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Evaluation of rhizobacteria as resistance inducers or bio-control agents for the control of *Meloidogyne incognita* in tomato

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Abstract

Bacterial strains *Bacillus brevis*, *B. cereus*, *B. firmus*, *Klebsiella planticola*, *Lactobacillus agilis*, *L. fermentum*, *Methylomonas methanica*, *Neisseria elongata*, *Obesumbacterium proteus* and *Pseudomonas aeruginosa* recovered from tomato rhizosphere and tested for their ability to induce systemic resistance or bio-control agent against *Meloidogyne incognita* in tomato under greenhouse condition. Results showed that all tested bacterial strains showed significant reduction in nematode development and reproduction. The most effective strains were *M. methanica*, *B. cereus*, *B. brevis* and *O. 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 as 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, sequentially. It was concluded that bacteria has induced tomato resistance or bio-control effects against *M. incognita* in tomato.

Keywords: Bacterial strains, resistance, *Meloidogyne incognita*, tomato, greenhouse

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 nematicide application have been used for the management of nematodes. Rhizosphere provides the first line of defense for roots against nematode attack and 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 that make them useful agents for nematode management. A number of plant-microbe interactions showed that such antagonistic rhizobacteria directly by competition and antibiosis (Buchenauer, 1998) and indirectly inducing systemic resistance (ISR) in the plant toward soil-borne pathogens. Hasky-Günther *et al*., (1998) reported first induced systemic resistance mechanism of action by rhizobacteria against nematode. Reitz *et al*., (2000) and Siddiqui & Shaukat (2002) confirmed occurring of ISR by rhizobacteria.

In this study the ability of some rhizobaterial strains was evaluated as ISR or bio-control agent toward root-knot nematode *Meloidogyne incognita* and to exploring the performance of different bacterial component as elicitor for plant resistance and impact on plant growth.

Materials and Methods

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

Single egg-mass culture of *Meloidogyne incognita* was mass reared in tomato plants growing in greenhouse conditions. Tomato cv. Castel rock cultivated in 25 cm diam. earthen pots filled with about one kg sterilized soil (3 sand : 1 clay V:V) were used in all experiments. Four weeks-old tomato seedlings susceptible to *Meloidogyne incognita* were transplanted in the pots kept in the greenhouse at 30±5 °C. Compound fertilizer and water applied to plants in green house as Egyptians Ministry of Agriculture. Bacterial suspensions were added as soil drench (100 ml/pot at 10^9 cfu) two days before nematode inoculation time with 1000 J_2 of *M. incognita* per pot. The treatments were replicated four times (4 pots) in a completely randomized block design. After forty five days of nematode inoculation, roots of 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 recorded. Also, peroxidase and polyphenol oxidase activity and total phenols were estimated. Total soluble phenols were determined by using Folin and Ciocalteu's Phenol Reagent (Daniel & George, 1972).

Enzymes extraction form rhizobateria treated and nematode infected roots only compared with healthy one were collected 7 days after nematode inoculation to estimate enzyme activity. Enzyme extract were prepared according to Maxwell & Batemen (1967). Assay of peroxidase activity (POX), changes in POX activity were determined following the procedure described by Sridhar & Ou (1974). POX activity was expressed as change in absorbance (Δ O.D 470 nm) per min/g fresh weight. Assay of polyphenol oxidase activity (PPO), changes in PPO activity were determined according to Maxwell & Batemen (1967). The activity of POX was expressed as (Δ O.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: *B. brevis*, *B. cereus*, *M. methanica* and *O. 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, VC and KC. All forms were added to soil (100 ml/pot) two days before nematode inoculation with 1000 *M. incognita* per plant. Pots were kept in a greenhouse for 45 days then plants were uprooted and nematode populations 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.

Results

The results in Table 1 showed that all selected strains could arrest *M. incognita* reproduction and development as compared to untreated control. The most effective isolate was *Methylomonas methanica* and impaired the different nematode stages, total population and fecundity (eggs/egg-mass) with 97 galls and 15 egg-masses/plant as compared to untreated control 678 galls and 201 egg-masses. Its effect was continued to diminish developmental stages (DS) to 75 and mature females (MF) to 17 as compared to 675 (DS) and 239 (MF) in untreated control. Consequently, the total population recorded 260 individuals as 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 weak isolate was *N. elongata* where recorded 1444 as total population.

