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Assessment of new rhizobacterial consortia to manage root knot nematode, Meloidogyne incognita infected banana plants

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Abstract

One of the most damaging plant parasitic nematodes affecting banana fields in Egypt is *Meloidogyne incognita*, which control is primarily performed with chemical nematicides. The aim of this study was to assess the effect of new native isolations of Plant growth-promoting rhizobacteria (PGBR) species isolated from El Sharqia, Egypt alone or rhizobacterial consortia on hatchability, mortality and their increasing banana plant growth parameters in vitro and potted plants. Results showed that the three rhizobacteria *Serratia*, *Bacillus* and *Pseudomonas* assay had potential effects on plant parasitic nematode, M. incognita eggs and infective juveniles with various significantly between rhizobacteria species. PGBR belong to *Serratia*, *Bacillus* and *Pseudomonas* tested species *in vitro* treatments were effective against egg hatching and mortality percentages of root knot nematode (RKN), *M. incognita* and *Pseudomonas* species showed more effectiveness than *Serratia* species. In greenhouse, all the growth and nematode induced parameters were significantly differed after PGBR treatment to banana plants. For instance, treated banana plants variety Grand Nain exhibited promoting growth parameters with comparable efficacy in reduction of *M. incognita* root galls and egg masses and the reproductive factor (RF) compared with non-treated plants with PGBR and gained similar results with oxamyl treatment. The current rhizobacteria assay confirmed previous results according to tested species also showing that *M. Incognita* reproduction decrease after treatment with the rhizobacterial consortia evaluated.

Keywords: control, egg hatching, J2 mortality, *Meloidogyne incognita* reproduction, Rhizobacteria, banana, plant growth

Full length article **Corresponding Author*, e-mail: ahmedscad2014@gmail.com

1. Introduction

Nematodes are a significant global pest of banana crops; in the subtropics, several phytonematodes attack bananas and other crops, producing economic harm that poses a huge risk to global crop productivity and the global economy [1] and impacts on crop yield with massive losses estimated by a billion of euros annually [2,3]. In addition, there is the loss incurred by the global community, particularly in regions that are grappling with shortages of food and raw materials, population expansion, and sluggish development [4]. Not just food crops are lost in these situations; they also have an impact on cash crops, which are a significant source of income for both farmers and the industry [2]. Plant parasitic nematodes are mostly controlled in agricultural settings by the use of resistant cultivars in conjunction with chemical, biological, physical, and cultural methods. Nematicides are hazardous and cause environmental damage, hence their usage is not recommended. In the context of Integrated Pest Management (IPM), biological management of nematodes is thought to be the best substitute for chemical control alone or in enhancing crop yields in conjunction with chemical control [5]. Among the countless microorganisms that live in the soil that makes up the environment are plant parasitic nematodes (PPNs). Nematodes are known to be present in large quantities in soil; an estimate of 100g of the bulk of soil contains 2000–4000 nematodes [6,7]. Most of the nematodes are obligate. Hence there must be a living plant tissue present to feed on to reproduce, grow and survive [8]. Recent taxonomy studies show over 25,000 species of nematodes though this number is still increasing with the progress of research and discovery of new species [9,10]. The majority of PPNs feed on roots although some nematodes feed on leaves and other upper parts of a plant. In general, depending on their feeding style, nematodes are classified as endoparasites for those that penetrate the host root to feed; and ectoparasites for those that feed externally by inserting their mouth stylet into the root cells [11].

Nematodes have been reported as beneficial organisms in some cases [12,13]. However, if poorly managed, PPNs can cause substantial damage to crops which results in yield and economic losses [14,15]. The annual yield loss caused by the plant parasitic nematode is estimated at 8.8% in developed countries and 14.6% in tropical and sub-tropical climates [16,17]. In vegetables only, damage by pathogenic nematodes can reach as high as 30% [18]. In addition to causing direct damage to crops, PPNs have been reported to accelerate diseases such as vesicular wilt and bacteria wilt [19]. As a result of most PPNs feed on roots, their symptoms may not appear in aboveground plant parts. This makes nematodes difficult to diagnose and reduces crop yield without plants showing any noticeable aboveground symptoms [20]. The other challenge is that most of the efforts to control plant pathogens, pests, and weeds have focused on the aboveground regions of plants such as stems, leaves, flowers, and fruits [21]. This makes prevention and control of PPNs so difficult as they are not integrated when controlling other pre-existing pathogens and pests. However, the consequences of synthetic chemicals have been detrimental to human health, animals, and environmental quality, resulting in agricultural and natural resource pollution [18]. Chemicals are also not reliable as they are unable to provide long-term protection and face so many strict regulatory measures [22]and [23]. As a result, researchers are working on finding the best alternative that will help to achieve sustainable management of plant parasitic nematodes. Many researchers have highlighted the potential of beneficial microbes to prevent and suppress the PPNs as part of biological control methods. The presence of beneficial microbes and their metabolites are enough to sustainably suppress or restrict the growth of PPNs [24]. In another research, rhizosphere microbiomes modulated by pre-crops were found to suppress plant parasitic nematodes and assist plants in developing immunity against PPNs [25].

Having looked at the importance of beneficial microbes and their formulated products to human health, environment as well as their effectiveness [14, 26] and [27], [28]. To guarantee that these bioproducts are fully utilized as the most effective substitute for pesticides made of synthetic chemicals, much work needs to be done. Thus, the processes by which advantageous microorganisms and their products inhibit the existence of PPNs are covered in this review. In Sharkia Governorate, Egypt, one of the major banana-producing regions in Egypt, the work intends to investigate the distribution and prevalence of phytonematode genera associated with bananas (*Musa* spp.) as well as the use of plant growth-promoting rhizobacteria (PGPR) species as a bio-control agent against PPNs, in particular Root Knot Nematode (RKN), *Meloidogyne incognita*.

2. Materials and Methods

This study was carried out in laboratory and greenhouse of Agric. Microbiology Dept. at the Faculty of Agriculture, Zagazig University, and Salahia Company fields, El Sharqia, Egypt, during the period of 2020 to 2023, in order to select efficient indigenous isolates of *Serratia*, *Pseudomonas* and *Bacillus* to be used in further studies as biological control of root Knot nematode in banana plants (*Musa* spp.).

2.1. Soil Core Samples

Soil core samples were collected following the soil core procedure with roots of banana plant grown in different locations in Sharkia governorate. The collected samples represented five locations namely: Abo kebesh (A), Elkhatara farm (K), wady elmolak (W), Belbase (B) and Salhia company (S). Soil and roots of banana grown in each soil sample were used for isolation of *Serratia, Pseudomonas* and *Bacillus* isolates. The isolation regions of soils are presented in Table (1).

2.2. Isolation and Purification of Bacterial Isolates

2.2.1. Isolation and Purification of Serratia

Several *Serratia* isolates were isolated using the pour plate method. One millilitre was plated on a nutrient agar plate following the dilution of multiple soil samples [29]. The plates were then incubated for 48 hours at 28±2°C. For additional research, the growing red colonies on the plates were removed from the plate under aseptic circumstances, filtered, and kept on the original medium at 4°C in a refrigerator. Using nutritional agar media, the chosen bacterial isolates were morphologically examined at various phases of growth. The general shape and colour of the colony, as well as its edge, elevation, surface, and pigment production, were recorded.

2.2.2. Isolation and Purification of Pseudomonas

Isolation of *Pseudomonas* was made by following the serial dilutions and pour plate method using the specific King's B medium **[**29**]**. The obtained soil was then shaken with 90 ml of sterile distilled water for 10-20 min. to obtain standard soil suspension. One ml of soil suspension from aliquot dilutions was aseptically added to sterile Petri dishes containing the sterile medium, and incubated at 28±2°C for 48 h. After incubation, well separated individual colonies with green and blue white pigments were marked and detected by viewing under UV light. The colonies were picked up and transferred to fresh King's B slants. For morphological characterization of the selected isolates, each was streaked on King's B Agar Petri dish and individual colonies were examined for shape, size, structure of colonies and pigmentation.

2.2.3. Isolation and Purification of Bacillus

Pour plate method was used for isolation of different *Bacillus* isolates. After the dilution of several soil samples, 1ml was plated in nutrient agar plate [30]. Plates were then incubated at 28±2°C for 24 h. The developing off Wight colonies on the plates were picked up under aseptic conditions, purified and maintained on the previous medium at 4°C in a refrigerator for further studies. The morphological examination of the selected bacterial isolates was carried out at different stages of growth on nutrient agar medium, and the general shape of colony, edge, elevation, and surface were recorded.

