Zagazig J. Agric. Res., Vol. 32 No. (4) 2005 1307 - 1326

ANTAGONISTIC EFFECT OF SOME ISOLATED BIOAGENTS ON MELOIDOGYNE JAVANICA AND THEIR POTENCY IN CONTROLING ROOT-KNOT DISEASE ON TOMATO PLANTS

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Accepted 28 / 6 / 2005

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

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

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

Under greenhouse conditions *B. subtilis* was more effective in reducing numbers of galls, egg-masses and eggs per plant and number of 2 nd stage juveniles in soil whereas, the least effective isolates were yeast *(Saccharomyces* spp.*)*.

Adding all antagonistic bacteria, fungi and yeast increased the fresh weight of root and shoot system on tomato plants at all used 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 *B. subtilis*, while the lowest effective was the isolate filtrate of *Saccharomyces* spp. The most effective isolate cell on juvenile mortality was *P. penetrans*, while the lowest effective was the isolate cell of *B. subtilis*. The tested *Saccharomyces* spp. was more harmful to the nematode juveniles than their filtrates of the same *Saccharomyces* spp.

Key words: Control, root-knot nematodes, *Bacillus subtilis*, *Pasteuria penetrans*, *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. (Epstein *et al.*, 1967; 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 *Pasteuria penetrans* were used as bioagent to control root knot nematode. Applying *Pasteuria penetrans* decreases the galls and nematodes number in soil and root (Hanna *et al.*, 1999; Rangaswamy *et al.*, 2001 and Ramesh and Chand, 2003). (Mankau, 1980; Sayre, 1980; Sterling, 1984; Shahazad *et al.*, 1990; Frederik *et al.*, 1995, and Jonathan *et al.*, 2000). Some fungi were recorded as bio-control agent to control 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) and *Verticillium chlamydosporium* (Sankaranarayanan, *et al.*, 2000).

A stable productive agroecosystem 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 caused by *M.*

javanica on tomato plants under laboratory and greenhouse conditions.

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 shacked for 20 min. Serial dilutions up to 10^{8} were used for the isolation of the different bio agents. 0.250 ml. of each dilution 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. In addition *Pasteuria penetrans* endospores were collected from infected females of *Meloidogyne* spp. and preserved as described by (Oostendorp *et al.*, 1991).

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 Ø). 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^{O} C. then the bacterial suspensions were centrifuged, washed and re-suspended in 20 m N buffer, pH 7.0. The slide-germination fungicidal bioassay technique (Sharvelle, 1979) was adapted 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, while the bio-agent candidates, which proved their antagonistic potency were identified according to (Rifai, 1969; Bissett, 1991 and Domsch 1980) in case of fungi, while 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 Bioagents Candidates

The bacterial isolates were inoculated in nutrient broth media, incubated with shaking at 150 rpm for 48 to 72 h. at 28°C., then the bacterial suspensions were centrifuged, washed and re-suspended in 20ml. 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* isolate except that 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 bioagents 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 bioagents candidates in *vivo* under greenhouse conditions

Four- week old tomato seedlings (cv. Supermarmande), were transplanted in pots 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 candidate, one of the last two groups was treated with Vydate nematicide at the recommended dose, and the second was left without any control agent to serve as cheek. Each pot was inoculated with suspension of egg masses of *M. javanica* containing about 3000 newly hatched second stage juveniles 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. Nematode populations in (250 gm.) soil and in roots were counted according to (Franklin & Goodey, 1957).

c- Mode biological action

In order to explain, how the bio-agents affect the nematode the different bio-agents 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. One ml. of each spore suspension or the diluted supernatants was 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.

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, Pasteuria penetrans, Trichoderma harzainum, Gliocladium virnes, Paecilomyces lilacinus* and three *Saccharomyces* spp. *(Saccharomyces cerevisiae, Saccharomyces ludwigii* and *Saccharomyces uvarum)*

II. Estimation the Antagonistic Potency of the Bioagents Candidates

However all tested candidates had remarkable antagonistic effect against the nematode juveniles (Table 1), no candidate overcame the tested nematicide vydate which resulted in 82.6% mortality. The percentage of mortality differed according to either the genus or the inoculum density of bacteria, fungi and yeast.

