Research Article

Effect of Certain *Pseudomonas fluorescens* Isolates on Root- Knot Nematode, *Meloidogyne incognita* on Eggplant as Affected by the Time of Addition

Wafaa M.A. El-Nagdi[1], Ahmed E.A. Mahgoob[2], Mahmoud M.A. Youssef[1]*, Entesar H. Taha[2], Mona M.S. Zayed[3] and Nora R.A. Saleh[1]

[1]Plant Pathology Department, Nematology Laboratory, National Research Centre, Dokki, 12622, Cairo, Egypt; [2]Plant Protection Department, Faculty of Agriculture, Ain Shams Univ., P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt; [3]Microbiology Department, Faculty of Agriculture, Ain Shams Univ., P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt.

Abstract | Under screen house conditions, four isolates of bacterium, *Pseudomonas fluorescens* (Pf_1, Pf_2, Pf_9 and Pf_{10}) were used for controlling of root-knot nematode, *Meloidogyne incognita* (Ne) on eggplant as influenced by time of addition. The obtained results proved that, the tested isolates adversely affected reproduction of root-knot nematode, *M. incognita,* either when bacterium was added before, after or at the same time with Ne inoculation. On the basis of the average overall percentages nematode reduction, bacterial isolates added before and after nematode inoculation were equal in decreasing *M. incognita* numbers, as they achieved higher average overall reduction (69.8%), when Pf isolates were added with the same time with Ne. Also, number of galls recorded the highest overall percentage reduction (60.3%), when Pf isolates were added before Ne followed by other groups. Also plant growth criteria improved according to the tested material at the time of addition and the highest percentage plant growth increase (55.5%) was achieved, when Pf isolates were added before Ne. As for enzyme activity, peroxidase and polyphenol oxidase activities increased at the different degrees as influenced by the different treatments. When comparing on the basis of average percentages potential of peroxidase activity, the highest potential (2.446%) was recorded, when *P. fluorescens* was added, 3 days after nematode inoculation than the other treatments. Bacterium and nematode applied at the same time were the first in increasing polyphenol activity (3.907%) than the other groups, but there were no significant differences among them.

Received | October 16, 2022; **Accepted** | December 16, 2022; **Published** | December 26, 2022
***Correspondence** | Mahmoud M.A. Youssef, Plant Pathology Department, Nematology Laboratory, National Research Centre, Dokki, 12622, Cairo, Egypt; **Email:** myoussef_2003@yahoo.com
Citation | El-Nagdi, W.M.A., Mahgoob, A.E.A., Youssef, M.M.A., Taha, E.H., Zayed, M.M.S. and Saleh, N.R.A., 2022. Effect of certain *Pseudomonas fluorescens* isolates on root- knot nematode, *Meloidogyne incognita* on eggplant as affected by the time of addition. *Pakistan Journal of Nematology*, 40(2): 111-119.
DOI | https://dx.doi.org/10.17582/journal.pjn/2022/40.2.111.119
Keywords | Bacterial isolates, *Pseudomonas fluorescens*, *Meloidogyne incognita,* Enzyme activity, Eggplant

Introduction

Root-knot nematodes, *Meloidogyne* spp. can cause root-knot symptoms and cause serious agricultural damage (Trudgill and Blok, 2001). Research trends directed efforts towards biological control and bio-protection for environmentally friendly management of root-knot nematodes (Timper, 2011); however, the

scientific knowledge on the efficacy of biocontrol agents for root- knot nematodes needs more investigation.

