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Evaluation of Rhizospheric-*Pseudomonas* spp. for the management of *Meloidogyne incognita* in tomato

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ABSTRACT

Root knot nematodes are commercially important plant parasites of many vegetable crops in Pakistan. This study investigate antagonistic effect of *Pseudomonas* spp. against *M. incognita* on tomato crop. Ten tomato cultivars were screened against RKN infection. Roma was found the most resistant against infection of *M. incognita* while money maker was found the most susceptible. Gall or egg mass number/indices are more resistant to RKN. *Pseudomonas* strains inoculated in the infected tomato plants reduced galling index and also improved the growth of the plant. So, it was obvious that bio-control agents also act as PGPR by stimulating plant growth and inducing resistance through the increase of total phenolic contents and salicylic acid. Tomato cv. Roma showed the greatest increase of total phenol contents and salicylic acid exhibiting tolerance against *M. incognita* infestation. Tomato cv. Roma showed significantly resistant response against root-knot nematode diseases. *Pseudomonas* showed significant antagonistic potential against *M. incognita in* tomato plant.

1. Introduction

Tomato, Lycopersicon esculentum (Family: Solanaceae) is an essential vegetable in Pakistan. It is a major source of vitamin A and C that is used in salads and for cooking purposes. In Pakistan, tomato is cultivated on 9000 ha area having 89,000 million tonnes annual crop production (FAO, 2020). Several pathogens including *Phytophthora infestans, Septoria lycopersici, Alternaria solani, Xanthomonas campestris* pv. *vesicatoria, Colletotrichum coccodes, Pseudomonas syringae* pv. *Tomato, Botrytis cinerea, Fulvia fulva, Verticillium dahlia, TSWV, Oidium neolycopersici* and *Meloidogyne* spp. are commonly known to infect tomato plants.

Meloidogyne cause drastic losses in tropical and sub-tropical vegetable crops. *Meloidogyne incognita*, is the polyphagus obligate parasite and the most predominant RKN species infecting tomato plants. *M. incognita* with a wide host range of about 770 plant species, are sedentary endoparasitic and the most damaging agricultural pests. *M. incognita* takes food from vascular vessels and causes hyperplasia and hypertrophy (Eid et al., 2018) however, in case secondary infestation of microorganisms like *Fusarium* spp. the plant root system gets completely damaged (Singh et al., 2015). About 22–30 % average tomato yield loss was recorded by *M. incognita* infestation having about 60 % crop losses in individual fields. Several tomato varieties are vulnerable to the RKN contributing major crop losses (Sasser and Carter, 1982; Singh et al., 2015). The yield losses of tomato cultivated in warm and dry regions having sandy soil can reach up to 95 % (Bourne et al., 2004). RKN found in almost all the cultivated parts of the Pakistan is more than developed countries because of its soil texture, warm

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and sandy soil conditions, tropical and subtropical sites and favorable climatic conditions for nematode development and reproduction. The nematode infestation was comparatively greater in irrigated soils due to continuous cultivation. (Khan et al., 2006; Shahid et al., 2007). More attention and immediate measures are required to minimize the alarming crop damage due to RKN.

Nematode infestation is comparatively difficult to manage as they are basically soil borne in nature and their infestation on root system mostly goes unnoticed for a long time until plant expresses foliar symptoms (Moens and Aicha, 1983). Currently, the most common way to manage nematodes is the use of chemicals however, high cost, health hazards and most importantly environmental concerns create challenging problems. Application of different sort of nematicides as granules is one of the general control method. But use of nematicides is not a good option as soil fumigation suppresses plant pathogenic nematode population along with suppression of beneficial or non-harmful organisms inhabiting the soil such as non-parasitic fungi and bacteria (Ahmadi and Bac, 2005). However, lack of resistance against *M. incognita* makes the worst situation for the researchers. In case of cultural practice, it is time consuming and do not provide satisfactory results (Abawi and Widmer, 2000). This emphasis the need of alternative methods. Another alternative approaches includes stimulation of plant defenses induction of resistance against M. incognita (Raddy et al., 1987). These practices inactivates the pathogen which may become active later on, alter the crop value and gross income. Conclusively, the use of antagonistic agent mostly endophytic bacteria, is an alternative approach for effectively managing *M. incognita* infestation. Biological control of nematode infestation has become successful in the last few years. Antagonistic bacteria like *Pseudomonas* spp. has been widely used in bio antagonism (Youssef and Korayem, 2006). So, this research aims to assess the antagonistic activity of various *Pseudomonas* spp. for the control of *M. incognita* including both *in-vivo* and *in-vitro* conditions.