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

Bacterial strains	No. of Galls	No. of Juveniles in soil	Developmental stages	Females	Egg-masses	Total population	Reduction (%)	Rf	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 planticola	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**

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

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 planticola	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 the ten selected bacterial strains which consider as inducers for the systemic resistance and bio agent on nematode reduction. The presence of *M. incognita* only and without any interference led to increase the total phenols (19.6 µg/g fwt) as compared to untreated and uninfected plant (healthy plant) which recorded 16.383 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* (27.088 µg/g fwt) followed by *B. cereus*, *B. brevis*, *O. proteus* and *B. firmus* and provided 26.3, 26.6, 25.5 and 24.2 µg/g fwt, respectively. The lowest value was observed in *N. elongata* (20.5 µg/g fwt). POX and 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 were recorded in all other 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 effect.

Enzyme activity of PPO indicated that *M. methanica* was high followed by *B. cereus* > *O. proteus* > *B. brevis* > *B. firmus* > *P. aeruginos* > *L. agilis* > *L. fermentum* > *N. elongate* > *K. planticola*. The enzyme activity of infected plants remained higher than uninfected.

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 fwt	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
Lactobacillus agilis	22.8	1.9	903.0	0.34	777.5
Klebsiella planticola	22.2	1.9	876.4	0.20	454.2
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**	-

Results presented in Table 4 noted that all bacterial components were effective on suppressing the different nematode development and reproduction. All treatments achieved high reduction in number of galls was related to the crude suspensions followed by filtrates (Fig. 1). 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* 96.2% reduction followed by *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 of *M. methanica* (90.4%), *B. cereus* (86.5%), *O. proteus* (85%) and *B. brevis* (84.5%).

No. of eggs/egg-mass reduced by *M. methanica* (74.3%) followed by *B. cereus* (70.9%), *B. brevis* (62.8%) and *O. proteus* (57.5%). 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.

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.

Bacteria (B)	No. of Galls	R (%)	No. of J_2	R (%)	No. of egg-mass	R (%)	No. of Develop-mental stages	R (%)	No. of females	R (%)	Total population	R (%)	No. of Eggs/ egg-mass	R (%)
Bacillus brevis														
Crude suspension (CS)	92	82.9	350	84.1	40	85.8	110	85.0	43	86.2	503	84.5	189	62.8
Filtrate (F)	186	65.3	523	76.3	65	76.0	263	63.9	68	77.8	854	73.6	203	60.1
Live cell suspension (LCS)	273	49.1	850	61.4	79	71.7	376	48.4	80	73.9	1306	59.7	331	34.9
Dead cell suspension (DCS)	298	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	-	308	-	3239	-	509	-
Mean	277.2	-	959	-	111.4		390.6	-	122.4	-	-	-	316.4	-
Bacillus cereus														
Crude suspension	94	82.5	330	85.0	32	88.5	73	89.9	34	88.9	437	86.5	148	70.9
Filtrate	126	76.6	468	78.8	42	85.0	76	89.5	43	85.9	587	81.9	186	63.4
Live cell suspension	141	73.7	630	71.4	68	75.6	80	89.0	69	77.5	779	76.0	233	54.3
Dead cell suspension	242	55.0	718	67.4	73	74.0	106	85.5	82	73.4	906	72.0	264	48.1
Untreated infested plant	537	-	2202	-	279	-	729	-	308	-	3239	-	509	-
Mean	228	-	869.6	-	98.8	-	212.8	-	107.2	-	-	-	268	-
Methylomonas methanica														
Crude suspension	65	87.8	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	148	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	58	79.3	122	83.3	60	80.5	895	72.4	241	52.7
Untreated infested plant	537	-	2202	-	279	-	729	-	308	-	3239	-	509	-
Mean	206.2	-	831	-	85	-	219.8	-	94	-	-	-	251.8	-
Obesumbacterium proteus														
Crude suspension	54	89.9	398	81.9	34	87.8	53	92.7	35	88.5	486	85.0	216	57.5
Filtrate	62	88.5	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	52.6
Dead cell suspension	187	65.2	826	62.5	91	67.4	160	78.0	93	69.7	1079	66.7	277	45.6
Untreated infested plant	537	-	2202	-	279	-	729	-	308	-	3239	-	509	-
Mean	191.2	-	943.4	-	107	-	218.4	-	114.4	-	-	-	293.4	-
CS	76.3	-	327.8	-	29.3	-	74.0	-	32.3	-	-	-	171.0	-
F	115.0	-	489.5	-	48.3	-	115.8	-	51.5	-	-	-	190.5	-
Mean LCS	167.5	-	703.0	-	67.3	-	167.0	-	68.8	-	-	-	258.8	-
Mean DCS	232.5	-	781.8	-	79.0	-	215.8	-	87.0	-	-	-	283.0	-
LSD 0.05 (B)	22.1	-	20.3	-	5,8	-	6.9	-	6.8	-	-	-	17.0	-
LSD 0.05 (F)	20	-	20.8	-	9.1	-	13.6	-	9.9	-	-	-	23.7	-
LSD 0.05 (BxF)	44.8		46.6		20.3		30.4		20.9	-	-	-	53.0	-