2.3. Microscopic examination of the isolated bacterial cells

The direct microscopic examination of stained smears of bacterial isolates was carried out for studying shape of the bacterial cells, gram staining, spore staining and motility (Table 2), were also examined according to the key of Bergey's Manual of Systematic Bacteriology [31] and [32].

2.4. Isolates characterization

Bacterial isolates screened for IAA production and chitin hydrolysis. Primary screening was performed by single line streak of their bacteria isolates in the center of CCA media [33].

2.4.1. Production of Indole Acetic Acid (IAA)

A modified colorimetric method was used for the determination of IAA [34]. Pure colonies of 30 isolates were obtained from Banana plants rhizosphere and grown in 100 ml Erlenmeyer flasks containing 25 mL nutrient broth with 5 ml (0.5%) of tryptophan (L-TRP) solution and incubated at 28 $^{\pm 2}$ °C for 24 h on a rotary shaker at 150 rpm. The cultures were then centrifuged at 4000 rpm for 20 min. Non-inoculated flasks were kept for comparison as control. One ml solutions of the supernatant were placed in test-tubes and mixed each with 2 mL Salkowski reagent (2% of 0.5M $FeCl_3$ in 35% perchloric acid). After 25-30 min incubation in the dark, the color of supernatant containing IAA turned red; the color absorbance was read using a spectrophotometer Model JENWAY No. 6405 UV/ Vis at 540 nm. Pure IAA was used for preparing the standards of 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 mg mL^{-}

2.4.2. Chitin hydrolysis

Chitin was studied as a major constituent of the outer layer of nematodes, and also protein (in the form of gelatin) is a major component of the nematode eggs, and its hydrolysis decrease the hatching from 98% to only 2%. Also, protein hydrolysis results in cell wall damage, which leaves the nematode vulnerable to attack by any biological, chemical and physical agents. Chitinase specific activity was determined according to the method of Singh et al. (1999). The activity was estimated based on the liberated N-acetyl-d glucosamine (NAGA) from colloidal chitin. Chitinase specific activity (U= 1 unit of chitinase) was defined as the amount of the enzyme releasing 1 µmol of NAGA/mg protein/h. Protease activity was determined according to the method of Fry et al., (1993). The unit of protease activity was defined as an increase in absorbance of 0.01nm [35].

2.5. Frequency of occurrence, population density and prominence value of plant parasitic nematodes infecting banana plants

Current survey has been planned and conducted in the investigated area (~ 67 ha) to throw light on the most important genera of plant parasitic nematodes (PPNs) infecting banana plants in the three main banana-producing areas of El Sharqia Governorate, Egypt

2.5.1. Banana (Musa spp.) Experimental Sites Description

The Grand Nain banana cultivar, found at Abo Kebesh, Fakous, and Elkhatara farms, is linked to soil-borne nematodes. This is where the survey site was established. The three areas under investigation are situated in Egypt's Sharqia Governorate, Elhalhia Elgadida. The primary focus of the study locations is bananas meant for both domestic and international markets. on order to prevent plant-parasitic nematodes, tested farms have agreed to using biopesticides like NemaStop®. The soils on these farms have also been free of chemical pesticides for 25 years. Both the conventional horticultural treatment and the primary drip irrigation system were put into practice.

2.5.2. Test plants

Soils and root samples of banana plants cv. Grand Nain were collected during two seasons (2019/ 2020 and 2020/2021). Infested soil samples were collected at the farm level. A total of 210 root and rhizosphere soil samples, as described by [36] were collected from the rhizosphere of each banana variety. According to [37], composite samples were collected from 40 Feddan from each banana plantations of surveyed varieties using a shovel directly kept in an icebox at approximately 18°C then sent to the laboratory for nematode extraction in Agriculture Microbiology Department, Faculty of Agriculture, Zagazig University, Egypt.

2.5.3. Analytical Reports

Reports about analysis of soils (Physical properties - granulometry, fertility, microelements, ratios of interest and cation ratios) of banana plants soils were scheduled in Tables (2). Using a shovel, composite samples were taken from banana crops that were contaminated. Three well-mixed subsamples made up each primary soil sample, and a uniform 1 kg sample was collected. Every soil sample was placed in clearly marked plastic bags, transported to the nematology lab in insulated chests, and kept there for further analysis at 12 °C. To study distribution and abundance of phytonematodes infecting banana cultivars in Sharkia Governorate, samples were collected from different cultivars i.e., Abo Kebesh (70 samples), Elkasara (75 samples) and Elkhatara Farm (65 samples. On the other hands, samples of irrigation systems were obtained from El Sharqia.

To study the population densities of plant parasitic nematodes (PPNs) and free-living nematodes (FLNs; Genus: *Rhabditis*), predacious nematodes (PNs; Order: Mononchida), a total of 210 root and rhizosphere soil samples, as described by [28], were collected from the rhizosphere of banana plantations. Composite samples were collected from infested vegetable fields using a shovel. Each main soil sample consisted of three subsamples mixed well, and a 1 kg sample was taken homogeneously. Each soil sample was put in labeled plastic bags and transferred in insulated chests to the nematology laboratory and stored at 12 °C for successive examination.

2.6. Source and inoculum of nematodes, RKN, M. incognita

As for the source and inoculum of nematodes, RKN, *M. incognita* second-stage juveniles (J2) were obtained by direct communication with [5], Department of Plant Protection, Faculty of Agriculture, Zagazig University, from an identified pure culture of *M. incognita* maintained on tomato seedlings planted in the greenhouse. RKN was then cultured and made ready for use in both *in vitro* and *in vivo* experiments.

To serve as an inoculum source, a pure culture of *M. incognita* was kept on the tomato -susceptible cultivar Super Strain B in a greenhouse. A nematode colony was established using a single egg mass. [38] state that the identification of species was based on juvenile measurements and an analysis of the adult females' perineal pattern system. Cut into 2-cm-long sections, infected tomato roots were added to a 600 ml flask along with 200 ml of 0.5% sodium hypochlorite (180 ml water with 20 ml Clorox). For three minutes, the flak with its tight cap was shook. Eggs were liberated from egg-masses as a result of the shaking partially dissolving the gelatinous matrix [39].

2.6.1. In vitro bioassay of rhizobacteria [(plant growth-promoting bacteria (PGPB)] used in the experiments on immobility and mortality of M. incognita juveniles

A 5-cm-diameter Petri plate with a total amount of 10 ml of the tested materials was used for the assessment. The three efficacious PGPB species of which there were eleven were made at the experimental concentration of 10^8 cfu/ml. This mixture was then put to 10 millilitres of distilled water (DW), along with 0.1 millilitres of nematode suspension, which included 100 newly hatched juveniles. The toxicity of the aforementioned strains and species of rhizobacteria, as well as the suggested application rate (RC) of oxamyl, were tested *in vitro* against *M. incognita* J2. Ten millilitres of the recommended rate (RC) of oxamyl and 10^8 cfu/ml of rhizobacteria were assessed. Additionally, 0.1 millilitres, or roughly 100 J2, were pipetted onto each Petri dish. The control treatment consisted of the 100 IJs maintained in 10 ml distilled water alone. Each treatment was replicated 5 times, and the dishes were kept at 24 ± 3 °C as optimum temperature for IJ survival [40]. Tables only included data from the first, third, fifth, seventh, and ten days after treatment. All dishes were carefully sealed with parafilm to prevent the solution from vaporising and were kept in a wet room. The number of emerging or dead juveniles was calculated daily using a research microscope (100 x magnifications). DW, or distilled water, served as the control. Using a research microscope set at ×100, the periodic examination was carried out by pipetting 0.5 ml of treatment solution into a Hawksley counting slide. Juvenile nematodes exhibiting inactive straight or (S) posture or not moving at all when prodded were scored as dead; all other forms of movement were classified as alive. [41]. Then treated nematodes were washed off on a 20-mm polyethylene sieve. After washing, a 5-ml nematode suspension was transferred to a clean dish and left at 24 ± 3 °C. After 24 h the nematodes were again monitored at 35x. Nematodes were considered immobile if they failed to respond to stimulation with a bristle. Dark blue staining indicated the death of every nematode that did not resurface in water after 24 hours. It was carefully observed that during evaluation, there should be a thin layer of extract to ensure that the nematodes have access to ample oxygen. The quantity of immobile nematodes in each treatment was recorded at different intervals of time. Egg immobility was measured three times, using four plates per experimental unit. Each measurement was conducted twice and examined separately. The following equation was utilized to compute the number of dead juveniles:

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

2.6.2. Ovicidal effect on free eggs of M. incognita

The same protocol was conducted to evaluate ovicidal and larvicidal effect of mentioned tested materials in vitro. Control treatment supplanted by 10 ml of distilled water only. *In vitro* tests of evaluation, 0.1 ml containing of 200 free eggs of *M. incognita* or 100 infective juveniles (IJs) were added to each Petri dish to test efficacy of bacterial species and oils as ovicidal or larvicidal.