2. Materials and methods

2.1. Sample collection and nematode extraction

Total 163 root and soil samples were collected from twenty different vegetable locations of Bahawalpur. After sample processing, these were weighed and visually rated for galling/plant on a 0 to 5 scale (Anwar et al., 2007; Quesenberry et al., 1989). Nematode was extracted from root and soil samples by following Baermann funnel and root maceration methods for soil and root samples respectively.

2.2. Nematode inoculum preparation

Tomato seedlings were raised in sterilized sandy loam soil for 30 days and transplanted to 25:75 sand and clay mixture. After 1 week of seedling transplantation, the nematodes were picked manually from galled roots of egg-plant and inoculated in each healthy tomato by creating a hole of about 3 cm depth at plant base that was immediately covered and watered to prevent the plant from drying out. The nematodes species were identified according to the perineal pattern (Eisenback et al., 1981; Hartman and Sasser, 1985).

2.3. Screening and reproduction of M. incognita on roots of tomato cultivars

2.3.1. Establishment of sick plot

All the nematode infested samples were multiplied according to localities. Each plot was planted with the susceptible eggplant cv. Dilnasheen. *M. incognita* was multiplied on the roots of tomato cv. Money maker (a susceptible tomato cultivar) by inoculating single egg mass in the pots.

2.3.2. Screening of tomato cultivars against RKN

Ten locally available tomato cultivars were screened against RKN infection in a glass house with five replications for each. From the tomato roots, eggs and J_2s were collected using 1 % NaOCl solution (Hussey and Barker, 1973). After egg collection, the egg suspension was allowed to settle down for 3–4 h and the excess water was drained out without upsetting the eggs. The nematode reproduction experiment were conducted for 48 days. The desired inoculum density of egg suspension (2000 eggs) was inoculated in a little volume in three holes around each plant. The holes were immediately covered and plants were lightly watered after 1 day of inoculation to minimize egg loss due to leaching or severe drying.

2.4. Evaluation of antagonistic effect of Pseudomonas spp. against M. incognita both in-vivo and in-vitro

2.4.1. Evaluation of Pseudomonas spp. for the potential to inhibit M. incognita J_2s

Four different strains of *Pseudomonas* spp. (NA12, NA4, 006 and 005) were assessed for their bio-control potential by using 100 ± 2 nematode larvae per mL plus bacterial isolates at 2×10^8 CFU per mL with 2 mL concentration for both. After 2 days of treatment, nematodes were shifted to tap water for 24 h to evaluate their mortality. Nematode Juveniles present in tap water were touched by probe to decide about their mortality. After 24 h nematodes were again transferred to clean petri dishes to make sure there is no recovery. The following formula was used to calculate juvenile inhibition:

Juvenile mortality = dead juveniles/total juvenile × 100.

2.4.2. Evaluation of antagonistic activity of Pseudomonas spp.

The antagonistic activity of selected *Pseudomonas* spp. was evaluated under greenhouse experimentation. After 1 week of seedling transplantation, the treatments were applied at the same concentration as above. All the treatments were applied through soil drenching at recommended rates but bacterial isolate was applied twice. Nematode culture (2000 J_2s) was applied after ten days. Irrigation and fertilization (1 g NPK per plant) was applied periodically under CRD.

Plants were uprooted after 50 days of inoculation for nematode extraction. Four egg-masses/root system were selected to count no. of eggs per egg mass. Other parameters such as reduction percentage of J_2s, no. of females, no. of egg masses, gall formation and total population including J_2s in soil + females + egg mass were also calculated. The nematode build up was calculated by subtracting initial population from final population. The individual fresh and the dry root weight along with length were recorded to measure the effect on plant growth. The decimal count technique was used to record the total microbial count in rhizosphere.

