Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt
28(1), 315-327, 2020
Website: http://ajs.journals.ekb.eg

EFFECT OF CERTAIN *PSEUDOMONAS FLUORESCENS* ISOLATES ON THE INFECTION OF ROOT-KNOT NEMATODE*, MELOIDOGYNE INCOGNITA* IN TOMATO AND EGGPLANT AND THE PLANT GROWTH

[23]

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

1- Plant Pathology Dept., Nematology Lab., National Research Center, Dokki, Giza, Egypt
2- Plant Protection Dept., Fac. of Agric. Ain Shams Univ., P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt
3- Microbiology Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

*Corresponding author: norasaleh7841@yahoo.com

Received 30 January, 2020 Accepted 19 March, 2020

ABSTRACT

Under screen house conditions, two experiments were carried out to evaluate certain bacterium, *Pseudomonas fluorescens* isolates regarding reproductive potential of root-knot nematode, *Meloidogyne incognita*, infecting tomato or eggplant. Results on tomato revealed that, on the basis of average total percentages nematode reduction, the over topped results were gained with *P. fluorescens* (Pf_2) which recorded the highest significant ($P \leq 0.05$) average nematode reduction (61.3%) and higher percentage reduction of females (77%) per plant. The second rank was obtained by Pf_3 which reduced all nematode numbers as an average of 56.9%.On the basis of average total percentages plant growth and weight of fruit increases, four bacterial treatments can be ranked in a descending order as follows: Pf9 > Pf_4>$Pf_1$and Pf_7, as they achieved the highest average total percentages increases of 96.0, 47.3, 38.2and 29.8%, respectively compared to other treatments and untreated check. Regarding to eggplant, the over topped results observed was achieved by *P. fluorescens* (Pf_{10}) which recorded the highest average total nematode reduction (66.2%) with higher reduction of (J_2s) in roots (89.9%) per plant and in soil (78.8%) per pot. The second rank was obtained by Pf_9 and Pf_2 where they reduced all nematode numbers as averages of 55.9% and 54.9%, respectively. Also *Pseudomonas* isolates enhanced the plant growth of eggplant, averages were found in a descending order as follows: Pf_1 (20.0%), Pf_9 (18.7%) and Pf_{10} (18.3%). It is worthy to note that the most distinct growth criteria was fresh weight of roots as it achieved higher percentage increase (58.1%) by using Pf_9 followed by Pf_1 as it caused 40.6% increase compared to untreated check. The highest average percentage of fresh weight of shoot were recorded for Pf_{10} (26.4%) and Pf8 (22.1%). Whereas for dry weight Pf_3 (29.8%) and Pf_2 (19.1%). In conclusion, the tested biocontrol agent was efficient in controlling the root-knot nematode on tested plants.

Keywords: *Pseudomonas fluorescens* isolates, Tomato, Eggplant, *Meloidogyne incognita.*

INTRODUCTION

Root-knot nematodes belonging to the genus of *Meloidogyne* known as an endoparasite cause root-knot symptoms and serious plant damage **(Trudgill and Blok 2001)**. Plant parasitic nematodes reducing production by 12–20% worldwide were studied widely **(Oka et al 2000)**, moreover losses can reach 30–60% **(Talavera et al 2012)** in protected cultivation. Efforts were directed to use the biological control as environmental friendly management of root - knot nematodes **(Timper 2011)**. Plant growth-promoting rhizobacteria (PGPR) can protect plants against nematodes (**El-Hadad et al 2010 and**

Oliveira et al 2007). Approximately, 7–10% of all rhizobacteria act as antagonistic agents against nematodes by more than mode of action, including competition, antibiosis and induced resistance **(Burkett-Cadena et al 2008).** Under pot experiments, several *Pseudomonas* species were effective in managing root- knot nematode **(Siddiqui et al 2009, Singh and Siddiqui 2010). Kavitha et al (2011)** mentioned that soil application by the native isolates Pft 20 (*P. fluorescens*) at 2.5 kg/ha significantly reduced the infestation of root-knot nematode, *M. incognita* in the treated tomato both in soil and roots as indicated by the reduction in number of the studied nematode criteria and increasing in the plant growth. **Mokbel and Alharbi (2014)** evaluated the efficacy of certain bacterial genera against *M. javanica* on eggplant as egg-hatch inhibition and reduction in 2nd stage juveniles percentages ranged from 50.5–90.3% were obtained by using *Bacillus subtilis*, *B. thuringiensis*, *P. fluorescens* and *Serratia marcescens*. Also, they observed that 56.5-86.8% reductions in the number of galls, egg-masses/root system, and number of J_2s. Subsequently, 50.9-73.7% increases in the root and shoot dry weights of eggplant occurred. The information about the efficacy of biocontrol agents in controlling root-knot nematodes needs more expanded researches. Therefore, this research was designed to evaluate certain *Pseudomonas fluorescens* isolates for their nematicidal efficacy on root-knot nematode, *M. incognita* infecting tomato and eggplant and subsequently on their plant growth.

