



Original article

## *In vivo* and *in vitro* management of *Meloidogyne incognita* (Tylenchida: Heteroderidae) using rhizosphere bacteria, *Pseudomonas* spp. and *Serratia* spp. compared with oxamyl



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### ABSTRACT

Biological control using rhizosphere bacteria, *Pseudomonas* spp. and *Serratia* spp. is a prospective alternative technique to overcome plant parasitic nematodes infection. So, the current study was conducted *in vitro* on five egg-masses, 100 free eggs and 100 infective juveniles (IJs) of *Meloidogyne incognita* as well as greenhouse treatments on *Luffa aegyptiaca* L. to evaluate the nematicidal potential of six strains belong to *Pseudomonas* spp. and *Serratia* spp. as compared to oxamyl.

Results showed that the inhibitory effect and juvenile mortality varied according to bacteria species, strains and exposure time. All the tested bacteria significantly ( $P \leq 0.05$ ) inhibited egg hatching and increased juvenile mortality *in vitro*. After 3 days of treatment, *Pseudomonas* spp. were more effective against eggs (48.31 to 55.15%) and IJs (20.98 to 25.30%) than *Serratia* spp. (44.55 to 49.75% with eggs) and (19.06 to 21.61% with IJs), respectively. In the pot experiment, *Luffa aegyptiaca* L. treated with *Serratia* spp. and *Pseudomonas* spp. displayed significantly higher ( $P \leq 0.05$ ) levels of growth (as indicated by root length, fresh roots weight and fresh shoots weight) compared to control plants and significantly ( $P \leq 0.05$ ) suppressed galling (number of galls) and reproduction (as indicated by number of egg-masses on roots and number of eggs and juveniles in pot soil). Meanwhile, among the treated plants, *Serratia* spp. and *Pseudomonas* spp. gave the best results in shoot weight of pots infected by eggs of *M. incognita* than those infected with IJs as compared with positive control. While, oxamyl treatment gave the best results in pots infected by eggs and IJs.

The lowest galling (gall index), number of eggs and juveniles in soil was observed in the treatment with mixture of *Serratia* spp. and *Pseudomonas* spp. as well as, enhanced growth of sponge gourd more than application each of them alone. Pots treated with oxamyl overwhelmed those treated with mixture of *Serratia* spp. and *Pseudomonas* spp.

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### 1. Introduction

Species of the root- knot nematodes (*Meloidogyne* spp.) are among the most damaging nematodes in light agriculture soils, causing an estimated \$100 billion loss/year worldwide (Oka et al., 2002) and they have high reproductive potential and protected in plant tissues of wide host range. Therefore, control of such nematodes is partly difficult. *Meloidogyne incognita* (Kofoid and White) Chitwood is the most destructive species in most agricultural crops and nearby one hundred valid species are in the

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genus *Meloidogyne* (Trinh et al., 2019) which cause serious damage worldwide (Moens et al., 2009). In Egypt, this species is one of the most common and economically important root-knot nematodes because considerable damage to majority of crops including vegetables and their production depends on the correct management of these pathogens (Sikora & Fernandez, 2005). Nowadays, Root-knot nematodes are one of the top five primary plant pathogens and the first of the 10 most important plant parasite genera in the world (Mukhtar et al., 2018; Ravichandra, 2018). More efficient management methods for root-knot nematodes are currently under consideration due to environmental issues and increased restrictions on the application of chemical nematodes (Nico et al., 2004). Therefore, a range of alternative techniques are urgently needed to replace chemical control such as antagonistic bacteria are among the biological control agents tested (Hegazy et al., 2019; Kiewnick & Sikora, 2005; Tariq-Khan, 2018; Khan et al., 2021; Heflish, 2021).

Bacteria are subject to a range of suppressive activities in nematodes by various methods, which generate or interfere with recognition of toxins, antibiotics and enzymes (Khan et al., 2004). Several rhizobacteria species such as *Pseudomonas* and *Serratia marcescens* B. have been isolated from soil water, plants and insects reported to play important role as biocontrol agents against *M. incognita* either by promoting plant growth or by inhibiting nematode infectivity factors (Akhtar & Siddiqui, 2009a,b; Khanna et al., 2019). *S. marcescens* is known to be a chitinase producer, which is considered a reason for its capability to inhibit the growth of several phytopathogenic and saprophytic fungi (Okay et al., 2013).

Therefore, the main option of the present research was designed to estimate the nematicidal effect of *Pseudomonas* spp. and *S. marcescens* on egg hatching and juvenile mortality of *M. incognita* *in vitro* and observe the suppressiveness effects of rhizobacteria on *M. incognita* reproduction and its damages on sponge gourd under greenhouse conditions comparing with oxamyl.

## 2. Materials and methods

### 2.1. Culturing of the Root-Knot Nematode, *M. incognita*:

Pure culture of *M. incognita* was maintained in the greenhouse on the tomato susceptible cultivar Super Strain B for using as a source of egg-masses, free eggs and second stage juveniles. A single egg-mass was used to establish a nematode population. The identification of species was depending on second stage juvenile measurements and the perineal pattern method of adult females according to Eisenback et al. (1981) and Jepson (1987).

### 2.2. Rhizobacteria inocula source:

The tested rhizobacteria (*Serratia* sp. and *Pseudomonas* spp.) were previously isolated from collected soil samples infested with root-knot nematodes from different areas in El-Sharqia Governorate, Egypt. The obtained bacteria isolates were identified by using 16S rDNA gene sequencing (Hegazy et al., 2019). The tested isolates used in the study were *Pseudomonas putida* (PP29 and PP22), *P. fluorescens* (PF131), and *Serratia marcescens* (A10, A15, and A20).

