Nematicidal activity and composition of some organic fertilizers and amendments

Taysir BADRA [1], Mahmoud A. SALEH and Bakir A. OTEIFA

Nematology Research Centre, Faculty of Agriculture, Cairo University, Giza, Egypt.

SUMMARY

Some organic amendments were screened for toxicity to the infective stages of the reniform *(Rotylenchulus reniformis)* and citrus *(Tylenchulus semipenetrans)* nematodes. Pigeon droppings, poultry droppings and cotton-seed cake were highly toxic, while rice straw, sheep dung and horse dung were low in toxicity. The effectiveness of tested materials deteriorated after a ten-day decomposition period. Chemical analysis revealed that acetic, propionic and butyric were the dominant low molecular weight fatty acids, while isovaleric and valeric volatile fatty acids were lower in amounts and even absent from some amendments. Phenols were present in organic amendments, especially higher in the plant-origin forms. GLC showed that hydrogen, hydrogen sulfide and methane evolved from tested materials at different levels.

In toxicological studies, the three dominant fatty acids, phenol and fermented gasses appeared nematotoxic. The LD_{50} values of acetic, propionic and butyric acids for reniform nematode were 135, 520 and 270 ppm, respectively ; and 120, 200 and 64 ppm on citrus nematode, respectively. The LD_{50} obtained with phenol on the infecting stages of reniform and citrus nematodes was 150 and 120 ppm, respectively. Lethal gasses from pigeon droppings, poultry droppings and the nematicide DBCP were lethal to immature females of *R. reniformis*, but were not toxic from sheep dung, horse dung, rice straw and cotton-seed cake. The organic compounds found in these fertilizers and amendments may account for the nematicidal activity displayed by tested organic amendments.

RÉSUMÉ

Activité nématicide et composition de quelques engrais et amendements organiques

Cette étude avait pour but de connaître l'efficacité de quelques amendements organiques dans la lutte contre les nématodes. Au cours des premiers essais toxicologiques *in vitro*, certains amendements ont présenté une remarquable toxicité à l'égard des femelles immatures de *Rotylenchulus reniformis* et des larves du deuxième stade de *Tylenchulus semipenetrans*. La mortalité causée par les déjections de pigeons et de poules et par les tourteaux de coton a été supérieure à celle causée par la paille de riz et les fumiers de mouton et de cheval. Les suspensions aqueuses vieilles de huit jours, ayant subi une décomposition avancée, étaient beaucoup moins ou plus du tout actives dans le cas des fumiers de cheval et de mouton ainsi que de la paille de riz et du tourteau de coton alors que cette diminution était beaucoup plus faible ou inexistante dans le cas des déjections de pigeon et de poulet.

La détection de cette toxicité des amendements à l'égard des nématodes a conduit à tenter de définir quels étaient leurs composants toxiques. Les études de chromatographie en phase gazeuse ont montré que, parmi les acides gras, les acides acétique, propionique et butyrique étaient plus abondants que les acides volatils, valérique et isovalérique. Leur abondance était plus forte dans les déjections de pigeon et de poule ainsi que dans les tourteaux de coton. Le contenu en mono- et polyphénols, estimé par colorimétrie, est généralement assez important dans les amendements organiques, spécialement ceux d'origine végétale. L'identification des gaz émis par les amendements a révélé la présence d'hydrogène, d'hydrogène sulfuré et de méthane à des concentrations variables.

La LD_{50} des acides acétique, propionique et butyrique sur les femelles immatures de *R. reniformis* a été de 135, 520 et 270 ppm respectivement et 120, 200 et 64 ppm sur les larves de deuxième stade de *T. semipenetrans*. La

(1) Now : I.I.T.A., Germ Plasm Unit, Oyo Road, P.M.B. 5320, Ibadan, Nigeria.

LD_{50} du phénol sur les stades infectants de *R. reniformis* et *T. semipenetrans* a été respectivement 150 et 120 ppm. Il est évident que le stade infectant de *T. semipenetrans* a été, en gros, plus sensible aux effets toxiques que celui de *R. reniformis*. Les gaz émis par les déjections de pigeon et de poule et le nématicide DBCP se sont montrés toxiques envers les femelles immatures de *R. reniformis*. Les auteurs émettent l'hypothèse que la présence de ces composants dans les amendements organiques pourrait expliquer leur toxicité envers les nématodes.

