Research Article

Impact of Orchard Age on Citrus Nematode Population Dynamics and Evaluation of Native Rhizobacteria for Nematode Management

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Abstract | Citrus slow decline disease caused by citrus nematode (*Tylenchulus semipenetrans* Cobb) is of significant importance and prevailing in all citrus growing areas. The concept of this study was to select citrus orchards with different age groups to assess the population dynamics of citrus nematode. Moreover, in-vitro nematicidal potential of rhizobacteria was evaluated. The results showed that ninety nine percent of the total soil and root samples collected were infested with citrus nematode. The data showed that population of citrus nematodes was significantly different across the citrus orchards with different age groups and cultivars. The average population of juveniles was recorded from 20 to 40850 per 100 grams soil and root females ranged from 3 to 1370 per 1 gram of root. The mean population of juveniles in all soil samples was 8030±463 per 100g soil and mean number of females were 364±23 per gram of root. The citrus cultivar 'kniow' showed significantly highest nematode infestation followed by 'sweet orange' and 'fruiter early' than all other citrus cultivars. Moreover, in-vitro potential of rhizobacteria revealed more than 50% nematode mortality at 24 hrs and more than 70% at 48hrs of incubation. Moreover, bacterial culture filtrates and volatiles also showed promising results by showing the nematode mortality by 40-95% in 48 hr. The rhizobacteria and their products can be good potential alternatives of chemical nematicides to manage plant parasitic nematodes.

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Introduction

Citrus belongs to family rutaceae that include large group of fruit trees and shrubs. The origin of citrus was considered to be north east of China and India but the exact origin is not clear (Gmitter *et al.*, 1990). Citrus cultivation is successfully well adopted in tropical and subtropical zone of the world (Shah, 2004). Citrus fruit and juice have high nutritive values and major source of vitamin C and keep antioxidant potential (Kamboh *et al.*, 2018). Fruit production is always an imperative part of Pakistan's economy. Citrus is an important fruit crop and occupying second position in fruit industry by producing good quality citrus for export purposes (Naqvi, 2005). Approximately, 137 countries are covering tropical and subtropical areas predominantly under citrus cultivation (Ismail *et al.*, 2004).

Plant parasitic nematodes are considered as hidden enemies of the plants that reduces the crop yield significantly. Citrus nematode (*Tylenchulus semipenetrans* Cobb.) is a serious and widespread pest of citrus crop all over the world and responsible to cause slow decline (SD) disease. Yield losses caused by *T. semipenetrans* reported up to 10-30% and subjected to level of infestation, population density, soil properties, types of root stock, presence and absence of other soil borne pathogens and cultural practices (Cohn and Duncan, 1990). In contrast, the situation is not different around the world for citrus nematode dynamics (Heald and O'Bannon, 1987). Citrus nematode is serious threat to citrus production in Pakistan because country lies in tropical and subtropical regions of the world where climate favor the development of citrus nematode (Parvez *et al.*, 2003). *T. semipenetrans* is reported from all citrus growing area of Pakistan but it is widely distributed in Punjab province than other provinces and possibly responsible for citrus decline in this area (Khanzada *et al.*, 2008). *T. semipenetrans* infestation provide infection sites on roots for secondary infection by other microorganisms that lead towards the complete destruction of plant (O' Bannon *et al.*, 1967). *T. semipenetrans* invasion and multiplication is varying according to soil type and rootstocks used to develop various cultivars (Hamid *et al.*, 2025). *T. semipenetrans* spreads within the orchard by poor management practices and to new plantations by using infested nursery stock (Duncan, 2009; Abd-Elgawad *et al.*, 2016) The economic threshold level of 1000 J2/100cm^3 soil was reported for citrus nematode (Bridge and Starr, 2007). The best season for the multiplication of *T. semipenetrans* occurs in spring and autumn when plant is flushing shoots and roots (Van Gundy, 1958; Shokoohi and Duncan, 2018). By keeping in view the increasing population of citrus nematode in these seasons, proper management practices need to be adopted in appropriate time manner.

The best integrated management strategy need to be adopted to manage *T. semipenetrans* is the adoption of resistant rootstocks but chemical control isalways dominated and is still the major approach to manage the population of nematode in fields (Jones, 2017, Hamid *et al.*, 2025). Although, the health hazardous effects of chemical pesticides especially nematicides are substantial and there is dire need to reduce the application of chemicals (Damalas and Koutroubas, 2016; Ahmad *et al.*, 2024; Shekhar *et al.*, 2024). Biological control of plant parasitic nematode is an effective, economical and eco-friendly approach that significantly reduces the damage occurred by plant parasitic nematodes (Ashoub and Amara, 2010; Soliman *et al.*, 2019; Hamid *et al.*, 2023). Many groups of bacteria have been reported and used as biological control agent against plant parasitic nematodes (Affokpon *et al.*, 2011; Geng *et al.*, 2016). Bacteria are known to inhibit the nematode population through diverse mechanisms; production of lytic enzyme, antibiotic production, attractant and repellent volatile organic compound and through direct parasitism (Li, 2015; Zhai *et al.*, 2018). These inhibition mechanisms by beneficial bacterial strains can directly kill the second stage juveniles and also reduce egg hatching (Davies, 1998; Siddique and Mahmood, 1999). Thus, the focus on the native bacterial species contributing to pest control and improving crop yield is imperative.