All treatments were incubated at 24± 3° C and numbers of emerged or dead juveniles were calculated daily using a research microscope (100 x magnifications), but tables contained only data of 1 ,3 ,5 ,7 and 10 days after treatment. Percentage of hatching inhibition or dead J_2 in comparison with negative treatment (control) was calculated according to according to Abbott (1925) formula:

$$\text{Egg hatching inhibition }(\%) = \frac{\text{Number of hatched eggs in Control} - \text{Number of hatched eggs in Treatment}}{\text{Number of hatched eggs in Control}} \times 100$$

2.7. Statistical analysis

The experimental units were arranged in a randomized complete block design in the field experiment. Data were subjected to statistical analysis using MSTAT version 4, where analysis of variance and means was compared using Duncan's multiple range test at $p \leq 0.05$ probability.

3. Results and Discussion

Laboratory experiments were conducted to assess the potential of 30 rhizobacterial isolates isolated from banana plants rhizosphere to IAA and chitinase production, in order to select effective PGPR to be used as bio-control for plant parasitic nematode's of banana in pot experiments in the greenhouse.

3.1. Cultural characteristics of the bacterial isolates

The results in Table (3) showed that some of these isolates had flat and rhizoid elevation of colonies shape. They differed in color was observed in the colonies grown on nutrient agar plates 11 isolates namely SB2, SB3, SB10, SW3, SW6, SW7, ST1, ST5, ST8, SS2 and SS9 which showed red pigments. There were 9 isolates showed without pigments namely BB1, BB2, BW2, BW6, BW9, BT5, BT8, BS1and BS6. The isolates namely PB2, PB4, PB8, PW5, PW9, PW10, PT5, PS2, PS5, PS10 showed the fluorescent pigments on kings medium plates. Gram negative isolates included 21 isolates presented in two genera were identified at the species level as *Serratia* sp. and *Pseudomonas* sp. the remaining nineteen isolates were un-identified. Gram positive isolates Th included 9 isolates presented in one genera were identified at the species level as *Bacillus*. the remaining eight isolates were un-identified. Thirty of the chosen isolates were motility and the obtained results showed that some of these isolates had spores.

3.2. Screened of the bacterial isolates

The results in the present study showed that 30 isolates were isolated from rhizosphere soils of banana plants cultivated in four districts in El Sharkia governorate, Egypt. These isolates were screened for assays which are: IAA production and chitinase enzymes production and scheduled in Table 3.

Table 1: The selected locations of soil samples for isolation of *Serratia, Pseudomonas and Bacillus* isolates

Locations	*Serratia* isolates			*Pseudomonas* isolates			*Bacillus* isolates		
	Total No. of isolates	No. of chosen isolates	Nomenation	Total No. of isolates	No. of chosen isolates	Nomenation	Total No. of isolates	No. of chosen isolates	Nomenation
Belbies (B)	10	3	SB 2,3,10	10	3	PB 2, 6,8	10	2	BB 1, 7
Wady Elmolak (W)	10	3	SW 3,6,7	10	2	PW 5,9,10	10	3	BW 2 ,6,9
Tal Elkebier (H)	10	3	ST 1, 5,8	10	1	PT 5	10	2	BT 5,8
Salhia Comp.(S)	10	3	SS 2,5,9	10	3	PS 2, 5,10	10	2	BS 1, 6

Table 2: Physical properties - granulometry, fertility, microelements, ratios of interest and cation ratios of El Sharqia banana farm of Salyhia Company for agriculture investment

Physical properties		Granulometry
Texture *		Sandy Loam
Clay		4.00 %
Silt		40.0 %
Sand		56.0 %
Fertility		
Parameter	Result	Units
pH (1/2.5 Extract)	8.75	-
Electrical Conductivity (1)	3,450	µS/cm a 20ºC
Oxidizable Organic Matte	< 0.17	%
Active Lime	1.7	% $CaCO_3$
Nitrogen	< 155	mg/kg
Phosphorus	19.7	mg/kg
Available Calcium	28.2	meq/100 g
Available Magnesium	1.49	meq/100 g
Available Potassium	1.24	meq/100 g
Available Sodium	37.4	meq/100 g
	Microelements	
Parameter	Result	Units
Iron	< 4.00	mg/kg
Manganese	< 1.00	mg/kg
Copper	0.27	mg/kg
Zinc	< 0.20	mg/kg
Ratios of Interest		
Parameter	Result	Units
C/N Ratio	< 0.08	-
Cation Ratios		
% Available Bases		

Ca D (65%/41)	Mg D (25%/2%)	K D (10%/2)	Na D (0%/55%)

Table 3: IAA and Chitinase production by Bacterial isolates isolated from different Banana fields in El Sharkia governorate

Isolates	IAA equivalent (mg L^{-1})	Chitinase (U/mg)	Isolates	IAA equivalent (mg L^{-1})	Chitinase (U/mg)
SB2	17.47	3.05	PB2	22.03	4.67
SB3	21.81	3.89	PB4	8.99	0
SB10	18.19	3.64	PB8	31.20	3.95
SW3	17.46	4.32	PW5	15.61	0
SW6	15.82	0	PW9	19.33	3.90
SW7	33.66	5.82	PW10	16.28	0
ST1	17.59	4.58	PT5	19.47	3.56
ST5	12.81	0	PS2	35.36	5.68
ST8	16.14	0	PS5	23.65	4.92
SS2	26.02	4.32	PS10	9.81	0
SS5	10.04	2.5	BB1	7.15	0
SS9	15.63	1.9	BB7	34.76	5.65
BT5	21.4	6.94	BW2	41.55	4.24
BT8	27.29	5.98	BW6	20.35	3.62
BS1	14.00	0	BS6	20.32	3.48

Table 4: *Meloidogyne incognita* hatchability (egg hatching) evaluated *in vitro* studies

Treatments/ Rhizobacteria species	Egg hatchability after various time intervals				
	Day 1	Day 2	Day3	Day 5	Day7
Control (DW)	47.40a	92.60 a	124.40a	156.20a	193.20a
Bacillus	7.20b	17.60b	23.00b	47.60b	54.20b
	(84.81)	(80.99)	(81.51)	(69.53)	(71.95)
Serratia	3.20c	12.00c	17.60c	41.00c	45.60c
	(93.25)	(87.04)	(85.85)	(73.75)	(76.40)
Pseudomonas	2.20c	7.00d	10.60d	33.80d	46.00c
	(95.36)	(92.44)	(91.48)	(78.36)	(76.19)

*Reported numbers represent means of five replicate counts.
**Tested nematodes were observed daily for mortality up to 7 days.
***Different rhizobacteria in the same column with the same letters are not significantly different from each other (Duncan's multiple range test = 0.05).

Table 5: Efficacy of newly plant growth promoting rhizobacteria (PGPR) species during 7 days against J2s of *Meloidogyne incognita in vitro*

Treatments	Mortality percentages (%) of *Meloidogyne incognita* J_2s at various time intervals				
	Day 1	Day 2	Day 3	Day5	Day 7
Distilled Water (DW)	0.143[d]	1.143[d]	1.857[d]	2.571[d]	3.714[d]
Bacillus sp.	9.00[c]	18.714[c]	29.714[c]	43.714[c]	64.857[c]
Serratia sp.	11.857[b]	33.00[b]	52.571[b]	62.571[b]	72.857[b]
Pseudomonas sp.	20.00[a]	41.00[a]	61.857[a]	73.429[a]	84.143[a]

*Reported numbers represent means of five replicate counts.
**Tested nematodes were observed daily for mortality up to 7 days.
***Different rhizobacteria in the same column with the same letters are not significantly different from each other (Duncan's multiple range test = 0.05).