B. subtilis and *P. penetrans* showed the highest antagonistic effect (62.1 and 53.6% respectively), followed by, *P. lilacinus, T. harzainum* then *G. virnes* resulting in 47.9, 41.2 and 32.8% mortality respectively. Yeast isolates showed the lowest antagonistic effect with 17.7, 16.1 and 12.9%.

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 bio-agents 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, P. penetrans),* fungi *(T. harzainum, G. virnes, P. lilacinus)* and yeast (*Saccharomyces* spp.) in three time periods (24,48,72 hours) at the highest concentration (1:10).

B. subtilis achieved the highest percentage of juvenile mortality after the used nematicides (vydate) whereas, the lowest percentage of juvenile mortality were the isolates of yeast especially at the highest concentration (1:10) during all exposure periods especially after 72 hours.

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	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
	Pasteuria penetrans	1:10	58.7	66.3	75.6
		1:25	45.3	50.1	58.2
		1:50	35.8	43.6	48.5
		Mean	46.6	53.3	60.8
Fungi	*Gliocladium virnes*	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
	Paecilomyces lilacinus	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
	Trichoderma harzainum	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
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)			72.9	84.5	90.3
Control with nematodes			2.2	2.7	3.8
L.S.D. A*			0.62	0.73	0.75
L.S.D. B**			0.33	0.37	0.46
L.S.D. AB***			1.11	1.19	1.47

A* Treatments B* Concentrations

AB***Treatments& Concentrations

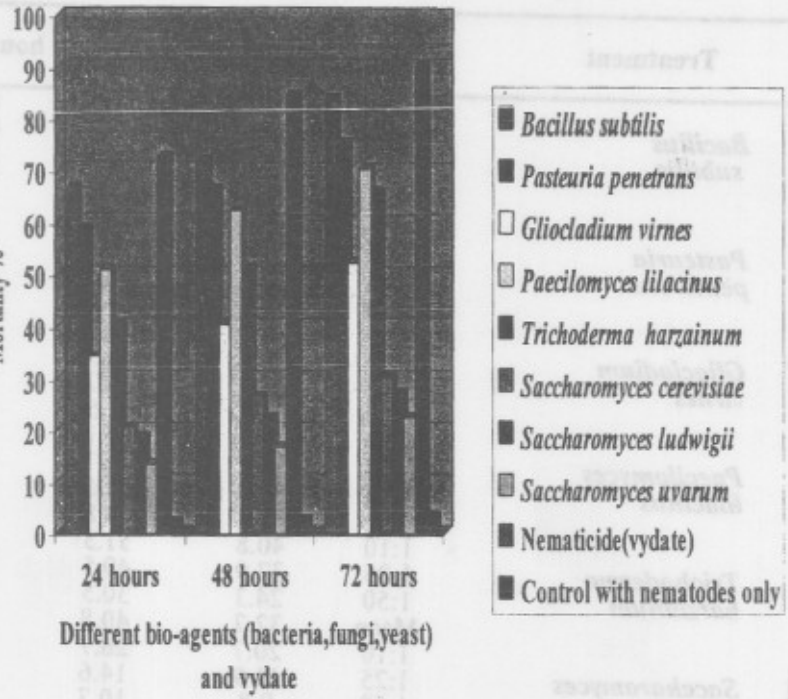

Fig. 1. Effect of different antagonistic bioagents (bacteria, fungi and yeast) comparing with nematicide (vydate) on % mortality of *Meloidogyne javanica* juveniles at the highest concentration (1:10) under laboratory conditions

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

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 a 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). Because the nematode egg layers containing a chitin and protein Layers, it may be lytic enzymes produced by *Paecilomyces*, *Trichoderma* and *Gliocladium* play an important role in dissolving the egg layers, consequently abortion the egg hatching.