Plant growth-promoting rhizobacteria (PGPR) can also protect plants from nematode infection (El-Hadad *et al*., 2010; Oliveira *et al*., 2007). Kavitha *et al*. (2011) reported that, soil application of the native isolates Pft20 @ 2.5 kg/ha of *Pseudomonas fluorescens*, significantly reduced root-knot nematode, *M. incognita* in the treated tomato both in soil and roots. They increased the plant growth, total soluble sugars and fruit lycopene content. Norabadia *et al*. (2014) showed that infection with *M. javanica* was significantly decreased in tomato by *P. fluorescens* (10^9 CFU/ml) and other parameters. Resistance-related enzymes, namely peroxidase and phenylalanine ammonia lyase, increased significantly in plants inoculated with *P. fluorescens*, especially at 5 days after inoculation. Thiyagarajan and Hari (2014) reported that, *P. fluorescens* was used as biological control agent for its antagonistic attributes in controlling root-knot nematode in tomato plants. According to this result, they stated that *P. fluorescens* proved comparatively to be more effective than chemical treatments. Zaghloul *et al*. (2015) evaluated nineteen rhizospheric bacterial isolates and three biocontrol agents (*Serratia marcescens*, *P. fluorescens* and *Bacillus thuringiensis* BT14) against root-knot nematode (*M. incognita*). It was observed that isolates, *P. fluorescens* B103 and *B. subtilis* B38 were the most potent. Youssef *et al*. (2017) conducted an experiment to test *P. fluorescens* as single treatment on *M. incognita* infecting sugar beet. The results revealed that, *P. fluorescens* could reduce the gall numbers by 60%, and caused percentage reduction of egg mass numbers by 61%. Abd-El-Khair *et al*. (2019) showed that, *P. fluorescens* (Pf_1) registered the highest yield increase of cowpea (97%) followed by Pf_2 (63.8%), when cowpea was inoculated with *M. incognita*. The same isolates of *P. fluorescens* Pf_1 and Pf_2 scored intermediate root-knot nematode reductions (69.8 and 62.3%, respectively) compared to untreated check.

The purpose of this research was to elucidate the effect of different isolates of *P. fluorescens* added at different times as biocontrol agents against root-knot nematode, *M. incognita* on eggplant.

Materials and Methods

Preparation of isolates of Pseudomonas fluorescens (Pf_1)
As described and cited by Saleh *et al*. (2020), isolates of *P. fluorescens* were isolated and identified according to Ghini *et al*. (2007); Schaad (1980); Lelliot and Stead (1987) and Goszczynska *et al*. (2000). Justification of bacterial inoculum for each isolate to 10^7-10^9 colony forming unit (CFU)/ml was made by turbidity method (Baid *et al*., 2000). This mixture of bacterial cells and cultural filtrate was used in the present study. Four isolates (Pf_1, Pf_2, Pf_9, and Pf_{10}) were selected for their higher efficiency against root-knot nematode, *M incognita* in previous study (Saleh *et al*., 2020).

Pot experiment
The experiment was carried out in pots at screen house of Plant Pathology Department, National Research Centre. Seeds of eggplant cv. Ice were sown in nursery pots for a month. After that, one eggplant seedling was transplanted in each permanent pot (25-cm diameter) containing 2 kg of solarized sandy loamy soil in October, 2018.

Treatments were carried out as follows: (1) plants were treated with the tested four isolates of *P. fluorescens* (Pf_1, Pf_2, Pf_9 and Pf_{10}). Each replicate contained a mixture of cultural bacterial cells and filtrate at the tested rate of 30ml/pot (10^{-7}-10^{-9} colony forming unit (CFU)/ml) in four holes around the plant. Three days later, the same pots were inoculated with 500 newly emerged second stage juveniles (J_2s) of *M. incognita* (Ne) in four holes made around the plant; (2) Bacterial isolates were added, 3 days after nematode inoculation; (3) Nematode inoculum and bacterial isolates were added at the same time; (4) Nematode only (Control 1) and (5) healthy plants (without nematode) served as control 2. Eight replicates for each treatment were distributed in a completely randomized design.

After 3 months from nematode inoculation (at harvesting stage), plants of eggplant were carefully uprooted and roots were washed thoroughly with running tap water to avoid debris. The number of J_2s in the soil was extracted using a sieving and decanting technique (Barker, 1985) and counted. Numbers of J_2s in soil, galls and egg-masses in one half of eggplant root system were recorded. Then, another half of root system was incubated in tap water by incubation method (Young, 1954) to help emerging J_2s from egg masses. Nematode J_2 numbers were counted under a light microscope Also, at the same time; plant growth criteria of eggplant including shoot length (cm), fresh and dry shoot weights (g) and fresh root weight (g) were recorded.

Measurements were compared to those of untreated check (control 1) and those plants without nematode inoculation were compared to healthy plants (control 2). Average total percentages of nematode reductions was calculated to compare among treatments within all groups. Also, an average overall percentages of reductions of nematode and increases in plant growth criteria in each group was calculated to compare among different groups.