Table 6: Biocontrol of Root Knot Nematode (RKN), M. incognita by newly plant growth promoting rhizobacteria (PGPR) species in comparison with oxamyl and promoting the growth of banana Plants (Musa spp.) cv. Grand Nain under greenhouse conditions

Treatments	Promoting the growth of banana plants (% Increase)			Suppression of *M. incognita* reproduction (% Decrease)		
	Stem diameter (mm)	Shoot weight (g)	Root weight (g)	Root galls	Egg masses /Root	No.IJs/100 g soil
Heathy banana plants (without PPN or bacteria, negative control)	13.932a	22.04 a	9.988a	0.00e	0.00 e	0.00e
Banana plants infected with J2 of *M. incognita* (positive control)	10.842d	17.10e	8.828b	61.80a	103.20a	64.80a
Infected banana plants treated with *Serratia*	11.794c	18.10d	8.990b	53.40b	80.60b	50.20b
	(8.780)	(5.847)	(1.835)	(13.592)	(21.899)	(22.530)
Infected banana plants treated with *Pseudomonas* .	12.714c	19.30c	9.290ab	41.20b	80.60b	45.60b
	(17.266)	(12.865)	(5.233)	(33.333)	(21.899)	(29.629)
Infected banana plants treated with *Bacillus* .	11.590c	18.00c	8.969b	50.80b	82.80b	52.20b
	(6.899)	(5.263)	(1.597)	(17.799)	(19.767)	(19.444)
Infected banana plants treated with mixture of PGPR species.	13.150b	20.78b	9.928a	29.60c	36.80c	22.80c
	(21.287)	(21.520)	(12.460)	(52.103)	(64.341)	(64.814)
Infected banana plants treated with RC of oxamyl	11.284d	17.19e	8.972b	9.60d	12.80d	4.20 d
	(4.076)	(0.526)	(1.631)	(84.466)	(87.596)	(93.518)

*Root-knot index was assessed using 0-5 scale 0 of: No galling;1: 1- 2 galls; 2: 3- 10 galls; 3: 11- 30 galls; 4: 31- 100 galls and 5: more than 100 galls (Taylor and Sasser, 1978).

**Number between parentheses refer to the reduction percentage resulted from treatment calculated from the number of hatched juveniles after define incubation period; The same letter (s) in each row indicate no significant difference ($P \leq 0.05$) between treatments according to Duncan's multiple range test.

Table 7: Galling and reproduction of *Meloidogyne incognita* on banana plants cv. Grand Nain treated with newly plant growth promoting rhizobacteria (PGPR) species under greenhouse conditions

Treatments	Banana root parameters		*M. incognita* reproduction in pot soils		
	Galls numbers/root and Root Gall Index (RGI)*	Egg masses numbers/root and egg masses index (EI)	No. eggs/ 100g soil (Reduction %)	No. of IJs/100 g soil (Reduction %)	Reproduction factor (Rf) and % reduction
Infected banana plants with J2 of *M. incognita*	61.80a	103.20a	200.00a	64.80a	2.640
	(4.0)*	(4.60)	(0.00)	(0.00)	(0.00)
Infected banana treated with *Serratia*	50.80b	82.80b	85.40b	52.20b	1.376
	(3.20)*	(4.00)	(57.3)	(19.44)	(47.87)
Infected banana treated with *Pseudomonas*	41.20b	80.60b	46.20c	45.60b	0.918
	(3.40)*	(3.80)	(76.9)	(29.62)	(65.22)
infected banana treated with *Bacillus*.	53.40b	80.60b	61.40b	50.20b	1.116
	(3.80)*	(4.00)	(69.3)	(22.53)	(57.72)
Infected banana treated with mixture of PGPR species.	29.60c	36.80c	30.20c	22.80c	0.530
	(3.20)*	(2.60)	(84.9)	(64.81)	(79.92)
Infected banana treated with RC of oxamyl	9.60d	12.80d	5.80 d	4.20 d	0.100
	(2.00)*	(2.40)	(97.1)	(93.51)	(96.21)

Rf = Final population/Initial population. Final population= No. J2/ 100g soil +No. eggs/100g soil/ Initial population.

*Root-gall index (RGI) or egg mass index (EI) calculated according to Taylor and Sasser (1978) as: 0 = no galls or egg masses, 1=1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100 and 5 = more than 100 galls or egg masses. (1750-gram soil/pot)

$$\text{Reduction \%} = \frac{\text{Control-treatment}}{\text{Control}} \times 100 \qquad \text{Increase \%} = \frac{\text{Treated - Control}}{\text{Control}} \times 100$$

3.3. Efficacy of plant growth promoting rhizobacteria (PGPR) against free eggs hatchability

The nematicidal activity of three species of plant growth promoting rhizobacteria (PGPR) on free eggs of Root Knot Nematode (RKN), *Meloidogyne incognita* hatchability (egg hatching) were evaluated *in vitro* studies, and the obtained data was presented in Table 4. Egg hatching was evaluated at various time of exposure to tested PGPR. At first day, three tested PGPR *Serratia*, *Pseudomonas* and *Bacillus* showed nematicidal activity by affecting the hatching of free eggs of *M. incognita* and varied according to PGPR species. Results revealed that *Pseudomonas* exhibited the highest effect on 200 free eggs of *M. incognita* after 1st day of treatment with egg hatching inhibition 95.36 % followed by *Serratia marcescens* (93.25 %) and *Bacillus* (84.81 %), respectively. After 2nd day post treatment, the greatest number of hatching eggs in control treatment was 92.60 compared with 7.00, 17.60 and 12.00 eggs with *Pseudomonas*, *Bacillus* and *Serratia* with percent of egg hatching inhibition 92.44, 80.99, 87.04 % with *Pseudomonas*, *Bacillus* and *Serratia,* respectively. As time elapsed, clearly variations were exhibited with three tested PGPR. For example , after 3rd day post treatment , impact toxicity of rhizobacteria on *M. incognita* egg hatching was decreased resulting in correspondent decrease in inhibition %, and minimum egg hatching inhibition rate (maximum egg hatching rate was observed in distilled water as control treatment , 0% concentration) compared to 91.48 %, 81.51 5 and 85.85 % with *Pseudomonas*, *Bacillus* and *Serratia* ,respectively and the number of hatching free eggs were 124.40 , 10.60 , 23.00 and , 17.60 in control treatment (distilled water , DW) , *Serratia* , *Bacillus* and *Pseudomonas* ,respectively (Table 4).

There were significant differences ($P \leq 0.05$) among PGPR species and DW treatment in number of hatched juveniles from treated *M. incognita* eggs and number of egg hatching in DW reached to 193.20 compared to 46.00, 71.95 and 45.60 after 7 days of treatment with percent egg hatching inhibition 76.19 ,71.95 and 76.40 after exposure to PGPR species, *Pseudomonas*, *Bacillus* and *Serratia*, respectively. Based on these findings, PGPR species, *Serratia*, *Bacillus* and *Pseudomonas* could be arranged in their toxicity as nematicidal activity against *M. incognita* eggs as stated by greatest inhibition percentage with high impact in *Pseudomonas* treatments followed by *Serratia* whereas *Bacillus* showed the least effect.

A previous study [28] mentioned that numerous microorganisms are showing promising biocontrol action against *M. incognita*, such as *Serratia* sp. and *Pseudomonas* sp. *in vitro* and *in vivo* treatments. Moreover, these isolates particularly A5 and A9 showed the highest percentages of juvenile mortality of *M. incognita* (96% and 98%, respectively) after 24 hr. [41] exhibited that *Pseudomonas* isolates species were more effective than *Serratia* isolates in egg masses hatching inhibition percentages and hatchability of egg masses was inhibited by 64.51% when treated with *Pseudomonas* isolates, while decreased to 39.34% with *Serratia* isolates treatment. While the larvicidal effect of *Pseudomonas* isolates was 99.34% and 88.36 in *Serratia* isolates treatments. The tested PGPR showed mediated ovicidal and larvicide lower than oxamyl with surpassing the larvicidal effect. The secreted bioactive molecules produce by PGPR included siderophores, phytohormones, and chelators [42,43]. Many degradable enzymes such as chitinases, gelatinase, and protease were mentioned by numerous authors like [44,45] enable PGPR causing *M. incognita* egg hatching failure due to liquidizing gelatin matrix surrounded egg masses and causing damage to infective juvenile nematode by degrading cuticle which chitin involved in composition causing mortality or water imbalance or bleeding. Toxic molecules (simple or complex) were found in secondary metabolites include ammonia, hydrogen cyanide [46] as simple toxic molecules. Besides complex molecules e.g. prodigiosin is red pigment secreted as a secondary metabolite, produced by *Serratia*. This pigment is a natural alkaloid that has three pyrrole rings in chemical structures. Prodigiosin has been described as a stronger antioxidant activity [47,42,49]. These secondary metabolites may be volatile or nonvolatile [50,56] with direct nematicidal, fumigant, and repellent activities on egg or juveniles with multiple modes of RKN control [40]. So, the cultural filtrate of PGPR approved the nematicidal effect [54]. The bacterial extraction treatment of *Pseudomonas* strains UTPF5 kills almost 100% of the larvae hatching after 24 h and a complete ban on egg hatch [57]. Therefore, the nematotoxic effect of PGPB was positively correlated with bacterial concentrations, exposure periods, and chitinases production [48,54]. Consequently, it becomes clear that the difference between isolates of the same bacterial species is due to the qualitative and quantitative difference between the different isolates in the secreted extracellular metabolites that define the activity range (breadth or narrow) of bacteria on pests. To ensure the maximum effectiveness of the bacteria, it is recommended to mix them upon application.