2.5. Effect on biochemical parameters

2.5.1. Salicylic acid and total phenolic contents

After 15 days of nematode inoculation, salicylic acid and total phenol contents were evaluated in one gram leaf sample collected from each treatment. The samples for the analysis of total phenolic contents were homogenized in 10 mL of 80 % methanol following 15 min agitation at 70 °C temperature (Zieslin and Ben-Zaken, 1993). The total phenol contents were extracted as described by Sharma and Sain (2005) and represented as µg catechol per gram fresh leaf of plant. To extract salicylic acid content, small discs of

leaf samples were dipped in water and filtered through Whatman filter paper no. 1 after keeping at room temperature overnight. The ethyl acetate was added in filtrate suspension to extract the phenol content. To remove water from the filtrate suspension, sodium sulphate was added. After water evaporation under water bath, the dried sample was obtained and the absorbance of the final solution was measured by a spectrophotometer (UV 2450) at 306 nm by adding 10 mL methanol in dried sample (Shane and Kowblansky, 1968). Various salicylic acid concentrations (0, 10, 20, 30, 40, 50, 100 ppm) were prepared in methanol to make a standard salicylic curve. Data recorded from absorbance was plotted and the best fit line was drawn passing by the origin. The salicylic concentration was measured from the drawn standard curve by using y = mx ± c formula (Loake and Grant, 2007).

2.5.2. Carotenoid and chlorophyll contents of leaves

After 15 days of nematode inoculation, one gram of fresh leaf tissue was collected from interveinal portion of tomato plant leaf to calculate the carotenoids, total chlorophyll, chlorophyll *a* and chlorophyll *b* contents. The collected leaf tissues were finely ground by adding 40 mL volume of 80 % acetone and then passed through two Whatman No. 1 filter papers. The suspension residues ground and filtered twice by following same process. After shifting the filtrate to a flask, acetone was added up to the 100 mL volume. Optical density was calculated by spectrometer at 470 nm, at 645 nm and at 663 nm for carotenoid, for chlorophyll *a* and for chlorophyll *b* respectively (Arnon, 1949; Maclachlan and Zalik, 1963).

3. Results

3.1. Screening and multiplication of M. incognita on roots of tomato cultivars

3.1.1. In vivo study

3.1.1.1. Occurrence of Meloidogyne spp. on the vegetables. 62 samples were determined to be infested with RKN. The rate of occurrence varied from 0 % to 85.71 percent, with a mean of 47.1935 percent (Table 1). The galling index mean was 3. Both the frequency and the galling index vary by location that might be due to soil type. The highest incidence was found in Chak 32 and Chak 13 (85.71 %), with a maximum galling index of 5, while the lowest incidence was found in Shah Bagh, Chak 222, and Chak 224 (0 %), with a minimum galling value of 0. *M. incognita* and *M. javanica* were recognized as RKN species.

3.1.1.2. Population of nematodes in plant roots and soil. In each site, the amount of nematodes in the roots was varied. In various places, the numbers of nematodes in the soil were varied. Chak 32 and Chak 13 were found to have more nematodes in the soil than the other locations. The three locations including Shah Bagh, Chak 22 and Chak 224 had not found nematodes in soil means RKN infection was not existed in these locations (Table 1).

3.1.1.3. M. incognita and M. javanica diagnostic characteristics. M. incognita features are Dorsal arch high and square, lateral field not marked by loop, striae smooth to wary, and fold striae while *M. javanica* features are Deep incisures clearly define the lateral line typically extending beyond the perineum.

3.1.1.4. Survey results. M. incognita was observed in 15 (68.18 %) sampling sites. In five locations (22.72 %) presence of *M. javanica* and *M. incognita* were determined. *M. javanica* alone was recovered from two locations (9.09 %). Separate processing of 100 cm^3 of soil and 20 g of roots documented that RKN population ranged between 0 and 461 with an average of 232 nematodes in soil and 0 to 283 with a mean of 141 nematodes in roots (Table 1). Last five years' data of area under vegetable cultivation and their yield was also collected to access the percentage losses of specific locality.

Seven additional plant parasitic nematode genera, *Aphelenchus, Xiphinema, Criconema, Pratylenchus, Helicotylenchus, Longidorus* and *Hoplolaimus*, were found in the root and rhizosphere soil analyses with very low root and soil population ranging from 0 to 2 in roots and 2–15 in soil. These nematode genera are of minor importance.

