

## Evaluation of Certain organic Manures as Carrier Substrates for Mass Production of some Nematophagous Fungi and Related Effects on *Meloidogyne incognita* Infecting Sugar beet Plants 1 – Animal manures

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### ABSTRACT

In a laboratory test, the fungi; *Aspergillus niger*, *Fusarium oxysporium*, *Penicillium nigricans*, *Trichoderma harzianum*, *T. viride* and *Verticillium chlamydosporium* were isolated, from eggmasses of *Meloidogyne* spp. root-knot nematodes or/and rhizosphere of sugarbeet roots. Potential growth of such fungi on some animal manures acted as carrier substrates for mass production of these nematophagous fungi was evaluated in comparison to wheat grains as a standard medium. Total nitrogen, carbon, potassium and phosphorus contents as well as pH values of all tested carrier substrates were determined. The results proved that broiler chicken, cow and layer chicken manures acted as good carrier substrate alternatives to wheat grain medium for mass production of the nematophagous fungi; *F. oxysporium*, *T. harzianum*, *T. viride* and *V. chlamydosporium*. However, broiler and layer chicken manures are only suitable for mass production of *P. nigricans*. In a greenhouse test, when broiler chicken, cow and layer chicken manures used as carrier substrates, the three tested nematophagous fungi; *F. oxysporium*, *T. viride* and *V. chlamydosporium* reduced the gall numbers of *M. incognita* per sugarbeet root. Moreover, percentages of the colonized nematode eggs by such fungi propagated on the tested substrates were more than those on wheat grains. Only *F. oxysporium* significantly ( $P>0.05$ ) reduced the gall numbers and increased colonized eggs percentages per sugar beet root, when introduced on broiler or layer chicken manures. However, *T. viride* and *V. chlamydosporium* caused significant decrease in root galling, and significant increase in colonized eggs percentage of *M. incognita* when added with broiler chicken, cow and layer chicken manures compared to their influence on nematode when added on wheat grain medium. By using layer chicken manure as the best carrier substrate, the three tested nematophagous fungi were very effective for controlling root-knot-nematode, *M. incognita* on sugarbeet plants as compared with the other two carrier substrates; broiler chicken and cow manures.

**Key Words:** Organic manures, Carrier substrates, Mass production, Propagation, Nematophagous, Fungi, Sugar beet.

### INTRODUCTION

Sugar beet (*Beta vulgaris* L.), is an important cash crop and mostly a cultivable crop in newly reclaimed soils. Sugarbeet is affected at various stages of growth, by the root-knot nematodes, *Meloidogyne* spp. (Ibrahim, 1982, Oteifa and El-Gindi, 1982 and Maareg *et al.* 1988, 1998 & 2005). Chemical control is usually used to eliminate such nematodes. However, it is very expensive and has hazardous effects on man and environment. Many organic amendments of plant or animal origin have been reported to possess nematicidal properties (Ahmad, 1977; Alam, 1987; Mian and Rodriguez-Kabana, 1982; Rodriguez-Kabana *et al.* 1987; and Ahmad and Karim, 1990; Amin and El-Shafeey, 1998, and Maareg *et al.*, 1999a & b). Also, Microbial bioagents, including nematophagous fungi, have, successfully been used to minimize nematode damage in plants. Grains of wheat (Jatala, 1981), rice (Shahzad and Ghaffor, 1989), Oat (Kerry *et al.*, 1984) alginate pellets (Cabanillas *et al.*, 1989) and other media have been used to propagate such fungi additions to soil. Usage of cereal grains for mass production of fungi for nematode control becomes impractical due to increased demand of cereals for human consumption. Therefore, search for other substrates for mass production of fungi is very insistent. Animal manures, for instance, are sheep, universally available substrates and commonly used in agriculture as fertilizers. The type and composition of the manure are important factors in hastening the development of fungi which are inimical to the nematode. Comparative experiments conducted by Oteifa *et al.* (1964) on different organic manures established that pigeon droppings promoted better fungoid growth. Also, they mentioned that manuring with goat dung promoted a rapid growth of the fungus *Arthrobotrys oligospora* Fres., horse dung with *Dactylaria thaumasia* Dr., facial matter with *Arthrobotrys conoids* Dr., and *Dactylaria brachopaga* Dr., grew well in compost. In addition, Maareg *et al.* (2008) reported that addition of organic soil amendments; chicken manure, pigeon – dung and poudrette best, cattle manure and rice straw to naturally infest sandy soil by *M. javanica* gave pronounced nematode suppression and improved sugar beet yields and quality.

