OPEN ACCESS Int. J. Phytopathol. 13 (03) 2024. 219-232 DOI: 10.33687/phytopath.013.03.5382

Check for updates

Available Online at EScience Press

International Journal of Phytopathology

ISSN: 2312-9344 (Online), 2313-1241 (Print)

https://esciencepress.net/journals/phytopath

EFFICACY OF ENTOMOPATHOGENIC NEMATODE (*STEINERNEMA PAKISTANENSE*) IN SUPPRESSING ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) ON TOMATO

[a]Muhammad Sohail Gondal, [a]Tariq Mukhtar, [a]Gulshan Irshad, [b]Tabassum Ara Khanum

[a] Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

[b] National Nematological Research Center, University of Karachi, Karachi, Pakistan.

ARTICLE INFO

Article History

Received: September 19, 2024

Revised: November 28, 2024

Accepted: December 30, 2024

Keywords

Entomopathogenic nematodes

Steinernema pakistanense

Root-knot nematode

Meloidogyne incognita

Tomato disease management

ABSTRACT

The present study assessed the biocontrol potential of *Steinernema pakistanense* (SG1 and SG2 strains) and cell-free culture filtrates (CFCFs) of their bacteria, *Xenorhabdus* spp., against *Meloidogyne incognita* in *in vitro* and greenhouse experiments, offering an eco-friendly alternative to costly and resistant-inducing nematicides. The application of *S. pakistanense* significantly influenced plant growth and nematode infestation parameters. Higher application rates improved plant growth, with (Nematicide + 1000 RKN) and (1000 EPN + 1000 RKN) treatments showing the highest shoot length, root length, and shoot weight. Contrariwise, nematode infestation was significantly reduced with increasing *S. pakistanense* application. The control treatment exhibited the highest infestation levels, while (Nematicide + 1000 RKN) treatment nearly eliminated nematode populations. SG1 strain demonstrated better plant growth promotion and comparable nematode suppression to the SG2 strain. Application timing also affected plant growth and nematode reproduction. 'EPNs applied one week before RKNs' resulted in the best plant growth and lowest nematode reproduction, while 'EPNs applied one week after RKNs' exhibited the weakest plant performance and highest nematode populations. 'Simultaneous application' had intermediate effects. These findings suggest that pre-application of *S. pakistanense* enhances plant growth and nematode suppression. Moreover, *Xenorhabdus* CFCFs demonstrated strong inhibitory effects on *M. incognita* egg hatching. Higher concentrations (90% CFCF) showed the greatest suppression, with up to 79.25% inhibition for SG1 and 77.12% for SG2. Lower concentrations resulted in progressively higher egg hatching rates, while the control exhibited no inhibition. These results highlight the potential of *S. pakistanense* and its bacterial symbionts as effective biocontrol agents against root-knot nematodes.

Corresponding Author: Muhammad Sohail Gondal

Email: misturmental8089@gmail.com

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.), a member of the solanaceae family, is one of the most economically and nutritionally significant vegetable crops worldwide. It ranks second in global vegetable production after potatoes and is rich in essential nutrients, including carotenoids, vitamins A and C, potassium, and iron (Azeem *et al*., 2021; Collins *et al*., 2022). Moreover, tomatoes offer medicinal benefits, such as cancer prevention and cardiovascular protection, making them a vital component of the human diet (Aslam *et al*., 2017; Mukhtar, 2018; Li *et al*., 2021).

Globally, tomatoes are cultivated on over 6.57 million hectares, with an annual production of 262.53 million metric tons. Major producers include China, Nigeria, India, Türkiye, Egypt, and the United States, while Pakistan ranks 14th in global tomato production (FAO, 2023). In Pakistan, tomatoes are grown on approximately 68.86 thousand hectares, primarily in Sindh, Punjab, and Khyber Pakhtunkhwa (GOP, 2023). Despite a production increase to 762.74 thousand tons in 2023, the national average yield (11.08 tons/ha) remains significantly below the global average (FAO, 2023).

Tomatoes are susceptible to over 200 diseases, leading to significant yield losses (Gondal *et al*., 2018; Mukhtar and Hussain, 2019; Yaseen *et al*., 2025). Among the major constraints, plant-parasitic nematodes, particularly root-knot nematodes (*Meloidogyne* spp.) are the most devastating pests (Haq *et al*., 2022; Yaseen *et al*., 2023). These nematodes cause economic losses exceeding $157 billion annually worldwide (Mukhtar and Kayani, 2019, 2020; Mendoza-de Gives, 2022). They damage crops by forming root galls, disrupting water and nutrient uptake, and reducing plant vigor and yield (Moens *et al*., 2009; Gondal *et al*., 2012; Khan *et al*., 2019). In Pakistan, *M. incognita* and *M. javanica* are the most prevalent species, occurring at rates of 58% and 31%, respectively (Maqbool *et al*., 1992; Tariq-Khan et al., 2017, 2020; Hussain and Mukhtar, 2019; Saeed and Mukhtar, 2024).

Root-knot nematodes often interact synergistically with other plant pathogens, such as fungi, bacteria, and viruses, leading to more severe disease complexes and greater crop damage than either pathogen alone (Asghar *et al*., 2020; Ahmed *et al*., 2021; Yaseen and Mukhtar, 2024). These interactions can alter host plant physiology, weaken immune responses, and create favorable conditions for secondary infections, complicating disease management strategies in agricultural systems (Yaseen *et al*., 2024).

For the management of root-knot nematodes, various strategies are employed, each with inherent limitations (Nazir *et al*., 2019; Saeed *et al*., 2023). Conventional approaches predominantly rely on chemical nematicides, which, while effective, raise concerns due to their environmental and health risks, as well as the potential for nematode resistance development (Luc *et al*., 2005). This underscores the urgent need for sustainable and eco-friendly alternatives.

Among viable alternatives, entomopathogenic nematodes (EPNs) from the families *Steinernematidae* and *Heterorhabditidae* have emerged as promising biocontrol agents against a wide range of pests and pathogens (Sharma *et al*., 2011; Rahoo *et al*., 2017, 2018a, 2019a; Gulzar *et al*., 2020). Their symbiotic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.) produce metabolites that inhibit nematode egg hatching and reduce infestations (Pérez and Lewis, 2002; Rahoo *et al*., 2011). The effectiveness of EPNs in controlling *M. incognita* has been demonstrated in both greenhouse and field conditions (El Aimani *et al*., 2022), though their success depends on factors such as species, application rate, and timing (Molina *et al*., 2007; Rahoo *et al*., 2018b, 2019b).

Given the destructive impact of *M. incognita* and the potential of EPNs as biological control, this study aimed to evaluate the efficacy of *Steinernema pakistanense* against *M. incognita* in tomato cultivation. The research focused on determining the optimal EPN application rate, assessing their effectiveness at different application times, and evaluating the *in vitro* nematicidal activity of mutualistic bacteria isolated from EPNs against *M. incognita*.

