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BIOLOGICAL CONTROL OF *Meloidogyne javanica* ON TOMATO PLANTS WITH SOME ISOLATED BIOAGENTS IN EGYPT

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ABSTRACT

Eight antagonists; *Bacillus subtilis*, *Bacillus thuringiensis*, *Trichoderma harzianum*, *Gliocladium virnes*, *Paecilomyces lilacinus* and three yeast *(Saccharomyces* spp.*)* isolated from Egyptian soil at different concentrations were used to control root-knot nematode, *Meloidogyne javanica* under laboratory and green house conditions on tomato plants.

The most effective isolate in controlling root-knot nematodes was the isolate of *T. harzianum* whereas the least effective were the isolates of yeast *(Saccharomyces* spp.*)* under both laboratory , greenhouse and field conditions.

Under laboratory conditions applying the antagonistic bacteria, fungi and yeast achieved high percentage of juvenile mortality at the highest concentration (1:10) during all exposure periods especially after 72 hours.

Under greenhouse conditions *T. harzianum* was more effective in reducing numbers of galls, egg-masses and eggs per plant and number of 2nd stage juveniles in soil whereas, the least effective isolates were those of the yeast *(Saccharomyces* spp.*)*. Adding all antagonistic bacteria, fungi and yeast increased the fresh weight of root and shoot system on tomato plants at all tested concentrations especially in the highest concentration (1:10).

Using the antagonistic bacteria, fungi and yeast in both (filtrates and cells) achieved high percentage of juvenile mortality during all exposure periods especially after 72 hours under laboratory conditions. The most effective isolate filtrate on juvenile mortality was *T. harzianum*, while the lowest effective was the isolate filtrate of *Saccharomyces* spp. The most highest effective isolate cell on juvenile mortality was *Saccharomyces cerevisiae* while the lowest effective was the isolate cell of *T. harzianum*. The tested *Saccharomyces* spp. cells were more harmful to the nematode juveniles than their filtrates of the same *Saccharomyces* spp. Also, adding all bioagents treatments decreased number of nematodes in both roots and soil. In addition the crop of tomato increased after adding the treatments at concentration (1:10) under field conditions.

Keywords: Control, root-knot nematodes, *Bacillus subtilis*, *B. thuringiensis*, *Trichoderma harzianum*, *Gliocladium virnes*, *Paecilomyces lilacinus*, *Saccharomyces* spp.

INTRODUTION

In the last few decades, the awareness of the pesticides hazards to human and environment directed the attention towards searching for other safe alternative methods. (Fawcett and Spencer, 1970; Dubey and Mall, 1972; and Javoraska, 1978) Biological control is gaining increasing role throughout the world as alternative method to pesticides for nematode suppression. Different bacteria such as *Bacillus subtilis and B. thuringiensis* were used as bioagents to control root knot nematode. Applying *B. thuringiensis* and *B. subtilis* decreases the galls and nematodes number in soil and root (Frederik *et al.*, 1995 ; Hanna *et al.*, 1999; Jonathan *et al.*, 2000 and Rangaswamy *et al.*, 2001) Some fungi were recorded as bio-control

agent on root knot nematode *i.e. Trichoderma harzianum* (Miller, 1976; Abd El-Moity, *et al.* 1985; Ali 1994; Ali and Barakat, 1991), *Paecilomyces lilacinus, Gliocladium virnes* (Rodriguez *et al.*, 1984; Abd El-Moity *et al.*; 1998). Also, *Saccharomyces* spp. were recorded as bio-control agent on root knot nematodes (Orion and Kritzman, 1991)

A stable productive agroecosy-stem with effective biological control of plant diseases can be equated with a natural ecosystem in biological balance (Cook and Baker, 1989). The present work is aiming to isolate some antagonistic soil microorganisms from the Egyptian agro-ecosystem and evaluate their influence on the root-knot nematode, *M. javanica* on tomato plants under laboratory, greenhouse and field conditions, as well as studying the mode biological action of the bioagents.

MATERIAL AND METHODS

I.Isolation the Different Bioagents from the Soil

In order to isolate the different bio-agents the standard dilution plating technique (Wollum, 1982) was followed. Rhizosphere soil samples with tomato roots were collected from different Egyptian tomato fields. The samples were crushed thoroughly, ten grams of each sample were suspended in 90 ml sterilized distilled water and shaked for 20 min. Serial dilutions up to 10^{8} were used for the isolation of the different bioagents. Of each dilution, . 0.250 ml. was spread on the surface of soil extract agar in petri dishes using sterile glass rod. Five petri dishes were used for each dilution. The dishes were incubated at 28°C for 2 days. The separated grown colonies were morphologically classified. The bacterial colonies were sub-cultivated on slant nutrient agar medium; the fungal colonies were sub-cultivated on PDA medium while the *Saccharomyces* colonies were sub-cultivated on Nutrient-yeast extract broth medium as described by (Oostendorp *et al.*, 1991).

All bioagents were collected and identified in Department of Agricultural Botany Identification, Faculty of Agriculture, Minufiya University, Egypt"".

