Volume Number 9 || Issue Number 3 || Year 2024 || Page 33 || * Corresponding Author: malikabubakar3831@gmail.com||

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746

WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY

Research Manuscript www.sciplatform.com Peer review

Nematicidal activity of *Pseudomonas spp.* against citrus nematode (*Tylenchulus semipenetrans*)

[a] Wafia Batool, [b] Muhammad Abubakar Siddique, [b] Adnan Akhter, [c] Muhammad Khurshid, [b] Hafiz Muhammad Tariq *, [b] Nasir Ali, [b] Umair Raza, [b] Muhammad Zia Ullah

[a] Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha,
[b] Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore Pakistan,
[c] School of Biochemistry and Biotechnology, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan.

Contribution | **Batool, W., M.A. Siddique, A. Akhter** & **M. Khurshid** conceptualized the study, provided resources, and performed experiments. **N. Ali., H.M. Tariq., M. Z. Ullah** & **U. Raza** performed data analysis. All authors contributed in drafting and approved the final manuscript.

Citrus slow decline caused by *Tylenchulus Semipenetrans* is a common disease deteriorating both quality and quantity of citrus fruits. The need to assess the citrus nematode population in citrus orchards is of fundamental importance in designing suitable management strategies. Population densities of nematode were assessed in different age groups and in different genera of citrus crops. Orchards reaching to the age of 20 years were most infested by *T. semipenetrans* as compared to new established orchards and very old orchards. Kinow genus was the most infested by nematodes as compared to sweet orange, fruiter and grapefruit. Plant parasitic nematodes are commonly managed by chemical control due to its rapid results but it is toxic to human health, causes resistance development in pathogens and exerts harmful effects on environment. So, there is a need of an alternatives to these noxious chemicals because world is shifting to organic farming. Bacterial isolates were collected from citrus rhizosphere and subjected to biochemical characterization. Nematicidal activity of isolates against citrus nematode was screened in laboratory conditions. Out of total 41 tested bacteria, 20 (48.5 % of total) displayed more than 50 % nematicidal activity against *T. semipenetrans*, among which 2 isolates CA26 and CA41 exhibited nematicidal activity more than 80%. Culture filtrate of tested bacteria also reduced the population of nematode. 5 strains from total tested bacteria exhibited nematicidal volatile effect on *T. semipenetrans*. Bacterium degrades multiple tissues of nematode body including, pharynx, intestine, cuticle, stylet, esophagus lobe and reproductive system of nematode.

Keywords: Biocontrol, rhizobacteria, filtrate, rhizosphere, volatile compound.

INTRODUCTION: Citrus belongs to the *Rutaceae* family, which includes a diverse range of fruit trees and shrubs such as sour orange (*Citrus aurantium*), lime (*C. aurantifolia*), lemon (*C. limonia*) wild orange (*C. macroptera*), mandarins (*C. reticulata*), grapefruit (*C. paradisi*), sweet orange (*C. sinensis*), and *Fortunella spp.* (Hazarika, 2023). Citrus growing is widely practiced across the world's tropical and subtropical zones (Zekri, 2011). *Tylenchulus semipenetrans* is a major citrus nematode of citrus crops worldwide (Verdejo-Lucas and McKenry, 2004) . Male of *T. semipenitrans* has a life cycle of 7-10 days while for female it is 6-8 weeks. They reproduce either by amphimixis and parthenogenesis. The citrus nematode is a semi endoparasite of fibrous root of citrus; the female anterior section of the worm penetrates the cells of the roots while the posterior part remains outside the roots (Noe and Bernard, 2004). *T. semipenetrans* has been discovered in every citrus-growing region in the world since its discovery (Bozbuga *et al.*, 2023). Citrus nematode poses a serious warning to the industry because of its widespread incidence throughout the major citrus producing countries (Hammam *et al.*, 2021). Citrus nematode *T. semipenetrans* causes 10-30% yield losses, depending on the extent of infection, population density, soil quality, root stock type, presence or absence of other soil-borne diseases, and cultural practices (Abd-Elgawad *et al.*, 2016; Abd-Elgawad, 2020). *T. semipenetrans* has been documented from all citrus growing areas in Pakistan; however, it is more prevalent in Punjab than in other provinces and may be to blame for the citrus loss in this region (Haseeb *et al.*, 2024). Citrus nematode among other pathogens, is wreaking destruction on the district of Sargodha, which accounts for half of Pakistan's entire national citrus production. *Bacillus spp.* is responsible for the production of a vast number of noxious compounds to plant parasitic nematodes (Subedi *et al.*, 2020; Antil *et al.*, 2023). *B. thuringiensis* generates toxin inclusion protein, which is extremely poisonous to nematodes (Fernández-Chapa *et al.*, 2019). *Bacillus cereus* strain S2 has also been reported to produce nematicidal toxin to decrease the quantity of plant parasitic nematodes (Gao *et al.*, 2016; Yin *et al.*, 2021). *Pseudomonas spp*. is a genus of gram-negative, aerobic, chemo heterotrophic, and rod-shaped bacteria (Midhat and Abed, 2023). The genome sizes of a number of Pseudomonas spp. are >5,500 ORFs (Stover *et al.*, 2000), that are smaller than the genome size of other plant associated bacteria (Van Sluys *et al.*, 2003). *Pseudomonas spp.* include plant growth promoting and large group of secondary metabolites producing bacteria that are found in the rhizosphere of plants, and are active against plant pathogens (Shahid *et al.*, 2018). The phylogeny and diversity of different *Pseudomonas spp.* is responsible for the natural control of *T. semipenetrans* (Loper *et al.*, 2012; Shokoohi and Duncan, 2018). *Pseudomonads* suppress disease through direct aggressive actions on the plant pathogen and create systemic resistance (Shaikh *et al.*, 2020). There is battle for nutrient acquisition and other effects like that of antibiosis plausibly impacting the plants, without any direct contact with pest (Boominadhan *et al.*, 2009). Expression of Pseudomonas antibiotic and siderophore biosynthesis genes in the rhizosphere has been demonstrate (Zboralski and Filion, 2020). Gram negative bacteria, such as Pseudomonas isolates and chitinolytic bacteria, have the ability to manage nematodes (Soliman *et al.*, 2019).

