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Research Article

A PRELIMINARY STUDY ON THE INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND SOME STRAINS OF *PSEUDOMONAS* SPP. ON GROWTH PERFORMANCE OF TOMATO UNDER GREENHOUSE CONDITIONS

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A B S T R A C T

The present study investigated the biological management of root-knot nematode (*Meloidogyne incognita*) in tomato plants using different strains of *Pseudomonas* spp. The experiment involved five treatments: a healthy control (T1), a nematode-inoculated control (T2), *Pseudomonas fluorescens* strain A (T3), *P. fluorescens* strain B (T4), and *P. putida* (T5). The results demonstrated significant differences in plant growth and root gall formation across treatments. The healthy control exhibited an average plant height of 12 cm and weight of 11 g. Nematode inoculation slightly reduced growth, with plants averaging 11 cm in height and 10 g in weight. Treatment with *P. fluorescens* strain A resulted in the highest growth, with plants reaching 13 cm in height and 12 g in weight. *P. fluorescens* strain B and *P. putida* treatments produced plants with average heights of 12 cm and 12.5 cm, and weights of 11 g and 10 g, respectively. Leaf and flower counts also varied, with T3, T4, and T5 treatments increasing leaf counts to 26, 40, and 33, respectively, compared to 15 in the nematode-inoculated control. The application of *P. fluorescens* strains A and B significantly reduced root galls to 8 and 15, respectively, compared to 29 in T2. *P. putida* treatment resulted in 21 root galls. These findings highlight the potential of *Pseudomonas* spp., especially *P. fluorescens* strains, in managing nematode infestation in tomato plants. Future studies should explore the mechanisms behind these microbial interactions and their long-term effects on plant health and yield.

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INTRODUCTION

Tomato plants are considered the most important fruit vegetables in the world and belong to the Solanaceae family (Raza et al., 2022). In Pakistan, people use tomatoes both as a fruit and a vegetable, with their consumption as a vegetable being more common. In Pakistan, the total field area under this crop was 60.5 hectares during 2016-17. Tomatoes are susceptible to over 200 diseases (Ali et al., 2023a), with soilborne diseases being a major concern. Soilborne pathogens especially plant pathogenic nematodes, bacteria and fungi are challenging to control due to their persistence in soil and wide host range (Hussain et al., 2012; Tariq-Khan et al., 2017; Hussain and Mukhtar, 2019; Asghar et al., 2020; Cheng et al., 2021; Aslam and Mukhtar, 2023a,b, 2024; Aziz et al., 2024; Yaseen et al., 2024).

Plant parasitic nematodes, such as root-knot nematodes, cause major yield losses that deteriorate both the quantity and quality of the tomato and other crops (Hussain et al., 2011; Mukhtar and Hussain, 2019; Yaseen et al., 2023). Root-knot nematodes are among the most important nematodes of crop plants, affecting a variety of hosts (Tariq-Khan et al., 2020; Saeed and Mukhtar, 2024). Root-knot nematode species are immobile endoparasites living in roots and are responsible for the development of specialized feeding structures known as giant cells. The infectious stage of the nematode is the second-stage juvenile (J2). J2 penetrates the roots and progresses to adult females or males through three successive stages. The process of feeding site development by root-knot nematodes is not well understood because it is dynamic and complex, involving genes from both the nematode and the host plant. *Meloidogyne incognita* is a root-knot nematode that severely affects tomato crops by retarding the growth of tomato plants (Kayani et al., 2017; Mukhtar et al., 2017; Mukhtar and Kayani, 2020) and is widespread in vegetable-growing regions of Pakistan significantly reducing growth and yield (Kayani et al., 2018). This species has a 47% global distribution and a 52% presence in Pakistan (Kayani et al., 2013). Reports indicate that root-knot nematodes can cause yield losses ranging from 50% to 80% in vegetable crops and production losses ranging from 24% to 38% in tomato crops. *M. incognita* solely is estimated to cause a 24% to 38% yield loss in tomatoes globally (Mukhtar, 2018). In terms of money, this nematode causes an annual loss of 157 billion dollars in production (Hassan et al., 2013).

Nematode-induced damage to plants often goes unnoticed, as diseases related to nutrition and water can cause symptoms such as slow growth, stunting, and yellowing. The use of nematicides to inhibit and restrict nematode growth has its drawbacks (Azeem et al., 2021).

Historically, chemical nematicides have been the primary method for controlling root-knot nematodes (Desaeger and Watson, 2019). However, due to the potential risks these chemicals pose to human health and the environment, there is a growing shift towards safer and more environmentally friendly alternatives (Mukhtar et al., 2013a; Hussain et al., 2016; Kayani and Mukhtar, 2018; Ntalli et al., 2018; Khan et al., 2019; Mukhtar and Kayani, 2019; Sayedain et al., 2021; Haq et al., 2022; Haque and Khan, 2022; Mukhtar et al., 2021). As a result, developing effective and eco-friendly nematode management strategies has become a critical focus in modern agriculture.

