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Nematicidal Effect of A Wild Type of *Serratia Marcescens* and Its Mutants Against *Meloidogyne Incognita* Juveniles

Kassab, S. A*.; Eissa, M. F. M. **; Badr, U. M. *; Ismail, A,E.**; Abdel Razik, A.B. **** and Gaziea, M. Soliman****

* Department of Plant Protection, Fac. of Agric., Ain Shams Univ., Shoubra El-Kheima, Cairo, Egypt.

** Nematology Lab., Plant Pathology Department, National Research Centre, Dokki, Giza, Egypt.

*** Department of Genetic and Cytology, National Research Centre, Dokki, Giza, Egypt.

**** Department of Genetic, Fac. of Agric., Ain Shams Univ., Shoubra El-Keima, Cairo, Egypt.

Abstract

Serratia marcescens (SM) is currently considered as a biocontrol agent against plant parasitic nematodes. It is one of the most effective bacteria for degradation of chitin. This lytic bacteria was evaluated on the survival of *Meloidogyne incognita* juveniles under laboratory conditions. The best treatment by (SM 36) mutant achieved zero viable juveniles in either S or S/10 i.e, highly effective on juveniles mortality compared to the wild type of SM and untreated control which recorded 51.8, 49.3 and 49 juveniles, respectively. The numbers of non viable juveniles of the best treatment were 6, 4 and 2.8 individuals after 24, 48 and 72 hrs, respectively compared to the SM wild type which achieved 26.3. 33.3 and 27.3 while the untreated control did not show any effect on the juveniles. There were positive relationships between the nematode mortality and each of the bacteria concentration and enzyme production from the mutants. The numbers of either viable juveniles or non viable juveniles were reduced at S or S/10 dilutions and exposure periods of 24, 48 and 72 hr compared to the untreated control. This reduction was attributed to the dead nematode bodies which were degraded and destroyed by these bacterial mutants. Mutation achieved increasing of chitinase and alkaline protease-over producing mutants, which produced two to three times more endochitinase activity than the wild type of *S.marcescens*.

Keywords: lytic bacteria, chitinase, protease, *Serratia marcescens*, mutation *Meloidogyne incognita*.

Introduction

Among the plant-parasitic nematodes, root knot nematodes *(Meloidogyne* spp.) are worldwide in distribution and attack many economically important crops **(Sasser, 1979, Jung and Wyss, 1999)**. *Meloidogyne* species are able to infect more than 2000 plant species all over the world.

The risk to humans and environments presented by using synthetic

pesticides and in turn emphasize the need for tools such as biological control in optimizing sustainable agricultural systems **(Patent, 2012).** Bacteria are extremely important in biological control of plant pathogens. The lytic activity of bacteria is one of the mechanisms that has been implicated in biocontrol for several years **(Mitchell and Hurwitz, 1964; Lim *et al*., 1991; Mavingui and Heulin, 1994; Chernin *et al*., 1995, Soliman, 2008 & 2014; Abd El Bary *et al*., 2007; Eissa et al., 2010 and Ismail *et al*., 2009 &2010).**

Nematode eggshell and cuticle are infection sites for microorganisms. Protease, chitinase, and lysozyme enzymes are commonly produced and secreted by bacteria to degrade the nematode eggshell and cuticle **(Frandberg and Schnurer, 1994).** The eggshell is the toughest part of nematode eggs and plays a role in their resistance to chemicals and bionematicides **(Wharton, 1980).** The eggshell may consist of one to five layers depending on the nematode order. The most common structure features are the inner lipid layer, the middle chitinous layer (major components) and the outer vitelline layer **(Bird and McClure, 1976).** For example, *M. javanica* eggshells contain 50% protein and 30% chitin **(Bird and Bird, 1991).**

The Gram-negative bacterium *Serratia marcescens* secretes a variety of extracellular enzymes including chitinases **(Hines *et al*., 1988).** It is one of the most effective bacteria for degradation of chitin **(Monreal and Reese, 1969).** When this bacteria is cultivated in the presence of chitin, a variety of chitinolytic enzymes and chitin-binding proteins can be detected **(Fuchs *et al*., 1986).**