OBJECTIVES: The present study has been designed to explore the bacterial species connected with citrus rhizosphere and play role in the inhibition of nematodes parasitizing the citrus roots.

MATERIALS AND METHODS: **Survey and sampling:** A survey of citrus orchards from District Sargodha was conducted for the collection of soil samples from at least 5 plants by digging up to 30 cm under the plant canopy from each location (Ullah *et al.*, 2023). The samples were packed appropriately in polythene bags, labelled and transferred to lab for further assessment (Stewart-Jones and Poppy, 2006).

Extraction of plant-parasitic nematodes: Before nematode extraction, soil samples were mixed thoroughly to remove stone and debris. The samples were subjected to nematode extraction by using tray method (Bhuiyan *et al.*, 2014). For extraction 100 g soil sample was placed on a perforated tray having towel paper (Haluschak, 2006). The perforated tray was placed on non-perforated tray having the water that just touches the surface of the perforated tray (Damodara, 2008). After 48 h, water was collected in a beaker. The excessive water was siphoned out when the juveniles settled (Bell and Watson, 2001).

Assessment of nematode population: Extracted nematodes were viewed and counted at magnification of 40X by using an inverted microscope (Murfin *et al.*, 2012). Then nematode suspension was collected in the pipette and placed in a counting dish. The nematodes were counted systematically following gridlines with each for three replicates. The average nematodes in a suspension of 1 ml were multiplied by the total suspension volume for the measurement of total population of nematodes. Total number of nematodes were counted from formula (Shapiro and Lewis, 1999).

Total no of Nematodes =
No. of nematode in mL of suspension X total volume of suspension.

After extraction, healthy nematodes were picked by micropipette, placed in a sterilized glass slide and covered with a cover slip. Physical and anatomical features of nematodes were identified under a camera fitted compound microscope.

Collection of rhizobacteria: The Pseudomonas strain was isolated from citrus rhizosphere and cultured on Nutrient Agar (NA) comprising of NaCl (0.05 g), agar (2 g), peptone (0.5 g) and beef extract (0.3 g) and distilled water up to 100 mL by following serial

dilution method (Nam, 2021). The colonies produced fluorescent pigments as siderophores (pyoverdine) and were carefully picked. The pure culture was transferred to nutrient broth media and stored at 28 °C for 24 h.

Culture filtrate preparation: Bacterial strains were grown on nutrient agar plates at 25 °C for 48 h, injected into 100 mL nutrient broth (0.05 g NaCl, 0.5 g peptone, and 0.3 g beef extract), centrifuged twice at 3000 rpm for 10 min., and filtered through a 0.4 µm to create cell-free cultures, then collected in sterilized falcon tubes.