The present study was designed to estimate the population dynamics of *T. semipenetrans* in relation to citrus growing age and cultivar selection and in-vitro evaluation of native rhizospheric bacteria to manage citrus nematode.

Materials and Methods

Orchards selection and sampling

The main citrus growing area of Pakistan (Sargodha, Punjab) was selected for the present study during 2019-2020. The total orchards selected were 24 with different ages and cultivars from different locations. Totally, 8 locations were selected from which 3 orchards of same cultivar were tagged for further sampling. From each orchard, 5 trees were selected by following the cross sampling method. A brief history of citrus orchards such as; citrus cultivar, age of trees, and application of pesticides was collected by farmers of selected orchards. Soil and roots sample were collected from different age groups and citrus cultivars (Kinnow, sweet orange, fruiter early, other citrus) of selected citrus orchards. Orchards are categorized on the basis of age in different group as defined in Table 1. Soil and root samples were collected from 5-30 cm depth around the canopy of tree using soil auger. The samples were collected in triplicate from each tree and pooled into single sample. The soil and root samples were transported to laboratory for further nematode extraction.

Table 1: *The selection of citrus orchards by age for sampling and nematode population densities.*

Sr. No.	Year range	Category
1	3-7	5
2	8-12	10
3	13-17	15
4	18-22	20
5	23-27	25
6	28-32	30
7	Above 32	35

Nematodes extraction from soil and roots

Soil sample collected from each orchard homogenized manually and 100 g of soil from each sample were measured. Second stage Juveniles were extracted from 100 g soil sample by decanting and sieving the soil suspension through a 43 µm diameter aperture sieve onto a 264 µm sieve, followed by sucrose floatation and centrifugation (Hamid *et al*., 2017). The extracted nematodes were observed and counted under inverted microscope (Olympus CK40). For the extraction of females and eggs, fibrous roots were separated for each sample and 1g of root from each sample was sliced into 2-3cm pieces, stirred in 3% NaOCl solution for 3-5 min and blended gently for 3-4 min. The root mass was collected in beaker and passed through nested 264-µm-pore and 25-µm-pore sieves. The females and eggs collected on the 25-µm-pore sieve were counted using the inverted microscope. Citrus nematode was identified morphologically under digital camera fitted compound microscope. Physical and anatomical feature of juvenile, female and egg were observed at different magnifications.

Table 2: *Rhizobacteria used in this in-vitro study against T. semipenetrans.*

Sr. No.	Isolates	Strains	Accessions
1	BA10	Oceanobacillus kimchi	MT380165
2	BA17	Duganella zoogloeoides	MT380163
3	BA18	Bacillus Subtilis	MT197385
4	BA21	Pseudomonas geniculata	MT197387
5	BA32	*Bacillus* sp.	MT197389
6	BA33	Pseudomonas Putida	MT197386
7	BA34	Pseudomonas fluorescens	MT197384
8	BA39	Rhizobium pusense	MT197388
9	BA44	Bacillus licheniformis	MT380164

Measuring the populations of citrus nematode in different age orchards

The nematode suspensions of processed soil and root sample were examined under inverted microscope in 12 well tissue culture plates in three replicates to measure the population densities. The females and eggs per 1 gram of roots were also counted. Total number of nematodes in each sample was calculated and data expressed from different cultivars separately. The extracted eggs were surface sterilized and subjected to hatching assay in 0.1% $ZnCl_2$ solution by using manually prepared micro-sieves. Eggs were surface sterilized in 0.5% sodium hypochlorite for 2 min and subsequently washed with sterile water trice. The assays were performed under optimum conditions of 25°C±2.

Isolation and characterization of bacteria from citrus rhizosphere

The rhizosphere soil from the roots of healthy citrus plant with more than 20 years of age was prepared from all the samples separately. The 1 g of roots were weighted and washed for the collection of rhizosphere soil. Briefly, small pieces of roots were placed in 9ml of sterilized distilled water and vigorously shaken on vortex at 1000 rpm. The root pieces were carefully removed from the suspension and dilutions were further developed for the isolation of bacteria. By using the sterilized micropipette, an aliquot of 100 µl from 6^{th} dilution were taken from each sample and placed on sterilized petri plates (90 mm) containing nutrient agar and King's B agar. The plates were examined after every 24 hours for the development of colonies. The morphologically different colonies were picked and purified on freshly poured nutrient agar and king's B agar medium. The cultures were morphological characterized by following the Bergey's manual of systematic bacteriology. The selected isolates with nematicidal properties were characterized by using 16S rRNA gene and sequences were submitted in NCBI database (Table 2).

