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Serratia marcescens and *Pseudomonas aeruginosa* are promising candidates as biocontrol agents against root-knot nematodes (*Meloidogyne* spp.)

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ABSTRACT

Numerous microorganisms are showing promising biocontrol action against plant-parasitic nematodes, where they exploit divers modes of action. In this research, the effect of two candidate biocontrol bacteria (*Serratia* sp. and *Pseudomonas* sp.) was evaluated against the root-knot nematode *Meloidogyne incognita*. *Serratia* sp. and *Pseudomonas* sp. were isolated from different areas in El-Sharqia Governorate, Egypt, identified on biochemical and 16S rDNA bases, then tested for their *In-vitro* nematicidal effect against *M. incognita*. The isolates were then screened for chitin and protein hydrolysis, IAA production, phosphate solubilization, followed by a Bio-control field experiment to examine their actual biocontrol capabilities. Among all isolates, A5 and A9 showed the highest percentages of juvenile mortality of *M. incognita* (96% and 98%, respectively) after 24hr. Ten isolates were selected for evaluation of their products, where, isolates A5 and A9 recorded high values of chitinase and protease activities , but were not as high in IAA production or phosphate solubilization. According to phenotypic and genotypic characterization of A5 and A9 isolates, they were identified as *Serratia marcescens* subsp. *Marcescens* and *Pseudomonas aeruginosa*, respectively. In the field experiment, the mixture of the two bacteria showed highly significant nematicidal effect ($P \leq 0.5$) compared with the individual species. Also, the killing efficiency was negatively proportional with time increase. The increase in bacterial concentration added to the soil, resulted in substantially increasing the nematode reduction percentage. Our conclusion is that *Serratia marcescens* and *Pseudomonas aeruginosa* are promising biocontrol agents for *Meloidogyne* spp., and that commercial product of both of them need to be further evaluated.

***Keywords*:** Biocontrol, *Serratia marcescens, Pseudomonas aeruginosa*, root-knot nematodes, chitin and protein hydrolysis, IAA production, phosphate solubilization.

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are among the most economically important groups of plant-parasitic nematodes, causing damage and yield losses in most cultivated plants (Sasser and Freckman, 1987). Nematodes are microscopic non segmented worms and are ubiquitous in soil. Certain nematodes are parasitic to plants and can cause extensive damage to crops (Stone, 1977). Cultural practices, use of resistant cultivars and application of chemical nematicides are primary strategies for disease management, but yield losses persist with numerous crops. In addition, chemical nematicides are highly toxic compounds, and their use, which is limited to high-value crops, is detrimental to the environment (Curtis *et al.,* 2011).

Application of microorganisms antagonistic to *Meloidogyne* spp., or of compounds produced by these microbes, could provide additional opportunities for managing disease. Research in this area has resulted in commercial biocontrol preparations reported to act against root-knot nematodes (Stirling, 1991; Fravel, 2000). Continued studies are needed to identify additional organisms that reduce diseases caused by these nematodes.

Biological control of plant-parasitic nematodes has long been considered as an alternative to nematicides, especially because of the environmental and health concerns associated with these chemicals. In the past, research into the biological control of plant-parasitic nematodes has focused mainly on predators (e.g. nematophagous fungi) and parasitic microorganisms (e.g. *Pasteuria*

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penetrans) (Stirling, 1991). The effect of rhizobacteria (bacteria living in the soil under the influence of roots) on plant-parasitic nematodes has been poorly investigated (Oostendorp and Sikora, 1990; Soler-Serratosa *et al*., 1994). Among these rhizobacteria, several species of *Pseudomonas* have been reported to play important role as biocontrol agents against *Meloidogyne incognita* either by promoting plant growth or by inhibiting nematode infectivity factors (Akhtar and Siddiqui, 2009; Khanna *et al*., 2019; Meena *et al*., 2019). Also, *Serratia marcescens* is a bacterium widely isolated from soil, water, plants and insects, and exhibits pathogenic or saprophytic characteristics (Ovcharenko *et al.,* 2010). It is known to be a chitinase producer, which is considered a reason for its capability to inhibit the growth of several phytopathogenic and saprophytic fungi (Okay *et al*., 2013). Other studies have reported the association of *Serratia* with nematodes, and its killing ability under laboratory conditions (Torres-Barragan *et al.,* 2011). Research has focused on other bacteria for control of parasitic nematodes, with the objective of proposing strategies for inhibition of egg hatch, degradation of the hatching factor, or production of metabolites toxic to juvenile nematodes (Meadows *et al.,* 1989; Oostendorp and Sikora 1990).