### 2.3. Preparation of egg-masses, free eggs and second juveniles stage needed for experiments:

Infected tomato roots are cut to 2-cm long pieces with 0.5% sodium hypochlorite in 200 ml of 600 ml bottle (180 ml of water + 20 ml of Clorox). Three minutes were shaken by a closely clasped pin and dissolved the gelatinous matrix then free eggs of

the egg mass (Hussey and Barker, 1973). A 200-mesh sieve nestled on a 500-mesh sieve was used for the liquid suspension of eggs. Eggs collected on the 500-mesh sieve were immediately washed under a slow stream of tap water free of residual sodium hypochlorite and incubated until the hatching on Petri dishes was at around  $25 \pm 2$  °C. Newly hatched juveniles were collected by using a micropipette. Egg-masses of equal size needed to study the effect of the tested oils on *M. incognita* egg hatching were hand-picked with fine forceps from small galls on the infected tomato roots obtained from previously maintained pure culture. The collected egg-masses were surface sterilized in 1:500 V/V aqueous solution of sodium hypochlorite for 5 min (Haseeb et al., 2005).

### 2.4. *In vitro* nematicidal effect of the tested bacterial isolates against *M. Incognita*

#### 2.4.1. Effect of six *Pseudomonas* spp. and *Serratia marcescens* on egg hatching and juvenile mortality of *M. Incognita* *in vitro*

**2.4.1.1. Effect on Egg-Masses.** Five fresh and uniform size egg-masses were transferred to 10-cm diameter Petri dishes contained 10 ml of each bacterial isolates were screened *in vitro* for their biocontrol efficiency against *M. incognita* at different time periods. Each bacteria species was adjusted to about  $1.8 \times 10^8$  cfu/ml, from which, 10 ml aliquots were mixed with mentioned egg-masses. The control treatment contained only nematode egg-masses in distilled water, and all treatments were replicated five times. Treatments were left under ambient temperature of  $25 \pm 2$  °C to determine the bacterial biocontrol efficiency.

All treatments were left under laboratory temperature  $25 \pm 2$  °C and all treatments were replicated five times. Numbers of hatched juveniles were counted using a research microscope (100X magnification). The cumulative number of hatched juveniles in each Petri dish was counted after 1, 2, 3, 5 and 7 days post treatment. Compared to control treatment, the percentage of the hatching inhibition was determined as follows:

$$\text{Egg hatching inhibition (\%)} = \frac{\text{Control} - \text{treatment}}{\text{Control}} \times 100$$

**2.4.1.2. Effect on free eggs.** According to Hussey and Barker (1973) as mentioned before, *M. incognita* free eggs were removed from infected roots of tomatoes. Extracted eggs were suspended by distilled water and counted by using a counting slide under a research microscope (100X magnification). The number of eggs per 1 ml was adjusted to about 1000 eggs by diluting the suspension. Approximately 100 free eggs in 0.1 ml water were exposed to  $1.8 \times 10^8$  cfu/ml bacterial cell free supernatant. The control treatment contained only distilled water. All treatments were replicated five times. The cumulative number of hatched juveniles was calculated in comparison with the control treatment and the percentage of egg hatching inhibition was determined by the following equation:

$$\text{Egg hatching inhibition (\%)} = \frac{\text{Control} - \text{treatment}}{\text{Control}} \times 100$$

**2.4.1.3. Effect on juvenile Mortality.** The suspension concentration of emerged juveniles was adjusted to 100 juveniles per 0.1 ml. Ten ml of the six bacterial isolates (*Pseudomonas* spp. and *S. marcescens*) were screened *in vitro* for their biocontrol efficiency against of *M. incognita* at different time periods. Bacteria species were adjusted to about  $1.8 \times 10^8$  cfu/ml, from which, 10 ml aliquots were mixed with 0.1 ml of nematode suspensions (about 100 juveniles). As mentioned before, control treatment contained only distilled water and all treatments were replicated five times. Treatments were left under ambient temperature of  $25 \pm 2$  °C to determine the bacterial biocontrol efficiency.

Tested materials were observed daily for juvenile mortality but tables only contain data of 1, 2, 3, 5 and 7 days as mentioned before. Juveniles showing inactive straight posture or did not show any movement after prodding were considered dead (Elizabeth et al., 2003). Mortality counts were observed using a research microscope under 100X magnification in 1 ml over the specified periods. The cumulative number of dead juveniles was calculated in comparison with the control treatment of distilled water. The mortality percentages were calculated as the following equation:

$$\text{Mortality}(\%) = \frac{\text{Dead juveniles}}{\text{Total number of juveniles}} \times 100$$

#### 2.4.2. Efficacy of *Pseudomonas* spp. And *S. Marcescens* on galling and reproduction of *M. Incognita* infecting *L. Aegyptiaca* L. Under greenhouse conditions

Pot experiment was maintained in the greenhouse, Faculty of Agriculture, Zagazig University, Egypt on the sponge gourd (*L. aegyptiaca*) as a host plant. Seeds of sponge gourd (Local cultivar) were soaked in distilled water in Petri dishes and kept in an incubator at  $27 \pm 1$  °C. After 48 h, seeds were sown in formalin sterilized 15-cm diameter plastic pots filled with about 1600 g steam sterilized soil (2:1 v/v sandy soil: clay soil) mixed with 60 g compost. Three weeks after sowing, seedlings were thinned to one plant per pot. 20 ml-mixture of *Pseudomonas* spp. or of *S. marcescens* species (at concentration of  $1.8 \times 10^8$  cfu/ml) were incorporated with the upper 3 cm of soil around each plant.