3.1.1.5. M. incognita reproduction on tomato cultivars. M. incognita caused galls on the roots of all 10 cultivars (Table 2). Money maker found to be the most vulnerable by generating the highest number of galls on its roots while Roma was statistically the least vulnerable cultivar regarding all the reproduction parameters.

3.1.1.6. Effect of M. incognita densities on growth of tomato. At four different inoculum stages, RKN's infestation impact was varied. A substantial (P = 0.05) decrease in plant growth was developed

Table 1
Incidence of Meloidogyne incognita, galling index, J2 population on vegetable crops plant at various locations.

Localities	Total Samples	Infested Sample	Incidence	Galling index*	J2 Population (100 cm^3 of soil)	Losses (%)
Poomi Farms	6	3	50	4	215	45
Fakhar Agriculture	5	3	60	4	237	53
Al-Haq Farms	8	3	37.5	3	152	35
Syed farms	9	5	55.55	4	216	45
Shah Garden	5	0	0	0	0	0
Bahoo Farms	5	3	60	4	254	55
Hafiz farms	6	4	66.67	4	315	60
Waseeb Farms	4	3	75	4	319	65
Chak # 156	4	3	75	4	334	60
Chak 32	7	6	85.71	5	430	80
Mussal pur	10	6	60	4	247	55
238 jb	8	3	37.5	3	144	35
Batiwala	6	2	33.33	3	122	45
Chak 222	4	0	0	0	0	0
Chahdar farms	8	4	50	3	209	65
Chak # 225	9	3	33.33	3	133	35
Chak 224	6	0	0	0	0	0
Chak 195	7	2	28.57	2	186	25
Chak # 164	6	3	50	3	215	45
Chak 13	7	6	85.71	5	419	80

*Gall and egg mass indices: 0–5 scale

Table 2
Meloidogyne incognita infection and reaction of 10 tomato cultivars.

Tomato Cultivars	Galls/root system	Galling index**	Egg masses/ root system	Egg mass index**	Root weight (g)	Rate of Reproduction	Final Population
UC-134	118b*	5	140b	5	18.54d	3.652 g	7300 g
UAE-I	109b	5	131b	5	16.53f	3.250i	6500i
MARRCHIA	124ab	5	153ab	5	18.78d	4.450f	8900f
Peelo	125ab	5	155ab	5	18.60d	4.650e	9300e
Money maker	181a	5	215a	5	25.48a	6.100a	12200a
Pasestter	151ab	5	214a	5	19.87c	5.150c	10300c
Pakit	159ab	5	190ab	5	20.68b	5.280b	10560b
Roma	45c	4	50c	4	10.15g	2.150j	4300j
Areletta	113b	5	135b	5	17.20e	3.420h	6840h
CHICO	148ab	5	178ab	5	18.96d	4.970d	9940d

**Gall and egg mass indices: 0–5 scale.
* Values following same letter are not different significantly at P = 0.05 according to DMRT.

due to massive production of root galls and egg masses. Increased inoculum affected nematode reproduction. The rate of nematode build up was highest at the initial inoculum level (Table 3) but it was decreased as the inoculum level increased.

All the observed parameters varies between four *M. incognita* densities. Some parameters such as foliage length, foliage weight, root length, whole plant fresh weight of leaves and number of flowers showed inverse relationship with inoculum level. All these parameters were significantly different ($P < 0.05$) at different inoculum levels.

Whereas, the root weight parameter was found to be higher at greater inoculum level and minimum at initial inoculum level indicating the direct relationship with the inoculum density (Table 3).

Fruit initiation was not started in any of the plant inoculated with different nematodes density. Fruit initiation had been observed in only control plants (Table 3).

3.1.1.7. Effect of M. incognita densities on nematode reproduction. The greatest gall indices were recorded at the highest inoculum level. At four levels of infestation, the ability of *M. incognita* to reproduce was clearly established by the formation of egg masses. At 250 eggs per plant, the output was significantly lower. At all inoculum levels, the egg mass indices were almost identical. The egg production per root system and per egg mass are directly related to inoculum level raised as the inoculum amount increased. At higher inoculum levels, the quantity of eggs per egg mass and egg per root system were substantially ($P = 0.05$) varied (Table 4). However, the reproduction rate was greatest at the first inoculum density (Table 4) and began to decline as inoculum concentrations increased. It's thought to be a density-related phenomena.