MATERIALS AND METHODS

1. Preparation of root-knot nematode pure culture and inoculum

The tested species of root-knot nematode was *Meloidogyne incognita,* identified from nematode adult female according to the morphological characteristics of the female perineal pattern **(Taylor and Sasser 1978).** Pure culture of *M. incognita* was reared on eggplant cv. Ice under screen house conditions at 30 ± 5ºC by using a single egg-mass of this nematode. Newly hatched second stage juveniles (J_2s) were used as inoculum.

2. Bacterial isolates

2.1. Source of *Pseudomonas fluorescens* isolates

Twelve soil samples (200g soil) were taken from the rhizospheres of eggplant and tomato plants cultivated in El-Beheira and Monoufyia Governorates, Egypt.In addition, 3 soil samples of pepper plant only were collected from El-Beheira Governorate. All samples were free from root-knot nematode infestation; the samples were transferred to the laboratory of PPD-NRC for isolation and identification of *Pseudomonas fluorescens*.

2.2. Isolation and identification of *P. fluorescens*

For isolation of *P. fluorescens*, the total plate counts technique and dilution method were carried out according to **(Ghini et al 2007)**. Each collective sample (Ten gram) was transferred into 250 ml conical flask containing 90-ml of sterile distilled water to prepare serial dilutions ranging from 10^{-1} to 10^{-7}. One ml of each sample dilution was pipetted onto the surface of sterile Petri-dish (9 cm diam.) containing KB (King's medium B)[Peptone 20.0g; Glycrol 15.0 ml; K_2HPO_4 (anhydrous) 1.5g; $MgSO_4$ x7H_20; Agar 15.0g in 1.0 liter of distilled water at pH 7.2 ± 0.2]. The soil suspension was spread on the surface of medium using glass rod (L-shape).The inoculated plates was incubated at 28°C for 48h. The growing bacterial colonies were examined with UV light for detection fluorescent pigment production. Subsequently, the bacterial colonies, which showed fluorescent pigment, were picked soon on slant of nutrient glucose 2% agar medium (NGA) [Beef extract 3.0g ; Peptone 5.0g; Glucose 20.0g; Agar 15.0g in 1.0 liter of distilled water at pH 7.2 ± 0.2].

Nine *P. fluorescens* isolates were isolated from the collected rhizosphere samples **(Table 1 and 2)**. The isolates were identified according to cultural characters on NGA medium and LOPAT test (Levan production, Oxidase test, pectolytic enzymes, arginine dihydrolase and hyper sensitivity reaction) using standard bacteriological methods **(Schaad 1980, Lelliot and Stead 1987 and Goszczynska et al 2000)**.

Justification of bacterial inoculums for each isolate reached 10^7-10^9 colony forming unit (CFU)/ml by turbidity method **(Baid et al 2000)** and was applied as mixture of bacterial cells and cultural filtrate.

Table 1. Location, source and name of the tested bacterial isolates

Governorate	Source plant	No. of samples	Bacterial isolate
El-Beheira	Egg plant	3	Pf_1
			Pf_2
	Pepper	3	Pf_3
			Pf_4
			Pf_5
	Tomato	3	Pf_6
			Pf_7
El-Monoufyia	Egg plant	3	Pf_8
	Tomato	3	Pf_9

Table 2. Identification of some green fluorescent *Pseudomonas* by the LOPAT scheme

P. flourescens Isolates (lvb)	LOPAT Test				
	Levan Production	Oxidase Test	Potato Soft Rot	Arginin Dihydrolase	Tobacco Hyper Sensitive Reaction
Pf1	-	+	+	+	-
Pf2	-	+	+	+	-
Pf3	-	+	+	+	-
Pf4	-	+	+	+	-
Pf5	-	+	+	+	-
Pf6	-	+	+	+	-
Pf7	-	+	+	+	-
Pf8	-	+	+	+	-
Pf9	-	+	+	+	-

3. Source of standard *P. fluorescens* isolate

A standard *P. fluorescens* Pf_{10} (NRC isolate) was obtained from (PPD-NRC). The inoculum of this isolate was prepared as mentioned previously.

4. Source of Micronema®

The commercial bio-nematicide**,** Micronema® (mixture of certain beneficial bacteria) was obtained from the Agricultural Research Center (Giza, Egypt) and used with the recommended dose.

The standard *P. fluorescens* Pf_{10} (NRC isolate) and Micronema® were used for comparison.

5. Tested plants

Seeds of tomato (*Lycopersicum esculentum*) cv. Castle Rock and eggplant (*Solanum melongena*) cv. Ice. were obtained from Agricultural Research Center, Giza, Egypt. Seeds were sown in a nursery for germination and maintained till the seedling reached one-month- old.

6. *In vivo* evaluation of the *P. fluorescens* isolates against *M. incognita*

Under screen house conditions, two experiments were conducted in PPD-NRC, in order to differentiate the potential of *P. fluorescens* isolates on *M. incognita* infecting tomato and eggplant.

