J. Agric. Sci. Mansoura Univ., 28 (4): 3093 - 3108, 2003

POTENTIAL OF CERTAIN BACTERIAL STRAINS AS ROOT- KNOT NEMATODE BIOCONTROL AGENTS

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ABSTRACT

Five bacterial strains i.e. *Bacillus subtilis*, *B. megatherium*, *Klebsiella pneumoniae*, *Pseudomonas* spp. and *Streptomyces* spp. were tested as biological control agents against root - knot nematode *Meloidoggne javanica* infecting sunflower plant under green house conditions. Their potential effect on development and reproduction of *M. javanica* was evaluated. Results indicated that most of the bacterial strains tested significantly reduced numbers of galls, developmental stages, egg masses in roots, and second stage juveniles (J2) in soil. Consequently, nematode rate of reproduction decreased. The degree of nematode suppression was proportional to inoculum size of the bacterial culture added to the pots, and was more pronounced when bacterial treatment was applied one week prior to nematode infection. Moreover, application of bacterial inoculants improved the growth of sunflower plants regardless to type or inoculum size.

Keywords: Nematode, *Meloidogyne javanica*, Rhizobacteria, *Bacillus*, *Kelbsiella*, *Pseudomonas*, *Streptomyces* and Biological control

INTRODUCTION

Organisms identified as potential biocontrol agents against plant-parasitic nematodes have widely been known all over the world. They mostly include fungi, bacteria, soil invertebrates, and predatory nematodes (Stirling, 1991). Although an encounter between a nematode and its antagonist may occur any where in soils, certain developmental stages of the most plant-parasitic nematodes are commonly found in the root zone of the host plants. For example, potential targets for disruption are nematode eggs and hatching, juvenile movement through soil, and juvenile attraction to roots. The presence of specific naturally occurring or introduced rhizobacteria can significantly modify the rhizosphere environment and affect directly or indirectly on the nematode or on the host parasite interrelationship. Consequently, rhizobacteria have been evaluated for their antagonistic effects on plant-parasitic nematodes including *Meloidogyne incognita* (Becker *et al.*, 1987 and 1988; Kloepper *et al.*, 1992 and Zavaleta-Mejia, 1985), *M. hapla* (Hongling *et al.*, 1995), *M. javanica* (Spiegel *et al.*, 1991), *Criconemella xenoplax* (Kluepfel *et al.*, 1993), *Heterodera glycines* (Kloepper *et al.*, 1992 and Honglin and Riggs, 2000); *H. schachtii* (Oostendrop and Sikora, 1989, 1990 and Neipp and Becker, 1999); *Globodera pallida* (Racke and Sikora, 1992), and *G. rostochiensis* (Cronin *et al.*, 1997).

Many rhizobacteria, including strains of *Serriata* spp. (Zavaleta-Mejia, 1985), *Pseudomonas* spp. (Becker *et al.*, 1989; Oostendrop and Sikora,

Zavaleta-Mejia *et al.*, 1989 and Neipp and Becker, 1999) have been recorded as effective antagonists to plant – parasitic nematodes. In addition, the actinomycetes have been considered as one of the main groups of interest, which produce antibiotics, with suppressive effects against other organisms (Omura, 1986). *Streptomyces avermitilis* reduced galling in plant roots by *M. incognita* (Becker *et al.*, 1988); and *Streptomyces* spp. was negatively effective on egg hatching and juvenile survival of *M. javanica* (El-Sherif *et al.*, 1994 and Ali, 1996).

Some bacterial metabolites, such as avermectins (Stretton *et al.*, 1987), valinomycin (Mishra *et al.*, 1987), and 2, 4-diacetylphloroglucinol (Cronin *et al.*, 1997) as well as volatile metabolites such as various organic acids, hydrogen sulfide, and ammonia have adverse effects on nematode development (Stirling, 1991).

The objective of this research was to evaluate the antagonistic potential of the five bacterial isolates identified as *Bacillus subtilis*, *B. megatherium*, *Klebsiella pneumoniae*, *Pseudomonas* spp. and *Streptomyces* spp. on development and reproduction of *M. javanica* parasitizing sunflower plants grown under green house conditions.