II. Prescreening of the Isolated Organisms for their Antagonistic Potency *in Vitro*

Because of the great number of the isolated microorganisms a prescreening test for their antagonistic potency was carried out. The separated colonies, which showed different morphological characters, were cute out with cork borer (5 mm). The colony disks were transferred into sterilized test tube containing 5 ml. sterilized distilled water, shaked thoroughly, and the resulted spore suspension was used in this test. At the same time, the different fungal isolates were sub-cultivated on PDA medium, while the bacterial and yeast isolates were sub-cultivated on nutrient-yeast extract medium in flasks, incubated with shaking at 150 rpm for 72 h. at 28° C. The bacterial suspensions were then centrifuged, washed and re-suspended in 20 m N buffer, pH 7.0. The slide-germination fungicidal bioassay technique (Sharvelle, 1979) was adopted since, cavity glass slides were used, the nematode (*M. javanica*) was used instead of the fungus spores and the bioagent suspensions were used instead of the fungicides.

The isolate cultures which did not show any antagonistic effect were discarded. The bioagent candidates of fungi that proved their antagonistic potency were identified according to (Rifai, 1969; Bissett, 1991 and Domsch *et al.*,1980) The bacterial and yeast isolates were identified according to Boone *et al.* (2001) and Holt *et al.*, (1994) then were subjected to further antagonistic potency estimation test.

III.Estimation the Antagonistic Potency of the Bioagent Candidates

The bacterial isolates were inoculated in nutrient broth media, incubated with shaking at 150 rpm for 48 to 72 h. at 28° C., and then the bacterial suspensions were centrifuged, washed and re-suspended in 20 ml N buffer, pH 7.0. Successive dilutions were prepared in water and colony forming units (cfu) were counted using the dilution plate technique and adjusted to 5.3×10^{11}.

The same procedure was applied to the fungal isolates and *Saccharomyces* isolates but Potato Dextrose broth medium and Glucose-Peptone-Yeast extract medium (Papavizas and Davey, 1959) were used to grow the fungal isolates and Saccharomyces isolate respectively, incubated for one week and the cfu were adjusted to 1.7×10^9 and 3.5×10^7 respectively.

a- Estimation the antagonistic potency of the bioagent candidates *in vitro*

To test the efficacy of bacteria isolates in inhibiting the activity of *M. javanica* juveniles in vitro, 1 ml of each of the bacterial isolates (4.77×10^{11}, 3.975×10^{11} and 2.64×10^{11} cfu), fungi isolate (1.53×10^9, 1.275×10^9 and 0.85×10^9 cfu) and yeast isolate (3.15×10^7, 2.625×10^7 and 1.75×10^7 cfu) were added separately to 1 ml of nematode suspension in glass vials. The numbers of active and non-active juveniles were examined and counted microscopically after 24, 48 and 72 hours.

b-Estimation the antagonistic potency of the bioagent candidates *in vivo* under greenhouse conditions

Four- week old tomato seedlings (cv. Casel rock), were transplanted in pots (20 cm.) each containing steam–sterilized loamy sandy soil. The pots were divided into 26 groups each containing 5 pots. Each group received one of the three inoculum levels of one of 8 bioagent candidates, one of the last two groups was treated with nematicide (Vydate) at the recommended dose, and the second was left without any control agent to serve as cheek. Each pot was inoculated with 3000 newly hatched second stage juveniles of *M. javanica* containing at depths of 2-3 cm. around the roots. All treatments received the same agricultural treatments. After 60 days, all plants were carefully uprooted. Root and shoot systems were weighted. Number of juveniles per 250 g. soil and nematode populations in roots were counted according to (Franklin & Goodey, 1957).

c- Mode biological action

In order to explain, how the bioagents effect on the nematodes the different bioagent candidates were cultured in on Czapek's medium with shaking at ±28 °C for two days (for bacteria and *Saccharomyces*) and at ±25°C for one week (for fungi), The resulted cultures were filtrated through

filter paper. The filtrates were centrifuged at 3000 rpm and the precipitated bacterial cells and fungi spores (cells) were separated from the supernatant. The bacterial cells and fungi spores were washed many times by re-suspending them in enough amounts of N buffer, pH 7.0 with repeated centrifuge then the suspensions were adjusted to (4.77×10^{11}, 3.975×10^{11} and 2.64×10^{11} cfu), fungi isolates (1.53×10^{9}, 1.275×10^{9} and 0.85×10^{9} cfu) and yeast isolates (3.15×10^{7}, 2.625×10^{7} and 1.75×10^{7} cfu). On the other hand the supernatants were filtrated through bacterial filter (G5) and three dilutions (90%, 75% and 50%) were prepared. Of each spore suspension or diluted supernatants one ml was added separately to 1 ml of nematode suspension per glass vial. The numbers of active and non-active juveniles were examined and counted microscopically after 24, 48 and 72 hours.