6.1. Tomato experiment

One-month-old of tomato seedlings were transplanted into 20-cm diameter plastic pots (one seedling/pot) containing 2 kg of solarized sandy loamy soil (1:1) in August 2-2015. A week later, second stage juveniles of *M. incognita* at the rate of 1000 individuals per pot were inoculated around the vicinity of each seedling. At the same time, the inoculation of each tested *P. fluorescens* isolates, inoculated to each seedling with a rate of 30ml/pot (10^{-7}-10^{-9} colony forming unit (CFU)/ml). The commercial product, Micronema® was used at the recommended rate (0.5 ml/pot) as comparison. Nematodes only in 30-ml distilled water were used as untreated check.

6.2. Eggplant experiment

The same procedures in tomato were carried out except that; eggplant seedlings were transplanted in May 15, 2016. A week later, each pot was inoculated with 500 newly hatched juveniles (J_2s) of *M. incognita*. Simultaneously, seedlings of eggplant were treated with 9 isolates of *P. fluorescens*. Also the standard *P. fluorescens* Pf_{10} (NRC isolate) isolate was used as comparison. In both experiments, pots were arranged in a completely randomized design with 6 replicates for each treatment on a bench and maintained, plants were irrigated as needed.

7. Determination of *M. incognita* and plant growth parameters

7.1. *M. incognita* parameters

Plants of tomato and eggplant were harvested after three months from inoculation with nematodes and carefully uprooted. Roots were washed thoroughly with running tap water to get rid of soil aggregation and debris. Then, roots were cut into two halves. In one half, acid fuchsin in cold lacto-phenol was used to stain and store roots for not less than 24 hr. After that, stained roots were put in water and cut into pieces to enable counting of galls, females, and egg masses. Incubation method described by **(Young 1954)** was used for the remaining half of roots by incubating in tap water for obtaining J_2s from egg masses. The number of (J_2s) in the soil was extracted using a sieving and decanting technique **(Barker 1985)** and counted. Numbers of nematodes (J_2s) were counted under a light microscope. Average total percentages nematode reduction was calculated to compare among treatments. Rate of nematode reproduction (Rr) was calculated by dividing final nematode population (Pf) by the initial population (Pi).

7.2. Plant growth parameters

Plant growth criteria including shoot length (cm), fresh and dry shoot weights (g) and fresh root weight (g) of tomato and eggplant were recorded. Also, weight of fruits (g) was registered.

8. Statistical analysis

Statistically data of the present experiment were subjected to analysis by (ANOVA) procedures. Comparison was made for treatment at 5% probability by Duncan's Multiple Range Test as reported by **Snedecor and Cochran (1989).** This was carried out by Computerized Statistical Package (COSTAT) User Manual Version 3.03, Barkley Co.

RESULTS

1- Influence of certain *P. fluorescens* isolates on root-knot nematode, *Meloidogyne incognita* infecting tomato

Under screen house conditions, nine bacterial isolates were tested, for their nematicidal efficacy to control *M. incognita* infecting tomato as indicated by number of nematode juveniles (J_2s) in soil and roots, females, galls and egg masses compared to a commercial product namely, Micronema® and untreated check. **Table (3)** illustrates mean numbers of treatments and untreated check. In general, all bacterial isolates had suppressive effect on *M. incognita*, when number of the studied nematode reproductive criteria and galls were significantly ($P \leq 0.05$) reduced at various degrees on the basis of average total percentages nematode reduction. The over topped results gained were achieved by *P. fluorescens* (Pf_2) which recorded the highest average total percentages nematode reduction (61.3%) and high percentage reduction of females (77%) per plant. The second rank was obtained by Pf_3, where it reduced all reproductive nematode numbers as an

average total percentages reduction (56.9%) with the highest percentage reduction of females (84.7%.) and 78.3% for number of second stage juveniles in roots. This was followed by Micronema® which caused 56% and was as effective as some treatments in reducing number of females by 58.1%. However, the percentages reduction of galls behaved an independent pattern, as they recorded the highest reduction (48.1%) caused by Pf_1 followed by Pf_4 (43.8%). On the other hand, control treatment (untreated infected plants) showed the highest numbers and galls of root knot nematode. Obviously, the isolates of Pf_2 and Pf_3 recorded the lowest reproduction rates (2.9 and 2.4), respectively followed by Micronema®. The reproduction rate of the other isolates ranged from 3.7-4.8.

2- Effect of certain *P. fluorescens* isolates on tomato growth and yield infected by *Meloidogyne incognita*

Concerning tomato growth, a significant ($P \leq 0.05$) augmentation of shoot length, fresh and dry weights, root fresh weight and weight of fruits as influenced by the tested isolates of *P. fluorescens* are illustrated in **Table (4)**. On the basis of average total percentages of plant growth, Pf_9 isolate was superior in increasing shoot length, shoot and root fresh weights as four bacterial treatments can be ranked in a descending order as follows: Pf_9 >Pf_4>$Pf_1$and Pf_7 achieved the highest percentages increase (96.0, 47.3, 38.2and 29.8%), respectively compared to other treatments and untreated check. As for weight of fruits, Pf_9 achieved the highest percentage increase (194.6%) followed by Pf_2 (95.7%) and Pf_4 (80.4%), whereas Micronema® caused 66.3% only.