The aim of this work was to evaluate some animal manures *e.g.*, broiler chicken, cow layer chicken and sheep manures and their mixture (compost) as alternative carrier substrates for fungal propagation and for

mass production of nematode antagonistic fungi, conserving grains of wheat, corn or sorghum for human and animal feeding.

## MATERIALS AND METHODS

### Tested fungi:

In a laboratory, the fungi, *Aspergillus niger*, *Fusarium oxysporium*, *Penicillium nigricans*, *Trichoderma harzianum*, *T. viride*, and *Verticillium chlamydosporium*, were isolated from egg-masses of *Meloidogyne* spp., root-knot nematodes or/and rhizosphere of sugarbeet roots. Growth and mass production of such fungi, as nematode antagonistic agents, on some animal manure were evaluated.

### Preparation of animal manures as carrier substrates:

Fresh broiler chicken, cow and layer chicken, and sheep manures and their fermented mixture (compost) were used as carrier substrates for fungal propagation. Wheat grains were used as a comparable standard medium. The animal manures (carrier substrates) were air dried each at 30° C, ground, thoroughly mixed and moistened with tap water at the ratio of 1:1 (w/w), then autoclaved at 121°C for 20 minutes. The wheat grains medium was prepared as described by Jatala (1981) and Crump and Irving (1992).

The total nitrogen, carbon, potassium and phosphorus contents of each substrate (Table 1) were determined using Kjeldahl method (Bremner, 1965), Mebius method (Nelson and Sommers, 1982), the ignition method and the flame photometer method (Page *et al.*, 1985), respectively. The pH values were also determined.

### Laboratory test:

For inoculation stock, spore suspensions of each fungus were prepared from a seven day old culture. One ml of the suspension was used to inoculate each carrier substrate in 250 ml flask. Three replicates were used for each fungus and substrate. The flasks were incubated at 25°C for 2 weeks, with daily shaking and then examined for fungal growth. A fine point fungal growth index was used to evaluate the suitability of substrates for each fungus (Villanueva and Davide, 1984). The number of spores / g that colonized substrate of each tested fungus was estimated by suspending 1 g of colonized substrate in 99 ml sterile distilled water followed by further dilutions to allow direct counting using a haemocytometer slide.

### Greenhouse test:

The fungi, *F. oxysporium*, *T. viride* and *V. chlamydosporium* were selected to be tested against *M. incognita* in a greenhouse using the most promising animal manures, viz., broiler chicken, cow and layer chicken manures as substrates, plus wheat grains as a standard substrate. They were all prepared using 500 ml flasks filled with 200 g moist carrier substrate. Flasks were autoclaved, inoculated with 1 cm agar disc from colonies of the respective fungus and incubated at 25°C for 2 weeks. The colonized carrier substrates of each fungus were used to inoculate pots at the rate of 10 g for each pot containing 1.5 kg sterilized sandy soil. Similar amounts of each fungus-free substrate were used to inoculate pots of the respective check treatment. In addition, some pots were left as untreated treatment (without fungi or substrates). Each pot was then planted with a 2 weeks old sugar beet seedling (cv. Chems). One week after transplanting, the pots were inoculated with 1500 eggs per plot of *M. incognita* extracted by Na O Cl (Ehwaeti *et al.*, 1998). Each treatment was replicated three times and all pots were kept in a greenhouse in a completely randomized block design receiving fertilizers and irrigation as needed. The experiment was terminated 6 weeks after nematode inoculation. Galls number per root system was counted and galling index was estimated.

After cutting up the roots and mixing the segments, eggs and newly hatched second stage juveniles were extracted from 5 g sample (Coolen and D'Herde, 1973). Because immature eggs are more susceptible to fungal infection than mature eggs (eggs contain developed juveniles) and second stage juveniles (Irving and Kerry, 1986), it was assumed that eggs and juveniles had, therefore, estimated according to De Leij *et al.* (1993) by comparing the numbers of mature eggs and newly hatched second stage juveniles in the untreated plants with those extracted from treated plants. To confirm egg-infection by each fungus, ten egg-masses were randomly picked from each root system of treated and untreated ones. Each egg-mass was examined at 200 X magnification for counting infected eggs and fungi spores.

The results were therefore transformed according to the following formula (Meynell and Meynell, 1970):

$$P = 1 - e^{-m}$$

$P$  = represents the observed

$m$  = the most probable number of infected nematodes.