The detrimental impacts of synthetic nematicides highlight the need for the development of environmentally friendly solutions to manage root-knot nematodes, which are effective for organic and sustainable farming (Stirling, 2011; Afzal and Mukhtar, 2024). Biocontrol methods for root-knot nematodes encompass biological organisms, including plant growth-promoting rhizospheric bacteria and fungi, which have been increasingly preferred and proven to be efficacious over many years (Nazir et al., 2019; Subedi et al., 2020; Saeed et al., 2021, 2023). Several bacteria and fungi found in the rhizosphere, such as *Xenorhabdus bovienii*, *Bacillus thuringiensis*, *Pasteuria penetrans*, *Pseudomonas fluorescens*, and *Bacillus amyloliquefaciens* have been proven effective in controlling root-knot nematodes (Mukhtar et al., 2013b; Khan et al., 2020; Liu et al., 2020). These microorganisms control root-knot nematodes through multiple mechanisms, such as inhibiting their hatching, eliminating root-knot nematode females and juveniles, and decreasing the overall nematode population in the soil (Ahmed et al., 2021; Liu et al., 2022). Biocontrol agents can exert their effects on infections through either direct or indirect antagonism. Direct antagonism arises through predation, the secretion of harmful metabolites like lytic enzymes, antibiotics, volatile chemicals, siderophores, or competition for nutrition and exclusion from a certain ecological niche. Indirect antagonism, on the other hand, occurs through the stimulation of systemic resistance or the release of chemicals that alter nematode behavior, such as feeding, recognition, and sex ratio (Antil and Kumar, 2019). One indirect approach for enhancing plant tolerance is the production of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC deaminase), which reduces ethylene levels in plants. Certain plant growth-promoting rhizobacteria enhance plant health by generating substances that trigger induced systemic resistance (ISR), thereby activating plant defense mechanisms to thwart worm infections.

The objective of this study was to evaluate the effectiveness of different strains of *Pseudomonas* spp., specifically *P. fluorescens* (A), *P. fluorescens* (B), and *P. putida*, in the biological management of root-knot nematode (*M. incognita*) infestations in tomato plants. The study also aimed to determine the impact of these bacterial strains on plant growth parameters of tomato.

MATERIALS AND METHODS

Media preparation and nematode extraction

The *Pseudomonas* strains were obtained from the Crop Diseases Research Institute (CDRI) at the National Agricultural Research Centre (NARC) in Islamabad. Nutrient agar medium was prepared by dissolving 28 g of the powder in 1 L of distilled water and autoclaving at 121°C for 15 min. The isolates were then reisolated and purified for further research. Nematodes were extracted using the techniques outlined by Chitruk et al. (2020).

Experimental design

Cultivation and transplantation of tomato seedlings

Tomato seedlings of the "Rio Grande" variety were transplanted into earthen pots containing 1 kg of sterilized soil. The soil was a mixture of sand, field soil, and farmyard manure (FYM). Prior to planting, the soil was watered to soften it, and 10 tomato seeds were sown per pot. The experimental setup included 5 different treatments; each replicated five times (Table 1).

Table 1. Description of treatments.

Treatment	Description
T1	Control (Healthy)
T2	Control (Nematode inoculated)
T3	*Pseudomonas fluorescence* (A)
T4	*Pseudomonas fluorescence (B)*
T5	*Pseudomonas putida*

After two to three weeks, the germinated tomato seedlings were transplanted into individual pots, ensuring one healthy plant per pot. To investigate the activity of *Pseudomonas* spp. strains, an adequate bacterial culture was mixed with wheat straw and applied to the rhizosphere of the tomato plants. Additionally, a 5 ml suspension of nematode culture, containing approximately 2000 *Meloidogyne incognita* juveniles, was inoculated into each tomato seedling. The pots were arranged in a completely randomized design under greenhouse conditions for 7 weeks and watered as needed.

Parameters studied

Plant growth parameters, including plant height, plant weight, and the number of leaves and flowers, were measured according to the methodology described by Costa, et al. (2021). The number of nematode-induced root galls was estimated using the procedure outlined by Radwan et al. (2012).

Statistical analysis

Results are presented as the mean ± standard error of the mean (SEM). Statistical analysis was conducted using ANOVA, followed by post hoc tests for further investigation. A p-value of less than 0.005 was considered statistically significance.

RESULTS

Plant height and plant weight

The experimental findings revealed significant variations in the growth patterns of tomato plants across different treatments (Figure 1). In the control group (T1), which maintained healthy plants, the average plant height reached 12 cm, with an average plant weight of 11 g. In contrast, the control group subjected to nematode inoculation (T2) experienced a slight reduction in growth, with an average height of 11 cm and a corresponding weight of 10 g.

For the treatments involving microbial inoculants, the application of *P. fluorescens* (Strain A) (T3) resulted in an average plant height of 13 cm and an average plant weight of 12 g. Similarly, the use of *P. fluorescens* (Strain B) (T4) produced plants with an average height of 12 cm and an average weight of 11 g. Interestingly, the application of *P. putida* (T5) resulted in an average plant height of 12.5 cm, although the plant weight was relatively lower at 10 g (Figure 2).

