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IMPACT OF CERTAIN ANIMAL WASTES AS SOIL AMENDMENTS ON *Tylenchulus semipenetrans* INFECTING *Citrus aurantium* L.

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ABSTRACT

A pot experiment was conducted to study the influence of six animal wastes as soil amendments, i.e. poultry, pigeon, horse, sheep manures as well as died bee workers and shrimp shell powders in comparison with oxamyl (Vydate 24% E.C.) against citrus nematode, *Tylenchulus semipenetrans* infecting sour-orange plant *Citrus aurantium* L. under greenhouse conditions at 25 ± 5°C. Obviously all tested components improved plant growth parameters of sour-orange plant and significantly reduced nematode population to certain extent, except that of shrimp shell treatment that showed a negative result of whole plant fresh weight with value of –12.94% with a slight increase in shoot dry weight (4.0%). Among the tested animal products, horse or pigeon or poultry manures appeared to be the most effective in increasing total plant fresh and shoot dry weights as well as reducing nematode population. Their values were recorded to be 198.82 and 160.00%; 188.24 and 136.00%; 144.71 and 112.00% as well as 70.33%, 70.16% and 70.19%, respectively.

Moreover, bee workers powder and sheep manure treatments showed the moderately percentage increase for the whole plant fresh and shoot dry weights with values of 95.29, 88.00% and 69.21 and 56.00%, respectively. Meanwhile, sheep manure, bee workers and shrimp shells powders gave considerable percentages of nematode reduction with values of 69.90, 69.63 and 66.84%, respectively. In addition, oxamyl treatment achieved the highest percentage of increase in plant growth parameters as well as rate of nematode population reduction.

Keywords: Animal waste products, citrus nematode, sour-orange plant, oxamyl (Vydate 24% E.C.).

INTRODUCTION

Citrus trees are considered to be the most produced fruit trees in Egypt and all over the world. About 339533 feddans are grown in Egypt producing 2350247 tons per year (Saad Allah and Meleegy, 2003).

Several plant parasitic nematodes are recorded to attack the root system of citrus plants. The citrus nematode, *Tylenchulus semipenetrans* (Cobb) is the most important one. Infestation by citrus nematode can result in the disease named "slow decline". The occurrence of high populations of this nematode caused the encrusted appearance of the root due to soil particles that adhere to egg matrix (dirty roots). However above ground symptoms are wilting, poor fruit production and die back (Jenkins and Taylor, 1967).

Losses caused by citrus nematode can be decreased effectively by adding certain chemical pesticides to the soil. However, environmental, health problems and disturbance in the biological balance of nature that caused as a result of extensive use of nematicides have enhanced scientists to search for another alternative nematode management.

The addition of organic matters to soils has been known to improve soil structure, aid in water retention, provide nutrients through its decomposition and might stimulate natural enemies. Thus improving soil conditions might reduce plant stress which in turn can make the stress caused by plant parasitic nematodes less severe or apparent, therefore, great attention has been given, among nematologists, to the use of animal organic manures, i.e. poultry, pigeon-dung, horse and sheep for the management of plant parasitic nematodes as alternative control strategies (Gamliel and Stapleton, 1993; El-Naggar *et al.*, 1994; Ali, 1995; Akhtar and Mahmood, 1997; Dias *et al.*, 2000; El-Sherif and Refaei, 2004; El-Zawahry, 2000; Jayakumar *et al.*, 2004; Kimenju *et al.*, 2004 and Raviv *et al.*, 2005).

A possible effect of adding dung from laying hens on *M. incognita* population was studied by Dias *et al.* (2000) who observed that number of galls was significantly lower in regard to control when the concentrated effluent after 45 days of biodigestion was used, and egg number reduced significantly when the concentrated effluent was used diluted 1:1 v:v or undiluted. They indicated that soluble toxic substance (s) from anaerobiosis may be responsible for the deleterious effects on the nematodes.

Moreover, the effect of different organic manures; farmyard, goat, rabbit, poultry and pigeon on root-knot nematode infesting faba bean was investigated and indicated that organic manure treatments increased plant growth and reduced nematode population in soil and roots (El-Zawahry, 2000).