$E$  = the base of the natural logarithms to the power of  $-m$

#### Statistical analysis of Data:

Obtained data were subjected to statistical analysis using MSTAT version 4 (1987). Significant differences among means of different treatments were carried out by Student-Newman-Keuls Test (0.05 probabilities).

## RESULTS AND DISCUSSION

The total nitrogen, carbon, potassium and phosphorus contents as well as pH values of wheat grains (standard medium) and the carrier substrates of broiler chicken, cow, layer chicken, sheep, and compost manures were determined as in Table (1). The highest value of total nitrogen content was recorded by both broiler and layer chicken manures (3.97 and 3.95 %, respectively) followed by cow manure (2.49%) and wheat grains (2.36 %); however, sheep and compost manures gave the lowest values (1.68 and 1.87%, respectively). Carbon content attained the highest percentage with wheat grains and sheep manure (46.6 and 42.6 %, respectively). Moderate total carbon values were estimated by broiler chicken (32.1%), layer chicken (32.1 %) and cow (30.2 %) manures, while the lowest value (15.2 %) was with compost manure. Also, the highest value of C/N ratio was found by sheep manure (22.8) and then with wheat grains (19.7), followed by cow (12.1) and compost (9.0) manures. The lowest value was recorded by both broiler and layer chicken manures (8.1). The maximum value of potassium content was estimated by sheep manure followed by compost, broiler chicken and layer chicken manures, and then cow manure and wheat grains. Whereas, phosphorus content was 1.71, 1.72, 4.19, 0.37, 1.27 and 0.36 % for broiler chicken, layer chicken, compost, cow and sheep manures as well as wheat grains, respectively. Data indicated also that pH values of broiler chicken, layer chicken and wheat grains were in limits of the acidity range (6.4 – 6.6), while, the pH values of compost, cow and sheep manures were in limits of the alkaline range (7.6 – 7.8).

#### Laboratory test:

##### Fungal growth on the substrates:

##### a- Mycelial growth:

Growth indices of six selected soil fungi, namely, *A. niger*, *F. oxysporium*, *P. nigricans*, *T. harzianum*, *T. viride* and *V. chlamydosporium* on different animal manures (broiler chicken, compost, cow, layer chicken and sheep manures) compared to wheat grains are listed in Table (2). The results indicated that the greatest growth index of *A. niger* (4.7) was observed on wheat grains, while the lowest one (1.7) was found on compost manure. Generally, growth of *A. niger* was abundant on wheat grains, but moderate on broiler chicken, compost, cow and layer chicken manures, while poor on sheep manure. Growth of *F. oxysporium* was abundant on broiler chicken (4.7), compost (4.0) and layer chicken (4.7) manures and wheat grains (4.0) with no significant differences among the four substrates. Slightly but significantly lower growth was observed on cow (3.7) and sheep (3.0) manures. Growth of *P. nigricans* on wheat grains (5.0) and broiler chicken manure (4.7) significantly surpassed other tested substrates. Moderate growth indices were observed on cow and layer chicken manures (3.3 and 3.7, respectively); while, poor growth indices were obtained by compost and sheep manures (2.7 and 2.3, respectively).

Growth of *T. harzianum* was abundant on wheat grains (4.3) and broiler chicken manure (4.0), followed by layer chicken (3.3) and cow manures (3.3). The compost (2.0) and sheep (2.3) manures were less suitable for growth of *T. harzianum*. Meanwhile, growth of *T. viride* on broiler chicken, cow, compost, layer chicken

Table (1): Mineral contents and pH values of six carrier substrates used for propagating some nematophagous fungi.

Evaluated substrates	Total N %	Total C %	C/N Ratio	Total K%	Total P%	pH
Broiler manure	3.97	32.1	8.1	1.44	1.71	6.6
Compost	1.68	15.2	9.0	1.45	4.19	7.8
Cow manure	2.49	30.2	12.1	0.81	0.37	7.6
Layer manure	3.95	32.1	8.1	1.44	1.72	6.4
Sheep manure	1.87	42.6	22.8	2.15	1.27	7.7
Wheat grains	2.36	46.6	19.7	0.36	0.36	6.4

and sheep manures was mostly greater than that observed on wheat grains. Growth of this fungus was abundant on broiler chicken, cow and layer chicken manures, but moderate on both compost and sheep manures. *V. chlamydosporium* had great growth on broiler chicken, cow and layer chicken manures and wheat grains, followed by sheep manure. Poor growth was observed on compost manure.

It could be concluded that growth of the three fungi, *F. oxysporium*, *T. viride* and *V. chlamydosporium* on broiler chicken, layer chicken, or cow manures was greater than, or similar to, that observed on wheat grains (Table 2).

