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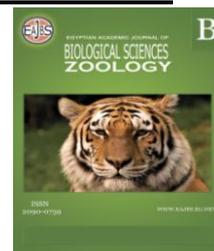


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## Suppress Root-Knot Nematode Infested Vegetable Fields Via Enhancing Rhizobacteria Inoculated in Composted Chicken Manure

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### ABSTRACT

In the last years, the concept of proper sustainability, cost potency of plant growth-promoting bacteria, and organic manures drive us to focus on their exploration in agriculture. Laboratory effectiveness of *Serratia* spp. and *Pseudomonas* spp. isolates against egg hatching and infective juvenile mortality of the root-knot nematode, *Meloidogyne incognita* was determined. As well as performance evaluation of composted chicken manure (CM) inoculated with bacteria under greenhouse and field conditions on vegetable plants, cucumber, pepper, potato and tomato besides banana. *In vitro* results after the 5<sup>th</sup> day of inoculation, *S. marcescens* (A10) and *Pseudomonas fluorescens* PF131 isolates gained the highest inhibition of egg hatching (85.18 and 75.36%). While juvenile mortality percentages were 43.30 and 35.10% with *S. marcescens* (A10) and *P. putida* (PP29), respectively. The nematicidal effect of the tested rhizobacteria on *M. incognita* inhibited egg hatching and juvenile mortality was directly proportional to isolates and exposure time. Under greenhouse conditions, the interaction between rhizobacteria and CM increased the fresh potato plant's shoot weight (31.78%) and root weight (28.94 %). Furthermore, the decrease in the number of galls, egg masses, IJs/100 g soil, and RF population significantly to 69.57%, 63.37%, 12.40, and 0.147, respectively. Under field conditions, the combination of chicken manure seems to be responsible for the sustainability of rhizobacteria *in situ* and extends their potency for a long period. After 20 days of the field application ( $1.8 \times 10^8$  cfu/ml; 15 L /Fed), the reduction percentages in IJs in blocks treated with the mixture of rhizobacteria and CM were 88.94, 87.92, 91.27, and 9276 % in tomato, cucumber, pepper, and banana plants, respectively. Our results indicate that the chicken manure inoculated with rhizobacteria is a promising biocontrol agent mixture for control of *M. incognita* in infected vegetables and orchard fields heavily infested.

### INTRODUCTION

Plant-parasitic nematodes play a major role in causing yield loss in many crops. Nearly one hundred valid species are in the genus *Meloidogyne* (Trinh et al., 2019) causing serious damage worldwide (Moens et al., 2009). *Meloidogyne* species especially root-knot nematodes (RKNs), *Meloidogyne incognita* (Kofoid & White)

Chitwood is the most damaging soil-borne disease. In agriculture soils of temperate and tropical climates, it causes an estimated \$100 billion loss/year worldwide (Oka *et al.*, 2000). Plant-parasitic nematode *M. incognita* is considered one of the main problems in tomato and other vegetables (cucumber and pepper) production mainly in the newly reclaimed sandy areas (Ibrahim, 1985). RKNs have high reproductive potential and a wide host range. Therefore, control of root-knot nematodes is partly difficult (Hussain *et al.*, 2016).

Since soil-borne diseases attack plant roots e.g., fungi and plant-parasitic nematodes are the common inhabitants of almost all the agricultural fields causing severe losses to crops. Many microbial have the capabilities of controlling these diverse groups of pathogens. Such as these bio-agents should be of practical significance and involved in crop production because of their safety and sustainability (El-Ashry *et al.*, 2019; Luc *et al.*, 2005).

Chemical nematicides are used effectively to manage RKNs. But chemical control has numerous problems, such as pollution of the environment, toxicity to man and animals besides natural balance disruption (El-Alfy and Schlenk, 2002). Nowadays, alternative methods for nematode management (Kerry, 2000) by using secondary metabolites, enzymes, and toxins of micro-organisms like rhizobacteria inhibits egg hatching and decline or directly kill infective juveniles (Siddiqui and Mahmood, 1999). Due to environmental concerns, the increased regulations limit the use of chemical nematicides, and currently encourage using nematicides alternatives for controlling RKNs (Nico *et al.*, 2004). Among the biological control agents that have been assessed are antagonistic bacteria for RKNs (Hegazy *et al.*, 2019; Kiewnick and Sikora, 2006; Mukhtar, 2018).

Bacterial biocontrol agents have a wide range of suppressive activities on phytonematodes by a variety of modes of action comprising producing toxins, antibiotics, and enzymes or interfering with nematode-plant-host recognition (Khan *et al.*, 2012). Several rhizobacteria species such as *Pseudomonas* spp. and *Serratia marcescens* have been isolated from soil, water, plants, and insects. These isolates reported playing an important role as biocontrol agents against *M. incognita* either by promoting plant growth or inhibiting nematode infectivity factors (Akhtar and Siddiqui, 2009; Khanna *et al.*, 2019; Ovcharenko *et al.*, 2010). Chitin is an important biopolymer mainly produced by fungi, arthropods, and nematodes (Merzendorfer and Zimoch, 2003). *Serratia marcescens* is a chitinase producer that degrades chitin to monomers “N-acetylglucosamine” (Kramer and Muthukrishnan, 1997) explaining its capability to inhibit the growth of several phytopathogenic and saprophytic fungi (Oka *et al.*, 2000, 1993). Under field conditions, bacterial biocontrol agents were tested against the juveniles of RKN, *M. incognita* associated with banana (Hegazy *et al.*, 2019) and vegetable crops (Mohamed *et al.*, 2009). Also, successful control by amending soil with phytocompost and chicken manure against RKNs (Akhtar, 1997; Ali *et al.*, 2018; Karmani *et al.*, 2011; Tanimola and Akarekor, 2014). The rhizosphere harbors a variety of microorganisms including bacteria, fungi, and arbuscular mycorrhizal fungi.

In view of the foregoing, the current study was conducted to investigate: a) the effects of these *Pseudomonas* spp. and *Serratia marcescens* isolates against egg masses and infective juveniles of the RKN, *M. incognita* under laboratory, b) confirmation of the nematicidal effect of *Serratia* spp. and *Pseudomonas* spp. inocula applied alone and mixed with chicken manure on potato growth and *M. incognita* reproduction under greenhouse and c) enhancing the ability of these bacterial isolates with well-composted chicken manure as a potentially curative treatment under field conditions. The obtained results from these treatments should donate a better

understanding of the complex interactions among southern root-knot nematode, *M. incognita* and the introduced combination of rhizobacteria and composted manure used under field conditions.

## MATERIALS AND METHODS

### Source and Culturing of the Root-Knot Nematode, *Meloidogyne incognita*:

The pure culture of *M. incognita*, isolated previously by El-Ashry et al. (2019), and maintained in the greenhouse on susceptible tomato cultivar “Super Strain B” as a source of inoculum. The species identification was verified based on the juvenile measurements and examination of the perineal pattern system of adult females according to Jepson (1987).

### Bacterial Isolates Source:

Three specific *Serratia marcescens* (A10, A15, and A20) and *Pseudomonas putida* (PP22 and PP29) and *P. fluorescens* (PF131) were obtained from Agric. Microbiology. Dept., Zagazig University. The bacterial isolates used in this study were tested previously by Hegazy et al. (2019) as promising candidates for control RKN, *M. incognita*.

### Extraction of Egg Masses and Second-Stage Juveniles:

A homolog egg masses were hand-picked with fine forceps from small galls on the infected tomato roots obtained from previously maintained pure culture and used to study the effect of the tested rhizosphere bacteria isolates on *M. incognita* egg hatching. The collected egg masses were surface sterilized in a 1:500 (v/v) aqueous solution of sodium hypochlorite for 5 min (Whitehead and Hemming, 2008).

A flask 600 ml volume contained small pieces of infected tomato roots in 200 ml of 0.5% sodium hypochlorite (180 ml water + 20 ml Clorox) was shaken vigorously for 3 minutes. The liquid suspension of free eggs resulted from a dissolving gelatinous matrix of egg masses (Hussey and Barker, 1973) that was poured through a 200-mesh sieve nested upon a 500-mesh sieve. The extracted free eggs were directly washed under a slow stream of tap water to remove sodium hypochlorite residues and incubated in Petri dishes containing distilled water and were incubated at 25±2 °C until hatching. Newly hatched juveniles were collected by using a micropipette.