MATERIALS AND METHODS

1- Microorganism

Five bacterial species isolated from sandy soils (Sedik, 1997) were used throughout the following experiment. The bacterial isolates were purified and identified to the species level according to API microtube system (API 20B, API 20E and API 50 CHE, Logan and Berkeley, 1984). Also, cells grown in nutrient broth and on agar plantes were studied for their Gram reaction, colony and cell characteristics and their antagonist effect against *M. javanica* infected sunflower plants.

2- Experimental design

Each strain was precultured in nutrient broth and grown separately at 30° C for 3-5 days with shaking rate at 140 rpm. Serial dilutions were prepared and the bacterial counts were estimated in a selective media as proposed by Haahtela *et al.* (1983) for *Pseudomonas* spp.; Youch and pengra (1966) for *Klebsiella* spp. and Martin (1950) for *Streptomyces* spp.. In addition, nutrient agar supplemented with yeast extract was used for growth of *Bacillus* spp.. Bacterial cells grown in nutrient broth were studied for their Gram reaction, colony and cell morphology. The bacterial inoculum was prepared for each isolates in a liquid culture containing ca 10^8 cells/ml.

The inoculum of each strain was added to the soil at the rate of 2.5, 5.0 and 10.0 ml/2.0 kg soil/pot. Application time for both bacteria and nematodes inocula was also studied. The bacterial inoculants were added to the soil seven days before nematode inoculation, at the same time or seven days after adding the nematode inocula to the pots.

Seeds of sunflower, *Helianthus annus* L. cv. Balady were planted in 16 cm diam. pots containing 2.0 kg autoclaved sandy clay soil (2:1, V/V). After germination, the plants were thinned to one seedling per pot. Then, the

pots were divided to five sets; each of three of them contained twenty pots. Four pots of the first set were inoculated with each bacterial culture inoculants 5 days after thinning at the rate of 2.5, 5.0 and 10.0 ml/pot and control. One week later, the pots were inoculated with 2000 newly hatched juveniles of *javanica* (J2) per pot.

In the second set of pots, both nematode and bacterial inoculants were applied simultaneously. Finally, nematode inoculation was introduced one week before bacteria inoculation in the third set of pots. Each of the other two sets contains three pots and served as check. One of them was inoculated with nematode only at the above-mentioned rate (check 1), and the plants of the other set received nematode and plain media (check 2).

Each treatment was replicated four times and all the pots were arranged in a completely randomized design in a green house, watered and received the normal agricultural practices.

After 50 days, the experiment was terminated. Plant growth parameters based on length and weight of shoots and roots were recorded. In addition, the number of galls, nematode counts in soil (Baerman pan technique, 1917; Cobb, 1918 and Christie and Peery, 1951) and roots (Franklin, 1949 and Goodey, 1957) were determined to calculate the nematode final population and rate of reproduction.

3- Analysis.

The least significant difference using MSTAT microcomputer statistical program (Power *et al.*, 1982) performed statistical analysis.

RESULTS

1- Application of bacterial inoculants prior to nematode infection

1-1- Effect on development of the nematode (*M. javanica*)

Based on microscopical, cultural and nutritional characteristics, the isolates were belonged to *Bacillus subtilis*, *B. megatherium*, *Klebsiella pneumonia*, *Pseudomonas* spp. and *Streptomyces* spp..

These bacterial isolates at different inoculum size and time of application had significant effect on development and reproduction of *M. javanica* infecting sunflower plants. The nematocidal activities of such bacterial species are presented in Table (1) when they had been added one week before nematode inoculation.

In general, all inoculum sizes tested for the five isolates significantly reduced the number of galls on sunflower roots comparing with those of the checks. Evidently, no significant differences in gall numbers was detected between both check treatments. Number of galls developed was negatively correlated with the bacterial cell concentration. By increasing cell concentration, number of galls was gradually decreased. In addition, all bacterial inoculants greatly reduced the nematode counts either in soil or in roots. Moreover, all inoculum sizes tested significantly reduced the nematode developmental stages and egg-laying females in roots as well as number of J2 in soil.

Table(1): Effect of five bacterial inoculants applied seven days before *M. javanica* infection on nematode development and reproduction.