Mercer *et al*. (1992) studied the effects of the chitinolytic bacteria, chitinase from *Serratia marcescens* on eggs and juveniles of *M. hapla*. They observed that, hatch rates of nematode eggs were increased if incubated with chitinase from *S. marcescens*. After treatment with chitinase from *S. marcescens*, many of the remaining eggs became spherical with a concomitant increase in the number of the dead juveniles. Their results suggest that, exogenous chitinases cause premature hatch of nematode eggs and could be used in the control of nematodes. Patents have been applied for the use of chitinases as nematode control agents in genetically modified rhizosphere bacteria *Serratia marcescens*, because of its ability to produce different chitinolytic enzymes.

Khan *et al*., (2004) found that, a serine protease and an enzyme preparation consisting of six chitinases, previously semi-purified from a liquid culture of *Paecilomyces lilacinus* strain 251, drastically altered the eggshell structures of *M. javanica* when applied individually or in combination. In the protease-treated eggs, the lipid layer disappeared and the chitin layer was thinner than in the control. The eggs treated with chitinases displayed large vacuoles in the chitin layer, and the vitelline layer was split and had lost its integrity. The major changes in the eggshell structures occurred by the combined effect of *P. lilacinus* protease and chitinases.

Lian *et al.*, (2007) isolated rhizobacteria with nematicidal activity from soil samples of five root knot nematode-infested farms. Further assays of nematicidal toxins from *Bacillus* sp. strain RH219 indicated an extracellular cuticle-degrading protease. The cuticle-degrading protease genes were also amplified from four other nematicidal *Bacillus* strains isolated from the rhizosphere. They suggested that, these enzymes likely play an important role in bacteria- nematode- plant interactions and may serve as important nematicidal factor in balancing nematode populations in the soil.

Reid and Ogrydziak (1981) succeeded in isolating a chitinase-over producing mutant of *Serratia marcescens* which produced two to three times more endochitinase activity than the wild type. The mutant also produced two to three times more than the wild type of the other components of chitinolytic enzyme systems. This study aimed to increasing production of alkaline ptotease and chitinase on *Serratia marcescens* by chemical mutation (MMS, EMS) and increasing suppress nematode population of *Meloidogyne incognita.*

Materials and Methods

Preparation of M. incognita inocula:

When nematode inocula as the second stage juveniles (J_2s) was needed, galled tomato roots were washed thoroughly with tap water, cut into pieces, then placed in mist chamber for egg hatching. The first catch was discarded, and the following emerged J_2s were collected daily and refrigerated at 6ºC for the experimental use. J_2s were placed in 0.5% sodium hypochlorite, agitated and rinsed with sterile water immediately before infestation.

Bacterial strains*: Serratia marcescens* code number ACGEB (Nimatos 105) was used in this study. It was obtained from Ain Shams Center for Genetic Enginering and biotechnology (ACGEBSR).

The isolate of bacteria used as a wild type in this study, were obtained from Faculty of Agriculture, Ain Shams Univ. This isolates were stored on slants of nutrient agar medium and used in the current studies.

Media:

Nutrient Agar Medium**:** It was used for assaying enzyme activity according to **Dowson (1957).**

Luria broth (LB)**:** Tryptone 10 g, Yeast extract 5 g, Nacl 5, agar 20g and up to 1000 ml of Distilled water.

Chitinase assay: 0.4% colloidal chitin and 0.02 M Sodium phosphate buffer (PH 6.0) and stained with 0.1% Congo red according to **Watanabe *et al*. (1990).**

Preparation of Colloidal Chitin:

Colloidal chitin was prepared by the method of **Roberts and Selitrennikoff (1988**) with certain modifications. Five gram of chitin powder (Himedia Chemicals Co. Mumbai) was added slowly into 60 ml of concentrated HCl (Sd. Fine Chemical) and left overnight at 4°C with vigorous stirring. The mixture was added to 2.0 L of ice-cold 95% ethanol with rapid stirring and kept overnight at room temperature (25°C). The precipitate was collected by centrifugation at 5000 rpm for 20 minutes at 4°C and washed with sterile distilled water until the colloidal chitin became neutral (pH 7.0). Colloidal chitin solution (5%) was prepared and stored at 4°C until further use(**Rodriguez-Kabana *et al.*, 1983).**