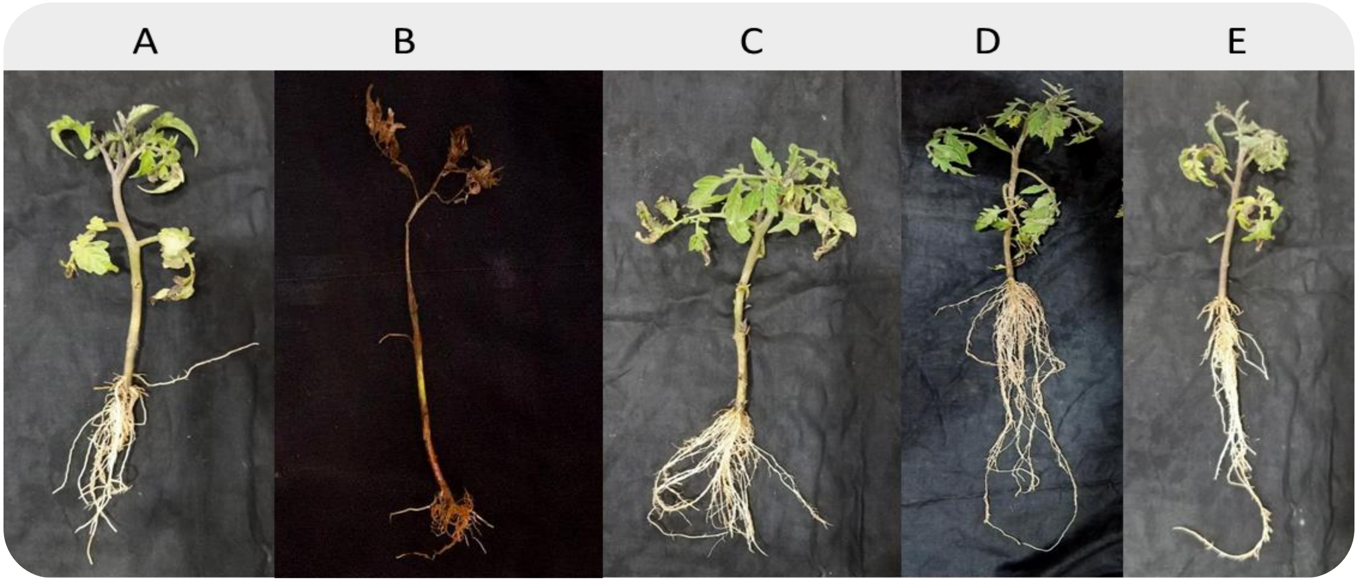

Figure 1. (A) healthy plant, (B) only nematode, (C) *Pseudomonas fluorescence* (A), (D) *Pseudomonas fluorescence* (B), and (E) *Pseudomonas putida.*

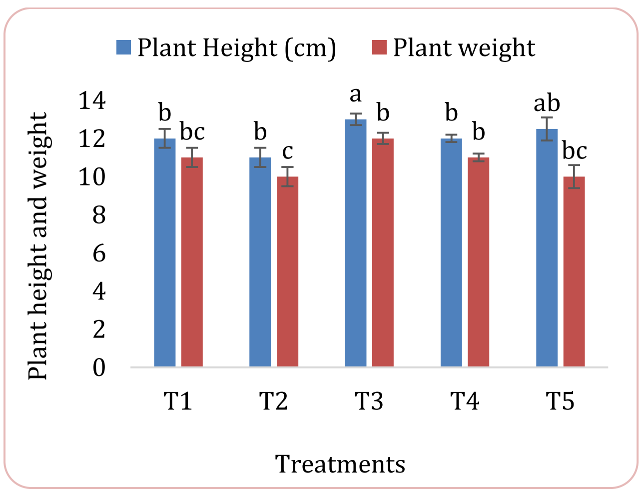

Figure 2. (T1) healthy plant, (T2) only nematode, (T3) *Pseudomonas fluorescence* (A), (T4) *Pseudomonas fluorescence* (B), and (T5) *Pseudomonas putida.*

Numbers of leaves and flowers

The findings of the study revealed distinct variations in the development of leaves and flowers across different treatments (Figure 3). In the control group (T1), which cultivated healthy plants, an average of 18 leaves and 2 flowers per plant were recorded. Conversely, the control group subjected to nematode infestation (T2) displayed a slight reduction in leaf count, averaging 15 leaves, accompanied by an increase in the average number of flowers to 3 per plant.

Among the microbial treatments, the application of *P. fluorescens* (Strain A) (T3) resulted in a notable increase in both leaf and flower numbers, averaging 26 leaves and 8 flowers per plant. Similarly, the use of *P. fluorescens* (Strain B) (T4) led to a significant increase in leaf count, averaging 40 leaves per plant, with an average of 5 flowers. Interestingly, treatment with *P. putida* (T5) produced an average of 33 leaves and 4 flowers per plant.

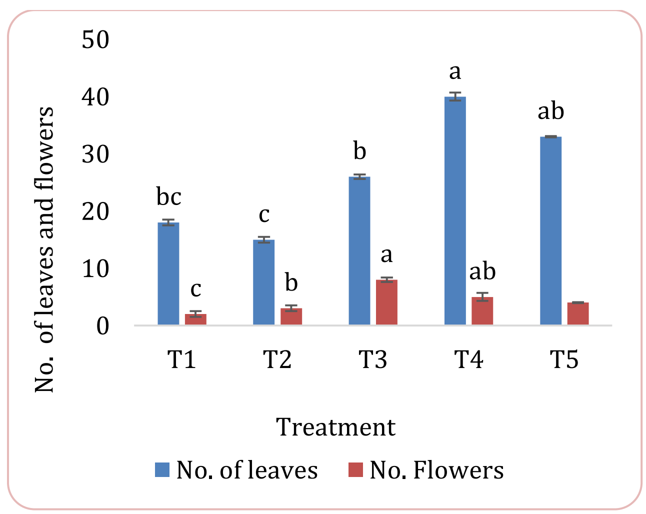

Figure 3. (T1) healthy plant, (T2) only nematode, (T3) *Pseudomonas fluorescence* (A), (T4) *Pseudomonas fluorescence* (B), and (T5) *Pseudomonas putida.*

Root galling

The study's outcomes revealed discernible trends in root gall formation across the distinct treatments (Figure 4). Within the control group (T1), characterized by the presence of healthy plants, and has no galls developments. In stark contrast, the control group exposed to nematode infestation (T2) demonstrated a marked escalation in root gall occurrence, registering an average of 29 root galls.