#### b- Sporulation:

Number of spores produced by each of the six tested fungi per gram of different animal manures compared with that of wheat grains is presented in Table (3). Data indicated that sporulation of *A. niger* fungus was greater on wheat grains than on all tested animal manures (broiler chicken, cow, compost, layer chicken and sheep manures). Also, broiler chicken, cow and layer chicken manures were best alternative to wheat grains for spores' production of the fungi, *F. oxysporium*, *T. harzianum* and *V. chlamydosporium*. Sporulation of *T. viride* on broiler chicken, cow, layer chicken and sheep manures was greater than that observed on wheat grains. Sporulation was, relatively, poor on compost manure. *P. nigricans* produced the highest numbers of spores only on broiler and layer chicken manures compared with those on wheat grain medium and the other animal manures.

Spores production of the five fungi, *F. oxysporium*, *P. nigricans*, *T. harzianum*, *T. viride* and *V. chlamydosporium* on broiler chicken manure was 417.5, 158.2, 137.6, 130.3 and 148.5 %, respectively, whereas, it was 468.4, 127.2, 116.6, 142.4 and 119.3 % on layer chicken manure, respectively, higher than that on wheat grain medium. Sporulation of *F. oxysporium*, *T. harzianum*, *T. viride* and *V. chlamydosporium* was greater on cow manure than on wheat grain medium by 191.2, 107.1, 123.9 and 135.5 %, respectively.

It could be noticed that, spores production of these fungi on both chicken manures ranged between 116.6 to 468.4 % and on cow manure between 107.1 to 191.2 % higher than on wheat grain medium. Variability in species of the fungi involved indicates a general suitability of the tested carrier substrates for fungal production. Organic matter with a low C/N ratio resulted in a broad spectrum stimulation of the soil micro flora (Rodriguez-Kabana *et al.*, 1987). The above mentioned manures had lower C/N ratio than wheat grains (Table 1). On these carrier substrates, the fungi produced more spores than on wheat grains. These results are

Table (2): Mycelial growth indices\* of six nematophagous fungi on different animal manures used as carrier substrates compared to that on wheat grain medium.

Investigated fungi	Broiler chicken	Compost	Cow manure	Layer chicken	Sheep manure	Wheat grain	L.S.D. <sub>0.05</sub>
<i>A. niger</i>	3.7	1.7	3.0	3.3	2.0	4.7	0.86
<i>F. oxysporium</i>	4.7	4.0	3.7	4.7	3.0	4.0	1.37
<i>P. nigricans</i>	4.7	2.7	3.3	3.7	2.3	5.0	1.18
<i>T. harzianum</i>	4.0	2.0	3.3	3.3	2.3	4.3	1.37
<i>T. viride</i>	4.3	3.7	4.0	4.7	3.7	3.3	1.31
<i>V. chlamydosporium</i>	5.0	2.7	4.7	4.3	3.3	5.0	0.88
Mean	4.4	2.8	3.7	4.0	2.8	4.4	0.77
L.S.D. <sub>0.05</sub>	1.15	1.09	1.11	1.01	1.31	1.00	
* 1 = No growth		2 = poor growth		3 = moderate growth			
4 = abundant growth		5 = very abundant growth.					

Table (3): Spores number of six nematophagous fungi produced per gram of different animal manures used as carrier substrates compared to that on wheat grain medium.

Investigated fungi	Number of spores /g X10 <sup>7</sup>						L.S.D. <sub>0.05</sub>
	Broiler chicken	Compost	Cow manure	Layer chicken	Sheep manure	Wheat grain	
<i>A. niger</i>	25.5	14.8	24.3	21.4	11.5	42.7	6.55
<i>F. oxysporium</i>	23.8	5.3	10.9	26.7	4.7	5.7	4.38
<i>P. nigricans</i>	33.7	2.7	17.8	27.1	5.3	21.3	5.61
<i>T. harzianum</i>	76.1	16.2	59.2	64.5	49.7	55.3	22.91
<i>T. viride</i>	46.4	11.9	44.1	50.7	39.3	35.6	12.60
<i>V. chlamydosporium</i>	95.5	53.0	87.1	76.7	41.1	64.3	14.70

in agreement with those reported by Abu-Laban and Saleh (1992). They found that spores production of *F. oxysporium*, *F. solani* and *P. lilacinus* on both chicken manures was 22–290% higher than on wheat grains.