Biochemical analysis

Extraction of enzymes: Determination of enzymes was carried out at one month after nematode inoculation.

Root tissues in gram were homogenized with 0.2 M tris HCL buffer at pH 7.8 containing 14 mM B-mercaptoethanol at the rate of 1/3 w/v. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was used to determine enzyme activities (Tuzun *et al*., 1989) by using spectrophotometer (Shimadzu UV-visible Recording Spectrophotometer UV-240). This analysis included:

Peroxidase activity assay: Peroxidase (POX) was assayed according to the method described by Lee (1973). The reaction mixture contained 0.5 ml of 0.05 M potassium phosphate buffer at pH 5.7 and 0.75 ml of 0.04 M Guaiacol solution; 0.2 ml of 0.02 M H_2O_2 and 0.05 ml of enzyme extract. The increasing in absorbance at 470 nm was recorded against blank with phosphate buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of enzyme which changes the amount of optical density at 470 nm per min. at 25°C under standard assay conditions. Peroxidase activity was expressed as the increase in absorbance at 470nm/g root fresh weight.

Polyphenol oxidase assay: Polyphenol oxidase (PPO) was assayed according to the method of Bashan *et al*. (1985). The reaction mixture contained 0.5ml of 0,03M potassium phosphate buffer at pH 6.5 and 0.4ml of 5 mM 3, 4 DOPA and 0.1 ml of enzyme extract. The increasing in absorbance at 470 nm was recorded against blank with phosphate buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of enzyme which changes the amount of optical density at 470 nm per min. at 25°C under standard assay conditions. Peroxidase activity was expressed as the increase in absorbance at 470nm/g root fresh weight.

Statistical procedures

The current experiment was analyzed separately. Duncan's Multiple Range Test (DMRT) was used to separate the treatment means (Duncan, 1955). ANOVA test was used for the analysis, with the crucial difference set at P≤0.05 (Gomez and Gomez, 1984). This was done by Computer Statistical (COSTAT) software.

Results and Discussion

Effect of Pf isolates on root knot-nematode M. incognita parameters

Table 1 illustrated the potential of tested bacterial isolates of *P. fluorescens* on eggplant, when they were added before, after and at the same time of nematode inoculation to eggplant. It was clearly noticed that, the tested isolates adversely affected reproduction of root-knot nematode, *M. incognita* (Ne) as indicated by reproductive characteristics (number of the second stage juveniles (J_2s) in soil per pot and roots, egg masses as well as number of galls per root system compared to untreated check). In Table 2, on the basis of average total percentages of nematode reduction, treatment of Pf_9 added before Ne, recorded the highest percentage nematode reduction (85.4%) followed by treatments of Pf_{10} and Pf_1, when added after Ne which recorded 71.8 and 70.6% nematode reductions, respectively. The least percentages of reduction (75.3 and 49.9%) were recorded by treatments of Pf_9 and Pf_2, added together with Ne, respectively.

On the basis of the average overall percentages of nematode reductions, bacterial isolates added before and after nematode inoculation were equal in decreasing *M. incognita* numbers and galls, as they achieved higher average overall reductions (69.8%) than the treatments, when bacterial isolates and Ne were added at the same time (54.4%) compared to untreated check. Number of galls recorded the highest average overall percentages of reductions (60.3%), when Pf isolates were added before Ne followed by 48.5 and 13.2%, when bacterial isolates were added after and at the same time, respectively.

Effect of Pf isolates and nematode on eggplant growth

Concerning eggplant growth as influenced by the tested four bacterial isolates (mean numbers of shoot length, fresh and dry weights and root fresh weight) was illustrated in Table 3. The results indicated that, the four bacterial treatments (Pf_1, Pf_2, Pf_9 and Pf_{10})

Table 1: *Effect of four isolates (Pf_1, Pf_2, Pf_9, Pf_{10}) of Pseudomonas fluorescens on reproduction potential and galls of Meloidogyne incognita infecting eggplant as affected by the time of inoculation.*