In 2004, Jayakumar *et al.* tested chitinous waste materials such as crab shell, prawn wastes and fish meal, each at 10 g/pot against root-knot nematode (*M. incognita*) infesting tomato crop under glass-house conditions. Plant growth parameters such as shoot length, shoot weight and root weight significantly increased by 45.07, 25.05 and 45.16%, respectively, over the uninoculated control in plants treated with prawn wastes. Prawn shell showed a maximum decrease of up to 55.5% in root-knot disease incidence compared to the control, followed by crab shell and fish meal.

Literature revealed the lacking of informations concerning the use of certain animal manures for controlling the citrus nematode, *Tylenchulus semipenetrans* infecting citrus plants, therefore, the aim of the present work was to determine the impact of certain animal waste products as soil amendments to sour-orange seedlings infected with *T. semipenetrans*, its development and plant growth response under greenhouse conditions.

MATERIALS AND METHODS

Source of the citrus nematode, *Tylenchulus semipenetrans* inoculum:

Second stage juveniles of *T. semipenetrans* were obtained from a pure culture propagated on sour-orange, *Citrus aurantium* in the greenhouse of

Nematology Research Unit, Agricultural Zoology Department, Faculty of Agriculture, Mansoura University. Nematodes were extracted from soil by sieving and modified Baermann technique (Goodey, 1957).

Impact of certain animal waste products on sour-orange plant infected with *T. semipenetrans*:

In order to determine the impact of certain animal wastes as soil amendments against citrus nematode on sour-orange plant, four animal manures i.e. horse, pigeon, chicken and sheep as well as shrimp shell and died bee workers were sun-dried and powdered. One dose of 10 g was used per 890 g soil. Each 890 g of steam sterilized sandy soil : loam soil (1:1) per plastic pot of 10-cm diam., was separately mixed with the particular dose of the manure or powder over a sheet of polyethylene, watered in order to keep it moist and to facilitate proper decomposition of soil components.

Eighteen plastic pots filled with sterilized soil treated with animal manure or powder was left on a greenhouse bench for one week, in addition to the untreated nine plastic pots for the same experiment.

A total of twenty seven sour-orange seedlings (one year old). *C. aurantium* were then transplanted to all pots, one each. Twenty four of the plastic pots with sour-orange seedlings were then artificially infested with 2000 second stage juveniles of *T. semipenetrans* (N), three of them received a 0.3 ml/pot of oxamyl (Vydate 24% E.C.) each at the recommended dose of this nematicide. Treatments were as follows:

1- N + Bee worker powder, 2- N + Chicken manure, 3- N + Horse manure, 4- N + Pigeon-dung, 5- N + Sheep manure, 6- N + Shrimp shell powder, 7- N + Oxamyl, 8- N alone (control) and 9- Plant free of N or manure or powder (Ck).

Each treatment was replicated three times. All pots were randomly arranged on a greenhouse bench at 25 $\pm$ 5°C. Plants were watered and regularly received the same horticultural treatments. During the period of the experiment, plants were protected against mite and insect attack by conventional pesticides. Sixty days after transplanting, seedlings were up-rooted. Observations on length and weight of shoots and roots as well as shoot dry weight were determined and recorded.

Infected sour-orange roots was stained in 0.01 hot acid fuchsin in lactic acid (Byrd *et al.*, 1983), examined for the number of developmental stages and females. Nematodes were then extracted from soil by sieving and modified Baermann technique (Goodey, 1957).

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) followed by Duncan's multiple-range test to compare means at $P < 0.05$ (Duncan, 1955).

Concerning nitrogen determination in animal wastes, 0.4 g of dry pigeon-dung, poultry, horse and sheep manures as well as shrimp shell and bee workers powders was subjected to chemical analysis. Total nitrogen content was determined by kjeldahl method (A.O.A.C., 1980).

RESULTS AND DISCUSSION

Data in Tables (1 & 2) presented the effect of certain animal manures i.e. poultry, pigeon-dung, horse, sheep manures as well as shrimp shells and bee worker powders in comparison with oxamyl on plant growth response of sour-orange infected with *T. semipenetrans*; as well as nematode development and reproduction under greenhouse conditions at 25 ± 5°C.