Organic fertilizers and amendments are used conventionally on many agricultural plantings. Their essentiel role as fertilizers has been recognized for centuries for their substantial increases in crop productivity. Many workers showed that organic materials of plant and animal origin may also be beneficial in agricultural production because of their depressant effect on plant-parasitic nematodes such as *Meloidogyne*, *Heterodera*, *Tylenchulus*, *Hoplolaimus*, *Xiphinema*, *Pratylenchus*, *Ditylenchus*, *Paratylenchus*, *Tylenchorhynchus* and *Belonolaimus* (Habicht, 1975 ; Mankau & Das, 1974 ; Miller, 1977 ; Mobin & Khan, 1969 ; Oostenbrink, 1960 ; Singh & Sitaramaiah, 1969 ; Tomerlin *et al.*, 1969 ; Van der Laan, 1956 ; Vlk, 1970). This paper reports the activity of some organic amendments against two important nematodes along with the identification and toxicity of some of their chemical constituents.

Materials and Methods

The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, 1940, and the citrus nematode, *Tylenchulus semipenetrans* Cobb, 1913, were used as test organisms. Six materials of organic nature were evaluated in this study along with three volatile fatty acids and phenol. The animal-origin materials were pigeon droppings, poultry droppings, sheep dung and horse dung ; while the plant-origin substances were cotton-seed cake and rice straw. A successive series of toxicological *in-vitro* tests were used to bioassay the nematicidal activity of the six amendments, three volatile fatty acids as well as phenol base against infective stages of *R. reniformis* and *T. semipenetrans*. The amendments were screened at six concentrations, while the fatty acids and phenol were tested at fourteen concentrations. Three subsamples of 50 ml each, were transferred from each concentration into 60 ml glass vials and subsequently three replicated populations of approximately 1,000 *R. reniformis* immature females or *T. semipenetrans* second stage larvae were exposed simultaneously. Another five nematode replications were retained in tap water to serve as control. A parallel experiment was conducted with the same dilutions and procedure after materials had decomposed for 10 days in their watery suspensions. Counts were made on live and dead nematodes after 48 h. Live nematodes were those that remained actively mobile while those that were immobile, with distorted and granulated cytoplasm were considered dead. Mortality was calculated against the tap water control by Abbott's (1925) formula. The effect of volatile compounds was studied in 14 cm desiccators at concentrations ranging from 1-10 g/500 ml water at 25 °C. In addition, a standard volatile nematicide, namely DBCP 75% EC (1,2-dibromo-3-chloropropane) was assessed in the same manner at 12, 24, 36 and 48 l/ha for comparison. Five replicated populations at each 1,000 *R. reniformis* immature females in seven ml water were deposited into small Petri dishes and placed into the desiccators. A fifth desiccator was filled with five replicate nematode populations to serve as controls. Mortality was measured at end of 72 h.

Chemical determinations

The low molecular weight fatty acids and gas constituents in organic amendments were determined using the gas chromatograph techniques of Erwin, Marco and Emery (1961), while phenol components were determined colorimetrically by the Snell and Snell (1953) method.

Results

Activity of organic amendments

Effects of the organic amendments at 1 and 2.5 g/liter on the infective stages of the reniform and citrus nematodes are given in Table 1. Concentrations of 5, 10, 15 and 20 g/liter gave 100% mortality for pigeon droppings, poultry droppings and cotton-seed cake. Horse dung, sheep dung and rice straw gave 100% mortality at concentrations above 10 g/liter. The lethal effects of these materials varied considerably within the 1 to 2.5 g/liter concentrations. Pigeon droppings, poultry droppings and cotton-seed cake were the most toxic, while rice straw, sheep dung and horse dung were least toxic. Horse dung, sheep dung, rice straw and cotton-seed cake that were decomposed for 10 days were generally less toxic than fresh materials (Table 1). Pigeon droppings and poultry droppings remained consistantly toxic during decomposition.