IV Estimation the bioagent candidates under field conditions:-

This experiment was conducted in naturally infested soil with root knot nematode; *M. javanica* to determine the effect of different bioagents i.e. bacteria, fungi and yeast on tomato plants at concentration (1:10) for each treatment under field conditions. All treatments were replicated three times, each replicate contained twenty seedlings. At the end of the experiment, data on nematode population in both soil and root were determined and recorded. Also, data on yield per feddan were also estimated and recorded.

Statistical analyses:-

Data obtained in this study were statistically analyzed according to the procedures "ANOVA" reported by Sendecor and Cochran (1980). Treatment means were compared by the Duncan's Multiple Rang Test at 5% level of probability.

RESULTS AND DISCUSSION

I. Prescreening of the Isolated Organisms for their Antagonistic Potency *in vitro*

A great number of fungi and bacteria colonies were obtained, prescreening of the different colonies resulted in only eight isolates that showed notable antagonistic effect to *Meloidogyne javanica*. These isolates were identified as *Bacillus subtilis*,, *B. thuringiensis, Trichoderma harzianum, Gliocladium virens, Paecilomyces lilacinus* and three *Saccharomyces* spp. *(Saccharomyces cerevisiae, S. ludwigii* and *S. uvarum)*

II. Estimation the Antagonistic Potency of the Bioagent Candidates

However all tested candidates had remarkable antagonistic effect against the nematode juveniles (Table 1), after 72 hours no candidate overcame the tested nematicide , Vydate which resulted in 87.3% mortality. The percentage of mortality differed according to either the genus or the inoculum density of bacteria, fungi and yeast. *T. harzianum* and *P. lilacinus* showed the highest antagonistic effect at the mean of the three concentrations (70.2 and 60.5% respectively), followed by *G. virnes(54.7%)* then *B. thuringiensis* and *B. subtilis* resulting in 50.3 and 41.9 % mortality respectively. Yeast isolates (*Saccharomyces cerevisiae, S. ludwigii* and *S. uvarum)* showed the lowest antagonistic effect with 21.7, 21.4 and 17.2% mortality respectively.

Table 1: Effect of the different bioagent candidates and vydate nematicide on *Meloidogyne javanica* juveniles after different exposure periods under laboratory conditions

Treatment		Conc.	Mortality% Exposure period (in hours) 24	48	72
Bacteria	*Bacillus subtilis*	1:10	34.1	40.3	52.3
		1:25	23.4	32.5	40.8
		1:50	17.2	21.7	32.6
		Mean	24.9	31.5	41.9
	Bacillus thuringiensis	1:10	40.8	51.3	66.2
		1:25	32.9	40.5	49.6
		1:50	24.3	30.5	35.1
		Mean	32.7	40.8	50.3
Fungi	*Gliocladium virnes*	1:10	50.9	62.1	70.5
		1:25	38.9	45.7	50.3
		1:50	30.8	38.6	43.3
		Mean	40.2	48.8	54.7
	Paecilomyces lilacinus	1:10	58.7	66.3	75.6
		1:25	45.3	50.1	58.2
		1:50	39.8	47.6	53.5
		Mean	47.9	54.7	62.4
	Trichoderma harzianum	1:10	66.8	72.3	83.9
		1:25	53.7	62.8	69.2
		1:50	42.6	50.4	57.6
		Mean	54.4	61.8	70.2
Yeast	*Saccharomyces cerevisiae*	1:10	20.7	26.7	30.3
		1:25	11.9	14.6	19.8
		1:50	9.8	10.7	15.1
		Mean	14.1	17.3	21.7
	Saccharomyces Ludwigii	1:10	18.8	22.7	27.3
		1:25	10.2	12.3	21.5
		1:50	7.3	9.7	15.5
		Mean	12.1	14.9	21.4
	Saccharomyces uvarum	1:10	13.5	16.8	22.5
		1:25	8.3	10.2	16.2
		1:50	6.9	8.4	13.0
		Mean	9.6	11.8	17.2
Nematicide (vydate 24% EC)			70.9	81.5	87.3
Control with nematodes			2.2	2.7	3.8
L.S.D. (5 %)			4.54	5.12	6.75

Increasing the inoculum density resulted in increasing the juveniles' mortality. That was clear in the case of all the bioagent candidates, since in all cases, the mortality was proportional to the bioagent inoculum density. On the other hand, prolonging the exposure time to the bioagents resulted in increasing the antagonistic effect in all cases.

Data obtained in figure (1) illustrated the percentage of mortality in all used antagonistic bacteria *(B. Subtilis, B. thuringiensis),* fungi (*T. harzianum*, *G. virnes*, *P. lilacinus*) and yeast (*Saccharomyces* spp.) in three time periods (24, 48 and 72 hours) at the mean of the three concentrations. *T. harzianum* achieved the highest percentage of juvenile mortality after the used nematicides (Vydate) whereas, the lowest percentage of juvenile mortality were the isolates of yeast during all exposure periods especially after 72 hours.

