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Evaluation of some Rhizobacteria as Induce Systemic Resistance or Bio-Control Agents in Controlling Root-knot Nematode, *Meloidogyne incognita* on Tomato

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Abstract

Ten bacterial strains, selected from a bulk of isolates recovered from tomato rhizosphere, were tested for their ability to induce systemic resistance or bio-control agents against *Meloidogyne incognita* in tomato under greenhouse condition. Results showed that all ten tested bacterial strains showed significant reduction in nematode development and reproduction. The most effective strains were *Methylomonas methanica, Bacillus cereus, Bacillus brevis* and *Obesumbacterium proteus*. They were achieving the highest reduction in nematode total population and fecundity. Plant growth was improved as a result of application of rhizobacteria. Antioxidant enzymes activity for both peroxidase and polyphenol oxidase were elevated in bacteriazied plants compared nematode infected plant as well as total phenol content. Results revealed that crude culture suspension of bacteria was more effective for reducing nematode population followed by cell-free culture filtrates, bacterial live cells and bacterial dead cells suspension, sequentially. It was concluded that these bacteria able to suppress *M. incognita* as resistance inducers for tomato plants or bio-control agents.

Keywords: Rhizobacteria, *Meloidogyne incognita,* induce systemic resistance, biological control, tomato.

Introduction

The root-knot nematodes, *Meloidogyne* spp. are one of the most economically important pest causing severe damages to a wide variety of crops particularly to tomato. Various techniques, including crop rotation, planting of resistant cultivars, and nematicides application have been used for the management of this nematode. Since the rhizosphere provides the first line of defense for roots against nematode attack, it is generally considered that rhizosphere bacteria are ideal bio-control agents. Their ability to multiply and spread in the rhizosphere environment, to colonize potential infection-sites on the root and possibly to act by direct contact with the parasites are characteristics that make them useful agents for nematode management. Studies on a number of plant-microbe interactions showed that such antagonistic rhizobacteria can function directly by competition and antibiosis **(Buchenauer, 1998)**. Also, indirectly by

inducing systemic resistance (ISR) in the plant toward soil-borne pathogens. **Hasky-Gunther *et al.* (1998)** were the first who demonstrate induced systemic resistance mechanism of action by Rhizobacteria against nematode. Fatherly, **Reitz *et al*., (2000) and Siddiqui and Shaukat, (2002&2004)** confirmed occurring of ISR by rhizobacteria.

This investigation was done to evaluate the ability of some rhizobaterial strains as ISR or bio-control agent toward root-knot nematode, *Meloidogyne incognita* and to exploring the performance of different bacterial component as elicitors for plant resistance and impact on plant growth.

Materials and Methods

Out of 35 bacterial strains were isolated from the rhizosphere of tomato plants only 10 isolates were consider as plant inducer after *in vitro* and *in vivo* screenings on tomato plant infested with *Meloidogyne incognita*. These isolates were identified as: *Bacillus brevis, Bacillus cereus, Bacillus firmus, Klebsiella planticolla, Lactobacillus agilis*, *Lactobacillus fermentum, Methylomonas methanica, Neisseria elongata, Obesumbacterium proteus* and *Pseudomonas aeruginosa*.

Single egg-mass culture of *Meloidogyne incognita* was mass rearing in tomato plants cultivated in disinfected soil and growing in greenhouse conditions. Tomato plants cv. Castel rock cultivated in 25 cm diam. earthen pots filled with about one kg sterilized soil (3 sand: 1clay V:V) were used in all experiments.

Four-weeks old tomato seedlings cv. Castol rock susceptible to *Meloidogyne incognita* were transplanted in the pots kept in the greenhouse at 30±5℃ for use in the experiments. Plants were fertilized with compound fertilizer and watered as needed.

Bacterial suspensions were added as soil drench (100 ml/ pot at 10^9 CFU/ml) two days before nematode inoculation with 1000 J_2 of *M. incognita* per pot. The plants under greenhouse conditions were irrigated and fertilized according to the recommendations of the Egyptians Ministry of Agriculture. The treatments were replicated four times (4 pots) in a completely randomized block design. Later, after forty five days of nematode inoculation, plants were carefully uprooted and nematodes in soil and roots were counted and recorded based on No of galls, No. of juveniles in soil, developmental stages, mature female, egg-masses numbers per plant and average eggs per egg-mass were recorded (average 10 egg-masses). The plants weights and lengths were registered. Also, peroxidase and polyphenol oxidase activity and total phenols were estimated in roots. Total soluble phenols were determined by using Folin and Ciocalteu's Phenol Reagent **(Daniel and George, 1972).**