Preparation of bacterial cultures and culture filtrates

Bacterial strains were maintained on nutrient agar plates for 48 hour at 25°C and stored at 4°C. Two loops full of bacteria from pure culture were taken and inoculated in 250 ml flask containing 100 ml of sterilized nutrient broth with shaking at 200 rpm for 48 hours. Nutrient broth was transferred to 50ml tube and centrifuged at 9000 rpm for 10 mints. For the preparation of cell free culture, broth was

passed through 0.4 um syringe filter and collected in sterilized 50 ml tubes. The culture filtrates were diluted to 20 % by adding sterilized distilled water for further experiments.

In-vitro nematode mortality assays

Freshly extracted juvenile by White head and Hemming tray method (Whitehead & Hemming, 1965) were collected and surface sterilized with 1.5% NaOCl solution for 2-3 mints. The bacterial cell pellet was collected by centrifugation at 10,000 rpm for 5 mints. The cell suspension was prepared by using sterilized distilled water to a concentration of 1×10^8 cells/ml. The cell culture and culture filtrates (20 %) were prepared and transferred to 24 well cell culture plates (Nest) and 100ul of nematode suspension (~1000 J2/ml) was added. In control treatment, nematodes were added in freshly prepared nutrient broth. Data of juvenile mortality was recorded after 24, 48 hours of incubation at optimum temperature (25). The culture filtrates were also evaluated against nematode eggs and hatching capacity was recorded at 48 hours of incubation. The freshly collected eggs (~100 eggs/ml) were added in 20% culture filtrates. The volatile effect of culture filtrates was also assessed against citrus nematode and eggs. The experiment was conducted in 90 mm petri plates by placing another 30 mm petri plate in the center with 3ml culture filtrate. The petri plates were sealed properly and data for nematode mortality was recorded at 24 and 48 hours of incubation. The nematode egg hatching capacity was also recorded at 48 hours of incubation. Each treatment was replicated thrice and experiments were repeated twice.

Data analysis

The nematode population densities and mortalities were subjected to analysis of variance (ANOVA) and followed by Tukey's HSD multiple comparison tests to compare the means values at $P < 0.05$ using Statistix 8.1 software (Tallahassee, FL, USA). The multiple correlations for nematode densities and orchard ages were developed by using the R script.

Results

Nematode morphology and populations in citrus orchards

Citrus nematode was present in all the citrus orchards sampled and found that heavy infestations were present in all soils and roots with *T. semipenetrans*. The anatomical feature of nematode was observed under compound microscope. The juveniles were vermiform with sharp and strong stylet. The excretory pore and vulva were present at the posterior end of juvenile. Pore is surrounded by small irregular lobed shape. Esophagus of female was longer than the male. Intestine was not amalgamated and separated in distinguished parts. Male has reduced stylet and esophagus, no bursa and male was greater in diameter, female were swollen at the posterior. Female was swollen, single ovary and eggs deposited in matrix. Eggs of nematode were transparent, capsule in shape and mobile juvenile was present inside the egg (Figure 1a). The number of second stage juveniles plus male in 100 g soil sample was ranged from 20 to 40850 and number of females ranged from 3 to 1370 per 1 g of root. The mean population density of juvenile plus male in infested sample were 8030±463 per 100 g of soil and mean number of female 364±23 per 1 g of root (Figure 1b, 1c). The result showed that heavy infestation of citrus nematode is present in all the orchards surveyed that may responsible for reduced production and slow decline of citrus orchards.

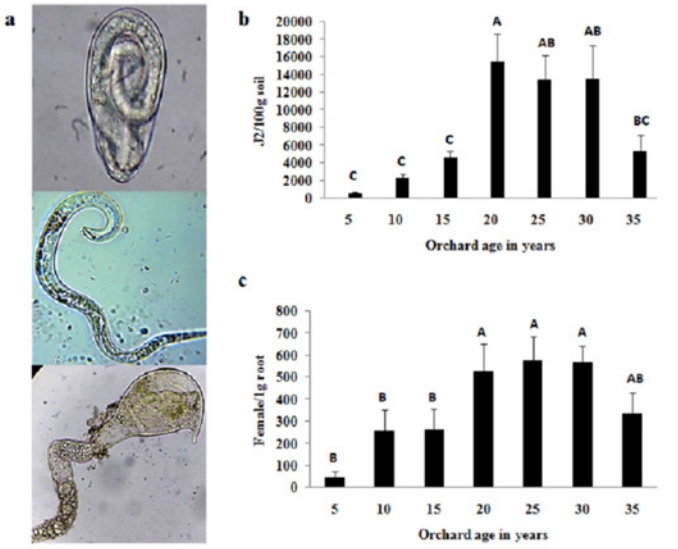

Figure 1: *Morphology and population densities of citrus nematode. (a) Morphology of citrus nematode egg, second stage juvenile and fourth stage female (b) Population of second stage juveniles in different age orchards (c) Population of T. semipenetrans females on 1 gram of root in relation to orchard age. ANOVA with Tukey's HSD test at* α=*0.05.*

Population dynamics of tylenchulus semipenetrans in different age group of citrus