Bacteria can be identified by using different methods: like phenotypic, biochemical, or nucleic acid and genetic assays. With biochemical identification, bacteria can be classified to species level, but the level of classification can vary between groups. The 16S rDNA gene sequencing technique has several advantages over phenotypic and biochemical identification. One of the advantages of using the 16S rDNA gene analysis is that this gene is present in all bacteria with conserved sequences that evolve slowly and correlate well with the evolutionary distance between species (Clarridge, 2004).
The main goal of the current study was isolation, evaluation, and identification of some potentially promising Egyptian strains of indigenous rhizobacteria by sequencing a fragment of the 16S rDNA gene and determines the ability of selected microbes to inhibitory effect on the root-knot nematode.

Materials and Methods

Samples collection:

Soil samples infested with Root-knot nematodes were collected from different areas in El-Sharqia Governorate, Egypt, in January 2018. The samples were kept in an ice box, and directly transferred to the laboratory for bacterial isolation.

Isolation of the bacterial species:

Isolation of Serratia:

Pour plate method was used for isolation of different *Serratia* species. After the dilution of several soil samples, 1ml was plated in nutrient agar plate (Atlas, 2004). Plates were then incubated at 28±2°C for 48h. The developing red 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 and color of colony, edge, elevation, surface, and pigment production were recorded.

Isolation of Pseudomonas:

The 10 cm rhizospheric soil particles loosely adhering to the roots were gently teased out and the roots. The obtained soil was then shaken with 90 ml of sterile distilled water for 10-20 min. to obtain standard soil suspension. Isolation of *Pseudomonas* was made by following the serial dilutions and pour plate method using the specific King's B medium (Kings *et al*., 1954). 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.

Maintenance and identification of Meloidogyne incognita:

Pure culture of *M. incognita*, isolated in a previous work (El-Ashry *et al*., 2018), was maintained in the greenhouse on the tomato susceptible cultivar Super Strain B for using as a source of inoculum. Species identification was verified based on the juvenile measurements and examination of perineal pattern system of adult females according to Jepson, (1987). Nematode eggs were collected from tomato roots in 0.5% sodium hypochlorite and purified using different size sieves, followed by hatching in Petri dishes at 25±1°C, according to Hussey and Barker (1973).

In-vitro nematicidal effect of the tested bacterial isolates against M. incognita:

A total of 340 isolates were obtained (150 as *Serratia* spp., and 190 as *Pseudomonas* spp.) by screening on their respective media. Ten bacterial isolates were screened *in-vitro* for their biocontrol efficiency against *M. incognita* at different time periods. Bacterial cell free supernatant was adjusted to about 1.8 x 10^8/ml, from which, 10ml aliquots were mixed with 0.1 ml of nematode suspensions (about 200 juveniles) in 9cm diameter Petri dishes. The control treatment contained only nematode in growth medium without bacteria, and all treatments were replicated three times. Treatments were left under ambient temperature of 25±2°C to determine the bacterial biocontrol efficiency. Observations of the juveniles mortality were recorded after 1, 6, 12, and 24 hr using a Hawksely counting slide and stereo microscope (AmScope 7X-90X). Juveniles showing inactive straight posture were counted as dead, and any other movement types were considered alive (Ishibashi and Takii, 1993). The percentages of mortality were calculated using the following equation:

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

Characterization of bacterial isolates:

Bacterial isolates were screened for several assays, which are: chitinase activity, indole acetic acid (IAA) production, phosphate solubilization and protease activity. A modified colorimetric method (Asghar *et al.,* 2000) was used for the determination of IAA. Non-inoculated flasks were used as control, and pure IAA was used for preparing the standard curve. The ability of the isolates to solubilize phosphate was assessed using Bunt and Rovira medium (1955), modified by Abd El-Hafez (1966). Clear zones appeared around the tested isolates were measured in mm. 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.