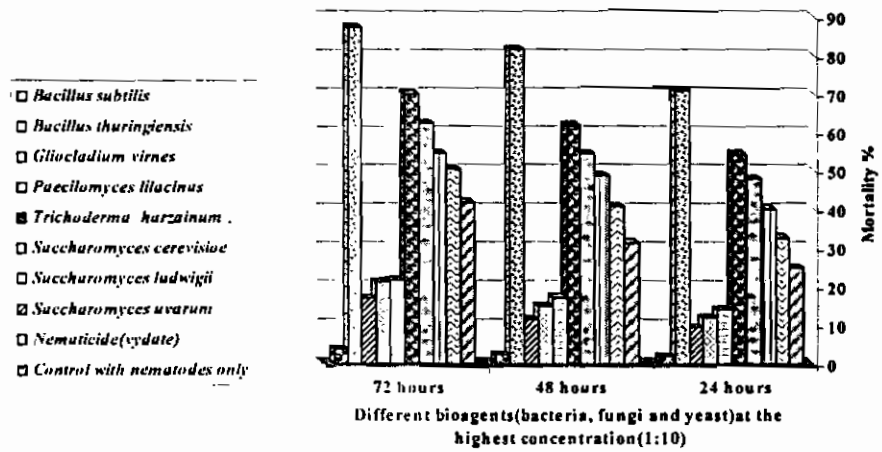

Fig. 1. Effect of different antagonistic bioagents (bacteria, fungi and yeast) comparing with nematicide (Vydate) on % mortality of *Meloidogyne javanica* juveniles at the highest concentration (1:10) under laboratory conditions

Data obtained in Figure (2) illustrated the effect of different antagonistic bioagents (bacteria, fungi and yeast) comparing with nematicide (Vydate) on % mortality of *Meloidogyne javanica* juveniles at different concentrations after 72 hours under laboratory conditions.

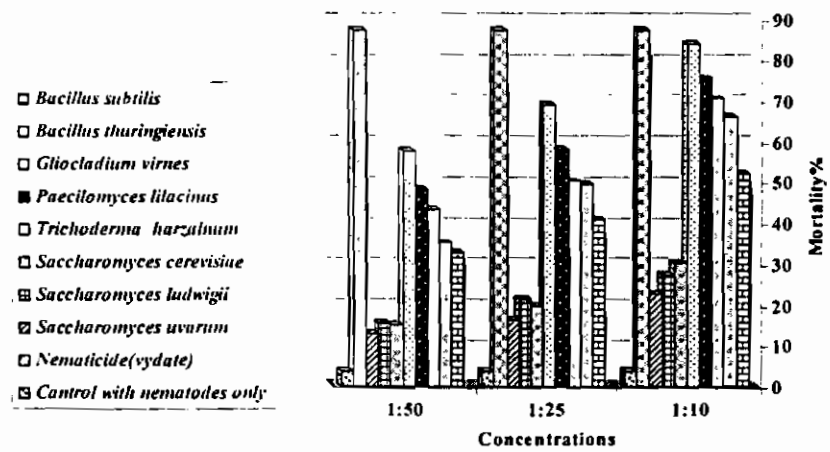

Fig. 2. Effect of different antagonistic bioagents (bacteria, fungi and yeast) comparing with nematicide (Vydate) on % mortality of *Meloidogyne javanica* juveniles at different concentrations after 72 hours under laboratory conditions

III. Estimation the Antagonistic Potency of the Bioagent Candidates *in Vivo* Under Greenhouse Conditions

a-Effect on number of root galls

However all tested bioagent candidates showed remarkable decrease of the number of root galls (Table 2), the nematicide (Vydate) resulted in the lowest number of root galls and the highest reduction percentage. *T. harzianum* and *P. lilacinus* performed the highest reduction of galls numbers compared with the other tested bioagent candidates. There were no significant difference between mean numbers of root galls resulted on plants treated with *T. harzianum* and such resulted on plants treated with vydate at concentration (1:10).

Table 2. Effect of the different bioagent candidates and nematicide on the number of nematode population of *m. Javanica* the causal of root- knot nematode of tomato under greenhouse