Table 1

In-vitro activity of organic amendments on *R. reniformis* and *T. semipenetrans* infective stages

Amendment				*Mortality Rate %*			
		pH		R. reniformis		T. semipenetrans	
	g/liter	*Instant*	*10-day*	*Instant*	*10-day*	*Instant*	*10-day*
Animal-origin :							
Pigeon droppings	1	7.7	6.8	83.59	70.88	100.00	100.00
	2.5	7.3	6.5	100.00	100.00	100.00	100.00
Poultry droppings	1	6.9	6.6	60.83	26.11	100.00	100.00
	2.5	6.8	6.5	100.00	100.00	100.00	100.00
Sheep dung	1	7.5	6.7	27.57	0	26.03	0.34
	2.5	7.8	6.7	62.82	5.63	100.00	28.51
Horse dung	1	7.0	7.4	49.48	0	0	0
	2.5	7.0	7.1	86.66	1.88	0.22	4.94
Plant-origin :							
Cotton-seed cake	1	7.7	6.6	100.00	13.42	100.00	75.53
	2.5	7.6	6.5	100.00	55.16	100.00	100.00
Rice straw	1	7.1	6.6	25.86	5.51	0	0.74
	2.5	7.1	6.4	62.20	40.75	16.82	13.85

Identification of fatty acids, phenols and gasses from organic amendments

Low molecular weight fatty acids

Five low molecular weight fatty acids found in most of the organic amendments are given in Table 2. Acetic, propionic, butyric, isovaleric and valeric acids in succession were detected in sheep dung, cotton-seed cake and rice straw. Pigeon droppings and poultry droppings, however, lacked isovaleric acid, while horse dung did not contain either isovaleric or valeric acids. Acetic, propionic and butyric contents were, on the average, found in greater concentrations than isovaleric and valeric acids. The three major fatty acids ranged from as little as 16.1 to as much as 50.1% of total acid content. The two minor isovaleric and valeric acids ranged from 0 to 11.2% in both animal- and plant-origin groups. Pigeon droppings, poultry droppings and cotton-seed cake had the highest total acid constituent at 11,174; 10,834 and 12,390 µg/g, respectively and were the most toxic to nematodes.

Table 2

Low molecular weight fatty acid content (μg/g fresh weight) of six organic amendments

Fatty acid	*Animal-origin* (1)				*Plant-origin* (1)	
	PG	PT	SP	HS	CT	RS
Acetic	3031 (27.1) (2)	2798 (25.8)	749 (24.6)	214 (45.0)	2980 (24.1)	1578 (41.9)
Propionic	4417 (39.5)	3459 (31.9)	489 (16.1)	82 (17.3)	3666 (29.6)	345 (9.2)
Butyric	3022 (27.0)	3361 (31.0)	1523 (50.1)	179 (37.7)	4974 (40.1)	1566 (41.6)
Isovaleric	0 (0)	0 (0)	59 (1.9)	0 (0)	146 (1.2)	132 (3.5)
Valeric	704 (6.3)	1216 (11.2)	220 (7.2)	0 (0)	623 (5.0)	147 (3.9)
Total amount	11,174	10,834	3039	476	12,390	3767
Grand Percent % (3)	1.12	1.08	0.30	0.05	1.24	0.38

(1) PG = pigeon droppings, PT = poultry droppings, SP = sheep dung, HS = horse dung, CT = cotton-seed cake and RS = rice straw.
(2) Values between parentheses represent receptive acid percent in the total amount of detected acids.
(3) Grand percent records arbitrary calculation of cumulative acids contents in one gram material.

Phenolic contents

Phenol fractions in amendments are given in Table 3. The animal- and plant-origin amendments were very different in their phenolic composition. The plant-origin materials contained higher amounts (135-255 μg/) of total phenols than those of animal-origin (28-56 μg/g).

Table 3

Determination of phenols in organic amendments (μg/g fresh weight)

Amendment	*Monophenols* (1)	*Polyphenols*	*Total phenols*
Animal-origin :			
Pigeon droppings	6	36	42
Poultry droppings	36	24	50
Sheep dung	14	14	28
Horse dung	16	40	56
Plant-origin :			
Cotton-seed cake	105	150	255
Rice straw	82.5	52.5	135

(1) Monophenols were calculated by substracting polyphenols from total phenols.