Biocontrol field experiment:

Banana trees were used as a host plant in a field experiment performed in the land of "El-Salheya Co. for Investment and Development" in El-Sharqia Governorate, Egypt during the period of April to June 2018. The numbers of root-knot nematodes in rhizosphere were counted before bacterial inoculation using the method mentioned before (Ishibashi and Takii, 1993) after preparing homogenized soil suspension. Bacterial suspensions of *S. marcescens*, *P. aeruginosa* or mixture of them were prepared at concentrations of 10^6, 10^7 and 10^8 cfu /ml, and applied to the soil with the rates of 5, 10, 15, or 20 L/feddan, while control treatment contained growth media without bacteria. Nematodes were counted after 10, 20, and 30 days. Total numbers of nematodes per 250 g soil were recorded as the percentage of nematode number decrease or increase, compared with the initial count in the soil.

Morphological and biochemical characteristics of the isolated bacteria:

Morphological assays of the isolated bacteria were done using direct microscopic examination of bacterial isolates for studying the shapes of the bacterial cells, Gram staining, spore staining and motility. Biochemical testes of these cultures were also performed according to the key of Bergey's Manual of Systematic Bacteriology (Garrity and Grimont, 2005).

Identification of the bacterial isolates using 16S rDNA gene sequencing:

Preparation of DNA samples for electrophoresis:

Bacterial DNA was extracted according to the standard procedures (Maniatis *et al.*, 1989). Exponentially growing cells (50 ml) were pelleted by centrifugation, re-suspended in 0.5 ml of lysis solution (25% sucrose, 50mM Tris – HCl, 100mM EDTA), and treated with 5 mg of lysozyme for 30 min at 37°C. Sodium dodecyl sulfate (SDS) and proteinase K were added to final concentrations of 1% and 100 µg/ml, respectively, and the samples were incubated at 56°C overnight. The bacterial DNA was extracted three times with phenol: chloroform: isoamyl alcohol (25:24:1) and twice with chloroform: isoamyl alcohol (24:1) then was precipitated and washed with 70% ethanol. The precipitate was re-suspended in 100 µl of Tris – EDTA buffer, and stored at -20°C until polymerase chain reactions (PCRs) were performed using PCR thermocycler (Biometra T-Gradient Thermoblock).

Amplification of the 16S rDNA gene fragment:

PCR amplification of the 16S rDNA gene was performed on the purified DNA extracted from the bacterial isolate (Clarridge, 2004). The forward primer was:5`- AGAGTTTG ATCCT GGCTCAG-3`, and the reverse primer was:5`-GGTTACCTTGTTACGACTT-3`.

Sequencing of the 16S rDNA Gene and Phylogenetic Analyses:

Sequencing of the PCR amplified product was conducted by GATC Company (USA) using ABI 3730xl DNA sequencer with forward/reverse primers. The traditional Sanger's technology was used combined with the new 454 technology (Thompson *et al.*, 1994). Phylogenetic tree was constructed using the neighbor-joining method. In this program, bootstrap analysis was used to evaluate the tree topologies by performing 1000 resemblances.

Statistical Analyses:

The data recorded in three replicates for the parameters in various treatments were subjected to the analysis of variance (ANOVA). The experimental design was a "factorial design 3x3" (Snedecor and Cochran 1980), and SPSS statistical package was used to evaluate the sources and magnitudes of variation. Duncan's multiple range test (DMRT) was applied to compare the mean performances of different treatments for the specific parameters under study and the rankings were denoted by superscripts in the relevant tables. Differences in means were compared at $P \leq 0.05$.

Results and Discussion

Screening of the bacterial isolates:

Out of the isolated bacteria, only 10 isolates (5 as *Serratia* spp., and 5 as *Pseudomonas* spp.) were selected on the basis of their nematode killing rates. They were screened *in-vitro* for their killing potency against *M. incognita* at different time periods, and the results are shown in Table (1). Isolates A5 and A9 showed the highest juveniles mortality percentages (96% and 98%, respectively) after 24hr, being significantly higher ($P \leq 0.05$) than the majority of the isolates in the killing rate. Following to these, isolates A4 and A6 resulted in 94% and 92% mortality rates, respectively. Isolate A9 showed early competitive results in a short time, scoring 40% juveniles mortality in only 1hr, and up to 74% after 6 h. This result was followed by isolate A5, while both of them (A9 and A5) reached 92% after 12h of inoculation.

