Utilization of the root-knot nematode, Meloidogyne incognita for monitoring nematicide movement in soil

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ABSTRACT

Leachability of carbofuran, oxamyl and terbufos in relation to its efficiency against the root-knot nematode, Meloidogyne incognita on tomato seedlings was studied in soil columns under laboratory conditions. In addition, the quantitiy of nematicide residues in leached water was biologically assayed using Culex pipiens larvae. The results showed that leaching of carbofuran or oxamyl was rapid through sandy clay loam soil than that of clay loam soil. However, these nematicides were easily leached out from both soils and had better effect against nematodes in the lower depths than on the surface. Conversely, terbufos acted better on the surface than in deeper layers of both soil types. Few quantities of carbofuran and oxamyl were detected in the leachate from both soils, while terbufos was only found in sandy clay loam soil leachate. The nematicides amounts recovered from column were proportionally related to the amounts of water applied and to the soil type.

Keywords: Nematicides, Leaching, Root-knot nematodes, Carbofuran, Oxamyl, Terbufos, Biomonitor.

INTRODUCTION

Agricultural use of pesticides should be part of an overall management strategy. Pesticides have been continually incorporated into soils. Some of these pesticides decompose readily into harmless by-products, but others are relatively inert to biological and chemical degradation. However, the bulk of pesticide residues in soil are generally confined to the upper 5 cm of the top, soil. Consequently, they move from the surface when they percolate down through the soil. Those that have infiltrated the soil will eventually reach the groundwater (FAO, 2000).

Leaching is a process whereby pollutants are flushed through the soil by rainfall or irrigation water as it moves downward. Leaching potential and persistence of non-fumigant nematicides in soil have been studied by several investigators. It is generally accepted that differences in chemical solubility, adsorptive capacity, volatility, degradability, the amount of water passing downward through the soil profile, as well as soil properties that effect water movement, biological activity and chemical retention, all affect the amount of a nematicide that will leach to groundwater (Helling and Gish, 1986, Kumari et al., 1988, Yen et al., 1997; Jaramillo et al., 1999/2000 and Fava et al., 2001).

Non-fumigant organophosphate and carbamate nematicides are among the current options for managing nematodes on numerous crops worldwide (Abu-Elamayem et al., 1989; Roberts, 1993; Radwan, 1999 and Shafiq and Khan, 2001).

Certain microbivorous nematodes have been described as excellent indicator organisms for detection of toxicants in aquatic, marine, and terrestrial habitats (Samoiloff, 1987). Plant parasitic nematodes have the potential for being used as biomonitors of presence and movement of aldicarb and phenamiphos in soil (Gourd et al., 1993).

The objective of the present study was to compare the mobility of carbofuran, oxamyl, and terbufos downward through the root zone of two soil types under laboratory conditions. The root galling criteria were used for monitoring the chemicals in soil depths. In addition, the amount of nematicide residues in leached water was biologically assayed using *Culex pipiens* larvae.

MATERIALS AND METHODS

Nematicides used: Carbofuran (=Furadan 10% G; analytical grade sample 99.7% purity) "2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate" oxamyl (=Vydate 10% G; analytical grade sample 98% purity) "Methyl N,N-dimethyl-N-[(methyl carbamoyl) oxy)]-1-thio-oxamimidate" and terbufos (= Counter 10% G; analytical grade sample 99.9 % purity) "S-(tert-butyl thio) methyl O,O-diethyl phosphorodithioate". The formulated samples were provided from FMC Corporation Agric. Chem. Group, DuPont de Nemours Co., and American Cyanamide Company, respectively.

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All analytical standards were supplied by Environmental Protection Agency (EPA), USA.

Soils used: Two different types of soil were chosen to represent the most common types of Egypt. 1- Sandy clay loam soil was collected from El-Nobaria region at the west of Alexandria. 2- Clay loam soil was collected from the Agricultural Research Station at Abis region, Alexandria, Egypt. Soils used in the present study were collected from soil pits at previously mentioned locations where there had been no pesticide application. The soil samples were air-dried, crushed and sieved using a 2 mm sieve and stored in a dry place until needed. The physical and chemical properties of these soils were determined and shown in Table 1.

Table (1): The physicochemical properties of the two soil types used.