Volatile gasses

The gasses that evolved from amendments as measured by differential chromatogram is given in Figure 1. Every amendment gave a multiband of three peaks ; hydrogen (H_2), hydrogen sulfide (H_2S) and methane (CH_4), in succession. Hydrogen sulfide and methane gasses were variable in occurrence. All amendments produced hydrogen sulfide, however, pigeon droppings, poultry droppings and horse dung gave the highest H_2S peaks. Sheep dung and cottonseed cake, gave the lowest level of H_2S. The concentration of methane was lower than H_2S. Methane was highest in pigeon droppings but reduced in both poultry droppings and horse dung and was absent in sheep dung and cottonseed cake.

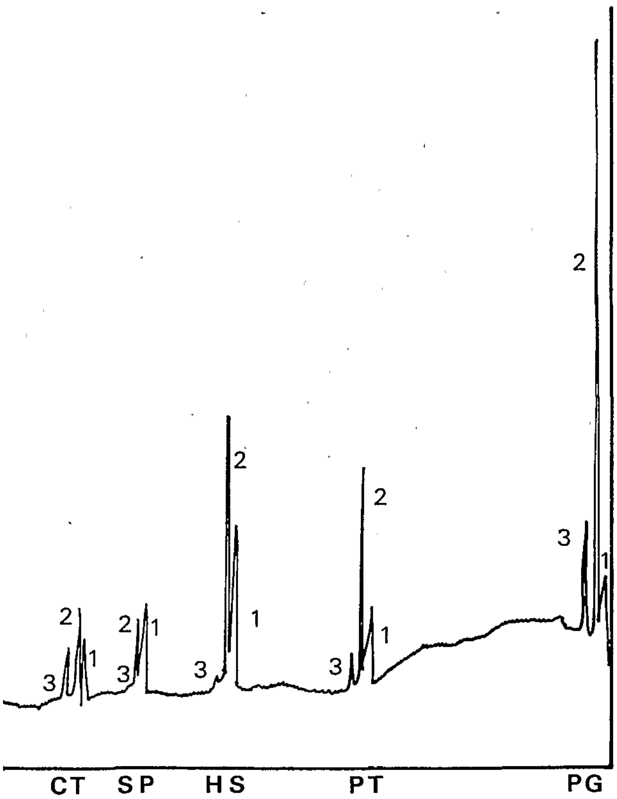

Fig. 1 : Gaseous chromatogram of pigeon droppings (PG), poultry droppings (PT), sheep dung (SP), horse dung (HS) and cotton-seed cake (CT). (1 = hydrogen, 2 = hydrogen sulfide and 3 = methane).