### Laboratory Experiments:

#### Nematicidal Effect of The Tested Bacterial Isolates against *M. incognita*:

##### Effect of the Tested Six Bacterial Isolates on Egg Hatching:

Bacterial concentration was adjusted to about  $1.8 \times 10^8$  cfu/ml (Hegazy et al., 2019). Five fresh and uniform size egg masses were transferred to 10 cm diameter Petri dishes containing 10 ml of the bacterial suspension for the tested bacterial isolates mixed with mentioned egg masses for screening the bacterial biocontrol efficiency against *M. incognita* at different periods. The check treatment contained the only nematode in the bacterial growth medium free of bacteria, and all treatments were replicated five times. Treatments were left under an ambient temperature of 25± 2°C. The 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. The percentage of hatching inhibition was calculated in comparison with the control treatment, according to the following equation:

$$\text{Egg hatching inhibition (\%)} = \frac{\text{No. of hatched IJ}_2 \text{ in Control} - \text{No. of hatched IJ}_2 \text{ in treatment}}{\text{No. of hatched IJ}_2 \text{ in Control}} \times 100$$

### Effect of Tested Bacteria on Juvenile Mortality:

The stock suspension of emerged juveniles (IJs) was adjusted to the concentration of 1000 IJs/ml. Ten ml of the six bacterial isolates (*Pseudomonas* spp. and *Serratia marcescens*) were screened *in vitro* for their biocontrol efficiency against the second infective juveniles of *M. incognita*. A 0.2 ml volume of nematode suspensions (about 200 juveniles) was mixed with bacterial suspension and complemented to obtain a concentration of  $1.8 \times 10^8$  cfu/ml. The control treatment contained the only nematode amended with a bacterial growth medium free of bacteria. All treatments were replicated five times. Treatments were left under an ambient temperature of  $25 \pm 2^\circ\text{C}$ . Juvenile mortality was observed daily. Juveniles were considered dead when showed inactive straight posture or did not show any movement after prodding (Hooper, 1990; Nardo and Grewal, 2003) using a research microscope under 100X magnification in 1 ml. The number of dead juveniles was calculated in comparison with the control treatment. The mortality percentages were calculated due to the following equation:

$$\text{Mortality (\%)} = \frac{\text{No. of dead juveniles}}{\text{No. of exposed of juveniles}} \times 100$$

### Greenhouse Experiments:

Potato plants (*Solanum tuberosum* L) were chosen in the present study because of the severely attacked by *M. incognita* besides regional economic importance, especially in the newly reclaimed soils.

### Experimental Design:

Potato tubers were germinated in plastic pots of 30 cm diameter, filled with 5 kg steam-sterilized sandy soil (95.7% sand; 1.2% silt and 3.1% clay). When the five seedlings were approximately 20 cm in height, plants were inoculated with 5000 newly hatched IJs of *M. incognita* per pot. The inoculum was obtained from available pure culture formerly prepared and propagated in the greenhouse. In each pot, IJs were added by pipetting 5 ml of the nematode inoculum stock (1000 IJs/ml) into ten holes around the root system, then, the holes were covered with moist sandy soil.

The experimental treatments included *Serratia marcescens* (S) isolates, *Pseudomonas* spp. isolates (P), the combination of both [(*Serratia marcescens* (S) + *Pseudomonas* spp. isolates (P), (SP)], the combination of both bacterial isolates impregnate on composted chicken manure (SPCM), chicken manure (CM) alone and control treatment (negative “healthy plants without RKN or rhizobacteria” and positive control “RKN infection”. Each pot 5 kg received 50 ml ( $1.8 \times 10^8$  cfu/ml) of the bacterial suspension in treatments comprise of bacterial inoculation. Inoculation was conducted by incorporating the upper 5 cm of soil around the plant in the pot. While CM was applied to potato plants due to the recommended dose (6.64 tons/ feddan) at approximately a rate of 15 g/pot or 3 g/plant.

Each treatment was replicated five times. The pots were arranged in a randomized complete design in the greenhouse at  $25 \pm 3^\circ\text{C}$ , and all pots received a similar horticultural treatment. Fifty-five days after inoculation, plants were removed carefully from pots and soaked in tap water for one hour to facilitate removing adhering soil and to keep egg masses on the root surface. Data about plant growth included root weight (g), root length (cm), and shoot weight (g) were recorded. While the recorded parameters related to nematode development were number of root galls /root, gall diameter, number of egg masses /root, number of IJs/100 g and RF (Final population /Initial population). The root-knot index (RGI) was assessed using Taylor and Sasser (1978) scale of 0 = No galling; 1 = 1:2 galls; 2 = 3: 10 galls; 3 = 11: 30 galls; 4 = 31:100 galls and 5 = more than

100 galls. Gall diameter was also measured at its greatest diameter (Ali and El-Ashry, 2021). Also, samples of 100 g soil were processed for nematode extraction using a combination of sieving and Baermann trays technique (Hooper, 1990). During the steps of evaluation, roots were wrapped in tissue paper to prevent drying out and numbers of galls and egg masses were counted per root system. The parameters changing percentage (I %: increasing and R%: reduction percentage) imputed to “negative or positive” control value and the current equations were used:

$$\text{Reduction (\%)} = ((\text{Control} - \text{Treated})/\text{Control}) \times 100$$

$$\text{Increase(\%)} = ((\text{Treated} - \text{Control})/\text{Control}) \times 100$$

**Field Evaluation of Tested Bacterial Isolates for Suppression of the Root-Knot Nematode:**

Three vegetable plants (Tomato, *Solanum lycopersicum* L.; Cucumber, *Cucumis sativus* L. and Pepper, *Capsicum annuum* L.), as well as banana, were cultivated in open fields in the land of "El-Salheya Co. for Investment and Development" in El-Sharqia Governorate, Egypt during the period of March to June 2019. The numbers of root-knot nematodes in the rhizosphere were enumerated before bacterial inoculation or adding composted chicken manure using Ishibashi and Takii (1993) method after preparing homogenized soil suspension.

Bacterial suspensions of *Pseudomonas* spp. and *Serratia marcescens* or a mixture of them were prepared at a concentration of  $1.8 \times 10^8$  cfu/ml and applied to the soil at the rates of 10 and 15 L/feddan delivered to plants by fertigation. While control treatment was amended with bacterial growth media free of bacteria. Nematode enumeration in soil samples was conducted after 10, 15 and 20 days of treatments. Moreover, composted chicken manure was added with a rate of 3 g/plant in vegetables and 3 kg/banana plant then incorporated with the upper 5 cm of soil around each plant and irrigated before being applied with bacteria isolates to check the compatibility effect in suppressing populations of RKN in the selected vegetables and banana fields, respectively. Plants of the control treatment were left without chicken manure or rhizosphere bacteria.

Nematode extraction from soil samples was conducted as mentioned above. To identify nematode species, one milliliter of nematode suspension was pipetted into the Burker Hawksley counting slide, and nematodes were examined with the aid of the research microscope under 100X magnification. Based on the morphology of eggs and juvenile forms of root-knot nematode were identified (Ibrahim, 1985; Mai et al., 1996; Siddiqi, 1986). The recorded enumerations were expressed as the percent control due to treatment was calculated (Henderson and Tilton, 1955):

$$\text{Control Efficiency (\%)} = \left(1 - \frac{T_a \times C_b}{T_b \times C_a}\right) \times 100$$

$T_b$  is the number of IJs extracted per sampling unit before treatment,  $T_a$  is the number extracted after treatment,  $C_b$  is the number extracted from the check plot before treatment, and  $C_a$  is the number extracted from the check plot after treatment of the tested plots.

**Analysis and structure of applied chicken manure**

The physical and chemical properties of applied chicken manure were determined. According to (Leege, 1998), pH, electrical conductivity, organic matter, organic carbon, total nitrogen, and total phosphorus were all measured.

**Statistical Analyses:**

Laboratory experiments were conducted using a complete randomized design. While greenhouse experiment treatments were distributed using a randomized block

design. Whereas the field experiments were laid out as a  $2 \times 5$  factorial in a randomized block design. All treatments were replicated five times, and the measured parameters were subjected to the analysis of variance ( $P \leq 0.05$ ) using Costat Statistical Software (Cohort software, Berkeley). Duncan's multiple range test was applied to compare the mean performances of different treatments.

## RESULTS

### *In vitro* Bioassay of Rhizobacteria Isolates:

The laboratory experiment guarantees an effective and rapid method for screening the effectiveness of different isolates and it is carried out under controlled conditions. Also, it measures the direct effect of tested rhizobacteria on target disease, *M. incognita* in the absence of plant or fluctuating environmental conditions.