Physicochemical properties		Clay loam	Sandy clay loam	
	Sand	27.88	59.00	
Texture	Silt	26.23	16,00	
1	Clay	45.89	25.00	
E.C. (m m	ohs/cm at 25°c) Soluble	7.9	8.6	
cations co	nc.			
	(meq/L.)			
	Ca [↔]	10.10	7.20	
	Mg [↔]	16,30	11.00	
	Na ⁺	70.60	15.20	
	K⁺	2.70	3.40	
Soluble an	tions conc.			
	(meq/L.)			
	CO ₃	12.2	31.7	
	HCO ₃	9.90	2.80	
	CF	63.30	24.00	
	SO ₄	33,80	8.00	
Oreanic n	natter content	1.38	0.31	
0	(%)			
	рH	8.60	7.82	

Nematode inocula: Root-knot nematode, Meloidogyne incognita (Kofoid & White) Chitwood was isolated from infected roots of local Guamia (Myoporum pictum) plants grown in the garden of Faculty of Agriculture, University of Alexandria Eggs were extracted from the infected roots with

a modified sodium hypochlorite extraction technique (Hussey and Barker, 1973).

Experimental procedure: Leachability in relation to the efficiency of certain nematicides against root-knot nematode, M. incognita was studied in soil column under laboratory conditions. Soil columns were prepared by sealing four PVC segments (5 cm × 7.5 cm in diameter) with silicone, and filled with the sieved soil (Ca. 1 Kg). A layer of 1 cm thickness was removed from the soil surface of the fourth ring, before the application of nematicides. Each granular nematicide (5 mg) was applied uniformly over the surface area of each column. Once again the layer of fine soil (1 cm) was added on the surface of the fourth ring. The columns were leached with two amounts of tap water (500 or 1000 ml) approximately equal and twice field capacity, to evaluate the effect of water volume applied on nematicide movement. Filter paper was used on the soil surface to ensure uniform distribution of water. The soil columns were tampled tightly and preventing any formation of holes in the surface of the soil during water or chemical treatments. After leaching, the columns were allowed to drain overnight. After 48 hours from treatments, the column segments were individually separated and the residues of nematicide were biomonitored using the rootknot nematode, M. incognita. Each soil segment (about 250 gm soil) was placed in a clay pot, inoculated with 100 eggs of M.incognita and transplanted with a pair of tomato seedlings. Each column treatment for each soil type was replicated two times. Nematicide-free, but infested checks were also included. Galling of tomato roots after 7 weeks indicated the depth to which nematicide had penetrated. Also, the amount of nematicide residues in leached water was directly assayed by mosquito larvae, Culex pipiens and the concentration of each nematicide in leached water at 20 cm deepth was calculated by refering to the standard Ld-pline.

RESULTS AND DISCUSSION

Movement of carbofuran, oxamyl and terbufos in relation to their efficiency against root-knot nematode, *M. incognita* on tomato seedlings was studied in soil columns under the laboratory conditions, at four depth levels; 0-5, 5-10, 10-15 and 15-20 cm in two soil types; sandy clay loam and clay loam soils. Two amounts of water, 500 and 1000 ml were applied to elute the nematicide through the column. The amount and rate of nematicide residues in the column depths were assessed based on root galling formed

after seven weeks. The effectiveness percentage at different depths in the two soil types were estimated and presented in Tables 2 & 3.

In general, results are listed in Tables 2 and 3, indicated that the galls formed considerably varied at the depths of the two soil types when two amounts of leaching water were applied. The three tested nematicides were differently reduced nematode population in the tomato roots, as a number of galls at all depths compared with the untreated check.

The data in Table 2, showed that carbofuran moved to the third depth (10-15 cm), indicating an effectiveness of 92.66 % when 500 ml of leaching water was added. In addition, carbofuran leached more rapidly to the last two depths (10-15 and 15-20 cm) when 1000 ml was added and its effectiveness was 87.77 and 96.23 %, respectively. Oxamyl leached at about the same rate as carbofuran when 500 ml water was used. Its effectiveness was found to be high in the third depth following either low or high leaching water volume. The high volume of leaching water (1000 ml) leached oxamyl to deeper zones. Oxamyl completely eliminated galls at the last depth (15-20 cm) giving effectiveness of 100 %. Lower leaching potential of terbufos was noted. As it is clear from the obtained results, this nematicide completely eliminated galls at the upper first depth (0-5 cm) and had the higher effectiveness of 89.27 % in the second depth (5-10 cm) when an amount of 500 ml of leached water was applied. When 1000 ml was added of eluted water, terbufos had an excellent activity against nematode at the first three depths which achieved an effectiveness of 93.04 % at depth from 0-5 cm, 97.93 % at depth from 5-10 cm, and 90.59 % at depth from 10-15 cm.