Enzymes extraction form rhizobateria-treated and nematode infected roots, nematode infected roots only and healthy one were collected 7 days after nematode

inoculation to estimate enzyme activity. Enzyme extract were prepared according to **Maxwell and Batemen (1967).** Assay of peroxidase activity (POX), changes in POX activity were determined following the procedure described by **Sridhar and Ou (1974).** POX activity was expressed as change in absorbance (Δ O.D 470 nm) per min/gram fresh weight. Assay of polyphenol oxidase activity (PPO), changes in PPO activity were determined according to **Maxwell and Batemen (1967).** The activity of PPO was expressed as (ΔO.D 495 nm)/1.0 ml of extract per min per gram fresh weight.

Crude culture suspension (CS), cell free filtrate (F), viable or life cell (LCS) suspension and heat-killed cell suspension (DCS) of the most vigorous four bacteria: *Bacillus brevis, Bacillus cereus, Methylomonas methanica* and *Obesumbacterium proteus* were evaluated separately for their ability to suppress *M. incognita* severity and induced systemic resistance in tomato plants against nematode. Four-week old tomato seedlings were transplanted in disinfected earthen pots. Different bacterial concertinos were adjusted at 10^9 CFU/ml for CS, LCS and DCS. All forms were added to soil (100 ml/pot) two days before nematode inoculation with 1000 *M. incognita* (J_{2s}) per plant. Pots were kept in a greenhouse for 45 days then plants were uprooted and nematode criteria were recorded.

The data were subjected to analysis of variance and means were separated by the least significant difference LSD at (p=0.05) using PLABSTAT program Version 3, Institute fuer Pflanzen zuechtung, Universitaet Hohenheim.

Results

The results in Table 1 conclude that all selected strains could arrest *M. incognita* reproduction and development compared to untreated control. The most effective isolate was *M. methanica* where it impaired the different nematode stages and total population and its fecundity (eggs/egg-mass). *M.methanica* gave 97 galls and 15 egg-masses/plant compared to untreated control which recorded 678 galls and 201 egg-masses. Its effect was continued to diminish developmental stages (DS) to 75 and mature females (MF) to 17 compared to 675 (DS) and 239 (MF) in untreated control. Consequently, the total population recorded 260 individuals compared to 3824 in untreated control. This bacterium could inhibit *M. incognita* fecundity (112) while untreated control favored nematode fecundity to 640 eggs/egg-mass. However, the most effective three strains followed *M. methanica* in suppression nematode total population were *B. cereus, O. proteus* and *B. brevis* followed by *B. firmus* and *P.aeruginosa*. On the other hand, the weakest isolate was *N. elongata* where recorded 1444 as total population.

Effect of rhizobacterial strains on plant growth presented in Table 2 showed that all bacterial strains enhanced tomato growth compared to nematode infected plants. Generally, all treatments exhibited an increment in total plant weight and length compared by untreated control. The minimum impact on growth criteria was recorded by both *K. planticolla* and *N. elongata*. The same trend was observed on plant length.

Table (1).Effect of some bacterial strains on development and reproduction of *Meloidogyne incognita* infected tomato plants under greenhouse conditions.

Bacterial strains	Galls	Juveniles in soil	Number of Develop-mental stages	Females	Egg-masses	Total population	%R*	Rf**	Number of Eggs/ egg- mass
Bacillus brevis	94	316	112	44	39	472	87.7	0.472	210
Bacillus cereus	116	280	90	41	38	411	89.3	0.411	138
Bacillus firmus	281	650	209	50	41	909	76.2	0.909	239
Klebsiella planticolla	191	948	164	96	93	1208	68.4	1.208	305
Lactobacillus agilis	100	766	223	62	57	1051	72.5	1.051	231
Lactobacillus fermentum	365	760	416	111	96	1287	66.3	1.287	319
Methylomonas methanica	97	168	75	17	15	260	93.2	0.260	112
Neisseria elongata	398	773	594	77	73	1444	62.2	1.444	517
Obesumbacterium proteus	147	283	143	43	40	469	87.7	0.469	182
Pseudomonas aeruginosa	339	633	319	82	69	1034	73.0	1.034	265
Control	678	2910	675	239	201	3824	-	3.824	640
LSD 0.05	9.0	120.0	20.8	9.6	9.6	-	-	-	18.9

*%R= Total population Reduction, **Rf= Reproduction factor

Table (2). Effect of some bacterial strains on growth parameters of tomato plants infected with *Meloidogyne incognita* under greenhouse conditions.