Bacterial species and inoculum level (cm)	No. of Galls	No. of egg-masses	Developmental stage	Average Eggs/Egg mass	No. of J2	Final pop. (Pf) of nematode	Rate of reproduction Pf/Pl
Bacillus subtilis							
2.5	186 cd*	153 d	56 b	367 b	3120 b	3329 b	1.66
5.0	160 e	142 d	27 d	360 b	2620 bc	2789 bc	1.39
10.0	99 g	88 f	22 defgh	240 f	1378 efg	1488 efg	0.74
Bacillus megatherium							
2.5	174 cd	154 cd	41 c	312 de	2459 cd	2654 cd	1.33
5.0	154 e	145 d	23 defg	305 e	1875 de	2043 de	1.02
10.0	125 f	119 e	13 ghi	200 g	1497 ef	1629 ef	0.81
Klebsiella pneumoniae							
2.5	202 b	192 b	21 defgh	359 bc	2760 bc	2973 bc	1.49
5.0	191 bc	183 b	19 defghi	335 cd	2318 cd	2520 cd	1.26
10.0	173 d	167 c	13 ghi	329 de	1913 de	2093 de	1.05
Streptomyces spp.							
2.5	87 ghi	76 fg	24 def	122 j	907 fgh	1007 fgh	0.50
5.0	80 hij	74 g	12 hi	128 ij	868 gh	954 gh	0.48
10.0	75 ij	68 g	14 fghi	169 h	676 h	758 h	0.38
Pseudomonas spp.							
2.5	91 gh	80 fg	25 de	168 h	1099 fgh	1204 fgh	0.60
5.0	71 j	64 g	15 efghi	150 hi	722 h	801 h	0.40
10.0	58 k	54 h	9 i	121 j	713 h	776 h	0.39
Check-1(nematode only)	1490 a	1386 a	158 a	600 a	28234 a	29778 a	14.89
Check-2(nematode + media)	1402 a	1296 a	159 a	570 a	26458 a	29913 a	13.96

*Data with the same letters within a column are not significantly different according to Duncan's new multiple range tests.

In addition, fecundity of the nematode based on number of eggs/egg mass was also affected by the bacterial strains. Consequently, the nematode final population was reduced when compared with those of the check. Accordingly, the rates of nematode reproduction were decreased due to application of all the bacterial isolates comparing to those of check.

A negative correlation was detected between bacterial inoculation rate and the nematode reproduction rate in all treatments. Comparatively, treatments of *Streptomyces* spp. and *Pseudomonas* spp. achieved the highest positive effect on nematode reproduction (Table, 1).

1-2- Effect on growth parameters of sunflower plants.

Growth response of *M. javanica* infected sunflower plants to bacterial treatments as measured by length and fresh weight of shoots and roots is shown in Table (2). In general, bacterial inoculants at all sizes tested succeeded in improving the plant growth of the infected plants.

Improvement in shoot length was pronounced in treatments of *B. subtilis*, or *B. megatherium* followed by those of *pseudomonas* spp. and finally *Streptomyces* spp.. While, the lowest response was noticed with that of *Klebsiella pneumoniae* comparing with untreated ones. In addition, application of *B. subtilis*, *Streptomyces* spp. or *Pseudomonas* spp. improved the shoot weight whereas *B. megatherium* or *K. pneumoniae* had a negative effect on such growth parameter.

As for the root reaction, it is worthy to notice that highest percent increase in root length and weight was obtained in plants treated with *K. pneumoniae*, *B. subtilis* or *B. megatherium* comparing with those of the control treatments.

2- Simultaneous application of bacterial inoculants and nematode infection.

2-1- Effect on *M. javanica* development.

The antagonistic effect of the bacterial inoculants on the biological activity of the nematode when the tested bacterial inoculants were simultaneously applied to the soil together with the nematode infection is listed in Table (3). The effects on gall formation and counts of egg-laying females were variously affected by the tested bacterial strain; *K. pneumoniae* was, however, the least effective among the tested bacterial strains.

Generally, number of galls and counts of egg laying females were negatively correlated with level of bacterial inocula in all tested treatments except for those of *K. pneumoniae*. Likewise, the bacteria species at all volume tested significantly reduced counts of the nematode developmental stages in roots, and (J2) in soil. The nematode fecundity was also, affected as expected by relatively low count of eggs/egg mass. Therefore, the nematode final population in all treatments was reduced and consequently, the rate of reproduction was decreased, in relative to the volume of bacterial inocula tested.