Treatments	Nematode parameters			No. of galls/root system
	No. of J_2s in soil/pot	No. of J_2s /root system	No. of egg masses/root system	
Group 1: Bacterium at the same time with Ne				
Pf_1 + Ne	53.0e	99.0b	3.0bc	13.0b
Pf_2+ Ne	128.0cde	71.0bc	6.0a	18.0a
Pf_9+ Ne	104.0de	29.0cd	5.0ab	12.0bc
Pf_{10}+ Ne	72.0e	106.0b	5.0ab	17.0a
Average	89A	76.3A	4.8A	15.0A
Group 2: Bacterium at 3 days before Ne				
Pf_1 +Ne	290.0b	49.0bcd	2.0cd	7.0de
Pf_2 +Ne	230.0bcd	51.0bcd	3.0bc	6.0de
Pf_9 +Ne	75.0e	29.0cd	1.0d	6.0de
Pf_{10} + Ne	162.0cde	32.0cd	3.0bc	8.0de
Average	189.3A	40.3AB	2.3B	6.8B
Group 3: Bacterium at 3 days after Ne				
Pf_1 + Ne	53.0e	46.0bcd	3.0bc	9.0d
Pf_2, +Ne	243.0bc	0.0d	3.0bc	5.0e
Pf_9 +Ne Ne	88.0e	48.0bcd	3.0bc	9.0cd
Pf_{10} +Ne	81.0e	29.0cd	3.0bc	12.0bc
Average	116A	30.8CDB	3.0B	8.8B
Nematode only (Control 1)	684.0a	225.0a	5.0a	17.0a

Values are means of 8 replicates. Means followed by same letter(s) are no significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test. Small letters to indicate the differences among treatments. Capital letters to compare among groups.

Table 2: *% Reduction of parameters of root-knot nematode, Meloidogyne incognita infecting eggplant as affected by the time of inoculation of four isolates (Pf_1, Pf_2, Pf_9, Pf_{10}) of Pseudomonas fluorescens.*

Treatment	% reduction of number of reproductive criteria of nematodes and galls				
	J_2s in soil/pot	J_2s in roots	Egg masses in roots	% Average total percentages reduction	Galls in roots
Group 1: Bacterium at the same time with Ne					
Pf_1+ Ne	92.3	56.0	40.0	62.8	23.5
Pf_2+Ne	81.3	68.4	-	49.9	-
Pf_9+Ne	84.8	87.1	0.0	57.3	29.4
Pf_{10}+Ne	89.5	52.9	0.0	47.5	0.0
Average overall percentages reduction	-	-	-	54.4	13.2
Group 2: Bacterium at 3 days before Ne					
Pf1	57.6	78.2	60.0	65.3	58.8
Pf2	66.4	77.3	40.0	61.2	64.7
Pf9	89.0	87.1	80.0	85.4	64.7
Pf_{10}	76.3	85.8	40.0	67.4	52.9
Average overall percentages reduction	-	-	-	69.8	60.3
Group 3: Bacterium at 3 days after Ne					
Pf_1 +Ne	92.3	79.6	40.0	70.6	47.1
Pf_2 +Ne	64.5	100.0	40.0	68.2	70.6
Pf_9 +Ne	87.1	78.7	40.0	68.6	47.1
Pf_{10} +Ne	88.2	87.1	40.0	71.8	29.4
Average overall percentages reduction	-	-	-	69.8	48.5
Nematode only (Control 1)	0.0	0.0	0.0	0.0	0.0

Table 3: *Effect of four isolates (Pf_1, Pf_2, Pf_9, Pf_{10}) of Pseudomonas fluorescens on growth of eggplant infected by root-knot nematode, Meloidogyne incognita as influenced by the time of inoculation.*

Treatments	Shoot parameters/plant			Root parameters/plant
	Length (cm)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)
Group 1: Bacterium at the same time with Ne				
Pf_1+ Ne	37.0ab	7.2ab	1.4c	2.2ab
Pf_2+ Ne	35.0ab	8.5ab	1.4c	1.8ab
Pf_9+ Ne	33.0b	6.7ab	1.7abc	1.3b
Pf_{10}+ Ne	35.3ab	8.5ab	2.0abc	2.0ab
Average	35.1A	7.7A	1.6B	1.8a
Group 2: Bacterium at 3 days before Ne				
Pf_1 +Ne	34.0ab	5.5b	1.8abc	2.1ab
Pf_2 +Ne	37.2ab	8.4ab	1.9abc	2.3ab
Pf_9 +Ne	35.7ab	8.4ab	2.4abc	3.0a
Pf_{10} +Ne	39.3a	10.4a	2.6ab	3.1a
Average	36.6A	8.2A	2.2A	2.6a
Group 3: Bacterium at 3 days after Ne				
Pf_1 +Ne	38.3ab	7.4ab	1.9abc	2.8ab
Pf_2 +Ne	36.3ab	5.9ab	1.3c	2.8ab
Pf_9 +Ne	33.3b	5.2b	1.4c	2.3ab
Pf_{10} +Ne	33.5b	7.0ab	1.8abc	1.6ab
Average	35.4A	6.4A	1.6A	2.4a
Nematode only (Control 1)	33.8ab	5.2b	1.2c	1.5ab