Population of *T. semipenetrans* varied in relation to age of citrus orchards. Mean number of juvenile and female from each of orchards were taken and

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subjected to analysis of regression. Regression studies showed positive and significant relationship between ageing and nematode population in citrus. The result showed that there was positive relationship (r^2=0.73) between the number of juvenile per 100 g of soil to the aging of citrus orchards. For female nematode there was also a positive (r^2=0.86) relationship was found between the age and population of nematode. The mean population of Juveniles in 100 g of soil were 576, 2310, 4640, 15480, 13400, 13580 and 5370 for five, ten, fifteen, twenty, twenty five, thirty and thirty five years old citrus orchard respectively (Figure 2a). Mean number of females for five, ten, fifteen, twenty, twenty five, thirty and thirty five years old citrus orchard were 45, 260, 265, 528, 580, 570 and 335, respectively (Figure 2b). Highest numbers of nematode juvenile were found in twenty year citrus orchard; however, nematode population started decreasing in the samples of twenty years and upper age orchards. The population of early age orchards was also low due to the new plantations. The results of mature females population showed an increase with the increase of orchard age but started decreasing from the orchards of more than twenty five years age (Figure 2). The result showed that nematode population was less in new orchards and starts increasing as orchard age more than ten years. Moreover, nematode population was observed decreasing in orchards with more than twenty years of age.

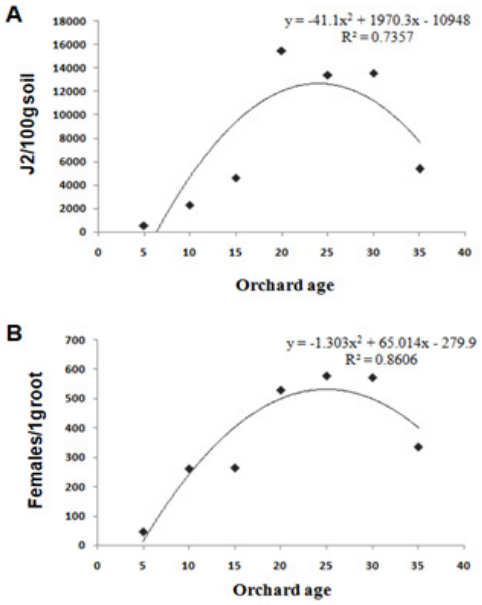

Figure 2: *Relationship between number of citrus nematodes and age of orchards. (A) Relation of number of second stage juveniles with citrus orchard age (B) The relationship between the numbers of females of citrus nematode with orchard age.*

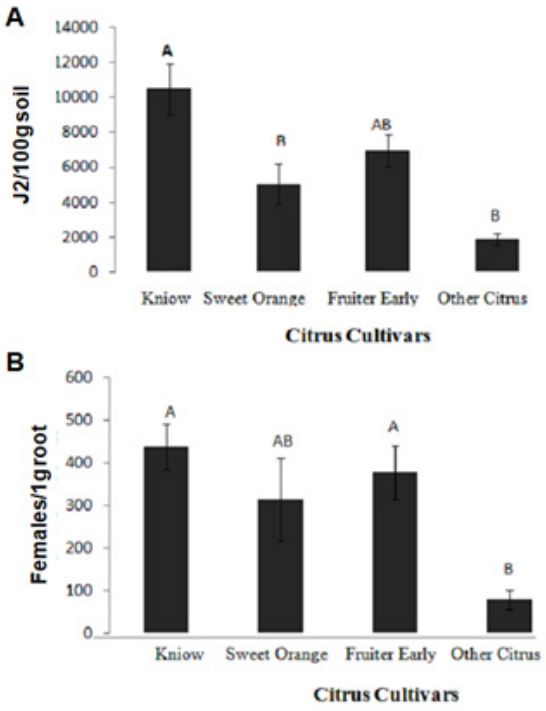

Figure 3: *Population densities of T. semipenetrans across various citrus cultivars. (A) Number of second stage juveniles per 100 g soil (B) Number of females per 1 gram of citrus root. ANOVA with Tukey's HSD test at* α*=0.05.*

Population density of citrus nematodes in different citrus cultivars

The populations of *T. semipenetrans* varied across the cultivar of citrus. Three main cultivars were considered for samples collection with rest of other citrus cultivars as fourth dataset. Maximum population of Juvenile plus male in 100 g of soil was observed in 'kinnow' orchards that were ranged from 425-37500 with the mean population of 10490 per100 g soil. The fruiter early orchards were also infested with nematode juvenile that were ranged from 7 to 18700 with mean number of 6965 per100 g soil. The orchards of sweat orange showed less infestation by nematode as compared to kinnow and fruiter early and population were ranged from 7 to 18700 with mean number 5040/100 g of soil. The rest of the cultivars showed least population of citrus nematode (Figure 3A). The population of females on roots was also observed maximum on kinnow followed by sweet orange, fruiter early and rest of cultivars respectively (Figure 3B). The results showed that nematode population densities vary with citrus cultivars and possibly presenting the role of rhizospheric microbes to manage the populations of nematodes.