Results in Table (1) indicated that most of tested treatments caused remarkable increase in sour-orange plant growth as compared to nematode alone. Among the six animal amendments examined, horse manure achieved the highest percentage of increase for whole plant fresh weight as well as shoot dry weight with values of 198.82% and 160%, respectively, followed by pigeon-dung treatment with values of 188.24% and 136%, respectively and poultry manure application with values of 144.71% and 112%, respectively. Moreover, bee workers powder and sheep manure treatments showed moderately percentage increase for the same criteria with values of 95.29% and 88%; and 69.21% and 56.00%, respectively, whereas shrimp shell treatment gave no percentage of increase for whole plant fresh weight and slight increase in shoot dry weight with values of –12.94% and 4.00%. On the other hand, oxamyl surpassed all treatments in percentage of increase for whole plant fresh weight and shoot dry weight with values of 200% and 180%, respectively. Length of root and shoot of sour-orange plants were significantly affected with horse manure application as well as oxamyl when compared with nematode alone (Table 1 and Fig. 1).

Table 1: Impact of four animal wastes as well as shrimp shell and bee worker powders in comparison with oxamyl on plant growth response of sour-orange seedlings infected with *Tylenchulus semipenetrans* under greenhouse conditions at 25 ± 5°C.

Treatments	*Plant growth response							
	Length (cm)		Fresh weight (g)		F.wt.of whole plant (g)	% of Increase	Shoot dry wt. (g)	% of Increase
	Shoot	Root	Shoot	Root				
Bee worker powder + N	10.93^{ab}	17.33^{a}	1.01^{cd}	0.65^{a}	1.66^{bc}	95.29	0.47^{ab}	88.0
Horse manure + N	13.00^{a}	17.87^{a}	1.69^{a}	0.86^{a}	2.54^{a}	198.82	0.65^{a}	160.0
Pigeon –dung + N	11.10^{ab}	12.27^{bc}	1.68^{a}	0.77^{a}	2.45^{a}	188.24	0.59^{a}	136.0
Poultry manure + N	10.93^{ab}	13.27^{abc}	1.55^{ab}	0.54^{ab}	2.08^{b}	144.71	0.53^{ab}	112.0
Sheep manure + N	11.50^{ab}	16.43^{ab}	0.83^{de}	0.62^{a}	1.44^{bc}	69.21	0.39^{b}	56.0
Shrimp shell powder + N	10.83^{ab}	10.47^{c}	0.47^{e}	0.27^{c}	0.74^{c}	-12.94	0.26^{c}	4.0
Oxamyl + N	10.53^{ab}	15.53^{ab}	1.73^{a}	0.83^{a}	2.55^{a}	200	0.70^{a}	180.0
N. alone	8.60^{bc}	11.33^{bc}	0.56^{de}	0.29^{c}	0.85^{c}	--	0.25^{c}	--
Plant free of N & untreated	8.00^{c}	11.27^{bc}	1.29^{bc}	0.88^{a}	2.17^{b}	--	0.64^{a}	--

N = *T. semipenetrans* at 2000 second stage juveniles (J2)/pot.
*** Each number presented the mean of three replicates.**
Means in each column followed by the same small letter didn't differ at P < 0.05 according to Duncan's multiple-range test..

Data in Table (2) presented the citrus nematode, *T. semipenetrans* population in soil and sessile females in roots of sour-orange plant as

affected by the six animal amendments tested comparing with oxamyl as a nematicide. Obviously, the total number of citrus nematode was significantly influenced by all tested animal waste products as well as oxamyl application when compared with that of the nematode alone (Table 2 and Fig. 3). Treatments of oxamyl or pigeon-dung or horse manure, or poultry manure gave the highest values of percentage of nematode population reduction that were amounted to 73.16%, 70.16%, 70.33% and 70.19%, respectively. Moreover, sheep manure and bee workers powder showed considerable percentage of reduction of nematode population with values of 69.90% and 69.63%, respectively, whereas shrimp shells treatment showed the moderate percentage of nematode population reduction with value of 66.84% (Table 2 and Fig. 2) comparing with nematode alone.

Table 2: Influence of certain animal wastes; shrimp shells; bee workers powders and oxamyl on percentage of reduction of *Tylenchulus semipenetrans* under greenhouse conditions (25 ± 5°C).