These results are in accordance with Mohamed, *et al*. (2009), who have tested the *in-vitro* effect of several bacterial isolates on the mortality of root-knot larvae of *Meloidogyne* spp. The mortality rate reached up to 100%, which was shown with *Pseudomonas fluorescens*. Similar results were also reported by Rubina *et al*., (2018). Also, Meena *et al*., (2019) stated that *P. aeruginosa* is one of the commonly isolated bacteria from the insects, and that its pathogenicity is correlated with the production of proteolytic enzymes.

Table 1: *In-vitro* effect of bacterial isolates on the mortality of *M. incognita* juveniles.

Bacterial isolate	Mortality(%) after 1 h	Mortality (%) after 6 h	Mortality (%) after 12 h	Mortality (%) after 24 h
Control	00.0 a	5.6 a	14.3 a	17.3 a
A1	14± 2.6 b	52± 10.1 b	66± 10.0 b	72± 8.6 b
A2	18± 3.1 bc	48± 12.5 b	72± 8.3 c	78± 7.9 bc
A3	14± 2.2 b	52± 9.6 b	80± 7.0 d	88± 8.7 c
A4	18± 2.7 bc	56± 8.1 bc	88± 8.5 de	94± 9.2 d
A5	28± 2.6 d	70± 12.3 d	92± 0.0 e	96± 4.6 d
A6	20± 1.3 c	66± 7.7 cd	84± 6.5 d	92± 11.0 cd
A7	20± 4.3 c	64± 10.6 c	76± 10.6 c	84± 8.9 c
A8	26± 3.3 d	70± 9.0 d	78± 6.9 cd	86± 5.7 c
A9	40± 5.7 e	74± 12.3 d	92± 4.6 e	98± 7.5 d
A10	24± 5.6 cd	60± 11.4 c	74± 9.1 c	84± 8.6 c

[1] Reported numbers represent means of triplicate counts. Data are presented ± standard deviation.

[2] Different letters (a through d) represent significant differences ($P < 0.05$) among different isolate's effect (in the same column).

Isolates characterization:

The selected isolates were tested for their characters that are significant for nematode inhibition (chitinase and protease activity) as well as characters important for supporting plant growth (IAA production and P-solubilization). 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 (Curtis *et al.,* 2011).

Ten isolates were selected for evaluation based on their nematode killing rates, and the results are presented in Table (2). Among them, isolates A3, A4, A5, A6, A7, and A9 showed chitinase activity, where isolate A5 showed the maximum specific activity (5.54 U/mg), followed by isolate A9 (3.92 U/mg), then isolates A3 and A4 (3.46 and 3.31 U/mg, respectively).

Regarding IAA production, the highest levels were recorded in the case of isolates A7, A8, and A2, as they produced 24.3, 21.5, and 20.7 mg/l, respectively. Also, isolates A4, A1, A9, and A10 showed the highest levels of phosphate solubilization zone, being 13, 12, 12, and 11, respectively. All isolates showed protease activity at different levels, with A4, A5, A6, and A9, being the highest.

Isolate A5 showed the maximum activity in chitinase and high level of protease activity, while isolate A9 showed moderate levels of chitinase and protease activity, in addition to high levels of IAA production and the highest of P-solubilization. Therefore, those two isolates were used as promising candidates for further studies.

By comparing the data of Table (1) and Table (2), it can be inferred that chitinase activity is more relevant than protease activity in the killing effect against nematodes. The reason is that isolates A5 and A9 were the highest in chitinase activity as shown in Table (2), and were also the highest in killing rate in Table 1, despite that isolate A4 was higher than both of them in protease activity. The obtained results are in accordance with Zaghloul *et al*., (2015) who showed that *Serratia* has more chitinase and protease activity than *Pseudomonas*. Previous researchers (Bhattacharya *et al*., 2007) have shown that the species of *Serratia* and *Pseudomonas* and many other bacteria have the ability to produce chitinase. Also, Mercer *et al*. (1992) studied the effect of bacterial chitinase on the eggs of *Meloidogyne helpa*, where they found that chitinase causes premature hatching of nematode eggs, and thereby, can be used in the biological control of nematodes. Additionally, Rubina *et al*. (2018) have reported phosphate solubilization capacity for different species of *Pseudomonas*. Since isolates A5 and A9 (*Serratia marcescens* and *Pseudomonas aeruginosa,* respectively) showed the best results in Tables (1) and (2), they were selected for evaluating their efficiency in killing nematodes in a field experiment.