MATERIALS AND METHODS

Isolation and Mass Multiplication of *Meloidogyne incognita*

Tomato roots exhibiting typical root-knot nematode symptoms, characterized by knots or galls, were collected from the culture maintained on tomato at Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. The surrounding soil was also collected and stored in polythene bags to preserve moisture for nematode survival. Female nematodes were isolated from infected roots and identified based on their perineal patterns, following the method described by Jepson (1987). To isolate the perineal pattern, the posterior half of a female nematode was excised on a glass slide, and a square-shaped incision was made on the lower cuticle to obtain the portion containing the perineal pattern, which was then examined under a microscope (Eisenback *et al*., 1981).

The infected roots were thoroughly washed to remove soil, cut into small pieces, and transferred to a jar containing 5% sodium hypochlorite (NaOCl) (Figure 1). The mixture was vigorously shaken for 2 minutes to release eggs from the roots into the bleach solution. This

solution was subsequently passed through sieves (200-500 mesh) to separate eggs from root debris. The collected eggs were counted on a counting dish under a microscope (Hussey and Barker, 1973).

For mass multiplication, 2000 *M. incognita* eggs were inoculated around 20-day-old tomato seedlings in pots containing 2 kg of sterilized soil. Three holes were made around each plant using a pointed wooden stick for inoculation. The pots were maintained at 27°C for six weeks, with daily watering to prevent excessive drying.

Figure 1. Extraction of *M. incognita* eggs by sodium hypochlorite method.

Mass Multiplication of Entomopathogenic Nematodes

Two strains of *Steinernema pakistanense* (SG1 and SG2) were obtained from the National Nematological Research Centre (NNRC), Karachi, Pakistan. The greater wax moth, *Galleria mellonella*, was reared on an artificial diet composed of wheat flour, corn flour, yeast, milk powder, glycerin, beeswax, and honey to facilitate normal growth across various instars.

For mass multiplication of *S. pakistanense*, the insect baiting technique was employed. A plastic container was filled with 200 g of sterilized sandy soil, into which last instar larvae of *G. mellonella* were added at a density of approximately 200 infective juveniles (IJs) per larva to minimize competition. After 24 hours, dead cadavers exhibiting brown or ochre coloration were collected (Bedding and Akhurst, 1975). These cadavers were surface-sterilized by rinsing with a 2% clorox solution, followed by two washes with sterilized distilled water (Shapiro-Ilan *et al*., 2001).

Infective juveniles were harvested using modified White trap, where infected cadavers were placed in a smaller Petri dish floating in a larger dish containing water. This setup was left undisturbed at room temperature for 10-25 days to allow IJs to emerge (Figure 2). The freshly hatched juveniles were stored at 15-17°C in an incubator until needed for experimentation (Javed *et al*., 2022).

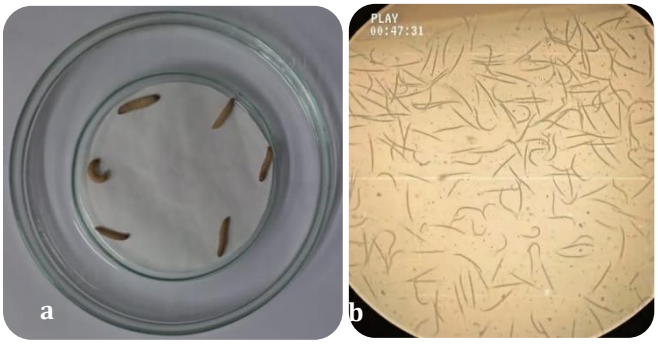

Figure 2. (a) Modified white trap for the harvesting of EPNs; (b) infective juveniles of *S. pakistanense*.

Evaluation of Optimal Application Rate of *S. pakistanense* against *M. incognita*

Nursery seedlings of the Rio Grande tomato variety were raised in a germination tray, maintaining moisture to ensure maximum germination. The planting medium consisted of 75% soil and 25% manure, which was wet-sterilized using a formalin solution (1:49 commercial formalin to water) as described by Fosler (1958). The sterilized soil was covered with a polythene sheet for three days to allow fumes to dissipate before use.

After 20 days, tomato seedlings were transplanted into plastic pots containing 1 kg of sterilized soil and left

undisturbed for one week before nematode inoculation. Six treatments were designed, including different application rates of EPNs, a control, and a nematicide treatment (Table 1).

Table 1. Treatments used for evaluating application rate of EPNs against *M. incognita*.

Treatments	EPNs (SG1 and SG2)
T1	250 EPNs + 1000 RKNs
T2	500 EPNs + 1000 RKNs
T3	750 EPNs + 1000 RKNs
T4	1000 EPNs + 1000 RKNs
T5	Nematicide + 1000 RKNs
T0	Control

Before inoculation, *M. incognita* eggs (approximately 29,333 eggs/ml) were counted using a counting dish (Hussey and Barker, 1973). Similarly, infective juveniles of *S. pakistanense* (SG1 and SG2) were counted (approximately 9,333 IJs/ml). Three holes were made around each plant at a distance of 2 inches from the stem for nematode eggs and 1 inch from the stem for EPNs. Using a micropipette, precise volumes of egg suspension (34 µl) and juvenile suspensions (28-107 µl for SG1 and 26-102 µl for SG2) were applied according to treatment specifications.

Plants were arranged in a completely randomized design in a greenhouse and watered regularly. After six weeks, plants were harvested for data collection. Root and shoot lengths were measured, and fresh weights were recorded. Roots were washed, and galls were counted under a magnifying glass, while egg masses were assessed under a stereomicroscope (Holbrook *et al*., 1983). Reproduction factors were determined by crushing egg masses and counting eggs per mass under a microscope.

Evaluation of Optimal Application Time of *S. pakistanense* against *M. incognita*

Based on the previous experiment, where a significant number of IJs were effective, 1000 IJs were used to evaluate the best inoculation time against *M. incognita*. The nursery raising, seedling transplantation, and nematode counting procedures followed the previously described methodology. Treatments designed to assess different application times are presented in Table 2.

M. incognita eggs and *S. pakistanense* juveniles were inoculated using a micropipette. A 6.8 ml egg suspension was applied, while SG1 and SG2 juvenile suspensions (196 µl and 228 µl, respectively) were used to provide 1000 IJs per treatment. After six weeks, plants were harvested, and parameters such as root and shoot lengths, fresh and dry weights, number of galls, egg masses, eggs per egg mass, and reproduction factors were recorded.

Table 2. Treatments used for evaluating application time of EPNs against *M. incognita*.

Treatments	EPNs
T1	EPNs 1 Week before RKNs
T2	EPNs and RKNs simultaneously
T3	EPNs 1 Week after RKNs

In Vitro* Efficacy of Mutualistic Bacteria of *S. pakistanense* against *M. incognita

Xenorhabdus spp. was isolated from EPN-infected cadavers using Nutrient Bromothymol Blue Agar (NBTA) at pH 7 (Johnigk, 1999). Nutrient broth was prepared to confirm the *in vitro* effectiveness of the isolated bacteria. Infected cadavers were surface sterilized in a 2% Clorox solution, and hemolymph was extracted and streaked onto NBTA media, which was incubated at 28 ± 2°C (Boemare and Akhurst, 1988) (Figure 3).