Bacterial strains	Fresh shoot weight	Fresh root weight	Shoot length	Root length	% plant weight increment	% plant length increment
Bacillus brevis	32.6	12.1	51	34	22.9	14.1
Bacillus cereus	35.2	14.5	52	35	30.5	16.1
Bacillus firmus	33.4	13.8	51	34	26.9	14.1
Klebsiella planticolla	29.4	10.3	47	30	13.2	5.2
Lactobacillus agilis	30.2	11.2	50	34	16.7	13.1
Lactobacillus fermentum	31.5	11.3	51	33	19.4	13.1
Methylomonas methanica	34.8	13.3	54	38	28.2	20.7
Neisseria elongata	27.2	11.0	48	32	9.6	8.8
Obesumbacterium proteus	32.4	12.4	50	36	23.1	15.1
Pseudomonas aeruginosa	32.1	11.6	49	32	21.0	9.9
Untreated	25.1	9.4	44	29	-	-
LSD 0.05	2.3	1.6	3.3	3.5	-	-

Data in Table 3 revealed that the activity of certain biological processes was enhanced as a result of using bacterial strains which considered as inducers for the systemic resistance of tomato plants and bio-control agents on nematode. The presence of *M. incognita* only and without any interference led to increase the total phenols (19.6 µg/g.fwt) compared to untreated and uninfected plant (healthy plant) which recorded 16.4 µg/g.fwt. On the other hand, the tomato plants treated with different selected bacterial strains showed increment in their total phenols. The highest value was related to *M. methanica* (27.1 µg/g.fwt) followed by *B. cereus, B.brevis*, *O. proteus* and *B. firmus*. They recorded 26.3, 26.6, 25.5 and 24.2 µg/g.fwt respectively. The lowest value was registered by *N. elongata*. (20.5 µg/g.fwt).

Table (3). Effect of some bacterial strains on peroxidase, polyphenol oxidase activities and total phenol content in tomato roots infected with *Meloidogyne incognita*.

Bacterial strains	Total phenols µg/g f wt	Enzymes			
		Peroxidase		Polyphenol oxidase	
		Activity	Relative activity	Activity	Relative activity
Bacillus brevis	26.3	2.2	1021.9	0.44	1031.6
Bacillus cereus	26.6	2.3	1062.6	0.55	1262.5
Bacillus firmus	24.2	2.1	979.7	0.43	985.5
Klebsiella planticolla	22.2	1.9	876.4	0.20	454.2
Lactobacillus agilis	22.8	1.9	903.0	0.34	777.5
Lactobacillus fermentum	21.2	1.8	826.3	0.32	746.7
Methylomonas methanica	27.1	2.3	1073.6	0.57	1308.7
Neisseria elongata	20.5	1.7	807.5	0.30	669.8
Obesumbacterium proteus	25.5	2.2	1011.0	0.50	1147.0
Pseudomonas aeruginosa	22.8	2.0	924.9	0.42	962.3
Check (infected control)	19.6	0.5	226.9	0.19	431.1
Healthy (Untreated)	16.4	0.2	100.0	0.04	100.0
LSD 0.05	**0.7**	**0.4**	**-**	**0.06**	-

Peroxidase (POX) and polyphenol oxidase (PPO) were increased by all bacterial strains treatments (Table 3). The maximum POX activity was induced by *M. methanica* (2.3 mg/g.fwt). No significant in the POX activity was recorded by the rested bacterial strains .On the other side, the nematode infected plants exhibited enzyme activity (0.5 mg/g.fwt) higher than healthy one (0.2 mg/g.fwt) which was the lowest value.

Enzyme activity of PPO indicated that *M. methanica*has the highest between all treatments followed by *B.cereus*> *O.proteus*> *B.brevis*> *B.firmus*> *P.aeruginosa*> *L.agilis*> *L.fermentum*> *N.elongata*> *K.planticolla*. The enzyme activity of infected plants remained higher than the healthy one that rested at the lowest value.