In short, the present investigation demonstrated that broiler chicken, layer chicken and cow manures were better alternatives to wheat grain medium for mass production of *F. oxysporium*, *T. harzianum*, *T. viride* and *V. chlamydosporium*; however, the broiler and layer chicken manures were only the best for mass production of the fungus *P. nigricans*.

#### Greenhouse test:

Results in Table (4) showed the efficacy of the three selected nematophagous fungi; *F. oxysporium*, *T. viride* and *V. chlamydosporium* propagated on broiler chicken, cow and layer chicken manures as well as wheat grains on gall numbers per root system and colonized eggs percentage of *M. incognita* infecting sugarbeet plants.

Interestingly, when all the tested carrier substrates were applied alone they did reduce number of galls per root system of sugarbeet plants as compared with that of untreated treatment (nematode inoculum alone). Comparatively, broiler chicken manure caused significant reduction in number of galls (26.1 %) followed by layer chicken (21.7 %), cow manures (11.6 %) and then wheat grains (6.1 %).

Table (4): Effect of the three nematophagous fungi; *Fusarium oxysporium*, *Trichoderma viride* and *Verticillium chlamydosporium*, propagated on different carrier substrates, on the root-knot nematode, *Meloidogyne incognita* infecting sugarbeet.

Treatment	Galls No. / root system		% Colonized eggs
	Average No.	Reduction %	
Nematode + broiler chicken manure inoculated with :			
<i>F. oxysporium</i>	45.0	60.9	2.3
<i>T. viride</i>	23.0	80.0	5.0
<i>V. chlamydosporium</i>	18.3	84.1	56.6
Check	85.0	26.1	--
Mean	42.8	62.8	11.33
Nematode + cow manure inoculated with :			
<i>F. oxysporium</i>	55.7	51.6	2.0
<i>T. viride</i>	26.7	76.8	4.3
<i>V. chlamydosporium</i>	30.3	73.7	35.3
Check	101.7	11.6	--
Mean	53.6	53.4	13.9
Nematode + layer chicken manure inoculated with :			
<i>F. oxysporium</i>	22.3	80.6	4.7
<i>T. viride</i>	10.0	91.3	11.5
<i>V. chlamydosporium</i>	5.7	95.0	73.3
Check	90.0	21.7	--
Mean	32.0	72.2	29.8
Nematode + wheat grain medium inoculated with :			
<i>F. oxysporium</i>	62.7	45.5	2.0
<i>T. viride</i>	36.7	68.1	3.3
<i>V. chlamydosporium</i>	40.3	65.0	26.6
Check	108.0	6.1	--
Mean	61.9	46.2	10.6
Untreated	115	--	--
L.S.D. 0.05	4.2	--	1.09

L.S.D. 0.05 between fungi species for gall number / root  
Carrier I Carrier II Carrier III Carrier IV

4.9 10.1 15.3 13.5

L.S.D. 0.05 fungus X substrate for gall number / root

*F. oxysporium* 7  
*T. viride* 3.78  
*V. chlamydosporium* 3.71

L.S.D. 0.05 between fungi species for % colonized eggs

Carrier I Carrier II Carrier III Carrier IV

2.28 1.48 1.10 1.22

L.S.D. 0.05 fungus X substrate for % colonized eggs

*F. oxysporium* 0.14  
*T. viride* 0.32  
*V. chlamydosporium* 2.63

On broiler chicken, cow or layer chicken manures, the three tested nematophagous fungi reduced the number of galls more than on wheat grains. *F. oxysporium* only significantly ( $P \leq 0.05$ ) reduced the number of galls when introduced on broiler or layer chicken manures. However, *T. viride* and *V. chlamydosporium* significantly ( $P \leq 0.05$ ) reduced gall numbers when propagated on cow, broiler chicken or layer chicken manures, more than they did when propagated on wheat grain medium.

By using layer chicken manure as carrier substrate, the three tested nematophagous fungi were very effective in reducing root galling on sugarbeet plants as compared with the other two carrier substrates (broiler chicken and cow manures).

*F. oxysporium* was less effective against the nematode when propagated on cow manure or wheat grains, while, *V. chlamydosporium* was the most effective when propagated on layer chicken manure medium.

In short, on layer chicken manure, as the best carrier substrate, *F. oxysporium*, *T. viride* and *V. chlamydosporium* reduced galling by 80.6, 91.3 and 95.0 %, respectively in relative to the untreated treatment. Also, % reduction in galls of treatments of the mentioned tested nematophagous fungi propagated on both broiler chicken and layer chicken manures were (134.1 – 177.1 %) and (117.5 – 133.8 %), respectively, higher than when they were introduced with wheat grains.