Purification involved sub-culturing until uniform-sized colonies were obtained (Akhurst, 1980). Fresh bacterial colonies were transferred to nutrient broth and incubated in a shaking incubator at 155 rpm for three days (Safdar *et al*., 2023) (Figure 4). The optical density index (ODI) was determined at 600 nm using a spectrophotometer (Alves *et al*., 2024).

Bacterial suspensions were centrifuged at 6000 rpm for 15 minutes, and the supernatant was filtered through a 0.22 µm syringe filter (Srivastava and Chaubey, 2022).

One milliliter of cell-free culture filtrate (CFCF) was added to different cavity blocks, followed by the addition of 234 µl of egg suspension (containing 301 eggs/ml) diluted with distilled water to achieve concentrations of 90%, 50%, 25%, and 10%. A control group containing only distilled water was included, with three replications for each treatment. The cavity blocks were incubated at 25 ± 2°C, and egg hatching inhibition was recorded after 24, 48, and 72 hours. The percentage of egg hatching inhibition was calculated using the formula described by (Abd El-Aal *et al*., 2021).

Egg Hatching Inhibition % = (C - T) / C × 100

Where:

C = control group count

T = treatment group count

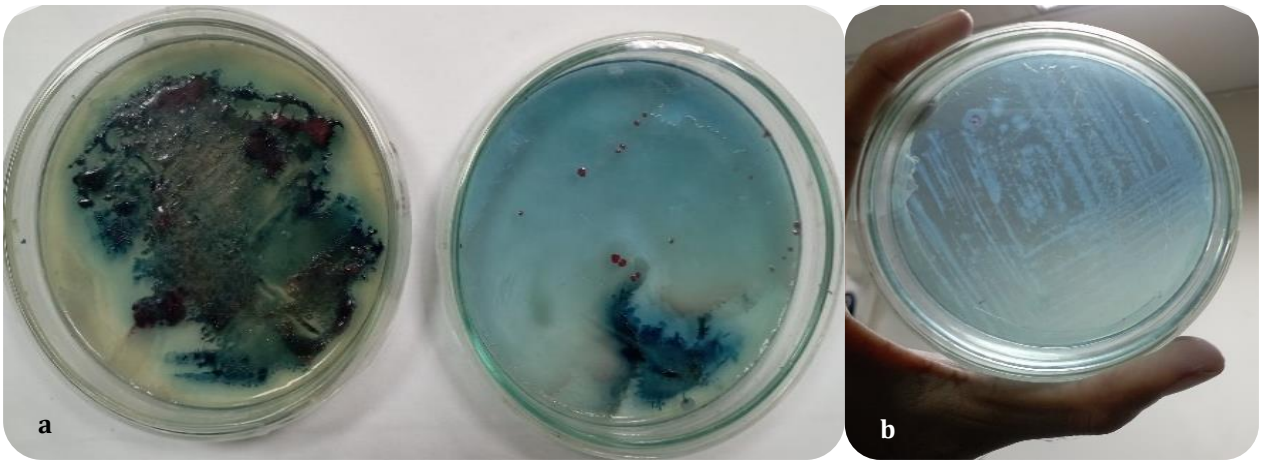

Figure 3. (a) Isolation of *Xenorhabdus* spp. from infected cadaver and (b) purified colonies of *Xenorhabdus* spp. on NBTA media.

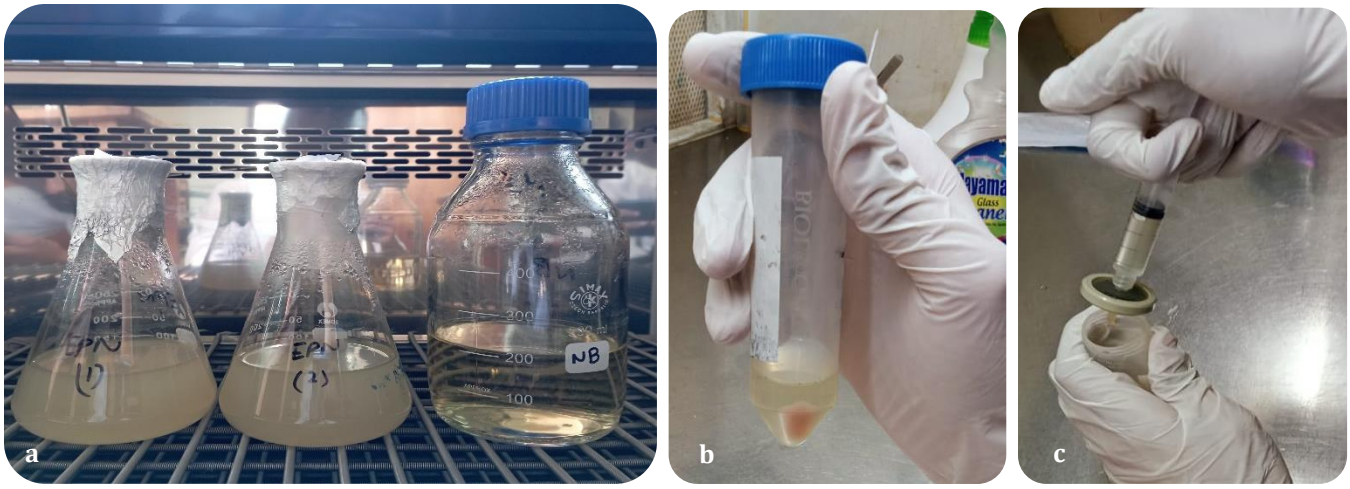

Figure 4. (a) Incubation of *Xenorhabdus* spp. suspension in a shaking incubator, (b) centrifugation of suspension to separate bacterial cells, and (c) separating cell debris through a 0.22 µm syringe filter to obtain pure cell-free culture filtrates.

Statistical Analysis

Data were analyzed using ANOVA (Steel *et al*., 1997), followed by the Least Significant Difference (LSD) test at a 0.05 significance level to compare treatment effects on plant growth and nematode management efficacy.

RESULTS

Effect of *S. pakistanense* Application on Plant Growth Parameters

The application of *S. pakistanense* (SG1 and SG2 strains) significantly influenced plant growth parameters, with variations observed across treatments (Table 3). Shoot length increased with higher application rates, with T4 (1000 EPN + 1000 RKN) and T5 (Nematicide + 1000 RKN) showing the highest values for both SG1 (14.680 cm and 13.480 cm) and SG2 (15.380 cm and 14.700 cm), respectively (Figure 5). Root length followed a similar trend; with T5 recording the highest values (8.200 cm for SG1 and 8.000 cm for SG2). Fresh shoot weight was significantly greater in T5 (3.558 g for SG1 and 2.732 g for SG2), followed by T4, while the control (T0) had the lowest values (Figure 6). Dry shoot weight showed a similar pattern; with T5 having the highest values (0.904 g for SG1 and 0.732 g for SG2). Fresh and dry root weights were generally higher in control (T0), with significant reductions in treated plants. Overall, T5 (Nematicide + 1000 RKN) resulted in the best plant growth performance, followed by T4, while the control exhibited the poorest growth across all parameters.