Results presented in Table 4 noted that all bacterial components were effective on suppressing nematode development and reproduction. All treatments

achieved high reduction in number of galls was related to the crude suspensions followed by filtrates. The lowest reduction in galls was exhibited by the dead cells suspensions. The less effective strain was *B. brevis* (44.5%). Egg-masses production was highly depressed by crude suspension of all strains. The most effective strain was *M. methanica* which registered 96.2% reduction. The rested bacterial strains were arranged as follows: *B. cereus*, *O. proteus* and *B. brevis*. Similarly, filtrate additions keep their efficiency as previously ranked. Viable and dead cell suspensions were less effective than (CS) or (F) in reducing egg-masses/plant. The reduction in nematode total population was related to crude form, values of *M. methanica* (90.4%), *B. cereus* (86.5%), *O. Proteus* (85%) and *B. brevis* (84.5%).

Due to the No. of eggs/egg-mass *M. methanica* was the most successful strain could reduce the reproductive potency of *M. incognita* (74.3 %) followed by *B. cereus* (70.9%), *B. brevis* (62.8) and *O. proteus* (57.6). The lowest effect was done by *B. brevis* (31.3%) as (DCS). The rates of build-up take the same trend, while CS was the most suppresser for nematode reproduction (Rf) in all tested bacteria (Fig. 1).

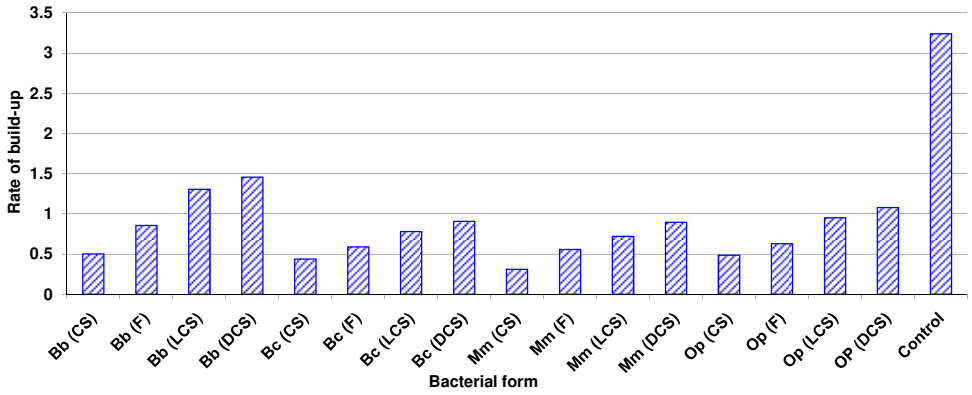

Fig. (1). Effect of some bacterial strains applied as crude suspension, culture filtrate, live and killed cells on *Meloidogyne incognita* reproduction infected tomato plants cv. Castle rock under greenhouse conditions.

CS= Crude suspension, F= Filtrate, LCS= Live cell, DSC= Dead cell, Bb= *Bacillus brevis*, Bc=*Bacillus cereus*, Mm=*Methylomonas methanica*, Op=*Obesumbacterium proteus*

Results in Table 5 pointed to the different forms of all bacterial strains and plant growth criteria. The most effective strain was *M. methanica* as (CS), which exhibited the maximum improvement for shoot and root fresh weight besides shoot dry weight which recorded 30.6, 6.3 and 4.4 gm. respectively. Dead cells of *M. methanica* had the priority than other strains.

Table (4). Effect of some bacterial strains applied as crude suspension, culture filtrate, live and killed cells on development and reproduction of *Meloidogyne incognita* infected tomato plants under greenhouse conditions.