Table 2: Effect of five bacterial inoculants on growth parameters of sunflower plants treated seven days before *M. javanica* infection.

Bacterial species and inoculum level (cm)	Shoot				Root			
	length (cm)	Increase %	weight (gm)	Increase %	length (cm)	Increase %	weight (gm)	Increase %
Bacillus subtilis								
2.5	96.75 abc*	21.7	21.88 cd	21.6	19.00 abcd	49.0	9.75 abcde	56.0
5.0	103.75 a	30.5	30.00 ab	66.70	19.25 abcd	51.0	10.25 abcd	64.0
10.0	104.50 a	31.4	31.25 a	73.60	20.50 abc	60.8	11.88 abc	90.0
Bacillus megatherium								
2.5	89.00 abcd	11.9	15.00 defg	-	15.75 cdefg	23.5	6.25 ef	0.0
5.0	91.00 abcd	14.5	18.00 cdefg	0.0	18.00 abcde	41.2	8.50 cdef	36.0
10.0	95.00 abcd	19.5	19.25 cdefg	6.9	20.75 ab	62.7	9.50 bcde	52.0
Klebsiella pneumoniae								
2.5	83.50 bcd	5.0	17.25 cdefg	-	20.00 abc	56.9	13.75 ab	120.0
5.0	84.00 bcd	5.7	15.00 defg	-	17.50 bcdef	37.3	11.25 abc	80.0
10.0	83.00 bcd	4.4	14.50 defg	-	16.75 bcdef	31.4	8.5 cdef	36.0
Streptomyces spp.								
2.5	82.25 cd	3.5	18.25 cdefg	1.4	14.00 efg	9.8	7.00 def	12.0
5.0	89.0 abcd	11.9	21.00 cdef	16.7	17.25 bcdef	35.3	8.75 cdef	40.0
10.0	97.00 abc	22.0	23.25 bc	29.2	17.50 bcdef	37.3	10.50 abcde	68.0
Pseudomonas spp.								
2.5	83.50 bcd	5.0	15.50 defg	0.0	11.50 g	-	4.50 f	-
5.0	91.75 abcd	15.4	21.50 cdef	19.4	15.00 defg	17.6	8.50 cdef	36.0
10.0	100.0 ab	25.8	21.75 cde	20.8	20.75 ab	62.7	11.50 abc	84.0
Check-1(nematode only)	79.50 d	-	18.0 cdefg	-	12.75 fg	-	6.25 ef	-
Check-2(nematode + media)	85.75 bcd	-	15.5 defg	-	16.00 bcdefg	-	6.25 ef	0

*Data with the same letters within a column are not significantly different according to Duncan's new multiple range tests.

Table 3: Effect of five bacterial inoculants simultaneously applied with *M. javanica* infection on nematode development and reproduction.

Bacterial species and inoculum level (cm)	No. of Galls	No. of egg-masses	Developmental stage	Average Eggs/Egg-mass	No. of J2	Final pop. (Pf)	Rate of reproduction Pf/Pi
Bacillus subtilis							
2.5	1043 [b*]	925 [b]	169 [a]	413 [d]	12291 [c]	13385 [c]	6.69
5.0	574 [c]	511 [c]	100 [c]	382 [de]	7195 [ef]	7806 [ef]	3.90
10.0	547 [c]	510 [c]	72 [cd]	370 [e]	6138 [fg]	6720 [fg]	3.36
Bacillus megatherium							
2.5	1132 [b]	1010 [b]	152 [ab]	464 [c]	9471 [d]	10633 [d]	5.32
5.0	1062 [b]	973 [b]	156 [ab]	398 [de]	3914 [h]	5043 [gh]	2.52
10.0	497 [cd]	445 [cd]	93 [c]	377 [de]	2181 [ij]	2719 [ij]	1.36
Klebsiella pneumoniae							
2.5	1452 [a]	1315 [a]	186 [a]	502 [bc]	15199 [b]	16700 [b]	8.35
5.0	1337 [a]	1220 [a]	152 [ab]	512 [b]	9160 [d]	10532 [d]	5.27
10.0	1002 [b]	928 [b]	110 [bc]	401 [de]	7924 [de]	8962 [de]	4.48
Streptomyces spp.							
2.5	337 [de]	297 [de]	83 [cd]	302 [f]	7120 [ef]	7500 [ef]	3.75
5.0	274 [ef]	235 [ef]	75 [cd]	277 [fg]	3801 [hi]	4111 [hi]	2.06
10.0	114 [f]	91 [f]	46 [d]	242 [gh]	1141 [j]	1278 [j]	0.64
Pseudomonas spp.							
2.5	582 [c]	512 [c]	105 [c]	303 [f]	6586 [f]	7203 [f]	3.60
5.0	561 [c]	506 [c]	90 [cd]	241 [gh]	4738 [gh]	5334 [gh]	2.67
10.0	550 [c]	484 [c]	104 [c]	226 [h]	2238 [ij]	2826 [ij]	1.41
Check-1(nematode only)	1500 [a]	1436 [a]	192 [a]	609 [a]	28711 [a]	30339 [a]	15.17
Check-2(nematode + media)	1378 [a]	1280 [a]	170 [a]	566 [a]	26004 [a]	27454 [a]	13.73

