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ACTIVITY OF *SERRATIA* SPP. AND *BACILLUS* SPP. AS BIOCONTROL AGENTS AGAINST *MELOIDOGYNE INCOGNITA* INFECTING TOMATO

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ABSTRACT

Background: Tomato (*Solanum lycopersicum*) is one of the most popular vegetable crops in Egypt. It is affected by a wide range of pests; prominent among them is root-knot nematode (RKN) (*Meloidogyne* spp.) which is considered as one of the major limiting factors affecting tomato productions worldwide, especially in Egypt. **Aim of study:** The present study was performed to evaluate the quantitative and qualitative chitionolytic potentials of *Serratia*, *Bacillus megaterium* and *B. subtilis* as well as their nematicidal effect against *Meloidogyne incognita* under both *in vitro* and *in vivo* conditions. **Methodology:** Qualitative and quantitative assays of bacterial chitinase of the applied bacteria were determined using Chitinase assay agar and liquid media, as well as the nelson-somogyi method. Nematicidal and ovicidal activities of bacterial treatments were evaluated by Test Tube Bio-assay technique. A greenhouse experiment was conducted for *in vivo* evaluation of bacterial treatments on nematode parameters. The most effective bacterial isolate was molecular identified based on the nucleotide sequencing of its 16S rRNA gene. **Results**: *Serratia* sp. (S2) showed the highest total and specific chitinase activities (0.620 U/mL and 0.079 U/mg), respectively, followed by S1 (0.560 U/mL and 0.061 U/mg) and S3 (0.434 U/mL and 0.057 U/mg). *B. megaterium* appeared the minimum total and specific chitinase activities (0.320 U/mL and 0.042 U/mg), and no chitinase activities was shown for *B. subtilis*. *Serratia* sp. (S2) recorded the highest ovicidal activity (53.61%) even when applied at low concentration (1%). Among greenhouse experiment, *Serratia* sp. (S2) recorded the minimum number of galls and egg masses. The nucleotide sequence analysis of 16S rRNA gene of *Serratia* sp. (S2) proved that this isolate could be a strain related to *S. marcescens,* therefore, it was documented in GenBank as *S. marcescens* strain ARC3 under the accession number MN533708. **Conclusion:** The results indicated that *S. marcescens* strain ARC3 showed the maximum nematicidal potentiality against *M. incognita* and its significant chitinolytic activity play an essential role in such bio-control effect. **Recommendation:** Chitonlytic bacteria could be suggested as effective and eco-friendly alternative approach for controlling the *M. incognita*.

Keywords: Tomato; Chitinases; Biocontrol, *Meloidog-yne incognita*; *Serratia*; *Bacillus.*

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a popular vegetable crop worldwide which considered an optimum source of micronutrients such as minerals, vitamins and antioxidants for a well-balanced human diet. Egypt is the fifth country in the world in terms of tomato production and the cultivated area reached 475.514 thousand Fadden produced approximately 7.94 million tons (FAO 2016). Tomato is the favourable host for various genera of plant parasitic nematodes including *Meloidogyne* spp. (El-Sherbiny et al., 2014 and Dahlin et al., 2019). Root-knot nematodes (RKNs) (*Meloidogyne* spp.) have been referred as the most well-known notorious phytophagous nematode infesting the majority of valuable plant species in the world. This genus has approximately 80 species (Karseen, 2002) and is expected to cause an estimated $100 billion loss each year (Oka 2010). In addition, RKNs interact with other phytopathogens, resulting in increased destroys caused by other diseases, affecting world food supplies (Kassie et al., 2019).

The difficulty in controlling the RKNs may be due to their wide host range, short generation times, worldwide distributions, high reproductive rates and their endoparasitic nature (Karssen and Moens, 2006). Chitin is known to be involved in the formation of the egg shell and cuticle of nematodes and acting as a protective barrier against chemical and biological nematicides (Thongkaewyuan and Chairin, 2018).

Bacterial metabolites are a rich source of bioactive compounds that can be exploited to produce new antimicrobial agents for crop protection against wide range of plant pathogens (Habash et al., 2020). Because of their antagonistic activity against pathogenic chitin-containing species, microbial chitinases have received interest in the field of biological control. As chitin is not found in plants or vertebrates, it is possible to consider it safe and

selective "target" molecule to control chitin-containing pathogenic organisms (Gortari and Hours, 2008).

Several rhizobacterial genera such as *Bacillus*, *Serratia*, *Pseudomonas* and *Streptomyces* could use chitin as an energy source and infect phytopathogens that containing chitin (AbdelRazek and Yaseen, 2020, Mohammed 2020 and Song et al., 2020). For example, *S. marcescens* is a potent chitinase producer with multiple applications in industrial, medical and agricultural fields (Wang et al., 2014).

The nematicidal potentiality of chitinases is based mainly on the hydrolysis of chitin found in the egg shell and cuticle of nematodes resulted in lysis and malformations (Chen et al., 2015). Extracellular crude chitinases and proteases secreted by *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Paenibacillus* have been shown to damage nematode juveniles and the egg shell, and significantly suppress the egg hatching (Soliman et al., 2019). Moreover, the medium components greatly influenced the microbial production of extracellular chitinase and its interaction plays a vital role in the synthesis of the chitinases (Wang et al., 2014).

Accordingly, the present study aimed at inducing and evaluating the chitinolytic behavior of five rhizobacterial isolates to introduce low-cost and environmentally sustainable control approach against *M. incognita*.

MATERIALS AND METHODS

Microbial isolates: Three *Serratia* isolates, *Bacillus subtilis* and *B. megaterium* were provided by Central Lab. of Organic Agriculture, Agricultural Research Center, Egypt.