3.4. Efficacy of PGPR against second stage juveniles ($J_{2}s$) of M. incognita

Nematicideal activity of mentioned plant growth promoting rhizobacteria (PGPR) against second stage juveniles (J_2s) of *M. incognita* was assessed *in vitro* treatments and their larvicidal effect were calculated by observation the mortality in J_2s at various time intervals after exposed to tested PGPR (Table 5). The effect of distilled water (DW), *Serratia*, *Bacillus* and *Pseudomonas*. A significant ($P \leq 0.05$) differences among tested treatments were observed. After one days, the least mortality percentage (0.143 %) of *M. incognita* J_2s was observed with control (distilled water, DW) followed by *Bacillus* (9.00 %) and *Serratia* (11.857 %) treatments application, while, the highest mortality percentage (20.00 %) of J2s was observed in treatment of *Pseudomonas* (15%). As time elapsed, the percentages of viability or mortality of the second stage juveniles (J_2s) was affected by rhizobacteria species (type) and their exposure times. In general, the mortality percentage increased with the progressive increase after exposure to tested PGPR. For instance, after 3 days of exposure, all bacteria species of tested PGPR were effective in causing larval mortality and larvicidal effect (the mortality percentages rates) was found to differ significantly ($P \leq 0.05$) among different PGPR species as illustrated in Table 5. After 5 days, the highest larvicidal effect was observed in treatment of *Pseudomonas* (78.36 %) being more efficacious and show high significant ($P \leq 0.05$) differences than the other PGPR species followed by *Serratia* (73.75 %) while the least mortality percent recorded in treatment of *Bacillus* (69.53 %). Larvicidal effect of tested PGPR species increased gradually to reach 76.19 %, 76.40% and 71.95 % compared to 2.571 (3.714) % in distilled water treatment after 7 days. From the obtained mortality, results are agreement with those obtained from ovicidal effect (egg hatching inhibition) and *Pseudomonas* ranked as the greatest larvicidal effect followed by *Serratia* while the least effect obtained from the second stage juveniles (J_2s) exposed to *Bacillus*.

Introducing plant growth-promoting bacteria (PGPR) enhance plant growth and increase tolerance against biotic and abiotic stresses as well as their role in reducing the Root-Knot Nematode population [55,37,4] alone or when use mixture of PGPR species besides combined PGPR species with other materials such as composted chicken and cow manures. Comparing the actual application (composted cattle manure), the accessible (oxamyl nematicide), and the desired (PGPR application) always determines how the actual application actually changes. Prior research established that combining various PGPR (species, isolates, and strains) was more feasible than using any one of them alone [56,57,58] because of the synergistic effect between combined bacteria. Treatment with *Serratia* alone resulted in the least effective low potency [59]. When comparing the nematicides applications with PGPB, research work showed initial incomparable potency [47,60] vanished soon as the active ingredient dissipated in the environment to return nematode population built-up in infested soil. As for application, PGPR ensures the relatively less potency, with the continuous protection as a result of colonization of the rhizosphere and built-up population gradually with some precautions. Although, some strains pseudomonas proved to be more

effective than fenamiphos nematicide in nematode egg production on olive seedlings roots [61]. This is maybe due to the presence of mutations that increase the production of secondary metabolites. The limited potency of compost may be due to the toxic levels of ammonium, [62,63] beside some metabolites include enzymes and minerals water-soluble resulted from fermentation during composting manure although the main role of manure by alterations in soil structure, the stimulation of antagonistic organisms, and improved plant tolerance.

3.5. Effect of isolates species on M. incognita Reproduction Parameters in comparison with oxamyl and promoting the growth parameters of banana Plants under greenhouse conditions

The measured plant growth parameters of infect banana plants with RNK, *M. incognita,* and received different PGPR species under greenhouse conditions are illustrated in Table 6. By scrutinizing data, all recorded plant growth parameters of banana were raised without exception when compared with control treatment (banana plants infected with J2 of *M. incognita* and non-treated with any of PGPR species. Stem diameter (mm) and shoot fresh weight in treatment of oxamyl treatment recorded minimum increase percentage 4.076 and 0.527%, when compared with PGPR species. While the mixture of PGPR species (*Serratia*, *Pseudomonas* and *Bacillus*) surpassed in stem diameter (21.287) and fresh shoot weight (21.520) the solitary application of PGPR species under greenhouse conditions. Although Fresh root weight (g) of banana plants showed insignificant differences ($P \leq 0.05$) between chemical nematicide, oxamyl (1.631%) and *Serratia* (1.835%) and *Bacillus* (1.597%), other PGPR species like *Pseudomonas* (5.333%) surpassed oxamyl effect when used alone. Moreover, maximum increase percentage in fresh root weigh was obtained in treatment of mixture of PGPR species to reach 12.460%. Therefore, the mixture of PGPR species showed prepotency with most growth parameters better than applied separately and pots treated with oxalyl. Therefore, the results confirm the feasibility and effectiveness of mixing PGPR species under field conditions better than the individual application or utilization of oxamyl recommended application rate (RC). There were significant differences ($P \leq 0.05$) between different PGPR treatments and oxamyl in infected root plant parameters (gall formation, egg masses and number of infective juveniles per 100 g Pot soil.

Current results revealed that all PGPR species or its mixture, reduced banana root galling (as shown by the number of galls and *M. incognita* reproduction as directed by the number of egg masses on roots and final number of IJs in soil). Under greenhouse conditions, pots treated with a mixture of *Serratia, Pseudomonas* and *Bacillus* gained the highest level in percent reduction of gall numbers (29.60) with percent reduction 52.103% after oxamyl treatment (9.60) with percent reduction 84.466 %, respectively. Among the tested rhizobacteria, a mixture of rhizobacteria (52.103) exceeded *Serratia* (13.592)*, pseudomonas* (33.333) and *Bacillus* (17.799) in percent reduction of galls. Moreover, pots treated with oxamyl exceeded pots treated with a mixture of rhizobacteria or each of them alone in suppression of egg masses numbers. For instance, while positive control gained the high number of egg masses (103.20), number of egg masses in plant treated with *Serratia* (80.60)*, Pseudomonas* (78.60) and *Bacillus* (82.80) with percent reduction 21.8995, 23.837 % and 19.767 % respectively. Whereas, mixture of PGPR species exhibited highest effect in suppression of number (36.80) and percentage reduction (64.341%) in egg masses. Regarding the efficiency of the tested PGPR species in comparison with oxamyl in the reduction number of *M. incognita* infective juveniles/100 g soil, results clearly showed that oxamyl effect (93.518%) followed by the mixture of rhizobacteria (64.814%) achieved the highest significantly effect in minifying numbers of infective juveniles/100 g soil compared to other tested treatments. Whereas *Bacillus* achieved the lowest significant effect (19.444%) compared to untreated Banana plants. Generally, PGPR species varied in their efficacy against galls and egg masses of *M. incognita* as well as in number of IJs/100 soil under greenhouse conditions. PGPR species reduced the production of *M. incognita* population (root galls, egg masses and IJs in pot soils) and increased the plant growth parameters of banana plants. Direct and indirect mechanisms described the promote in plant parameters. The direct mechanism describes PGPR as biofertilizers producing organic compounds that promote plant growth by increasing soil nutrient uptake. Indirect mechanisms refer to PGPR-dependent biocontrol, including the production of antibiotics, Fe chelators (called siderophores), and external cell wall-degrading enzymes (e.g., chitinase and glucanase) that perhaps hydrolyze the pathogen (i.e., nematode) cell wall [64]. The increase in plant tolerance via 1-aminocyclopropane-1-carboxylic acid deaminase expression, lowering the ethylene level in plants, is included among indirect mechanisms [65,66] Also, most PGPR isolates can produce hydrogen cyanide (HCN) as a potent inhibitor of *M. incognita* and Agrobacterium tumefaciens.