*Data with the same letters within a column are not significantly different according to Duncan's new multiple range tests.

Among the tested bacterial treatments, treatment received *Pseudomonas* spp. achieved the lowest values of the nematode reproduction rate followed by *Streptomyces* spp., while treatment received *K. pneumoniae* had the highest rates followed by *Bacillus subtilis*.

2-2 Effect on growth parameters of sunflower plants.

Data presented in Table (4) describe the effect of the five bacterial inoculants on growth of sunflower plants when added to the soil at the same time of nematode infection. Improvements in all growth parameters occurred and were progressively correlated with type of bacteria inoculants and their inoculum sizes. It was evident that the highest inoculation rate of the bacterial inoculants remarkably improved growth of both shoots and roots. The highest percentages of increase in shoot weight and length were achieved in treatments of *Streptomyces* spp. or *Pseudomonas* spp.. While, those of *K. pneumoniae* and *B. megatherium* achieved the highest percentage of increase in root weight and length (Table 4). Unexcepectedly, plants in check- 1 treatment had the growth parameter increment.

3- Application of bacterial inoculants after nematode infection.

3-1- Effect on *M. javanica* development and reproduction.

The effect of the bacteria strains on development and reproduction of *M. javanica* when applied one week after nematode infection is presented in Table (5). Data reveal that all the tested strains of bacteria suppressed the nematode development and reproduction when compared with those of the check treatments, but to a lesser extent than when they were applied either before or with nematode inoculation.

In general, most bacterial sizes gave significant reduction in number of galls, egg-laying females, nematode population in roots and soil. Also, the average number of eggs/egg mass, and consequently, the nematode final population rates of reproduction were 3.59, 3.84, 8.07, 9.52 and 10.3 for *Pseudomonas* spp, *Streptomyces* spp., *B. megatherium*, *K. pneumoniae* and *B. subtilis*, respectively, vs. 14.27 and 15.51 for the check treatments.

3-2- Effect on growth parameters of sunflower plants.

Growth response of sunflower plants to the tested bacterial strains when they added to the soil seven days before the nematode inoculation applied is listed in Table (6). In general, all the bacterial genera at all volume tested improved growth of the plants. Plant growth parameters were greatly increased when compared with those of check treatments. Percentages of increase were more pronounced in roots than in shoots.

DISCUSSION

Data of this study indicate that, the majority of the tested bacterial inoculants were suppressive to the *M. javanica* populations occurred on sunflower plants when added to the soil one week before the nematode inoculation. The positive effect was noticeable in reduced galling formation as well as the rate of reproduction. Moreover, the degree of suppression of nematode was proportional to the bacterial inoculum size added to the soil.

Table 4: Effect of five bacterial species on growth parameters of sunflower plants when simultaneously applied with *M. javanica* infection.