In the realm of microbial interventions, the administration of *P. fluorescence* (Strain A) (T3) correlated with a noteworthy decrease in root gall formation, with an average of 8 root galls per plant. Likewise, the employment of *P. fluorescence* (Strain B) (T4) resulted in an average of 15 root galls per plant. Interestingly, the application of *P. putida* (T5) yielded an average of 21 root galls per plant.

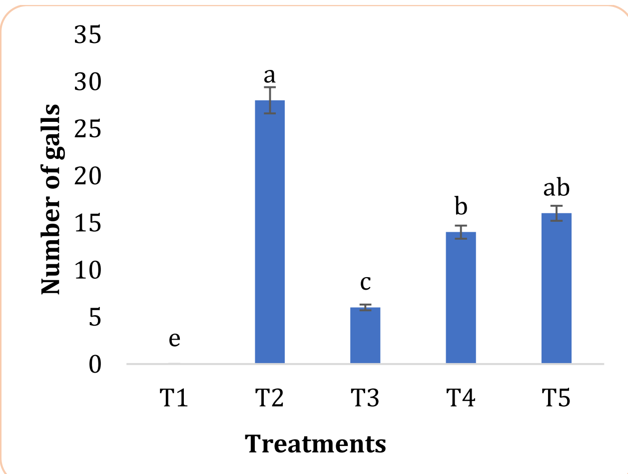

Figure 4. (T1) healthy plant, (T2) only nematode, (T3) *Pseudomonas fluorescence* (A), (T4) *Pseudomonas fluorescence* (B), and (T5) *Pseudomonas putida.*

DISCUSSION

The application of several *Pseudomonas* spp. strains are one of the promising biological management options that could be used to suppress root-knot nematodes (*Meloidogyne incognita*) in tomato crops and in several other crops (Azeem et al., 2020; Ali et al., 2022; Tabbasum et al., 2022; Ali et al., 2023b; Nauman et al., 2023). Numerous studies have examined the effectiveness of various *Pseudomonas* spp. strains in reducing nematode infestations through mechanisms such as direct antagonistic relationships, systemic resistance induction, and hormone regulation that promotes plant growth. For instance, research by Khanna et al. (2021) demonstrated that certain strains of *Pseudomonas* spp. effectively suppressed nematode population densities in tomato plants by colonizing the rhizosphere, competing for resources, and producing nematocidal compounds. Sharma and Sharma, (2017) used pseudomonad rhizobacteria filtrates to prevent root-knot disease both *in vitro* and in the greenhouse. Specifically, the *P. jessenii* strains R62 and R81 controlled root-knot nematodes (*M. incognita*) on tomato plants. All treatments with R62 and R81 (25%, 50%, 75%, 100%) and all R62 + R81 dilutions killed second-stage juveniles in the lab. There was no effect on J2 at 25%, and some mortality at 50%. Combining R62 and R81 in greenhouse conditions resulted in significant variations in plant growth characteristics. Plants under nematode stress showed a significant increase in growth metrics after receiving the same treatment (R62 + R81), unlike nematode-inoculated plants.

Chinheya et al. (2017) investigated 70 *Bacillus* isolates against *M. javanica* J2 on soybean *in vitro*. After successive dilutions, the primary spore suspension was 10^8 per ml, and five isolates viz. BC 27, BC 29, BC 31, BC 56, and BC 64 caused more than 50% mortality. Three of these five isolates namely BC 27, BC 29, and BC 31 from the grass rhizosphere in goat pastures induced 80% larval mortality after 24 hours and were selected for a second screening. In a second *in vitro* screening, BC 27 produced 100% mortality after 3 h, while BC 29 induced more mortality than BC 31. BC 27 and BC 29 were more effective than BC 31 after 24 h. In glasshouse tests, bacterial isolates BC 27 and BC 29 significantly reduced gall formation and eggmasses. *Bacillus* spp. are also widely employed to control plant-parasitic nematodes (Lee et al., 2021). Nematicidal metabolites from *Bacillus* spp. minimize chemical use. A possible biocontrol agent, *B. subtilis*, may generate spores and has other traits that help it survive in the rhizosphere (Rao et al., 2017).

The endospore-forming parasites of *Pasteuria* reduce root-knot nematodes on tomato, grapevine (*Vitis vinifera* L.), tobacco (*Nicotiana tabacum* L.), and peanut (*Arachis hypogaea* L.) (Mukhtar et al., 2013b). This host-specific root-knot nematode parasite is very virulent and resistant to nematicides. Cetintas and Dickson (2004) found that *P. penetrans* reduced *M. arenaria* race 1 root galls on peanut. Bakr et al. (2017) found that *P. penetrans* controlled *M. arenaria* on tomato. Additionally, *Serratia plymuthica*, a ubiquitous bacterium, can control *M. incognita*.