Effect of *S. pakistanense* on Nematode Infestation parameters

The effect of varying application rates of *S. pakistanense* (SG1 and SG2 strains) on nematode infestation parameters has been shown in Table 4.

Figure 5. Difference of shoot length in control (T0) and 1000 EPNs treated plant (T4).

The control (T0) had the highest values across all parameters, including the number of galls (21.8 for SG1, 19.4 for SG2), egg masses (21.8 for SG1, 19.4 for SG2), soil population (2560 for SG1, 3360 for SG2), root population (13407 for SG1, 11931 for SG2), total population (15967 for SG1, 15291 for SG2), and reproduction factor (15.967 for SG1, 15.291 for SG2) (Figure 7a and b). As the application rates of *S. pakistanense* increased, nematode infestation decreased significantly. T4 (1000 EPN + 1000 RKN) and T5 (Nematicide + 1000 RKN) showed the most effective suppression, with T5 nearly eliminating nematode populations (0.0 for SG1, 0.4 for SG2 in galls; 0.0 for SG1, 0.4 for SG2 in egg masses; and 0.0 for SG1, 326 for SG2 in total population). Both SG1 and SG2 demonstrated similar trends, with SG1 mostly showing slightly better suppression. Overall, higher application rates of *S. pakistanense* and the nematicide treatment significantly reduced nematode infestation parameters compared to the control.

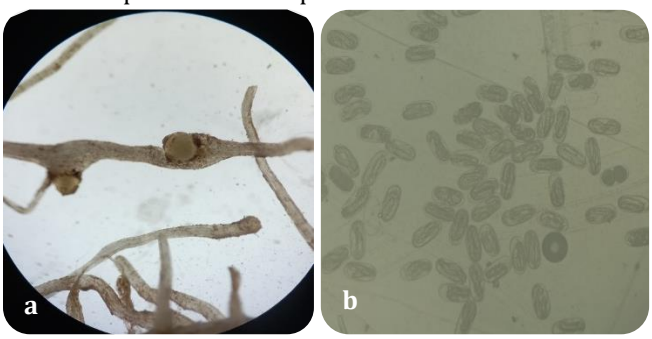

Figure 7. (a) Egg masses of *M. incognita* on tomato roots; (b) Eggs of *M. incognita* under microscope.

Figure 6. Effect of EPNs and nematicide on tomato roots. (a) control, (b) T1, (c) T2, (d) T3, (e) T4 and (f) nematicide.

Table 3. Effect of varying application rates of *S. pakistanense* (SG1 and SG2) on plant growth parameters.

Treatments	Shoot Length		Root Length		Fresh Shoot Weight		Dry Shoot Weight		Fresh Root Weight		Dry Root Weight	
	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)
T1	12.500 B	14.680 A	5.260 BC	5.3200 BC	3.0440 AB	1.9640 A	0.8440 AB	0.5340 A	1.0900 B	1.0020 B	0.0694 B	0.0602 B
T2	13.040 B	14.700 A	5.320 BC	6.9200 AB	2.5820 BC	2.0100 A	0.7180 BC	0.5600 A	0.9600 BC	0.8800 C	0.0426 C	0.0456 C
T3	13.060 B	14.900 A	5.460 BC	7.3000 AB	3.2260 A	2.1200 A	0.7660 AB	0.6440 A	0.9060 BC	0.8320 C	0.0414 C	0.0348 D
T4	14.680 A	15.380 A	6.580 B	7.6800 AB	3.5420 A	2.2880 A	0.9080 A	0.6420 A	0.8340 C	0.8080 C	0.0326 D	0.0238 E
T5	13.480 AB	14.700 A	8.200 A	8.0000 A	3.5580 A	2.7320 A	0.9040 A	0.7320 A	0.8180 C	0.7200 D	0.0276 A	0.0182 F
T0	8.600 C	10.880 B	4.600 C	5.2600 C	2.1840 C	1.9380 A	0.5760 C	0.5300 A	1.4740 A	1.2420 A	0.0760 A	0.0720 A

T1 = 250 EPN + 1000 RKN, T2 = 500 EPN + 1000 RKN, T3 = 750 EPN + 1000 RKN, T4 = 1000 EPN + 1000 RKN, T5 = Nematicide + 1000 RKN, T0 = Control.

Table 4. Effect of varying application rates of *S. pakistanense* (SG1 and SG2) on nematode infestation parameters.

Treatments	No. of Galls		No. of Egg Masses		Soil Population		Root Population		Total Population		Reproduction Factor	
	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)
T1	8.8000 B	8.400 B	8.8000 B	8.200 B	1440.0 B	1120.0 B	5412.0 B	5043.0 B	6852.0 B	6163 B	6.8520 B	6.616 B
T2	4.8000 C	5.200 C	4.8000 C	5.200 C	1360.0 B	960.0 B	2952.0 C	3198.0 C	4312.0 C	4158 C	4.3120 C	4.158 C
T3	4.0000 C	4.000 CD	3.8000 C	4.000 CD	1120.0 B	960.0 B	2337.0 C	2460.0 CD	3457.0 C	3420 CD	3.3470 C	3.420 CD
T4	2.2000 D	3.000 D	2.2000 D	3.000 D	640.00 C	720.0 BC	1353.0 D	1845.0 D	1993.0 D	2565 D	1.9930 D	2.565 D
T5	0.0000 E	0.400 E	0.0000 E	0.400 E	0.0000 D	80.0 C	0.0000 E	246.0 E	0.0000 E	326 E	0.0000 E	0.326 E
T0	21.800 A	19.400 A	21.800 A	19.400 A	2560.0 A	3360.0 A	13407 A	11931.0 A	15967 A	15291 A	15.967 A	15.291 A

T1 = 250 EPN + 1000 RKN, T2 = 500 EPN + 1000 RKN, T3 = 750 EPN + 1000 RKN, T4 = 1000EPN + 1000 RKN, T5 = Nematicide + 1000 RKN, T0 = Control.

Effect of *S. pakistanense* Application Timing on Plant Growth

Table 5 shows the effect of *S. pakistanense* (SG1 and SG2 strains) application time on plant growth parameters. In general, T1 (EPNs applied one week before RKNs) resulted in the highest values for shoot length, root length, fresh and dry shoot weight, and fresh and dry root weight. Treatments T2 (simultaneous application) and T3 (EPNs one week after RKNs) showed progressively lower values for most parameters, with T3 mostly exhibiting the lowest values. This suggested that applying *S. pakistanense* one week before RKNs is most effective for promoting plant growth.