Bacterial species and inoculum level (cm)	Shoot				Root			
	length (cm)	Increase %	weight (gm)	Increase %	length (cm)	Increase %	weight (gm)	Increase %
Bacillus subtilis								
2.5	75.25 ^{g*}	-	12.50 ^{e}	-	22.00 ^{cde}	22.22	5.75 ^{ef}	-
5.0	86.50 ^{f}	13.07	17.25 ^{cde}	32.69	25.50 ^{abc}	41.67	6.75 ^{def}	-
10.0	95.75 ^{cde}	25.16	26.25 ^{a}	101.92	27.5 ^{ab}	52.78	10.75 ^{bc}	34.40
Bacillus megatherium								
2.5	77.75 ^{g}	1.63	17.00 ^{cde}	30.77	20.00 ^{ef}	11.11	9.25 ^{cd}	15.60
5.0	93.75 ^{de}	22.55	17.00 ^{cde}	30.77	25.00 ^{abcd}	38.89	9.25 ^{cd}	15.60
10.0	101.25 ^{bc}	32.35	23.00 ^{abc}	76.92	27.50 ^{ab}	52.78	12.50 ^{b}	56.25
Klebsiella pneumoniae								
2.5	86.50 ^{f}	13.07	18.00 ^{cde}	38.46	19.50 ^{ef}	8.33	6.75 ^{def}	-
5.0	91.75 ^{def}	19.93	18.25 ^{cde}	40.38	22.00 ^{cde}	22.22	7.50 ^{def}	-
10.0	98.00 ^{cd}	28.10	22.50 ^{abcd}	73.08	29.00 ^{a}	61.11	14.75 ^{a}	84.40
Streptomyces spp.								
2.5	89.50 ^{ef}	16.99	20.50 ^{abcd}	57.69	21.00 ^{def}	16.67	7.75 ^{de}	-
5.0	98.50 ^{cd}	28.76	16.50 ^{de}	26.92	24.50 ^{bcd}	36.11	9.00 ^{cd}	12.50
10.0	107.75 ^{ab}	40.85	25.25 ^{ab}	94.23	27.25 ^{ab}	51.39	11.00 ^{bc}	37.50
Pseudomonas spp.								
2.5	88.75 ^{ef}	16.01	19.75 ^{bcd}	51.92	17.00 ^{f}	-	5.75 ^{ef}	-
5.0	97.50 ^{cd}	27.45	20.00 ^{abcd}	53.85	22.00 ^{cde}	22.22	8.00 ^{de}	0.00
10.0	109.50 ^{a}	43.14	25.25 ^{ab}	94.23	26.25 ^{ab}	45.83	12.25 ^{b}	53.10
Check-1 (nematode only)	76.50 ^{g}	-	13.0 ^{e}	-	18.00 ^{ef}	-	8.00 ^{de}	-
Check-2 (nematode + media)	105.50 ^{ab}	37.91	26.50 ^{a}	103.85	28.00 ^{a}	55.56	15.00 ^{a}	87.50

*** Data with the same letters within a column are not significantly different according to Duncan's new multiple range tests.**

Table 5: Effect of five bacterial inoculants applied seven days after *M. javanica* infection on nematode development and reproduction.

Bacterial genera and inoculum level (cm)	No. of Galls	No. of egg-masses	Developmental stage	Average Eggs/Egg-mass	No. of J2	Final pop. (Pf)	Rate of reproduction Pf/Pi
Bacillus subtilis							
2.5	1317 abc*	1277 ab	63 c	503 b	19258 b	20598 b	10.30
5.0	1157 cdef	1119 bcde	62 c	443 c	8249 g	9430 f	4.72
10.0	1067 efgh	996 defg	94 b	412 cde	4961 ijk	6051 ghi	3.03
Bacillus megatherium							
2.5	1281 bcd	1231 abc	77 bc	496 b	14836 d	16144 c	8.07
5.0	1203 cde	1166 bcd	61 c	450 c	12169 ef	13396 de	6.70
10.0	1090 defg	1052 cdef	61 c	388 de	10621 f	11734 e	5.87
Klebsiella pneumoniae							
2.5	1447 ab	1390 a	80 bc	520 b	17575 c	19045 b	9.52
5.0	1317 abc	1266 ab	73 bc	422 cd	14611 d	15950 c	7.98
10.0	1283 bcd	1224 abc	75 bc	377 e	12949 e	14248 cd	7.12
Streptomyces spp.							
2.5	996 fghi	948 efg	68 c	328 f	6657 gh	7673 fg	3.84
5.0	926 ghij	868 fgh	78 bc	298 f	4090 jkl	5036 hij	2.52
10.0	791 j	742 h	74 bc	246 g	3731 kl	4547 ij	2.27
Pseudomonas spp.							
2.5	926 ghij	889 fgh	61 c	294 f	6238 hi	7188 gh	3.59
5.0	878 hij	837 gh	59 c	242 g	5685 hij	6581 ghi	3.29
10.0	847 ij	806 gh	69 c	227 g	2529 l	3404 j	1.70
Check-1(nematode only)	1543 a	1469 a	200 a	590 a	29342 a	31011 a	15.51
Check-2(nematode + media)	1497 a	1344 a	168 a	558 a	27019 a	28531 a	14.27