3- Influence of certain *P. fluorescens* isolates on root-knot-nematode, *Meloidogyne incognita* infecting eggplant

Also, the nine bacterial isolates in addition the standard isolate (Pf_{10}) were tested, for their efficacy on *M. incognita* infecting eggplant. Averages number of nematode juveniles in soil and roots, females, egg masses per root system as well as number of galls were used as indicators. In general, on the basis of these indicators, data in **Table (5)**, indicated that all tested bacterial isolates had significantly ($P \leq 0.05$) suppressed *M. incognita* (J_2s) in the above mentioned nematode criteria compared to the untreated check. The over topped results gained was achieved by *P. fluorescens* (Pf_{10}) which recorded the highest average total nematode reduction (66.2%) with higher reduction of (J_2s) in roots (89.9%) and in soil (78.8%) per plant. The second rank was obtained by Pf_9 and Pf_2 as they reduced all nematode numbers with averages 55.9% and 54.9%, respectively. The highest reductions of J_2s in soil (77.1%) and female (72.4%) in root were recorded by Pf_1. However, the percentages reduction of galls behaved an independent pattern, as they were reduced by 71.9 % caused by Pf_6 followed by Pf_9 (69.2%). On the other hand, the lowest reproduction rates (Rr) were recorded by the isolates Pf_{10} (3.4), Pf_2 (5.8), Pf_9 (6.97) and Pf_1 (9.1). In contrast, control treatment (untreated infected plants) registered the highest numbers in all nematode parameters.

4- Effect of certain *Pseudomonas fluorescens* isolates on eggplant growth parameters infected by *Meloidogyne incognita*

Concerning eggplant growth as influenced by the tested bacterial isolates, mean numbers of shoot length, fresh and dry weights and root fresh weight and untreated check are illustrated in **Table (6)**. Results indicated that, three treatments significantly ($P \leq 0.05$) promoted plant growth criteria than the other treatments and un-infected untreated plants as follows: averages total percentages plant growth increase of Pf_1 (20.0%), Pf_9 (18.7%) and Pf_{10} (18.3%) were found in a descending order. It is worthy to note that the most distinct growth criteria was fresh weight of roots, as it achieved higher percentage increase (58.1%) by using Pf_9 followed by Pf_1 caused increase 40.6% compared to untreated check. The highest average percentages increase recorded for fresh weight of shoot were achieved by Pf_{10} (26.4%) and Pf_8 (22.1%), while for dry weight, the most effective isolates were Pf_3 (29.8%) and Pf_2 (19.1%).

Table 3. Effects of certain *Pseudomonas fluorescens* isolates on reproduction potential of *Meloidogyne incognita* infecting tomato plants

Treatments	Nematodes reproductive parameters and galls (No./plant/pot)										Reproduction rate (Rr)
	J_2s in soil	% Red.	J_2s in roots	% Red.	Females	% Red.	Egg - masses	% Red.	Galls	% Red.	% Average total percentages reduction	
Pf_1	1451b	53.3	2970ab	10.0	330ab	29.0	74c	63.0	166c	48.1	38.8	4.8
Pf_2	1358 b	56.3	1430cd	56.7	107c	**77.0**	90c	55.0	219bc	31.6	61.3	2.9
Pf_3	1599 b	48.5	715d	78.3	71 c	84.7	168ab	16.0	350a	-	56.9	2.4
Pf_4	1958ab	37.0	2236abc	32.2	167bc	64.1	80c	60.0	180c	43.8	48.3	4.4
Pf_5	1708b	45.0	2310abc	30.0	167 bc	64.1	117bc	41.5	258abc	19.4	45.2	4.2
Pf_6	1466b	52.8	2786ab	15.6	174 bc	62.6	117bc	41.5	260abc	18.8	43.1	4.4
Pf_7	2325ab	25.1	2255abc	31.7	243 bc	47.7	73c	63.5	220abc	31.3	42.0	4.8
Pf_8	1769 b	43.0	2035bc	38.3	212 bc	54.4	161ab	19.5	329ab	-	38.8	4.0
Pf_9	1558b	49.8	1925bc	41.7	235 bc	49.5	127bc	36.5	202c	36.9	44.4	3.7
Micronema®	1371 b	55.9	1393cd	57.8	195 bc	58.1	96c	52.0	245abc	23.4	56.0	3.0
Nematode only	3106a	0	3300a	0	465a	0	200a	0	320ab	0	0	6.9

Averages followed by same letter(s) within each column are not significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test. Reproduction rate (Rr) = Final population (Pf)/Initial population (Pi).