**Table 1.** Effect of different bacterial isolates of *Serratia marcescens* and *Pseudomonas* spp. on the number of juveniles emerged from egg masses of *M. incognita* at different intervals of exposure *in vitro*.

Exposure period (Days)	Control	<i>Serratia marcescens</i>			<i>Pseudomonas</i> spp.		
		<i>S. marcescens</i> A10	<i>S. marcescens</i> A15	<i>S. marcescens</i> A20	<i>P. putida</i> PP22	<i>P. putida</i> PP29	<i>P. fluorescens</i> PF131
1	220.40 <sup>a</sup>	42.20 <sup>c</sup> (80.85)	57.80 <sup>b</sup> (73.77)	43.80 <sup>bc</sup> (80.13)	52.80 <sup>bc</sup> (76.04)	54.80 <sup>bc</sup> (75.14)	40.40 <sup>c</sup> (81.67)
3	436.60 <sup>a</sup>	59.40 <sup>d</sup> (86.39)	79.40 <sup>c</sup> (81.81)	59.20 <sup>d</sup> (86.44)	97.20 <sup>b</sup> (77.74)	88.40 <sup>bc</sup> (79.75)	83.00 <sup>bc</sup> (80.99)
5	595.00 <sup>a</sup>	88.20 <sup>e</sup> (85.18)	113.00 <sup>d</sup> (81.01)	142.60 <sup>c</sup> (76.03)	227.20 <sup>b</sup> (61.82)	166.00 <sup>c</sup> (72.10)	146.60 <sup>c</sup> (75.36)
7	946.20 <sup>a</sup>	115.40 <sup>d</sup> (87.80)	295.0 <sup>bc</sup> (68.82)	283.40 <sup>c</sup> (70.05)	312.80 <sup>b</sup> (66.94)	313.60 <sup>b</sup> (66.86)	298.80 <sup>bc</sup> (68.42)
10	1243.40 <sup>a</sup>	249.40 <sup>c</sup> (79.94)	363.8 <sup>b</sup> (70.74)	392.00 <sup>b</sup> (68.47)	363.80 <sup>b</sup> (70.74)	350.80 <sup>b</sup> (71.79)	353.40 <sup>b</sup> (71.58)
Mean	688.32	110.92 (83.89)	181.80 (73.59)	184.20 (73.24)	210.76 (69.38)	194.72 (71.71)	184.44 (73.20)

Reported numbers represent means of 5 replicates; Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water; Different letters in the same row indicate significant differences ( $P \leq 0.05$ ) according to Duncan's multiple range test.

### a- Inhibition Effect on Egg Masses:

Results in Table (1) demonstrated the inhibition of three *Serratia marcescens* and three *Pseudomonas* spp. isolates of rhizosphere bacteria on egg hatching of RKN, *M. incognita in vitro*. The nematicidal properties of the rhizosphere bacteria on egg hatching (Percentage inhibition in egg hatching) of *M. incognita* under laboratory conditions was studied after 1, 3, 5, 7 and 10 days. Laboratory bioassay on *M. incognita* eggs varied according to rhizobacteria isolates. All tested bacteria isolates showed a superior inhibition ( $P \leq 0.05$ ) after one-day incubation with nematode egg mass ranging from 73.77 to 80.85 % reduction with *S. marcescens* isolates while, *Pseudomonas* showed a fluctuated potency recorded 75.14 and 76.04 % inhibition percentage with *p. putida* isolates, whereas *P. fluorescens* (PF131) reduced hatching with 81.67 surpassed all *Pseudomonas* isolates and its potency equivalent to *S. marcescens* (A10) the most efficient isolates. On the other hand, increasing the incubation period to three days, the percentage of emerged juveniles was decreased obviously, and all treatments showed increasing in hatching inhibition recorded 86.44 % reduction in *S. marcescens* (A20) treatment.

After the 5<sup>th</sup> day of exposure, all bacterial isolates showed a slight reduction in egg hatching inhibition continued to the end of the experiment. The treatments showed

the high significant effectiveness of *Serratia* spp. especially *S. marcescens* (A10) general mean which surpassed all other tested bacterial isolates recorded 83.89 % hatching reduction as shown in Table 1 and illustrated in Figure 1. Other bacterial isolates attained lower general mean of egg hatching inhibition ranging from 69.38 to 73.59 % after 10 days incubation. It was true with the six tested bacteria at the five times exposure significantly ( $P \leq 0.05$ ) decreased numbers of emerged juveniles when compared to distilled water treatment. On the other hand, the general mean for egg hatching inhibition percentage with all *S. marcescens* isolates (76.90) was higher than *Pseudomonas* spp. isolates (71.43) as shown in Figure 2.

**Table 2.** Effect of the tested *Serratia marcescens* and *Pseudomonas* spp. isolates on juvenile mortality of the RKN, *M. incognita* at different interval periods of exposure *in vitro*.

Exposure period (Days)	<i>Serratia marcescens</i> (% Mortality)			<i>Pseudomonas</i> spp. (% Mortality)-		
	<i>S. marcescens</i> (A10)	<i>S. marcescens</i> (A15)	<i>S. marcescens</i> (A20)	<i>P. putida</i> (PP22)	<i>P. putida</i> (PP29)	<i>P. fluorescens</i> (PF131)
1	26.00 <sup>bc</sup> (12.00)	23.60 <sup>c</sup> (10.80)	37.40 <sup>a</sup> (17.70)	40.20 <sup>a</sup> (19.10)	36.40 <sup>a</sup> (17.20)	33.00 <sup>ab</sup> (15.50)
3	47.40 <sup>bc</sup> (22.10)	44.00 <sup>c</sup> (20.40)	52.00 <sup>b</sup> (24.40)	61.20 <sup>a</sup> (29.00)	50.60 <sup>b</sup> (23.70)	60.60 <sup>a</sup> (28.70)
5	92.60 <sup>a</sup> (43.30)	68.20 <sup>d</sup> (31.10)	70.20 <sup>cd</sup> (32.10)	74.80 <sup>bc</sup> (34.40)	76.20 <sup>b</sup> (35.10)	72.80 <sup>bcd</sup> (33.40)
7	124.40 <sup>a</sup> (58.10)	108.80 <sup>bc</sup> (50.30)	101.80 <sup>d</sup> (46.80)	104.00 <sup>cd</sup> (47.90)	114.80 <sup>b</sup> (53.30)	109.60 <sup>bc</sup> (50.70)
10	156.20 <sup>a</sup> (73.50)	128.20 <sup>b</sup> (59.50)	117.00 <sup>d</sup> (53.90)	120.40 <sup>cd</sup> (55.60)	126.00 <sup>bc</sup> (58.40)	116.80 <sup>d</sup> (53.80)

Reported numbers represent means of 5 replicates; Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water; Different letters in the same row indicate significant differences ( $P \leq 0.05$ ) according to Duncan's multiple range test.

**b- Nematode Juveniles Activity:**

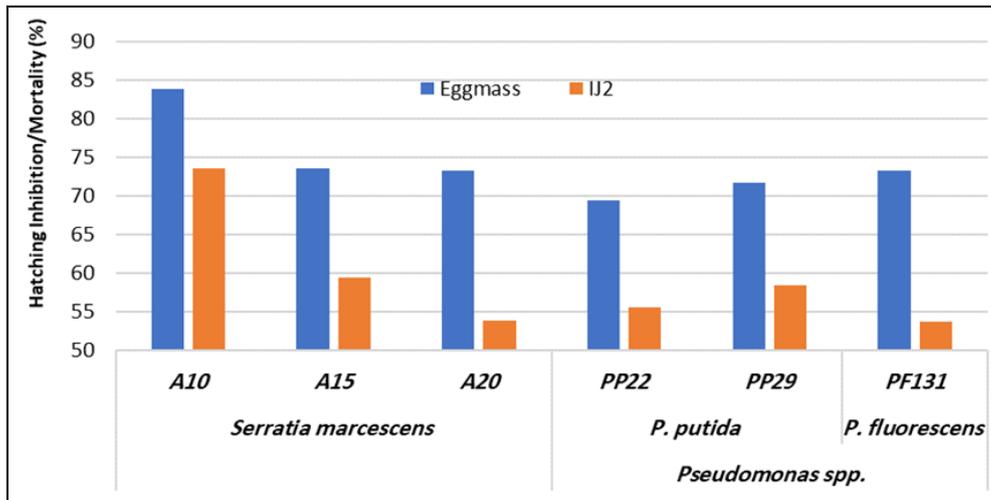
Second stage juvenile mortality of *M. incognita* incubated with the tested rhizosphere bacteria isolates *in vitro* was demonstrated in Table (2). The bacterial isolates effect on juvenile mortality of *M. incognita* varied greatly according to *Serratia* and *Pseudomonas* isolates. *Pseudomonas putida* (PP22) was the most effective after one and three days of incubation followed by *P. fluorescens* (PF131) while *S. marcescens* (A15) was the lowest effective one. After 5 days of exposure, the highest juvenile mortality was recorded with *S. marcescens* (A10) with 43.3% while, other rhizobacteria isolate recorded mortality ranged from 31.10 to 35.10 %.