III. Estimation the Antagonistic Potency of the Bio-agents 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, *B. subtilis* performed the highest reduction of galls numbers compared with the other tested bioagent candidates. There was no significant difference between mean numbers of root galls resulted on plants treated with *B. subtilis* and such resulted on plants treated with vydate. *P. penetrans* ranked on the second place resulting in 64.3 % reduction, whereas *P. lilacinus* resulted in lower reduction of galls numbers (56.6%), followed by, *T. harzianum* with 50.9% then *G. virnes* with 43.9%. The least effective bio-agents candidates were the three yeast isolates *S. cerevisiae*,*S. ludwigii* and *S. uvarum* which recorded 42.3, 38.5 and 36.2% respectively. The root galls reduction was affected greatly with the inoculum density of the bioagent. Decrease the inoculum density of *Bacillus subtilis* from (1:10) to (1:50) resulted in decrease the reduction efficiency from 74.6 to 64.8%. Similar reaction was found in the case of all the other bioagent candidates.

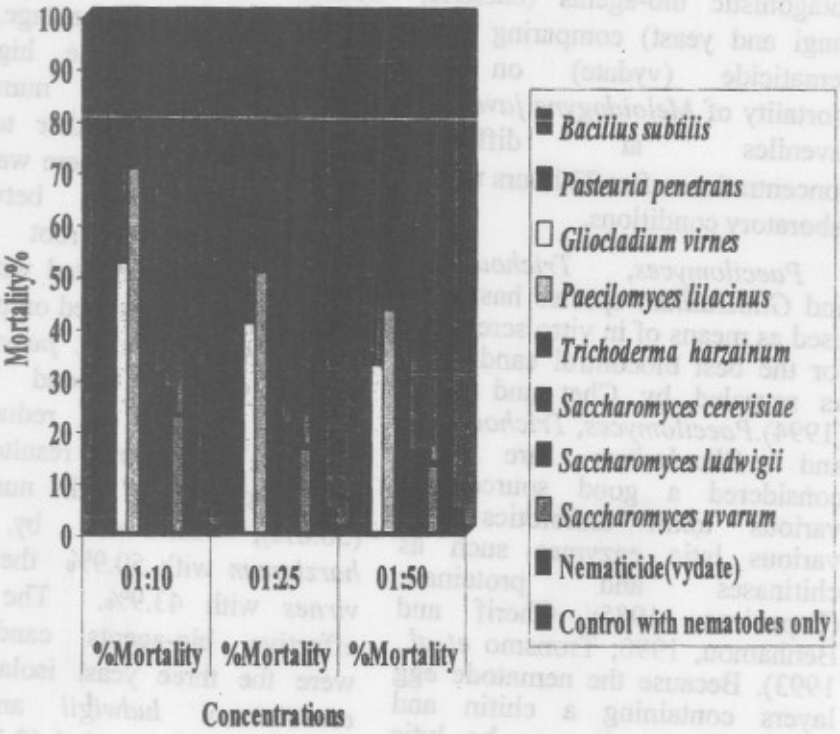

Fig. 2. Effect of different antagonistic bioagents (bacteria, fungi and yeast) comparing with nematicide (vydate) on % mortality of *Meloidogyne javanica* juveniles at different concentrations after 72 hours under laboratory conditions