Table 4. Effects of certain *Pseudomonas fluorescens* isolates on growth and yield parameters of tomato infected with *Meloidogyne incognita*

Treatments	Plant growth and yield parameters/plant									
	Shoot length(cm)		Shoot weight(g)			Root weight(g)		Fruit weight (g)		% Average total percentages inc.
	Length	% inc.	Fresh	% inc.	Dry	% inc.	Fresh	% inc.	Weight	% inc.	
Pf_1	29.7d	-	19.2bc	26.3	3.9cd	25.8	21.3ab	67.7	15.8bcd	71.1	38.2
Pf_2	31.3cd	-	16.3c	7.2	3.8cd	22.6	15.2bc	19.7	18.0b	95.7	29.0
Pf_3	30.5cd	-	17.2bc	13.2	3.6cd	16.1	15.8bc	24.4	13.3bcde	44.6	19.6
Pf_4	35.7ab	11.5	20.9bc	37.5	5.2ab	67.7	17.7bc	39.4	16.6bc	80.4	47.3
Pf_5	33.7abc	5.3	16.6c	9.2	2.9d	-	13.8c	8.7	13.2bcde	43.5	13.3
Pf_6	32.0bcd	0	16.0c	5.3	3.3de	6.5	13.6c	7.1	11.5cde	25.0	11.0
Pf_7	37.5a	17.2	23.0ab	51.3	4.3bc	38.7	16.6bc	30.7	10.2de	10.9	29.8
Pf_8	34.5abc	7.8	18.9bc	24.3	4.4bc	41.9	15.0bc	18.1	13.1bcde	42.4	26.9
Pf_9	36.2ab	13.1	27.4a	80.3	6.1a	96.8	25.3a	99.2	27.1a	194.6	96.8
Micronema®	34.3abc	7.2	16.7c	10	3.8cd	22.6	14.6bc	15.0	15.3bcde	66.3	24.2
Nematode only (control)	32.0bcd	0	15.2c	0	3.1d	0	12.7c	0	9.2e	0	0

Averages followed by same letter(s) within each column are not significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test, -Decrease less than control.

Table 5. Effects of certain *Pseudomonas fluorescens* isolates on reproduction potential of *Meloidogyne incognita* infecting eggplant

Treatments	Nematodes reproductive parameters and galls (No./plant/pot)										Reproduction rate (Rr)
	J_2s in soil	% Red.	J_2s in roots	% Red.	Females	% Red.	Egg - masses	% Red.	%Average total percentages Red.	Galls	% Red.	
Pf_1	1179b	77.1	3311ab	31.2	85b	72.4	114ab	21.9	50.7	221ab	33.2	9.1
Pf_2	1540b	70.1	1205b	75.0	171ab	44.5	104ab	28.8	54.6	110b	66.8	5.8
Pf_3	1907b	63.0	2662ab	44.7	118b	61.7	98ab	32.9	50.6	228ab	31.1	9.4
Pf_4	6914a	-	2340ab	51.4	190ab	38.3	81b	44.5	33.6	115b	56.3	18.9
Pf_5	1987b	61.4	2420ab	49.7	201ab	34.7	125ab	14.4	40.1	213ab	35.6	9.3
Pf_6	2472b	52.0	2960ab	38.5	130b	57.8	75b	48.6	49.2	93b	71.9	11.1
Pf_7	5164a	-	1819b	62.2	146ab	52.6	74b	49.3	41.0	127b	61.6	14.3
Pf_8	2070b	59.8	2314ab	51.9	235ab	23.7	96ab	34.2	45.4	166b	49.8	9.2
Pf_9	1535b	70.2	1764b	63.3	189ab	38.6	71b	51.4	55.9	102b	69.2	6.97
Pf_{10} (Standard)	1093b	78.8	487b	89.9	135b	56.2	88b	39.7	66.2	104b	68.6	3.4
Nematode only (control)	5150a	0	4811a	0	308a	0	146a	0	0	331a	0	20.5

Averages followed by same letter(s) within each column are not significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test. Reproduction rate (Rr) = Final population (Pf)/Initial population (Pi).

Table 6. Effects of certain *Pseudomonas fluorescens* isolates on growth parameters of eggplant infected with *Meloidogyne incognita*

Treatments	Plant growth and yield parameters/plant								%Average total percentages inc.
	Shoot Length		Shoot weight (g.)				Root weight (g.)		
	Length (cm)	% inc.	Fresh weight (g)	% inc.	Dry	% inc.	Fresh	% inc.	
Pf_1	46.3abc	7.7	26.8a	16.0	5.5ab	17.0	22.5ab	40.6	20.3
Pf_2	45abc	4.7	23.3ab	0.9	5.6ab	19.1	19.1ab	19.4	11.0
Pf_3	44.0bc	2.3	27.2a	17.7	6.1a	29.8	17.0b	6.3	14.0
Pf_4	48.0ab	11.6	25.4a	10.0	3.7bc	-	20.1ab	25.6	11.8
Pf_5	41.0c	-	19.6ab	-	5.2ab	10.6	19.6ab	22.5	8.3
Pf_6	47.5ab	10.4	26.3a	13.9	4.9abc	4.3	17.0b	6.3	8.7
Pf_7	31.0d	-	13.3b	-	3.0c	-	22.0ab	37.5	9.4
Pf_8	47.0ab	9.3	28.2a	22.1	5.2ab	10.6	18.6ab	16.3	14.6
Pf_9	43bc	0	23.5ab	1.7	5.4ab	14.9	25.3a	58.1	18.7
Pf10 (Standard)	50.0a	16.3	29.2a	26.4	5.4ab	14.9	18.5ab	15.6	18.3
Nematode only (control)	43.0bc	0	23.1ab	0	4.7abc	0	16b	0	0