Bacterial strains	Bacterial form (B.F)	No of Galls	%R	No of Juveniles	%R	No of egg-mass	%R	No. of Larval stages	%R	No. of females	%R	Total Population	%R	No. of Eggs/ egg-mass	%R
Bacillus brevis	Crude suspension (CS)	92	52.9	350	54.1	40	55.6	110	55.0	42	56.2	502	54.5	159	62.5
	Filtrate (F)	156	65.3	523	76.2	65	76.0	263	63.9	66	77.6	854	73.6	203	60.1
	Live cell suspension (LCS)	272	49.1	850	61.4	79	71.7	376	45.4	80	72.9	1306	59.7	331	34.9
	Dead cell suspension (DCS)	295	44.5	870	60.5	94	66.3	475	34.9	113	63.2	1458	55.0	350	31.3
	Untreated infested plant	537	-	2202	-	279	-	729	-	306	-	3239	-	509	-
	Mean Bacteria	277.2	-	959	-	111.4		398.6	-	122.4	-	-	-	316.4	-
Bacillus cereus	Crude suspension	94	82.5	330	85.0	32	85.5	73	89.9	34	88.9	427	86.8	145	70.9
	Filtrate	126	76.6	466	78.8	42	85.0	76	89.5	43	85.9	587	81.9	166	63.4
	Live cell suspension	141	73.7	630	71.4	66	75.6	80	89.0	69	77.5	779	76.0	233	54.3
	Dead cell suspension	242	55.0	715	67.4	73	74.0	106	85.5	82	73.4	906	72.0	264	45.1
	Untreated infested plant	537	-	2202	-	279	-	729	-	306	-	3239	-	509	-
	Mean Bacteria	228	-	869.6	-	98.8	-	212.8	-	107.2	-	-	-	268	-
Methylomonas methanica	Crude suspension	65	87.5	233	89.4	11	96.2	60	91.7	17	94.6	310	90.4	131	74.3
	Filtrate	86	83.9	455	79.3	27	90.2	69	90.6	33	89.4	557	82.8	145	70.9
	Live cell suspension	140	73.9	552	74.9	50	82.0	117	83.9	52	83.2	721	77.7	230	54.8
	Dead cell suspension	203	62.1	713	67.6	56	79.3	122	83.3	60	80.5	895	72.4	241	52.7
	Untreated infested plant	537	-	2202	-	279	-	729	-	306	-	3239	-	509	-
	Mean Bacteria	206.2	-	631	-	85	-	219.8	-	94	-	-	-	251.8	-
Obesumbacterium proteus	Crude suspension	54	89.9	395	81.9	34	87.8	53	92.7	35	88.5	486	85.0	216	57.5
	Filtrate	62	88.8	512	76.8	59	79.0	55	92.5	62	79.9	629	80.6	225	55.7
	Live cell suspension	116	78.3	780	64.6	72	74.3	95	86.9	74	76.0	949	70.7	241	52.8
	Dead cell suspension	157	85.2	526	82.5	91	67.4	160	78.0	93	69.7	1079	66.7	277	45.6
	Untreated infested plant	537	-	2202	-	279	-	729	-	306	-	3239	-	509	-
	Mean Bacteria	191.2	-	943.4	-	107	-	218.4	-	114.4	-	-	-	293.4	-
Mean Bacterial form (CS)		76.3	-	327.8	-	29.3	-	74.0	-	32.3	-	-	-	171.0	-
Mean Bacterial form (F)		115.0	-	489.5	-	48.3	-	115.8	-	51.5	-	-	-	190.5	-
Mean Bacterial form (LCS)		167.5	-	703.0	-	67.3	-	187.0	-	68.8	-	-	-	258.8	-
Mean Bacterial form (DCS)		232.5	-	781.8	-	79.0	-	215.8	-	87.0	-	-	-	283.0	-
LSD 0.05 Bacteria (B)		22.1		20.3		5.8		6.9		6.8				17.0	
LSD 0.05 Bacterial form (BF)		20.0		20.8		9.1		13.8		9.9				23.7	
LSD 0.05 (BxBF)		44.8		46.6		20.3		30.4		20.9				53.0	

Table (5). Effect of some bacterial strains applied as crude suspension, filtrate, live and killed cells on growth parameters of tomato infected by *Meloidogyne incognita* under greenhouse conditions.