Treatments	*Nematode population in		Final population (Pf)	% Reduction
	Soil	Root		
	Juveniles + Males	Females		
Bee workers powder + N	895.00^{b}	28.33^{b}	923.33^{b}	69.63
Horse manure + N	877.33^{b}	24.67^{b}	902.00^{b}	70.33
Pigeon –dung + N	879.33^{b}	27.67^{b}	907.00^{b}	70.16
Poultry manure + N	882.00^{b}	24.33^{b}	906.33^{b}	70.19
Sheep manure + N	888.00^{b}	27.00^{b}	915.00^{b}	69.90
Shrimp shells + N	982.33^{b}	25.67^{b}	1008.00^{b}	66.84
Oxamyl + N	797.67^{b}	18.33^{b}	816.00^{b}	73.16
N. alone	2990.00^{a}	50.00^{a}	3040.00^{a}	--

N = *T. semipenetrans* at 2000 second stage juveniles (J2)/pot.
*** Each number presented the mean of three replicates.**
Means in each column followed by the same small letter didn't differ at P < 0.05 according to Duncan's nultiple-range test.

$$\% \text{ of nematode reduction} = \frac{\text{N alone} - \text{N of each treatment}}{\text{N alone}} \times 100$$

Data in Table (3) presented the chemical analysis of total nitrogen percentage within the organic animal wastes tested i.e. pigeon-dung, horse, poultry and sheep manures as well as powders of bee workers and shrimp shells. Data revealed that the pigeon-dung possessed the highest percentage of nitrogen (7.28%), followed by poultry manure (4.04%), then horse manure (2.13%), and sheep manure (1.8%) whereas the lowest values resulted from bee worker (0.9%) and shrimp shell powders (0.85%), respectively (Table 3).

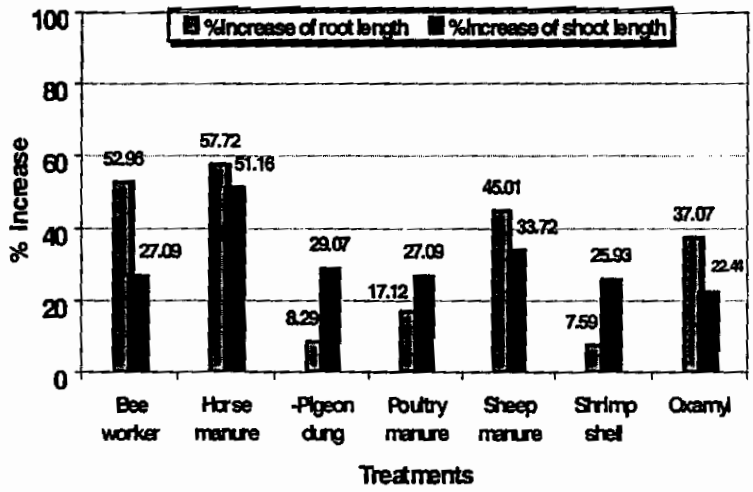

Fig. 1: Impact of four animal wastes as well as shrimp shell and bee worker powders in comparison with oxamyl on increase of shoot and root length of sour-orange infected with *Tylenchulus semipenetrans* under greenhouse conditions (25 ± 5°C).

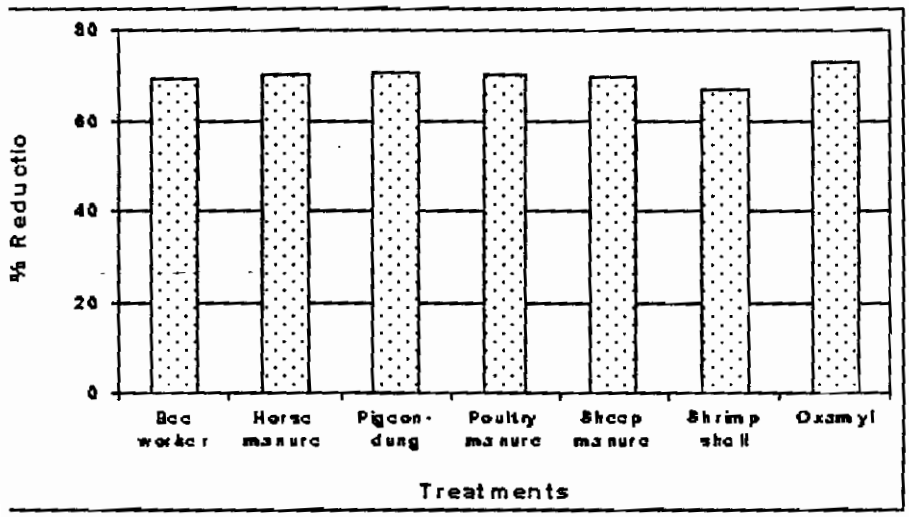

Fig. 2: Impact of four animal wastes as well as shrimp shells and bee workers powders in comparison with oxamyl on percentage of reduction of *Tylenchulus semipenetrans* under greenhouse conditions (25 ± 5°C).