3.2. In vitro study

3.2.1. Effect of Pseudomonas spp. on M. incognita J_2 mortality

In-vitro assays using lytic enzymes were used to assess nematicidal activity in the identified bacterial strains. After 48 h of exposure, the results indicated that all culture filtrates induced death in *M. incognita* J2 (significant p 0.05). The mortality rate varied from 100 to 84.29 percent. When compared to control, *Pseudomonas* spp. 005 showed the greatest percentage (100 %) mortality, followed by *Pseudomonas* spp. NA-12 (97.25 %), *Pseudomonas* spp. NA-4 (94 %), *Pseudomonas* spp. 006 (93 %), and *Pseudomonas* spp. NA-20 (92 %). (Table 5).

3.2.2. Effect of Pseudomonas spp. on nematode parameters

3.2.2.1. Effect on root galls and egg masses. Stunted growth with mild chlorotic symptoms on leaves was observed in *M. incognita* inoculated tomato plants. All the four inoculated *Pseudomonas* strains showed high antagonistic potential by significantly ($P \leq 0.001$) reducing the nematode parameters as compared to the negative control (plant inoculated with just *M. incognita*). Among all four *Pseudomonas* strains the maximum galling index and egg mass index were observed in NA-12 i.e., 2.33 and 3 respectively (Table 6). Maximum reduction in gall index ($P \leq 0.001$) and egg mass index ($P \leq 0.01$) was found in NA-4 treated plants.

3.2.2.2. Final density of J_2 population in soil. The population density of J_2 *M. incognita* was high in the soil of negative control at initial inoculation level as compared to *Pseudomonas* inoculated plants. The highest population of J_2 per kg of pot soil was found in negative control. However, all the treatment plants were found to suppress the population of J_2/kg of pot soil at initial population density ($P \leq 0.001$). The highest nematode suppression in J_2 population was observed by NA-4 (350 %) followed by Strain-005 (103 %) in response to initial population density significantly different at $P \leq 0.001$ (Table 6).

3.3. Effect of Pseudomonas spp. on plant growth parameters

In addition to nematode reproduction, all the tested *Pseudomonas* strains significantly stimulated the plant growth parameters viz., plant height, shoot height, root and shoot weight, number of leaves and number of flowers. After 60 days of inoculation, plants were harvested and data was recorded.

Table 3
Effect of M. incognita densities on plant growth parameters.

Inoculum level (Juveniles)	Length (cm)		Fresh Weight (g)		No of leaves	No of flowers	Weight of whole plant	Initiation of fruits
	Root	Shoot	Root	Shoot				
500	18b*	23.4a	10.6b	33.72b	10.000	3ab	25.22c	Not
1000	16.b	17.6b	5.18b	28.16c	14.400	1c	20.11d	Not
1500	16b	14.8b	11b	23.56d	13.800	2bc	18.56d	Not
2000	16b	13.8b	23.9a	21.74d	11.400	2bc	40.02b	Not
Control	29a	25.2a	11.2b	40.38a	15.400	4a	59.98a	Yes

*Means followed by the same letter are not significantly different from each other at P = 0.05 according to DMRT.

Table 4
Nematode reproduction at different inoculum densities.

Inoculum Level (Juveniles)	Root Galls	Galling index**	Egg masses	Egg mass index**	Eggs per egg mass	Egg per root system
500	71.00d*	4	86.00d	4	60.00c	5105d
1000	155.0c	5	179.0c	5	92.00b	10470c
1500	218.0b	5	251.0b	5	106.0b	23610b
2000	553.0a	5	608.0a	5	155.0a	94250a
Control	0.0000e	0	0.0000e	0	0.0000d	0.0000e

**Gall and egg mass indices: 0–5 scale.
*Values following same letter were not different significantly at P = 0.05 according to DMRT.

Table 5
Juvenile mortality after 48 h exposure to cultural filtrate of Pseudomonas spp. (% Mortality*).