Then all plants were inoculated with 1000 free eggs or 1000 freshly hatched infectious juveniles of *M. incognita* by pipetting 2 ml of the inoculum suspension into three holes around a root system and directly covered with moist soil immediately. The chemical pesticide oxamyl (Vydate 24% EC), methyl 2- (dimethylamino) -N- [(methylcarbamoyl) oxy] -2-oxoethanimidothioate, was applied to sponge gourd seedlings at the rate of 0.3 ml/plant. Plants of the control treatment were inoculated with *M. incognita* alone. Five replications have been made for each treatment. The randomized complete design of all pots was arranged at  $25 \pm 3$  °C and similar horticultural treatments have been received.

The plants were carefully removed from the cans 55 days after inoculation and plant growth data were recorded according to length and fresh weight of the shoots. An aliquot sample of 100 g of soil was treated using a mixture of sieving and Baermann trays technique for the extraction of nematodes (Hopper et al., 2005). Roots were immersed in tape water for one hour to help eliminate

soil adherence and maintain egg root masses. The roots were wrapped into tissue paper during assessment phases to avoid drying and the root mass was counted for a numbers of galls and egg masses.

#### 2.5. Statistical Analysis:

The experiments were conducted in a completely randomized design. Data were subjected to analysis of variance (ANOVA) using MSTAT VERSION 4 (1987). Means were compared by Tukey's test:  $\alpha = 0.05$ .

### 3. Results

#### 3.1. In vitro experiments

##### 3.1.1. Ovicidal activity of *Serratia* spp. And *Pseudomonas* spp. On egg masses hatching of *M. Incognita*

Different strains of *Serratia* spp. and *Pseudomonas* spp. significantly ( $P \leq 0.05$ ) inhibited egg hatching of *M. incognita* (Table 1). The inhibitory effect varied according to bacteria species, strains and exposure time. Between tested bacteria, the highest values were detected with *S. marcescens* A10 and *Pseudomonas fluorescens* M. PF131 after the third day post treatment with percent inhibition reached 86.61 and 80.84%, respectively, while the lowest value was observed with *S. marcescens* A15 and *Pseudomonas putida* M. PP22 with percent reduction of 77.33 and 77.38%, respectively. On the other hand, Petri dishes treated with recommended dose of oxamyl overwhelmed those treated with bacteria strains. For instance, after one, three, five and seven-days post treatment; numbers of emerged juveniles in oxamyl treatment were 1.33, 4.66, 7.66 and 8.33 juveniles with percent inhibition of 99.41, 98.92, 98.71 and 99.16%, respectively.

As the period of the tested bacteria was increased, numbers of emerged juvenile were obviously increased. For instance, numbers of emerged juveniles with the highest effect bacteria, *S. marcescens* A10 and *P. fluorescens* PF131 were 89.0, 145.33; 112.33,294.67 after 5 and 7 days of exposure with percent inhibition of 85.08,75.64 and 88.68,70.64%, respectively. The same trend was found after 10 days post treatment. It was true with the six tested bacteria at the five times exposure, the distilled water treatment (control) had significantly higher numbers of eggs that hatched compared to other treatments. Ten days after treatment, numbers of emerged juveniles per egg masses was 1193.67 juveniles in dis-

**Table 1**  
Effect of certain bacteria species and oxamyl on number of juveniles emerged from *M. incognita* egg-masses at different intervals during 10 days of exposure *in vitro*.

Treatments	Days after treatment					Mean hatch per egg-mass
	1 day	3 days	5 days	7 days	10 days	
Control (distilled water)	226.66 a	433.33 a	596.66 a	993.00 a	1193.67 a	688.66
<i>Serratia marcescens</i> A10	41.00b (81.91)	60.00c (86.61)	89.00 d (85.08)	112.33c (88.68)	265.67b (77.74)	113.60
<i>S. marcescens</i> A20	40.00b (82.35)	58.00c (86.15)	147.33c (75.30)	269.67b (72.84)	391.67b (67.18)	181.33
<i>S. marcescens</i> A15	60.33b (73.38)	77.33 bc (82.15)	113.33 cd (81.00)	322.33b (67.53)	360.00b (69.84)	186.66
<i>Pseudomonas putida</i> PP29	55.66b (75.44)	87.33 bc (79.84)	155.66c (73.91)	308.00b (68.98)	343.00b (71.26)	189.93
<i>P. putida</i> PP22	54.00b (76.17)	98.00b (77.38)	226.66b (62.01)	309.33b (68.84)	364.33b (69.47)	210.46
<i>P. fluorescens</i> PF131	40.10b (82.30)	83.00 bc (80.84)	145.33c (75.64)	294.67b (70.32)	345.00b (74.09)	181.62
Vydate 24% SL (Oxamyl)	1.33c (99.41)	4.66 d (98.92)	7.66 e (98.71)	8.33 d (99.16)	9.33c (99.21)	6.26

\*Reported numbers represent means of 5 replicates.

\*\*Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water.

\*\*\*Treatments in the same column with the same letters are not significantly different from each other (Tukey's test:  $\alpha = 0.05$ ).

**Table 2**  
Effect of certain *Serratia* spp. and *Pseudomonas* spp. on hatching of *M. incognita* free eggs at five intervals during 10 days of exposure.