Concerning the infestation of eggs, the results in Table (4) reveal that the colonization of eggs by *F. oxysporium*, *T. viride* and *V. chlamydosporium* when added on broiler chicken, cow and layer chicken manures was either greater than or similar to that observed when added on wheat grains. On the other hand, the colonization of eggs by the inoculation of fungi was greater when these were introduced on layer chicken manure than on the other two carrier substrates (cow and broiler chicken manures). By using layer chicken manure as a carrier substrate, *V. chlamydosporium* gave the highest colonized eggs percentage (73.3%) followed by *T. viride* (11.5%), while *F. oxysporium* gave the lowest percentage (4.7%) in this respect. The same trend was observed when these fungi were added on either broiler chicken, cow manures or wheat grains. On all tested carrier substrates, *V. chlamydosporium* significantly gave the highest values of % colonized eggs (56.6, 35.5, 73.3 and 26.6 %) when added on broiler chicken, cow, layer chicken and wheat grains substrates, respectively. While, *F. oxysporium* gave lower percentages in this respect. Generally, the highest percentages of colonized egg masses were obtained by *V. chlamydosporium* fungus when introduced on layer and broiler chicken manures (73.3 and 56.6%, respectively). However, the lowest percentage was obtained by *F. oxysporium* when added on cow manure or on wheat grain medium (2.0% of both).

From the previous results, it could be noticed that the three mentioned nematophagous fungi were very effective for controlling *M. incognita* when propagated on layer chicken manure more than their effects when propagated on wheat grains as carrier substrates.

## REFERENCES

- Abu-Laban, A.Z. and H.M. Saleh 1992. Evaluation of animal manures for mass production, storage and application of some nematode egg parasitic fungi. *Nematologica* 38: 237-244.
- Ahmad, M. U. 1977. A review of plant parasitic nematodes in Bangladesh ; A paper presented in a seminar in the Imperial Coll. Sci. and Tech., Univ. London, U. K., pp :3.
- Ahmad, M. U. and M. R. Karim. 1990. Effect of extract of indigenous plants on galling and growth of brinjal infected with *M. javanica*. *Int. Nematol. Network Newsl.*, 7: 5 – 7.
- Alam, M. M. 1987. Pollution free control of plant parasitic nematodes by soil amendments with plant wastes. *Biol. Wastes*, 22: 75 – 79.
- Amin, A. W. and E. I. El Shateey 1998. Effect of certain soil organic amendments and nematicides on citrus nematode, *Tylenchulus semipentrans* infecting orange trees. *Egypt. J. Agronematol.*, 2 (2): 229 – 244.
- Bremner, J. N. 1965. Total nitrogen: In *Methods of soil analysis. Part II. Chemical and microbiological properties*. Ed. Black. American society of Agronomy. Madison, Wisconsin. pp 1149-1176.
- Cabanillas, E.; K.R. Barker and L. A. Nelson 1989. Survival of *Paecilomyces lilacinus* in selected carriers and related effects on *Meloidogyne incognita* on tomato. *Journal of Nematology*, 21: 121-130.
- Coolen, W. A. and C. L. D'Herde 1973. A method for quantitative extraction of nematodes from plant tissue. Ghent State Agriculture Center. 77pp.
- Crump, D. H. and F. Irving 1992. Selection of isolates and methods of culturing *Verticillium chlamydosporium* and its efficacy as a biological control agent of beet and potato cyst nematodes.