TOXICITY OF ORGANIC AMENDMENTS COMPONENTS

Toxicity of fatty acids to nematodes

Acetic, propionic and butyric volatile fatty

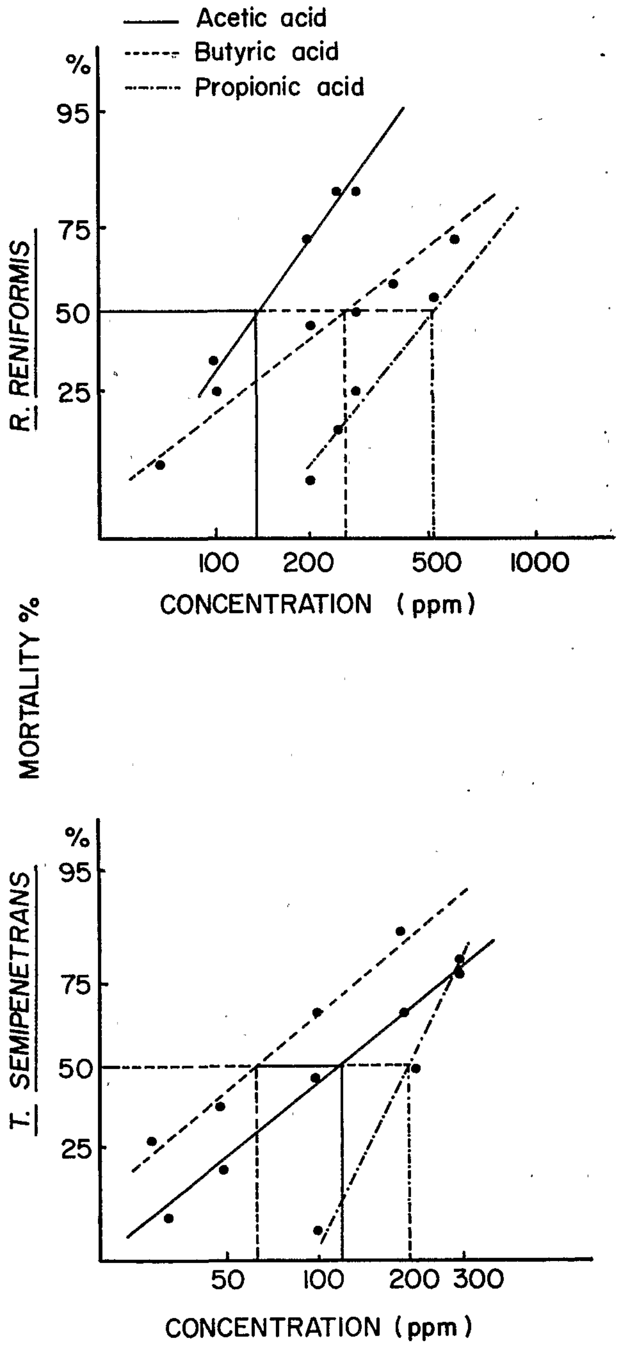

Fig. 2 : Toxicity of three volatile fatty acids to *R. reniformis* and *T. semipenetrans* infective stages.

acids were prepared in a 14-graded series of concentrations : 25 ; 50 ; 100 ; 200 ; 250 ; 300 ; 400 ; 500 ; 600 ; 1,000 ; 2,000 ; 3,000 ; 4,000 and 5,000 ppm and biossayed *in vitro* against infective stages of *R. reniformis* and *T. semipenetrans*. These concentrations were selected on the basis of the actual amounts found in the organic amendments. The dead counts were plotted on logarithmic papers and the LD_{50} of each acid was calculated for both nematodes (Fig. 2).

Concentrations of 1,000 ppm and above gave 100% mortality to both nematodes. Lower concentrations, gave a variety of effects which varied with regard to nematode type and acid tested. Exposure of *R. reniformis* immature females to acetic, butyric and propionic acids yielded LD_{50} values of 135, 270 and 520 ppm acid, respectively. Acetic was the most toxic followed by butyric and propionic acids. The second stage larvae of *T. semipenetrans* were more sensitive to all the organic acids than *R. reniformis*. The LD_{50} of butyric, acetic and propionic acids to the citrus nematode was 64, 120 and 200 ppm, respectively.

Toxicity of phenol to nematodes

The infective stages of *R. reniformis* and *T. semipenetrans* were treated in concentrations of 10, 20, 40, 60, 80, 100, 140, 160, 180, 200, 220, 240 and 260 ppm phenol and checked for mortality after 48 h.

The LD_{50} values for *R. reniformis* and *T. semipenetrans* are illustrated in Figure 3. Phenol

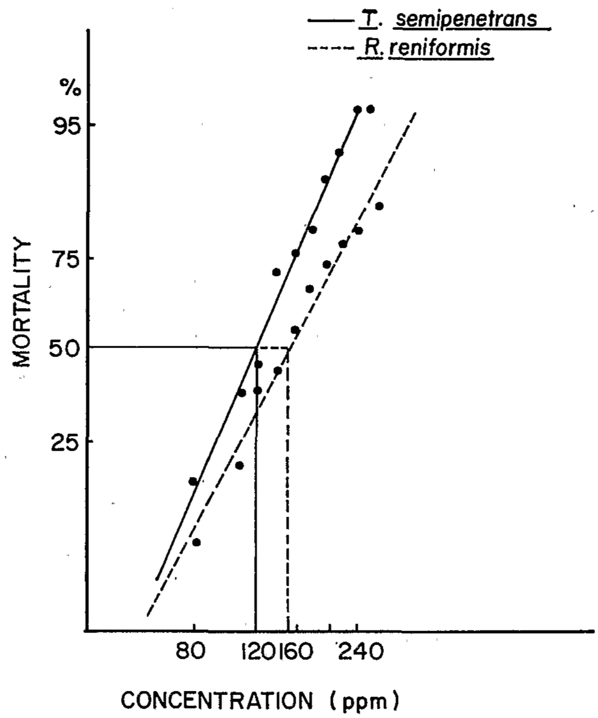

Fig. 3 : Toxicity of phenol base to *R. reniformis* and *T. semipenetrans* infective stages.