Protease assay:

Petri dishes 150 mm diameter each containing 50 ml of each gelatien medium (0.4 % Gelatien) were inoculated from the 24 – hours age slants of bacteria under tested in single colonies. Inoculated plates were then incubated for 48 hours at 30 ^{o}C after which 7- 10 ml of the test solution (100 ml Dist. Water + 15 gr. $Hgcl_2$ +20 ml Hcl) were pipetted on the surface of agar. Relationships among diameters of the grown colonies itself were calculated and taken as an indication for the enzyme activity produced for each culture **(Smith *et al.,* 1952)**

Preparation of bacterial inocula:

For each bacterial strain, a conical flask (250 ml) containing 100 ml of nutrient broth medium consisting of 5.0 g peptone, 3.0 g beef extract, 1000 ml tap water, (pH adjusted to 7.0) was inoculated and incubated at 28-30°C with shaking at 150 rpm for two days prior to application.

Genetic improvement by:

1. Improvement of bacteria via mutagenesis by:

MMS treatments: Methyl Methan Sulfonate was obtained from Eastman Organic Chemicals and was vacuum-redistilled before use. A 0.05 µ solution was prepared by dissolving 0.085 ml of MMS in 20 ml of cold 0.05 m phosphate buffer immediately before use and incubated on the shaker for 60 min **(Prakash and Bernard, 1970).**

EMS treatments: 0.4 ml of ethyl methansulfonate (Eastman Kodak co.) was incubated on the shaker for 60 min.

Effect of a wild type and different mutants of lytic bacteria, *Serratia marcescens* on activity of *Meloidogyne incognita juveniles:*

Two dilutions (S and S/10) from cell suspension of wild type of *Serratia marcescens* (parentals or pre-genetic improvement bacteria) and 4 mutants were used. One ml of freshly hatching surface-sterilized juvenile suspension (45 ± 5

juveniles/1 ml) with 2 ml of culture filtrate were transferred to cavity glasses. Juveniles were kept in 1 ml of *Luria broth* (LB) without bacteria and left as control. There were five replications of each treatment. Cavity slides were incubated at room temperature (28± 2 ^{o}C) .After 24h, 48h and 72hr, the number of dead juveniles were counted with the aid of microscope. The juveniles which did not regain their activities and did not move when probed with fine needle were considered "dead". Juveniles were considered active when they moved Handling tools were cleaned with sterilized water throughout the experiment. The nematode number were recorded after different exposure times.

Results

Mutation studies:

First step of MMS treatments: The strains reported above were used in mutation inductions as first step treatment by chemical mutant: methyl methan sulphonate (MMS). In Table (1) 209 isolates appeared and comparing the enzymes production *Serratia marcescens*, proved that, the alkaline protease productions of 5 isolates had the highest level productions (isolates number 91, 130, 141, 144, 167) had the highest which lost production were (34, 36, 105, 131, 184, 192). Also chitinase was produced 35 isolates the greatest of isolates were (24, 31, 82, 168, 190) had the greatest chitinase production. Please, use correct terms and grammar to clarify the obtained data! On the other hand 20 isolates lost their ability for production (Table 1).

Table (1): Enzymes production from *Serrtia marcescens* mutant by MMS treatment.

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
1	120.12	85.91
2	105.37	152.73
3	120.12	198.55
4	165.96	95.46
5	107.48	210.00
6	118.54	93.33
7	76.77	109.10
8	120.12	137.45
9	120.12	220.10
10	89.92	0.00
11	120.12	168.00
12	10537	218.10
13	120.12	76.37

Cont.