In-vitro efficacy of bacterial cell culture on nematodes: Culture filtrates from all selected isolates were produced and passed through a 0.4 µm bacterial micro-filter (Khanal, 2019). The culture filtrates (20%) were made by diluting the filtrate in 1:4 ratios with sterilized distilled water (Cayrol *et al.*, 1989). About 5 mL of 20% filtrate was placed in sterilized petri plates and 1mL of nematode suspension (150-200 J 2/ mL) was added. In the control treatment, 5 mL of nutrient broth was mixed with 1 mL of nematode suspension. The data of percentage nematode mortality was recorded after 24, 48, and 72 h of incubation at room temperature (Mahar *et al.*, 2005). Each treatment had three replicates in the experiment.

$$Percentage\ of\ Mortality\ =\ \frac{n}{N}X\ 100$$

N= Total number of nematodes
n= Total number of dead nematodes

Nematicidal activity of cultural filtrate: *B. subtilis* and *P. fluorescens* bacteria were grown separately at 30°C for 1 week in conical flasks containing 100 mL of nutrient broth medium (Sharma *et al.*, 2023). The cultural filtrate was obtained by filtering each biocontrol agent and then centrifuged at 3000 rpm for 3 min. (Ganaie *et al.*, 2011; Pan and Jash, 2011). The cell filtrate and nematode suspension with 1:1 ratio was transferred into 24 well plates and observed under microscope to check the mortality rate and morphological changes of nematode (Aballay *et al.*, 2017).

Activity of bacterial volatiles on nematode: The nematicidal activity of bacterial volatile organic compounds(VOCs) was studied using a petri plate method (Fernando *et al.*, 2005; Xu *et al.*, 2015). In one compartment, 3 mL of the culture filtrate was added, and in the other, 0.2L nematode suspension was introduced. To prevent the volatiles from escaping, the plate lids were immediately sealed with para-film, and the plates were incubated at 28°C in the dark. Each treatment had three replicates and the trials were performed twice. The numbers of alive and dead nematodes were counted under an inverted microscope at 40X magnification after 24, 48, and 72 h to confirm the nematicidal activity of VOCs produced by different bacterial strains (Li *et al.*, 2024).

Examination of filtrate affected nematodes: After 48 h of treatment, anatomical modifications were studied under a microscope to identify the mechanism of action of potent strains. A micropipette was used to remove dead and straight nematodes from the treatment, which were then placed on a clean glass slide and covered with a cover slip (Hunt, 2020). The internal structure of fresh and treated nematodes was examined using a 40x lens in a camera-equipped compound microscope (Florman, 2020).

Data analysis: Nematicidal activity of *Pseudomonas spp*. was estimated by nematode mortality % during predacious test (El-Nagdi *et al.*, 2021). Data was collected as % nematode mortality against cell filtrates and volatiles of isolated bacteria. Collected data was subjected to analyses of variance, multiple comparison test, standard error and standard deviation using Microsoft Excel software. Graphs were plotted to show the means of percentage mortality.

RESULTS: Extraction and examination of *T. semipenetrans*: Soil samples were collected from 4 locations of district Sargodha. Soil samples collected from 97% of citrus orchards were contaminated with *T. semipenetrans.* Soil was sampled at the rate of 5 samples per orchard. Location 1 had 1104, location 2 had 884, location 3 had 1062 and location 4 had 1106 nematodes / 100 g of soil respectively.

Microscopic examination of nematode: Following anatomical features of nematodes were noted; nematodes are vermiform at the juvenile stage, and female nematode had a sharp and robust stylet (figure 1). The excretory orifice and vulva were found at the juvenile's posterior end. A tiny irregular lobed structure surrounds the pore. The female esophagus is longer than the male. The intestine was not merged and was segregated into distinct segments. There was no bursa, smaller stylet, smaller esophagus and larger diameter in males while females were swallowed, and enlarged with solitary ovary.

Figure 1: Microscopic examination of plant parasitic nematodes (*T.semipenetrans*). Nematodes exhibited vermiform shape at juvenile stage of growth (a), male with smaller esophagus (b), female with longer esophagus than male (c).

Identification of isolated bacteria: *Pseudomonas spp.* had an irregular colony that was dull white to blue in color, short rod cells and were gram-negative and migratory. In the presence of light, the colony exits yellow to blue pigment. Pseudomonas bacterium did not produces gas, and sugar fermentation was similarly negative in the majority of strains (figure 2). The cell motility of Pseudomonas bacteria was observed to be positive under a compound microscope. Most bacteria discovered in the citrus rhizosphere belong to *Bacillus spp.* It accounted for 31% of all bacteria identified from citrus rhizosphere. Colonies were typically white in color, rhizoid to filamentous, and irregular in shape in rare cases. Except for a few strains, there was no gas generation in this group, and glucose fermentation was negative. There was no pigment production in this group of bacteria.