Treatments	Conc.	Nematode numbers / root plant					
		Root galling		Egg- masses		Eggs / egg-mass	
		No. of galls	Red. %	No. of egg-masses	Red. %	No. of eggs	Red. %
Bacillus subtilis	1:10	70	50.7	55	52.2	190	60.4
	1:25	82	42.3	61	46.9	210	56.3
	1:50	87	38.7	72	37.4	272	43.3
	Mean	80	43.9	63	45.5	224	53.3
Bacillus thuringiensis	1:10	61	57	49	57.4	216	55.0
	1:25	70	50.7	55	52.2	245	48.9
	1:50	78	45.1	61	46.9	277	42.3
	Mean	70	50.9	55	52.2	246	48.7
Gliocladium virnes	1:10	55	61.3	40	65.2	125	73.9
	1:25	62	56.3	48	58.3	142	70.4
	1:50	68	52.1	52	54.8	168	65.0
	Mean	62	56.6	47	59.4	145	69.8
Paecilomyces lilacinus	1:10	42	70.4	35	69.6	95	80.2
	1:25	51	64.1	38	66.9	116	75.8
	1:50	59	58.5	42	63.5	132	72.5
	Mean	51	64.3	38	66.7	114	76.2
Trichoderma harzianum	1:10	36	74.6	25	78.3	85	82.3
	1:25	44	69	30	73.9	98	79.6
	1:50	50	64.8	37	67.8	120	75.0
	Mean	44	69.5	31	73.3	101	78.9
Saccharomyces cerevisiae	1:10	75	47.2	63	45.2	225	53.1
	1:25	83	41.6	66	42.6	256	46.7
	1:50	88	38	73	36.5	270	43.8
	Mean	82	42.3	68	41.4	250	47.9
Saccharomyces Ludwigii	1:10	79	44.4	63	45.2	238	50.0
	1:25	89	37.3	68	40.9	267	44.3
	1:50	94	33.8	75	34.8	286	40.4
	Mean	88	38.5	69	40.3	264	44.9
Saccharomyces uvarum	1:10	82	42.3	70	39.1	274	42.9
	1:25	92	35.2	76	33.9	290	39.6
	1:50	98	31	82	28.7	330	31.3
	Mean	91	36.2	76	33.9	298	37.9
Nematicide(vydate24% EC)		32	77.5	22		70	85.4
Control (nematodes)		142		115		480	
L.S.D. (5 %)		1.57		1.45		12.60	

P. lilacinus ranked on the second place resulting in 70.4 % reduction, whereas *G. virnes.* resulted in lower reduction of galls numbers 61.3%, followed by, *B. thuringiensis* with 57.0% then *B. subtilis* with 50.7%. The least effective bioagents candidates were the three yeast isolates *S. cerevisiae,S. ludwigii* and *S. uvarum* which recorded 47.2, 44.4 and 42.3% respectively. The root galls reduction was affected greatly with the inoculum density of the bioagent. Increase the inoculum density of *T. harzianum* from (1:50) to (1:10) resulted in decreasing the reduction efficiency from 74.6 to 64.8 %. Similar reaction was found in the case of all the other bioagent candidates.

b- Effect on egg masses and eggs numbers

The same trend obtained with the effect on number of root galls was also obtained with the effect on egg masses and egg numbers.

Data in figure (3) illustrated the effect of different antagonistic bio-agents (bacteria, fungi and yeast) compared with the nematicide (Vydate) on reducing numbers of *M. javanica* juveniles in 250 g. soil at the highest concentration (1:10) under greenhouse conditions. Data show that the most effective isolates in controlling root knot nematode were the isolates of *(T. harzianum* and *P. lilacinus*) whereas the least effective were the isolates of yeast (*Saccharomyces cerevisiae, S. ludwigii* and *S. uvarum).* Moreover, *B. subtilis, B. thuringiensis* occupied an intermediate position in controlling *M. javanica.*

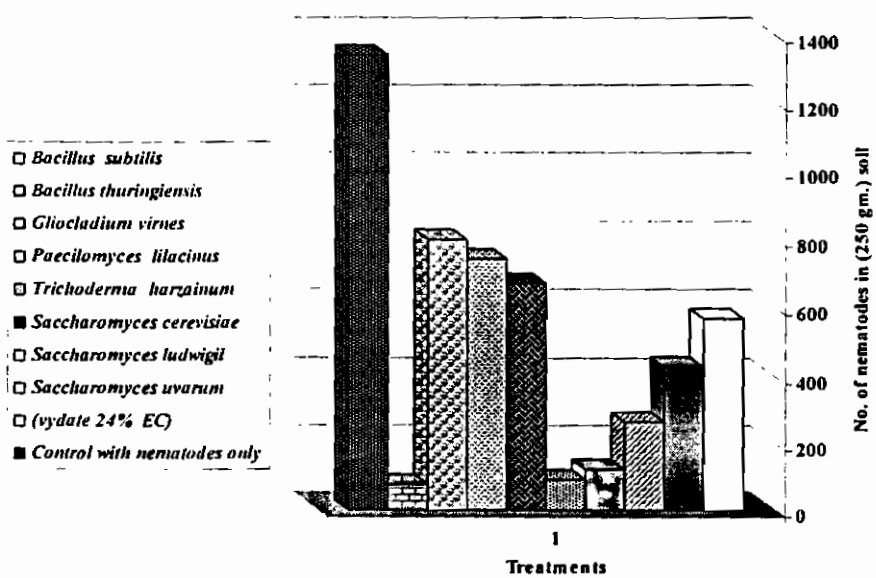

Fig. 3. Effect of different antagonistic bioagents (bacteria, fungi and yeast) compared with nematicide (vydate) on number of *Meloidogyne javanica* nematodes in soil at the highest concentration (1:10) under greenhouse conditions

c-Effect on the vegetative growth of the treated plants

The side effect of the different bioagents candidates, compared with Vydate nematicide, on the root and shoot fresh weight of the treated plants was studied (Table 3).