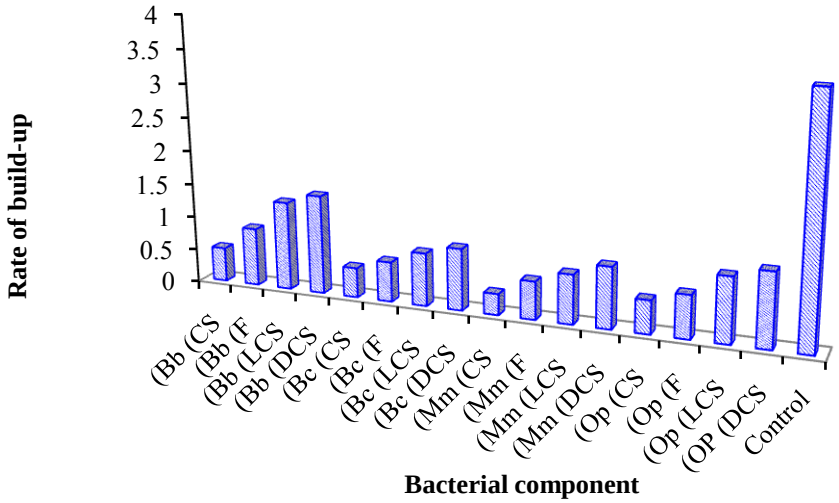
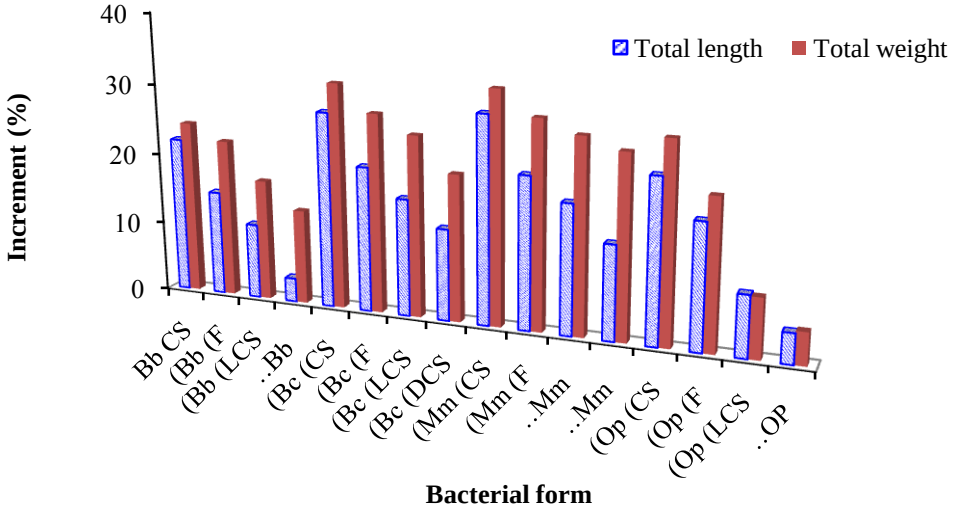


Fig. 1. Effect of selected bacterial strains as crude suspension, culture filtrate, live and killed cells on development and reproduction of *Meloidogyne incognita* infected tomato plants under greenhouse conditions.

Results in Table 5 pointed to the different forms of all bacterial strains and plant growth. 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 (Fig. 2).



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

Fig. 2. Effect of some bacterial strains as crude suspension, culture filtrate, live and killed cells on total fresh weight and height of tomato plants infected with *Meloidogyne incognita* under greenhouse conditions.