Averages followed by same letter(s) within each column are not significantly ($P \leq 0.05$) different according to Duncan's Multiple Range Test, - =Decrease less than control

DISCUSSION

As a result of cellular metabolism, fluorescent pseudomonad isolates can produce exotoxic compounds and also can affect nematode juveniles as reported by **Wescott and Kluepfel (1993)** which conform to the present study regarding efficacy of certain *P. fluoresens* isolates for controlling *M. incognita* on tomato or eggplant. This effect may refer to the selective permeability changes of juvenile's cuticle and this effect is more pronounced with molting inside eggs. These results are similar to those obtained by **Ashoub and Amara (2010).** Plant growth promoting pseudomonads, antibiotic production, and competition with pathogens for essential nutrients such as iron and more, may act to induce direct antagonism against pathogens **(Gamliel and Katan1993).** The efficacy of Micronema® against *M. incognita* may refer to that it contain some beneficial bacterial isolates which are well known to suppress nematodes as follows: **El- Hadad et al (2010)** stated that the nematode numbers were significantly reduced particularly, 60 days after inoculation by nitrogen fixing bacterium, *Azotobacter* sp. and the potassium solubilizing bacterium, *Bacillus circulans*. **Zavaleta-Mejia and Van Gundy (1989)** reported that *Serratia marcescens* reduced juveniles of *M. incognita* which may be due to volatile materials produced during its metabolic activity (**Ali 1996 and El-Sherif et al 1999**). **Eklund (1970)** showed that some Pseudomonads convert amino acids present in root exudates to ammonia suppressive to pathogens. Also, **El-Nagdi and Youssef (2015)** clarified that gall reduction reached 69.3% by Micronema® on sugar beet infected by *M. incognita*. The same percentages nematode gall reduction occurred when Micronema treated on date palm which may be due to that juveniles were unable to penetrate the host root as reported by **Youssef et al (2014).**The obtained present results conform with those reported by **Stirling and Sharma (1990) and El-Nagar et al (1998)** who showed that, *Pasteuria penetrans* reduced number of root-knot nematodes which led to decreased infectivity of the juveniles. In accordance**,** the product, agerin which contains *Bacillus thuringiensis* reduced the number of root knot nematode (**Noweer and Hasabo 2005)** by releasing toxins that suppressed synthesis of proteins and nucleic acids in nematode **(Sebesta et al 1969)**.Coinciding with these results**, Sohrabi et al (2018)** reported that tomato growth criteria infected by *M. javanica* was affected by four plant growth-promoting rhizobacteria (PGPR) and indicated that the PGPR significantly affected the reproductive factor of the nematode by *P. fluorescens* which was reduced from 112.15 to 24.94 and significantly improved the plant growth parameters. Also, **El-Nagdi et al (2019)** reported that *P. fluorescens* caused the highest percentage nematode reduction (89%) of *M. incognita* on cowpea and caused average increase of 55.6% in the studied plant growth parameters which conform to the present study. Finally, the different effects of the tested bacterial isolates on *M. incognita* as affected by eggplant and tomato may be due to the differences in genetic composition and degree of host susceptibility against root-knot nematode between these plants

REFERENCES

Ali A.H. 1996. Biocontrol of reniform and root-knot nematodes by new bacterial isolates. **Bull. Fac. Agric., Cairo Univ., Giza, Egypt, 47, 487-497.**

Ashoub A.H. and Amara M.T. 2010. Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. **J. Am. Sci., 6(10), 321-328.**

Baid R.M., Hodges N.A. and Denyer S.P. 2000. Handbook of Microbiolgy Quality Control: Pharmaceuticals and Medical Devices. London; UK, **280 p.**

Barker K.R. 1985. Nematode Extraction and Bioassays. In: An Advanced Treatise on *Meloidogyne*, Vol. II: Methodology, Barker K.R., Carter and Sasser J.N. (Ed.). North Carolina State Univ. Graphics, USA. **pp. 19-35.**

Burkett-Cadena M., Kokalis-Burelle N., Lawrence K.S., VanSanten E. and Kloepper J.W. 2008. Suppressiveness of root-knot nematodes mediated byrhizobacteria. **Biol. Control, 47, 55-59.** doi:10.1016/j.biocontrol.2008.07.008