In-vitro interaction of cell suspension of bacterial isolates against citrus nematode: The tested bacteria showed a wide range of nematicidal activity against *T. semipenetrans* (figure 3). After 24h. of incubation, bacterial isolate CA26 showed considerable nematode mortality (63.03%), followed by CA41 (55.7%), CA09 (50.6%), CA27 (48.8%), and CA62 (48.8%). After 48h of incubation, CA26 (88.02%) was followed by CA41 (70.8%), CA27 (70.52%), and 62(69.14%). From 5 isolated bacteria, 2 demonstrated more than 30% nematicidal activity against *T. semipenetrans.*

Microscopic examination of filtrate-treated nematodes: The nematicidal activity of the culture filtrate caused anatomical variations and death of nematodes. Isolates exhibiting significant nematicidal activity were studied for their mechanism of action against *T. semipenetrans*. Pathological characteristics of bacterial culture filtrate were assessed against *T. semipenetrans*. The nematodes (30-40 for each petri dish of *T. semipenetrans)* were treated with culture filtrate of isolates for 48 h. The sterilized nutrient broth was used as positive control. Then the differences between the culture filtrate treated group and the control group were examined and matched with compound microscope. The results indicated that the pathological characteristics of CA09 include accumulated intestinal tissue(a) and pharyngeal segment (b) and induced the reproductive system of nematode (c). CA26 affected on pharyngeal (d) and reproductive parts of nematodes that were distinct from control (f). Filtrate CA27 induced amalgamated structure of all parts of nematode (g, h, i). CA41 turned the intestine into granule (k), disrupted pharyngeal tissue (j) and malformed the genital area (l). CA62 disappeared from the pharyngeal parts (figure 4).

Volatile activity of bacteria against citrus nematode: The nematicidal effects of selected isolates (CA09, CA26, CA27, CA41, CA62) exhibiting substantial nematicidal activity were examined in a Petri plate assay for volatile effect against *T. semipenetrans* and data were recorded after 24, 48, and 72 h of incubation (figure 5).

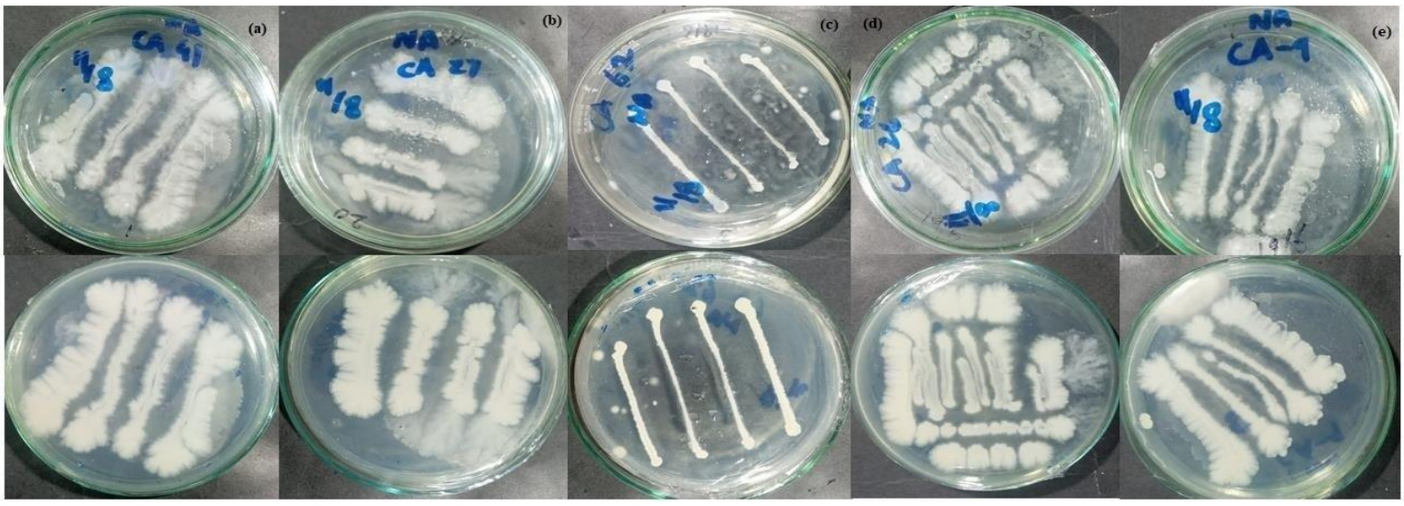

Figure 2: (a) *Bacillus* spp. (b) *B. subtilis* (c) *P. geniculata* (d) *P. putida* (e) *P. licheniformis* as bacterial strains.

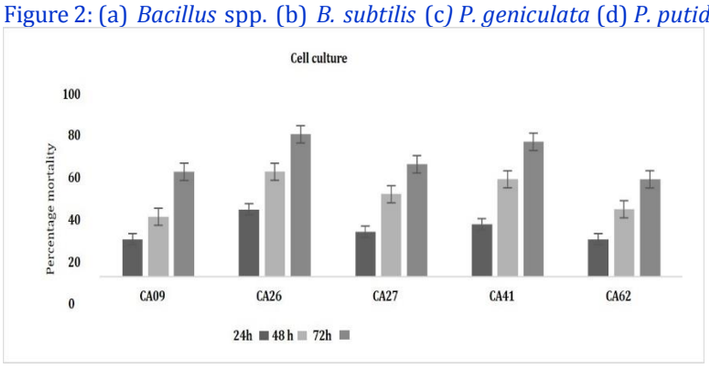

Figure 3: Nematicidal activity of cell suspensions of bacteria strains at 24 h, 48 h and 72 h against *T. semipenetrans*.