Bacteria strains	Form	Fresh shoot weight	%I*	Dry shoot weight	%I	Shoot length	%I	Root weight	%I	Root length	%I
Bacillus brevis	Crude suspension	27.4	24.2	3.8	14.2	51.7	24.5	5.5	25.3	35.0	18.1
	Filtrate	26.7	22.3	3.6	9.9	45.3	14.0	5.3	22.0	34.0	15.7
	Live cell suspension	25.4	18.2	3.6	9.6	45.3	14.0	4.6	10.7	30.3	5.
	Dead cell suspension	24.2	13.9	3.4	3.2	40.3	3.3	4.6	10.2	29.7	3.4
	Untreated infested plant	20.8	-	3.3	-	39.0	-	4.12	-	28.7	-
	Mean Bacteria	**24.9**	-	**3.5**	-	**44.3**	-	**4.8**	-	**31.5**	-
Bacillus cereus	Crude suspension(CS)	30.5	31.9	4.4	25.7	57.3	32.0	5.9	30.3	36.0	20.7
	Filtrate (F)	29.0	28.3	4.0	19.3	50.3	22.5	5.5	25.3	34.7	17.3
	Live cell suspension (LCS)	28.3	26.6	3.9	17.3	48.7	19.9	5.1	18.8	32.3	11.3
	Dead cell suspension (DCS)	26.5	21.6	3.8	15.4	47.0	17.0	4.9	15.1	30.7	6.5
	Untreated infested plant	20.8	-	3.3	-	39.0	-	4.1	-	28.7	-
	Mean Bacteria	**27.0**	-	**3.9**	-	**48.5**	-	**5.1**	-	**32.5**	-
Methylomonas mechanica	Crude suspension	30.6	32.0	4.4	26.3	58.3	33.1	6.3	34.5	37.0	22.5
	Filtrate	29.6	29.7	4.1	21.2	51.0	23.5	5.5	25.7	35.0	18.1
	Live cell suspension	28.9	28.0	4.1	19.9	49.0	20.4	5.3	22.6	33.7	14.9
	Dead cell suspension	28.3	26.5	3.9	17.1	47.3	17.6	5.2	20.4	30.7	6.5
	Untreated infested plant	20.8	-	3.3	-	39.0	-	4.1	-	28.7	-
	Mean Bacteria	**27.6**		**3.95**	-	**48.9**	-	**5.3**	-	-	-
Obesumbacterium proteus	Crude suspension	28.9	28.0	4.0	18.4	52.0	25.0	5.6	26.4	35.7	19.6
	Filtrate	26.2	20.6	3.9	16.2	47.7	18.2	5.3	22.2	34.3	16.5
	Live cell suspension	22.5	7.5	3.6	10.0	43.3	10.0	4.7	12.6	30.7	6.5
	Dead cell suspension	21.5	3.1	3.5	6.1	40.3	3.3	4.7	11.4	30.3	5.5
	Untreated infested plant	20.8	-	3.3	-	39.0	-	4.1	-	28.7	-
	Mean Bacteria	**24.0**	-	**3.6**	-	**44.5**	-	**4.9**	-	**31.0**	-
Mean Bacterial form (CS)		**29.4**	-	**4.1**	-	**54.8**	-	**5.8**	-	**35.9**	-
Mean Bacterial form (F)		**27.9**	-	**3.9**	-	**48.6**	-	**5.4**	-	**34.5**	-
Mean Bacterial form (LCS)		**26.3**	-	**3.8**	-	**46.6**	-	**4.9**	-	**31.8**	-
Mean Bacterial form (DCS)		**25.1**	-	**3.7**	-	**43.8**	-	**4.8**	-	**30.3**	-
LDS 0.05 Bacteria (B)		**0.7**		**0.4**		**1.6**		**0.6**		**2.5**	
LDS 0.05 Bacterial form (BF)		**1.8**		**0.4**		**1.8**		**0.5**		**1.7**	
LDS 0.05 (BxBF)		**3.5**		**0,8**		**3.7**		**1.0**		**3.4**	

* % I = Increment percentage, B= Bacteria, BF = Bacterial form

Discussion

These results were in agreement with **Valerie *et al.*, (2001)** where they investigated the possibility of soil-born *Pseudomonas* spp. and *Bacillus cereus* for induction resistance. They added that the application of these bacteria reduce nematodes fecundity, increase the proportions of distorted females and produced females with fewer eggs. Studies of **Marleny *et al.*, (2008)** led to the hypothesis that induction of soil suppressiveness against *M. incognita* using inoculants is related to soil microbial activity and rhizosphere bacterial populations. They added that the selected microbial inoculants increase rhizosphere bacterial populations. Besides the previous effects, **Vetrivelkalail *et al.*, (2010)** pointed to the nematicidal action of *Pseudomonas* spp., *Bacillus* spp. and *Methylobacterium* spp. against *M. incognita*. Also results present here can be clarify as a network form with complex interactions among bacteria, nematodes, plants and environment to control populations of plant-parasitic nematodes in natural conditions **(Kerry,2000).** Rhizobacteria have many different modes of action in the soil, their effects directly through antagonizing by means of the production of toxins, lytic enzymes and other anti-nematode products **(Siddiqui and Mahmood, 1999 and Giannakou *et al.,* 2007).** Also, rhizobacteria-mediated induced systemic resistance-ISR- **(Van Loon *et al.*, 1998 and Ramamoorthy *et al.*, 2001).**