In regard to the leaching potential of the tested nematicides through clay loam soil, the results are shown in Table 3, indicated that carbofuran was distributed in the soil depths and its high effectiveness of 94.21 % occurred in the second depth when 500 ml of leaching water was applied. When 1000 ml of water applied to elute it, the high effectiveness on galling was found in the intermediate depths from 5-10 cm and from 10-15 cm, which have an effectiveness values of 84.14 and 91.43 %, respectively. Oxamyl had nearly the same result as carbofuran when 500 ml of leaching water was used. Its high nematicidal activity of 93.36 % was found in the second depth. Also, it had an excellent efficacy at the two intermediate depths, which have an effectiveness values of 92.71 and 98.28 %, respectively. On the other hand, terbufos completely control was achieved at the surface depth and had high

Nematicide Volume No of No. of	able (2): Efficie	ncy of certain gra	granular nen	naticides in relation	to depths in san	in relation to depths in sandy clay loant soil on tomato veedling	on tomato see	dling		
of water 0.5 15.16 16.15 15.2 te volume No of volume No. of salls root No. of salls		leaching				Depths of lea	Ching (cm)			
No of No o				0.5		1.10	7	9-15		02
\$00 96.00 63.86 67.50 74.59 19.50 92.66 137.00 1000 119.00 55.20 60.50 77.23 32.50 87.77 10.00 500 61.00 77.04 37.50 85.88 8.00 96.99 110.00 1000 99.00 62.73 45.50 82.87 12.50 95.29 0.00 1000 100.00 100.00 28.50 89.27 176.50 33.56 209.00 1000 18.50 93.04 5.50 97.93 25.00 90.59 134.50	Nematicide	volume (ml.)	No of galls root	. 1	No. of galls/root system	Effectiveners (*o)	Vo. of galls mot	Effectivences (*)	No of galls mot evetern	Effectivates (**)
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1 500 0.00 100.00 28.50 89.27 176.50 33.56 209.00 100.00 18.50 93.04 5.50 97.93 25.00 90.59 134.50		9	8	62.73	45.50	82.87	17.50	75.47	5	45.00
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Namaticide	on water volume (mi.)	No. of galls/root system	Effectiveness (%)	No. of galls/root system	Effectiveness (°5)	No. of galls root system	Effectiveness (%6)	No of galkroot system	Effectiveness (*6)
1	8	16.4	97 78	13.5	94.21	\$6.5	75,78	102.0	\$6.28
	3 5	6	66.78	17.0	84.14	20.0	91.43	29.00	41.99
	3 5	0.10	76 60	· ·	91.10	\$ 15	77.93	91.00	61.00
	3	76.0	90.50	22	92.71	4.0	98.28	95,00	82.65
	36	0.04	2001		89.07	\$ 1.3	70,77	111.0	52.43
	000	5.0	97.86	1.5	96.96	45.0	80.71	00'66	57.57

Theaverage number of galls/root system in the untreated check was 233.33

effectiveness of 89.07 % in the second depth, when 500 ml was applied, while it suppressed galling by 99.36 % followed by the first depth with the activity of 97.86 %.

Oxamyl and carbofuran were considered to be very mobile through soils due to their water solubility, 2.8 X 103 ppm and 700 ppm, respectively. However, terbufos was slightly mobile downward into soils because of its low water solubility, 10-15 ppm, (Anonymous, 2000). From the previous results, it could be concluded that the nematicides applied to sandy clay loam soil were highly leachable than that found in clay loam soil. Carbofuran and oxamyl in both soils had a better effect on nematode in deep layers than on the surface one. Conversely, terbufos acted better on the surface layer than in deeper layers of both soils. This could be explained by the leaching of carbofuran and oxamyl in sandy clay loam soil and clay loam soil. Terbufos was very slow in its mobility downward in both soils. This result support the statement of Helling (1971) that the observation that pesticides applied to coarse-textured, sandy soils are subject to greater leaching than those found in soils of higher clay and organic content. For example, carbofuran leached significantly, although leaching may not occur in highly organic soils (Yen et al., 1997). Also, carbofuran was reported to move rapidly through the first meter of soil, and its short persistence prevented accumulation groundwater (Gupta, 1994). Furthermore, the adsorptive capacity of nematicides to clay particles represented a differentiated factor in controlling nematode at different depths of both soil types. These results are in a good agreement with that reported by Taha et al. (1973). They showed that the soil type was an important factor for controlling root-knot nematode, Meloidogyne spp. in the upper or lower depths. Also, Osman (1979) found that oxamyl and carbofuran were effective in the lower depths than in the upper in comparison with phenamiphos. Oxamyl and carbofuran are weakly sorbed by soil and so readily available for leaching (Bromilow, 1973 and Singh et al., 1994). Terbufos, while displayed low mobility and poor leaching, strongly sorbed by soil and the highest adsorption coefficient "K" was shown in the soil rich in organic matter content (Felsot et al., 1982). On the other hand, terbufos was rapidly oxidized in soil to sulfoxide and then to sulfone and both metabolites clearly showed the characteristics of leachers (Bowman and Sans, 1982)

Efficient translocation of systemic pesticides seems to be a function of their water solubility. Materials of low water solubility take much longer to be absorbed in toxic amounts from the soil than more water soluble systemics (Lichtenstein and Schulz, 1965). Abdel-Latif et al., (1967) found that translocation of carbofuran was controlled by both the organic matter and clay content. Also, Felsot et.al., (1982) reported that terbufos was generally immobile and its translocation pattern was similar to that of dieldrin, whereas translocation of carbofuran is at least 100 times more than terbufos or dieldrin.