*Data with the same letters within a column are not significantly different according to Duncan's new multiple range tests.

Table 6: Effect of five bacterial inoculants on growth parameters of sunflower plants when applied seven days after *M. javanica* inoculation.

Bacterial species and inoculum level(cm)	Shoot				Root			
	length (cm)	Increase %	weight (gm)	Increase %	length (cm)	Increase %	weight (gm)	Increase %
Bacillus subtilis								
2.5	94.00 cd*	6.8	19.25 bc	38.69	17.75 bcd	-	8.25 d	-
5.0	96.00 bcd	9.1	19.75 abc	42.29	18.50 abcd	-	13.25 abc	53.53
10.0	99.00 bcd	12.5	21.75 abc	56.70	19.00 abcd	0.0	15.00 a	73.80
Bacillus megatherium								
2.5	100.25 abcd	13.92	22.25 abc	60.30	17.00 bcd	-	6.75 d	-
5.0	102.0 abcd	15.91	22.25 abc	60.30	18.75 abcd	-	10.25 cd	18.77
10.0	103.5 abcd	17.6	25.25 ab	81.92	22.00 ab	15.79	14.75 a	70.92
Klebsiella pneumoniae								
2.5	97.50 bcd	10.80	20.75 abc	49.50	14.25 d	-	9.50 cd	10.08
5.0	97.75 bcd	11.08	20.75 abc	49.50	19.00 abcd	0.0	13.00 abc	50.64
10.0	98.25 bcd	11.65	25.75 ab	85.52	20.50 abc	7.89	14.50 ab	68.00
Streptomyces spp.								
2.5	104.00 abcd	18.18	24.50 ab	76.50	17.25 bcd	-	10.50 bcd	21.67
5.0	111.25 ab	26.42	26.50 ab	90.90	19.25 abcd	1.30	11.00 abcd	27.46
10.0	116.00 a	31.82	28.50 a	105.30	20.00 abc	5.26	13.00 abc	50.64
Pseudomonas spp.								
2.5	100.5 abcd	14.20	23.25 ab	67.51	15.50 cd	-	10.25 cd	18.77
5.0	104.0 abcd	18.18	26.00 ab	87.30	16.25 cd	-	11.25 abcd	30.36
10.0	109.00 abc	23.86	27.00 ab	94.52	20.25 abc	7.89	12.75 abc	47.74
Check-1(nematode only)	88.00 d	-	13.88 c	-	19.00 abcd	-	8.63 d	-
Check-2(nematode + media)	100.5 abcd	14.20	18.00 bc	29.68	24.00 a	26.32	14.50 ab	68.02

*** Data with the same letters within a column are not significantly different according to Duncans new multiple range tests.**

Thus, the antagonistic effect of bacterial isolates to nematode might be due to production of certain antimicrobial metabolites, which act as bio-agents.

These results are in agreement with those of many investigators (Sochroth and Becker, 1990; Hasky-Gunther *et al.*, 1998; Martinez-Ochoa *et al.*, 1995 and Hackenberg *et al.*, 2000). They found that, certain rhizobacteria were good as bio-agents because they produce antimicrobial metabolites. These metabolites have had major important role in the biological control of many pathogens. For example, some rhizosphere-inhabiting bacteria produce toxic metabolites, which affect the motility of nematodes in vitro (Becke *et al.*, 1987, 1988 and Stirling and Sharma, 1990). The nematicidal activity is, therefore, resulted from accumulation of some metabolites such as organic acids, hydrogen sulphide and ammonia, which affect adversely on nematode development. Ammonia released by bacteria growing on nitrogen-rich media was found to be toxic to most of nematode species (Rodriguez-Kabana, 1986).