Values are means of 5 replicates. -Values are means of 5 replicates. Values followed by the same letters are not significantly different according to Duncan's Multiple Range Test (P≤0.05). Small letter (s) to indicate the differences among treatments. Capital letter to compare among groups.

promoted plant growth criteria significantly (P≤0.05) than inoculated untreated and untreated uninoculated plants (Healthy). On the basis of average total percentages of plant growth increases, Pf_{10} added before Ne was superior in increasing plant growth parameters (84.9%) followed by treatment of Pf_9 before Ne (66.9%) and 50.2% was recorded by Pf_1 added after Ne (Table 4). The highest average overall percentages of plant growth increases (55.5%) was achieved, when bacterial isolates preceded inoculation by root-knot nematode. This was followed by 29.9 and 28.4%, when nematode was inoculated after and at the same time with bacterial isolates, respectively.

Effect of Pf isolates and nematode on enzyme activity in eggplant roots

Table 5 clarified peroxidase and polyphenol oxidase activities as influenced by the different treatments of *P. fluorescens* before, after and at the same time of nematode inoculation. It was noticed that, the highest percentage increase potential of peroxidase (4.344%) over control occurred by treatment of Pf_2 after Ne followed by (3.662%) occurred by Pf_1+Ne together. The least increase 0.656% occurred by Pf_{10}+Ne together. When comparing on the basis of average percentages potential of peroxidase activity, the highest potential (2.446%) was recorded in the group 3, when *P. fluorescens* was added, 3 days after nematode inoculation followed by 1.490 and 1.062%, when *P. fluorescens* was added at the same time (group1), and at 3 days before nematode inoculation (group 2), respectively. As for polyphenol oxidase activity, the highest percentage of increase (6.135%) was recorded by treatment of Pf_2 + Ne together (group 1) followed by Pf_1 before Ne (5.321%) (group 2). The least increase (1.242%) was occurred by Pf_1+Ne together (group 1). Based on the average percentages potential, group 1 (bacterium and Ne at the same time) was the first in increasing polyphenol activity (3.907%) followed by 2.787% and 2.479%, when bacterium and Ne were added either before (group 2) or after (group 3) nematode inoculation, but there were no significant differences among groups.

The findings in the present study regarding efficacy of certain *P. fluorescens* isolates for controlling *M. incognita* on eggplant conformed to the results by Wescott and Kluepfel (1993) who reported that,

Table 4: *% increase in the growth of eggplant infected by root knot nematode, Meloidogyne incognita as affected by the time of inoculation of four isolates (Pf_1, Pf_2, Pf_9, Pf_{10}) of Pseudomonas fluorescens.*

Treatment	% Increase in shoot parameters			% Increase in root parameter	% Average total percentages plant growth increases
	Length (cm)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	
Group 1: Bacterium at the same time with Ne					
Pf_1+ Ne	9.0	38.5	16.7	46.7	27.7
Pf_2+ Ne	4.0	63.5	16.7	20.0	26.1
Pf_9+ Ne	-	28.8	41.7	-	17.6
Pf_{10}+ Ne	4.4	63.5	66.7	33.0	41.9
Average overall percentages increase	-	-	-	-	**28.4**
Group 2: Bacteriun at 3 days before Ne					
Pf_1 +Ne	0.1	5.8	50.0	40.0	24.2
Pf_2 +Ne	10.1	61.5	58.3	53.3	45.8
Pf_9 +Ne	6.0	61.5	100.0	100.0	66.9
Pf_{10}+Ne	16.3	100	116.7	106.7	84.9
Average overall percentages increase	-	-	-	-	55.5
Group 3: Bacterium at 3 days after Ne					
Pf_1 +Ne	13.3	42.3	58.3	86.7	50.2
Pf_2 +Ne	7.4	13.5	8.3	86.7	29.0
Pf_9 +Ne	-	0.0	16.7	53.3	17.5
Pf_{10} +Ne	-	34.6	50.0	6.7	22.8
Average overall percentages increase	-	-	-	-	29.9
Nematode only (Control 1)	0	0	0	0	0

