5%. But, when it is used in combination with Oxamyl then it decreases the nematode population up to 64.6 to 71.4% (Swelam *et al*.). *Pleurotus Ostreatus* is also considered a nematophagous fungus for *T. semipenetrans* that cause the death of the nematode by paralyzing it (Kanaujiya *et al*.).

Bacterial Control

Citrus rhizospheres contained a variety of strains of bacteria and each bacterial strain had a unique impact on the development of citrus roots, J2 mortality and *Tylenchulus semipenetrans* egg hatch (M Labiadh *et al*.). *P. fluorescens* bioagent-treated plants showed the fewest number of nematodes as compared to the untreated control. *P. fluorescens* increases the fruit yield of the citrus as compared to the control by limiting the no. of citrus nematodes in the soil (Deepa *et al*.). Some bacterial strains like *Bacillus weihenstephanensis*, *Lysinibacillus sphaericus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus pumilus* and *Bacillus cereus* cause effective damage to second-stage juveniles of *T. semipenetrans* and leads to 85% mortality J2 juveniles of nematode. Out of these *B. weihenstephanensis*, *B. cereus*, *B. mycoides*, *B. thuringiensis* and *L. sphaericus* increases the fruit yield of the sour orange (Manel Labiadh *et al*.). The bacterial strains such as *B. cereus* X30 and *B. thuringiensis* B27 are responsible for inhibiting the process of egg hatching of *T. semipenetrans* while some strains of bacteria such as *B. cereus* X30, *B. thuringiensis* B4, B18 and *Brevundimonas bullata* X20 kills J2 juvenile of *T. semipenetrans* up to 90 % (M Labiadh *et al*.). Plant Growth Promoting Rhizobacteria (PGPR) in combination with *Tilapia* Fish Powder (TFP) also shows nematicidal properties against *T. semipenetrans* and is responsible for egg-hatching imbibition of the nematode from 42.25 to 75.12% in the soil and roots of Navel Orange (El-Ashry *et al*.).

Mites Predator

C. capreolus, a predatory mite feeds on three different types of juveniles: J2 of *T. semipenetrans*, J2 of *M. incognita*, and egg mass of *M. incognita*. (Al-Azzazy and Al-Rehiayani). *Diplogaster* is another predatory mite which feeds on j2 juveniles of *T. semipenetrans* and decreases its population in the roots of sour orange (Osman). These predatory mites are used to make organic manure and compost to increase soil resistance against *T. semipenetrans* (Ntalli *et al*.). *C. simplex* also has a nematicidal property and suppresses the reproduction of *T. semipenetrans* and when it uses with aldicarb it increases the length of shoot up to 60 cm of the plant as compared to nematode untreated control (38.7 cm) (Al Rehiayani and Fouly).

Hybrid Root Stock

The population of *T. semipenetrans* increases slowly in the resistant rootstock as compared to the resistant rootstock because these resistant rootstocks give a negative response to nematode infestation and only a few individuals reach the mature stage (Verdejo-Lucas) Forner-Alcaide 5 is a hybrid of *Cleopatra mandarin* and *Poncirus trifoliata* is considered as a resistant rootstock against *T. semipenetrans.* The efficiency of their resistance is by pre-plant fumigation by 1,3 Dichloropropene which delays the infestation and reduces the number of nematodes (Verdejo-Lucas *et al*.), *Poncirus trifoliata* and the hybrid Swingle citrumelo also shows the resistance against *T. semipenetrans* but their use is limited due their intolerance to alkaline soils (Galeano *et al*.). The rootstocks like Cox mandarin hybrid and Trifoliate orange also show effective resistance against *T. semipenetrans* and show a minimum reduction in root weight, shoot weight, root length, shoot length and no. of leaves during the infestation of nematode (Afzal *et al*.). *Poncirus trifoliata* hybrids with Cleopatra mandarin and King mandarin show tolerance against *T. semipenetrans* (Aleza *et al*.).

Conclusion

One of the most significant fruit crops, both in India and around the world, is citrus. According to the report of FAO, 89409.1 tons of citrus is produced in India from the year of 2011 to 2018. One of the most significant nematodes of citrus is *Tylenchulus semipenetrans*. It causes a slow decrease in citrus plantations. *T. semipenetrans* infest the feeder roots of the citrus plant so the plant is unable to absorb water and nutrients from the soil which leads to its slow decline. In India, the states like Assam, Himachal Pradesh, Madhya Pradesh, Haryana, and Tamil Nadu, and the moderately infested states are Punjab, Gujrat, and West Bengal. At the global level, the case of *T. semipenetrans* is reported from the Florida of USA, Egypt, Pakistan, and Iraq. Various management strategies are implemented to manage the infestation of plant parasitic Nematodes which involves the effective collection and proper sampling of enhanced PPN quarantine, the development of certification procedures, and the evaluation of the limiting factor concept. Botanicals such as desert and medicinal plants are considered a limiting factor for plant parasitic nematodes including *T. semipenetrans.* Some fungi such as *Paecilomyces lilacinus, Fusarium solani, Cylindrocarpon cylindroides, Sepedomium chrysospermum, Veronea botryosa and Exophiala pisciphila* also used to control the citrus nematode. Some species of bacteria such as *P. fluorescens, Bacillus weihenstephanensis*, *Lysinibacillus sphaericus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus pumilus* and *Bacillus cereus* show nematicidal properties against *T. semipenetrans. C. capreolus, C. simplex* and *Diplogaster* are predatory mites that feed on the egg mass and J2 stage female of *T. semipenetrans* which is considered the most important biological control for *T. semipenetrans*. Yet, plant parasitic nematodes are one of the most important crop parasites but researchers tend to ignore the most. Their mode of damage is underestimated. So, there is a need to further work on the infestation of PPNs.

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