Treatments	Mean number of juveniles emerged after				
	1	3	5	7	10
Control (distilled water)	399.33 a	691.33 a	888.67 a	983.67 a	993.00 a
<i>Serratia marcescens</i> A10	67.00 d (83.22)	110.00 e (84.08)	148.00 d (83.34)	174.00c (82.31)	183.00 d (81.57)
<i>S. marcescens</i> A20	122.00c (69.44)	147.33 d (78.68)	170.00c (80.87)	180.00 d (81.70)	184.67 d (81.40)
<i>S. marcescens</i> A15	145.67b (63.52)	183.33b (73.48)	194.67b (78.09)	195.67b (80.10)	198.67b (79.99)
<i>Pseudomonas putida</i> PP29	137.00 bc (65.69)	159.67c (76.90)	175.00c (80.30)	188.67c (80.81)	195.67 bc (80.29)
<i>P. putida</i> PP22	132.33 bc (66.86)	157.33 cd (77.24)	173.33c (80.49)	186.67c (81.02)	193.67 bc (80.49)
<i>P. fluorescens</i> PF131	124.00c (68.94)	154.67 cd (77.62)	171.33c (80.72)	185.00 cd (81.19)	189.67 cd (80.79)
Vydate 24% SL (Oxamyl)	1.33 e (99.66)	4.66f (99.32)	7.66 e (99.13)	8.33f (99.15)	9.33 e (99.06)

\*Reported numbers represent means of 5 replicates.

\*\*Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water.

\*\*\*Treatments in the same column with the same letters are not significantly different from each other (Tukey's test:  $\alpha=0.05$ ).

**Table 3**  
Effect of certain *Serratia* and *Pseudomonas* species on juvenile mortality of *M. incognita* at different interval periods during 10 days of exposure compared with oxamyl.

Treatments	Mortality (%) after exposure time				
	1 day	3 days	5 days	7 days	10 days
<i>Serratia marcescens</i> (A10)	4.45c	19.06 d	30.60c	41.71c	51.46c
<i>S. marcescens</i> (A15)	4.65c	16.79 de	34.53c	42.88c	55.71 bc
<i>S. marcescens</i> (A20)	5.87b	20.56c	40.40b	46.97b	59.56b
<i>Pseudomonas fluorescens</i> (PF131)	3.75 d	21.61c	33.65c	45.08c	56.37b
<i>P. putida</i> (PP22)	6.84b	25.30b	41.36b	47.52b	57.66b
<i>P. putida</i> (PP29)	4.60c	20.98c	35.60c	46.40b	58.40b
Vydate 24% SL (Oxamyl)	18.80 a	41.60 a	95.60 a	100.00 a	100.00 a
Control (Distilled water)	0.50 e	1.10f	3.40 d	4.10 d	4.96 d

<sup>1</sup>Reported numbers represent means of five replicate counts.

<sup>2</sup>Tested nematodes were observed daily for mortality up to 10 days.

<sup>3</sup>Different bacterial isolates in the same column with the same letters are not significantly different from each other (Tukey's test:  $\alpha=0.05$ ).

tilled water treatment compared to 265.67, 345.00 and 9.33 juveniles in treatments of *S. marcescens* A10, *P. fluorescens* and oxamyl, respectively.

3.1.2. Effect of *Serratia* spp. And *Pseudomonas* spp. On free eggs hatching of *M. Incognita*

Six bacteria species were found to be significantly ( $P \leq 0.05$ ) effective against free eggs of *M. incognita* (Table 2). The egg viability of *M. incognita* was influenced by the time of exposure as well as species of bacteria. Among which, *S. marcescens* A10 was the most effective one followed by *S. marcescens* A20 and *P. fluorescens* PF131 while *S. marcescens* A15 was the lowest effective one. One day after exposure, numbers of hatched juveniles and percentage of hatching inhibition in *S. marcescens* A10, *S. marcescens* A20 and *P. fluorescens* PF131 treatments at were 67.00 (83.22%), 122.00 (69.44%) and 124.00 (68.94%) respectively. The same arrangement was noticed after 3 and 5 days post treatment.

At the seventh day of treatment, cumulative numbers of hatched juveniles and percent of hatching inhibition in Petri dishes treated with *S. marcescens* A10, *S. marcescens* A20 and *P. fluorescens* PF131 and *P. putida* PP29 were 148.00 (82.31%), 180.0 (81.70%), 185.00 (81.19%) and 188.67 (80.81%), correspondingly as compared to 983.67 and 8.33 juveniles hatched in distilled water and oxamyl treatments. It was found that as the exposure time increased, percentage of hatching inhibition was decreased with some tested bacteria. For instances, percent of reduction in *Serratia marcescens* A10 treatment were 84.08, 83.34, 82.31 and 81.57 after 3, 5, 7 and 10 days post treatment. Generally, the nematicidal effect

of the tested bacteria showed relatively highest egg hatching inhibition on *M. incognita* eggs, but it was lower as compared to oxamyl.