Control (water only)	0.00e
Pseudomonas spp. 005	100.00a
Pseudomonas spp. NA-20	92.0 0c
Pseudomonas spp. NA-4	94.50bc
Pseudomonas spp. NA-12	97.24ab
Pseudomonas spp. 006	93.00c

*Values following same letter(s) were not different significantly according to DMRT. There were five replicates.

3.3.1. Pseudomonas spp. NA-12

The plant growth parameters including total plant weight, total plant height, shoot height, number of leaves and number of flowers were found maximum at positive control followed by untreated control, treatment plant and negative control respectively. Unlike total plant weight, the root weight was recorded highest in negative control g) followed by treatment plant, positive control and untreated control. (Table 7). So, the positive control showed the best plant growth indicating the PGPR activity and antagonistic efficacy of Pseudomonas strain NA-12.

3.3.2. Pseudomonas spp. 006

The plant growth parameters including total plant weight, total plant height, shoot height, number of leaves and number of flowers were positive control > untreated control > treatment plant > negative control respectively. Unlike other growth parameters, the order of root weight was negative control followed by treatment plant, positive control and untreated control. However, the results indicated that *Pseudomonas* strain-006 was better than strain NA-12 for nematode management (Table 7).

3.3.3. Pseudomonas spp. 005

The plant growth parameters including total plant weight, plant height, shoot height, number of leaves and number of flowers were arranged as positive control > untreated plant > treatment plant > negative control respectively. Unlike other parameters, the root weight was maximum in negative control followed by treatment plant, positive control and untreated control. The antagonistic efficacy of strain-005 is better than NA-12 and closely related with strain-006 (Table 7).

3.3.4. Pseudomonas spp. NA-4

The plant growth parameters including total plant weight, total plant height, shoot height, number of leaves and number of flowers were arranged as positive control > untreated control > treatment plant > negative control respectively. Unlike other growth parameters, the root weight was maximum in the negative control. All the four tested Pseudomonas strains significantly promoted the plant growth and reduced the nematode diseases but among all these NA-4 strain showed the best results (Table 7).

3.4. Effect on Pseudomonas strains on biochemical properties of plants

3.4.1. Contents of salicylic acid and total phenol of leaves

Content of salicylic acid and total phenol in plant leaves of negative control was recorded to be increased by 9.6 % and 17 % respectively as compared to untreated control. Total phenol content was recorded to be highly increased (20–34 %) in treatment plants comparing with untreated control. Treatments inoculated with NA-12, NA-4, Strain-005 and Strain-006 significantly ($P \leq 0.001$) increased salicylic acid content to 4.4 %, 4.9 %, 9.2 % and 9 % comparing with untreated control respectively. The salicylic acid or total phenol levels was lower in plants with more root galls and egg masses (Table 8).

3.4.2. Carotenoid and chlorophyll contents of leaves

Total chlorophyll content of plant leaves was recorded to be reduced by 11.6 %. Chlorophyll *a* and b content varied in tomato cultivars from 2.4 % to 12.4 %. The negative impact of *M. incognita* on leaf pigments was reduced by *Pseudomonas* strains. The chlorophyll and carotenoids contents were increased (6 % to 9 % and 4 % to 5 %) in NA-12 and NA-4 strain ($P \leq 0.05$), respectively than control. Leaf pigments in treatments inoculated with Strain-005 and Strain-006 was recorded to be increased 4 % to 12 % and 4 % to 10 % respectively. Total effect of different treatments was significant for total chlorophyll ($P \leq 0.01$) and chlorophyll *a* ($P \leq 0.05$) but not in case of carotenoids and chlorophyll *b* (Table 8).

Table 6
Effect of Pseudomonas strains on root-gall and egg mass indices and final J2 population in soil of tomato cultivar inoculated with Meloidogyne incognita at initial population (2000 J_2 per kg soil). Each value is the average of 3 replicate values.

Treatments	Galls/ Root	Galling Index	Egg masse/root	Egg mass Index	Soil Population (J_2Kg^{-1})
Pseudomonas spp. NA-12	15b	2.33	30b	3	1445b
Pseudomonas spp. NA-4	2d	1	2c	1	445cd
Pseudomonas spp. 005	8c	1.33	10c	2	985bc
Pseudomonas spp. 006	10c	2	8c	1.66	1003bc
Untreated control	0d	0	0c	0	0d
Positive control	0d	0	0c	0	0d
Negative control	85a	4	95a	4	5814a

Table 7
Effect of Pseudomonas spp. on plant growth parameters of tomato crop.