After 10 days of exposure, *S. marcescens* (A10) recorded the highest significant mortality 73.50 %, followed by *S. marcescens* (A15) 59.50 % with no significant difference with *P. putida* (PP29) which recorded 58.40%. while the percent mortality reached 55.60, 53.90 and 53.80% with *P. putida* (PP22), *S. marcescens* (A20) and *P. fluorescens* (PF131), respectively. Among *Pseudomonas* spp. isolates, *P. putida* (PP29) was the most effective one.

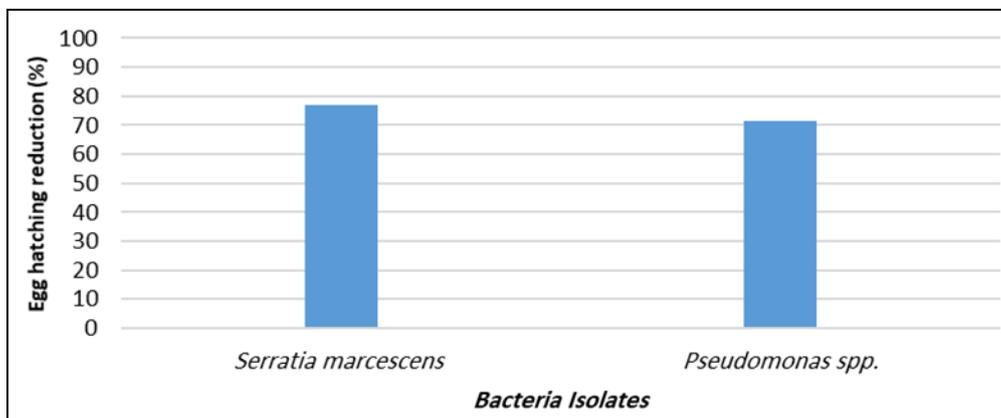
All tested rhizobacteria showed a significant percentage of juvenile immobilization of RKN. The high ovicidal effect (83.89%) of *S. marcescens* (A10) bacteria in reducing the number of J2 which surpassed the other entire tested isolates close up the potency with a little difference with those arises from the registered and marketed chemical nematicides. On the other hand, J2 showed a tolerant effect toward the tested isolated which achieved lower larvicidal potency compared with the ovicidal effect. *S. marcescens* (A10) overwhelmed all other testes isolates in juvenile mortality

recording 73.5% followed by *S. marcescens* (A15), 59.50 and *S. marcescens* (PP29) 58.40 %, while other isolates recorded mortality from 53.80 to 55.60 % mortality, as shown in Table (2) and Figure 1.

The tested rhizobacterial isolate achieved a lower larvicidal effect with little potency compared with registered and marketed chemical nematicides. Generally, the nematicidal effect of the tested bacteria on *M. incognita* juveniles was directly varied according to bacterial species, isolate and exposure time. Taken together, the six rhizosphere bacteria have larvicidal activity against J2 of RKN, *M. incognita* and can be regarded as a potential biocontrol agent.



**Fig.1:** Hatching inhibition of the egg masses and IJs mortality percentages of RKN, *M. incognita* after incubation for ten days at  $1.8 \times 10^8$  cfu/ml concentration of the tested rhizobacteria isolates.



**Fig.2.** *In vitro* general mean of egg hatching percentages in egg masses of RKN, *M. incognita* as influenced by rhizobacterial cell suspension after ten days.

### Greenhouse Experiment:

Based on physical and chemical analysis of applied chicken manure (CM) showed in Table (3), exhibited the lower moisture content recorded 13.60 %. CM nitrogen is present in the nitrate form with a balanced distribution of nitrogen, which reflects the long-term effect of nitrogen source. The tested manure showed significant convergence of the organic matter/carbon (C/N ratio) and ash content percentage. However, a low carbon content and high content of nitrogen (11.6: 1) in CM, extra content of nitrogen content (3.55%) was sufficient to microbial growth and plant

requirements. A high content of phosphorous (0.73%) in CM with the optimum option as a result of balanced NPK ratios which strongly able to meet the demand of soil flora growth and colonization besides enhance plant growth features without nutrition deficiency as showed in Table (4).

**Table 3. Physical and chemical characteristics of chicken manure.**

Parameter	Chicken manure
Density (kg/m <sup>3</sup> )	450
Moisture content (%)	13.60
pH (1: 10)	8.41
EC (1: 10) (dS/m)	3.66
Total – nitrogen (%)	3.55
Ammoniacal – N (ppm)	9.82
Nitrate – N (ppm)	86
Organic matter (%)	68.19
Organic carbon (%)	39.62
Ash (%)	31.92
C/N ratio	11.6: 1
Phosphorus (%)	0.73
Potassium (%)	1.42
NPK	1.1: 0.8 : 0.5

Data are based on a 105 °C dry weight basis.

Different treatments of rhizobacteria and chicken manure (CM) were demonstrated on potato plant growth and RKN, *M. incognita* development parameters under greenhouse conditions. The results indicate the friendly synergism between chicken manure and rhizobacteria as a biocontrol agent against the *M. incognita*. The interaction between rhizobacteria and CM as a source of organic matter and has the potential to decrease nematode parameters and promote plant growth parameters by manipulating the rhizosphere numbers of microorganisms as well as available nutrients in the soil of the rhizosphere. The increase in potato growth parameters varied according to the drenching application of bacterial isolates and CM treatments (Table 4).

For shoot weight, treated plants with *Pseudomonas* spp. (P treatment) and *S. marcescens* (S treatment) alone caused the least fresh shoot weight (8.77% & 8.84%). On the other hand, adding *S. marcescens* + *Pseudomonas* spp.+ chicken manure (SPCM treatment) to the infected potato plants improved root growth condition and significantly promoted shoot weight compared with infected plant + S+P (SP treatment) and infected plants treated only with CM treatment and increase percentages were 31.78, 30.56 and 16.21%, respectively. The highest significant increase ( $P \leq 0.05$ ) in root weight of potato plants was obtained with treatments of SPCM (28.94 %) and SP (28.73%), respectively. While P treatment was displayed the least increase (15.85 %) after CM treatment (18.18%). In concern of root length, percentages of increase were 31.40, 26.44, 18.18 and 8.26% with SPCM, CM, P and S treatment, respectively. Treatments with the rhizobacteria isolates combination of each genus displayed the least increase in potato plant growth parameters compared to check control infected with the nematode.

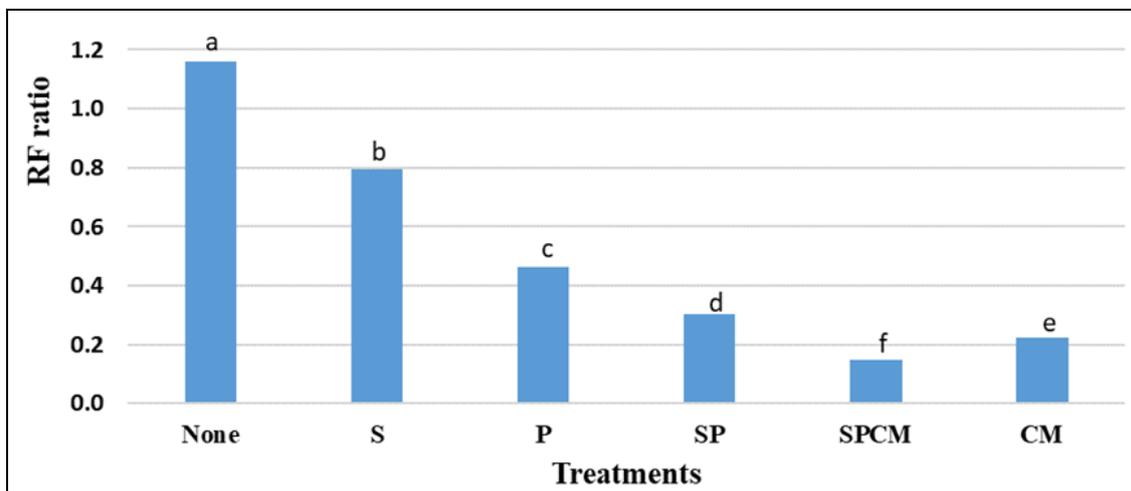
Potato plants are grown in the soil inoculated with different rhizobacteria genera (*Serratia* spp. and *Pseudomonas* spp.) and chicken manure significantly suppressed *M. incognita* infective juveniles in 100 g soil, root galling, and RF population, when compared with the control (Table 4). Treatments with each bacterial genus showed less effect on parameters of nematode infection and reproduction compared to those under

the dual effect of chicken manure and a mixture of rhizobacteria under greenhouse conditions but had a positive effect compared to the control. With treatments of CM and rhizobacteria (S+P), the number of galls, egg masses, IJs/100 g soil and RF population decreased significantly to 69.57, 63.37, 12.40 and 0.147%, respectively.