- Nematologica, 38:367-364.
- De Leij, F. A. A. M.; B. R. Kerry and J. A. Dennehy 1993. *Verticillium chlamydosporium* as biological control agent for *Meloidogyne incognita* and *M. hapla* in pot and microplot tests. Nematologica, 39: 115-126.
- Ehwaeti, M. E.; M. S. Phillips and D. L. Trudgill. 1998. The viability of *Meloidogyne incognita* eggs released from egg-masses of different ages using different concentrations of sodium hypochlorite. Nematologica, 44: 207-217.
- Ibrahim, I. K. A. 1982. Species and races of root-knot nematodes and their relationships to economic host plants in Northern Egypt. In: Proceedings of the Third Research and Planning Conference on Root-Knot Nematode, *Meloidogyne* spp. September 13-17, Coimbra, Portugal, pp. 66-84.
- Irving, F. and B. R. Kerry 1986. Variation between strains of nematophagous *Verticillium chlamydosporium* Goddard. II Factors affecting parasitism of cyst nematode eggs. Nematologica, 32: 474-485.
- Jatala, P. 1981. Biological control of *Meloidogyne* species: Methodology for preparation and establishment of *Paecilomyces lilacinus* for field inoculation. International *Meloidogyne* Project. Proceedings of the 3<sup>rd</sup> Research Planning Conference on Root-knot Nematodes, *Meloidogyne* spp. Jakarta. Region VI: pp. 228-231.
- Kerry, B. R.; A. Simon and A. D. Rovira 1984. Observation on the introduction of *Verticillium chlamydosporium* and other parasitic fungi into soil for control of cereal cyst nematode, *Heterodera avenae*. Ann. Appl. Biol., 105: 509-516.
- Maareg, M. F.; M. H. El-Deeb and A. M. Ebieda 1988. Susceptibility of ten sugar beet cultivars to root-knot nematode, *Meloidogyne* spp.. Alex. Sci. Exch., 9(3): 293-302.
- Maareg, M. F.; M. A. Hassanein.; A. I. Allam and B. A. Oteifa 1998. Susceptibility of twenty six sugarbeet varieties to the root-knot nematodes *Meloidogyne* spp in the newly reclaimed sandy soil of Al-Bostan region. Egypt. J. Agronematol., 2 (1): 111-125.
- Maareg, M.F.; I.M.A. Goher and A. Abd Elaal 2005. Susceptibility of twenty one sugar beet varieties to the root-knot nematode *Meloidogyne incognita* at west Nubariya district. Egypt. J. Agric. Res., 83 (2): 789- 802 .
- Maareg, M. F.; Sohir T. Badr and A. I. Allam 1999a. Controlling of root-knot nematode, *Meloidogyne incognita* using organic soil amendments, nematicides and their mixtures in sugar beet. Egypt. J. Agronematol., 3 (1/2): 75-94.
- Maareg, M. F.; Sohir T. Badr and B. A. Oteifa 1999b. Effect of two waste organic composts, fenamifos and ammonium nitrate on controlling *Meloidogyne javanica* and productivity of sugarbeet. Egypt. J. Agronematol., 3 (1/2): 95-111.
- Maareg, M. F.; I. M. A. Gohar and Sahar F. Tawfik. 2008. Effect of certain organic soil amendments on sugarbeet (*Beta vulgaris* L.) infested with root- knot nematode, *Meloidogyne javanica* under field conditions. 2<sup>nd</sup> Arab Conference for Applied Biological Pest Control Cairo, Egypt 6-10 April 2008 (in press)
- Meynell, G. G. and E. Meynell. 1970. Theory and practice in experimental bacteriology. Cambridge Univ. Press., England, 347 pp.
- Mian, I. H. and R. Rodriguez-Kabana 1982. A survey of the nematicidal properties of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria*. Nematologica, 12: 154-157.
- Nelson, D. w. and L. E. Sommers 1982. Total carbon, organic matter and organic carbon. Methods of soil analysis, Part II, Chemical and microbiological properties. Ed. A. L. Page; R. H. Miller and D. R. Keeny. American Society of Agronomy & American Society of soil Science: Madison, Wisconsin, pp. 539-579.
- Oteifa, B. A. and D. M. El-Gindi 1982. Relative susceptibility of certain commercially important cultivars to existing biotypes of *Meloidogyne incognita* and *M. javanica* in Nile-Delta. Egypt. In: Proceeding of the 3<sup>rd</sup> Research and Planning. Conf. of Root-knot Nematodes, *Meloidogyne* spp.. 13-17 September, 1982. Coimbra, Portugal. Pp. 66-84.
- Oteifa, B. A.; D. M. El-Gindi and H. Z. Aboul-Eid 1964. Egyptian organic manures favor natural enemies of nematodes. Plant Dis. Repr., 48: 894.
- Page, A. L.; D. R. Keeny and R. H. Miller 1985. Methods of soil analysis. Part II. Chemical and total nitrogen. In microbiological properties. 2<sup>nd</sup> Ed. Black, American society of Agronomy. Madison, Wisconsin. pp.1149-1176.
- Rodriguez-Kabana, R.; G. Morgan-Jones and I. Chet 1987. Biological control of nematodes: Soil amendments and microbial antagonists. Plant and Soil, 100: 237-247.

- Shahzad, S. and A. Ghaffor 1989. Use of *Paecilomyces lilacinus* in the control of root rot and root-knot disease complex of okra and mung bean. Pak. J. Nematol., 7: 47-55.
- Villanueva, L. M. and R. G. Davide, 1984. Evaluation of several isolates of soil fungi for bio-control of root-knot nematodes. Philip. Agric., 67: 361-371.