Media Used: Nutrient Glucose Agar medium (Dowson, 1957), chitinase assay medium (Agrawal and Kotasthane, 2009), and Nutrient Broth medium (Ramaley and Burden, 1970) were used. This medium was modified by supplementation with colloidal chitin as a sole carbon source for induction of bacterial chitinolytic activity.

Qualitative assay of chitinase: Tested bacterial cultures were spotted on colloidal chitin agar plates and incubated at 30ºC up to 7 days. Ability of the tested bacterial isolates to produce chitinase was determined according to the ratio of hydrolysis based on the appearance of clear halo around each colony, as follows: Ratio of hydrolysis = Halo Diameter/Colony Diameter (Murthy and Bleakley, 2012). Colloidal chitin was prepared from crab shells chitin according to Roberts and Selitrennikoff (1988).

Quantitative assay of chitinase: The reducing sugars released from colloidal chitin degradation was measured colorimetrically as described by Neish (1952) using chitinase assay medium for bacterial chitinase induction. One chitinase activity unit was defined as 1 µmol of N-acetyl glucosamine released per one hour reaction at specified conditions.

Determination of protein and specific chitinase activity: Total protein was measured using the method described by Lowry et al., (1951). Specific chitinase activity is calculated by dividing the total chitinase activity in U/mL by the protein concentration in mg/mL.

Preparation of bacterial cultures: Different bacterial isolates were grown separately on modified nutrient broth medium for 7 days with shaking at 120 rpm and 30ºC±2. Different bioagents were prepared as suspension that adjusted to be containing 10^7 CFU/ 1 mL.

Bioassay of Egg hatching: Test Tube Bio-assay was conducted to investigate the efficacy of different concentrations (1, 10 and 50%) of homogenized growth culture suspensions of the selected bacterial species on hatching of *M. incognita* eggs under *in vitro* conditions. Eggs were extracted from galled tomato roots infected with *M. inco-gnita* using 1.5 % sodium hypochlorite solution by the method modified by Sikora and Greco (1990). The reduction in egg hatching was calculated according to the formula:

$$\textbf{Red. (\%)} = \textbf{(C-T)/C} \times \textbf{100}$$

Where, Red.: Reduction of the egg hatching, T: Number of hatched eggs in treatment and C: Number of hatched eggs in control.

Bioassay of Juveniles (J2) mortality: Three concentrations (1, 10, and 50%) of each bacterial isolates were screened for their antagonistic activity against second stage juveniles (J2) of *M. incognita* according to the method described by Naserinasab et al., (2011). On incubation for 24 hours, the immobile juveniles were counted in each test tube. Morphological changes and distortions noticed in treated juveniles were also studied by the aid of a compound microscope.

Juveniles mortality was calculated according to the formula:

$$\textbf{JM (\%)} = \textbf{(T/C)} \times \textbf{100}$$

Where, JM: The mortality of J2, T: Number of dead J2 in treatment; C: The total number of J2 used in test.

Greenhouse experiment

Site of the Experiment: A greenhouse experiment was carried out at Central Lab. of Organic Agriculture, Agricultural Research Center (ARC), Egypt. The experiment was conducted from August to

October, 2018 under minimum and maximum temperatures of about 28°C and 35°C, respectively.

Preparation of root-knot nematode inoculum: The eggs of root-knot nematode were obtained from galled roots of *M. incognita*-infected tomato plants using the maceration extraction method modified by Sikora and Greco (1990).

Preparation of bacterial inoculum: Three isolates of *Serratia* spp., *B. megaterium* and *B. subtilis* were grown on modified nutrient broth medium at 30±2ºC and 120 rpm for 7 days. Different bioagents were prepared as homogenized culture suspension that adjusted to be containing 10^7 CFU/mL.

Design of the Experiment: *Solanum lycopersicum* Mill (tomato) cv. Castle Rock seedlings (30-days old) were transplanted to 25 cm diameter plastic pots filled with autoclaved sand and peat moss (1:1, v:v) each pot contained one tomato seedling. The experiment was designed as reported by Abd El-Monem et al., (2016). Three plastic pots with tomato seedlings inoculated with *M. incognita* but without any microbial treatment were served as control. There were three replicates for each bacterial treatment and all treatments were arranged in a complete randomized block design. All pots were irrigated, fertilized periodically using the same amount of water and fertilizers per each pot. Sixty days after nematode inoculation, tomato pla-nts were uprooted for further examination. Plant growth criteria in terms of shoot and root lengths and weights as well as dry shoot weight were measured. Moreover, the numbers of juveniles in soil, number of galls, egg masses and root developmental stages were counted. Root gall index (RGI): and egg masses index (EI) was determined according to the scale given by Taylor and Sasser (1978**)** as follows:

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.

Molecular characterization of the most effective bacterial isolate: For molecular identification of the most effective bacterial isolate, bacterial DNA extraction was performed from 2 mL bacterial culture collected at the exponential growth phase using MicroSeq® 500 16S rDNA according to manufacturing instructions. The used primers were F 27 and R 1492. The two universal primers (27F: 5' AGA GTT TGA TCM TGG CTC AG'3 and 1492R: 5' TAC GGY TAC CTT GTT ACG ACT T'3) were used for PCR-isolation of 16S rRNA gene. PCR program was conducted as foll-ows: Initial denaturation / enzyme activation (95 ºC for 5 min at 1 cycle; 30 cycle each consists of denaturation at 94ºC for 1 min, annealing at 55ºC for 1 min and extension at 72ºC for 1.5 min), and the final cycle was extended for 5 min. The PCR product was cleaned up by using GeneJET™ PCR Purification Kit (Thermo K0701). The sequencing of the PCR product has been determined on GA-TC Company by using ABI 3730xl DNA seque-ncer. The sequenced PCR product was blasted using The National Centre for Biotechnology Information (NCBI) Mega Blast for the species identification. According to the percentage of identities between the isolate under investigation and strains documented in GenBank, the names of genus and species were defined.