Table 2: Effect of the different bioagent candidates and nematicide on the number of root galls, egg-masses and eggs per egg-mass 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 X 10^3	Red. %
Bacillus subtilis	1:10	36	74.6	25	78.3	3.5	81.3
	1:25	44	69	30	73.9	3.8	79.7
	1:50	50	64.8	37	67.8	4.2	77.5
	Mean	44	69.5	31	73.3	3.8	79.5
Pasteuria penetrans	1:10	42	70.4	35	69.6	3.9	79.1
	1:25	51	64.1	38	66.9	4.4	76.5
	1:50	59	58.5	42	63.5	5.2	72.2
	mean	51	64.3	38	66.7	4.5	75.9
Gliocladium virnes	1:10	70	50.7	55	52.2	6	67.9
	1:25	82	42.3	61	46.9	6.9	63.1
	1:50	87	38.7	72	37.4	7.3	60.1
	mean	80	43.9	63	45.5	6.7	63.7
Paecilomyces lilacinus	1:10	55	61.3	40	65.2	4.7	74.9
	1:25	62	56.3	48	58.3	5.3	71.1
	1:50	68	52.1	52	54.8	5.9	68.4
	mean	62	56.6	47	59.4	5.3	71.5
Trichoderma harzainum	1:10	61	57	49	57.4	5.5	70.6
	1:25	70	50.7	55	52.2	5.9	68.9
	1:50	78	45.1	61	46.9	6.5	65.2
	Mean	70	50.9	55	52.2	6.0	68.2
Saccharomyces cerevisiae	1:10	75	47.2	63	45.2	7.2	61.5
	1:25	83	41.6	66	42.6	7.8	58.3
	1:50	88	38	73	36.5	8.4	55.1
	Mean	82	42.3	68	41.4	7.8	58.3
Saccharomyces Ludwigii	1:10	79	44.4	63	45.2	7.9	57.8
	1:25	89	37.3	68	40.9	8.6	54.0
	1:50	94	33.8	75	34.8	9.2	50.1
	mean	88	38.5	69	40.3	8.6	54.0
Saccharomyces uvarum	1:10	82	42.3	70	39.1	8.3	55.6
	1:25	92	35.2	76	33.9	8.9	52.4
	1:50	98	31	82	28.7	9.7	48.1
	mean	91	36.2	76	33.9	9.0	52.0
Nematicide		32	77.5	22		3.3	82.4
Control(nematodes)		142		115		18.7	
L.S.D. A		1.52		1.27		1.14	
L.S.D. B		0.38		0.35		0.22	
L.S.D. AB		1.21		1.12		1.05	

A* Treatments B* Concentrations AB***Treatments &Concentrations

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 masse and egg numbers.

Data in figure (3) illustrated the effect of different antagonistic bio-agents (bacteria, fungi and yeast) compared with nematicide (vydate) on reducing numbers of *M. javanica* juveniles in soil (250 gm.) 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 *(B. subtilis, P. penetrans)* whereas the least effective were the isolates of yeast. *P. lilacinus T. harzianum* and *G. virnes* occupied an intermediate position.

d- Effect on the vegetative growth of the treated plants

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

1. Effect on shoot weights

All the treatments provoked the growth of the treated plants compared with the untreated and infected plants with *Meloidogyne incognita*. Increasing % in fresh weight of healthy plants reached to 61.1% compared with the infected plants. The maximum increasing % at (1:10 concentration) was recorded on the treated plants with the bio-agent *B. subtilis* and *P. penetrans* (with 55.4 and 51.4%) respectively, followed by *P. lilacinus* with 46.9%, nematicide (vydate) with 44.8% ,*T. harzianum* with 40.0%, then *G. virnes* with 34.3%. *S. cerevisiae, S. ludwigii* and *S. uvarum* showed the lowest shoot weight increasing %, which reached 17.7, 12.0 and 0.5% respectively.

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 Bioagents Cells and their Filtrates in the Biological Control Process

The effect of certain bio-agents and their filtrates on the *M. javanica* juveniles at different exposure times is demonstrated in Table (4). In the case of *B. subtilis*,