Effect of *S. pakistanense* Application Timing on Nematode Infestations

The application timing of *S. pakistanense* (SG1 and SG2 strains) significantly influenced nematode reproduction parameters as shown in Table 6. Treatment T1 (EPNs applied one week before RKNs) resulted in the lowest number of galls, egg masses, nematode populations, and reproduction factors with SG1 and SG2 showing similar effects. In T2 (EPNs and RKNs applied simultaneously), there was a slight increase in all parameters, particularly in the soil population, where SG2 had a higher count than SG1. The highest nematode reproduction was observed in T3 (EPNs applied one week after RKNs), where both SG1 and SG2 recorded significantly greater numbers of galls, egg masses, total population and reproduction factors, indicating that delayed application of *S. pakistanense* was less effective in suppressing nematode reproduction.

Table 5. Effect of different application times of *S. pakistanense* (SG1 and SG2) on plant growth parameters.

Treatments	Shoot Length		Root Length		Fresh Shoot Weight		Dry Shoot Weight		Fresh Root Weight		Dry Root Weight	
	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)
T1	33.300 A	33.780 A	13.600 A	14.760 A	7.2780 A	7.2460 A	0.8142 A	0.7346 A	1.4040 B	1.1300 B	0.1158 B	0.0933 B
T2	28.340 B	31.020 B	12.260 A	13.320 A	6.3800 B	6.3960 B	0.7242 A	0.7004 AB	1.5840 AB	1.4600 AB	0.1305 AB	0.1202 AB
T3	25.960 C	25.440 C	8.860 B	9.800 B	5.0980 C	5.3380 C	0.6772 A	0.6210 B	1.7520 A	1.6800 A	0.1440 A	0.1382 A

T1= EPNs 1 week before RKNs, T2 = EPNs and RKNs, T3 = simultaneously EPNs 1 week after RKNs.

Table 6. Effect of different application times of *S. pakistanense* (SG1 and SG2) on nematode reproduction parameters.

Treatments	No. of Galls		No. of Egg Masses		Soil Population		Root Population		Total Population		Reproduction Factor	
	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)	(SG1)	(SG2)
T1	5.0000 B	5.0000 B	5.0000 B	5.0000 B	640.0 B	800.0 A	2890.0 B	2890.0 B	3530.0 B	3690.0 B	3.5300 B	3.6900 B
T2	5.6000 B	6.0000 B	5.6000 B	6.0000 B	800.0 AB	1120.0 A	3236.8 B	3468.0 B	4036.8 B	4588.0 B	4.0368 B	4.5880 B
T3	8.6000 A	7.6000 A	8.4000 A	7.6000 A	1120.0 A	1200.0 A	4855.2 A	4392.8 A	5975.2 A	5592.8 A	5.9752 A	5.5928 A

T1= EPNs 1 week before RKNs, T2 = EPNs and RKNs, T3 = simultaneously EPNs 1 week after RKNs.

Inhibitory Effect of *Xenorhabdus* CFCFs on Egg Hatching of *M. incognita*

The application of CFCFs from *Xenorhabdus* spp. isolated from *S. pakistanense* (SG1 and SG2 strains) significantly inhibited *M. incognita* egg hatching (Table 7). Higher concentrations of CFCFs resulted in greater inhibition, with T1 (90% CFCF) showing the lowest number of hatched eggs across all days (0 on Day 1, 7-8 on Day 2, and 8-17 on Day 3) and the highest inhibition percentage (79.25% for SG1 and 77.12% for SG2). T2 (50% CFCF) also exhibited strong inhibition, while T3 (25%) and T4 (10%) showed a gradual increase in egg hatching with decreasing inhibition percentages. The control (water) had the highest egg hatching rates across all days (8.67-11.33 on Day 1, 24-27 on Day 2, and 27-59 on Day 3) with no inhibition, confirming the effectiveness of CFCFs in suppressing *M. incognita* egg hatching.

DISCUSSION

The present study assessed the biocontrol potential of *Steinernema pakistanense* (SG1 and SG2 strains) and CFCFs of their symbiotic bacteria, *Xenorhabdus* spp., against *M. incognita* in *in vitro* and greenhouse experiments. Root-knot nematodes (*Meloidogyne* spp.) pose a significant economic threat to global agriculture, causing substantial yield losses in a wide range of crops, including vegetables, cereals, and fruit trees. Their ability to form complex feeding sites within plant roots leads to stunted growth, reduced nutrient uptake, and increased susceptibility to secondary infections (Kayani and Mukhtar, 2018; Kayani *et al*., 2018; Ayub *et al*., 2024; Shahid *et al*., 2024; Yaseen and Mukhtar, 2024). Conventional management strategies rely heavily on synthetic nematicides, which are not only expensive but also contribute to environmental contamination and the development of resistant nematode populations (Mukhtar *et al*., 2021; Saeed *et al*., 2021; Afzal and Mukhtar, 2024).

Given these challenges, *S. pakistanense* and its bacterial symbionts present a promising eco-friendly alternative for sustainable nematode control. Entomopathogenic nematodes like *S. pakistanense* have shown great potential due to their ability to efficiently parasitize and kill plant-parasitic nematodes through the release of bacterial toxins. The CFCFs of *Xenorhabdus* spp. contain secondary metabolites with nematicidal properties, further enhancing the biocontrol efficacy of *S. pakistanense*. By providing a biological control strategy that reduces dependency on chemical nematicides, this approach aligns with integrated pest management (IPM) principles and supports environmentally sustainable agricultural practices.

Table 7. Effect of CFCFs of *Xenorhabdus* spp. isolated from *S. pakistanense* (SG1 and SG2) on egg hatching of *M. incognita.*

Treatment	Description	Eggs Hatched						Inhibition Percentage	
		Day 1		Day 2		Day 3			
		SG1	SG2	SG1	SG2	SG1	SG2	SG1	SG2
T1	90%	0.0000B	0.0000C	7.0000C	8.0000D	17.000D	8.0000D	79.2452A	77.1235A
T2	50%	0.0000B	0.0000C	8.0000C	11.000CD	18.000D	11.000CD	77.35849A	74.5098A
T3	25%	0.0000B	0.3333BC	17.000B	14.667BC	25.000C	14.667BC	64.15094B	60.7843B
T4	10%	1.0000B	1.6667B	16.000B	18.333B	30.000B	18.333B	54.7169C	47.0588C
Control	Water	8.6667A	11.3333A	24.000A	27.000A	59.000A	27.000A	0D	0D

Our results demonstrated the significant suppressive effects of *S. pakistanense* and its symbiotic bacteria against *M. incognita*. SG1-treated plants in T4 achieved a maximum shoot length of 14.68 cm, compared to 8.60 cm in control, while SG2-treated plants showed even greater improvement, reaching 15.38 cm versus 10.88 cm in control. Similarly, fresh shoot weights were significantly enhanced, with SG1 in T4 showing the highest fresh weight at 3.54 g. The number of root galls was drastically reduced to 2.2 in T4 for SG1 and 3.0 for SG2, compared to 21.8 and 19.4 galls, respectively, in control plants. These findings align with previous studies that emphasize the efficacy of EPNs in reducing nematode populations and promoting plant health (Javed *et al*., 2012; Kepenekci *et al*., 2016).