Statistical analysis: Experiments were carried out in a completely randomized design with nine treatments and three replications. Data were subjected to analysis of variance (ANOVA). In order to provide a picture on significant treatments, means were compared with Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Qualitative and quantitative assay of chitinolytic activity in tested bacterial isolates: The results revealed that all tested bacterial isolates except *Bacillus subtilis* showed chitinolytic activity (Table 1, Fig. 1). All of tested *Serratia* isolates effectively hydrolyzed chitin and produced large clear zone diameters followed *Bacillus megaterium*. *B. subtilus* showed no chitinase activity. Results in Table 2 showed that all *Serratia* isolates and *Bacillus megaterium* exhibited chitinase activity. *Serratia* sp. S2 recorded the highest total and specific chitinase activity (0.620 U/mL and 0.079 U/mg) followed by S1 (0.560 U/mL and 0.061 U/mg); S3 (0.434 U/mL and 0.057 U/mg), respectively. *B. megaterium* recorded the minimum total and specific chitinase activity (0.320 U/mL and 0.042 U/mg), respectively.

Some of bacterial genera such as *Aeromonas, Serratia, Vibrio, Streptomyces* and *Bacillus* have been reported for their chitinolytic potentiality (Cody et al., 1989). Some rizobacterial strains were reported to have chitinolytic activity which plays a significant role in their antagonistic activity (Chang et al., 2010, Shende et al., 2014, Prakash et al., 2015 and Abdel Razek and Yaseen 2020). Lamine et al., (2012) confirmed the strong chitiniloytic activity of *S. marcescens* when tested on medium containing chitin as a main carbon source.

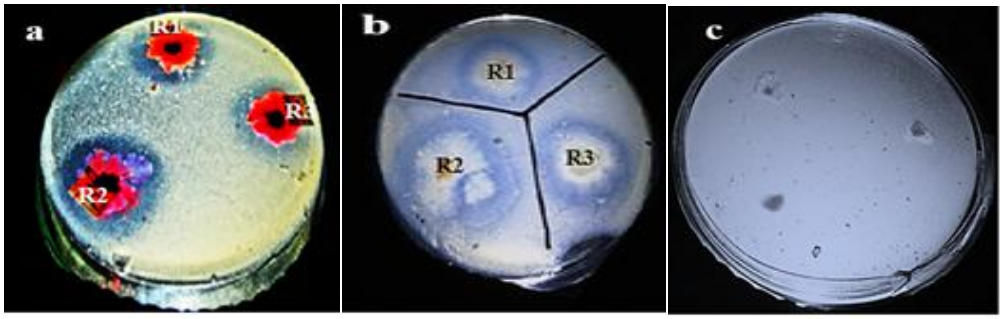


Fig. 1: Chitinolytic activity of the tested bacterial isolates. a: ***Serratia*** **sp., b: *B: megaterium*, b: *B. subtilus.***

(Chang et al., 2010, Shende et al., 2014, Prakash et al., 2015 and Abdel Razek and Yaseen 2020). Lamine et al., (2012) confirmed the strong chitiniloytic activity of *S. marcescens* when tested on medium containing chitin as a main carbon source.

Table 1: Qualitative assay of chitinolytic activity in bacterial species.

Bacterial species	Diameter of clear zone(mm)	Diameter of bacterial colony(mm)	Ratio of hydrolysis
Serratia sp. (S1)	29.66[a]	5.5	5.392
Serratia sp. (S2)	31.33[a]	5.0	6.266
Serratia sp. (S3)	30.66[a]	5.0	6.132
Bacillus megaterium	22.33[b]	10	2.233
B. subtilis	00.00[c]	5.0	0.000
L.S.D at 0.01	3.340	-	-

Table 2: Quantitative assay of chitinolytic activity.

Bacterial isolates	Final pH	Chitinase activity (U/mL)	Total protein (mg)	Specific activity (U/mg)
Serratia sp. (S1)	8.00	0.560	9.130	0.061
Serratia sp. (S2)	7.80	0.620	7.818	0.079
Serratia sp. (S3)	8.00	0.434	7.600	0.057
Bacillus megaterium	7.35	0.320	7.560	0.042

Initial pH was 4.7.

In vitro **evaluation of the tested bacterial isolates against *M. incognita:* Ovicidal potentiality of the tested bacterial isolates:** Data in Table 3 indicated that all tested bacterial cultures significantly inhibited egg hatching of *M. incognita,* and the effectiveness of inhibition was concentration dependent. Isolate of *Serratia* sp. (S2) recorded significant ovicidal activity by 53.61% at the lowest concentration (1%) of bacterial culture; followed by S3 (39.92 %), S1 (39.16 %), *B. megaterium* (21.28 %) and *B. subtilis* (15.2 %). No significant difference in the reduction of egg hatching was recorded at high bacterial culture concentrations. The microscopic studies indicated that *Serratia* sp. and *Bacillus megaterium* caused lysis in eggshell and coagulation of egg components. *B. subtilis* caused distortion and death of first stage juvenile and thus prevent normal hatching (Fig. 2). Lee et al., (2015) found that the exposure of M. incognita eggs to *P. fluorescens* chitinase, suppressed the egg hatching. The purified chitinase LPCHI1 degraded the chitinous layer of *M. incognita* eggs and significantly influenced their development and hatching (Chen et al., 2006). Moreover, eggs of *M. javanica* eggs became swollen when treated with chitinase and the structure of the eggshells was completely destroys (Lee et al., 2014).