Table 2: Evaluation of the bacterial isolates as biocontrol agents and plant growth promoters

Bacterial isolates	Chitinase (U/mg)	IAA equivalent (mg L^{-1})	P-solubilization (diameter-mm)	Protease (U/mg)
A1	0	13.4	1.212.0	1.51
A2	0	20.7	10.0	1.37
A3	3.46	00.0	8.0	3.75
A4	3.31	00.0	13.0	4.60
A5	5.54	00.0	0.0	4.42
A6	2.55	00.0	7.0	4.25
A7	2.43	24.3	9.0	2.16
A8	0.00	21.5	9.0	2.87
A9	3.92	19.6	12.0	4.04
A10	0	11.9	11.0	1.83

Identification using 16S rDNA gene:

To substantiate the results of the phenotypic identification, two genera: *Serratia* sp. (isolate A5) and *Pseudomonas* sp. (isolate A9) were selected for molecular identification. These two species have previously shown the best results in killing nematode, as well as, active products secretion.

The molecular identification was performed by sequencing 785-bp of the 16S rDNA gene. The phylogenetic trees of the identified strains were inferred by the neighbor-joining distance method (Thompson *et al*., 1994) and presented in Fig. (1).

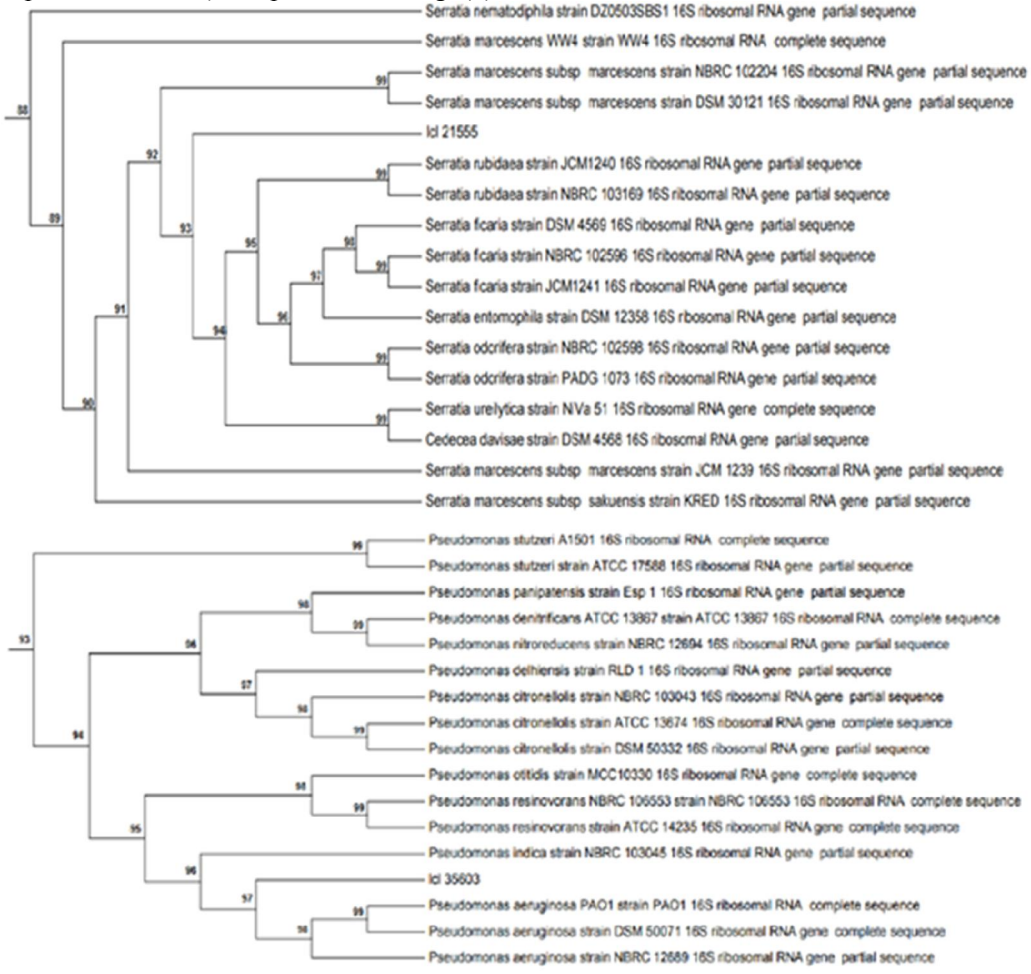

Fig. 1: Phylogenetic tree of *Pseudomonas aeruginosa* and *Serratia marcescens* based on the16S rDNA sequence.Effect of bacterial treatments on nematodes reduction in field:

Based on the 16S rDNA gene sequence and the comparison with other related sequences in the NCBI (National Center for Biotechnology Information) database, BLAST search showed maximum similarity for *Serratia* sp. with *Serratia marcescens* subsp *marcescens*, and for *Pseudomonas* sp. with *Pseudomonas aeruginosa* strain NBRC. The molecular strain identifications coincided with those obtained by the phenotypic approach. In one of our previous research (Hegazy and Mahgoub, 2013), there was some discrepancy between the identification results of the phenotypic and the molecular approach, which was not found in the current research. Probably, if the number of species that were subjected to 16S rDNA identification in this research was higher than the current situation (2 species), such discrepancy, similar to the other paper would exist.

The efficiency of various bacterial treatments in killing nematodes in field is shown in Table (3) and presented as the percentage of decrease or increase, compared with the initial nematode count in the soil. In general, the mixture of the two bacteria showed the highest efficiency compared with the individual species. That was prominently clear in the 15 and 20 L/feddan treatments, where the increase was mostly significant ($P \leq 0.5$). Also, *S. marcescens* was much more efficient than *P. aeruginosa* in reduction of nematodes numbers, with significant increase ($P \leq 0.5$) in almost all treatments.

The obtained results might contradict with those obtained by Mohamed*et al*., (2009) in a greenhouse experiment, where they showed that *P. fluorescens* had higher effect than *S. marcescens* in reduction of *Meloidogyne incognita* population (350/1000 *vs*. 250/1000). However, the obtained results are in harmony with our findings presented in Table (1), where *Serratia* showed much more chitin hydrolysis than *Pseudomonas* (5.54 vs. 3.31U/mg), with additionally, considerable increase in protease activity for *Serratia* over *Pseudomonas* (4.42 vs. 4.04 U/mg, respectively). Also, the obtained results are in harmony with the findings of El-Tarabily *et al*., (2000), since they stated that *S. marcescens* has an obvious potential as biological control agent for *Sclerotinia minor*, a plant pathogenic fungus, known with chitin as a major constituent of its cell wall.
The obtained results are in accordance with other reports (Zaghloul *et al*., 2015) where they showed that *S. marcescens* was the most effective (*in vitro*) among other bacterial isolates, as biocontrol agent for Root-Knot nematodes.

Regarding the effect of time, the killing efficiency was decreased as the time increased, where the highest reduction rate was after 10 d., followed by the 20 then 30 d. treatment, respectively. This result may be due to a lower survival rate of the bacteria in soil compared to the nematodes, thus a fraction of the nematode populations was able to multiply and increase their numbers, despite the increase in bacterial population. The increase in bacterial concentration per milliliter of culture resulted in substantially increasing the nematode killing efficiency. For instance, by looking at the column of “Average bacterial effect" after 20 d., and calculating the average of each three numbers pertaining to each bacterial concentration, the results would be 51.5, 26.6. and 23.6 for the concentrations of 10^6, 10^7, and 10^8cfu /ml, respectively. Apparently, the concentration of 10^7cfu /ml showed a substantially better effect over its previous concentration (10^6) compared to the improving effect of 10^8 concentration, over its previous concentration (10^7). In terms of the inoculation volume, there was a proportional increase in reduction percentage with increasing the numbers of liters used. Increasing the added liters above 15 L/fed, however, did not show significant ($P \leq 0.5$) reduction rate increase for almost all microbial concentrations of $10^7$and 10^8cfu /ml. In microbial concentration of 10^6 cfu/ml, increasing the added liters above 15 L/fed, showed significant ($P \leq 0.5$) reduction rate increase in almost all treatments.

The higher biocontrol efficiency of *S. marcescens* compared to *P. aeruginosa* is most likely due to the activity of its chitinase as shown in Table (1). Even though another isolate (A4) possessed higher protease activity, it did not show the same efficacy in killing nematode *in-vitro* (Table 2). This conclusion was also reached by Kobayashi *et al*., (1995) in their study about the capability of *S. marcescens* to protect plant hosts against fungal infection .Previous reports have shown that the suppressive effect of pseudomonads against plant pathogens is thought to be a result of their consumption of the amino acids found in the root exudates, which are converted into ammonia, and contribute to amicro-zone around the roots that would suppress pathogens (Eklund, 1970).