Nematicidal activity of bacterial isolates

The rhizosphere soil from the healthy citrus plants

with more than twenty years of age was prepared for the isolation of bacteria. The differentially appeared colonies on agar plates were picked and totally 43 pure culture were generated. The bacterial cell cultures were prepared from all isolates to test the nematicidal activity against citrus nematode. The results showed that some of the isolates showed more than 85 % nematode mortality in plate assays. The number of isolates showed more than 50 % nematode mortality was selected for further assays. These selected isolates (09) were characterized morphologically and with molecular approaches (Table 2) and tested for nematicidal activity by using different approaches. The cell cultures of selected isolates (totally 09) showed a maximum of 50 % nematode mortality at 24 hrs and more than 70 % mortality at 48 hrs of incubation (Figure 4A). The nematode egg hatching was significantly reduced with the treatments of bacterial cell cultures as compare to the untreated control. The egg hatching was recorded between 18-35 % in bacterial treatments while 74 % in untreated control (Figure 5A). The results showed that rhizospheric bacteria especially from *Pseudomonas* and *Bacillus* group have the potential to kill the citrus nematodes in short duration and also reduced the egg hatching capacity.

Effect of bacterial culture filtrates on citrus nematode and egg hatching

The selected bacterial culture filtrates were prepared from pure cultures and nematicidal activity was tested in sterilized petri plates at 24 and 48 hrs of incubation at 25^{0}C. The nematodes (J2) treated with culture filtrates of bacteria gradually reduced their movement at 24 hrs and mostly died at 48 hrs of incubation. The evaluated bacterium displayed significant nematode mortality as compare to the control treatment. The all selected bacterial strains showed more than 30 % nematode mortality at 24 hrs of incubation and more than 60 % nematode mortality at 48 hrs of incubation. Individually, bacterial strains (BA21, BA44) showed more than 80 % nematode mortality at 48 hrs of incubation (Figure 4B). The culture filtrates of selected bacterial strains also exhibited significant reduction in nematode egg hatching. The nematode egg hatching was recorded in the range of 17.5-32 % for all tested bacterium while the positive control showed 74 % hatching of eggs. The bacterial strains BA 21 and BA44 exhibited most promising results with 17.5 and 13.75 % egg hatching followed by B32, and BA39 with less than 25 % egg hatching (Figure 5B). The anatomical changes in nematode juveniles were observed while treated with bacterial culture filtrates as compare to the healthy nematodes (Figure 6). The results showed that bacterial culture filtrates have potential compounds to kill the nematode directly and also significantly reduced the egg hatching capacity of nematode.

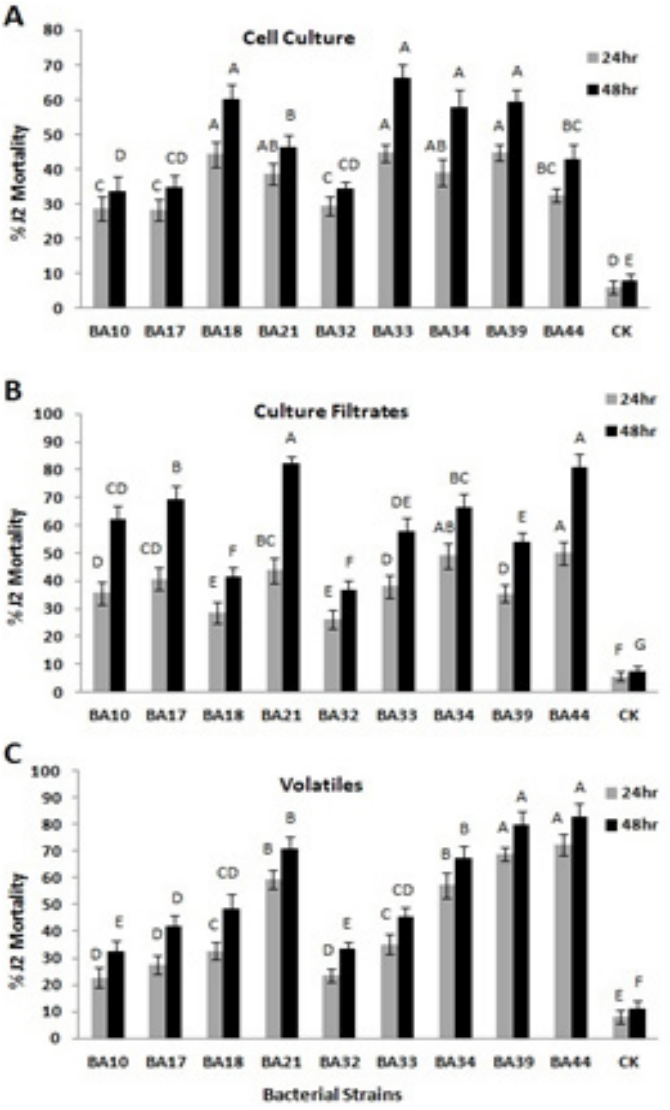

Figure 4: *Mortality assays for T. semipenetrans (J2) with bacterial cell culture, culture filtrates and volatiles. (A) Percentage mortality of second stage juveniles by different bacterial strains under 24 and 48 hrs of incubation. (B) Mortality percentage of second stage juveniles by bacterial culture filtrates at 24 and 48 hrs of incubation. (C) Mortality percentage of second stage juveniles by bacterial volatiles at 24 and 48 hrs of incubation. ANOVA with Tukey's HSD test at* α*=0.05.*