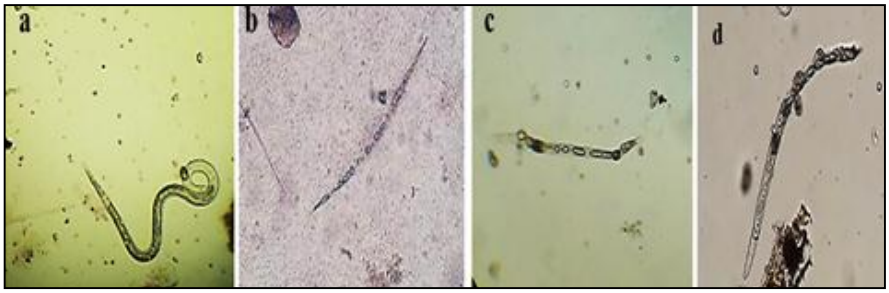


Fig. 2: Efficacy of homogenized growth culture suspensions of the tested bacterial species on egg hatching of M. incognita. a: Untreated control, b: *Serratia* sp., c: *B. megaterium*, d: *B. subtilus*.

Table 3: Ovicidal potentiality (Reduction in egg hatching) of the tested bacterial isolates.

Treatments/ Concentrations (%)	1		10		50	
	No. of hatched eggs	Red. (%)	No. of hatched eggs	Red. (%)	No. of hatched eggs	Red. (%)
Control (Nematode alone)	87.66^{a}	-	87.66^{a}	-	87.66^{a}	-
Serratia sp. (S1)	53.33^{c}	39.16	0.66^{d}	99.24	0.33^{b}	99.62
Serratia sp. (S2)	40.66^{cd}	53.61	0.33^{d}	99.62	0.00^{b}	100
Serratia sp. (S3)	52.66^{c}	39.92	0.66^{d}	99.24	0.00^{b}	100
Bacillus megaterium	69.00^{b}	21.28	14.33^{bc}	83.65	0.00^{b}	100
B. subtilis	74.33^{b}	15.20	18.66^{b}	78.71	1.66^{b}	98.10
L.S.D at 0.01	11.65	-	5.01	-	2.90	-

Mean in each column followed by the same letter(s) did not differ at P ≤ 0.01 according to Duncan`s multiple range test.

Nematicidal potentiality of the tested bacterial isolates: The experimental results showed that all *Serratia* isolates effectively inhibited second stage juveniles vitality of *M. incognita* when applied at different concentrations; followed by *B. subtilius* and *B. megaterium*, respectively **(**Table 4, Fig. 3). Few hours after treatment, juveniles lose their vitality and became straight and immobile. The immobilization of juveniles was irreversible and the death of the juveniles was confirmed when they were transferred to distilled water for 24 hr and showed no vitality. Moreover, the paralysed juveniles showed malformations and lysis after 24 hr from treatment time. The distortion effect was found to be microbial treatment dependent. Results of this study are in agreement with that reported by Zaghloul et al., (2015) who found that *S. marcescens* and *P. fluorescens* recorded the highest nema-ticidal activity against the second stage juveniles of *M. incognita* exceeding 94%. Further, Kassab et al., (2017) indicated that the *S. marcescens* caused *M. incognita* juveniles (J2) mortality up to 100%.

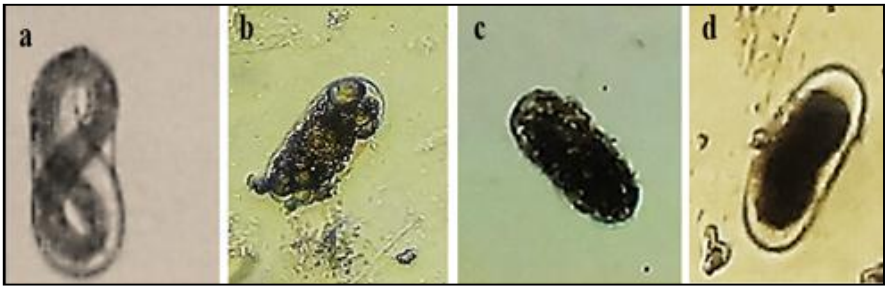


Fig. 3: Distortion effect of homogenized growth culture suspensions of the tested bacterial species on J_2 of *M. incognita*. a: Untreated control, b: *Serratia* sp., c: *B. megaterium*, d: *B. subtilus*.

Table 4: Nematicidal potentiality of the tested bacterial isolates

Treatments/ Concentrations (%)	1		10		50	
	No. of immobile juveniles	Mortality (%)	No. of immobile juveniles	Mortality (%)	No. of immobile juveniles	Mortality (%)
Control (Nematode alone)	00.00^{c}	-	00.00^{c}	-	00.00^{c}	-
Serratia sp. (S1)	43.00^{b}	43.00	99.33^{a}	99.33	100.00^{a}	100.00
Serratia. sp. (S2)	46.33^{b}	46.33	100.00^{a}	100.00	100.00^{a}	100.00
Serratia. sp. (S3)	47.00b	47.00	97.33a	97.33	100.00a	100.00
Bacillus megaterium	24.66b	24.66	41.00b	41.00	80.66b	80.66
B. subtilis	32.00b	32.00	46.33b	46.33	89.66ab	89.66

L.S.D at 0.01	9.97	-	7.88	-	15.86	-

Means in each column followed by the same letter(s) did not differ at P ≤ 0.01 according to Duncan`s multiple range test.

Evaluation of the selected bacterial isolates on promoting tomato growth under greenhouse conditions: All plant growth parameters (shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight and NPK content) were improved remarkably in all treatments at different rates (Tables 5, 6). Different genera of bacteria such as *Bacillus, Pseudomonas* and *Serratia* have the capacity to solubilize phosphorus from raw phosphate rock (Mohamed et al., 2018 and Blanco -Vargas et al., 2020). Patil (2014) reported that *B. subtilis* is a potent phosphate solubilizer that showed remarkable tolerance when applied in soil with high salinity. It has been reported that *B. megaterium* is a powerful phosphate solubilizing bacteria and regulates the endogenous plant carbohydrates and amino acids contents resulting in obvious growth enhancement in Mustard plant growth (Kang et al., 2014).