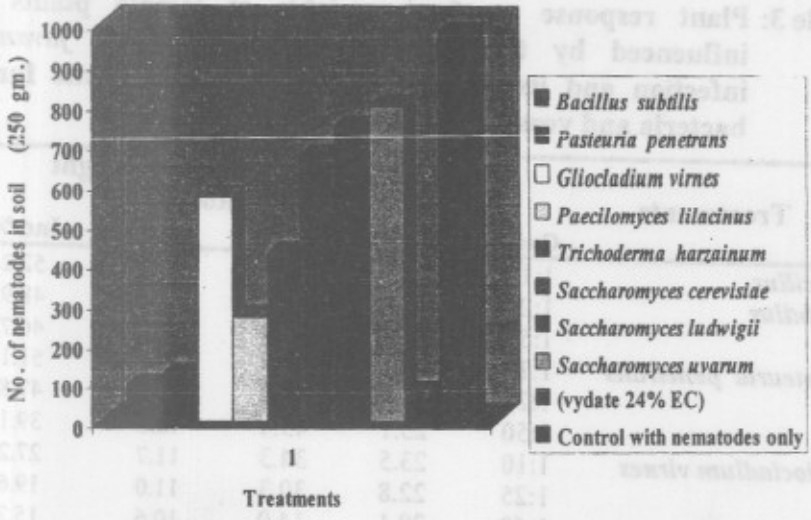

Fig. 3. Effect of different antagonistic bioagents (bacteria, fungi and yeast) compared with nematicide (vydate) on number of *Meloidogyne javanica* nematodes in soil at the highest concentration (1:10) under greenhouse conditions

Table 3: Plant response on fresh weights of tomato plants 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	1:10	27.2	55.4	14.0	52.2
subtilus	1:25	26.4	50.9	13.7	48.9
	1:50	25.7	46.9	13.5	46.7
Pasteuria penetrans	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	1:10	25.7	46.9	12.7	38.0
lilacinus	1:25	24.8	41.7	12.1	31.5
	1:50	24.2	38.3	11.8	28.3
Trichoderma	1:10	24.5	40.0	12.2	32.6
harzainum	1:25	23.8	36.0	11.7	27.2
	1:50	22.7	29.7	11.5	25.0
Saccharomyces	1:10	20.6	17.7	10.9	18.5
cerevisiae	1:25	19.9	13.7	10.5	14.1
	1:50	18.5	5.7	10.1	9.8
Saccharomyces	1:10	19.6	12.0	10.4	13.0
Ludwigii	1:25	18.3	4.6	9.9	7.6
	1:50	17.6	0.6	9.4	2.2
	1:10	18.3	0.5	9.8	6.5
Saccharomyces	1:25	18.0	0.3	9.5	3.3
uvarum	1:50	17.7	0.1	9.3	1.1
Nematicide (vydate24% 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. A		0.15		0.14	
L.S.D. B		0.07		0.06	
L.S.D. AB		0.22		0.20	

A* Treatments B* Concentrations AB*** Treatments &Concentrations

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

Treatments		Conc.	Filtrates % Mortality of *M. javanica* after			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
	Pasteuria penetrans	1:10	35.5	38.3	41.2	74.2	76.7	79.9
		1:25	30.2	33.4	36.4	68.5	70.4	74.2
		1:50	24.3	26.8	29.1	55.1	58.4	63.4
	Mean		30.0	32.8	35.6	65.9	68.5	72.5
Fungi	*Gliocladium virnes*	1:10	50.6	53.6	57.4	40.3	44.9	49.6
		1:25	44.2	47.5	50.1	35.6	37.8	40.3
		1:50	35.3	37.6	40.8	30.4	34.3	37.2
	Mean		43.3	46.2	49.4	35.4	39.0	42.4
	Paecilomyces lilacinus	1:10	58.3	62.2	68.3	70.5	73.6	76.8
		1:25	50.1	54.3	60.4	62.3	65.4	69.2
		1:50	41.5	46.1	52.3	50.4	53.7	57.1
	Mean		49.9	54.2	60.3	61.1	64.2	67.7
	Trichoderma harzianum	1:10	55.7	59.4	62.2	58.2	62.3	66.7
		1:25	48.2	51.2	52.4	51.4	54.8	59.2
		1:50	39.8	42.8	45.1	42.6	45.1	48.6
	Mean		47.9	51.1	53.2	50.7	54.1	58.2
Yeast	*Saccharomyces cerevisiae*	1:10	50.8	54.3	57.3	80.3	82.4	86.3
		1:25	45.3	48.4	52.5	75.2	77.9	80.1
		1:50	39.8	42.8	47.2	65.4	69.5	73.4
	Mean		45.3	48.5	52.3	73.6	76.6	79.9
	Saccharomyces Ludwigii	1:10	44.7	49.3	53.7	76.7	79.2	80.6
		1:25	36.5	38.9	43.4	69.3	72.3	75.2
		1:50	29.4	33.7	37.6	65.1	68.6	71.6
	Mean		36.8	40.6	44.9	70.4	73.4	75.8
	Saccharomyces uvarum	1:10	37.9	40.1	44.7	73.5	75.1	78.8
		1:25	30.2	36.7	39.8	65.4	69.4	71.2
		1:50	23.7	27.3	32.3	59.2	63.7	67.2
	Mean		30.6	34.7	38.9	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. A*			0.79	0.96	0.53	0.65	0.84	0.93
L.S.D. B**			0.47	0.40	0.35	0.30	0.23	0.30
L.S.D. AB***			1.49	1.29	1.12	0.96	0.75	0.97