Effect of PGPR species on galls (root gall index, GI) and egg masses (egg masses index, EI) on banana roots parameters and *M. incognita* reproduction in pot soils under greenhouse conditions were illustrated in Table 7. Current results revealed that all PGPR species or its mixture reduced banana root galling (as shown by the number of galls and root gall index, GI) and *M. incognita* reproduction (as directed by the number of egg masses on roots and final number of IJs in soil or reproduction factor, RF). Under greenhouse conditions, pots treated with a mixture of *Serratia, pseudomonas* and *Bacillus* gained the highest level in percent reduction of gall numbers (50.52) after oxamyl treatment. Among the tested PGPR species, a mixture of rhizobacteria showed least root gall numbers (29.60) and root gall index (3.20) when compared with *Serratia* (50.80, 3.20), *pseudomonas* (41.20, 3.40) and *Bacillus* (53.40, 3.80. oxamyl exhibited highest effect in reduction of number of galls (9.60) and RGI (2.00) exceeded of all PGPR species under greenhouse conditions. Egg masses Index (EI) for treated PGPR species were 4.60, 4.0., 3.80, 4.00 and 2.60 with positive control plants, *Serratia, pseudomonas*, *Bacillus* and mixture of PGPR species, respectively. There were significant differences ($P \leq 0.05$) in J2 population density in pot soils between various treatments of rhizobacteria in comparison with chemical nematicide, oxamyl. Treatment with a mixture of PGPR species recorded the least number of J2 of *M. incognita* populations (22.80/100 soil cc (g)) with a

percent reduction 64.81% while, pots treated with the recommended dose (RC) of oxamyl produced 4.20 in J2 of *M. incognita* populations with percent reduction 93.51%. The reproduction factor (RF) of *M. incognita* decreased significantly in pots treated with oxamyl (0.100) with percent reduction 96.21%. Mixture of PGPR species next oxamyl in reduction of reproduction factor (Rf) and % reduction and achieved 0.530 & 79.92%, while treatments of *Serratia, pseudomonas*, *Bacillus* caused the moderate decrease as compared with positive control treatment with 1.376 (47.87%), 0.918 (65.22%) and 1.116 (57.72%), respectively.

Generally, the mixture of treated PGPR species surpassed rhizobacteria species alone in the reduction of gall formation, egg masses, and reproduction of *M. incognita* as well as, next oxamyl in suppression of *M. incognita* reproduction (RF) under greenhouse conditions. HCN-producing rhizobacteria increased all growth parameters of Banana [67]. Thus, extracellular secretion explained the culture filtrate potency tool for the management of RKN [68], and differentiation between PGPR isolates depends on extracellular secretion molecules and their amount. Interestingly, the potency of *pseudomonas*, and *Bacillus subtilis* isolates [69,70] may depend on secondary metabolites [71] The application of PGPR plays a vital role in the inoculum amount delivered to the rhizosphere. Therefore, it is not surprising that inoculation of seeds with *Bacillus* combined with nitrogen fertilization had no effect on RKN or common bean growth [72]. In line with this scenario, PGPR are nonpathogenic bacteria known to enhance plant growth and development in both non-stressed and stressed conditions by direct and indirect mechanisms [73]. Also, root colonization and rhizosphere competency are critical prerequisites for selecting successful PGPR candidate. Furthermore, PGPR in the rhizosphere can induce plant systemic resistance against RKN in Banana [71], increasing phenolic compounds, osmoprotectants, and organic acids [74]. Aside from the available nutrients needed for growth, either fixative nitrogen or solubilized phosphorus is delivered to colonized root plants besides producing phytohormones, e.g., auxins, cytokinin, and gibberellins [75], leading to plant vigor. The combination of PGPR and composted animal manures under field conditions enhanced the growth of Banana. This is maybe due to plant exudates released into the rhizosphere responsible for raising the parasite nematodes of microbes.

4. Conclusions

The current rhizobacteria assay confirmed previous results according to tested species also showing that *M. incognita* reproduction decrease after treatment with the rhizobacterial consortia evaluated. All the growth and nematode induced parameters were significantly differed after PGBR treatment to banana plants. For instance, treated banana plants variety Grand Nain exhibited promoting growth parameters with comparable efficacy in reduction of *M. incognita* root galls and egg masses and the reproductive factor (RF) compared with non-treated plants with PGBR and gained similar results with oxamyl treatment.

References

[1] M.M.M. Abd-Elgawad & T.H. Askary. (2015). Impact of Phytonematodes on Agriculture Economy. In: Askary, T.H. and Martinelli, P.R.P., Eds., Biocontrol Agents of Phytonematodes, CABI, Wallington, 1-49. https://doi.org/10.1079/9781780643755.0003

[2] R.A. Sikora & E. Fernandez. (2005). Nematode parasites of vegetables, Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, CABI Publishing, Wallingford, UK, p. 319-392.

[3] A.M. El-Sagheer. (2020). Status of phytonematodes in a main commercial banana production of Upper Egypt. Pakistan Journal of Nematology, 38(2):156-160.

[4] Y. Oka. (2010). Mechanisms of nematode suppression by organic soil amendments—A review. Appl. Soil Ecol., 44:101–115. https://doi.org/10.1016/j.apsoil.2009.11.003Get

[5] R.M. El-Ashry, M.A.S. Ali, A.E.A. Elsobki & A.A.A. Aioub. (2021). Integrated management of *Meloidogyne incognita* on tomato using combinations of abamectin, *Purpureocillium lilacinum*, rhizobacteria, and botanicals compared with nematicide. Egypt J. Biol. Pest Control, 31:93. https://doi.org/10.1186/s41938-021-00438-x

[6] E. Gamalero & B.R. (2020). Glick The Use of Plant Growth-Promoting Bacteria to Prevent Nematode Damage to Plants. Biology (Basel). Nov 7;9(11):381. doi: 10.3390/biology9110381. PMID: 33171782; PMCID: PMC7695023

[7] O. Topalović, M. Hussain & H. Heuer. (2020). Plants and Associated Soil Microbiota Cooperatively Suppress Plant-Parasitic Nematodes," Front. Microbiol., 11:1– 15. DOI: 10.3389/fmicb.2020.00313.

[8] L.E. Escobar, S.J. Ryan, A.M. Stewart-Ibarra, J.L. Finkelstein, C.A. King, H. Qiao & M.E. Polhemus. (2015). A global map of suitability for coastal Vibrio cholerae under current and future climate conditions. Acta Trop. 2015 Sep;149:202-11. doi: 10.1016/j.actatropica.2015.05.028. Epub 2015 Jun 3. PMID: 26048558.

[9] Z.Q. Zhang. (2013). Animal biodiversity: An update of classification and diversity in Zootaxa. 2013;3703(1):5–11. DOI: 10.11646/zootaxa.3703.1.3.

[10] A.A. Elling. (2013). Major emerging problems with minor *Meloidogyne* species," Phytopathology. 2013;103(11):1092–1102. DOI: 10.1094/PHYTO-01-13-0019-RVW.

[11] E.Van Den Berg, M. Marais & A. Swart. (2017). Nematode Morphology and Classification; 2017. DOI: 10.1007/978-3-319-44210-5_3.

[12] I. Hiltpold, M. Baroni, S. Toepfer, U. Kuhlmann1 & Ted C. J. Turlings. (2010). Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. The Journal of Experimental Biology, 213, 2417-2423

[13] B. Kurtz, I. Hiltpold, T.C.J. Turlings, U. Kuhlmann & S. Toepfer. (2009). Comparative susceptibility of larval instars and pupae of the western corn rootworm to infection by three entomopathogenic nematodes, BioControl, 4(2):255–262. DOI: 10.1007/s10526-008-9156-y

[14] M.M. Sikder & M. Vestergård (2020). Impacts of Root Metabolites on Soil Nematodes," Front. Plant Sci., 10:1–18. DOI: 10.3389/fpls.2019.01792.