As for *M. incognita* development-related parameters, the application of rhizobacteria in combination with CM significantly decreased the number of galls, IJs and RF ratio as compared with other treatments. For instance, the percentage of decrease in root galls in treatments of SPCM, CM and SP were 69.57, 55.66 and 52.10%, respectively. While the application of rhizobacteria or CM alone was less effective against *M. incognita*, Table 4. As well a remarkable reduction resulted from the dual effect of CM and rhizobacteria in terms of IJs/100 g soil and RF ratio as compared to the control under greenhouse conditions. With treatments of SPCM, the number of galls, egg masses, IJs/100 g soil and RF population decreased significantly to 69.57, 63.37, 12.40 and 0.147%, respectively.

Regarding galls diameter measurements ascertained that SPCM treatment was a pioneering treatment avoiding completely the formation of galls greater than 4mm diameter accompanied with delaying and reduction of galls formation lower than 4 mm to achieve gall index 3.20. Treatments of SP, SPCM and CM showed a significant reduction in egg masses with 64.34, 63.37 and 57.55 %, while a dramatic reduction in RF ratio was obtained from SPCM (0.147) followed by CM (0.224) and SP (0.303), respectively. Treatments of P and S were the least effect and RF ratios increased to 0.462 and 0.795 as shown in Table 4 and Figure3, respectively.

Results obtained from greenhouse treatments indicate the friendly synergism between chicken manure and rhizobacteria individually and/or in combinations as a biocontrol agent against the *M. incognita* under greenhouse conditions. Both bacteria and chicken manure have the potential to decrease galls, egg masses and IJs of *M. incognita* and increase plant growth parameters such as fresh shoot weight root weight and root length.



**Fig. 3.** RF (Final population /Initial population) of different treatments of rhizobacteria on RKN in soil planted with potato plants in the greenhouse. (None: infected potato plants, S: *Serratia marcescens*; P: *Pseudomonas* spp.; SP: *S. marcescens* + *Pseudomonas* spp.; SPCM: *S. marcescens*+ *Pseudomonas* spp. + chicken manure; and CM: chicken manure).

**Table 4.** Potato plants growth response and *M. incognita* parameters (development and reproduction) after treatment of *S. marcescens* and *Pseudomonas* spp. isolates alone and in combination with chicken manure in greenhouse experiments.

Treatments	Shoot weight (g) (R%)	Root weight (g) (I %)	Root length (cm) (I%)	No. of root galls /root (R%)	Gall diameter			No. of egg-masses /Root (R%)	No. IJs /100 g	RF ratio
					> 4 mm	≤4-2mm	< 2 mm			
Potato plants without infection	8.932 <sup>a</sup>	5.788 <sup>a</sup>	17.04 <sup>a</sup>	0.0 <sup>c</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.00 <sup>d</sup>	0.00 <sup>f</sup>	0.00 <sup>e</sup>
<b>Infected potato plants</b>	6.242 <sup>d</sup>	3.828 <sup>c</sup>	12.10 <sup>c</sup>	61.80 <sup>a</sup>	6.00 <sup>a</sup>	40.0	15.8	103.20 <sup>a</sup>	78.20 <sup>a</sup>	1.16 <sup>a</sup>
Infected potato plants + <i>Serratia marcescens</i>	6.794 <sup>c</sup> (8.84)	4.630 <sup>bc</sup> (20.95)	13.10 <sup>d</sup> (8.26)	53.40 <sup>b</sup> (13.59)	1.60 <sup>b</sup>	30.4	21.4	80.60 <sup>b</sup> (21.89)	60.40 <sup>b</sup>	0.795 <sup>b</sup>
Infected potato plants + <i>Pseudomonas</i> spp.	6.790 <sup>c</sup> (8.77)	4.435 <sup>bc</sup> (15.85)	14.30 <sup>c</sup> (18.18)	48.40 <sup>b</sup> (21.68)	1.60 <sup>b</sup>	36.4	10.4	79.60 <sup>b</sup> (22.86)	53.60 <sup>c</sup>	0.462 <sup>c</sup>
Infected potato plants + <i>Serratia marcescens</i> + <i>Pseudomonas</i> spp.	8.150 <sup>b</sup> (30.56)	4.928 <sup>bc</sup> (28.73)	15.78 <sup>b</sup> (30.41)	29.60 <sup>c</sup> (52.10)	0.40 <sup>b</sup>	10.4	18.8	36.80 <sup>c</sup> (64.34)	35.20 <sup>d</sup>	0.303 <sup>d</sup>
Infected potato plants + <i>Serratia marcescens</i> + <i>Pseudomonas</i> spp. + chicken manure	8.226 <sup>b</sup> (31.78)	4.936 <sup>b</sup> (28.94)	15.90 <sup>b</sup> (31.40)	18.80 <sup>d</sup> (69.57)	0.00 <sup>c</sup>	8.60 <sup>b</sup>	10.2	37.80 <sup>c</sup> (63.37)	12.40 <sup>e</sup>	0.147 <sup>f</sup>
Infected potato plants + chicken manure	7.254 <sup>c</sup> (16.21)	4.524 <sup>bc</sup> (18.18)	15.30 <sup>b</sup> (26.44)	27.40 <sup>b</sup> (55.66)	0.20 <sup>b</sup>	10.6	16.6	43.80 <sup>c</sup> (57.55)	8.20 <sup>e</sup>	0.224 <sup>e</sup>

Each figure is the average of five replicates. Mean in each column followed by a different letter (s) significantly different from each other at P <0.05 according to Duncan's multiple range test. The number between parentheses refers to the parameters changing percentage (I %: increasing and R%: reduction percentage).

**Field Experiments:**

**Suppression of Root-Knot Nematode:**

The field trials are in harmony with successful treatments in laboratory and greenhouse experiments. Under field conditions, many changed factors such as soil fauna (Protozoa, earthworms and nematode species) and abiotic factors (soil physical and chemical properties) interact with the dynamics of root microbial communities which affect plant growth and resistance to pathogens and its potential for the management of root-knot nematodes. Results in Table (5) showed the population density of root-knot nematode in the field trials infested naturally by nematodes and treated with 5 treatments including S, P, SP and composted chicken manure inoculated with SP combination and naturally infected plants as check treatment. The rhizobacteria were applied at two rates of applications (10 and 15 L/fed) and chicken manure (CM) with some crops (3 g/plant with treatments of cucumber, tomato and pepper besides 3 kg/plant in treatments of banana.

Based on the statistical analysis of the field experiments, the application rate of 10 L/fed was no significant difference with 15 L/fed in most observation periods except pepper plant.

The highest augmentation was achieved in tomato block amended with CM and the mixture of rhizobacteria with application rate 10 and 15 L/Fed followed in descending order by those treated with the mixture of rhizobacteria alone, *Pseudomonas* spp. and *S. marcescens* after 20 days post-treatment. The numbers of infective juveniles per 250 g soil in rhizobacteria + CM treatment and rhizobacteria alone at 20 days post-treatment were 17.9 and 24.1, respectively.