Table 4

Toxicity of gaseous atmosphere on the infective stage of the reniform nematode, *R. reniformis*

Organic amendment	*Doses (g/500 ml water)*			
	1	*2.5*	*5*	*10*
Animal-origin :				
Pigeon droppings	0	7.67	99.41	100.00
Poultry droppings	0	0	30.18	100.00
Sheep dung	0	0	0	0
Horse dung	0	0	0	0
Plant-origin :				
Cotton-seed cake	0	0	0	0
Rice straw	0	0	0	0
Fumigant nematicide :	*Liter/ha*			
	12	*24*	*36*	*48*
DBCP 75% EC	100.00	100.00	100.00	100.00

nemastasis first appeared at 80 ppm and increased with the supplemental dosages until 87% to 100% were killed at the final 260 ppm dose. The LD_{50} value as constructed in Figure 3 for the reniform and citrus nematodes were 150 and 120 ppm phenol, respectively.

Toxicity of gasses to nematodes

Data on the effects of the volatile compounds on *R. reniformis* immature females are given in Table 4. The tested materials, except for pigeon droppings, poultry droppings and DBCP, exhibited no detectable toxicity in these tests. In these tests, it appeared that DBCP was sufficiently volatile to kill 100% of nematodes at all concentrations. Pigeon droppings were slightly toxic at 2.5 g and killed nearly all the nematodes at 5 and 10 g. Poultry droppings was slightly less toxic than pigeon droppings.

Discussion

The investigated organic amendments and their constituents were toxic againts nematodes in varying degrees. In descending order of toxicity, one gram organic material/liter of suspension contained the following µg amounts of fatty acids and phenol respectively ; cotton-seed cake, 12,390+255 ; pigeon droppings, 11,174+42 ; poultry droppings, 10,834+50 ; sheep dung, 3,039+28 ; rice straw, 3,767+135 and horse dung, 476+56. Studies on toxicity of three specific fatty acids and phenol base supported the hypothesis that these components are the toxic properties of the organic amendments. Others workers have also shown that low-molecular weight fatty acids were toxic to nematodes (Johnston, 1959 ; Banage & Visser, 1965 ; Hollis & Rodriguez-Kabana, 1966 ; Elmiligy & Norton, 1973 ; Husain & Masood, 1973 ; Pillai & Desai, 1975). Similarly, the lethal role of phenols was noted by Taylor and Murant (1966) and Singh and Sitaramaiah (1973) against *Longidorus elongatus* and *Meloidogyne javanica*. Therefore it may be possible to link the appreciable quantities of these constituents present in cotton-seed cake, pigeon droppings and poultry droppings with their toxicity to *R. reniformis* and *T. semipenetrans*.

The volatile compounds of these amendments, especially hydrogen sulfide and methane, have also been shown to be toxic to nematodes (Rodriguez-Kabana, 1965 ; Fortuner & Jacq, 1976). The conventional heavy dressing of organic matter maintained by farmers in Egypt probably provides some degree of nematode control as well as fertilization. The organic amendments, pigeon droppings, cotton-seed cake, poultry droppings, sheep dung, horse dung and rice straw contain 5.02, 4.04, 3.14, 1.63, 1.41 and .39% nitrogen, respectively (Abdel-Rahman, 1977). The nitrogen-deficient sheep dung, horse dung and rice straw amendments should be supplemented with an inorganic nitrogenous fertilizer in order to counteract their high carbon/nitrogen ratio. This could improve the C/N ratio, thus helping to eliminate the undesirable effects of nitrogen starvation to plants while maintaining their benefit for nematode control.

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