Table 5: Impact of different bacterial treatments on growth parameters of tomato infected with *M. incognita* under green-house conditions.

Treatments	Growth parameters				
	Shoot			Root	
	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)
Control (Nematode alone)	34.66^{d}	15.60^{d}	3.20^{b}	18.32^{c}	4.46^{a}
Serratia sp. (S1)	56.30^{a}	24.00^{b}	3.70^{ab}	26.30^{b}	5.83^{a}
Serratia. sp. (S2)	55.30^{ab}	32.40^{a}	6.20^{a}	30.30^{a}	7.20^{a}
Serratia sp. (S3)	52.60^{bc}	21.20^{c}	3.90^{ab}	30.60^{a}	6.70^{a}
B. megaterium	53.33^{bc}	30.40^{a}	4.50^{ab}	29.00^{b}	5.90^{a}
B. subtilis	51.80^{c}	19.90^{c}	3.70^{ab}	27.30^{b}	5.80^{a}
L.S.D at 0.05	04.29	02.58	02.29	01.77	01.93

Table 6: Impact of different bacterial treatments on NPK content of nematode-infected tomato under greenhouse conditions.

Treatment	N%	P%	K%
Control (Nematode alone)	1.83	0.42	0.31
Serratia sp. (S1)	1.87	0.54	0.35
Serratia. sp. (S2)	2.59	0.47	0.43
Serratia. sp.(S3)	2.51	0.45	0.41
Bacillus megaterium	2.59	0.54	0.46
B. subtilis	1.88	0.51	0.38

N: nitrogen, P:phosphorus , K: potassium

Impact of different microbial treatments on nematode parameters: The present greenhouse experiment revealed that tomato plants that were treated with tested bacterial species were less attacked by *M. incognita*, the total nematode population, root galling, number of egg masses and number of eggs/egg mass were suppressed with all treatments. However, there were significant differences among the bacterial treatments. Thus, it has been suggested that the inhibition was bacterial species dependent (Tables 7, 8). The hig-hest reduction in total nematode population and root galling was achieved by *Serratia* sp. (S2).

Competition for an ecological niche or a substrate, production of inhibitory substances, and induction of systemic resistance in host plants to a wide range of biotic and abiotic stresses are all well known mechanisms of PGPR as bio-control agents (Compant et al., 2005, Khan et al., 2008 and Sidhu 2018). Bacterial species belong to *Bacillus megaterium, B. subtilis, B. thuringiensis, Serratia marcescens* and *Pseudomonas fluore-scens* were investigated *in vivo* against root-knot nematode (Mokbel and Alharbi, 2014; Patel and Patel, 2019). It was found that the highest reduc-tion in root galling was observed in plants treated with *S. marcescens*. Mostafa et al., (2018) proved the nematicidal effect of *B. megaterium* against RKNs infecting sugar beet.

The biocontrol activity of *Serratia* spp. against *M. incognita* under greenhouse conditions as the plant treated with tested *Serratia* sp. recorded significant root galling inhibition and had a positive impact on plant growth parameters (Ketabchi et al., 2016 and Hegazy et al., 2019). The current results are in harmony with those of El-Sayed and

Edrees (2014) who demonstrated that rhizobacteria are good biocontrol agents against soil borne pathogens.

Table 7: Impact of different bacterial treatments on the population density of *M. incognita.*

Treatments	No. of juveniles /250 g soil	No. of females/5 g of root	No. of developmental stages/5 g of root	Final population	Red. %
Control (Nematode alone)	338.33[a]	82.66[a]	21.00[a]	441.99	-
Serratia sp. (S1)	070.00[d]	45.33[c]	11.00[c]	126.33	71.41
Serratia sp. (S2)	013.33[f]	12.00[f]	5.00[d]	030.33	93.13
Serratia sp. (S3)	033.33[e]	16.33[e]	11.00[c]	060.66	86.27
Bacillus megaterium	085.00[c]	32.33[d]	11.33[c]	128.66	70.89
B. subtilis	214.00[b]	80.00[b]	17.00[b]	311.00	29.63
L.S.D at 0.05	001.67	01.77	01.77	-	-

- Final population is calculated as the sum number of juveniles, females and developmental stages.
- Red. (%) (Reduction percentage) = (F.C-F.T)/F.C × 100 where, F.C: final population in untreated control and F.T: final population in treated plant
- Each value presented the mean of three replicates.
- Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan`s multiple range test

Table 8: Impact of different bacterial treatments on the development and reproduction of *Meloidogyne incognita.*

Treatments	No. of galls / 5 g of root	Red. %	Root gall index (RGI)	No. of egg masses/ 5 g of root	Red. %	Egg masses index (EI)	No. of eggs/ egg mass	Red. %
Control (Nematode alone)	167.00[a]	-	5	70.00[a]	-	4	418.00[a]	-
Serratia sp. (S1)	071.33[d]	57.28	4	16.00[c]	77.14	3	191.33[c]	54.30
Serratia sp. (S2)	038.00[f]	77.24	4	12.00[d]	82.85	3	111.00[f]	73.44
Serratia sp. (S3)	047.00[e]	71.85	4	15.00[cd]	78.57	3	132.00[e]	68.42
Bacillus megaterium	075.00[c]	55.08	4	17.33[c]	75.24	3	262.33[b]	37.24
B. subtilis	099.00[b]	46.10	4	36.00[b]	48.57	4	167.00[d]	60.04
L.S.D at 0.05	002.51	-	-	03.08	-	-	005.87	-

- (Red.) : Reduction; (RGI): Root gall index and (EI): egg masses index was determined according to the scale given by Taylor and Sasser, 1978 as follows:
  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.
- Each value presented the mean of three replicates.
- Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan`s multiple range test.