### الملخص العربي

تقييم بعض أسمدة المتخلفات العضوية للإنتاج الموسع للفطريات المضادة للنيماطودا وتأثيراتها على نيماطودا تعقد الجذور (*Meloidogyne incognita*) التي تصيب بنجر السكر

#### ١ - أسمدة المتخلفات الحيوانية

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تم تقويم نمو الفطريات المعزولة من كتل البيض لنيماطودا تعقد الجذور، *Meloidogyne spp* و المجال الجذري لنباتات بنجر السكر معملياً، وذلك على بعض سماد المتخلفات الحيوانية {سماد متخلفات دواجن التسمين - سماد متخلفات دواجن البيض و سماد متخلفات البقر و سماد متخلفات الأغنام و الخليط المتخمّر منهم جميعاً (سماد كمبوست)} مقارنة بنموها على حبوب القمح كبيئته قياسية و هذه الفطريات حصرياً هي: *Aspergillus niger*, *Fusarium oxysporium*, *Penicillium nigricans*, *Trichoderma harzianum*, *T. viride*, and *Verticillium chlamydosporium* وتم تقدير النتروجين الكلي والكربون والبوتاسيوم والفوسفور وكذلك رقم الحموضة pH لكل بيئة. وكان الغرض من هذه الدراسة تقييم هذه المواد وخطيها المتخمّر في تنمية وإكثار الفطريات المضادة للنيماطودا بغرض الإنتاج الموسع لهذه الفطريات، استبدالاً لحبوب القمح والذرة أو السورجم وتوفيرها لغذاء الإنسان والحيوان وأيضاً من أجل تعزيز استخدام وسائل أخرى غير المبيدات النيماطودية الكيميائية أكثر اماناً للبيئة و الصحة العامة و توفيراً للنقد وزيادة الدخل. وجد من النتائج المعملية أن سماد متخلفات دواجن التسمين وسماد متخلفات دواجن البيض وسماد متخلفات البقر أفضل البدائل لبيئة حبوب القمح في الإنتاج الموسع لكل من الفطريات المضادة للنيماطودا (*F. oxysporium*, *P. nigricans*, *T. harzianum*, *T. viride*, and *V. chlamydosporium*) أما سماد متخلفات دواجن التسمين وسماد متخلفات دواجن البيض فقط كانتا الأفضل للإنتاج الموسع لفطر *P. nigricans* المضاد للنيماطودا. وجد إن إنتاج الفطريات المضادة للنيماطودا الثلاثة المختبرة (*F. oxysporium*, *T. viride*, and *V. chlamydosporium*) على كل من سماد متخلفات دواجن التسمين وسماد متخلفات دواجن البيض وسماد متخلفات البقر وإضافتها إلى التربة الملوثة بنيماطودا تعقد الجذور (*Meloidogyne incognita*) تحت ظروف تصوبة لزراعية حققت خفضاً كبيراً في تعداد التعقيدات الجذرية وكذلك حققت زيادة كبيره في نسبة تطفلها على بيض النيماطودا على جذور بنجر السكر عن مثيلتهما عند إنتاجها على بيئة حبوب القمح. - ووجد أن فطر *F. oxysporium* فقط أعطى خفضاً معنوياً وزيادة معنوية في تعداد التعقيدات الجذرية ونسبة التطفل على البيض عند إنتاجه على سماد متخلفات دواجن التسمين وسماد متخلفات دواجن البيض بينما كل من فطري *T. viride* و *V. chlamydosporium* أعطى خفضاً معنوياً في تعداد التعقيدات الجذرية وزيادة معنوية في نسبة تطفلها على بيض النيماطودا عند إنتاجهما على سماد متخلفات دواجن التسمين وسماد متخلفات دواجن البيض وسماد متخلفات البقر مقارنة بتأثيرهما على النيماطودا عند إنتاجهما على بيئة حبوب القمح. عموماً الفطريات المضادة للنيماطودا الثلاثة المختبرة المنتجة على سماد متخلفات دواجن البيض كانت أكثر فاعلية في مكافحة نيماطودا تعقد الجذور على جذور بنجر السكر مقارنة بإنتاجها على سماد متخلفات دواجن التسمين وسماد متخلفات البقر و بذلك يمكن استخدام المتوفر من سماد هذه المتخلفات للإنتاج الموسع لهذه الكائنات كمضادات حيوية لنيماطودا تعقد الجذور وتوفير القمح و الحبوب للغذاء الأدمي والحيواني.