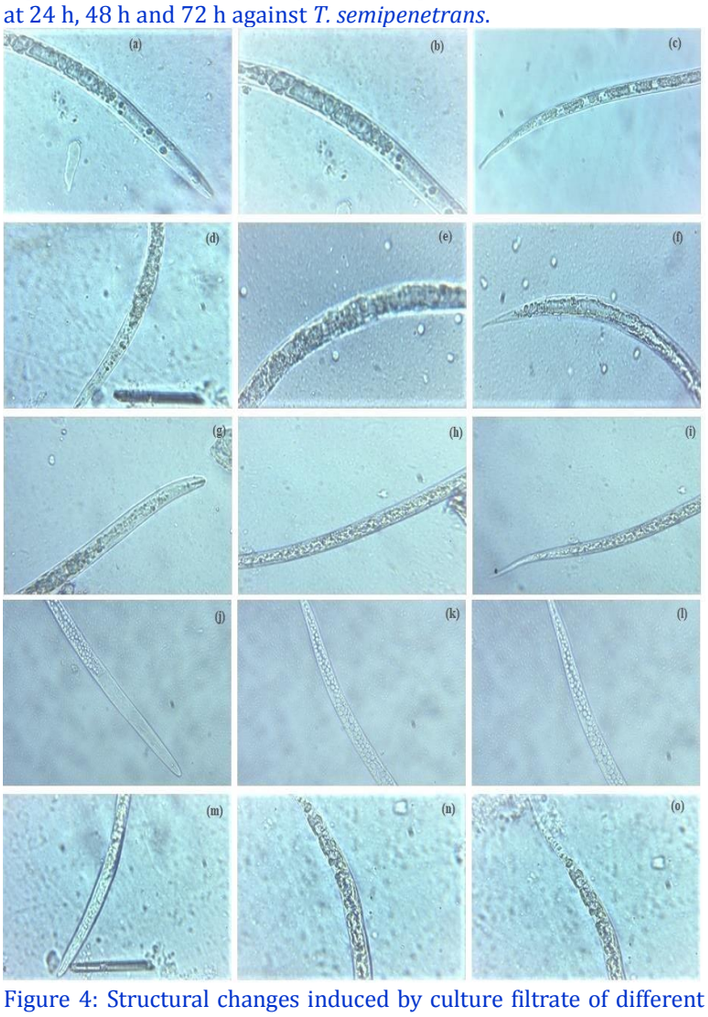

Figure 4: Structural changes induced by culture filtrate of different bacteria on the nematodes were observed under an inverted microscope after 72 h of incubation at 40X magnification. Nematode treated with sterilized nutrient broth accumulates different changes in its body. Isolate CA09 of *B. licheniformis* affects intestinal tissue (a) and induces pharyngeal area of nematode (b). Isolate CA26 of *P. putida* affects pharyngeal and reproductive parts of nematode (c, d). Isolate CA27 of *B. subtilis* induces amalgamated structure of all parts of nematode (e, f). Isolate CA41 of *P. putida* turns the pharyngeal parts of nematodes into granules (g to l). CA62 of *B. subtilis* also induces amalgamated structure of all segments of nematode body (m to o).

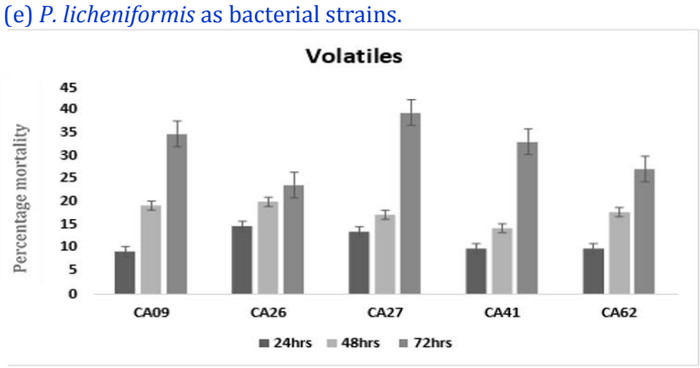

Figure 5: Nematicidal effects of different bacterial volatiles on *T. semipenetrans* at 24, 48 and 72 h.

In three replicates, the experiment was carried out in sealed petri plates with a plastic cup in the middle of the plate containing bacterium filtrate. Out of the five studied bacteria, two isolates CA26 and CA41 showed the highest mortality of 90% after 72 hours of incubation. In a sealed petri plate assay, isolates of CA26 caused (58.3%) mortality after 48 h and (79.2%) after 72 h. CA09, CA27, and CA62 isolates all demonstrated considerable mortality, with 58.3%, 62.5% and 54.2%, respectively.