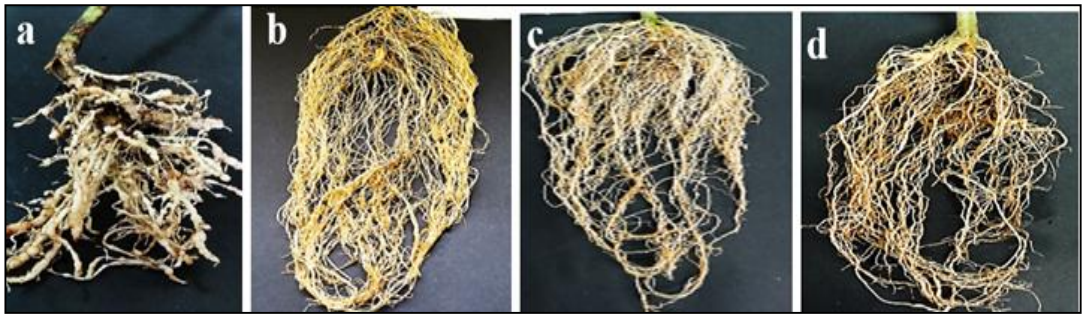


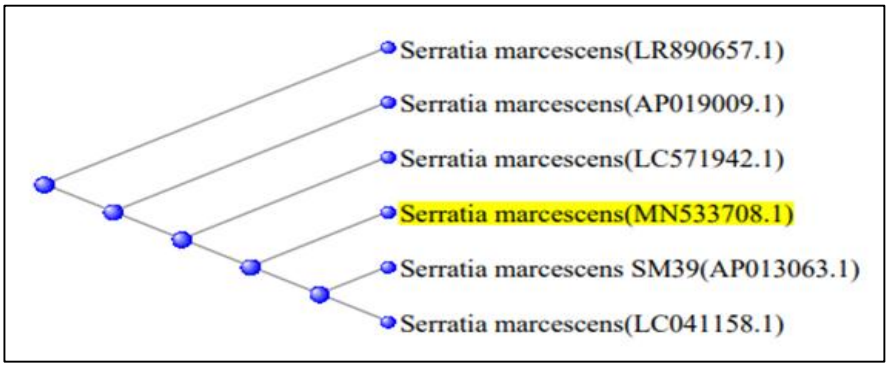
Fig. 4: Impact of different bacterial species on root fitness of tomato infected with *M. incognita*. a: Untreated control, b: *Serratia* sp., c: *B: megaterium,* d: *B. subtilis.*

Molecular characterization of the most effective bacterial isolate against *M. incognita:* The present *in vitro* and *in vivo* investigations revealed that both maximum chitinolytic, as well as nematicidal activities, were recorded by *Serratia* sp. (S2). This isolate was subjected to molecular identification by DNA-sequencing based method. PCR amplification of 16S rRNA gene was successfully obtained and then sequenced. The nucleotide sequence analysis indicated that *Serratia* sp. (S2) was showed percentage identities ranged from 98.28 to 98.52 when compared with the most five *S. marcescens* documented in GenBank (Table 9, Fig. 5). The isolate was given the name of *S. marcescens* strain ARC3 and accession num-ber MN533708.1. Rapid advances in DNA sequencing technology have resulted in a significant change in the manner prokaryotes are classified. Sequence analysis of highly conserved regions of the bacterial genome, such as the small subunit rRNA gene, provide a universal approach to estimate the evolutionary relationships among all organisms. Phylogenetic classification is now a broadly accepted method of representing taxonomic relationships among prokaryotes (Petti, 2007).

Table 9: Identities percentage of 16S rRNA of *Serratia marcescens* strain ARC3 (MN533708.1) compared to the most similar *S. marcescens* strains documented in GenBank with E-value (0.0).

Description	Query Cover (%)	Identities (%)	Accessions
Serratia marcescens strain B3R3, complete genome	100	98.52	CP013046.2
Serratia marcescens strain JW-CZ2 chromosome, complete genome	100	98.28	CP055161.1
Serratia marcescens strain FY chromosome, complete genome	100	98.28	CP053378.1
Serratia marcescens strain FDAARGOS_659 chromosome	100	98.28	CP050960.1
Serratia marcescens strain MWU13-2543 16S ribosomal RNA gene, partial sequence	100	98.28	MT101739.1

Fig. 5: Phylogenetic tree of *S. marcescens* strain ARC3 (MN533708.1) show that the strain under investigation was lied in the same cluster includes S. *marcescens* strains B3R3 and JW-CZS.



Conclusions

As a conclusion, all rhizobacterial species showed more or less effects in the control of root-knot nematode. Amongst, chitinolytic *Serratia marcescens* strain ARC3 exhibited maximum reduction in the root-knot multiplication. Also, application of *S. marcescens* did not cause any negative impact on plant growth and yield of tomato and by keeping the importance of this bacteria, chitinolytic *S. marcescens* can be recommended in the use of root-knot disease management and plant growth promotions of tomato.

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REFERENCES

Abd El-Monem M.A.S., Atef M.K., Mohamed S.A.and Mohamed M.N. Induced resistance in tomato plants against root knot nematode using biotic and abiotic inducers. *International Journal of Advanced Research in Biological Sciences* 3(11): 31-46 (2016).

AbdelRazek G.M. and Yaseen R., Effect of some rhizosphere bacteria on root-knot nematodes. *Egypt J Biol Pest Control* 30: 140 (2000).