Table 3: Total nitrogen percentage of certain animal wastes.

Animal wastes	Total N%
Bee workers powder	0.902
Horse manure	2.131
Pigeon-dung	7.276
Poultry manure	4.042
Sheep manure	1.800
Shrimp shells powder	0.851

Among the six animal wastes tested against *T. semipenetrans* infecting sour-orange plants, horse manure (2.13%) or pigeon-dung (7.27%) or poultry manure (4.04%) appeared to be the most effective one in increasing plant fresh weight and shoot dry weight as well as reducing nematode population. These data agree with findings of Oduor-Owino and Waudo (1995); Akhtar and Mahmood (1997); Marull *et al.* (1997); Conn and Larzarovits (1999) and Rajesh *et al.* (1999) in respect to poultry manure and El-Naggar *et al.* (1994) and El-Zawahry (2000) in respect to pigeon-dung and Nour El-Deen (2002) in respect to horse manure.

Organic amendments enhance soil fertility, improve biological and physical properties of soil, help in controlling citrus nematode and increasing plant growth. Since the majority of nitrogen in pigeon-dung and poultry manure or horse manure is in the form of uric acid or urea and can be rapidly converted to ammonium nitrogen if temperature, pH and moisture are suitable for microbial activity (Sims and Wolf, 1994). The ammonium produced has been shown to kill plant parasitic nematodes (Eno *et al.*, 1955). More research is needed in order to clarify such phenomenon in this respect.

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تأثير بعض المخلفات الحيوانية كمخصبات تربة على نيماتودا الموالح التى تصيب نبات النارنج تحت ظروف الصوبة.

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تم إجراء تجربة فى أصص بلاستيكية لدارسة تأثير ستة مخلفات حيوانية كمخصبات تربة هى مخلفات الدواجن، الحمام، الخيل، الأغنام، ومسحوق كل من شغالات النحل الميت وقشور الجمبرى على نيماتودا الموالح التى تصيب نباتات النارنج تحت ظروف الصوبة عند درجة حرارة ٢٥ ± ٥ مئوية وأسفرت النتائج عن:

١- أدت جميع المخلفات الحيوانية الجافة للحمام والدواجن والأغنام والخيل وكذلك مسحوق شغالات النحل إلى تحسن مقاييس نمو نباتات النارنج مقارنة بالنباتات غير المعاملة بها وكذلك فى خفض تعداد النيماتودا.

٢- كما أعطت المعاملة بمخلفات الخيل والحمام والدواجن ارتفاع ملحوظ فى زيادة الوزن الرطب والجاف للمجموع الخضرى لشتلات النارنج بمعدلات (١٩٨,٨٢% & ١٦٠%) و(١٨٨,٢٤% & ١٣٦%) و(١٤٤,٧١% & ١١٢%) وأعطت نسب مرتفعة فى خفض تعداد النيماتودا بمعدلات هى ٧٠,٣٣%، ٧٠,١٦%، ٧٠,١٩% على التوالى.

٣- جاءت المعاملة بالمخلف الحيوانى للأغنام ومسحوق شغالات نحل العسل بنسبة متوسطة فى زيادة الوزن الرطب والجاف للمجموع الخضرى بقيم تصل إلى ٦٩,٢١%، ٥٦%، و٩٥,٢٩% و٨٨% على التوالى وكذلك فى خفض تعداد النيماتودا بمعدل ٦٩,٩٠% و٦٩,٦٣% على التوالى.

٤- أعطت المعاملات بمسحوق قشور الجمبرى أقل القيم من حيث نقص الوزن الرطب وزيادة طفيفة فى الجاف للمجموع الخضرى للشتلات بمعدلات -١٢,٩٤% و٤,٠%، أعطت نسب متوسطة فى خفض تعداد النيماتودا بنسبة ٦٦,٨٤%.

٥- أدت معاملة الشتلات بمبيد الأوكساميل (٢٤% سائل) ارتفاع ملحوظ فى الوزن الرطب والجاف للمجموع الخضرى للشتلات بنسبة ٢٠٠% و١٨٠%، كما أعطى أعلى معدل خفض فى تعداد النيماتودا بنسبة ٧٣,١٦%.