Continued Table (1)

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
14	76.77	111.61
15	127.44	168.00
16	120.12	168.00
17	71.12	95.45
18	134.34	198.55
19	85.80	168.00
20	85.80	152.73
21	66.76	152.73
22	132.76	178.19
23	165.96	171.82
24	94.83	**316.37**
25	106.41	198.55
26	94.83	140.00
27	120.12	126.77
28	10537	106.91
29	101.15	91.64
30	84.30	250.91
31	76.77	**316.37**
32	100,10	150.73
33	65.33	168.00
34	**0.00**	150.73
35	165.96	61.10
36	**0.00**	120.00
37	90.31	126.77
38	85.80	159.67
39	100.00	168.00
40	100.00	126.77
41	120.12	101.82
42	158.05	267.28
43	127.44	76.37
44	158.05	109.09
45	120.12	98.18
46	118.54	130.91
47	158.05	133.64

Cont.

Continued Table (1)

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
48	165.96	168.00
49	142.27	120.00
50	127.44	137.56
51	132.76	137.56
52	127.44	150.73
53	173.89	210.00
54	151.03	250.91
55	144.01	168.00
56	132.76	210.00
57	158.05	171.82
58	110.64	101.82
59	120.12	98.18
60	150.15	91.64
61	173.86	76.00
62	165.96	280.00
63	126.44	229.20
64	189.66	101.82
65	139.09	137.56
66	120.12	168.00
67	120.12	114.55
68	100.00	168.00
69	100.00	190.91
70	158.05	101.82
71	105.37	98.18
72	94.84	190.91
73	76.77	198.55
74	110.64	120.00
75	117.48	267.28
76	94.84	0.00
77	81.28	0.00
78	134.34	168.00
79	136.98	210.00

Cont.

Continued Table (1)

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
80	134.34	150.74
81	179.13	150.74
82	158.01	**330.92**
83	126.44	229.10
84	142.25	99.86
85	142.25	137.46
86	150.15	101.82
87	158.01	127.28
88	107.48	137.46
89	158.01	0.00
90	118.54	190.91
91	**200.20**	120.00
92	165.96	114.55
93	158.01	137.46
94	126.44	168.00
95	158.05	210.00
96	158.05	190.91
97	120.12	122.18
98	179.13	137.46
99	94.83	76.37
100	89.56	173.10
101	94.83	173.10
102	120.12	0.00
103	158.01	137.46
104	73.76	190.91
105	**0.00**	280.00
106	107.48	229.10
107	110.64	190.91
108	94.83	190.91
109	118.54	61.10
110	142.25	0.00
111	142.25	152.73
112	94.83	190.91

Cont.

Continued Table (1)

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
113	158.05	127.28
114	120.12	127.28
115	107.48	280.00
116	82.15	127.28
117	120.12	114.55
118	145.40	0.00
119	142.25	133.64
120	126.44	190.91
121	120.12	171.82
122	120.12	30.55
123	158.05	76.37
124	132.76	152.73
125	173.86	229.10
126	142.25	127.28
127	179.67	76,37
128	158.05	168.00
129	120.12	76.37
130	**210.74**	152.73
131	**0.00**	50.91
132	150.15	137.46
133	113.79	229.10
134	126.44	280.00
135	56.90	152.73
136	158.05	168.00
137	107.47	54.55
138	150.15	127.28
139	142.25	0.00
140	107.47	76.37
141	**221.27**	120
142	94.83	98.18
143	107.47	0.00
144	**200.20**	0.00
145	94.83	0.00

Cont.

Continued Table (1)

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
146	134.34	0.00
147	142.25	0.00
148	158.05	101.82
149	84.30	152.73
150	88.51	152.73
151	110.64	0.00
152	142.25	106.11
153	158.05	54.55
154	44.25	0.00
155	44.25	137.46
156	63.22	198.55
157	110.64	168.00
158	189.66	152.73
159	150.15	140.00
160	158.01	210.10
161	73.73	229.10
162	113.79	67.88
163	110..64	67.88
164	84.29	61.10
165	79.03	66.82
166	94.83	30.55
167	**221.27**	229.10
168	100.10	**305.46**
169	76.73	106.91
170	94.83	152.73
171	142.25	0.00
172	126.44	0.00
173	76.77	168.00
174	94.83	171.82
175	94.83	183.28
176	79.03	137.46
177	94.83	133.64
178	73.76	229.10

Cont.