Table 5: *Estimation of peroxidase and polyphenol oxidase in the eggplant roots treated with Pseudomonas fluorescens (Pf) isolates (Pf_1, Pf_2, Pf_9, Pf_{10}) and root-knot nematode, Meloidogyne incognita at different times.*

Treatment	Peroxidase (mg/g)	% Increase potential over control	Polyphenol oxidase (mg/g)	% Increase potential over control
Group 1: Bacterium at the same time with Ne				
Pf_1+ Ne	0.714b	3.662	0.267f	1.242
Pf_2+ Ne	0.172ghi	0.882*	1.319a	6.135
Pf_9+ Ne	0.148hi	0.759*	0.840c	3.907
Pf_{10}+ Ne	0.128i	0.656*	0.934c	4.344
Average	0.291A	1.490	0.840A	3.907
Group 2: Bacterium at 3 days before Ne				
Pf_1 +Ne	0.160ghi	0.821*	1.144b	5.321
Pf_2 +Ne	0.168ghi	0.862*	0.277d	1.288
Pf_9 + Ne	0.286d	1.467	0.696d	3.238
Pf_{10}+ Ne	0.214efg	1.097	0.279f	1.298
Average	0.207A	1.062	0.599A	2.787
Group 3: Bacterium at 3 days after Ne				
Pf_1 + Ne	0.254def	1.303	0.493e	2.293
Pf_2+ Ne	0.847a	4.344	0.500e	2.326
Pf_9+ Ne	0.274de	1.405	0.510c	2.372
Pf_{10}+ Ne	0.533c	2.733	0.629d	2.926
Average	0.477A	2.446	0.533A	2.479
Nematode only (Control 1)	0.195fgh	100.0	0.215f	100.0
Healthy plant (Control 2)	0.270de	100.0	0.470e	100.0

*Values are means of 5 replicates. -Values are means of 5 replicates. Values followed by the same letters are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05). *= Less than control. Small letters to indicate the differences among treatments. Capital letter to compare among groups.*

fluorescent pseudomonads isolates can produce exoteric compounds as a result of cellular metabolism, and also can affect nematode juveniles. Plant growth promoting pseudomonads can antagonize pathogen through antibiotic production, and competition for essential nutrients such as iron and, through plant growth promotion (Gamliel and Katan, 1993). In this study, some bacterial isolates inoculated before and after nematode inoculation were equal in inducing higher average overall percentages nematode reductions than added at the same time of nematode inoculation. This may be due to a systemic resistance by certain enzymes that can be induced by fluorescent pseudomonads, which was also considered to be a mechanism for the biocontrol of pathogens (Wei *et al*., 1996). This assumption was confirmed; when peroxidase and polyphenol oxidase activities in this study increased in plants inoculated by bacterial isolate suspensions. The differences among the tested isolates in producing resistance enzymes ascertained their different abilities in inducing systemic resistance at different times and consequently reducing nematode parameters. Khan and Haque (2011) reported that, *P. fluorescens* recorded the greatest reduction in the studied nematode parameters and increased plant growth in tobacco compared with controls. They added that increases in total phenol and salicylic acid recorded by *P. fluorescens* were negatively correlated with numbers of galls and egg masses/root system. Coinciding with this conclusion, Norabadia *et al.* (2014) stated that, *P. fluorescens* caused destruction in nematode egg mass matrices and significantly decreased nematode egg hatching level. They added that, specific activities of resistance-related enzymes, namely peroxidase and phenylalanine ammonia lyase, increased significantly in *P. fluorescens* inoculated plants, especially at 5 days after inoculation, which indicated to that systemic resistance may be induced better by *P. fluorescens* against nematode at early stage of plant growth.