Activity of bacterial volatiles on J2 mortality nematode and egg hatching

The volatile effect of selected bacterial strains was also evaluated against citrus nematode (J2) and egg

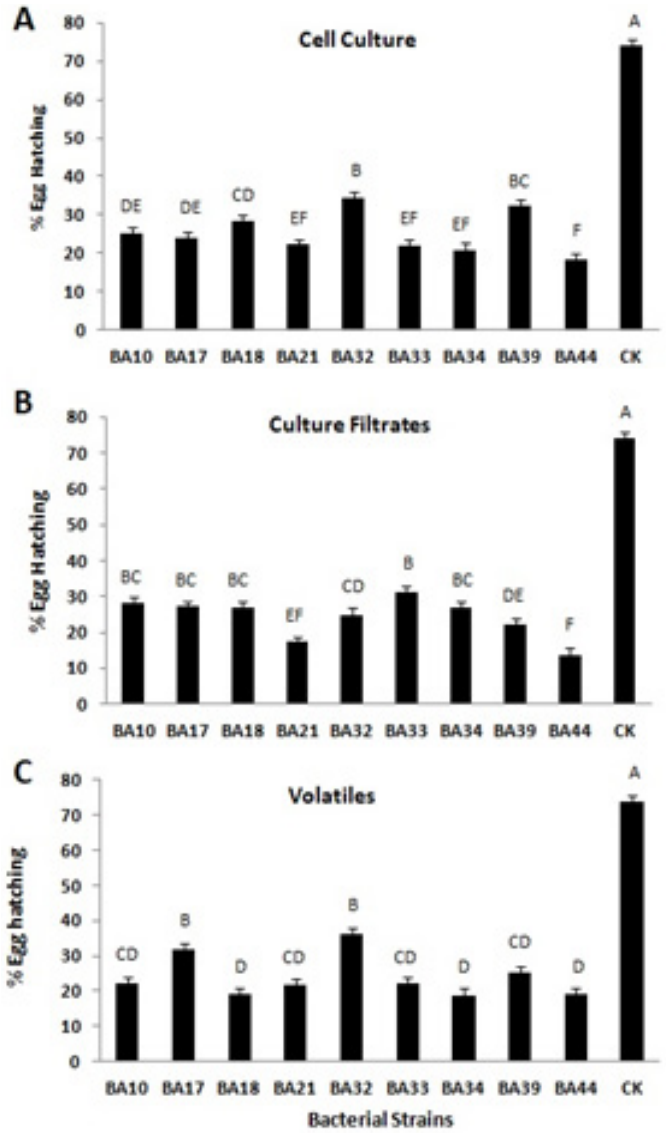

Figure 5: *Effects of bacterial cell culture, culture filtrates and volatiles on egg hatching of T. semipenetrans at 7 days of treatments (A) Percentage inhibition in egg hatching of citrus nematode by bacterial cell cultures (B) Percentage inhibition in egg hatching by culture filtrates (d) Inhibition in egg hatching by bacterial volatiles at 7 days of incubation. ANOVA with Tukey's HSD test at* α=*0.05.*

hatching. The bacterial volatiles exhibited significant J2 mortality and also reduced the nematode egg hatching capacity as compare to the control. The tested bacterial volatiles (BA39 and BA44) showed more than 70 % J2 mortality at 24 hrs and 80 % and 83 % J2 mortality at 48 hrs of incubation respectively. The other bacterial volatiles showed nematode mortality in the range of 30-70 % as compare to the control treatment which presented only 11.25 % J2 mortality (Figure 4C). The bacterial volatiles also exhibited significant reduction in nematode egg hatching as compare to the control. The bacterial strains BA18, BA34 and BA44 showed least egg hatching capacity of 19.25 %, 18.75 % and 19 % respectively. Moreover, other bacterial strains showed reduction in egg hatching in the range of 21 % to 40 % as compare to the control in which egg hatching was observed 74 % (Figure 5C). The result showed that bacterial volatiles contain such active compounds which exhibited nematicidal properties by killing the nematode juveniles in short period and also reduced the egg hatching capacity. Moreover, characterization of volatile compounds with nematicidal activity will be considered in future studies.