3.1.3. Larvicidal activity of *Serratia* spp. And *Pseudomonas* spp. On infective juveniles of *M. Incognita* in vitro

Under laboratory conditions, effectiveness of certain *Serratia* and *Pseudomonas* species on juvenile mortality of the root-knot nematode, *M. incognita* were evaluated. Data in (Table 3 & 4) and (Fig. 1) showed significant difference ( $P \leq 0.05$ ) between tested bacteria against infective juveniles of *M. incognita*. Among which,

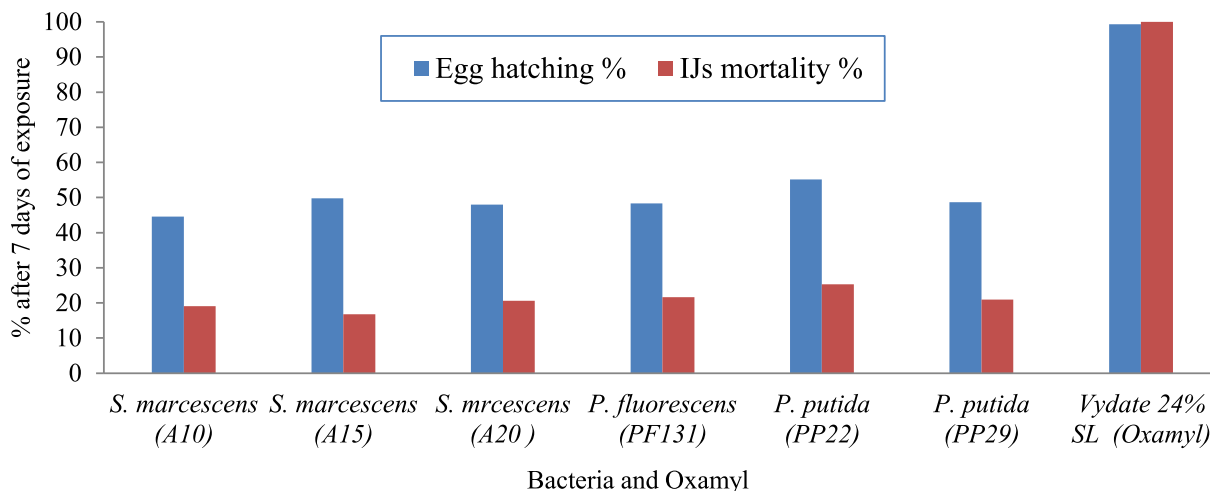
**Table 4**  
Effect of *Serratia* spp., *Pseudomonas* spp. and oxamyl on hatchability and mortality of *M. incognita* after 96 hrs.

Treatments	Hatching %	Mortality %
Control (Distilled water)	691.33 a	1.10f
<i>Serratia marcescens</i> (A10)	383.33b (44.55)	19.06 d
<i>Serratia marcescens</i> (A15)	347.33 d (49.75)	16.79 de
<i>Serratia marcescens</i> (A20)	359.67c (47.97)	20.56c
<i>Pseudomonas fluorescens</i> (PF131)	357.33 cd (48.31)	21.61c
<i>Pseudomonas putida</i> (PP22)	310.00 e (55.15)	25.30b
<i>Pseudomonas putida</i> (PP29)	354.67 cd (48.69)	20.98c
Vydate 24% SL (Oxamyl)	4.66f (99.32)	100.00 a

<sup>1</sup>Reported numbers represent means of five replicate counts.

<sup>2</sup>Tested nematodes were observed daily for hatching and mortality but only table contains data of 96 hrs.

<sup>3</sup>Different bacterial isolates in the same column with the same letters are not significantly different from each other (Tukey's test:  $\alpha=0.05$ ).



**Fig. 1.** Comparison between percentages of egg hatching inhibition in free eggs and dead infective juveniles of *M. incognita* after immersion by *Serratia* spp. and *Pseudomonas* spp. compared with oxamyl at the seventh day post treatment.

**Table 5**  
Effect of *Serratia* spp. and *Pseudomonas* spp. in comparison with oxamyl on galling and reproduction of *M. incognita* in relation to growth of *Luffa aegyptiaca* under greenhouse conditions.

Treatments	Shoot weight (% increase)	Root weight (g) (% increase)	Root length (cm) (% increase)	Number of galls (% reduction)	Root Gall Index (GI)	Number of egg-masses (% reduction)	Egg mass Index (EI)	infective juveniles / 100 g soil (% reduction)
Control- Luffa (without PPN or bacteria)	6.98 a	5.22 a	16.34 a	0.00 d	(0)	0.00c	(0)	0.00 g
Control -2 Luffa Treated with Infective juveniles of <i>M. incognita</i>	3.21 d	3.89 d	11.91 d	67.00 a	(4.0)	108.00 a	(5.0)	78.0 a
Control-3 Luffa Treated with eggs of <i>M. incognita</i>	4.30c	3.75 d	12.17 d	62.66 a	(4.0)	98.33 a	(4.3)	67.00b
Luffa + infective juveniles and bacteria ( <i>Serratia</i> spp.)	5.39b (67.91)	4.55c (16.96)	14.01 bc (17.63)	53.66b (19.91)	(4.0)	92.66 ab (14.20)	(4.0)	51.33c (34.19)
Luffa + eggs and bacteria ( <i>Serratia</i> spp.)	5.41b (25.18)	4.76 bc (22.36)	14.10 bc (15.85)	42.33 bc (32.44)	(4.0)	59.33c (39.66)	(4.0)	32.00 d (52.23)
Luffa + infective juveniles and bacteria ( <i>Pseudomonas</i> spp.)	5.99 ab (86.60)	4.57c (17.48)	12.16 d (2.09)	52.33b (21.89)	(4.0)	83.66 ab (45.06)	(4.6)	49.33c (36.75)
Luffa + eggs and bacteria ( <i>Pseudomonas</i> spp.)	5.16b (20.00)	4.78 bc (22.87)	12.33 d (1.31)	47.00b (24.99)	(4.0)	51.67c (47.45)	(4.0)	36.00 d (46.26)
Vydate 24% SL (Oxamyl) + of <i>M. incognita</i>	6.07 ab (89.09)	4.95b (27.24)	14.73 ab (23.67)	27.66 d (58.71)	(3.3)	31.66 d (70.68)	(4.0)	17.33 e (77.78)
Vydate 24% SL (Oxamyl) + eggs of <i>M. incognita</i>	6.48 a (50.69)	4.87b (29.86)	14.60 ab (19.96)	31.00 d (50.52)	(3.3)	32.66 d (66.78)	(4.0)	9.33 ef (86.07)

\*Each value is a mean of five replicates.