In previous studies, Burg *et al.* (1979) and Stretton *et al.* (1987) reported that a strain of *Streptomyces avermitilis* could produce a group of new biocides (avermectins), and a group of macrocyclic lactones that inhibit plant parasitic nematodes. *Streptomyces anulatna* produces valinomycin, which has adverse effects against nematodes; however, most of the nematoxic compounds produced by *Actinomycetes* were not identified.

In addition, *Pseudomonas* spp. is proved to be an effective bio-agent against *M. javanica*. This result was confirmed with similar study on *Pseudomonas a ureofaciens* isolated form soil, which inhibited *Criconemella xenoplax* population in green house and egg hatch in vitro (Westcott and Kluepfel, 1993). *Pseudomonas* spp. and *Bacillus megatherium* suppressed the cyst nematode, *Heterodera schachtii* on sugar beet in a greenhouse trail (Neipp and Becker, 1999).

Both *Bacillus megatherium* and *B. subtilis* produce volatile metabolites that significantly reduced activity of *M. incognita* juveniles when they had exposed for at least 48 hrs. (Zavaleta-Mejia *et al.*, 1989). Moreover, *B. megatherium* was reported to produce antibiotic compounds (Vary, 1992).

The obtained results confirm that the degree of nematode suppression was proportional to concentration of bacterial cells per kg of soil. Application of bacteria one week prior to nematode infection was most detrimental effective on nematode reproduction, when compared to the other two treatments. However, the least effective treatment was recorded when applying bacteria one week after nematode infection. These results are in agreement with those of Ali (1996).

In conclusion, this study proved the potential activity of the four bacterial genera against the root-knot nematodes, *M. javanica* and offered a promising bio-control tool in nematode management programs.

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كفاءة بعض العزلات البكتيرية فى مكافحة نيماتودا تعقد الجذور

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تم دراسة تأثير التلقيح بخمسة عزلات مختلفة من البكتريا أسماؤها كالتالى: *Bacillus subtilis, Bacillus megatherium, Klebsiella pneumoniae, Pseudomonas* spp *and Streptomyces* spp. وذلك بكل عزلة منها على حدة على نمو وتكاثر نيماتودا تعقد الجذور *Meloidogyae javanica* المتطفلة على جذور نباتات عباد الشمس صنف بلدى والمنزرعة تحت ظروف الصوبة. حيث أضيفت العزلات البكترية السابقة بثلاث أحجام مختلفة (٢,٥ ، ٥,٠ ، ١٠,٠ ملل/أصيص) فى ثلاثة مواعيد مختلفة (قبل إضافة عدوى النيماتودا بأسبوع، إضافة كل من البكتريا و النيماتودا معا، بعد إضافة عدوى النيماتودا بأسبوع).

أوضحت النتائج المتحصل عليها أن التلقيح بغالبية الأنواع البكترية المختبرة أدى إلى خفض عدد العقد النيماتودية المتكونة على جذور نباتات عباد الشمس، الأطوار النيماتودية المختلفة، عدد كتل البيض المتكونة على الجذور، الطور اليرقى الثانى الموجود فى التربة. ونتيجة لهذا انخفض معدل تكاثر النيماتودا مقارنة بالنباتات الغير ملقحة بالبكتريا. كذلك وجد ارتباط عكسى بين حجم اللقاح البكتيرى المضاف و معدل تكاثر النيماتودا.

أيضا أثبتت النتائج أن إضافة البكتريا قبل أجراء عدوى لنباتات عباد الشمس بالنيماتودا بأسبوع كانت أكثر ايجابية خاصة عند مقارنتها بالمعاملتين الأخريين (إضافة كل من البكتريا والنيماتودا معا، بعد أجراء العدوى بالنيماتودا بأسبوع). كذلك ثبت وجود تحسن ملحوظ فى نمو نباتات عباد الشمس الملقحة بالبكتريا عن النباتات الغير ملقحة حتى فى وجود العدوى بالنيماتودا من تقدير كل من أطوال وأوزان كل من السيقان و الجذور لنباتات عباد الشمس.