DISCUSSION: Current analysis of citrus slow decline provides insights about incidence of *T. semipenetrans* in the rhizosphere of citrus plants of different age groups present in citrus orchards of Sargodha District of Punjab, Pakistan (Haseeb *et al.*, 2024). Our data analysis revealed that few of orchards were free from infection by the slow decline nematode and those were all properly managed and sanitized orchards. The population of nematodes is not consistent from per 100 g of soil as change observed in the number and type of nematode recovered (Gao *et al.*, 2020). Factors associated with the decline of nematode population in citrus orchards was attributed to the application of chemical pesticides including nematicides, followed by cultural and soil solarization measures as well on the type of root stock, soil and on the incidence or prevalence of other bacterial and fungal phytopathogens (Duncan and Cohn, 1990). Some citrus root stocks of Sunki, Tuzku 891, C-35 and Citrumelo 4475 are resistant to *T. semipenetrants*, thus offer genetic resistance against nematode. However, microbial composition in rhizosphere also suppresses the plant parasitic nematode to damage. *B. cereus* commonly found in rhizosphere of crops suppresses plant parasitic nematodes by adopting aggressive strategy (Gao *et al.*, 2016). *P. fluorescens*, *P. aeruginosa*, *P. striata,* and *P. stutzeri* isolated from citrus rhizosphere and play a vital role in disease suppression, growth promotion and nodulation (Ezrari *et al.*, 2021). In this study we demonstrated that 70% of the total 40 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 modification. Chemical nematicide can also guide to suppression of beneficial microflora in the environment, and negative impact on biological equilibrium. Such shift in biological balance may produce a microbial vacuum, leading to increase in nematode population in future & cause even more spoilage than originally under attack. Thus, non-synthetic chemical methods that efficiently control plant parasitic nematodes are highly desired. Bacterial secondary metabolites and volatile compounds may be similar in their structure and mode of action.

Results obtained from current study show that more than one nematicidal compounds are present in bacterial filtrates with diverse modes of action against plant parasitic nematodes. The volatile compounds released by these bacteria have different nematicidal mechanisms and are more efficient for the management of nematodes as compared to direct exposure. The mixture of potential bacteria could be more effective for nematode control and less chance of development of resistance than exposure of chemical nematicides composed of a single active compound. Among the mechanisms of action examined in this study, degradation of intestine is the most important because main digestive system of nematode gets damaged and nematode will not able to parasitize and will ultimately lead to death of nematode. Disintegration of the reproductive system leads to sterility in the nematode. *Pseudomonas putida* was reported to degrade multiple intestinal factors of *T. semipenetrans*. In this study we identified 2 isolates with more than 90% nematicidal activity towards *Tylenchulus semipenetrans*. These screened isolates could have the possible biocontrol efficacy in opposition to *T. semipenetrans*. The next step would be to search the potential of bacterium, secondary metabolite, and their volatile organic compounds in the control of citrus nematode in lab conditions using bacteria, either bacterial product, bacterial-bacterial combination and use of bacteria with organic amendment.

CONCLUSION: In comparison to sweat orange, fruiter, and grape fruit, the Kinow genus was the most affected by nematodes. Bacterial stains (CA26 and CA41) exhibited nematicidal activity more than 80% and can be used for the development potential biocontrol agents against citrus nematode.

ACKNOWLEDGEMENT: The authors are thankful to the University of the Sargodha for providing the resources to conduct the study.

FUNDING: This research was conducted without any funding.

CONFLICT OF INTEREST: All the authors declared no conflict of interest.

LIFE SCIENCE REPORTING: In current research article no life science threat was reported

ETHICAL RESPONSIBILITY: This is original research, and it is not submitted in whole or in parts to another journal for publication purpose.

INFORMED CONSENT: The author(s) have reviewed the entire manuscript and approved the final version before submission.

REFERENCES:

Aballay, E., S. Prodan, A. Zamorano and C. Castaneda-Alvarez, 2017. Nematicidal effect of rhizobacteria on plant-parasitic nematodes associated with vineyards. World journal of microbiology and biotechnology, 33(7): 1-14.

Abd-Elgawad, M. M., 2020. Managing nematodes in Egyptian citrus orchards. Bulletin of the national research centre, 44(1): 41.

Abd-Elgawad, M. M., F. F. Koura, S. A. Montasser and M. M. Hammam, 2016. Distribution and losses of *Tylenchulus semipenetrans* in citrus orchards on reclaimed land in Egypt. Nematology, 18(10): 1141-1150.

Antil, S., R. Kumar, D. Pathak and A. Kumari, 2023. Recent advances in utilizing bacteria as biocontrol agents against plant parasitic nematodes emphasizing *Meloidogyne* spp. Biological control, 183: 105244.