A* Treatments B* Concentrations AB*** Treatments& Concentrations

the filtrate resulted in 70.7% juveniles mortality compared with 31.4% only in the case of the cells of the same organism after 72 hr. of exposure. Contrary data were obtained in the case of *P. penetrans* since; the filtrate resulted in 35.6% mortality compared with 72.5% in the case of the cells of the same organism after 72 hr. of exposure. However, the filtrate of the three tested fungi, *G. virnes, P. lilacinus* and *T. harzianum,* showed juveniles mortality ranged from 60.3 to 49.4% compared with juveniles mortality ranged from 67.7 to 42.4% in the case of the cells after three tested fungi. 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 bio-agents, *B. subtilis* and *P. penetrans,* 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 bio-agents candidates proved to be harmful to *M. javanica* juveniles, egg masses and eggs numbers however this effect differed from one candidate to another. Similar data were recorded too under greenhouse conditions. *B. subtilis* 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, Rangaswamy *et al., 2001 and* Ramesh and Chand ,2003. 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 bio-agents 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. Juveniles mortality ranged from 67.7 to 42.4%in the case of the cells of the three tested fungi. The cells of the tested *Saccharomyces* spp. were more harmful to the nematode juveniles than filtrates

REFERENCES

Abd El-Moity, T.H., F.W. Raid and S. El-Eraki. 1985. Biological control of root-Knot nematode, *Meloidogyne javanica* of tomato. The first conf. of Pests and Dis. of Veg& field crop in Egypt. Ismailia Oct. 1985, 1013 - 1020.

Abd El-Moity, T.H., M.H. Ali, El-Hamawi, T. El-sharkawy and K. Tiilikkala. 1998. Effect of some biological agents on reproduction of *Meloidogyne incognita* on tomato plants Egyptian- Journal- of Agricultural Research. 1998, 76 : 1, 51-62; 28 ref.

Ali, E.M. 1994. Advanced studies to control plant parasitic nematodes by non-chemical methodes. Ph. D Thesis, Fac. Agric., Cairo Univ.

Ali, H.H. and M.I.E. Barkat. 1991. Biocontrol and interaction between soil- borne fungi and root-Knot nematode associated with cantaloupe plants. Bull. Fac. Agric.Univ. of Cairo, Vol. 42: 279-292.

Boone, D.R., R.W. Castenholz and G.M. Garrity. 2001. Bergey's manual of systemic bacteriology. 2nd ed. Springer pp 721 vol. (1).

Bissett, J. 1991. A revision of the Genus *Trichoderma*. Infragenetic classification. Can. J. Bot. 69: 2357 - 2372.

Cherif,M. and N. Benhamou.1990. Cytochemical aspects of chitin breakdown during the parasitic action of a *Trichoderma* sp. on *Fusarium oxysporum* f.sp. radicislycopersici. Phytopathology, 80 : 1406- 1414.

Chet, I. and J. Inbar.1994. Biological control of fungal pathogens. Appl. Biochem. Biotechnnol. 48:37-43.