Table 3: The efficiency of various bacterial treatments in reduction of nematodes population in field experiment.

Treatments		After 10 d (%)					After 20 d (%)					After 30 d (%)				
		5 L /fed	10 L /fed	15 L /fed	20 L /fed	Avg. bact.eff.	5 L /fed	10 L /fed	15 L /fed	20 L /fed	Avg. bact.eff.	5 L /fed	10 L /fed	15 L /fed	20 L /fed	Avg. bact.eff.
	Control	108.0	115.0	107.0	110.0	110.0	130	128	125	125	127.0	170.0	155.0	175.0	160.0	165.0
10^6 cfu /ml	***S. marcescens***	66.0	50.0	45.4	27.2	47.6	58.6	50.2	45.4	27.9	47.0	60.0	56.0	50.0	45.4	53.6
	P. aeruginosa	72.9	71.3	66.9	58.9	66.5	72.7	71.1	55.9	61.2	67.7	76.6	71.9	70.3	65.9	71.2
	Mix. S. and P.	53.3	46.8	32.8	24.0	39.5	52.5	47.6	34.4	25.0	39.9	56.7	53.3	46.8	32.8	47.4
	Inoc. Vol. Avg.	60.4	55.7	47.7	29.7		60.9	56.0	47.2	41.0		64.4	60.4	55.7	47.7	
	LSD (0.05)	**Inoc. vol.**	**Bacteria**				**Inoc. vol.**	**Bacteria**				**Inoc. vol.**	**Bacteria**			
		4.4	5.6				4.9	6.1				4.5	6.0			
10^7 cfu /ml	***S. marcescens***	30.6	22.8	18.8	17.7	22.5	31.3	22.8	18.2	18.4	22.7	32.5	30.6	22.8	18.8	26.2
	P. aeruginosa	45.5	33.3	33.3	30.3	33.8	45.5	33.3	33.1	29.5	35.8	50.6	43.8	33.3	33.3	40.0
	Mix. S. and P.	28.8	19.5	17.2	15.4	20.2	30.4	19.5	18.8	16.2	21.2	33.6	28.8	19.5	17.2	24.8
	Inoc. Vol. Avg.	34.3	25.2	32.8	21.1		36.7	25.2	23.4	21.4		38.2	34.2	25.2	22.8	
	LSD (0.05)	**Inoc. vol.**	**Bacteria**				**Inoc. vol.**	**Bacteria**				**Inoc. vol.**	**Bacteria**			
		4.5	7.6				5.3	7.4				5.8	7.4			
10^8 cfu /ml	***S. marcescens***	27.2	23.5	20.9	19.9	22.9	29.4	25.0	21.6	20.6	24.1	29.4	27.2	23.5	20.9	25.2
	P. aeruginosa	34.7	31.0	24.3	20.7	27.7	36.1	21.0	24.3	21.4	28.2	37.5	34.7	31.0	24.3	31.9
	Mix. S. and P.	33.0	15.2	14.6	14.3	16.8	24.4	19.7	15.4	14.3	18.4	25.9	23.0	15.2	14.6	19.7
	Inoc. Vol. Avg.	29.3	23.2	19.9	18.3		30.0	25.2	20.4	18.8		30.9	28.3	23.2	19.9	
	LSD (0.05)	**Inoc. vol.**	**Bacteria**				**Inoc. vol.**	**Bacteria**				**Inoc. vol.**	**Bacteria**			
		3.6	4.8				4.6	6.1				3.9	5.7			

[1]Numbers represent the percentage of decrease or increase, compared with the initial nematode count in the soil
[2]Numbers represent means of triplicate counts.
[3] Initial nematode numbers ranged between 1150 and 1620 /250 g soil

Conclusion

Among all the isolates that were screened, *Serratia marcescens* and *Pseudomonas aeruginosa* showed the highest capabilities in the mortality rate of *Meloidogyne incognita*. The two species were capable of producing some nematicidal products (chitinase and protease) and plant growth promoters (IAA and phosphate solubilizers). In the field trial, the mixture of the two species showed the best nematicidal effect, followed by *Serratia marcescens.*

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