The application rate of 10 L/fed may be the most suitable choice and economic threshold level with all the tested plants except pepper which was treated with 15 L/fed. So, Different rhizobacteria treatments exhibited the same trend with different crops. Check treatments recorded the highest significant population density of nematodes. Whereas S treatment showed the second significant rank followed by P treatment with a significant the combination of the chicken manure seems to be responsible for the sustainability of rhizobacteria and extends their potency for a long period. Therefore, at 10 days post-treatment in tomato crop, SP treatment (25.1) overwhelmed SPCM treatment (33.2). However, after 15 and 20 days pronounced decrease in numbers of IJs of *M. incognita* treated with rhizobacteria+ CM was detected. For instance, after 20 days

of application, in tomato, cucumber, pepper and banana plants, the mean number of IJs in blocks treated with rhizobacteria and CM were 17.9, 16.9, 15.1 and 20.3, respectively. Whereas the parallel values with mixture rhizobacteria alone were 24.1, 21.3, 19.1 and 24.5.

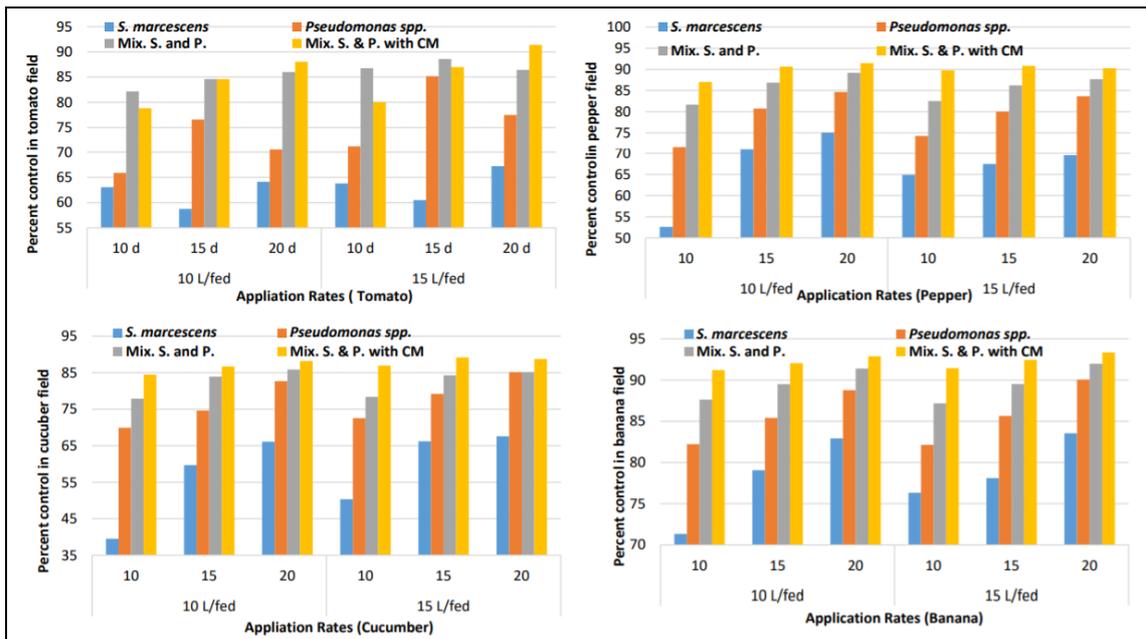
The probability of rhizobacteria treatment in reduction RKN population represented as control (%) which used initial and final density (before and after treatment) for each treatment, as shown in Figure 4.

t difference. On the other hand, the mixture of SP treatments significantly surpassed SPCM treatment without chicken manure.

**Table 5:** RKN, *M. incognita* population in the naturally infested field treated with two application rates of rhizobacteria *Pseudomonas* spp. and *S. marcescens*.

Crop	Nematode sampling period (d)	Application rate (L/fed)	Treatments					Mean	Interaction
			Control	<i>S. marcescens</i>	<i>Pseudomonas</i> spp.	<i>S. marcescens</i> + <i>Pseudomonas</i> spp.			
						Alone	manure		
Tomato	10	10	153.0	60.0	55.4	29.0	34.4	66.36 <sup>a</sup>	ns
		15	152.0	58.0	46.0	21.2	32.0	61.84 <sup>b</sup>	
		Mean	152.5 <sup>a</sup>	59.0 <sup>b</sup>	50.7 <sup>c</sup>	25.1 <sup>e</sup>	33.2 <sup>d</sup>		
	15	10	151.6	63.8	36.2	23.8	23.8	59.84 <sup>a</sup>	*
		15	159.6	64.4	24.2	18.6	21.2	57.60 <sup>a</sup>	
		Mean	155.6 <sup>a</sup>	64.1 <sup>b</sup>	30.2 <sup>c</sup>	21.2 <sup>d</sup>	22.5 <sup>d</sup>		
	20	10	157.2	60.6	49.8	23.6	20.2	62.28 <sup>a</sup>	*
		15	166.6	59.4	40.8	24.6	15.6	61.40 <sup>a</sup>	
		Mean	161.9 <sup>a</sup>	60 <sup>b</sup>	45.3 <sup>c</sup>	24.1 <sup>d</sup>	17.9 <sup>e</sup>		
Cucumber	10	10	111.6	77.2	38.4	28.2	19.8	55.04 <sup>a</sup>	*
		15	119.6	66.2	36.6	28.8	17.4	53.72 <sup>a</sup>	
		Mean	115.6 <sup>a</sup>	71.7 <sup>b</sup>	37.5 <sup>c</sup>	28.5 <sup>d</sup>	18.6 <sup>e</sup>		
	15	10	123.4	54.6	34.4	21.8	18.0	50.44 <sup>a</sup>	*
		15	130.4	49.4	30.4	23.0	15.8	49.80 <sup>a</sup>	
		Mean	126.9 <sup>a</sup>	52 <sup>b</sup>	32.4 <sup>c</sup>	22.4 <sup>d</sup>	16.9 <sup>e</sup>		
	20	10	136.6	49.4	25.2	20.6	17.2	49.80 <sup>a</sup>	*
		15	143.2	48.0	22.0	22.0	16.6	50.36 <sup>a</sup>	
		Mean	139.9 <sup>a</sup>	48.7 <sup>b</sup>	23.6 <sup>c</sup>	21.3 <sup>c</sup>	16.9 <sup>d</sup>		
Pepper	10	10	139.6	70.8	42.4	27.4	19.4	59.92 <sup>a</sup>	*
		15	154.8	57.0	41.8	28.4	16.6	59.72 <sup>a</sup>	
		Mean	147.2 <sup>a</sup>	63.9 <sup>b</sup>	42.1 <sup>c</sup>	27.9 <sup>d</sup>	18 <sup>e</sup>		
	15	10	170.4	52.2	34.6	23.6	16.8	59.52 <sup>a</sup>	*
		15	151.4	52.4	32.2	22.2	14.8	54.60 <sup>b</sup>	
		Mean	160.9 <sup>a</sup>	52.3 <sup>b</sup>	33.4 <sup>c</sup>	22.9 <sup>d</sup>	15.8 <sup>e</sup>		
	20	10	183.6	46.2	28.4	20.0	15.8	58.80 <sup>a</sup>	*
		15	162.2	45.0	24.2	18.2	14.4	52.80 <sup>b</sup>	
		Mean	172.9 <sup>a</sup>	45.6 <sup>b</sup>	26.3 <sup>c</sup>	19.1 <sup>d</sup>	15.1 <sup>e</sup>		
Banana	10	10	238.8	76.4	47.4	33.0	23.4	83.80 <sup>a</sup>	*
		15	254.6	62.0	46.8	33.6	22.4	83.88 <sup>a</sup>	
		Mean	246.7 <sup>a</sup>	69.2 <sup>b</sup>	47.1 <sup>c</sup>	33.3 <sup>d</sup>	22.9 <sup>e</sup>		
	15	10	272.0	57.4	40.0	28.8	21.8	84.00 <sup>a</sup>	*
		15	255.4	56.8	37.2	27.2	19.6	79.24 <sup>b</sup>	
		Mean	263.7 <sup>a</sup>	57.1 <sup>b</sup>	38.6 <sup>c</sup>	28 <sup>d</sup>	20.7 <sup>e</sup>		
	20	10	284	50.8	33.4	25.6	21.2	83.00 <sup>a</sup>	ns
		15	277	48.0	29.0	23.4	19.4	79.36 <sup>a</sup>	
		Mean	280.5 <sup>a</sup>	49.4 <sup>b</sup>	31.2 <sup>c</sup>	24.5 <sup>cd</sup>	20.3 <sup>d</sup>		

Mean in each column or row followed by a different letter (s) significantly different from each other at P <0.05 according to Duncan's multiple range test.



**Fig.4:** Results of controlling RKN, *M. incognita* in a naturally infested field planted with tomato, cucumber, pepper and banana received the tested bacterial isolates (*S. marcescens* “S”+ *Pseudomonas* spp. “P”) applied with two recommended rates (10 and 15 L/fed) alone or mixed after different application periods.