Continued Table (1)

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
179	94.83	137.46
180	68.49	137.46
181	120.12	152.73
182	79.03	152.73
183	73.76	106.91
184	**0.00**	229.10
185	113.79	229.10
186	94.83	0.00
187	110.64	137.46
188	158.05	305.46
189	94.83	178.19
190	120.12	**381.83**
191	50.58	114.55
192	**0.00**	152.73
193	94.83	0.00
194	126.44	101.82
195	126.44	137.46
196	150.15	190.91
197	126.44	152.73
198	158.05	152.73
199	120.12	114.55
200	126.44	168.00
201	126.44	229.10
202	113.80	152.73
203	94.83	190.91
204	158.05	0.00
205	126.44	168.00
206	126.44	95.46
207	158.05	152.73
208	56.90	229.10
209	94.83	213.82

In the first step, isolating two mutants by using MMS treatments. mutants No.190 a chitinase-over producing mutant which produced chitinase activity four times more than the wild type. Also, The mutant No.167 a Alkaline protease -over

producing mutant which produced two folds more than the wild type.

Second step EMS treatment:

The mutant No.190 (*Serratia marcescens*) In the second step were treated by EMS, and isolated three mutants (24, 36 and 39) its production are 199.25, 298.24 and 210.53 Table (2) Chitinase production of 6 mutants were completely lost enzyme production and all the mutants are approximately lowest chitinase production as compared to mentioned production by the mutant No.190 as shown in (Table 1).

Also, in the second step EMS treatments succeeded to isolate two mutants no. 36 which producing three folds of alkaline protease compared to the wild type, and mutant no. 21 which producing two folds (Table 2).

Table (3) showed that the mutant of *Serratia marcescens* no. 167 which treated by EMS in second step produced forty isolates. Nine isolates produced zero level chitanase production, and the isolate no. 8 was 109.12%. But in alkaline protease, 10 isolates produced zero level production. In high level, productions of 4 isolates (4, 5, 8 and 21) their productions were (124.28, 101.69, 124.28 and 135.58) respectively.

Table (2): Enzymes production of mutant No.190 of *Serratia marcescens* by using EMS treatment.

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
1	144.74	32.74
2	105.26	15.00
3	114.03	26.19
4	94.74	32.74
5	74.56	15.00
6	100.00	13.09
7	94.74	41.16
8	94.74	32.74
9	94.74	41.16
10	151.32	32.74
11	144.74	39.23
12	100.00	26.19
13	87.72	22.92
14	105.26	20.37
15	121.05	32.74

Cont.

Continued Table (2)

Serial No.	Alkaline protease based on wild type%	Chitinase production based on wild type%
16	87.72	26.19
17	87.72	26.19
18	151.32	63.60
19	89.47	21.46
20	94.74	17.46
21	87.72	32.01
22	144.74	29.09
23	151.32	15.71
24	**199.25**	26.19
25	164.47	26.19
26	144.74	29.09
27	144.74	32.74
28	115.79	26.19
29	115.79	36.01
30	151.74	48.64
31	100.88	41.16
32	126.32	39.28
33	126.32	26.19
34	105 26	32.01
35	105.26	23.57
36	**298.24**	0.00
37	145.61	0.00
38	131.58	0.00
39	**210.53**	39.28
40	173.68	41.16
41	105.26	26.19
42	138.16	17.47
43	110.53	32.74
44	105.26	0.00
45	105.26	0.00
46	94.74	0.00
47	131.58	22.92
48	110.53	23.57

Table (3): Enzymes production of mutant No.167 of *Serratia marcescens* by using EMS treatment.