Conclusions and Recommendations

It could be concluded that the tested *P. fluorescens* isolates adversely affected reproduction of root-knot nematode, *M. incognita,* either when bacterium was added before, after or at the same time with Ne inoculation and increased eggplant growth criteria. The differences among the tested isolates in producing resistance enzymes ascertained their different abilities in inducing systemic resistance at different times and consequently in reducing nematode parameters.

Novelty Statement

The current study indicated the nematicidal properties of the combined use of the antagonistic bacterium, *P. fluorescens* and root-knot nematode, *M. incognita* at different times on eggplant. This combination proved to be acted as a resistance inducer, plant growth promoter and biocontrol agent within sustainable pest management.

Author's Contribution

WMAE-N, AEAM, and MMAY were equal in the supervision of this work, design, writing and execution of this manuscript. EHT and MMSZ supervised the work and reviewed the manuscript. NRAS carried out the experiment in screen house and examined it in the laboratory. All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

References

Abd-El-Khair, H., El-Nagdi, Wafaa, M.A., Youssef, M.M.A., Abd-Elgawad, M.M.M. and Dawood, M.G., 2019. Protective effect of *Bacillus subtilis, B. pumilus, and Pseudomonas fluorescens* isolates against root knot nematode, *Meloidogyne incognita* on cowpea. Bull. Nat. Res. Centre, 43: 1-7. https://doi.org/10.1186/s42269-019-0108-8

Baid, R.M., Hodges, N.A. and Denyer, S.P., 2000. Handbook of microbiology quality control: Pharmaceuticals and medical devices. London; New York, NY; Taylor and Francis, pp. 280. https://doi.org/10.4324/9780203305195

Barker, K.R., 1985. Nematode extraction and bioassays. pp. 19-35. In: An advanced treatise on *Meloidogyne*, Vol. II: Methodology, Ed. by K. R. Barker, J. N. Sasser and C.C. Carter. North Carolina State University Graphics: Raleigh NC, pp. 19-35.

Bashan, Y., Okon Y. and Henis, Y., 1985. Peroxidase, polyphenol oxidase and phenols in relation to resistance against *Pseudomonas syringae* pv. Tomato in tomato plants. Can. J. Bot., 65: 366-372. https://doi.org/10.1139/b87-047

Duncan, D.B., 1955. Multiple range and multiple F-test. Biometrics, 11: 1-41. https://doi.org/10.2307/3001478

El-Hadad, M.E., Mustafa, M.I., Selim, S.M., Mahgoob, A.E.A., El-Tayeb, T.S. and Abdel-Aziz, N.H., 2010. *In vitro* evaluation of some bacterial isolates as biofertilizers and biocontrol agents against the second stage juveniles of *Meloidogyne incognita*. World J. Microbiol. Biotechnol., 26: 2249–2256. https://doi.org/10.1007/s11274-010-0413-8

Gamliel, A. and Katan, J., 1993. Suppression of major and minor pathogens by Fluorescent pseudomonads in solarized and non-solarized soil. Phytopathology, 83: 68–75. https://doi.org/10.1094/Phyto-83-68

Ghini, R.F., Patrico, R.A., Bettiol, W., de Almeida, M.G. and Maia, N.H.A., 2007. Effect of sewage sludge on suppressiveness to soil-borne plant pathogens. Soil Biol. Biochem., 39: 2797–2805. https://doi.org/10.1016/j.soilbio.2007.06.002

Gomez, K.A. and Gomez, A.A., 1984. Statistical procedures for agricultural research. John Wiley and Sons.

Goszczynska, T., Serfontein, J.J. and Serfontein, S., 2000. Introduction to practical phyto-bacteriology. Sponsored by the Swiss Agency for Development and Cooperation (SDC), Switzerland, pp. 83.

Kavitha, P.G., Jonathan, E.I. and Sankari-Meena, K., 2011. *Pseudomonas fluorescens* for the management of root-knot nematode *Meloidogyne incognita* in tomato. Madras Agric. J., 98: 176-177.

Khan, M.R. and Haque, Z., 2011. Soil application of *Pseudomonas fluorescens* and *Trichoderma harzianum* reduces root-knot nematode, *Meloidogyne incognita*, on tobacco. Phytopathol. Medit., 50: 257–266.