## DISCUSSION

The RKN, *Meloidogyne* spp. is one of the most economically important pests causing severe damage by the migratory stages (IJs) to a wide variety of crops. These nematodes affect crop yields directly through the alteration of the morphology of the root system that results from their feeding on, or invasion of, root tissues (Mukhtar, 2018; Mukhtar *et al.*, 2017). In this study, vegetables (tomato, cucumber and pepper) growth cycles were carried out in the same soil, and treatments of rhizosphere bacteria (*Pseudomonas* spp. and *Serratia* spp.) and animal manures like CM were applied at the start of each cycle might be suppressiveness of RKN to changes in structure and composition of soil fauna in the rhizosphere and within cultivated plant roots (Alam *et al.*, 1977; Rodriguez-Kabana *et al.*, 1987; Siddiqui *et al.*, 2001).

Plant growth-promoting bacteria (PGPB) were effective against a broad range of soilborne pathogens causing plant diseases like fungi (Rangeshwaran *et al.*, 2012) also, nematode disease is complex and reaping maximum yield in tomatoes under greenhouse conditions (Cetintas *et al.*, 2018; Ketabchi *et al.*, 2016; Khan *et al.*, 2012; Siddiqui, 2004; Viljoen *et al.*, 2019), or in tomato (Zhao *et al.*, 2018) and okra (Rao *et al.*, 2017) under field condition. Other natural enemies have specialized infection structures such as traps or adhesive spores or produce toxins to immobilize their prey or affect the behaviour of active nematodes to reduce their ability to attack roots (Kerry, 2000) and they can reduce nematode populations to density levels below the economic damage threshold.

### The Ovicidal and Larvicidal Effect of The Tested Bacteria:

Concerning the larvicidal effect of *S. marcescens* was approved in literature but with a fluctuated percentage on RKN IJs ranging from 54.7% to 96.25% (Mohamed *et al.*, 2009). While *S. marcescens* achieved the highest mortality percentages by more than 94% (Zaghloul *et al.*, 2015). Treatments in the current study with *Pseudomonas fluorescens* and *Serratia marcescens* each alone or in a mixture caused 50.5-90.3% inhibition on *M. javanica* egg hatch and second-stage juveniles (J2) activity.

*In vitro* studies revealed that the tested isolates of *Serratia* spp. and *Pseudomonas* spp. inhibit egg hatching of *M. incognita*. According to *M. incognita* life cycle, using rhizosphere bacteria to manage egg masses before hatching is an imperative method to avoid hatching up to 500 juveniles in the same root or roots nearby and start a new life cycle (Dropkin, 1989). (Mohamed *et al.*, 2009) elucidated the higher nematocidal activity of *S. marcescens* and *P. fluorescens*. Effect of rhizobacteria caused by direct parasitism on *M. incognita* eggs and IJs through the high production of certain enzymes i.e., protease, chitinase and gelatinase (Zaghloul *et al.*, 2015).

The potency of tested PGPB on egg hatching and IJs mortality may be related to the release of toxic metabolites (Siddiqui and Shaukat, 2004) amended with the production of gelatinase, protease, and chitinase enzymes, and were considered as biopesticides (Safni *et al.*, 2018).

Also, the reduced hatching and increased mortality of *M. incognita* second-stage juveniles were positively correlated with bacterial concentrations and exposure periods (Channppa *et al.*, 2008; Rangeshwaran *et al.*, 2012). Similarly, *in vitro* bioassay using juveniles of *M. incognita*, nematocidal properties of rhizobacteria varied according to tested bacterial isolates (Aravind *et al.*, 2009). As well as many mechanisms to suppress egg masses produced by *Meloidogyne* spp. other than parasitism for instance antibiosis, induction of host defense, etc. (Khan *et al.*, 2009). *Serratia marcescens* is identified to be a chitinase producer, which can inhibit the growth of several phytopathogenic (Phytonematodes) and saprophytic fungi (Okay *et al.*, 2013).

Six bacteria isolates had high larvicidal and ovicidal activity *in vitro* as a biocontrol agent against *M. incognita*, causing 99.17% juvenile mortality and 61.11% egg mortality (Zhai *et al.*, 2018). Also, (Kassab *et al.*, 2017) stated that *S. marcescens* was the most effective bacteria for degradation of chitin, under laboratory conditions against *M. incognita* juveniles and found positive relationships between the nematode mortality and each of the bacteria concentration and enzyme production (chitinase and alkaline protease) as well as its mutants.

A complete ban on egg hatch by bacterial extraction was almost 100% after 24 h (Bagheri *et al.*, 2014) or caused a 35-48% reduction in egg hatching and 32.2-48.8% increase in the mortality of IJs using the filtered culture of five isolates of *Pseudomonas fluorescens* and one isolate of *P. putida*, and *Serratia* sp. However, considerable variability was observed in the degree of virulence among different isolates/strains when tested *in vitro* (Haque *et al.*, 2018) as a biocontrol agent.

#### **Effectiveness of Rhizobacteria and Chicken Manure (CM):**

Six rhizobacteria isolates namely *Serratia marcescens* (A10), *S. marcescens* (A15), *S. marcescens* (A20), *P. putida* (PP22), *P. putida* (PP29) and *P. fluorescens* (PF131) have been reported in this study to play an important role as biocontrol agents against *M. incognita* either by promoting plant growth or by inhibiting nematode infectivity factors

Rhizosphere bacteria significantly increased fresh shoot weight, fresh root weight and root length (Akhtar and Siddiqui, 2009; Aravind *et al.*, 2009; Ketabchi *et al.*, 2016; Khanna *et al.*, 2019; Siddiqui, 2004; Siddiqui and Akhtar, 2008; Zhao *et al.*, 2018) and reduced galling, egg masses and reproduction of RKN, *M. incognita* IJs in greenhouse soils (No. of J2), from their extensive testing on vegetable crops including tomato, cucumber and pepper (El-Ashry *et al.*, 2020).

PGPB produced secondary metabolites such as hydrogen cyanide (*P. fluorescens*), ammonia, siderophore, and indole acetic acid, and solubilized phosphorus (Khan *et al.*, 2016; Siddiqui *et al.*, 2006; Zavaleta-Mejia and Van Gundy, 1989). As well as enzymes (gelatinase, protease, and chitinase) that are considered as biopesticides (Safni *et al.*,

2018) and numerous volatile metabolites (nematicidal properties) have potential against nematode stages or repellent activities (Zhai *et al.*, 2018).

Available organic matter for bacteria as a source of nutrition increased PGPB potency. application of *Serratia* sp. in combination with urea fertilizer had the greatest effect of galls and RF of *M. incognita*, as compared to other treatments (Ketabchi *et al.*, 2016). A combination of *P. fluorescens* with copper sulfate, ammonium molybdate, and urea was the best treatment in increasing tomato growth and decreasing nematode parameters (Saedi *et al.*, 2017).

Chicken manure was applied together with the tested rhizobacteria as curative treatments on potato plants infected with *M. incognita*, they reduced significantly ( $P \leq 0.05$ ) the number of galls, number of eggs, final nematode population and increased plant growth parameters such as fresh weight of root and stem above mixture of rhizobacteria. On the other hand, the most significant decrease in nematode parameters or the increase in plant growth parameters was achieved in the treatment of chicken manure with a mixture of rhizobacteria.

Particularly, animal manure such as poultry and goat when combined with *P. fluorescens* gave good results on tomatoes (Siddiqui, 2004). Integrating biocontrol agents like rhizobacteria with other cultural methods to achieve good results through multidisciplinary studies, especially in warm developing countries (Egypt) which have soil conditions (Physico-chemical and climatic) that differ from those encountered in temperate regions (Dong and Zhang, 2006).

Sikora and Hoffmann-Hergarten (1993) suggested 3 mechanisms for the reduction in nematode infection after soil treatment with rhizobacteria: release of toxic metabolites that reduce hatch and attraction, degradation of specific root exudates and enhancement mechanism leading to systemic resistance in plants. The primary mechanism for the control of RKN following application with rhizobacteria in the production of secondary metabolites. (Siddiqui and Shaukat, 2004) when used *P. fluorescens* together with *T. harzianum* in unsterilized sandy loam soils increased the production of nematicidal compounds in the crop rhizosphere and improved the antagonists against *M. javanica*.