Bell, N. and R. Watson, 2001. Optimising the whitehead and hemming tray method to extract plant parasitic and other nematodes from two soils under pasture. Nematology, 3(2): 179-185.

Bhuiyan, S. A., B. J. Croft, G. Stirling, L. M. Meagher and E. Wong, 2014. Development of methods for screening sugarcane and erianthus germplasm for resistance to plant-parasitic nematodes. In: Proceedings of the Australian Society of Sugarcane Technologists. pp: 166-176.

Boominadhan, U., R. Rajakumar, P. K. V. Sivakumaar and M. Joe, 2009. Optimization of protease enzyme production using *Bacillus* sp. Isolated from different wastes. Botany research international, 2(2): 83-87.

Bozbuga, R., S. Yildiz, E. Yuksel, G. Özer, A. A. Dababat and M. İmren, 2023. Nematode–citrus plant interactions: Host preference, damage rate and molecular characterization of citrus root nematode *T. semipenetrans*. Plant biology, 25(6): 871-879.

Cayrol, J.-C., C. Djian and L. Pijarowski, 1989. Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. Revue de nematologie, 12(4): 331-336.

Damodara, E. K. C., 2008. Clinical trial to determine the accuracy of prefabricated trays for making alginate impressions. All ETDs from UAB. 6615.

El-Nagdi, W., M. A Youssef, H. Abd-El-Khair, M. Abd Elgawad and M. Dawood, 2021. Effectiveness of *Bacillus subtilis*, *B. Pumilus*, *Pseudomonas fluorescens* on *Meloidogyne incognita* infecting cowpea. Pakistan journal of nematology, 37(1): 35-43.

Fernández-Chapa, D., J. Ramírez-Villalobos and L. Galán-Wong, 2019. Toxic potential of bacillus thuringiensis: An overview. Protecting rice grains in the post-genomic era. ntech open publishers, pp: 1-22.

Fernando, W. D., R. Ramarathnam, A. S. Krishnamoorthy and S. C. Savchuk, 2005. Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil biology and biochemistry, 37(5): 955-964.

Florman, J. T., 2020. Neuroendocrine modulation of complex behavior and physiology in c. Elegans. Morningside GSBS Dissertations and Theses.

Ganaie, M. A., A. A. Rather and M. A. Siddiqui, 2011. Pathogenicity of root knot nematode *Meloidogyne incognita* on okra and its management through botanicals. Archives of phytopathology and plant protection, 44(17): 1683-1688.

Gao, H., G. Qi, R. Yin, H. Zhang, C. Li and X. Zhao, 2016. Bacillus cereus strain s2 shows high nematicidal activity against *Meloidogyne incognita* by producing sphingosine. Scientific reports, 6(1): 28756.

Haluschak, P., 2006. Laboratory methods of soil analysis. Canada-Manitoba soil survey, 3: 133.

Hammam, M., M. Abdel Gawad, W. Ruan and A. El-Bahrawy, 2021. Management of pests and pathogens affecting citrus yield in Egypt with special emphasis on nematodes. Egyptian journal of agronematology, 20(2): 64-84.

Haseeb, A., Y. Iftikhar, M. A. Zeshan, S. Ali, R. Binyamin, S. Ghuffar and M. U. Ghani, 2024. A study on population diversity of citrus nematodes in District Sargodha. Sarhad journal of agriculture, 40(1): 149-157.

Hazarika, T., 2023. Citrus. In: Fruit and nut crops. Springer: pp: 1-44.

HuntT, J. D. E. D. J., 2020. Handling, fixing, staining and mounting nematodes. Techniques for work with plant and soil nematodes: 71.

Khanal, R., 2019. Pathogen removal form domestic wastewater using membrane filter. Master thesis. University of Stavanger, Norway.

Li, J., X. Wei, Z. Pei, J. Sun, J. Xi, X. Li, D. Shapiro-IIan and W. Ruan, 2024. Volatile organic compounds released from entomopathogenic nematode-infected insect cadavers for the biocontrol of meloidogyne incognita. Pest management science.

Loper, J. E., K. A. Hassan, D. V. Mavrodi, E. W. Davis, C. K. Lim, B. T. Shaffer, L. D. Elbourne, V. O. Stockwell, S. L. Hartney and K. Breakwell, 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: Insights into diversity and inheritance of traits involved in multitrophic interactions. PLoS genetics, 8(7): e1002784.

Mahar, A. N., M. Munir, S. Elawad, S. R. Gowen and N. G. M. Hague, 2005. Pathogenicity of bacterium, *Xenorhabdus nematophila* isolated from entomopathogenic nematode (*Steinernema carpocapsae*) and its secretion against *Galleria mellonella* larvae. Journal of zhejiang university science B, 6(6): 457-463.