Serial No.	Alkaline protease based on wild type%	Chitinaseproductionbased on wild type%
1	63.27	87.30
2	75.30	76.49
3	54.23	93.53
4	**124.28**	0.00
5	**101.69**	0.00
6	90.39	0.00
7	63.27	0.00
8	**124.28**	**109.12**
9	81.35	99.77
10	63.27	70.30
11	11.30	87.30
12	18.08	96.03
13	0.00	87.30
14	12.33	43.65
15	0.00	87.30
16	0.00	65.47
17	0.00	72.75
18	0.00	29.205
19	0.00	0.00
20	0.00	34.12
21	**135.58**	61.11
22	90.39	87.30
23	81.35	87.30
24	67.79	58.54
25	90.39	43.65
26	90.39	24.94
27	54.23	85.37
28	90.395	43.65

Cont.

Continued Table (3)

Serial No.	Alkaline protease based on wild type%	Chitinaseproductionbased on wild type%
29	63.27	43.65
30	69.84	80.02
31	0.00	49.11
32	0.00	58.20
33	39.54	0.00
34	90.39	87.30
35	90.39	0.00
36	63.27	43.65
37	18.08	72.75
38	45.19	0.00
39	0.00	0.00
40	45.19	76.49

Effect ofa wild type of lytic bacteria, *Serratia marcescens* and its mutants on mortality of *M. incognita* juveniles under laboratory conditions.

As illustrated in Table (4) wild types of lytic bacteria *Serratia marcescens*, and their ten mutants namely mutant no. 190, mutant no. 167, mutant no 36 and. mutant no. 21 from *Serratia marcescens*, were evaluated in this studies as affected survival of *M. incognita* juveniles J_2.The mortality percentages of the nematode were dependent on the bacterial concentration, enzyme production from mutants and exposure time. Data indicated that the best mortality by mutant SM. no. 36 which achieved the least number of both viable juveniles (VJ) and non viable juveniles (NVJ) due to this effect for high production of Alkaline protease 298.24 and chitinase from the first step of mutation which achieved 381.83 and this effect of least number due to degrading chitin and protein of juveniles nematodes. For example, the numbers of VJ of the best treatment were achieved zero in either S or S/10 (highly effective on juveniles mortality) comparable to the wild type SM. as compared to untreated control which reached 51.75, 49.25 and 49.55 viable nematode individuals. The numbers of non viable juveniles (NVJ) of the best treatments were 6, 4 and 2.27 individual after 24, 48 and 72 hrs; respectively compared with the SM wild type which achieved 26.25, 33.25 and 27.25 while the untreated control which achieved zero. There were positive relationships between the nematode mortality and each of the bacterial concentration and enzyme production from the mutant.

Table(4): Effect of the wild type of lytic bacteria, *Serratia marcescens* and its mutants on mortality of *M. incognita* juveniles under laboratory conditions.

Treatments		Bacterial Conc.	Nematode mortality after different exposure periods					
			Juveniles					
			Viable			Non Viable		
Strains	**Mutants Number**		**24 hr**	**48 hr**	**72 hr**	**24 hr**	**48 hr**	**72hr**
Serratiamarcensens (SM)	**190**	**S**	0	0	0	12.75	10.75	7.75
		S/10	0	3.00	0	16.75	15.00	13.00
	167	**S**	0	0	5.25	6.75	6.25	5.25
		S/10	5.00	3.25	2.25	12.5	12	2.25
	36	**S**	0	0	0	2.25	0.75	0
		S/10	0	0	0	**6.00**	**4.00**	**2.75**
	21	**S**	0	0	0	10.75	9.00	8.50
		S/10	6.50	3.25	2.50	19.25	15.75	14.00
Wild Type	**SM**	**S**	8.00	6.50	6.25	34.00	31.00	25.00
		S/10	8.25	7.25	6.25	**26.25**	**33.25**	**27.25**
Distilled water (Control)			**51.75**	**49.25**	**49.50**	0	0	0

Discussion

Results of the laboratory experiment (in case of using juveniles) proved that the wild type of *Serratia marcescens* and its mutants with their two dilutions have higher nematicidal activity against second stage juveniles (J_2) of *M. incognita*. Amongst the tested treatments, mutant no SM 36 at two dilutions achieved the highest mortality of *M. incognita* juveniles followed by mutant no. SM 190 and the wild type (SM).