Similarly, improvement in plant growth and decrease in galls, egg masses and  $J_2$  due to the release of toxic levels of ammonium, alterations in soil structure, stimulation of antagonistic organisms, and improved plant tolerance (Lazarovits *et al.*, 2001; López-Pérez *et al.*, 2005). Raw manures may be more effective than composted manure due to simultaneously increasing beneficial organisms or microbial activities and nematode populations as proposed by (Nahar *et al.*, 2006).

The nematicidal effect of organic manures on plant-parasitic nematodes could be attributed to producing ammonia, hydrogen sulfide, methane, fatty acids with low molecular weight like acetic, propionic, dimethylamine, trimethylamine, butyric acids and phenols (Oka *et al.*, 2000) and due to the increased number of antagonists in the rhizosphere (Shaukat and Siddiqui, 2001). Moreover, augmented rhizosphere defense due to the increase in plant enzymes including various peroxidase, chitinase,  $\beta$ -1,3-glucanases, lipoxxygenase and phenylalanine ammonia-lyase (Evans *et al.*, 2003; Howell *et al.*, 2000; Yedidia *et al.*, 1999).

#### **Effectiveness of Rhizobacteria and CM In Certain Infested Plants Against *Meloidogyne Incognita* Under Field Conditions:**

It should be noted that most studies on rhizobacteria as biocontrol agents against plant-parasitic nematodes combined with CM, were conducted under greenhouse conditions and there were rare studies that confirmed the efficacy of rhizobacteria in combination with animal manure under field conditions (Ali *et al.*, 2022; Siddiqui and Akhtar, 2008).

Under fluctuated field conditions, the efficacy of PGPB is altered according to numerous biotic and abiotic factors. Also, define the suitable application rates based on the host plant and the effective strains in reducing the infestation of root-knot nematode under field conditions (Korejo *et al.*, 2019) and could build high populations in soil infested with nematodes (Khatamidoost *et al.*, 2015).

The literature demonstrated that optimum inoculum densities were  $2.0 - 3.5 \times 10^6$ ,  $10^7$ ,  $10^8$ , and  $10^9$  cfu/ml according to (Haque *et al.*, 2018); (Condemarin *et al.*, 2018); (Amin, 2014) and (Norabadi *et al.*, 2014), respectively. Plant responses toward the inoculum amounts (application rates) vary according to the plant characteristics.

Curative treatments with biocontrol agents (Nameless, containing a specific strain of *Serratia marcescens*) required a long latent period (15 and 45 days of inoculation) to reduce of RKN population in infested soil compared with chemical nematicides in banana cv. Williams field and significantly increased plant growth parameters compared to the untreated plants (Eissa *et al.*, 2007).

Successful rhizosphere colonization by *P. aeruginosa* appears to be governed by high initial inoculum level which caused the greatest reduction in gall formation caused by *M. javanica*, and severity of the root-knot disease furthermore, 50 or 75% of soil moisture enhanced their biological control and growth-promoting potential (Siddiqui *et al.*, 2001).

In infested plots, the percentage J2 immobilization of RKN increased with the increase in concentration (Aatif *et al.*, 2012) and the rhizobacteria *P. fluorescens* appears to be an inexpensive cost-effective treatment as compared with nematicides and increased yield after fenamiphos in chickpea (Khan *et al.*, 2012).

Among used fertilizers, poultry manure resulted in less galling and nematode multiplication and poultry manure with *P. fluorescens* was the best combination for the management of *M. incognita* on tomatoes under field conditions (Siddiqui, 2004). As well, the maximum increase in the growth of nematode inoculated plants was detected in treatments of composted cow manure which was used with *Paecilomyces lilacinus* (Siddiqui and Akhtar, 2008). Moreover, it caused a high reduction in galling and nematode multiplication and may be used with composted organic manure for biocontrol of *M. incognita*. Similarly, *P. fluorescens* and *P. putida* may be used with cattle manure for the biocontrol of *M. incognita* on tomatoes (Siddiqui and Futai, 2009) and increased crop yield (Khan *et al.*, 2012).

### Conclusion

The mixture of *S. marcescens* and *Pseudomonas* spp. at rate 15 l/fed alone or/and the tested plant growth promoters and chicken manure capable of suppressing plant-parasitic nematodes, *M. incognita* and increasing beneficial soil fauna in vegetable crops. This method of nematode control is less expensive, safe and improves soil structure and fertility. The proposed mechanisms of chicken manure may be due to the release of toxic levels of ammonium which have nematicidal activities, although alterations in soil structure, the stimulation of antagonistic organisms, and improved plant tolerance also may play a role in the suppression of RKNs.

The current study provides evidence that the antagonistic effect of plant growth-promoting rhizobacteria and composted chicken manure under greenhouse and field conditions effectively reduced the number of galls and juveniles of *M. incognita* and consequently enhanced vegetable plants and banana growth and may be successful tools to use in place of oxamyl.

Further studies are required to verify these results, and other studies are needed to evaluate environmental factors and competition with other soil microorganisms to use suitable combinations of these biocontrol agents, to increase plant growth and resistance

to pathogens and its potential for the management of root-knot nematodes.

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## ARABIC SUMMARY

قمع نيماتودا تعقد الجذر في حقول الخضروات المصابة من خلال تعزيز فعالية تنشيط بكتيريا المجال الجذري عند تحميلها علي مخلفات الدجاج المتحلل

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<sup>1</sup>قسم وقاية النبات - كلية الزراعة - جامعة الزقازيق- مصر

في السنوات الأخيرة دفعتنا مفاهيم التنمية المستدامة والتكلفة والفاعلية لبكتيريا الجذور المشجعة لنمو النبات والأسمدة العضوية لاستكشاف دورها في الزراعة. لذلك تم تقييم فاعلية عزلات بكتيريا رايزوسفير (معزولة من قبل) وتعظيم كفاءتها لمكافحة نيماتودا تعقد الجذور *Meloidogyne incognita*. قيمت فاعلية عزلات جديدة من بكتيريا *Serratia spp.* و *Pseudomonas spp.* ضد فقس البيض وموت الطور المعدي لنيماتودا تعقد الجذور ميلودوجينا انكوجينينا معمليا بالإضافة الى التفاعل التنشيطي المأزر لمخلفات الدجاج المتحلل تحت ظروف الصوبة وظروف الحقل على نباتات الخضر مثل الكوسة والفلفل والبطاطس والطماطم بالإضافة إلى نباتات الموز.

أظهرت كلا من عزلة بكتيريا *S. marcescens* (A10) و *Pseudomonas fluorescens* (PF131) بعد اليوم الخامس من المعاملة نسبة عالية من تثبيط فقس البيض مقدارها 85.18 و 75.36 % على التوالي، بينما بلغت النسبة المئوية لموت الطور المعدي مقدارها 43.30 و 35.10 % مع كل من *S. marcescens* (A10) و *P. putida* (PP29) على التوالي. ويتوقف التأثير الإبادي لعزلات بكتيريا الريزوسفير والذي يتمثل في تثبيط فقس البيض وموت الطور المعدي لنيماتودا تعقد الجذور مباشرة على العزلات المستخدمة ومدة التعرض.

تحت ظروف الصوبة، أدى التفاعل بين بكتيريا الريزوسفير ومخلفات الدجاج لزيادة الوزن الغض للساق (31.78 %) والوزن الغض للجذور (28.94 %) وعلاوة على ذلك انخفضت معنويا عدد العقد الجذرية وكتل البيض وعدد الأطوار المعدي لكل 100 جرام تربة ومعامل تكاثر أعداد النيماتودا بنسبة مقدارها 69.57 % و 63.37 % و 12.40 و 0.147 على التوالي.

تحت ظروف الحقل أدى الجمع بين مخلفات الدجاج المتحلل الى تعزيز بكتيريا الريزوسفير وزيادة فاعليتها لتمتد لفترة طويلة، فبعد 20 يوم من المعاملة بمعدل تطبيق حقلي مقداره 15 لتر للفدان كانت النسبة المئوية للحفض في تعداد الطور المعدي في القطاعات المعاملة بكل من مخلوط بكتيريا الريزوسفير ومخلفات الدجاج المتحلل مقدارها 88.94 و 87.92 و 91.27 و 92.76 % في كلا من نباتات الطماطم والكوسة والفلفل والموز على التوالي. وتؤكد النتائج أهمية تلقيح مخلفات الدجاج المتحلل ببكتيريا الريزوسفير كأحد عوامل مكافحة البيولوجية الواعدة لمكافحة نيماتودا تعقد الجذور في حقول الخضر والفاكهة المصابة بشدة.