Table 3: Plant response of tomato plants on fresh weights as influenced by the root- knot nematode; *M. javanica* infection and its control by different antagonistic fungi, bacteria and yeast under greenhouse conditions.

Treatments	Conc.	Plant response fresh weight			
		Shoot weight(gm.)	Inc. %	Root weight(gm.)	Inc.%
Bacillus subtilus	1:10	27.2	55.4	14.0	52.2
	1:25	26.4	50.9	13.7	48.9
	1:50	25.7	46.9	13.5	46.7
Bacillus thuringiensis	1:10	26.5	51.4	13.9	51.1
	1:25	25.9	48.0	13.6	47.8
	1:50	25.1	43.4	12.8	39.1
Gliocladium virnes	1:10	23.5	34.3	11.7	27.2
	1:25	22.8	30.3	11.0	19.6
	1:50	20.1	14.9	10.6	15.2
Paecilomyces lilacinus	1:10	25.7	46.9	12.7	38.0
	1:25	24.8	41.7	12.1	31.5
	1:50	24.2	38.3	11.8	28.3
Trichoderma harzainum	1:10	24.5	40.0	12.2	32.6
	1:25	23.8	36.0	11.7	27.2
	1:50	22.7	29.7	11.5	25.0
Saccharomyces cerevisiae	1:10	20.6	17.7	10.9	18.5
	1:25	19.9	13.7	10.5	14.1
	1:50	18.5	5.7	10.1	9.8
Saccharomyces Ludwigii	1:10	19.6	12.0	10.4	13.0
	1:25	18.3	4.6	9.9	7.6
	1:50	17.6	0.6	9.4	2.2
Saccharomyces uvarum	1:10	18.3	0.5	9.8	6.5
	1:25	18.0	0.3	9.5	3.3
	1:50	17.7	0.1	9.3	1.1
Nematicide (vydate 24% EC)		25.3	44.8	13.7	48.9
Control (healthy)		28.2	61.1	14.9	61.9
Control(nematodes)		17.5		9.2	
L.S.D. (5 %)		1.12		1.06	

1. Effect on shoot weights

All the treatments provoked the growth of the treated plants compared with the untreated and infected plants with *Meloidogyne javanica*. Increasing percentage in fresh weight of healthy plants reached to 61.1% compared with the infected plants. The maximum increasing percentage at 1:10 concentration was recorded on the treated plants with the bioagents *T. harzianum* and *P. lilacinus* (with 55.4 and 51.4%) respectively, followed by *G. virnes* with 46.9%, *B. thuringiensis B. thuringiensis* with 40.0%, then *B. subtilis* with 34.3%. *S. cerevisiae, S. ludwigii* while, *S. uvarum* showed the

lowest shoot weight increasing%, which reached 17.7, 12.0 and 0.5 % respectively. Even though Vydate ranked the first in increasing percentage fresh weight of plant with value 57.1%.

2. Effect on root weights

Same trend obtained with effect on tomato shoot weights were also obtained on the effect on tomato root weights.

IV- Role of Certain Bioagent Cells and their Filtrates in the Biological Control Process

The effect of certain bioagents and their filtrates on the *M. javanica* juveniles at different exposure times is demonstrated in Table (4). In the case of *T. harzianum,* , the filtrate resulted in 77.2% juveniles mortality compared with 38.1 % only in the case of the cells of the same organism after 72 hr. of exposure. Contrary data were obtained in the case of *B. thuringiensis* since; the filtrate resulted in 57.3 % mortality compared with 76.8 % in the case of the cells of the same organism after 72 hr. of exposure. However, the filtrate of *P. lilacinus* , *G. virnes* and *B. Subtilis,* showed juveniles mortality ranged from 60.3 to 49.4% compared with juveniles mortality ranged from 68.3 to 57.4% in the case of the cells after three tested bioagents. The tested *Saccharomyces* spp. cells were more harmful to the nematode juveniles than their filtrates. Biological control is gaining increasing role throughout the world as alternative method to pesticides. From great number of soil microorganisms isolated from different localities in Egypt, two bacterial bioagents, *B. subtilis* and *B. thuringiensis,* three fungal bioagents *G. virnes, P. lilacinus* and *T. harzianum* and three *Saccharomyces* spp., *S. cerevisiae S. Ludwigii* and *S. uvarum* showed promising antagonistic effect on *M. javanica.* Under laboratory conditions, all the bioagent candidates proved to be harmful to *M. javanica* juveniles, egg masses and eggs number however this effect differed from one candidate to another. Similar data were recorded too under greenhouse conditions. *T. harzianum* was found to be the most effective candidate for controlling root-knot nematodes, whereas the least effective candidates were the three *Saccharomyces* spp. These data are in agreement with those obtained by Hanna *et al.*, 1995; Abd El-Moity *et al.*,1998; Hanna *et al.,* 1999 and Rangaswamy *et al 2001.* In addition, adding any of the antagonistic bacteria, fungi and yeast increased the fresh weight of root and shoot system of tomato plants especially in high concentrations. Most of the tested bioagents are saprophytic organisms that produce variety of enzymes that enable them to degrade a variety of natural substrates and contribute to renew nutrient cycling in the soil. The cells of the tested *Saccharomyces* spp. were more harmful to the nematode juveniles than filtrates.