Lee, N.T., 1973. On extraction and quantitation of plant peroxidase enzymes. Physiol. Planta., 29: 198-203. https://doi.org/10.1111/j.1399-3054.1973.tb03092.x

Lelliott, R.A. and Stead, D.E., 1987. Methods on plant pathology. Volume 2, Methods for the diagnosis of bacterial diseases of plants. Br. Soc.Plant Pathol. Blackwell Sci. Publ., Oxford London Edinburgh, Boston, Palo, Alto, Melbourne, pp. 216.

Norabadia, M.T., Sahebania, N. and Etebarianb, H.R., 2014. Biological control of root-knot nematode (*Meloidogyne javanica*) disease by *Pseudomonas fluorescens* (Chao). Arch. Phytopathol. Prot., 47: 615-621. https://doi.org/10.1080/03235408.2013.816102

Oliveira, D.F., Campos, V.P., Amaral, D.R., Nunes, A.S., Pantaleão, J.A., and Costa, D.A., 2007. Selection of rhizobacteria able to produce metabolites active against *Meloidogyne exigua.* Eur. J. Plant Pathol., 119: 477–479. https://doi.org/10.1007/s10658-007-9176-y

Saleh, N.R.A., Mahgoob, A.E.A., Taha, E.H., El-Nagdi, W.M.A., Youssef, M.M.A. and Zayed, M.M.S., 2020. Effect of certain *Pseudomonas fluorescens* isolates on the infection of root-knot nematode, *Meloidogyne incognita* in tomato and eggplant and the plant growth. Arab Univ. J. Agric. Sci., 28(1): 315-327.

Schaad, N.W., 1980. Laboratory guide for identification of plant pathogenic bacteria. Bacteriol. Comm. Amer. Phytopathol. Soc., Saint Paul, Minnesota, pp. 72.

Thiyagarajan, S.S. and Hari, K., 2014. Tomato root knot nematode control through biocontrol agent *Pseudomonas fluorescens*. Int. J. Res. Agric. Sci., 1: 2348–3997.

Timper, P., 2011. Utilization of biological control for managing plant-parasitic nematodes. In: Biological control of plant-parasitic nematodes: Building coherence between microbial ecology and molecular mechanisms (eds. Davies, K. and Spiegel, Y.). Dordrecht: Springer. pp. 259–287. https://doi.org/10.1007/978-1-4020-9648-8_11

Trudgill, D.L. and Blok, V.C., 2001. Apomictic, polyphagous root-knot nematodes: Exceptionally successful and damaging biotrophic root pathogens. Ann. Rev. Phytopathol., 39: 53–77. https://doi.org/10.1146/annurev.phyto.39.1.53

Tuzun, S., Rao, M.N., Vogeli, U., Shardi C.L. and Kuć, J., 1989. Induced systemic resistance to blue mold: Early induction and accumulation of β-1,3 glucanase, chitinase and other pathogenesis protein (b-proteins) in immunized tobacco. Phytopathology, 35: 979-983. https://doi.org/10.1094/Phyto-79-979

Wei, G., Kolepper, J.W. and Tuzun, S., 1996. Induced systemic resistance to encounter diseases and increased plant growth by plant growth promoting bacteria under field conditions. Phytopathology, 86: 221–224. https://doi.org/10.1094/Phyto-86-221

Wescott, S.W. and Kluepfel, D.A., 1993. Inhibition of *Criconemella xenoplax* egg hatch by *Pseudomonas aureofaciens*. Phytopathology, 83: 1245-1249. https://doi.org/10.1094/Phyto-83-1245

Young, T.W., 1954. An incubation method for collecting migratory- endoparasitic nematodes. Plant Dis. Reptr., 38: 794-795.

Youssef, M.M.A., Abd-El-Khair, H. and El-Nagdi, W.M.A., 2017. Management of root knot nematode, *Meloidogyne incognita* infecting sugar beet as affected by certain bacterial and fungal suspensions Agric. Eng. Int.: CIGR J., Special issue: pp. 293–301.

Zaghloul, R.A., Neweigy, N.A., Abou-Aly, H.E., El-Sayed, S.A. and Bahloul, A.M., 2015. Nematicidal activity of some biocontrol agents against root-knot nematodes *in-vitro*. Res. J. Pharm. Biol. Chem. Sci., 6: 429-438.