This plant encouragement is due to the microbial residents of the rhizosphere, those represent a potential reservoir of biological agents which can suppress nematode multiplication consequently the nematode damage diminishes. In the otherwise an induction resistance occurs within host that can decrease nematode infection. **Siddiqui *et al.,* (2007)** support our results; they found that inoculation of any PGPR species alone or together with *Rhizobium* increased plant growth in *M. javanica* inoculated plants. Also **Ali *et al.* (2002)** stated that, soil drench with some strains of *P. aeruginosa* and *Pseudomonas* sp. significantly reduced populations of *M. javanica* and subsequent root-knot disease severity with enhanced protein contents and yield of mungbean plants. Similarly another investigation confirmed that *B. cereus* S18 is an effective bio-control agent towards *M. incognita* on a broad spectrum of hosts' plant. **Mahdy (2002)** demonstrated that all crops treated with *B. cereus* S18 combined with *M. incognita* showed plant growth enhancement when compared with the bacteria untreated crops.

The mechanisms by which plant growth is improved may be similar to those exhibited by rhizosphere microorganisms and include the production of phytohormones, promotion through enhanced availability of nutrients, reduction of ethylene levels, production of antibiotics and induced systemic resistance **(Holland, 1997).** Suppressing nematode damage with rhizobacterial strains increased tomato root weight, which could also account for some of the observed suppression; as reducing galls, stopping revitalization root tips. This may stop their growth or cause excessive branching of roots, paving the way to normal function of roots such as

uptake and transport water and nutrients. Positive impact extended to improve plant biomass and height, **(Marleny *et al.,* 2008).**

This effective role of the total phenols was investigated since **1959** where **Clark *et al.***, related the mechanism of disease resistance to the phenolic compounds. They added that this activity due to the quinicacid or caffeic acid parts of chlorogenic acid which are released by the action of hydrolytic enzymes such as esterases. Also, certain phenolic compounds like acetylenes, terpenoid aldehydes, sesquiterpenoids and phenoxypropionic acid derivatives are known to have nematicidal activity **(Veech, 1979; Mori *et al*., 1982; Hayashi *et al.,* 1983).** In **1985 Mahajan *et al***., indicated that quinones are involved in imparting nematicidal activity.

These previous results are due to the synthesis and accumulation of these enzymes which are frequently associated with plant defense against various pathogens where they are catalysts for the oxidation of substrates like phenol and its derivates by hydrogen peroxide **(Buonario & Montalbini, 1993 and Lebeda *et al*., 1999).** The role of the peroxidase in plant defense systems is to remove the toxic effect of hydrogen peroxide from tissues and to participate in the synthesis of phenolic compounds and the building of the intermolecular bonds to fortify cell walls at the sites of pathogen invasion **(Repka & Lovakova, 1994 and Passardi *et al.,* 2004).** So peroxidase is a key enzyme in the biosynthesis of lignin **(Bruce and West, 1989).** Remarkable increases were observed in the peroxidase activity of all the cellular compounds, viz. soluble fraction, mitochondria and microsomes. It is previously suggested that peroxidase is an ISR marker.

These results demonstrated that efficacy of whole bacterial culture (CS) have a pronounced ability to ISR against nematode. However, (CS) represent rhizobacteria in viable state and their metabolites; such antibiotics, siderophores or/and other compounds like hormones, acids and other toxic compounds become more lethal to nematode or by another meaning (CS) gathered two advantages rerated to viable cells and metabolites so showed greater impact than other component. Several bacterial identified as ISR elicitation in different plant species as follow: lipopolysaccharides: lipid A; O-antigenic sidechain, siderophores: pseudobactins; pyochelin; salicylic acid (SA), flagella, antibiotics: pyocyanin, 2,4-diacetylphloroglucinol N-acylhomo serine lactones and volatile of systemic resistance in tomato plants **(Van Loon and Bakker, 2005).**

Live and dead cells also have the ability to induce systemic resistance. This was clearly observed by reduction of total nematode population and supported by the results of **(Reitz *et al.,* 2000)** which demonstrated that living and heat-killed cells of *R.etli* induced potato systemic resistance against *Globodera pallida* infection. They suggested that heat-stable surface structures such as exopolysaccharides (EPS) and/or lipopolysaccharides (LPS) of *R. etli* G12 act as inducing agents. The

highest effect resulted from *M. methanica* may due to it is gram-negative bacterium and may have lectin binding structures in the LPS and EPS layers of the cell wall membrane as in *Pseudomonads* **(Lotan *et al.,* 1975).** The resistance inducing activity of bacterial metabolites to diseases has been described in literature **(Schonbeck *et al.*, 1980).** Also, culture filtrate of rhizobacteria could ISR against nematode **(Hasky-Günther *et al.,* 1998).** This ability may due to certain compounds including siderophores, 2,3-butanediol, the compound 2,3-butanediol that produces by *Bacillus* spp. and it is not only elicits ISR, but it also involved in promoting growth **(Ryu *et al.*, 2003).**