\*\*Treatments in the same column with the same letters are not significantly different from each other (Tukey's test:  $\alpha = 0.05$ )  
Reduction (%) =  $\frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$  Increase (%) =  $\frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$

*P. putida* (PP22) was the most effective one followed by *S. marcescens* (A20) while *S. marcescens* (A10) was the lowest effective one after 7 days of exposure. The highest juvenile mortality was found with *P. putida* (PP22) after 7 days of exposure (47.52%) followed by *S. marcescens* (A20) (46.97%) at the same concentration and exposure time while, *P. fluorescens* (PF131) gained the least mortality after one day of exposure (3.75%). Generally, the nematocidal effect of the tested bacteria on *M. incognita* juveniles was directly proportion to bacteria species and exposure time. After 7 and 10 days of exposure, juvenile mortality reached to 100% in oxamyl. On contrarily, juvenile mortality ranged from 42.0 to 59.0 in the tested bacteria after 7 and 10 days of exposure, respectively.

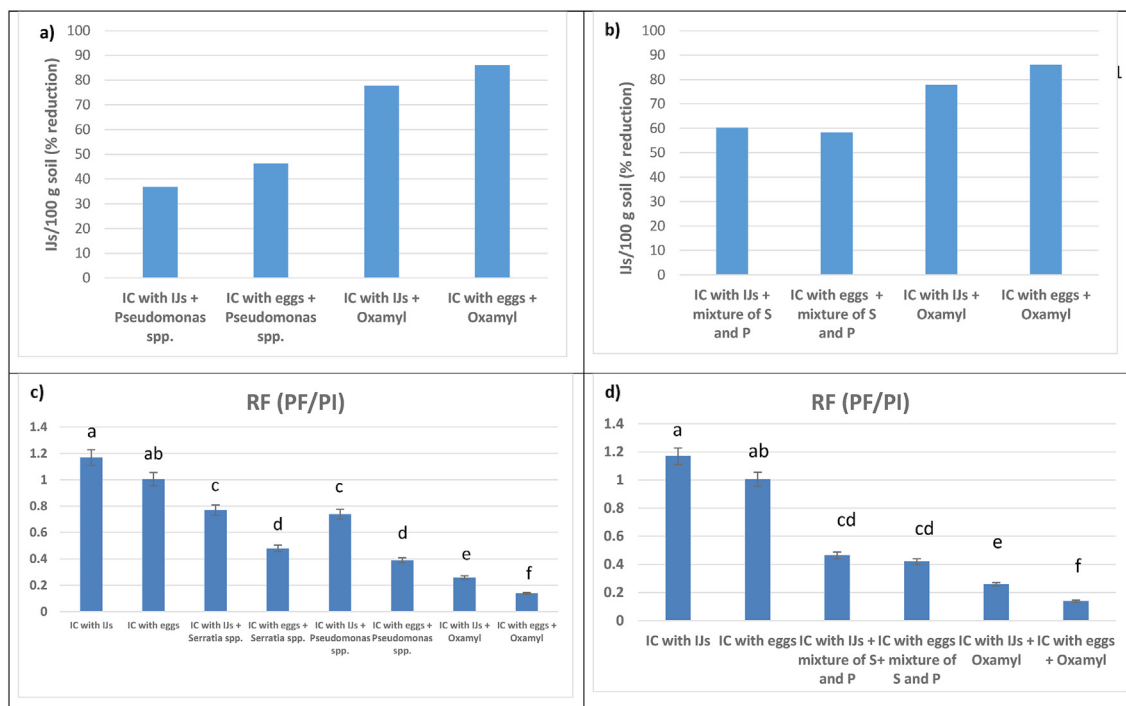
### 3.2. Greenhouse experiments

#### 3.2.1. Greenhouse evaluation of *Serratia* spp. And *Pseudomonas* spp

The effect of *Serratia* spp. and *Pseudomonas* spp. compared to oxamyl on *M. incognita* infected sponge gourd (*L. aegyptiaca*) under

greenhouse conditions is presented in (Table 5). The obtained results revealed that all treatments significantly ( $P \leq 0.05$ ) reduced galling (as indicated by number of galls) and reproduction (as indicated by number of egg-masses on roots and number of juveniles in soil) and enhanced growth of sponge gourd (as indicated by root length, fresh roots weight and fresh shoots weight) compared to plants inoculated with *M. incognita* alone. Pots treated with oxamyl overwhelmed those treated with *Serratia* spp. and *Pseudomonas* spp. Among the tested plants, *Serratia* spp. and *Pseudomonas* spp. gave the best results in shoot weight (5.41 and 5.16 g) in pots infected by eggs of *M. incognita* as compared with positive control (4.30). While, *Serratia* spp. and *Pseudomonas* spp. were less effective in pots infected by infective juveniles of *M. incognita*. While, oxamyl gave the best results in pots infected by eggs and infective juveniles but, *Serratia* spp. and *Pseudomonas* spp. showed best results with plants infected with eggs only.