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	Increase (%)	Dry shoot weight	Increase (%)	Shoot length	Increase (%)	Root weight	Increase (%)	Root length	Increase (%)
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 (BCS)	26.5	21.6	3.8	15.4	47.0	17.0	4.9	15.1	30.7	6.5
Untreated infested plant (control)		20.8	-	3.3	-	39.0	-	4.1	-	28.7	-
Mean		27.0	-	3.9	-	48.5	-	5.1	-	32.5	-
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 (control)		20.8	-	3.3	-	39.0	-	4.1	-	28.7	-
Mean		24.0	-	3.6	-	44.5	-	4.9	-	31.0	-
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 (control)		20.8	-	3.3	-	39.0	-	4.12	-	28.7	-
Mean		24.9	-	3.5	-	44.3	-	4.8	-	31.5	-
Methylomonas methanica	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 (control)		20.8	-	3.3	-	39.0	-	4.1	-	28.7	-
Mean		27.6		3.95	-	48.9	-	5.3	-	-	-
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 (F)		1.8		0.4		1.8		0.5		1.7	
LDS 0.05 (BxF)		3.5		0.8		3.7		1.0		3.4	

Discussion

These results were in agreement with Beneduzi *et al*., (2001) where they investigated the possibility of soil-born *Pseudomonas* spp., and *Bacillus cereus* for induction resistance. Application of bacteria reduced nematodes fecundity, increases the proportions of distorted females and produced females with fewer eggs. Studies of Burkett-Cadena *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, Vetrivelkalai *et al*., (2010) pointed to the nematicidal action of *Pseudomonas* spp., *Bacillus* spp., and *Methylobacterium* spp., against *M. incognita*. Results indicated as complex interactions among bacteria, nematodes, plants and environment to control populations of plant-parasitic nematodes in natural conditions (Kerry, 2000). These effective through directly antagonizing with the production of toxins, lytic enzymes and other anti-nematode products (Siddiqui & Mahmood, 1999; Giannakou *et al*., 2007). Also, Rhizobacteria-mediated induced systemic resistance-ISR- (van Loon *et al*., 1998; 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. Otherwise, an induction resistance occurs within host that can decrease nematode infection. Siddiqui *et al*., (2007) 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 *P. aeruginosa* strains 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 host plants. 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 improved 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 accounted for some of the observed suppression; as reducing galls, stopping revitalization root tips. Growth stopped or caused 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 (Burkett-Cadena *et al*., 2008). This effective role of the total phenols was investigated by Clark *et al*., (1959) related to the mechanism of disease resistance to the phenolic compounds. They added that this activity due to the quinic acid or caffeic acid parts of chlorogenic acid which released by the action of hydrolytic enzymes such as esterases. Also, certain phenolic compounds like acetylenes, terpenoid aldehydes, sesquiterpenoids and phenoxypropionic acid derivatives have nematicidal activity (Veech, 1979; Mori *et al*., 1982; Hayashi *et al*., 1983). Mahajan *et al*., (1985) indicated that quinines were involved in imparting nematicidal activity.

These previous results were due to the synthesis and accumulation of these enzymes which 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; 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; Passardi *et al*., 2004). So peroxidase was a key enzyme in the biosynthesis of lignin (Bruce & West, 1989). Remarkable increases were observed in the peroxidase activity of all the cellular compounds, viz. soluble fraction, mitochondria and microsomes. Peroxidase previously suggested as ISR marker. These results demonstrated that efficacy of 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 related to viable cells and metabolites so showed greater impact than other component. Several bacterial strains identified as ISR elicitation in different plant species as lipopolysaccharides: lipid A; O-antigenic sidechain, siderophores: pseudobactins; pyochelin; SA, flagella, antibiotics: pyocyanin, 2,4-diacetylphloroglucinol N-acylhomo serine lactones and volatile of systemic resistance in tomato plants (van Loon & Baker, 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 *Rhizobium 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 its 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 (Schonbeck *et al*., 1980). Also, culture filtrate of rhizobacteria (ISR) against nematode was reported by Hasky-Günther *et al*., (1998). This ability was due to certain compounds including siderophores, 2,3-butanediol, the compound 2,3-butanediol that produces by *Bacillus* spp., and not only elicited ISR, but also involved in promoting growth (Ryu *et al*., 2003). Enhancement of plant growth were due to the microbial metabolites of the rhizobacteria under study which have double impact; indirectly by suppress nematode reproduction resulting in relief the adverse impact on plant fitness or directly via releasing some beneficial matters such nutrients, hormones and others which improve plant health.

Enzyme activities elevation in bacterial treated roots over infected control only suggested that rhizobacteria indirectly suppress the nematode reproduction through ISR of tomato and supported by the adverse effect of such component tested and especially heat-killed cells which inhibited the nematode reproduction. Results from these studies contributed understanding of the complex interactions among root-knot nematodes, introduced rhizobacteria and host plant. Such information valuabled 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 will be suggested to determine their exact mode of action against nematodes, their survival in soil and efficient formulation and application methods.

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