Moreover, these beneficial bacteria have been observed to enhance plant vitality and root growth, thereby reducing tomato plants' vulnerability to nematode damage. As a sustainable and eco-friendly method of controlling root-knot nematodes in tomato farming, using a variety of *Pseudomonas* spp. strains shows great promise as an alternative to chemical pesticides. This approach not only improves soil health but also increases crop yield.

The results from this study highlight the significant impact of microbial inoculants on the growth of tomato plants. Both strains of *P. fluorescens* (Strain A and Strain B) demonstrated a positive effect on plant growth, as evidenced by increased plant height and weight compared to the control groups. This growth enhancement can be attributed to the microbial strains' ability to improve nutrient assimilation and stimulate the secretion of plant growth-regulating hormones. Notably, the application of *P. putida* (T5) resulted in increased plant height, although the corresponding increases in plant weight was less pronounced compared to the other treatments. This discrepancy might be due to the unique interactions between *P. putida* and the tomato plants, which differ from those established by *P. fluorescens*.

In contrast, the control group subjected to nematode inoculation (T2) exhibited inhibited growth, underscoring the detrimental effects of nematode infestation on tomato plants. The findings of the study underscore the potential of microbial treatments to positively influence the development of tomato plants. Specifically, the microbial treatments, particularly the strains of *P. fluorescens* (Strain A and Strain B), significantly enhanced both leaf and flower production, as indicated by the higher counts compared to the control groups. This increase in developmental parameters can be attributed to the microbial strains' ability to improve nutrient availability and induce factors that promote plant growth. It is noteworthy that the application of *P. putida* (T5) led to an increase in the number of leaves; however, the corresponding rise in flower count was relatively moderate. This divergence could potentially result from the unique interactions between *P. putida* and tomato plants, which differ from those mediated by *P. fluorescens*.

Bacteria are commonly observed to possess both nematicidal and plant growth-enhancing characteristics (Liu et al., 2022; Bhat et al., 2023; Nagrale et al., 2023). Multiple studies have demonstrated that bacteria can enhance growth and productivity in plants afflicted by nematodes in both greenhouse and field settings (Liu et al., 2022). However, some bacteria can significantly enhance plant development without affecting nematode control (Aballay et al., 2011). Xiang et al. (2017) found that *B. velezensis* Bve12 and *B. weihenstephanensis* Bwe15 can enhance the initial development and productivity of cotton plants in field settings, particularly when affected by *M. incognita*. *S. plymuthica* M24T3 exhibited potent nematicidal activity, achieving a 100% kill rate. Additionally, it demonstrated a remarkable ability to colonize plants and promote their growth (Proença et al., 2019). The combination of nematicidal activity and plant growth-stimulating capacity enables farmers to enhance their crop yield, leading to economic advantages and alleviating the strain on soil fertility.

This research makes a distinct contribution by conducting a thorough comparative examination of the impacts of various *Pseudomonas* strains on the growth parameters of tomato plants. This work differs from prior research that typically concentrated on a single strain or the overall impacts of *Pseudomonas* spp. Instead, it provides precise information on several strains, including *P. fluorescens* Strain A and Strain B, as well as *P. putida* T5.

Furthermore, tomato plants treated with *P. fluorescens* Strain A showed a height increase of 15%, while those treated with Strain B showed an increase of 18% compared to the control. In contrast, plants treated with *P. putida* (T5) exhibited a 12% increase in height. *P. fluorescens* Strain A and Strain B exhibited a weight gain of 20% and 22% respectively, while *P. putida* (T5) resulted in a 10% increase in weight. This sophisticated understanding can guide more precise applications of microbial therapies in sustainable agriculture.

Upon comparing these findings with other research, it becomes apparent that the distinct interactions between different strains of *Pseudomonas* and tomato plants can result in diverse outcomes in terms of growth and nematode suppression. This research provides a more sophisticated understanding of how various bacterial strains might be employed to enhance the health and productivity of tomato plants, contributing valuable knowledge to the field of biological plant protection and sustainable agriculture.

CONCLUSION

This study demonstrates that several strains of *Pseudomonas* spp. are effective in managing root-knot nematodes (*M. incognita*) in tomato crops through mechanisms such as antagonistic interactions, systemic resistance induction, and hormone regulation. Research highlights illustrate the nematode suppression and plant growth benefits provided by these bacterial strains. The findings underscore the potential of microbial inoculants like *P. fluorescens* and *P. putida* to enhance tomato plant growth and yield, offering a sustainable alternative to chemical pesticides and contributing to soil health and agricultural productivity.

AUTHORS' CONTRIBUTIONS

HMA and NF designed the study; HMA conducted the experiments; HMA collected and analyzed the data; MAA, NF, HMA, AA, MAA, MI, AB, AR, MA, RS, WS, MA, AM, MT, II, MA and MU wrote the manuscript; MA proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aballay, E., Mårtensson, A., Persson, P., 2011. Screening of rhizosphere bacteria from grapevine for their suppressive effect on *Xiphinema* index Thorne & Allen on *in vitro* grape plants. Plant and Soil 347, 313-325.

Afzal, A., Mukhtar, T., 2024. Revolutionizing nematode management to achieve global food security goals- An overview. Heliyon 10, 25325.

Ahmed, M.H., Ashfaq, M., Mukhtar, T., Khan, M.A., 2021. Categorization of available cucumber genotypes against Zucchini yellow mosaic virus and root-knot nematode (*Meloidogyne incognita*). International Journal of Agriculture and Biology 25, 955-961.