Agrawal T. and Kotasthane A.S., A simple medium for screening chitinase activity of *Trichoderma* spp. In: Methods of Molecular Identification and lab. Protocols. International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy (ISTH) (2009).

Andrea Blanco-Vargas A., Rodríguez-Gacha L. M., Castro, N.S., Jaramillo R.G., Camacho L. D.P., Pinales R.A.P., Claudia Hoyos C.M.R., Ariza L.A.D. and Rodríguez A.M.P., Phosphate-solubilizing *Pseudomonas* sp., and *Serratia* sp., co-culture for Allium cepa L. growth promotion. Heliyon (6): e05218. (2020).

Chang W., Chen, M. and Wang, S. An antifungal chitinase produced by *Bacillus subtilis* using chitin waste as a carbon source. *World Jour-nal of Microbiology and Biotechnology* 26(5): 945-950 (2010).

Chen L., Jiang H., Cheng Q., Chen J., Wu G., Kumar A., Sun M. and Liu Z., Enhanced nematicidal potential of the chitinase *pachi* from *Pseudomonas aeruginosa* in association with *Cry21Aa*. *Scientific Reports* 5: 14395 (2015).

Chen J., Moore, W.H., Yuen, G.Y., Kobayashi, D. and Caswell-Chen, E.P., Influence of Lysobacter *enzymogenes* Strain C3 on Nematodes. J. Nemato 8(2): 233–239 (2006).

Cody R.M., Distribution of chitinase and chitobiase in *Bacillus*. *Current Microbiology* 19(4): 201-205 (1989).

Compant S., Duffy B., Nowak J., Clement C. and Barka E.A., Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology* 71(9): 4951-4959 (2005).

Dahlin P., Eder, R., Consoli, E., Krauss, J. and Kiewnick, S., Integrated control of *Meloidogyne incognita* in tomatoes using fluopyram and Purpureocillium lilacinum strain 251. *Crop Protection 124*: 104874. (2019).

Dowson W.J., Plant disease due to bacteria .Second Edition, Cambridge the University Press, London, Pp. 23 (1957).

Duncan D.B., Multiple range and multiple, F-test. Biometrics 11(1):1- 42 (1955).

El-Sayed I.A. and Edrees N. O., Using of plant growth promoting rhizobacteria as biocontrol agent for root-knot nematode under greenhouse. *Natural Science* 12(12): 41-49 (2014).

El-Sherbiny A. and Awad Allah, S., Management of the root-knot nematode, Meloidogyne incognita on tomato plants by pre-planting soil Biofumigation with harvesting residues of some winter crops and waste residues of oyster mushroom cultivation under Field conditions Amr A. *Egyptian Journal of Agronematology 13*(1): 189-202 (2014).

FAO. Food and Agriculture Organization of the United Nations. Rome (2016).

Gortari M.C. and Hours R.A., Fungal chitinases and their biological role in the antagonism onto nematode eggs. A review. Mycol. Prog. 7: 221–238 (2008).

Habash S.S., Brass H.U.C., Klein A.S., Klebl D. P., Weber T.M., Classen T., Pietruszka J., Grundler F.M.W. and Schleker A.S.S., Novel Prodiginine Derivatives Demonstrate Bioactivities on Plants, Nematodes, and Fungi. Front. Plant Sci. 11:579807 (2020).

Hegazy M.I., Salama A.S.A., El-Ashry R.M. and Othman A.I., *Serratia marcescens* and *Pseudomonas aeruginosa* are promising candidates as biocontrol agents against root-knot nematodes (*Meloidogyne* spp.). *Middle East Journal of Agriculture Research* 8(3): 828-838 (2019).

Kang S.M., Radhakrishnan R., You Y.H., Joo G. J., Lee I.J., Lee K.E. and Kim J.H., Phosphate Solubilizing *Bacillus megaterium* mj1212 Regulates Endogenous Plant Carbohydrates and Amino Acids Contents to Promote Mustard Plant Growth. *Indian Journal of Microbiology* 54(4): 427–433 (2014).

Karssen G. and Moens, M., Root-knot nematodes. In, Plant Nematology. Perry, R.N.; Moens, M. and Starr, J. (Eds). CABI, Wallingford, UK. Pp. 59-90 (2006).

Kassab S.A., Eissa, M.F. M., Badr, U.M., Ismail, A.E., Abdel Razik, A.B. and Gaziea, M. Soliman, Nematicidal Effect of A Wild Type of *Serratia Marcescens* and Its Mutants Against *Meloidogyne incognita* Juveniles. *Egyptian Journal of Agronematol* 16(2): 95-114 (2017).

Kassie Y.G., Status of root-knot nematode (*Meloidogyne Species*) and Fusarium wilt (*Fusarium oxysporum*) disease complex on tomato (*Solanum lycopersicum* L.) in the central Rift Valley, Ethiopia. *Agricultural Sciences 10*(8): 1090-1103 (2019).

Ketabchi S., Charehgani H. and Majzoob S., Impact of rhizosphere antagonistic bacteria and urea fertilizer on root-kno nematode (*Meloidogyne incognita*) under greenhouse condition. *The Journal of Animal and Plant Sciences* 26(6): 1780-1786 (2016).

Khan Z., Kim S.G., Jeon Y.H., Khan H.U., Son S. H. and Kim Y.H., A plant growth promoting rhizobacterium, *Paenibacillus polymyxa* strain GBR-1, suppresses root-knot nematode. *Bioresource Technology* 99(8): 3016-3023 (2008).

Kloepper J.W. and Schroth M.N., Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. *Phytopathology* 71(6): 590-592 (1981).

Kloepper J.W., Leong J. and Schroth M.N., *Pseudomonas* siderophores: A mechanism explaining disease suppressive soils. *Current Microbiology* 4(5): 317-320 (1980).