Pseudomonas spp.		Total Weight (g)	Root Weight (g)	Total Height (cm)	Shoot Height (cm)	No. of Leaves	No. of flowers
NA-12	Untreated Control	8.50ab	1.55c	29a	22a	50ab	12a
	Positive Control	10.12a	1.58bc	30.8a	24.5a	56a	15a
	Negative Control	5.21c	2.50a	20.5b	13.8b	31c	03b
	Treatment Plant	6.56bc	1.87b	24.7ab	17.13ab	40bc	06b
Pseudomonas spp. 006	Untreated Control	8.50b	1.55b	29.0ab	22.0ab	50b	12b
	Positive Control	14.12a	1.62b	33.0a	26.3a	60a	18a
	Negative Control	5.21c	2.50a	20.5c	13.8c	31d	03d
	Treatment Plant	7.00bc	1.78b	26.0b	18.6bc	44c	07c
Pseudomonas spp. 005	Untreated Control	8.50b	1.55a	29.0b	22.0b	50b	12b
	Positive Control	15.0a	1.65a	35.0a	26.8a	63a	20a
	Negative Control	5.21b	2.50a	20.5c	13.8c	31c	03d
	Treatment Plant	7.8b	1.70a	26.8b	19.8b	46b	07c
Pseudomonas spp. NA-4	Untreated Control	8.50b	1.55b	29.0b	22.0b	50b	12b
	Positive Control	17.0a	1.70b	40.0a	30.2a	66a	24a
	Negative Control	5.21c	2.50a	20.5c	13.8c	31c	03c
	Treatment Plant	8.0b	1.62b	27.8b	20.7b	48b	9b

Means in the same column followed by the same letter for each Pseudomonas species are not significantly different ($P > 0.05$).

Table 8
Effects of four Pseudomonas strains on contents of salicylic acid (SA), total phenol (TP), carotenoids and chlorophyll in response to tomato cultivars inoculated with Meloidogyne incognita at initial population density (2000 J2/kg soil).

Treatments	TP (ug catechol g^{-1} FW)	SA (ppm/g FW)	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Pseudomonas spp. NA-12	121.5	15.52	0.978	0.958	1.936	0.131
Pseudomonas spp. NA-4	136.5	15.58	0.998	0.899	1.897	0.132
Pseudomonas spp. 005	131.5	16.24	0.972	0.953	1.925	0.129
Pseudomonas spp. 006	126.5	16.00	0.957	0.941	1.898	0.128
Untreated control	86.5	13.55	1.028	0.999	2.027	0.134
Negative control	101.5	14.86	0.897	0.894	1.791	0.125
$P \leq 0.05$	9.534	1.08	0.078	0.143	0.140	0.0152
$P \leq 0.01$	13.070	1.65	0.108	0.197	0.192	0.021
$P \leq 0.001$	17.790	2.39	0.147	0.271	0.262	0.0285
F-value	39.94^{z}	36.80^{z}	5.81^{x}	–	7.98^{y}	–

* ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) are significantly control difference.
[a] Significantly different from the respective inoculated control ($P \leq 0.05$), [b]($P \leq 0.01$) and [c]($P \leq 0.001$).
[x] F-values followed by × are significant for ($P \leq 0.05$), [y] ($P \leq 0.01$) and [z] ($P \leq 0.001$), otherwise not significant (NS) at $P \leq 0.05$.