Table 4: The role of the bioagent cells and filtrates on mortality of *M. javanica* juveniles at different exposure times under laboratory conditions

Treatments		Conc.	Filtrates			Cells		
			% Mortality of *M. javanica* after					
			24 h	48 h	72 h	24 h.	48 h.	72 h.
Bacteria	*Bacillus subtilis*	1:10	71.3	73.8	77.2	30.3	35.6	38.1
		1:25	66.2	69.7	70.3	24.4	27.3	30.5
		1:50	56.4	60.1	64.5	19.1	22.4	25.6
	Mean		64.6	67.9	70.7	24.6	28.4	31.4
	Bacillus thuringiensis	1:10	58.3	62.2	68.3	40.3	44.9	49.6
		1:25	50.1	54.3	60.4	35.6	37.8	40.3
		1:50	41.5	46.1	52.3	30.4	34.3	37.2
	Mean		49.9	54.2	60.3	35.4	39.0	42.4
Fungi	*Gliocladium virnes*	1:10	50.6	53.6	57.4	58.2	62.3	66.7
		1:25	44.2	47.5	50.1	51.4	54.8	59.2
		1:50	35.3	37.6	40.8	42.6	45.1	48.6
	Mean		43.3	46.2	49.4	50.7	54.1	58.2
	Paecilomyces lilacinus	1:10	55.7	59.4	62.2	74.2	76.7	79.9
		1:25	48.2	51.2	52.4	68.5	70.4	74.2
		1:50	39.8	42.8	45.1	55.1	58.4	63.4
	Mean		47.9	51.1	53.2	65.9	68.5	72.5
	Trichoderma harzianum	1:10	50.8	54.3	57.3	70.5	73.6	76.8
		1:25	45.3	48.4	52.5	62.3	65.4	69.2
		1:50	39.8	42.8	47.2	50.4	53.7	57.1
	Mean		45.3	48.5	52.3	61.1	64.2	67.7
Yeast	*Saccharomyces cerevisiae*	1:10	44.7	49.3	53.7	80.3	82.4	86.3
		1:25	36.5	38.9	43.4	75.2	77.9	80.1
		1:50	29.4	33.7	37.6	65.4	69.5	73.4
	Mean		36.8	40.6	44.9	73.6	76.6	79.9
	Saccharomyces Ludwigii	1:10	37.9	40.1	44.7	76.7	79.2	80.6
		1:25	30.2	36.7	39.8	69.3	72.3	75.2
		1:50	23.7	27.3	32.3	65.1	68.6	71.6
	Mean		30.6	34.7	38.9	70.4	73.4	75.8
	Saccharomyces uvarum	1:10	35.5	38.3	41.2	73.5	75.1	78.8
		1:25	30.2	33.4	36.4	65.4	69.4	71.2
		1:50	24.3	26.8	29.1	59.2	63.7	67.2
	Mean		30.0	32.8	35.6	66.0	69.4	72.4
Nematicide (vydate24%EC)			88.6	90.2	93.1	88.6	90.2	93.1
Control(nematodes)			1.3	1.9	2.4	1.3	1.9	2.4
L.S.D. (5 %)			5.71	6.22	5.43	5.87	6.11	4.85

V- Field experiments:-

Evaluation of different bioagent candidates and nematicide on *M. javanica* population population infecting tomato under field conditions:-

a- Effect on number of root galls:-

However all tested treatments showed remarkable reduction for the number of galls (Table 5), both of *T. harzianum* and *P. lilacinus* performed the highest decrease of galls numbers compared with the other treatments at

(1:10) with three weeks doses of each treatment. *G . virnes, B. subtilis* and *B. thuringiensis* occupied an intermediate position in decreasing number of root galls. whereas *Saccharomyces* spp. resulted the lowest decrease in the number of root galls.

b- Effect on juveniles number in soil, egg masses and eggs / egg-mass numbers:-

Likewise, similar trend was recorded with respect to juveniles numbers in soil, egg -masses as well as eggs / egg-mass numbers

Paecilomyces, *Trichoderma* and *Gliocladium* species has been used as means of in vitro screening for the best biocontrol candidates as revealed by Chet and Inbar (1994). *Paecilomyces*,*Trichoderma* and *Gliocladium* are also considered good sources of various toxin antibiotics and various lytic enzymes such as chitinases and proteinase (Papavizas, 1985; Cherif and Benhamou, 1990; Tronsmo *et. al.*, 1993 and Sankaranarayanan *et al.*, 2000). Because the nematode egg layers containing chitin and protein layers, lytic enzymes maybe produced by *Paecilomyces*, *Trichoderma* and *Gliocladium* play an important role in dissolving the egg layers, consequently abortion the egg-hatching.