EPNs enter the host through natural openings and release symbiotic bacteria, which proliferate and produce secondary metabolites that lead to host mortality within 24 to 48 hours. This process not only eliminates the nematode threat but also prevents microbial competitors from colonizing the cadaver, thus creating a conducive environment for nematode reproduction (El-Deen *et al*., 2016; Kim *et al*., 2024). In our study, both SG1 and SG2 stains effectively suppressed nematode populations, with the reproduction factor reduced to 1.99 for SG1 and 2.57 for SG2 in T4. This suppression aligns with previous reports on the nematicidal potential of *Xenorhabdus* spp., whose bioactive compounds inhibit nematode reproduction and development (Abd-Elgawad, 2022; Srivastava and Chaubey, 2022).

Although EPNs primarily target insects, evidence suggests they can directly parasitize or prey upon RKNs. The reduction in gall formation and egg masses observed in our study highlights the dual role of EPNs in suppressing nematodes and promoting plant growth. The application of SG1 in T4 resulted in the lowest gall count (2.2) and egg mass count (2.2), while the nematicide treatment (T5) completely inhibited gall formation. These findings suggest that *S. pakistanense* and its symbiotic bacteria can serve as sustainable alternatives to chemical nematicides.

The timing of EPN applications significantly influenced their efficacy. Applying EPNs one week before nematode inoculation (T1) had the most substantial impact on plant growth and nematode suppression. Shoot lengths were highest in T1, with SG1-treated plants reaching 33.30 cm and SG2-treated plants 33.78 cm, compared to the lowest values observed in post-inoculation treatments (25.96 cm for SG1 and 25.44 cm for SG2). Similarly, the number of root galls and egg masses was significantly reduced in T1, with only 5.0 galls and egg masses for both SG1 and SG2. These findings support previous research by Kenney and Eleftherianos (2016), which emphasizes the importance of optimizing the timing of EPN applications to maximize their effectiveness.

The use of CFCFs of *Xenorhabdus* spp. also presents a promising approach for nematode management. Higher concentrations of CFCFs (90%) exhibited the most significant inhibitory effects on egg hatching, with inhibition rates of 79.24% for SG1 and 77.12% for SG2. In contrast, lower concentrations (10%) were less effective, achieving inhibition rates of 54.72% for SG1 and 47.06% for SG2. These results confirm previous studies highlighting the nematicidal potential of *Xenorhabdus* spp. bioactive compounds (Srivastava and Chaubey, 2022).

EPNs and RKNs occupy similar ecological niches in the soil. The introduction of EPNs leads to competition for space and resources, potentially suppressing RKN populations. Additionally, the presence of EPNs may induce systemic resistance in plants, further deterring RKN establishment (Kenney and Eleftherianos, 2016). The dual functionality of EPNs and their bacterial symbionts in promoting plant growth and reducing nematode pressure was evident in our study. Fresh root weights were

significantly reduced in T4 (0.8340 g for SG1 and 0.8180 g for SG2) compared to 1.4740 g in the control, indicating effective suppression of nematode damage. These findings align with those of Tian *et al*. (2007) and Fabiyi *et al*. (2024), which demonstrate the ability of EPNs to enhance plant growth by mitigating nematode infestations.

CONCLUSION

The present study demonstrated the effectiveness of *Steinernema pakistanense* (SG1 and SG2 strains) and its symbiotic bacteria (*Xenorhabdus* spp.) as biocontrol agents against *Meloidogyne incognita*. The application of *S. pakistanense* significantly enhanced tomato plant growth, reduced root gall formation, and suppressed nematode populations. The highest application rate (1,000 infective juveniles) exhibited significant effects, improving shoot length and weight while reducing gall counts and egg masses. Pre-inoculation treatments proved more effective than post-inoculation treatments, highlighting the importance of timing in nematode suppression and plant growth promotion. *In vitro* experiments further confirmed the nematicidal potential of *Xenorhabdus* spp. CFCFs, which inhibited egg hatching by up to 80% at higher concentrations. These findings emphasize the dual role of EPNs and their symbiotic bacteria in controlling nematode infestations and enhancing plant health. This research highlights the potential of *S. pakistanense* and its symbiotic bacteria as sustainable alternatives to chemical nematicides. Future studies should focus on optimizing EPN application strategies and integrating them with other biological control methods for comprehensive root-knot nematode management in agriculture.

AUHORS CONTRIBUTIONS

MSG and TM conceptualized the idea and designed the study; MSG conducted experiments, collected and analyzed the data; GI assisted in data collection; TAK provided culture of entomopathogenic nematodes; MSG wrote the first draft of the manuscript; TM supervised the work and proofread the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Abd El-Aal, E. M., M. Shahen, S. Sayed, H. Kesba, M. J. Ansari, R. M. El-Ashry, A. A. Aioub, A. S. Salma and A. M. Eldeeb. 2021. In vivo and *in vitro* management of *Meloidogyne incognita* (*Tylenchida*: *Heteroderidae*) using rhizosphere bacteria, *Pseudomonas* spp. and *Serratia* spp. compared with oxamyl. Saudi Journal of Biological Sciences, 28: 4876-83.

Abd-Elgawad, M. M. 2022. *Xenorhabdus* spp.: an overview of the useful facets of mutualistic bacteria of entomopathogenic nematodes. Life, 12: 1360.

Afzal, A. and T. Mukhtar. 2024. Revolutionizing nematode management to achieve global food security goals-An overview. Heliyon, 10: e25325.

Ahmed, M. H., M. Ashfaq, T. Mukhtar and M. A. Khan. 2021. Categorization of available cucumber genotypes against Zucchini yellow mosaic virus and root-knot nematode (*Meloidogyne incognita*). International Journal of Agriculture & Biology, 25(5): 955-961.

Akhurst, R. 1980. Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. Microbiology, 121: 303-09.

Alves, F., P. J. T. Nakada, M. J. d. A. M. Marques, L. da Cruz Rea, A. A. Cortez, V. d. O. A. Pellegrini, I. Polikarpov and C. Kurachi. 2024. Complete photodynamic inactivation of *Pseudomonas aeruginosa* biofilm with use of potassium iodide and its comparison with enzymatic pretreatment. Journal of Photochemistry and Photobiology B: Biology, 257: 112974.

Asghar, A., T. Mukhtar, M. U. Raja and A. Gulzar. 2020. Interaction between *Meloidogyne javanica* and *Ralstonia solanacearum* in chili. Pakistan Journal of Zoology, 52(4): 1525-1530.