Ali, A., Fatih, Ö., Tatar, M., Tabbasum, I., Azeem, H., Sarwar, R., Ali, F., 2023a. Mycotoxin production by *Fusarium* species and a recent deep insight into management through biocontrol approaches. Journal of Global Innovations in Agricultural Sciences 11, 455-480.

Ali, A., Iftikhar, Y., Mubeen, M., Ali, H., Zeshan, M.A., 2022. Antagonistic potential of bacterial species against fungal plant pathogens (FPP) and their role in plant growth promotion (PGP): A review. Phyton-International Journal of Experimental Botany 91(9), 1859-1877.

Ali, A., Tabbasum, I., Azeem, H., Ölmez, F., Deveci, G., Khalid, B., Mehtab, M., 2023b. Bacterial endophytes, a resilient way toward sustainable agriculture: provide plant growth promotion and biocontrol of plant pathogens. Journal of Global Innovations in Agricultural Sciences 11(2), 153-174.

Antil, S., Kumar, R., 2019. Biocontrol of root-knot nematodes through PGPR. International Journal of Current Microbiology and Applied Sciences 8(11), 510-514.

Asghar, A., Mukhtar, T., Raja, M.U., Gulzar, A., 2020. Interaction between *Meloidogyne javanica* and *Ralstonia solanacearum* in chili. Pakistan Journal of Zoology 52, 1525-1530.

Aslam, M.N., Mukhtar, T., 2023a. Characterization of *Ralstonia solanacearum* causing bacterial wilt from major chili growing areas of Pakistan. Bragantia 82, e20230001.

Aslam, M.N., Mukhtar, T., 2023b. Distributional spectrum of bacterial wilt of chili incited by *Ralstonia solanacearum* in Pakistan. Bragantia 82, e20220196.

Aslam, M.N., Mukhtar, T., 2024. Evaluation of virulence among Pakistani isolates of *Ralstonia solanacearum* inducing bacterial wilt in chilies across different agro-ecological zones. Bragantia 83, e20230181.

Azeem, H., Ali, A., Zeshan, M.A., Ashraf, W., Ghani, M.U., Sajid, A., Sajid, M., 2020. Biological control of plant pathogens by using antagonistic bacteria: a review. Pakistan Journal of Phytopathology 32(2), 273-290.

Azeem, W., Mukhtar, T., Hamid, T., 2021. Evaluation of *Trichoderma harzianum* and *Azadirachta indica* in the management of *Meloidogyne incognita* in tomato. Pakistan Journal of Zoology 53, 119-125.

Aziz, S., Jamshed, S.A., Mukhtar, T., Irshad, G., Ijaz, S.S., Raja, M.U., 2024. Evaluation of *Bacillus* spp. as biocontrol agents against chili leaf spot caused by *Xanthomonas vesicatoria*. Journal of Plant Diseases and Protection 131, 987-997.

Bakr, R.A., Mahdy, M.E., Mousa, E.S.M., 2017. Evaluation of *Pasteuria penetrans* application methods on controlling of root-knot nematode infecting eggplant. Egyptian Journal of Crop Protection 2(1), 1-8.

Bhat, A.A., Shakeel, A., Waqar, S., Handoo, Z.A., Khan, A.A., 2023. Microbes vs. nematodes: Insights into biocontrol through antagonistic organisms to control root-knot nematodes. Plants 12(3), 451.

Cetintas, R., Dickson, D.W., 2004. Persistence and suppressiveness of *Pasteuria penetrans* to *Meloidogyne arenaria* race. Journal of Nematology 36(4), 540.

Cheng, H., Zhang, D., Ren, L., Song, Z., Li, Q., Wu, J., Cao, A., 2021. Bio-activation of soil with beneficial microbes after soil fumigation reduces soil-borne pathogens and increases tomato yield. Environmental Pollution 283, 117160.

Chinheya, C.C., Yobo, K.S., Laing, M.D., 2017. Biological control of the root-knot nematode, *Meloidogyne javanica* (Chitwood) using *Bacillus* isolates, on soybean. Biological Control 109, 37-41.

Chitruk, A., Yadav, A., Rode, P., Mohite, S., Magdum, C., 2020. Microwave assisted synthesis, antimicrobial and anti-inflammatory potential of some novel 1, 2, 4-triazole derivatives. International Journal of Scientific and Technology Research 7(4), 360-367.

Costa, S.R., Ng, J.L.P., Mathesius, U., 2021. Interaction of symbiotic rhizobia and parasitic root-knot nematodes in legume roots: from molecular regulation to field application. Molecular Plant-Microbe Interactions 34(5), 470-490.

Desaeger, J.A., Watson, T.T., 2019. Evaluation of new chemical and biological nematicides for managing *Meloidogyne javanica* in tomato production and associated double-crops in Florida. Pest Management Science 75, 3363-3370.

Haq, M.A., Mukhtar, T., Haq, M.I., Khalid, A., 2022. Reproduction of root-knot nematode, *Meloidogyne incognita*, on *Solanum melongena* genotypes determines their host status. Pakistan Journal of Zoology 54, 2097-2103.

Haque, Z., Khan, M.R., 2022. Evaluation of fluopyram against single and concomitant inoculation of *Rhizoctonia solani* and *Meloidogyne graminicola* infesting rice crop. Phytoparasitica 50 (4), 811-822.