Table (5): Effect of different bioagent candidates and nematicide on the number of nematode population of *M. javanica* infecting tomato under field conditions:-

Treatments	Conc.	Nematode population				weight of fruit (ton/ feddan)	Inc. %
		In soil (250 gm.)	In roots				
			No. of galls	No. of egg-masses	Eggs / egg-mass		
Bacillus subtilis	1:10	660	15	13	112	21.5	56.9
Bacillus thuringiensis		720	21	19	153	20.2	47.5
Gliocladium virnes		1310	36	32	240	17.1	24.8
Paecilomyces lilacinus		940	26	24	177	19.5	42.3
Trichoderma harzianum		1020	29	27	210	18.4	34.1
Saccharomyces cerevisiae		1450	41	36	280	16.5	20.4
Saccharomyces Ludwigii		1700	47	40	320	15.4	12.4
Saccharomyces uvarum		2100	56	52	355	14.6	6.6
Nematicide (Vydate24% EC)		420	10	4	85	22.2	62.0
Control (nematodes)		2250	72	67	495	13.7	
L.S.D. (5 %)		144.3	5.76	5.54	32.86	1.090	

The effect of *B. subtilis* and *B. thuringiensis* may be also attributed to the accumulation of nematoxic metabolites of these bioagents in soil. These metabolites may have a direct lethal effect on nematodes(Diclow *et al.*,1993) or have some physiological and behavioral effects such as disorder of neuromuscular junctions, or through suppression of hatching movement, capabilities of feeding and invasion to host tissue(Mishra *et al.*, 1987 and

Kluepfel *et al.*,1993). Furthermore, ammonia produced by ammonifying bacteria during natural decomposition of intergenous products has often been implicated in control plant parasitic nematodes(Rodriguez,1986). Volatile compounds, fatty acides, hydrogens sulfide, enzymes, hormones, alcohol and phenolic compounds are among the bacterial metabolites implicated in the control of plant parasitic nematodes(Mishra *et al.*, 1987). These products may be toxic to nematode directly or they indirectly suppress nematode population by modifying the rhizosphere environment of nematode vitality.

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المكافحة الحيوية لنيماتودا تعقد الجذور(ميلـــودوجين جافنيكـــا) علـــى نباتـــات الطماطم باستخدام عزلات كائنات حيوية تحت الظروف المصرية

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تم اختبار ثمانية عوامل حيوية و هى : الفطر باسيليوميس ليلينس و الفطر تريكودرما هارزيـــانم و الفطر جلوكلاديم فيرنس و نوعين من البكتريا باسيلس ساتلس و بكتريا باسيلس سيرنجينزس و ثلاثة عزلات من الخمائر(سكارومايسيس) معزولة من البيئة المصرية و استخدمت بثلاثة تركيزات مختلفة لمقاومة نيماتودا تعقد الجذور من النوع ميلودوجين جافنيكا فى المعمل و كذلك فى الصوبة على نباتات الطماطم.

كانت اكثر العزلات تاثيرا تحت ظروف كلا من المعمل و الصوبة فى مكافحة نيماتودا تعقد الجذور هى عزلة البكتريا (باسيلس ساتلس) بينما كانت عزلات الخمائر(سكارومايسيس) اقلهم تاثيرا .

اظهر استخدام التركيز الاعلى(١٠:١) تاثيرا فعالا فى زيادة النسبة المئوية للموت على جميع الكائنـــات الحيوية المستخدمة خاصة بعد ٧٢ ساعة تحت ظروف المعمل .

كان استخدام العزلات البكتيرية تاثيرا فعالا فى خفض اعداد العقد النيماتودية و كتل البـــيض و عـــدد البيض على النبات الواحد فى الجذور و كذلك تعداد اليرقات من العمر الثانى فى التربة و اقلهم تاثيرا كانت عزلات الخمائر تحت ظروف الصوبة .

ادى اضافة الكائنات الحيوية من فطريات و بكتريا و خمائر و كذلك المبيد الى زيادة فى الوزن الرطب لكل من المجموع الجذرى و الخضرى لنباتات الطماطم فى جميع التركيزات المستخدمة خاصـــة التركيـــز العالى (١٠:١).

اظهر استخدام الكائنات الحيوية على الصورتين (الراشح- الخلايا بعد ترشيحها) تاثير فعالا فى زيـــادة النسبة المئوية للموت لليرقات خاصة بعد ٧٢ ساعة تحت ظروف المعمل. اظهر استخدام العزلـــة البكتيريـــة (باسيلس ساتلس) اكبر تاثيرا بينما اظهرت عزلات الخمائر اقل تاثيرا على صورة الراشح.

كما اظهر اضافة الكائنات الحيوية من فطريات و بكتريا و خمائر و كذلك المبيد كفاءة فـــى تقليـــل تعـــداد نيماتودا تعقد الجذور فى كلا من التربة و الجذور بالاضافة لتحسين وزن المحصول لنباتات الطماطم تحـــت ظروف الحقل.