Aslam, M. N., T. Mukhtar, M. A. Hussain and M. Raheel. 2017. Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. Journal of Plant Diseases and Protection, 124(6): 585-590.

Ayub, H. M., M. Akram, A. Mehak, M. Ajmal, I. Ilyas, W. Seerat, M. Tatar, A. Ali, R. Sarwar, M. Abbasi and A. Rahman. 2024. A preliminary study on the interaction between *Meloidogyne incognita* and some strains of *Pseudomonas* spp. on growth performance of tomato under greenhouse conditions. Plant Protection, 8(2): 341-350.

Azeem, W., T. Mukhtar and T. Hamid. 2021. Evaluation of *Trichoderma harzianum* and *Azadirachta indica* in the management of *Meloidogyne incognita* in tomato. Pakistan Journal of Zoology, 53(1): 119-125.

Bedding, R. and R. Akhurst. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil.

Boemare, N. and R. Akhurst. 1988. Biochemical and physiological characterization of colony form variants in *Xenorhabdus* spp. (*Enterobacteriaceae*). Microbiology, 134: 751-61.

Collins, E. J., C. Bowyer, A. Tsouza and M. Chopra. 2022. Tomatoes: An extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. Biology, 11: 239.

Eisenback, J. D., H. Hrischmann, J. N. Sasser and A. C. Triantaphyllou. 1981. A guide to the four most common species of root-knot nematodes (*Meloidogyne* spp.), with a pictorial key. pp.48.

El Aimani, A., A. Houari, S.-E. Laasli, R. Mentag, D. Iraqi, G. Diria, S. Khayi, R. Lahlali, A. A. Dababat and F. Mokrini. 2022. Antagonistic potential of Moroccan entomopathogenic nematodes against root-knot nematodes, *Meloidogyne javanica* on tomato under greenhouse conditions. Scientific Reports, 12: 2915.

El-Deen, N. A., A.-B. F. Amal, D. Y. Hadeer and A. Al-Ghamdi. 2016. Eco-friendly management of root-knot nematode, *Meloidogyne incognita* infecting pomegranate at Taif Governorate, KSA. Research Journal of Pharmaceutical Biological and Chemical Sciences, 7: 1070-76.

Fabiyi, O. A., O. O. Adebisi, S. O. Falore and A. O. Claudius-Cole. 2024. Potential inhibition of entomopathogenic nematodes and plant growth-promoting bacteria with exposure to selected herbicides and insecticides. Vegetos, 37: 1503-12.

FAO. 2023. FAOSTAT (Crops and livestock products). Food and Agriculture Organization of the United Nations.

Fosler, G. M. 1958. Soil sterilization methods for the indoor gardener. Circular (University of Illinois (Urbana-Champaign campus). Extension Service in Agriculture and Home Economics); 793.

Gondal, A. S., C. A. Rauf, F. Naz and N. A. Abbasi. 2018. Surveillance of tomato foot rot caused by *Rhizoctonia solani* in Pothohar Region of Pakistan. MYCOPATH, 15: 47-50.

Gondal, A. S., N. Javed, S. A. Khan and S. Hyder. 2012. Genotypic diversity of potato germplasm against root knot nematode (*Meloidogyne incognita*) infection in Pakistan. International Journal of phytopathology, 1: 27-38.

GOP. 2023. Pakistan Economic Survey 2022-23. . Ministry of Finance.

Gulzar, A., T. Mukhtar and D. J. Wright. 2020. Effects of entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on the fitness of a Vip3A resistant subpopulation of *Heliothis virescens* (Noctuidae: Lepidoptera). Bragantia, 79(2): 281-292.

Haq, M. A., T. Mukhtar, M. I. Haq and A. Khalid. 2022. Reproduction of root-knot nematode, *Meloidogyne incognita*, on *Solanum melongena* genotypes determines their host status. Pakistan Journal of Zoology, 54(5): 2097-2103.

Holbrook, C., D. Knauft and D. Dickson. 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria*.

Hussain, M. A. and T. Mukhtar. 2019. Root-knot nematodes infecting okra in major vegetable growing districts of Punjab, Pakistan. Pakistan Journal of Zoology, 51(3): 1137-1143.

Hussey, R. and K. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique.

Javed, K., H. Javed, T. Mukhtar and D. Qiu. 2019. Efficacy of *Beauveria bassiana* and *Verticillium lecanii* for the management of whitefly and aphid. Pakistan Journal of Agricultural Sciences, 56(3): 669-674.

Javed, N., S. A. Khan, M. Atiq and M. Kamran. 2012. Effect of *Steinernema glaseri* and *Heterorhabditis indica* on the plant vigour and root knot nematodes in tomato roots at different densities and time of applications. Pakistan Journal of Zoology, 44.

Javed, S., T. A. Khanum and A. Ali. 2022. Storage and efficacy of entomopathogenic nematode species as a biocontrol agent against the armyworm, *Spodoptera litura* (Fabricius) (*Lepidoptera*: *Noctuidae*). Egyptian Journal of Biological Pest Control, 32: 6.

Jepson, S. B. 1987. Identification of root-knot nematodes (*Meloidogyne* species).

Johnigk, S. 1999. Frequently used standard methods in

entomopathogenic nematode-bacterium research. Kriel: Christian Albrechts University.

Kayani, M. Z. and T. Mukhtar. 2018. Reproductivity of *Meloidogyne incognita* on fifteen cucumber cultivars. Pakistan Journal of Zoology, 50(5): 1717-1722.

Kayani, M. Z., T. Mukhtar and M. A. Hussain. 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(3): 897-902.

Kenney, E. and I. Eleftherianos. 2016. Entomopathogenic and plant pathogenic nematodes as opposing forces in agriculture. International Journal for Parasitology, 46: 13-19.

Kepenekci, I., S. Hazir and E. E. Lewis. 2016. Evaluation of entomopathogenic nematodes and the supernatants of the in vitro culture medium of their mutualistic bacteria for the control of the root-knot nematodes Meloidogyne incognita and M. arenaria. Pest Management science, 72: 327-34.

Khan, M. T. A., T. Mukhtar and M. Saeed. 2019. Resistance or susceptibility of eight aubergine cultivars to *Meloidogyne javanica*. Pakistan Journal of Zoology, 51(6): 2187-2192.

Kim, J.-H., B.-M. Lee, H. C. Lee, I.-S. Choi, K.-B. Koo and K.-H. Son. 2024. Antagonistic Efficacy of Symbiotic Bacterium *Xenorhabdus* sp. SCG against Meloidogyne spp. Journal of Microbiology and Biotechnology, 34: 1627.

Li, N., X. Wu, W. Zhuang, L. Xia, Y. Chen, C. Wu, Z. Rao, L. Du, R. Zhao and M. Yi. 2021. Tomato and lycopene and multiple health outcomes: Umbrella Review. Food Chemistry, 343: 128396.