Lamine B.M., Lamine B.M. and Bouziane A., Optimisation of the Chitinase Production by *Serratia Marcescens* DSM 30121^T and Biological Control of Locusts. *Journal of Biotechnology and Biomaterials* 2(3): 1-5 (2012).

Lee Y.S., Anees, M., Park, Y.S., Kim, S. B., Jung, W.J. and Kim, K.Y., Purification and properties of a Meloidogyne-antagonistic chitinase from *Lysobacter capsici* YS1215. Nematology 16: 63–72 (2014).

Lee Y.S., and Kim, K.Y., Statistical optimization of medium components for chitinase production by Pseudomonas fluorescensstrain HN 1205: Role of chitinase on egg hatching inhibition of root-knot nematode. *Biotechnology & Biotechnological Equipment 29*(3): 470-478 (2015).

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry* 193(1): 265-275 (1951).

Mohamed E.A.H., Farag A.G. and Youssef S.A., Phosphate solubilization by *Bacillus subtilis* and *Serratia marcescens* isolated from tomato plant rhizosphere. *Journal of Environmental Protection* 9(3): 266-277 (2018).

Mohammed A.F., Optimization of cellulase and chitinase enzymes production by plant growth promoting rhizobacteria. *Novel Research in Microbiology Journal* 4(1): 641-652(2020).

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

Murthy N. and Bleakley B., Simplified method of preparing colloidal chitin used for screening of chitinase-producing microorganisms. *International Journal of Microbiology* 10(2): 1-5 *(*2012).

Naserinasab F., Sahebani N. and Etebarian H.R., Biological control of *Meloidogyne javanica* by *Trichoderma harzianum* BI and salicylic acid on tomato, *African Journal of. Food Science* 5(3): 276-280 (2011).

Neish A.C., Analytical methods for bacterial fermentations. Report 46-8-3, 2nd revision. Nat.Research Council of Can., Saskatoon. (1952).

Oka Y., Mechanism of nematode suppression by organic soil amendments – A review. *Applied Soil Ecology* 44: 101–115 (2010).

Patel N.A. and Patel A.D., Management of root-Knot Nematodes (*Meloidogyne* spp.) Using Different Bio-Agents in Papaya Nursery. International Journal of Current *Microbiology and Applied Sciences* 8(8): 1934-1940 (2019).

Patil V.S., *Bacillus subtilis*: A potential Salt Tolerant Phosphate Solubilizing Bacterial Agent. *International Journal of Life Sciences Biotechnology and Pharma Research* 3(2): 141-145 (2014).

Petti C.A., Detection and identification of microorganisms by gene amplification and sequencing. Clin. Infect. Dis. 44: 1108-1114 (2007).

Prakash B., Perumal P., Gowrishankar J., Sivasankari P., Ashokkumar L. and Tamilmani P. Optimization of cultural Conditions for Production of Chitinase by *Bacillus* sp. Isolated from Agriculture Soil using Substrate as Marine Crab Shell Waste. *International Journal of Current Microbiology and Applied Sciences* 4(11): 192-198 192 (2015).

Ramaley R.F. and Burden L., Replacement sporulation of *Bacillus subtilis* 168 in a chemically defined medium. *Journal of Bacteriology* 101(1): 1–8 (1970).

Roberts W.K. and Selitrennikoff C.P., Plant and bacterial chitinases differ in antifungal activity. *Journal of general microbiology* 134: 169–176(1988).

Shende M.H., Ingle A.B. and Kumbalwar, M.M. Study of enzyme chitinase produced by *Bacillus subtilis* and its antifungal activity against *Aspergillus species*. *Research Paper* 3 (5): 2277- 8179 (2014). .

Sidhu H.S., Potential of plant growth-promoting rhizobacteria in the management of nematodes: A review. *Journal of Entomology and Zoology Studies* 6(3): 1536-1545 (2018).

Sikora R.A. and Greco T., Nematode parasites of legumes. In: M Luc, Sikora, R. A. and J Bridge (eds). Plant parasitic nematodes of subtropical and tropical agriculture.CABI Wallingford, U K. CABI, Pp. 621 (1990).

Soliman G.M., Ameen H.H., Abdel-Aziz S.M. and El-Sayed, G.M., In vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. *Bulletin of the National Research Centr* 43 article number: Pp. 171 (2019).

Song W., Zhang N., Yang M., Yuling Zhou, Nisha H.E. and Guimin Zhang, Multiple stra-tegies to improve the yield of chitinase a from *Bacillus licheniformis* in *Pichia pastoris* to obtain plant growth enhancer and GlcNAc. *Microbial Cell Factories* 19: 181 (2020).

Taylor A.L. and Sasser J.N., Identification and control of root-knot nematodes (*Meloidogyne* spp.) crop. Publ. Dep. Plant Pathol, North

Carolina State Univ. and U.S. Agrency Int. Dev. Raliegh, N.C. PP111 (1978).

Thongkaewyuan A. and Chairin T., Biocontrol of *Meloidogyne incognita* by *Metarhizium guizhouense* and its protease. *Biological Control 126: 142–146* (2018).

Wang K., Yan P.S. and Cao L.X., Chitinase from a novel strain *of Serratia marcescens* JPP1 for biocontrol of aflatoxin: molecular characterization and production optimization using response surface methodology. *BioMed Research International* 482623 (2014).

Westcott S.W. and Kluepfel D.L., Inhibition of *Criconemella xenoplax* egg hatch by *Pseudomonas aureofaciens*. *Phytopathology* 83: 1245 -1249 (1993).

Zaghloul R.A., Neweigy, N.A., Abou-Aly, H.E., El-Sayed, S.A. and Bahloul, A.M., Nematicidal activity of some biocontrol agents against root-knot nematodes *in vitro*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 6(1): 429-438 (2015).