Discussion

Citrus slow decline is a threatening disease for the citrus industry of Pakistan and the nematode, *Tylenchulus semipenetrans* is considered to be responsible for the cause. The nematode penetrates into citrus roots during juvenile stage and complete rest of phases on plant (Duncan, 1991; Shokoohi and Duncan, 2018). By considering the aggravated intensityof this disease, a comprehensive survey was conducted to judge the population densities of *T. semipenetrans* in relation to citrus growing age and cultivars in the main citrus growing area of Sargodha, Pakistan. Survey results showed that *T. semipenetrans* occurrence was recorded in almost all the orchards (90-95 %) being sampled. It was also found that 94.7 % infestation of *T. semipenetrans* was recorded in citrus growing areas of China (Zhu *et al.*, 1992). Citrus orchards in southern California were infested up to 90-95 % by *T. semipenetrans* (Thorne, 1961). A survey of citrus nurseries in Andhra pradesh showed 75 % infestation of *T. semipenetrans* in India (Mani *et al.*, 1988). The citrus growing areas of KPK province of Pakistan were surveyed during the year 2005-2006 that showed the nematode infestation rate up to 81.66 % (Khan *et al.*, 2010*)*. A survey was conducted during 2002 in Sargodha district of Pakistan and reported 61.11 % infestation of citrus nematode (Parvez *et al.*, 2003). A recent study showed the incidence of citrus nematodes, which was recorded as maximum as 86 % and minimum as 44 % in Khyber Pakhtunkhwa province of Pakistan (Nasir *et al.*, 2021). The data represents an increase in nematode population with the passage of time. Tree age has a great effect on nematode population and distribution (Bellow *et al.*, 1986). In this survey, highest population of citrus nematode was found in twenty year citrus orchard, however, nematode numbers found in soil samples

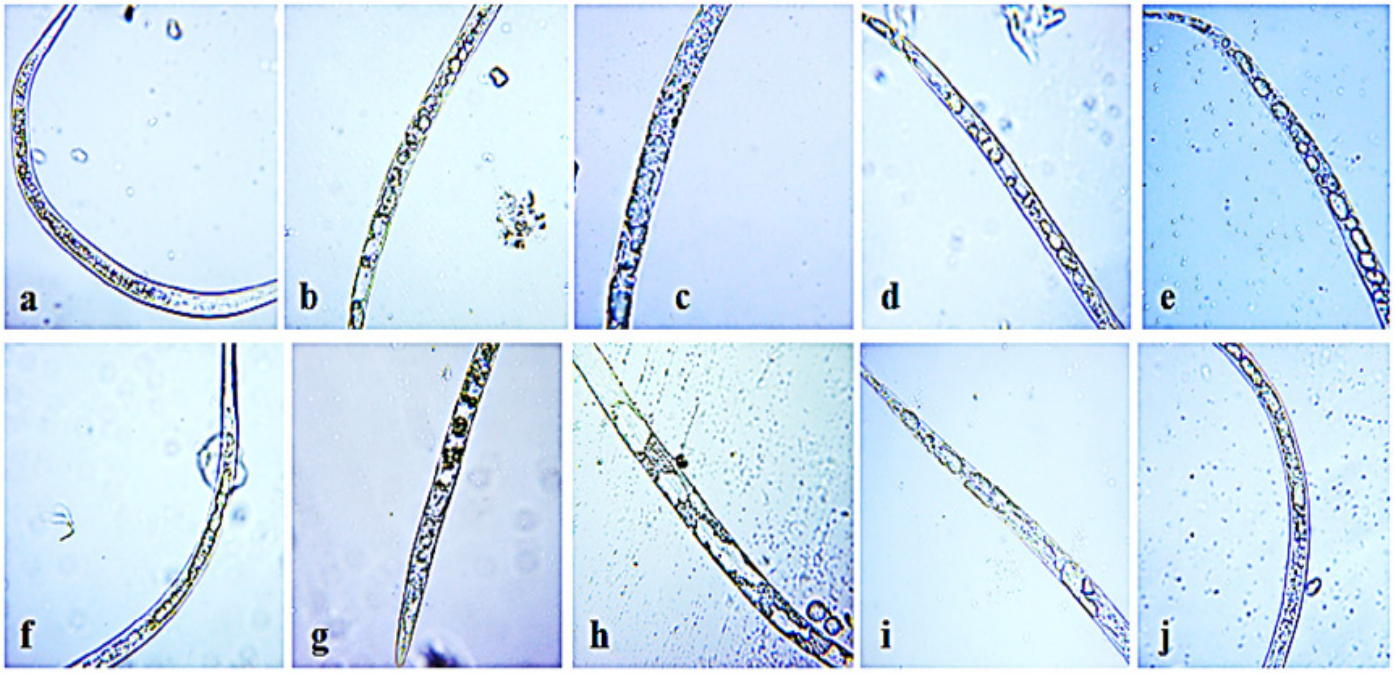

Figure 6: *Anatomical changes in body parts of citrus nematode with the treatment of bacterial filtrates. (a) Healthy second stage juvenile of T. semipenetrans; (b, c, d, e, f, g, h, i, j) second stage juveniles after the treatment with filtrates of different bacterial strains.*

of older orchards decreased gradually. This trend of population may also be influenced by root system of tree as more feeder roots are found in middle age mature trees as compared to old and very young trees. Moreover, microbial consortia of older trees may also contribute in the suppression of nematode. Long-term mono-culturing and intercropping of crops also affects the microbial community assemblage for the management of nematode densities (Hamid *et al.*, 2017; Bai *et al.*, 2018, Hussain *et al.*, 2018). By considering the citrus cultivars and nematode population, maximum population of nematode in 100 g of soil were found in the cultivar 'kinnow', followed by 'fruiter early' and 'sweet orange' respectively. A survey of citrus growing areas of Punjab and maximum incidence of citrus nematode was observed 56 % in Kinnow followed by 40 % for lemon and 35 % for sweet orange while least incidence was observed in grapefruit (20 %) (Khanzada *et al.*, 2008).