Hassan, M.A., Pham, T.H., Shi, H., Zheng, J., 2013. Nematodes threats to global food security. Acta Agriculturae Scandinavica, Section B-Soil and Plant Science 63(5), 420-425.

Hussain, M.A., Mukhtar, T., 2019. Root-knot nematodes infecting okra in major vegetable growing districts of Punjab, Pakistan. Pakistan Journal of Zoology 51, 1137-1143.

Hussain, M.A., Mukhtar, T., Kayani, M.Z., 2011. Assessment of the damage caused by *Meloidogyne incognita* on okra. Journal of Animal and Plant Sciences 21(4), 857-861.

Hussain, M.A., Mukhtar, T., Kayani, M.Z., 2016. Reproduction of *Meloidogyne incognita* on resistant and susceptible okra cultivars. Pakistan Journal of Agricultural Sciences 53(2), 371-375.

Hussain, M.A., Mukhtar, T., Kayani, M.Z., Aslam, M.N., Haque, M.I., 2012. A survey of okra (*Abelmoschus esculentus*) in the Punjab province of Pakistan for the determination of prevalence, incidence and severity of root-knot disease caused by *Meloidogyne* spp. Pakistan Journal of Botany 44 (6), 2071-2075.

Kayani, M.Z., Mukhtar, T., 2018. Reproductivity of *Meloidogyne incognita* on fifteen cucumber cultivars. Pakistan Journal of Zoology 50, 1717-1722.

Kayani, M.Z., Mukhtar, T., Hussain, M.A., 2017. Effects of southern root-knot nematode population densities and plant age on growth and yield parameters of cucumber. Crop Protection 92, 207-212.

Kayani, M.Z., Mukhtar, T., Hussain, M.A., 2018. Interaction between nematode inoculum density and plant age on growth and yield of cucumber and reproduction of *Meloidogyne incognita*. Pakistan Journal of Zoology 50, 897-902.

Khan, M.T.A., Mukhtar, T., Saeed, M., 2019. Resistance or susceptibility of eight aubergine cultivars to *Meloidogyne javanica*. Pakistan Journal of Zoology 51, 2187-2192.

Khan, R.A.A., Najeeb, S., Hussain, S., Xie, B., Li, Y., 2020. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms 8(6), 817.

Khanna, K., Kohli, S.K., Sharma, P., Kour, J., Singh, A.D., Sharma, N., Ohri, P., Bhardwaj, R., 2021. Antioxidant potential of plant growth-promoting rhizobacteria (PGPR) in agricultural crops infected with root-knot nematodes. Antioxidants in Plant-Microbe Interaction 339-379.

Lee, J.P., Sekhon, S.S., Kim, J.H., Kim, S.C., Cho, B.K., Ahn, J.Y., Kim, Y.H., 2021. The pine wood nematode *Bursaphelenchus xylophilus* and molecular diagnostic methods. Molecular and Cellular Toxicology 17, 1-13.

Liu, G., Lin, X., Xu, S., Liu, G., Liu, F., Mu, W., 2020. Screening, identification and application of soil bacteria with nematicidal activity against root-knot nematode (*Meloidogyne incognita*) on tomato. Pest Management Science 76(6), 2217-2224.

Liu, M., Philp, J., Wang, Y., Hu, J., Wei, Y., Li, J., Yang, H., 2022. Plant growth-promoting rhizobacteria *Burkholderia vietnamiensis* B418 inhibits root-knot nematode on watermelon by modifying the rhizosphere microbial community. Scientific Reports 12(1), 8381.

Mukhtar, T., 2018. Management of root-knot nematode, *Meloidogyne incognita*, in tomato with two *Trichoderma* species. Pakistan Journal of Zoology 50, 1589-1592.

Mukhtar, T., Hussain, M.A., 2019. Pathogenic potential of Javanese root-knot nematode on susceptible and resistant okra cultivars. Pakistan Journal of Zoology 51, 1891-1897.

Mukhtar, T., Hussain, M.A., Kayani, M.Z., 2013a. Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. Phytopathologia Mediterranea 52, 66-76.

Mukhtar, T., Hussain, M.A., Kayani, M.Z., 2017. Yield responses of twelve okra cultivars to southern root-knot nematode (*Meloidogyne incognita*). Bragantia 76(1), 108-112.

Mukhtar, T., Kayani, M.Z., 2019. Growth and yield responses of fifteen cucumber cultivars to root-knot nematode (*Meloidogyne incognita*). Acta Scientiarum Polonorum Hortorum Cultus 18, 45-52.

Mukhtar, T., Kayani, M.Z., 2020. Comparison of the damaging effects of *Meloidogyne incognita* on a resistant and susceptible cultivar of cucumber. Bragantia 79, 83-93.

Mukhtar, T., Kayani, M.Z., Hussain, M.A., 2013b. Response of selected cucumber cultivars to *Meloidogyne incognita*. Crop Protection 44, 13-17.

Mukhtar, T., Tariq-Khan, M., Aslam, M.N., 2021. Bioefficacy of *Trichoderma* species against Javanese root-knot nematode, *Meloidogyne javanica* in green gram. Gesunde Pflanzen 73, 265-272.

Nagrale, D.T., Chaurasia, A., Kumar, S., Gawande, S.P., Hiremani, N.S., Shankar, R., Prasad, Y.G., 2023. PGPR: the treasure of multifarious beneficial microorganisms for nutrient mobilization, pest biocontrol and plant growth promotion in field crops. World Journal of Microbiology and Biotechnology 39(4), 100.