The concentration of each nematicide recovered from the two soil types in leached water at 20 cm depth was estimated using *Culex* larvae in respect to its standard curve (Fig.1) and presented in Table 4. No residues of carbofuran and terbufos were recovered from clay loam soil using 500 ml

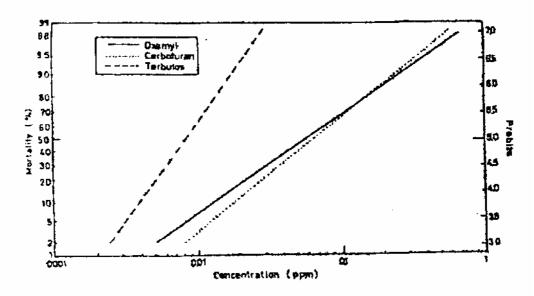


Fig. (1): Dosage-mortality regression lines for the contact toxicity of carbofuran, oxamyl and terbufos against *Culex pipiens* larvae.

water, while 0.008 ppm of oxamyl were detected. On the other hand, low quantities of carbofuran and oxamyl (0.012 and 0.013 ppm) were detected from columns packed with sandy clay loam soil, while terbufos was not detected when the same amount of water was applied. The concentration of carbofuran and oxamyl were a little bit higher using 1000 ml elution volume especially from sandy clay loam soil except terbufos, which was not detected in the case of clay loam soil. The concentrations of the tested nematicides in leached water are too much lower below the maximum

admissible level set by EPA (1998) for drinking water (0.04 ppm for carbofuran and 0.2 ppm for oxamyl).

Table (4): Nematicide residues in leached water at 20 cm depth of the two tested soil types.

Nematicide	Leaching water	Residue recovered Culex pip	
	volume (ml.)	Sandy clay loam soil	Clay loam soil
Carbofuran	500	0.012	0.000
	1000	0.018	0.012
Oxamyl	500	0.013	0.008
•	1000	0.021	0.013
Terbufos	500	0.000	0.000
	1000	0.003	0.000

In conclusion, the systemic action of the tested nematicides for controlling nematode depends upon soil type, the amount of water applied to the soil and the physicochemical properties of nematicides. Also, it could be concluded that both solubility and adsorptivity of the nematicides are important factors which affect the soil or water pollution.

Although some variability was encountered in similar experiment, nematodes such as *M. incognita* have considerable potential as biomonitors for quantifying nematicide movement in soil. Also, this study confirms the characteristics of contaminants of these nematicides, as reported in the literature.

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إستخدام نيماتودا تعقد الجذور "مليودوجين أنكوجنيتا" لرصد حركة المبيدات النيماتودية في التربة

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تم دراسة غسيل ثلاثه من المبيدات النيماتودية (كاربوفيوران، أوكساميل، تربيوفوس) من التربة وعلاقة ذلك بالكفاءة ضد نيماتودا تعقد الجذور على محصول الطماطم بإستخدام النيماتودا ككائن حيوى لرصد حركة المبيدات النيماتودية في التربة وقد تم ذلك في الأعمدة وتحت الظروف المعملية، بالإضافة إلى أنه قد تم تقدير متبقيات هذه المبيدات منفصله في ماء الغسيل بيولوجيا باستخدام يرقات البعوض. وقد أوضحت النتائج أن الكاربوفيوران والأوكساميل قد تم غسيلهم بسر عه من التربة الطوية الطينية اللومية ومع ذلك فإن هذين المبيدين يغسلا بسهولة من نوعي التربة وقد أعطت مكافحة ضد النيماتودا في القطاعات السفلية عن القطاعات العلوية، والتربيرفوس على العكس من ذلك وقد تم كشف كميات بسيطة من الكاربوفيوران العلوية، والتربيرفوس على التربة بينما التربيوفوس موجودا بكميات ضئيلة في ناتج غسيل التربة الرملية اللومية، وأن التركيز المتحصل عليه من العمود له علاقة طردية مع كميات المياه التربة على عمود التربة.