Eklund E. 1970. Secondary effect of some Pseudomonads in the rhizoplane of peat grown cucumber plants. **Suppl. Acta Agric. Scandinavica, 17, 1-57.**

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.** doi: 10.1007/s11274-010-0413-8

El-Nagar H.I., Farahat A.A., Hendy H.H. and El-Hadidy A.A. 1998. The extended effect of *Pasteuria penetrans* as a biocontrol agent of the root-knot nematodes. **Egypt. J. Agronematol., 2, 57-65.**

El-Nagdi W.M.A. and Youssef M.M.A. 2015. Nematicidal effect of some aqueous extracts of botanicals and a commercial bacterial byproduct for biocontrolling root knot nematode, *Meloidogyne incognita* infecting sugar beet. **Sci. Agric., 10(2), 55-58.**

El-Nagdi W.M.A., Youssef M.M.A., Abd-El-Khair H., Abd-Elgawad M.M.M. and Dawood M.G. 2019. Effectiveness of *Bacillus subtilis, B. pumilus, Pseudomonas fluorescens* on *Meloidogyne incognita* infecting cowpea. **Pak. J. Nematol., 37(1), 35-43.**

El-Sherif M.A., Ali A.H. and Barakat M.I. 1999. Suppressive bacteria associated with plant parasitic nematodes in Egyptian agriculture. **Jap. J. Nematol., 24, 55-59.**

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.**

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.**

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, **83 p.**

Kavitha P.G., Jonathan E.I. and SankariMeena K. 2011. *Pseudomonas fluorescens* for the management of root-knot nematode *Meloidogyne incognita* in Tomato. **Madras Agric. J., 98 (4-6), 176-177.**

Lelliot R.A. and Stead D.E. 1987. Methods on Plant Pathology Volume 2, Methods for the Diagnosis of Bacterial Diseases of Plants. British Society for Plant Pathology, Blackwell Scientific Publications, Boston, USA, **216 p**.

Mokbel A.A. and Alharbi A.A. 2014. Suppressive effect of some microbial agents on root-knot nematode, *Meloidogyne javanica* infected eggplant. **Aust. J. Crop Sci., 8(10), 1428-1434.**

Noweer E.M.A. and Hasabo S.A. 2005. Effect of different Management practices for controlling root-knot nematode *Meloidogyne incognita* on squash. **Egypt. J. Phytopathol., 33, 73-81.**

Oka Y., Koltai H., Bar-Eyal M., Mor M., Sharon E., Chet I. and Spiegel Y. 2000. New strategies for the control of plant parasitic nematodes. **Pest Manag. Sci., 56, 983-988.** doi: 10.1002/1526-4998(200011)56:11<983::AID-PS233>3.0.CO;2-X

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*. **Europ. J. Plant Pathol., 119, 477-479.** doi: 10.1007/s10658-007-9176-y

Schaad N.W. 1980. Laboratory guide for identification of plant pathogenic bacteria.Bacteriology Committee of American Phytopathological society St. Paul, Minnesotia, USA, **72 p.**

Sebesta K, Harsksand K. and Vankora J. 1969. Inhibition of de novo RNA synthesis by the insecticidal exotoxin of *Bacillus thuringiensis* var. Gelechiae. **Collect. Gzech. Chem. Communi., 34, 1786-1791.**

Snedecor G.W. and Cochran W.G. 1989. Statistical Methods. 8th ed. Ames, Iowa: Iowa State University Press, USA.

Siddiqui Z.A. and Akhtar M.S. 2009. Effects of antagonistic fungi, plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi alone and in combination on the reproduction of *Meloidogyne incognita* and growth of tomato. **J. General Plant Pathol., 75, 144-153.** doi:10.1007/s10327-009-0154-4

Singh P. and Siddiqui Z.A. 2010. Biocontrol of root-knot nematode *Meloidogyne incognita* by the isolates of *Bacillus* on tomato. **Arch. Phytopathol. Plant Protect, 43, 552-561.** doi:10.1080/03235400801939904

Sohrabi F., Sheikholeslami M., Heydari R., Rezaee S. and Sharifi R. 2018. Evaluation of four rhizobacteria on tomato growth and suppression of root-knot nematode, *Meloidogyne javanica* under greenhouse conditions, a pilot study. **Egypt. J. Biol. Pest Cont., 28, 1-5.**

Stirling G.R. and Sharma R.D. 1990. Attachment of *Pasteuria penetrans* spores to the root-knot nematode *Meloidogyne javanica* in soil. **Nematologica, 36, 246-252.**

Talavera M., Sayadi S., Chirosa-Rios M., Salmerón T., Flor-Peregrin E. and Verdejo-Lucas S. 2012. Perception of the impact of root-knot nematode-induced diseases in horticultural protected crops of south-eastern Spain. **Nematology, 14, 517–527.** doi: 10.1163/156854112X635850

Taylor A.L. and Sasser J.N. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh (NC): IMP, North Carolina State University Graphics.