On the other hand, number of galls, egg-masses and juveniles in soil in treatments of Luffa infected by eggs and treated with *Serra-*



**Fig. 2.** Percent reduction in infective juveniles of *Meloidogyne incognita* as influenced by tested Rhizobacter (*S. marcescens* and *Pseudomonas* spp.) applied alone or mixture compared with oxamyl (a & b) as well as, effect of different bacteria isolates (alone or mixture) on RF ratio in pot soil of *Luffa aegyptiaca* infected with eggs and infective juveniles (c & d). Bars with same letters are not differ significantly from each other (Tukey's test:  $\alpha = 0.05$ ).

*tia* spp. and *Pseudomonas* spp. were 42.33(47.00), 59.33 (51.67), and 32.00 (36.00), respectively. However, these values in treatment of *Luffa* infected by infective juveniles were 53.66 (52.33), 92.66 (83.66) and 51.33 (49.33), respectively. The parallel values in control treatment were 67.00, 108.00, and 78.0 with number of galls, egg-masses and juveniles in soil, respectively. Percent reduction in galls, egg-masses and soil juvenile number in treatments of *Serratia* spp. and *Pseudomonas* spp. were 32.44 (24.99), 39.66 (47.45) and 52.23(46.26) % in pots infected by eggs, respectively. For plant growth parameters, it was clear that all tested treatments ameliorated growth of the sponge gourd to certain extend as compared to plants inoculated with *M. incognita* alone.

Percent increase in fresh shoot weight and root length in treatments of *Serratia* spp. and *Pseudomonas* spp. were 67.91(86.60) and 17.63 (2.09) %, respectively in pots infected by infective juveniles. The parallel values in oxamyl treatment were 89.09 and 23.67%, respectively. Values of reproduction factor (RF) clearly displayed effect of *Serratia* spp. and *Pseudomonas* spp. in suppressing number of eggs and infective juveniles in soil. For example, in treatments of *Serratia* spp. and *Pseudomonas* spp., RF values were 0.769 (0.480) and 0.739 (0.390) in plants infected by eggs and infective juveniles of *M. incognita*. The parallel values in positive control were 1.170 and 1.005, respectively. Generally, oxamyl treatment surpassed *Serratia* spp. in improving plant growth parameters and soil parameters (Fig. 2).

### 3.2.2. Greenhouse evaluation mixture of *Serratia* spp. And *Pseudomonas* spp

The obtained results in (Table 6) revealed that, mixture of *Serratia* spp. and *Pseudomonas* spp. treatments significantly ( $P \leq 0.05$ ) reduced galling and reproduction and number of juveniles in soil and enhanced growth of sponge gourd more than application each of them alone. As well as, plant growth parameters were increased to reach nearby those in pots treated with oxamyl. As well, soil parameters such as number of galls, egg-masses and infective juve-

niles were decreased significantly ( $P \leq 0.05$ ) to close those in oxamyl treatments.

Percent reduction in galls, egg-masses and soil juvenile number in treatments of mixture of *Serratia* spp. and *Pseudomonas* spp. were 65.43 (56.71), 44.74(39.50) and 58.20 (60.25) % in pots infected by eggs and IJs, respectively. For plant growth parameters, mixture of *Serratia* spp. and *Pseudomonas* spp. ameliorated growth of the sponge gourd to certain extend as compared to control treatments. Percent increase in fresh shoot weight and root length in treatments of mixture *Serratia* spp. and *Pseudomonas* spp. and oxamyl were 91.90 (22.85) %, and 89.09 (23.67) %, respectively in pots infected by IJs with insignificant differences ( $P \leq 0.05$ ). Values of reproduction factor (RF) were 0.465 (0.420) and 0.259 (0.139) in treatments of tested bacteria and oxamyl, respectively (Fig. 2). Generally, mixture of *Serratia* spp. and *Pseudomonas* spp. surpassed *Serratia* spp. or *Pseudomonas* spp. alone in improving plant growth parameters and suppression of galls, egg-masses and infective juveniles in soil of pots.

## 4. Discussion

The root-knot nematode has an important effect on crop growth and productivity. The protection of plants from root-knot nematode, particularly for increasing crop yields, has thus become an important task. Control of root-knot nematodes, *Meloidogyne* spp. with synthetic nematicides is expensive and cause many problems to environment and human health. On the other hand, as organic agriculture increased, new alternative control methods need to be developed because chemical nematicides are not acceptable in organic farms. Nowadays, bioagents such as rhizosphere bacteria are important to the use for the management of nematodes, since they are eco-friendly, ease to apply, not expensive and are available to farmers (Chitwood, 2002; Oka et al., 2002; Prasad et al., 2002). The potential of applying rhizosphere bacteria in management of root – knot nematodes has been reported by many authors

**Table 6**Effect of mixture of *Serratia* spp. and *Pseudomonas* spp. in comparison with oxamyl on galling and reproduction of *M. incognita* in relation to growth parameters of *Luffa aegyptiaca* under greenhouse conditions.