Luc, M., D. Hunt and J. Machon. 2005. Identification, Morphology and Biology of Plant-parasitic Nematodes In: Luc M., Sikora RA and Bridge J.(eds) Plant parasitic nematodes in subtropical and tropical agriculture. CABI Publishing, Wallingford, UK.

Maqbool, M. A., B. Zarina, K. Nasira and K. Firoza. 1992. Distribution and host association of plant parasitic nematodes in Pakistan.

Mendoza-de Gives, P. 2022. Soil-Borne nematodes: Impact in agriculture and livestock and sustainable strategies of prevention and control with special reference to the use of nematode natural enemies. Pathogens, 11: 640.

Moens, M., R. N. Perry and J. L. Starr. 2009. *Meloidogyne* species-a diverse group of novel and important plant parasites. In, Root-knot nematodes CABI Wallingford UK.

Molina, J., C. Dolinski, R. Souza and E. Lewis. 2007. Effect of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on *Meloidogyne mayaguensis* Rammah and Hirschmann (Tylenchida: Meloidoginidae) infection in tomato plants. Journal of nematology, 39: 338.

Mukhtar, T. 2018. Management of root-knot nematode, *Meloidogyne incognita*, in tomato with two *Trichoderma* species. Pakistan Journal of Zoology, 50(4): 1589-1592.

Mukhtar, T. and M. A. Hussain. 2019. Pathogenic potential of Javanese root-knot nematode on susceptible and resistant okra cultivars. Pakistan Journal of Zoology, 51(5): 1891-1897.

Mukhtar, T. and M. Z. Kayani. 2019. Growth and yield responses of fifteen cucumber cultivars to root-knot nematode (*Meloidogyne incognita*). Acta Scientiarum Polonorum Hortorum Cultus, 18(3): 45-52.

Mukhtar, T. and M. Z. Kayani. 2020. Comparison of the damaging effects of *Meloidogyne incognita* on a resistant and susceptible cultivar of cucumber. Bragantia, 79(1): 83-93.

Mukhtar, T., M. Tariq-Khan and M. N. Aslam. 2021. Bioefficacy of *Trichoderma* species against Javanese root-knot nematode, *Meloidogyne javanica* in green gram. Gesunde Pflanzen, 73(3): 265–272.

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

Pérez, E. E. and E. E. Lewis. 2002. Use of entomopathogenic nematodes to suppress *Meloidogyne incognita* on greenhouse tomatoes. Journal of Nematology, 34: 171.

Rahoo, A. M., T. Mukhtar, A. M. Jakhar and R. K. Rahoo. 2018a. Inoculum doses and exposure periods affect recovery of *Steinernema feltiae* and *Heterorhabditis bacteriophora* from *Tenebrio molitor*. Pakistan Journal of Zoology, 50(3): 983-987.

Rahoo, A. M., T. Mukhtar, B. A. Bhugio and R. K. Rahoo.

2019a. Comparison of infectivity and productivity of *Steinernema feltiae* and *Heterorhabditis bacteriophora* in *Galleria mellonella* and *Tenebrio molitor*. Pakistan Journal of Zoology, 51(2): 717-724.

Rahoo, A. M., T. Mukhtar, B. A. Bughio and R. K. Rahoo. 2019b. Relationship between the size of *Galleria mellonella* larvae and the production of *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Pakistan Journal of Zoology, 51(1): 79-84.

Rahoo, A. M., T. Mukhtar, S. I. Abro, B. A. Bughio and R. K. Rahoo. 2018b. Comparing the productivity of five entomopathogenic nematodes in *Galleria mellonella*. Pakistan Journal of Zoology, 50(2): 679-684.

Rahoo, A. M., T. Mukhtar, S. R. Gowen and B. Pembroke. 2011. Virulence of entomopathogenic bacteria Xenorhabdus bovienii and Photorhabdus luminescens against Galleria mellonella larvae. Pakistan Journal of Zoology, 43(3): 543-548.

Rahoo, A. M., T. Mukhtar, S. R. Gowen, R. K. Rahoo and S. I. Abro. 2017. Reproductive potential and host searching ability of entomopathogenic nematode, *Steinernema feltiae*. Pakistan Journal of Zoology, 49(1): 229-234.

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

Saeed, M., T. Mukhtar, M. I. Haq and M. A. Khan. 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(5): 1555-1561.

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

Safdar, H., N. Javed, S. A. Khan, M. Z. Majeed, A. Mehmood and M. Arshad. 2023. *In-vitro* pathogenicity of entomopathogenic nematodes associated symbiotic bacteria and their metabolites against armyworm *Spodoptera litura* fabricius. Sarhad Journal of Agriculture, 39: 13-20.

Shahid, M., S. R. Gowen, M. Burhan, M. Z. Niaz, M. Anwar-ul-Haq and K. Mehmood. 2024. Differential responses of *Meloidogyne* spp. to *Pasteuria* isolates over crop cycles. Plant Protection, 8(2): 257-267.

Shapiro-Ilan, D. I., E. E. Lewis, R. W. Behle and M. R. McGuire. 2001. Formulation of entomopathogenic nematode-infected cadavers. Journal of invertebrate pathology, 78: 17-23.

Sharma, M. P., A. N. Sharma and S. S. Hussaini. 2011. Entomopathogenic nematodes, a potential microbial biopesticide: mass production and commercialisation status–a mini review. Archives of Phytopathology and Plant Protection, 44: 855-70.

Shehzad, M., M. Tariq, Q. Ali, A. Aslam, T. Mukhtar, M. F. Akhtar, A. Gulzar and M. Faisal. 2022. Evaluation of insecticidal activity of *Beauveria bassiana* against different instar larvae of *Plutella xylostella* by using two different methods of application. International Journal of Tropical Insect Science, 42: 1471-1476.

Shehzad, M., M. Tariq, T. Mukhtar and A. Gulzar. 2021. On the virulence of the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales), against the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Egyptian Journal of Biological Pest Control, 31(1): 86.

Srivastava, S. and A. K. Chaubey. 2022. *In vitro* study on the nematicidal activity of entomopathogenic bacteria against the root knot nematode *Meloidogyne incognita*. Journal of Applied and Natural Science, 14: 1-8.

Tariq-Khan, M., A. Munir, T. Mukhtar, J. Hallmann and H. Heuer. 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(3): 201-212.

Tariq-Khan, M., T. Mukhtar, A. Munir, J. Hallmann and H. Heuer. 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.

Tian, B., J. Yang and K.-Q. Zhang. 2007. Bacteria used in

the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. FEMS microbiology ecology, 61: 197-213.

Yaseen, I. and T. Mukhtar. 2024. Impact of sequential and concurrent inoculations of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on the growth performance of diverse okra cultivars. Plant Protection, 8(2): 303-313.

Yaseen, I., T. Mukhtar, A. Mubarik, B. Arshad, N. Sahu and U. Somaddar. 2025. Management of *Ralstonia solanacearum-Meloidogyne incognita* complex with *Trichoderma harzianum* in tomato. Bragantia, 84: e20240060.

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

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