The microbial composition in rhizosphere of plant contributes in the suppression of fungal pathogens and plant parasitic nematode to decrease damage. *Bacillus cereus* commonly found in rhizosphere of crop suppresses plant parasitic nematode by adopting aggressive strategy (Gao *et al.*, 2016). *Pseudomonas fluorescens, Pseudomonas aeruginosa*, *Pseudomonas striata* and *Pseudomonas stutzeri* isolated from tomato rhizosphere that played a vital role in root knot disease suppression, growth promotion and nodulation (Davies, 1998; Khan *et al.*, 2016). In this study, we demonstrated that 70 % of the total 43 bacteria exhibited more than 30 % nematicidal activity to *T. semipenetrans*. This suggested that some of the bacteria could play a skeleton for development of potential biocontrol agents of plant parasitic nematode by further research (Tian *et al.*, 2007; Zhai *et al.*, 2018). However, chemical nematicide can also lead to suppression of beneficial micro-flora in the environment, and negative impact on biological equilibrium. Such shift in biological equilibrium may create a microbial vacuum, leading to increase in nematode population in future and cause even more damage than originally targeted (Gamliel *et al.*, 2000). Thus, non-synthetic chemical methods especially microbial products and phytochemicals that efficiently control plant parasitic nematode are highly desired (Alves *et al.*, 2020). Bacterial secondary metabolites and volatile compounds may be similar in their structure and mode of action. However, results of this study revealed that bacterial filtrate contain more than one nematicidal compound and have diverse mode of action to control plant parasitic nematode. Similarly, volatile compound produced from bacteria had variety of mechanism and more efficacies to control nematode than direct exposure (Bogner *et al.*, 2017; Cheng *et al.*, 2017). Besides, microbe used as volatile biological control could produce lasting effect

in the environment once introduced in the soil. It inhibits the growth of pathogen by the production of diverse antibacterial and antifungal compounds, such as zwittermicin, kanosamine, and lipopeptides for disease suppression (Aslam *et al*., 2021; Bonaterra *et al*., 2022; Aslam *et al*., 2022; Ayaz *et al*., 2023). Iturins and fengycins display strong antifungal activities, and inhibit the growth of a wide range of plant pathogens (kim *et al*., 2013; Guo *et al*., 2014; Aslam *et al*., 2023). *Pseudomonas putida* strain BA19 and BA21 showed 96 and 76% disease suppression respectively, during in vitro assays. Pyoluteorin, pyrrolnitrin, 2,4-diacetylphloroglucinol and phenazine are the major determinants of the biocontrol activity of Pseudomonads (Bernal *et al*., 2017). Organic amendments were successfully used to control plant soil borne disease by non-chemical approach. Combination of bacterial products with organic amendments could serve as potential biocontrol agents of plant disease and could be alternative of chemical nematicides. Among the mechanism of action examined in this study, degradation of intestine is most important as the damage of main digestive system unable the nematode in movement and parasitism of host plant and ultimately lead to the death. *Pseudomonas putida* was reported to degrade multiple intestinal factor of *Meliodogyne incognita* (Bachate *et al*., 2013; Gao *et al*., 2016; Huang *et al*., 2016; Ju *et al*., 2016).

Conclusions and Recommendations

In this study, population dynamics of *T. semipenetrans* was measured and correlated the nematode populations with orchard age and cultivars selected. *T. semipenetrans* is always considered to beresponsible for slow decline syndrome of citrus all over the world and different nematode management approaches are being focused. The potential of rhizosphere bacterial strains and their metabolites were tested against *T. semipenetrans* and found promising results with more than 70 % J2 mortality and also decreased the egg hatching ability of nematode. Furthermore, exploration of active compounds in bacterial filtrates and volatiles with nematicidal activity will be the focus of future research.

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Novelty Statement

This study illustrates the importance and functional attributes of native rhizobacteria for the management of citrus nematode (*T. semipenetrans*), a ubiquitous parasite causing slow decline of citrus.

Author's Contributions

M. Imran Hamid: Planned the experiments, analyzed the data and write manuscript.
Ehsan Abdul Qadir, **Saman Aslam** and **Adil Mahmood:** Performed experiment.
Waqas Raza: Analyzed the data and write manuscript, developed illustrations and reviewed the manuscript.
Asma Safdar and **Waqas Raza:** Developed illustrations and reviewed the manuscript.

Generative AI or AI assisted technology statement
The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest
Authors declare no conflict of interest.

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