The number of either viable juveniles or non - viable juveniles clearly were reduced after treatments at their two dilutions of different exposure periods (24, 48 and 72 hr) compared to untreated control. This reduction in number of viable juveniles or non - viable juveniles may be attribute to the most of the dead nematode bodies were degraded and destroyed by these treatments. This effect may be attribute to the higher percentage production of chitinase and alkaline protease enzymes which were produced at a high amount by the mutant no. SM 36 alkaline protease which was about 298.24%, equal about three folds of the wild type.

Similar results were reported by **Khan *et al.*, 2003; khan *et al.*, 2004; Lian *et al.*, 2007; El- Hadad and Mustafa, 2010 and Lian *et al.*, (2007)** found that rhizobacteria *Bacillus* sp strain RH219 showed a remarkable nematicidal activity. RH219 strain Killed 80% tested nematode within 2 h and completely destroyed and

digested all tested nematodes targets after 12 h. It is well known that the cuticle of nematodes is rigid composed and composes of proteins and **chitins (Cox *et al.,* 1981 and Åhman, 2000).** The results suggested that the hydrolytic enzymes are involved in the penetration process for bacteria killing the hosts **(Åhman, 2000).**

Also, the present in – vitro bioassay on *M. incognita* juveniles cleared that all the tested mutants and the wild types of strains with their dilutions caused morphological effects on the dead juveniles. These results apparently agree with **Miller and Sands (1977)** who showed that a chitinase preparation from *serratiaantibioticus* killed juveniles of *Tylenchorhynchus dubius* and to a lesser extent *Pratylenchus penetrans,* as determined by percent decrease in mobility of juveniles. **Jaworski *et al.* (1986)** have reported these changes in cuticle.

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الملخص العربي

التأثير الإبادي لبكتيريا سيراتيا مارسينس وطفراتها لمقاومة يرقات نيماتودا التعقد الجذري (موليدوجين أنكوجنيتا)

عبد الله شحاته كساب*، محمد فهمي عيسى**، أسامة محمد بدر***، أحمد السيد إسماعيل**، أشرف بكري عبد الرازق***، وجازية سليمان**.

* قسم وقاية النبات –كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة – مصر.

** قسم أمراض النبات – المركز القومي للبحوث – الدقي – جيزة – مصر.

*** قسم الوراثة – المركز القومي للبحوث – الدقي – جيزة – مصر.

**** قسم الوراثة –كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة – مصر.

تعتبر بكتيريا سيراتيا مارسينس حاليًا مقاوم حيوي للآفات النيماتودية وهي من أكثر البكتيريا المحللة للكيتين فاعلية. وقد تم تقييم أثرها وطفراتها علي مقاومة يرقات آفة التعقد الجذري (موليدوجين أنكوجنيتا) تحت الظروف المعملية.

وأثرت الطفرة **SM36** أفضل تأثير منتجة صفر يرقات حية في تركيزات المعلق البكتيري **(S)** أو **(S/10)** مقارنة بالسلالة الأم. وبلغت أعداد اليرقات في الغير معامل ٥١.٨، ٤٩.٣، ٤٩ يرقة بعد ٢٤، ٤٧ و٧٢ ساعة بالترتيب ومقارنة بالسلالة الأم التي وجد بها ٢٦.٣، ٣٣.٣ و٢٧.٣ بينما بالمقارنة الغير معاملة لم تتأثر يرقاتها. وقد وجدت علاقة موجبة بين موت يرقات النيماتودا وتركيز البكتيريا وإنتاج الإنزيمات من الطفرات فقد انخفض عدد اليرقات الحية والغير حية في تركيز **S**، وكذا **S/10** لفترات ٢٤، ٤٧ و٧٢ ساعة مقارنة بالغير معامل. ويعزي هذا الانخفاض إلى موت وتحلل يرقات النيماتودا في معاملات السلالة المطفرة بسبب زيادة إفرازها لكل من إنزيم الكيتنيز والألكالين بروتييز من الطفرات التي تنتج ٢-٣ أمثال كمية الإنزيم التي تنتجها السلالة الأم من بكتيريا سيراتيا مارسينس.