Cook, J.R. and K.F. Baker.1989. The nature and practice of biological control of plant pathology. APS press St. Paul, Minnesota 539 pp.

Domsch, K.H., W. Gams and T.H. Anderson.1980. Compendium of soil fungi. Academic Press, London, 859 pp.

Dubey, P.S. and L.P. Mall.1972. Herbicidal pollutive, pollen damage by herbicide vapours. Sc. Cult., 39: 556-558

Epstein, S.S., J. Andreae, H. Jaffec, S. Joshu, H. Folk and N. Mantel. 1967. Carcinogenicity of the herbicide meleic hydrazide. Nature, 215: 1388-1390.

Fawcett, C.H. and D.M. Spencer. 1970. Plant chemotherapy

with natural products. Rev. Phytopath., 8: 403 - 418.

Franklin, M.T. and J.B. Goodey. 1957. A cotton - blue lactophenol technique for mounting plant parasitic nematodes. J. Helminthological abstracts, 23 : 175-178.

Frederik, L., G. Borgonie, G. Amaut and D.De-Waele. 1995. Nematoicidal activity of *Bacillus thuringiensis* isolates. Fundam Appt. Nematol., 18 (3) : 211-218.

Hanna, A.I., T.H. Abd El-Moity and F.W. Raid. 1995. Evaluation of some biocontrol agents in controlling root- knot nematodes and its effects in useful microorganism (*Rhizobium leguminosarum Pv. Vicia).* Egypt J. Appl. Sci., (10) No. (3).

Hanna, A.I., F.W. Riad and A.E. Tawfik.1999. Efficacy of antagonistic rhizobacteria on the control of root-knot nematode, *Meloidogyne incognita* in tomato plants. Egyptian-Journal of Agricultural Research. 1999, 77: 4, 1467-1476; 26 ref .

Holt, J.G., N.R. Krieg, P.H.A. Sneath, Staley and S. T. Williams. 1994. Bergey's manual of determinative bacteriology 9th Ed. Baltimore, Mary Land 21202, USA., 787 pp.

Javoraska, T. 1978. Effect of combined herbicides on the occurrence of morphoses in the spikes of spring barley. Agrochemia, 18: 37-42.

Jonathan, E.I., K.R. Barker, F.F. Abdel Alim, T.C. Vrain and D.W. Dickson. 2000. Biological control of *Meloidogyne incognita* on tomato and banana with rhizobacteria, actinomycetes, and *Pasteuria penetrans.* Nematropica. 2000, 30: 2, 231-240; 16 ref .

Miller, P.M.1976. Supperssion of *Pratylenchus penetrans* by *Tylenchorynchus dubius* by *Trichoderma viride* J. Nematol. 9:182-183.

Mankau, R. 1980. Biological control of nematode pests by natural enemies.Annu Rev. Phytopathol, 18: 415-440.

Oostendorp, M., D.W. Dickson, and D.J. Mitchell. 1991. Host range and ecology of isolates of Pasteuria spp. from the southeastern United States. Journal of Nematology 22, 524-531.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: Biology, Ecology and Potential

for biocontrol. Annu. Rev. Phytopatol, 23:23-54

Papavizas, G.C. and C.B. Davey. 1959. Evaluation of various media and antimicrobial agents for isolation of soil fungi. Soil Sci. 88: 112 – 117.

Ramesh, Chand, Gill-J.S. and R. Chand. 2003. Effect of *Pasteuria penetrans* on the life cycle of root-knot nematode, Meloidogyne incognita on tomato crop .Indian-Journal of Nematology. 2003, 33: 2, 129-131; 11 ref.

Rangaswamy, S.D., P.P. Reddy, D.N. Gowda and M. Nagesh. 2001. Influence of *Pasteuria penetrans* Sayre and Starr on the life cycle of *Meloidogyne incognita* (Kofoid and White) Chitwood infecting tomato. Pest Management in Horticultural Ecosystems. 2001, 7: 2, 168-170; 5 ref.