Treatments	Shoot weight (% increase)	Root weight (g) (%increase)	Root length (cm) (% increase)	Number of galls (% reduction)	Root Gall Index (GI)	Number of egg-masses (% reduction)	Egg mass Index (EI)	infective juveniles /100 g soil (% reduction)
Control- Luffa (without PPN or bacteria)	6.98 a	5.22 a	16.34 a	0.00 d	(0)	0.00c	(0)	0.00 g
Control –2 Luffa Treated with Infective juveniles of <i>M. incognita</i>	3.21 d	3.89c	11.91c	67.00 a	(4.0)	108.00 a	(5.0)	78.0 a
Control-3 Luffa Treated with eggs of <i>M. incognita</i>	4.30c	3.75c	12.17c	62.66 a	(4.0)	98.33 a	(4.3)	67.00b
Luffa + infective juveniles of root-knot nematode and mixture of <i>Serratia</i> spp. and <i>Pseudomonas</i> spp.	6.19 ab (91.90)	4.70b (20.82)	14.00b (22.85)	29.00b (56.71)	3.00b	65.33b (39.50)	3.66b	31.66c (60.25)
Luffa + eggs of root-knot nematode and mixture of <i>Serratia</i> spp. and <i>Pseudomonas</i> spp.	6.23 ab (44.88)	4.89b (30.40)	14.60b (19.96)	21.66 d (65.43)	3.00b	54.33c (44.74)	3.66b	28.00 d (58.20)
Vydate 24% SL (Oxamyl) + infective juveniles of <i>M. incognita</i>	6.07 ab (89.09)	4.95b (27.24)	14.73 ab (23.67)	27.66 d (58.71)	(3.3)	31.66 d (70.68)	(4.0)	17.33 e (77.78)
Vydate 24% SL (Oxamyl) + eggs of <i>M. incognita</i>	6.48 a (50.69)	4.87b (29.86)	14.60 ab (19.96)	31.00 d (50.52)	(3.3)	32.66 d (66.78)	(4.0)	9.33 ef (86.07)

\*Each value is a mean of five replicates.

\*\*Treatments in the same column with the same letters are not significantly different from each other (Tukey's test:  $\alpha = 0.05$ ). Reduction (%) =  $\frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$  Increase (%) =  $\frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$ 

(Ketabchi et al., 2016; Zavaleta-Mejia and Van Cundy, 1982). In addition to *Pseudomonas*, the bacteria belonging to the genus *Serratia* may make polyketides and strains of *P. fluorescens* are dominant plant rhizobacteria that promote growth in several cultivars (Beneduzi et al., 2012; Hokeberg et al., 1997), Bacterial nematodes which are attracted to feed on *P. fluorescens* in the rhizosphere may indirectly contribute to crop defense from abiotic or biotic stressors (Blanc et al., 2006). Paecilomyces *lilacinus* L. is known to be a colonizer of the rhizosphere and thus only infested by sedentary stage (eggs and females) from the cyst and the root-knot nematode. Therefore, this fungus will therefore not prevent initial damage to the root (Siddiqui et al., 1999; Siddiqui, 2000).

Microbial proteases have been suggested as nematode virulence factors (Siddiqui & Akhtar, 2009c; Tian et al., 2007). nematophagous fungal extracellular serine protease prevents nematode infection through the degrading cuticle defense (Meyer et al., 2004; Morton et al., 2004). Sikora (1992) reported that the rhizobacteria have great ability to control plant parasite nematodes. Also, plant growth-promoting rhizobacteria are beneficial bacteria colonizing the rhizosphere and the vegetable roots which enhance plant growth or plant pathogens protection (Kloepper and Ryu, 2006; Marleny et al., 2008; Sharma and Sharma, 2017). Our results indicate that the nematicidal properties of *Serratia* spp. and *Pseudomonas* spp. *in vitro* bioassay using *M. incognita* juveniles caused mortality and reduced egg hatching and suppressed number of galls, egg-masses and infective juveniles in soil to a lower degree and had positive effect on plant growth parameters under glass-house conditions. As well as, rhizosphere bacteria were more effective against eggs of *Meloidogyne* spp. than infective juveniles *in vitro* and under greenhouse conditions. The obtained results might agreement with those obtained by Mohamed et al., (2009) in a greenhouse experiment, where they showed that *P. fluorescens* had higher effect than *S. marcescens* in reduction of *M. incognita* population. Also, these findings corroborate the results obtained by Zhao et al., 2018 who showed that *P. putida* (Sneb 821), *P. fluorescens* (Sneb 825) and *Serratia proteamaculans* (Sneb 851) had high potential as a biocontrol agent against *M. incognita*, causing 99.17% juvenile mortality and 61.11% egg mortality. As well as, in the pot experiment displayed significantly higher levels of growth in root and shoot compared to control plants and reduced the number of galls and juveniles in the soil.

Moreover, application of *Serratia* sp., *Pseudomonas* spp. caused the higher mortality of second stage juvenile and had positive effect on plant growth parameters, besides decreased the nematode-related parameters, such as number of gall, egg and egg mass, as well as the reproduction factor (Mahgoob and El-Tayeb, 2010; Ketabchi et al., 2016; and Khan et al., 2012). Khan et al., 2012 showed that the suppressive effects of *Serratia* sp., *Pseudomonas* spp. on the nematode were less than that of fenamiphos. Although chemical nematicides such as carbofuran can have a high degree of effectiveness against root-knot nematodes, inducing resistance from microorganisms such as *S. marcescens* and *Pseudomonas* spp. may be considered a more natural and environmentally acceptable alternative to such chemicals. Viljoen et al., 2019 mentioned that plant growth-promoting rhizobacteria plant growth-promoting rhizobacteria strains caused second-stage juvenile paralyzes and have potential as biological control agents of *M. incognita* on carrots and tomatoes.

## 5. Conclusion

Inhibition of egg hatching and increasing juvenile mortality of *M. incognita* *in vitro* as well as reduction of galling and reproduction of such nematode *in vivo* strongly suggest the presence of compounds that possess ovicidal and larvicidal properties. On the other hand, the mechanisms of development, including nematicidal compounds and/or enhanced bacterial-induced defense mechanisms in plants can operate simultaneously or sequentially and can be important in the abolition of root-knot nematode. Moreover, it is recommended the use of organic modifications with productive strains of plant growth-promoting rhizobacteria because organic materials promote growth of pathogens competing with or destruction of species.

## 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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