4. Discussion

Diseases are among the major limiting factors for better crop and vegetable production (Ramírez and Anderson, 2019; Ashfaq et al. 2021, Asad et al. 2022; Hassan et al. 2022) Current and previous study suggested that RKN are the most destructive parasites of vegetable crops (Sasser, 1989). About 50–60 % loss of crop production was reported by Shepherd and Barker (1990) in tomato plant infected with RKN. Being the sedentary parasite, the nematodes feed on vascular systems of the plants. The plants despite of growing in the normal conditions show suppressive growth due to stress imposed by the nematode and necrotic symptoms appear as obvious in current results (Wallace, 1974). Development of abundant galls on several tomato cultivars were reported by Charles et al (2005) with the fewest galls on cv. Roma. Pseudomonas involve various mechanisms such as competition for food and shelter and production of volatile compounds lethal to nematode like coronatine, phaseolotoxins, tabtoxin, and syringopeptin for nematode suppression (Patil et al., 1964). In *Pseudomonas*, proteases deteriorate the *M. incognita* eggs while chitinases destroy chitin (the main component of nematode cell wall) leading to pathogen suppression. *Pseudomonas* also aided in the plants development and growth showing that the parasitic effect of nematode is over ruled by the activity of the bacterium (Santhi and Sundararabu, 1995). This significant output opens the gateway for many other options in which the bacterium can be used as a potential bio-control agent (Siddiqui & Ehteshamul-haque, 2001).

M. incognita was widely spread over the District Bahawalpur vegetable production regions, according to the findings of current study. Roots infested with nematodes are also more vulnerable to fungal, bacterial, and viral infections (Powell, 1971). There was no nematode-resistant cultivar discovered. Kamran (2008); Anwar and Khan (1992); Abbas et al., (2008); Khan et al., (2000); Zia (2008); Khan et al., (2006); Ubaid (2009) and Sahi (2008) studies were in agreement to our results but contrary with Shahzad et al., (1999) and Darban et al., (2003). All 10 cultivars were found to be sensitive, with gall and egg mass indices greater than three and reproduction rate > 1 (Buenna et al., 2007). Money Maker was the most vulnerable cultivar based on these factors. Because of the disparity in gall and egg mass indices, as well as egg production consistency, researchers concluded that the number of eggs is a better predictor of RKN resistance than gall or egg mass number/indices (Luzzi et al., 1987). Money Maker was chosen as per indices of galls and egg masses. When comparing the four Pi of *M. incognita* with the control, all Pi showed lower plant growth (Table 3). When Pi levels rose, root galls and GI levels rose as well (Ogbuji, 2004; Yadav and Mathur, 1993; El-Sherif et al., 2007; Anwar et al., 1996). The egg masses and index rose considerably when Pi raised from 500 to 2000 J2s of *M. incognita* showing a link between increasing Pi and egg mass index (Table 4).

Pseudomonas reduced gall index and improved the growth of the tomato plant as well. Similar effects of *P. fluorescens* on various crops infected with *Meloidogyne* spp. were observed (Hamid et al., 2003; Khan, 2007; Khan et al., 2005; Khan et al., 2007). In plant

photosynthesis chlorophylls content are basic desires for the absorption of light to fix CO_2 (Wallace, 1987). Our study also found that chlorophyll pigments are extremely susceptible to changes in host plant physiology due to *M. incognita* (Willcox-Lee and Lorea, 1987). The concentration of carotenoid and chlorophyll in leaves is extremely sensitive to water stress and decreases (Khan and Khan, 1987). As a result, the concentration of leaf pigments in infected plants decreases, resulting in a drop in photosynthetic activity in tomato cultivars, resulting in poorer biomass output. Due to nematode inoculation, the total phenol content of leaves rose in the negative control. Total phenol content contributes to the chemical defense mechanism of the host plant against RKN (Nicholson and Hammerschmidt, 1992 and Jones et al., 2013). Salicylic acid has also been reported to be an important component to boost resistance in plants against several pathogens by playing a role in signal transduction pathway (Ryals et al., 1996; Moret and Munoz, 2007; Wobbe and Klessig, 1996). Results of this study regarding salicylic acid and phenolic acid content are in agreement with other researchers revealing that salicylic acid activates the systemic acquired resistance and there is negative association of salicylic acid and root galling produced by *M. incognita* (Nandi et al., 2000; Gheysen and Mitchum, 2009). Though, a little study has been done on the activation of total phenol and salicylic acid levels in nematode-infested plant leaves using a biocontrol agent. Considering all the results the current study reveals that *Pseudomonas* application provides an effective control against *M. incognita* in tomato plant and improves host health as well.

5. Conclusion

Root knot nematodes cause destructive losses of vegetables. *Pseudomonas* species can be an effective alternative to control the RKN disease in an eco-friendly manner. In addition to bio-control agent, *Pseudomonas* also serve as plant growth promoting bacteria by effectively enhancing the plant growth.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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