Rifai, M.A. 1969. A revision of the genus *Trichoderma* Mycol. Papers. CMI Association of Applied. Biologists: Kew Surrey, UK, 116: 1-56.

Rodriguez, Kabana, R., G. Morgan-Jones, G. Godoy and B.O. Gintis. 1984. Effectiveness of species of *Gliocladium, Paecilomyces* and *Verticillium* for control of *Meloidogyne arenaria* in filed soil. Nematropica 14:155.

Sankaranarayanan, C., S.S. Hussaini, P.S. Kumar and R. Rangeshwaran. 2000. Granular application of antagonistic fungi for the biological control of *Meloidogyne incognita* on tomato. Indian Journal of Nematology. 2000, 30: 2, 157-161; 8 ref.

Sayre, R.M.1980. Biocontrol *Bacillus penetrans* and related parasites of nematodes.J. Nematol., 12:260-270.

Sendecor,G.W. and W.G. Cochran. 1980. Statistical methods. Oxford&J.BH Publishing com. 7 th. edition

Shahzad, S., S. Ehteshamul- Haque and A. Ghaffar.1990. Efficacy of *Pasteuria penetrans* and *Paecilamyces lilacinus* in the biology control of *Meloidogyne javanica* on mung bean. Int. Nematol. Network, 7(3):34-35.

Sharvelle, E.G. 1979. Plant disease Control. AVI Publishing Company, I. N. C. Westport. 331 pp.

Sterling, G.R.1984. Biological control of *Meloidogyne javanica* with *Bacillus*

penetrans. Phytropathology, 74: 55-60.

Tronsmo, A., S.S. Klemsdal; C. K. Hayes, M. Lorito and G.F. Hannan. 1993. The role of hydrolytic enzymes produced by *Trichoderma harzianum* in biological control of plant diseases. *Trichoderma reesei* cellulases and other hydrolases enzyme structures, Biochemistry, Genetics and Applications (P. Suominen and T. Reinikainen, eds.) Foundation for Biotechnical and Industrial Fermentation Research, Helsinki, Finland, vol. 8, PP. 159-168.

Wollum, A.G. 1982. Cultural methods for soil microorganisms. Pages 751–802 in : Methods of soil Analysis, Part 2, Chemical and Microbiological Processes. American Society of Agronomy and Soil Science. Madison, WI.

تاثير بعض الكائنات الحيوية على مكافحة نيماتودا ميلودوجين جافنيكا على نباتات الطماطم المصابة بمرض تعقد الجذور تحت ظروف المعمل و الصوبة

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تم اختبار ثمانية عوامل حيوية وهى: الفطر باسيلوميسس ليلينس والفطر تريكودرما هارزيانم و الفطر جلوكلاديم فيرنس و نوعين من البكتريا باسيلس سانتلس وبكتريا باستيوريا بنيترنس و ثلاثة عزلات من الخمائر(سكارومايسيس) معزولة من البيئة المصرية واستخدمت 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 سانتلس) بينما كانت عزلات الخمائر (سكارومايسيس) اقلهم تاثيرا.

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

كان استخدام العزلات البكتيرية تاثيرا فعالا فى خفض اعداد العقد النيماتودية وكتل البيض وعدد البيض على النبات الواحد فى الجذور وكذلك 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 خاصة التركيز العالى (١٠:١).

اظهر استخدام الكائنات الحيوية على الصورتين (الراشح- الخلايا بعد ترشيحها) تاثير فعالا فى زيادة النسبة المئوية للموت لليرقات خاصة بعد ٧٢ ساعة تحت ظروف المعمل. اظهر استخدام العزلة البكتيرية(باسيلس سانتلس) اكبر تاثيرا بينما اظهرت عزلات الخمائر اقل تاثيرا على صورة الراشح. اظهر استخدام العزلة البكتيرية(باستيوريا بنيترنس) اكبر تاثيرا بينما اظهرت العزلة البكتيرية(باسيلس سانتلس) اقل تاثيرا على صورة الخلايا.