Timper P. 2011. Utilization of biological control for managing plant-parasitic nematodes. In: Biological control of plant-parasitic nematodes. Davies K. and Spiegel Y. (Eds.) **pp. 259-287**. Building coherence between microbial ecology and molecular mechanisms. Dordrecht: Springer.

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

Wescott S.W. and Kluepfel D.A. 1993. Inhibition of *Criconemellaxenoplax*egg hatch by *Pseudomonas aureofaciens.* **Phytopathology, 83, 1245-1249.**

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

Youssef M.M.A., El-Nagdi W.M.A. and Eissa M.F.M. 2014. Population density of root knot nematode, *Meloidogyne incognita* infecting date palm under stress of aqueous extracts of some botanicals and a commercial bacterial by product. **Mid. East J. Appl. Sci., 4(4), 802-805.**

Zavaleta Mejia E., Seymour D.V. and Van Gundy S.D. 1989. Effect of the bacterium, *Serratia marcescens* Bizioon *Meloidogyne incognita* (Kofoid and White) Chiwood. **Rev. Mex. Fitopatol., 7, 178-187.**

مجلة اتحاد الجامعات العربية للعلوم الزراعية، جامعة عين شمس، القاهرة، مصر
مجلد(28)، عدد(1)، 315-327، 2020
Website: http://ajs.journals.ekb.eg

تأثير بعض عزلات *سيدوموناس فلوريسنس* علي نيماتودا تعقد الجذور *ميلويدوجين انكوجنيتا* والنمو الخضري علي نباتي الطماطم والباذنجان

[23]

نورا رمضان عبدالعاطي صالح[1*] – أحمد عيد عبدالمجيد محجوب[2] – وفاء محمد عبدالحميد النجدي[1] – انتصار حلمي طه السيد[2] – منى محمد سعيد زايد[3] – محمود محمد أحمد يوسف[1]

1- قسم أمراض النبات – معمل النيماتودا – المركز القومي للبحوث – دقى – جيزه – مصر

2- قسم وقاية النبات – كلية الزراعة – جامعة عين شمس – ص.ب 68 – حدائق شبرا 11241 – القاهرة – مصر

3- قسم الميكروبيولوجى – كلية الزراعة – جامعة عين شمس – ص.ب 68 – حدائق شبرا 11241 – القاهرة – مصر

*Corresponding author: norasaleh7841@yahoo.com

Received 30 January, 2020 Accepted 19 March, 2020

الموجـــــــــز

أجريت تجربتان تحت ظروف الصوبه على نباتي الطماطم والباذنجان لتقييم قدرة عزلات بكتريا *سيدوموناس فلوريسنس* التي تم عزلها من ريزوسفير بعض نباتات الفصيلة الباذنجانية على القدرة المرضية لنيماتودا تعقد الجذور *ميلويدوجين انكوجنيتا* (*Meloidogyne incognita*).

أوضحت النتائج في هذا البحث علي أن جميع العزلات المستخدمة أدت الى خفض أعداد نيماتودا تعقد الجذور. واتضح من النتائج ان العزلة Pf_2 قد سجلت أعلى نسبة مئوية لموت اعداد النيماتودا (61.3%) وسجلت أعلى نسبه خفض للإناث (77%) لكل نبات ويليها عزله Pf_3 حيث سجلت 56.9%. وكانت أفضل العزلات علـى المجموع الخضرى ووزن الثمار على التوالي عزلـة Pf_9 (96.8%) يليها العزلـة Pf_4 (47.3%) ويليها العزلة Pf_1 (38.2%) والعزلة Pf_7 (29.8%).

وعلى الباذنجان سجلت العزلة Pf_{10} أعلى نسبة مئوية لموت اعداد النيماتودا (66.2%) وكذلك أعلى نسبة خفض للطور اليرقي الثاني فى الجذور (89.9%) وفى التربة (78.8%) يليها العزلة Pf_9 (55.9%) ثم العزلة Pf_2 (54.6%).

كذلك بالنسبة للنمو الخضرى سجلت العزلة Pf_1 حيث سببت اعلى نسبة مئوية للزيادة قدرها (20.3%) يليها العزلة Pf_9 (18.7%) ثم العزلة Pf_{10} (18.3%). وسجلت العزلة Pf_9 اعلى نسبة زيادة في وزن الجذور بمقدار (58.1%) ويليها العزلة Pf_1 بنسبة (40.6%). وكانت أعلى نسبة فى الوزن الخضري Pf_{10} (26.4%) يليها Pf_8 (22.1%) وبالنسبة للوزن الجاف سجلت عزلة Pf_3 (29.8%) ويليها Pf_2 (19.1%). ويستنتج من ذلك أن *السيدوموناس فلوريسنس* فعالة في مكافحة نيماتودا تعقد الجذور.

الكلمات المفتاحية: عزلات بكتيريا *سيدوموناس فلوريسنس*، الطماطم، الباذنجان، *ميلويدوجين انكوجنيتا*

تحكيم: ا.د. محمد عبدالرحيم
ا.د. هدى أمين