Nauman, M., Mushtaq, S., Khan, M.F., Ali, A., Naqvi, S.A.H., Haq, Z., Umar, U.U.D., 2023. Morphological, biochemical, and molecular characterization of *Xanthomonas citri* subsp. *citri*, cause of citrus canker disease in Pakistan. Pakistan Journal of Botany 55(6), 2409-2421.

Nazir, K., Mukhtar, T., Javed, H., 2019. *In vitro* effectiveness of silver nanoparticles against root-knot nematode (*Meloidogyne incognita*). Pakistan Journal of Zoology 51(6), 2077-2083.

Ntalli, N., Monokrousos, N., Rumbos, C., Kontea, D., Zioga, D., Argyropoulou, M.D., Menkissoglu-Spiroudi, U., Tsiropoulos, N.G., 2018. Greenhouse biofumigation with *Melia azedarach* controls *Meloidogyne* spp. and enhances soil biological activity. Journal of Pest Science 91, 29-40.

Proença, D.N., Schwab, S., Vidal, M.S., Baldani, J.I., Xavier, G.R., Morais, P.V., 2019. The nematicide *Serratia plymuthica* M24T3 colonizes *Arabidopsis thaliana*, stimulates plant growth, and presents plant beneficial potential. Brazilian Journal of Microbiology 50, 777-789.

Radwan, M.A., Farrag, S.A.A., Abu-Elamayem, M.M., Ahmed, N.S., 2012. Efficacy of some granular nematicides against root-knot nematode, *Meloidogyne incognita* associated with tomato. Pakistan Journal of Nematology 30(1), 41-47.

Rao, M.S., Kamalnath, M., Umamaheswari, R., Rajinikanth, R., Prabu, P., Priti, K., Gopalakrishnan, C., 2017. *Bacillus subtilis* IIHR BS-2 enriched vermicompost controls root knot nematode and soft rot disease complex in carrot. Scientia Horticulturae 218, 56-62.

Raza, H.Y., Madni, M., Malik, Z., Zahra, A., Mansoor, T., 2022. Efficacy of *Pseudomonas* spp. NA-4 against *Meloidogyne incognita*. Phytopathogenomics and Disease Control 1(1), 55-59.

Saeed, M., Mukhtar, T., 2024. Root-knot nematodes (*Meloidogyne* spp.) infecting peach (*Prunus persica* L.) in the Pothwar region of Pakistan. Journal of Agricutltural Science and Technology 26(4), 897-908.

Saeed, M., Mukhtar, T., Ahmed, R., Ahmad, T., Iqbal, M.A., 2023. Suppression of *Meloidogyne javanica* infection in peach (*Prunus persica* L.) (Batsch) using fungal biocontrol agents. Sustainability 15(18), 13833.

Saeed, M., Mukhtar, T., Haq, M.I., Khan, M.A., 2021. Assessment of nematicidal potential of *Cannabis sativa* and *Azadirachta indica* in the management of root-knot nematode (*Meloidogyne javanica*) on peach. Pakistan Journal of Agricultural Sciences 58, 1555-1561.

Sayedain, F.S., Ahmadzadeh, M., Fattah-Hosseini, S., Bode, H.B., 2021. Soil application of entomopathogenic nematodes suppresses the root-knot nematode *Meloidogyne javanica* in cucumber. Journal of Plant Diseases and Protection 128, 215-223.

Sharma, I.P., Sharma, A.K., 2017. Effective control of root-knot nematode disease with *Pseudomonad* rhizobacteria filtrate. Rhizosphere 3, 123-125.

Stirling, G.R., 2011. Biological control of plant-parasitic nematodes: an ecological perspective, a review of progress and opportunities for further research. Biological control of plant-parasitic nematodes: building coherence between microbial ecology and molecular mechanisms 1-38.

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

Tabbasum, I., Ali, A., Shafique, U., Bibi, I., Khalid, B., Ijaz, N., Naseer, M.U., 2022. Role of endophytic fungal species in plant growth promotion and in sustainable agricultural productions: a review. The Journal of Microbiology and Molecular Genetics 3(1), 1-21.

Tariq-Khan, M., Mukhtar, T., Munir, A., Hallmann, J., Heuer, H., 2020. Comprehensive report on the prevalence of root-knot nematodes in the Poonch division of Azad Jammu and Kashmir, Pakistan. Journal of Phytopathology 168, 322-336.

Tariq-Khan, M., Munir, A., Mukhtar, T., Hallmann, J., Heuer, H., 2017. Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. Journal of Plant Diseases and Protection 124, 201-212.

Xiang, N., Lawrence, K.S., Kloepper, J.W., Donald, P.A., McInroy, J.A., Lawrence, G.W., 2017. Biological control of *Meloidogyne incognita* by spore-forming plant growth-promoting rhizobacteria on cotton. Plant Disease 101(5), 774-784.

Yaseen, I., Mukhtar, T., Kim, H.T., Arshad, B., 2023. Quantification of resistance to *Melidogyne incognita* in okra cultivars using linear and nonlinear analyses of growth parameters and nematode infestations. Bragantia 82, e20230114.

Yaseen, I., Mukhtar, T., Kim, H.T., Arshad, B., 2024. Interactive effects of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. v*asinfectum* on okra cultivars. Bragantia 83, e20230266.