[15] M.Z. Kayani, T. Mukhtar & M.A. Hussain. (2017). Effects of southern root knot nematode population densities and plant age on growth and yield parameters of cucumber," Crop Prot., 92:207–212. DOI: 10.1016/j.cropro.2016.09.007.

[16] J.M. Nicol, S.J. Turner, D.L. Coyne, L. Den Nijs & S. Hockland. (2011)."Genomics and Molecular Genetics of Plant-Nematode Interactions," Genomics Mol. Genet. Plant-Nematode Interact., DOI: 10.1007/978-94-007-0434-3.

[17] J.E. Palomares-Rius & T. Kikuchi (2013). Omics fields of study related to plant-parasitic nematodes," J. Integr. OMICS., 3(1): 1–10. DOI: 10.5584/jiomics.v3i1.120.

[18] T. Mukhtar, M.Z. Kayani & M.A. Hussain. (2013). Response of selected cucumber cultivars to *Meloidogyne incognita*," Crop Prot., 44:13–17. DOI: 10.1016/j.cropro.2012.10.015.

[19] J.F.W. Begum, N.M.I. Haque, T. Mukhtar & S.M. Naqvi. (2012). "Status of Bacterial Wilt Caused by Ralstonia Solanacearum in Pakistan," Pakistan J. Phytopathol., 2(3):11– 20.

[20] S.R. Gowen, P. Quénéhervé & R. Fogain. (2005). Nematode parasites of bananas and plantains, no; 2005.

[21] J.G. Ali, H.T. Alborn & L.L. Stelinski. (2011). Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes," J. Ecol., 99(1):26–35. DOI:

[22] C. Khanal & D. Harshman. (2022). Evaluation of summer cover crops for host suitability of *Meloidogyne enterolobii*," Crop Prot., vol.151, p. 105821, 2022, doi: 10.1016/j.cropro.2021.105821.

[23] C. Khanal & J.A. Desaeger. (2019). On-farm evaluations of non-fumigant nematicides on cucurbits," Crop Prot. 2019;133]:?105152, 2020, DOI: 10.1016/j.cropro.2020.105152.

[24] T. Kaur, S. Jasrotia & P. Ohri. (2016). Evaluation of in vitro and in vivo nematicidal potential of a multifunctional streptomycete, *Streptomyces hydrogenans* strain DH16 against *Meloidogyne incognita*," Microbiol. Res., vol. 192, pp. 247–252, DOI: 10.1016/j.micres.2016.07.009.

[25] A. Elhady, S. Adss, J. Hallmann & H. Heuer. (2018). Rhizosphere microbiomes modulated by pre-crops assisted plants in defense against plant-parasitic nematodes," Front. Microbiol., 9;1–9. DOI: 10.3389/fmicb.2018.01133.

[26] G.M. AbdelRazek and R. Yaseen. (2020). Effect of some rhizosphere bacteria on root-knot nematodes," Egypt. J. Biol. Pest Control, 30:1.DOI: 10.1186/s41938-020-00340-y.

[27] N. Thakur, S. Kaur, P. Tomar, S. Thakur & A.N. Yadav. (2020). Microbial biopesticides: Current status and advancement for sustainable agriculture and environment. Elsevier Inc; 2020.

[28] M.I. Hegazy, A.S. Salama, R.M. El-Ashry & A. Elrazik. (2019). *Serratia marcescens* and *Pseudomonas aeruginosa* are promising candidates as biocontrol agents against root-knot nematodes (*Meloidogyne* spp.). Middle East Journal of Agriculture Research, 8, 828–838

[29] R.M. Atlas. (2004). Handbook of microbiological media (3rd Edition). Boca Raton: CRC Press. 2051. doi: 10.1201/9781420039726.

[30] E.D. King, M.K,Ward, D.E. Raney. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. Journal of Laboratory and Clinical Medicine 44, 301–7.

[31] N.R. Krieg & J.G. Holt. (1984). Seen in Bergey's Manual of Systematic Bacteriology.Vol.1, Williams &Wilkins, Baltimore, U.S.A.

[32] P.H.A. Sneath, N.S. Mair, M. Elisabeth Sharpe, J.G. Holt, R.G.E. Murray, D.J. Chairman, M.P. Bryant, N.R. Krieg, J.W. Moulder, N. Pfennig & J.T. Staley. (1986). Section 12 gram-positive cocci, Bergey's Manual of Systemic Bacteriology, Vol. 2, Williams& wikins.USA**.**

[33] C. Priya, N. Jagannathan, P. Kalaichelvan. (2011). Production of chitinase by Streptomyces hygroscopicus VMCH2 by optimisation of cultural conditions. Int. J. Pharma Bio. Sci., 2 (2).

[34] H.N. Asghar, Z.A. Zahir, A. Khaliq & M. Arshad. (2000). Assessment of auxin production from rhizobacteria isolated from different varieties of rapeseed. Pakistan J. Agric. Sci., 37(3-4): 101-104.

[35] H. Curtis, J. Rosane Jones, K. Davies, E. Sharon & Y. Spiegel. (2011). Plant nematode surfaces, p. 116. In: Biological control of plant-parasitic nematodes: building coherence between microbial ecology and molecular mechanisms. K. G. Davies & Y. Spiegel, eds. Springer, Dordrecht.

[36] D.L. Coyne, J.M. Nicol & B. Claudius-Cole. (2014). Practical Plant Nematology: A Field and Laboratory Guide., 2nd ed. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin

[37] R.M. El-Ashry. (2021). Application of animal manure and plant growth promoting rhizobacteria as effective tools to control soil nematode population and increase crop yield in grapevine orchards. Egypt J. Agronematol., 20:34–52. https://doi.org/10.21608/ejaj. 2021.141311

[38] J.D. Eisenback. (1985). Detailed morphology and anatomy of second-stage juveniles, males, and females of the genus *Meloidogyne* (root-knot nematodes). An advanced treatise on Meloidogyne, 1: 47–77.

[39] R.S. Hussey & K.R. Barker. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep., 57:1025–1028.

[40] G.B. Dunphy & J.M. Webster. (1986). Temperature effects on the growth and virulence

of *Steinernema feltiae* strains and *Heterorhabditis heliothidis*. J. Nematolol., 18:270–272

[41] E. Nardo & P. Grewal. (2003). Compatibility of *Steinernema feltiae* (Nematoda: Steinernematidae) with Pesticides and Plant Growth Regulators Used in Glasshouse Plant Production. Biocontrol Sci. Technol., 13:441–448.

[42] R.M. El-Ashry, A.A.I. Ali & S.E. Awad. (2020). Enhancing application efficiency of pseudomonas spp. and *serratia marcescens* isolates against *Meloidogyne incognita* in tomato plants. Egypt Acad. J. Biolog. Sci. (f. Toxicology & Pest Control), 12:127–145. https://doi.org/10.21608/eajbsf.2020.116752.

[43] S. Chun, C.K. Sarpong, H. JinSong, S. Fei, Z. Jing, Y. Gang, L. LuLu, T. Dong, Z. Ying & D. ShiHuai. (2020). Accelerating phytoremediation of degraded agricultural soils utilizing rhizobacteria and endophytes: a review. Environmental Reviews, 28, 115– 127.

[44] R. Mandal, A. Adhikari, G. Rana & T. Mandal. (2017). Study of the useful characteristics of the red pigments of *Serratia marcescens* strains isolated from the soil. Journal of Applied Pharmaceutical Science, 7: 142–148.

[45] Z. Khatamidoost, S. Jamali, M. Moradi & R. Saberi Riseh. (2015). Effect of Iranian strains of *Pseudomonas* spp. on the control of root-knot nematodes on Pistachios. Biocontrol science and technology, 25: 291–301.

[46] I. Safni, L. Lisnawita, K. Lubis, A.R. Tantawi & S. Murthi. (2018). Isolation and characterization of rhizobacteria for biological control of root-knot nematodes in Indonesia. Journal of the International Society for Southeast Asian Agricultural Sciences, 24: 67–81.

[47] M.R. Khan, F.A. Mohidin, U. Khan & F. Ahamad. (2016). Native *Pseudomonas* spp. suppressed the root-knot nematode in *in vitro* and *in vivo*, and promoted the nodulation and grain yield in the field grown mungbean. Biological control, 101: 159–168.