Midhat, M. S. and S. M. Abed, 2023. Isolation and identification of pathogenic species of the genus pseudomonas and study of antibiotic resistance. GSC biological and pharmaceutical sciences, 23(1): 087-098.

Murfin, K. E., J. Chaston and H. Goodrich-Blair, 2012. Visualizing bacteria in nematodes using fluorescent microscopy. Journal of visualized experiments)(68): e4298.

Nam, J. H., 2021. Multidisciplinary approach to evaluate the effect of plant growth promoting rhizobacteria (pgpr) on soil microbiota and quality of strawberries. MSc Dissertation. Oregon State University.

Noe, J. P. and E. Bernard, 2004. Plant-parasitic nematodes. Trigiano, R. N .Plant pathology: concepts and laboratory exercises, 3rd edition. CRC Press, Boca Raton.

Pan, S. and S. Jash, 2011. Variability in biocontrol potential and microbial interaction of *Trichoderma* spp. With soil inhabiting antagonistic bacteria *Pseudomonas fluorescens*. Indian phytopathology, 63(2): 158-164.

Shahid, I., K. A. Malik and S. Mehnaz, 2018. A decade of understanding secondary metabolism in *Pseudomonas* spp. For sustainable agriculture and pharmaceutical applications. Environmental sustainability, 1: 3-17.

Shaikh, S., N. Yadav and A. R. Markande, 2020. Interactive potential of *Pseudomonas* species with plants. Journal of applied biology and biotechnology, 8 (6): 101-111.

Shapiro, D. I. and E. E. Lewis, 1999. Comparison of entomopathogenic nematode infectivity from infected hosts *Versus aqueous* suspension. Environmental entomology, 28(5): 907-911.

Sharma, L., S. K. Shukla, V. P. Jaiswal and A. Gaur, 2023. Novel strains of *Pseudomonas fluorescens* and *Bacillus cereus* and their integrated use with inorganic fertilizers enhancing p availability, crop growth parameters, and sugarcane yield in subtropical india. Sugar tech, 25(6): 1467-1485.

Shokoohi, E. and L. W. Duncan, 2018. Nematode parasites of citrus. In: Plant parasitic nematodes in subtropical and tropical agriculture. CAB International Wallingford UK: pp: 446-476.

Soliman, G. M., H. H. Ameen, S. M. Abdel-Aziz and G. M. El-Sayed, 2019. In vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. Bulletin of the national research centre, 43: 1-7.

Stewart-Jones, A. and G. M. Poppy, 2006. Comparison of glass vessels and plastic bags for enclosing living plant parts for headspace analysis. Journal of chemical ecology, 32: 845-864.

Stover, C., X. Pham, A. Erwin, S. Mizoguchi, P. Warrener, M. Hickey, F. Brinkman, W. Hufnagle, D. Kowalik and M. Lagrou, 2000. Complete genome sequence of *Pseudomonas aeruginosa* pao1, an opportunistic pathogen. Nature, 406(6799): 959-964.

Subedi, P., K. Gattoni, W. Liu, K. S. Lawrence and S.-W. Park, 2020. Current utility of plant growth-promoting rhizobacteria as biological control agents towards plant-parasitic nematodes. Plants, 9(9): 1167.

Ullah, M. I., M. Arshad, A. Abdullah, N. Altaf, S. M. A. Zahid and M. Afzal, 2023. Diversity of arthropods in citrus orchards of Sargodha, Pakistan: Use of different trapping techniques. International journal of tropical insect science, 43(5): 1797-1809.

Van Sluys, M.-A., M. De Oliveira, C. B. Monteiro-Vitorello, C. Y. Miyaki, L. Furlan, L. E. A. Camargo, A. Da Silva, D. H. Moon, M. A. Takita and E. Lemos, 2003. Comparative analyses of the complete genome sequences of pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. Journal of bacteriology, 185(3): 1018-1026.

Verdejo-Lucas, S. and M. McKenry, 2004. Management of the citrus nematode, tylenchulus semipenetrans. Journal of nematology, 36(4): 424.

Xu, Y. Y., H. Lu, X. Wang, K. Q. Zhang and G. H. Li, 2015. Effect of volatile organic compounds from bacteria on nematodes. Chemistry & biodiversity, 12(9): 1415-1421.

Yin, N., J.-L. Zhao, R. Liu, Y. Li, J. Ling, Y.-H. Yang, B.-Y. Xie and Z.-C. Mao, 2021. Biocontrol efficacy of *Bacillus cereus* strain bc-cm103 against *Meloidogyne incognita*. Plant disease, 105(8): 2061-2070.

Zboralski, A. and M. Filion, 2020. Genetic factors involved in rhizosphere colonization by phytobeneficial *Pseudomonas* spp. Computational and structural biotechnology journal, 18: 3539-3554.

Zekri, M., 2011. Factors affecting citrus production and quality. Trees. Citrus industry, 6-9.