Enhancement of plant growth are due to the microbial metabolites of the rhizobacteria under study which have double impact; indirectly by suppress nematode reproduction resulting in relief the adverse effect on plant fitness or directly via releasing some beneficial matters such nutrients, hormones and others which improve plant health.

Our study indicated that the potent rhizobacteria isolated and identified as *Bacillus brevis, Bacillus cereus, Methylomonas methanica* and *Obesumbacterium proteus*, could antagonistic to root-knot nematodes and could be developed into a valuable crop management tool to reduce the deleterious impact of these nematodes on plant growth. Also, enzyme activities elevation in bacterial treated roots over infected-untreated control suggested that these rhizobacteria can also indirectly suppress the nematode reproduction through ISR of tomato, this suggestion was supported by the adverse effect of such component tested, and especially Heat-killed cells which cloud inhibited the nematode reproduction. Results from these studies should contribute to a better understanding of the complex interactions among root-knot nematodes, introduced rhizobacteria and host plant. Such information would be valuable for the isolation and characterization of the active nematicidal agents or inducers agent or double impacts organisms. Also improving the performance of different bacterium by many procedures must be considered. However, to better use these isolates, more research is needed to determine their exact mode of action against nematodes on different hosts, their survival in soil, and efficient formulation and application methods.

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الملخص العربي

تقييم بعض أنواع بكتريا التربة كمستحثات لمقاومة نباتات الطماطم أو كعوامل حيوية لمكافحة نيماتودا تعقد الجذور (ميلودوجيني انكوجنيتا)

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**قسم وقاية النبات– مركز بحوث الصحراء– القاهرة.

بعد مجموعة من تجارب التقييم في المعمل و الصوبة للعديد من بكتريا التربة المعزولة من منطقة ريزوسفير جذور الطماطم في مناطق مختلفة تم انتخاب أفضل عشر سلالات بكتيرية معزولة من التربة والتي أظهرت كفاءة في خفض تعداد نيماتودا تعقد الجذور وكانت أكثرها كفاءة هي الأربع سلالات ***Bacillus brevis, Bacillus cereus, Methylomonas methanica*** **and** ***Obesumbacterium proteus*** حيث حققت نسب خفض عالية في التعداد الكلي للنيماتودا تتراوح ما بين ٨٧,٧ إلى ٩٣,٢% مقارنة بالكنترول المعدي الغير معامل بالبكتريا، كذلك كانت ذات تأثير ايجابي على نمو نباتات الطماطم صنف كاسل روك. وقد أظهر تقدير نشاط إنزيمي البروكسيديز والبولي فينول اوكسيديز والمحتوي الكلي للفينولات زيادة ملحوظة عن الكنترول السليم والمعدي، مما يظهر قدرة هذه البكتريا علي إحداث تغييرات بيوكيميائية داخل نبات الطماطم وتعتبر دلائل على استحثاث مقاومة النبات ضد النيماتودا.

في تجربة استخدام صور مختلفة من مكونات السلالات البكترية الأربع لمعرفة أكثرها تأثيرًا وكذلك تأكيد قدرتها علي التأثير الغير مباشر علي النيماتودا، فقد أظهرت النتائج أن إضافة المزرعة كاملة (خلايا حية+ راشح) كانت الأفضل في قدرتها علي خفض معدل تكاثر النيماتودا تلاه الراشح ثم الخلايا الحية ثم الخلايا الميتة. مما يشير إلى قدرة هذه السلالات البكتيرية علي تثبيط معدل تكاثر النيماتودا من خلال تنشيط مقاومة النبات.

يستنتج من هذه النتائج أن تلك السلالات من الريزوبكتريا استطاعت أن تحد من تكاثر النيماتودا من خلال قدرتها على استحثاث المقاومة لنباتات الطماطم، ويقترح إجراء المزيد من التجارب الحقلية عليها وتقييم كفاءتها على أنواع نباتية أخرى لإدخالها في برامج مكافحة النيماتودا.