[48] J.C.L. Lapenda, V.P. Alves, M.L. Adam, M.D. Rodrigues & S.C. Nascimento. (2020). Cytotoxic effect of prodigiosin, natural red pigment, isolated from *Serratia marcescens* UFPEDA 398. Journal of Microbiology, 60: 182–195.

[49] S. Rahul, P. Chandrashekhar, B. Hemant, N. Chandrakant, S. Laxmikant & P. Satish. (2014). Nematicidal activity of microbial pigment from *Serratia marcescens*. Natural Product Research, 28: 1399–1404.

[50] Y. Zhai, Z. Shao, M. Cai, L. Zheng, G. Li, D. Huang, W. Cheng, L.S. Thomashow, D.M. Weller, Z. Yu & J. Zhang. (2018). Multiple modes of nematode control by volatiles of *Pseudomonas putida* 1A00316 from Antarctic soil against *Meloidogyne incognita*. Front Microbiol, 9, 253–253. https://doi.org/10.3389/fmicb.2018.00253

[51] R. Rangeshwaran, G. Shivakumar & M. Nagesh. (2012). In vitro potency evaluation of *Pseudomonas* spp. against root-knot nematode, *Meloidogyne incognita*. Indian Journal of Nematology, 42: 156–160.

[52] N. Bagheri, Ahmadzadeh & R. Heydari. (2014). Effects of *Pseudomonas fluorescens* strain UTPF5 on the mobility, mortality and hatching of root-knot nematode *Meloidogyne javanica*. Archives of Phytopathology and Plant Protection, 47: 744– 752.

[53] B.S. Channppa, A.N. Srivastava & S.C. Dhawan. (2008). Effect of cell concentrations of four strains of rhizobacteriunl, *Pseudomonas fluorescens* on hatching and mortality of root-knot nematode. Meloidogyne incognita. Indian Journal of Nematology, 38: 189–195.

[54] S. Kassab, M. Eissa, U. Badr, A. Ismail, A. Abdel Razik & G. Soliman. (2017). Nematicidal effect of a wild type of *Serratia marcescens* and its mutants against *Meloidogyne incognita* juveniles. Egyptian Journal of Agronematology, 16: 95– 114.

[55] H.A. Osman, H.H. Ameen, M. Mohamed, R. El-Mohamedy & U.S. Elkelany. (2018). Field control of *Meloidogyne incognita* and root rot disease infecting eggplant using nematicide, fertilizers, and microbial agents. Egypt. J. Biol. Pest Control, 28(1): 40.

[56] J. Ara & S. Ehteshamul-Haque. (2016). Role of mungbean root nodule associated fluorescent *Pseudomonas* and rhizobia in suppressing the root rotting fungi and root knot nematodes in chickpea (*Cicer arietinum* L.). Pakistan Journal of Botany, 48: 2139–2145.

[57] W.M.A. El-Nagdi, M.M.A. Youssef, H. Abd-El-Khair, M.M.M.A. Elgawad & M.G. Dawood. (2019). Effectiveness of *Bacillus subtilis*, *B. pumilus*, *Pseudomonas fluorescens* on *Meloidogyne incognita* infecting cowpea. Journal of Nematology, 37: 35–43.

[58] Z.A., Siddiqui & M.S.Akhtar. (2008). Effects of organic wastes, *Glomus intraradices* and *Pseudomonas putida on* the growth of tomato and on the reproduction of the Rootknot nematode Meloidogyne incognita. Phytoparasitica, 36, 460.

[59] H.H. Ameen & M.M.A. Youssef. (2006). Effect of different management practices on *Meloidogyne incognita* root-knot nematode infecting cowpea. Pakistan Journal of Nematology, 24, 183–189.

[60] H.M. Raddy, A.F.A. Fouad, S.A. Montasser, M.F. Abdel-Lateef, A.M. & El-Samadisy. (2013). Efficacy of six nematicides and six commercial bioproducts against root-knot nematode, *Meloidogyne incognita* on tomato. Journal of Applied Sciences Research, 9: 4410–4417.

[61] S. Khalighi & G. Khodakaramian. (2012). Biocontrol of Meloidogyne javanica inducing olive root-knot under green-house conditions and by use of Fluorescent Pseudomonads. Iran J. Plant Prot. Sci., 43(2):323–32.

[62] G. Lazarovits, M. Tenuta, K.L. Conn. (2001). Organic amendments as a disease control strategy for soilborne diseases of high value agricultural crops. Australasian Plant Pathology, 30: 111–117.

[63] J.A. López-Pérez, T. Roubtsova & A. Ploeg. (2005). Effect of three plant residues and chicken manure

used as biofumigants at three temperatures on *Meloidogyne incognita* infestation of tomato in greenhouse experiments. Journal of nematology, 37, 489.

[64] P.H. Mhatre, C. Karthik, K. Kadirvelu, K. Divya, E. Venkatasalam, S. Srinivasan, G. Ramkumar, C. Saranya & R. Shanmuganathan. (2019). Plant growth promoting rhizobacteria (PGPR): a potential alternative tool for nematodes bio-control. Biocat Agricul Biotechnol 17:119–128. https://doi.org/10.1016/j.bcab.2018.11.009

[65] B.R. Glick. (2014). Beneficial Plant-Bacterial Interactions, 2nd ed.;Springer: Berlin/Heidelberg, Germany, p. 383. 2. https://doi.org/10.1007/978-3-030-44368-9

[66] A. Kumar, S. Singh, A.K. Gaurav, S. Srivastava & J.P. Verma. (2020). Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. Front Microbiol., 11:1216. https://doi.org/10.3389/fmicb.2020.01216

[67] A.F.A. El-Rahman, H.A. Shaheen, R.M.A. El-Aziz & D.S.S. Ibrahim. (2019). Influence of hydrogen cyanide-producing rhizobacteria in controlling the crown gall and root-knot nematode, *Meloidogyne incognita*. Egypt J. Biol. Pest Control, 29:1–11. https:// doi.org/10.1186/s41938-019-0143-7

[68] I.P. Sharma & A.K. Sharma. (2017). Effective control of root-knot nematode disease with Pseudomonad rhizobacteria filtrate. Rhizosphere, 3: 123–125. https://doi.org/10.1016/j.rhisph.2017.02.001

[69] J. Ludwig, A.B. Moura & C.B. Gomes. (2013). Potential of microbiolization of rice seeds with rhizobacteria for root-knot nematode biocontrol. Trop. Plant Pathol., 38:264–268. https://doi.org/10.1590/ s1982-56762013005000007.

[70] F. Sohrabi, M. Sheikholeslami, R. Heydari, S. Rezaee & R. Sharifi. (2018). Evaluation of four rhizobacteria on tomato growth and suppression of root-knot nematode, *Meloidogyne javanica* under greenhouse conditions, a pilot study. Egypt J. Biol. Pest Control, 28:56. https://doi.org/10.1186/s41938-018-0059-7

[71] J.J.F. Viljoen, N. Labuschagne, H. Fourie & R.A. Sikora. (2019). Biological control of the root-knot nematode Meloidogyne incognita on tomatoes and carrots by plant growth-promoting rhizobacteria. Trop. Plant Pathol., 44:284–291. https://doi.org/10.1007/s40858-019-00283-2

[72] B.H.C. Santos, R.C.F. Ribeiro, A.A. Xavier, J.A. Santos Neto and E.H. Mizobutsi. (2019). Nitrogen fertilization and rhizobacteria in the control of Meloidogyne javanica in common bean plants. J. Agric. Sci., 11:430–437. https://doi.org/10.5539/jas.v9n11p99

[73] B. Lugtenberg, and F. Kamilova, (2009). Plant-Growth-Promoting Rhizobacteria. Annual Review of Microbiology, 63, 541-556. http://dx.doi.org/10.1146/annurev.micro.62.081307 .162918

[74] K. Khanna, V.L. Jamwal, S.K. Kohli, S.G. Gandhi, P. Ohri, R. Bhardwaj, L. Wijaya, M.N. Alyemeni & P. Ahmad. (2019). Role of plant growth promoting Bacteria (PGPRs) as biocontrol agents of *Meloidogyne incognita* through improved plant defense of *Lycopersicon esculentum*. Plant and soil, 436, 325–345.

[75] A.H. Wani. (2015). Plant growth-promoting rhizobacteria as biocontrol agents of phytonematodes, in: biocontrol agents of phytonematodes. CABI, pp. 339–362. https://doi.org/10.1079/9781780643 755.0339.