Effects of Selected Insecticides and Nematicides on the In Vitro Development of the Entomogenous Nematode Neoaplectana carpocapsae[1]

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Abstract: The effects of organophosphates (mevinphos, phenamiphos, trichlorfon), carbamates (carbofuran, methomyl, oxamyl), a formamidine (chlordimeform), a synthetic pyrethroid (fenvalerate), a chlorinated hydrocarbon (methoxychlor), and an insect growth regulator (diflubenzuron) on in vitro development and reproduction of *Neoaplectana carpocapsae* were tested by incorporating each chemical into a nematode rearing medium. Organophosphates and carbamates adversely affected development and reproduction at concentrations $\geq$ 0.1 mg/ml. Phenamiphos was the most toxic, with no nematode reproduction at 0.01 mg/ml. Inoculated infective juveniles developed to adults with some of the organophosphates and carbamates, but limited or no reproduction occurred. Chlordimeform inhibited development at 1.0 mg/ml, while diflubenzuron, fenvalerate, and methoxychlor did not significantly ($P > 0.05$) reduced reproduction at 1.0 mg/ml. The organophosphate and carbamate nematicides in use for control of plant-parasitic nematodes may be toxic to *N. carpocapsae* in the soil. *Key words:* organophosphates, carbamates, integrated pest management, biological control.

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The use of chemical pesticides and biological control agents in integrated pest management (IPM) has received much interest. IPM attempts to maximize the effectiveness of chemical pesticides against the target organism, while minimizing their adverse effects on biological control agents and other nontarget organisms. A potential biological control agent that could be used in IPM programs is the entomogenous nematode, *Neoaplectana carpocapsae,* and its mutualistic associated bacterium, *Xenorhabdus nematophilus. Neoaplectana carpocapsae* infects an insect host when the infective juveniles enter the mouth, anus, or spiracles, penetrate into the hemocoel, and release the bacterial cells from their intestinal lumen. The bacterium multiplies rapidly and kills the host. The nematodes feed upon the bacterial cells and host tissues and develop to adults which reproduce sexually. The infective juveniles leave the host cadaver and infect new insect hosts.

Ideally, chemical pesticides should have no toxic effects on *N. carpocapsae* for the most effective use of this nematode in IPM programs. Dutky (2) and Welch (21) reported that the infective juveniles of *N. carpocapsae* were compatible with many chlorinated hydrocarbon and organophosphate insecticides in water solution or suspension: certain fungicides, herbicides, miticides, and nematicides had little or no adverse effect. Dutky (2) concluded that pesticide tolerance of *N. carpocapsae* enhances its use in integrated control programs. However, not all chemical pesticides are compatible with *N. carpocapsae.* Prakasa Rao et al. (17) tested the toxicity of various organophosphate insecticides to infective juveniles in water suspension and obtained 90–100% mortality after 24 h. Similarly, Fedorko et al. (4,5) found that certain carbamates were toxic. Furthermore, Kamionek (11) observed that *N. carpocapsae* did not develop in larvae of the Colorado potato beetle, *Leptinotarsa decemlineata,* and the greater wax moth, *Galleria mellonella,* when an organophosphate nematicide was applied to the host insects 48 h after invasion by *N. carpocapsae.* She emphasized that while some chemical pesticides do not cause direct mortality to the infective juveniles, they may influence the development of *N. carpocapsae* in treated hosts in the field. Except for Kamionek's important observations of nondevelopment in pesticide-treated hosts, we are unaware of any reports of the effects of chemical pesticides on the development of *N. carpocapsae.* The selective nature of the various classes of insecti-

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cides and nematicides on *N. carpocapsae* may determine whether a particular chemical can be integrated with *N. carpocapsae*, so both can be used in IPM programs. This is particularly important if *N. carpocapsae* is used as a long-term biological control agent, where recycling of the nematode in the environment is desired. Studies were thus initiated on the effects of various classes of insecticides and nematicides on the in vitro development and reproduction of *N. carpocapsae*.

MATERIALS AND METHODS

Chemicals: Chemicals representing different classes of insecticides and nematicides were tested against *N. carpocapsae*. The following technical grade insecticides (I) and nematicides (N) provided by the manufacturers were tested: organophosphates—mevinphos (I) (2-carbomethoxy-1-methylvinyl dimethyl phosphate), phenamiphos (N) (ethyl 3-methyl-4-[methylthio] phenyl [1-methylethyl] phosphoramidate), trichlorfon (I) (dimethyl [2,2,2-trichloro-1-hydroxyethyl] phosphonate); carbamates—carbofuran (I and N) (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), methomyl (I) (S-methyl-N-[(methyl carbamoyl) oxy] thioacetimidate), oxamyl (I and N) (methyl N′, N′-dimethyl-N-[(methyl carbamoyl) oxy]-1-thiooxamimidate); formamidine—chlordimeform (I) (N′-[4-choloro-o-tolyl]-N,N-dimethylformamidine); chlorinated hydrocarbon—methoxychlor (I) (2,2-bis [p-methoxphenyl]-1,1,1-trichloroethane); synthetic pyrethroid—fenvalerate (I) (cyano [3-phenoxyphenyl] methyl 4-chloro-alpha-[1-methylethyl] benzeneacetate); benzoylphenyl urea—diflubenzuron (insect growth regulator) (N-[[[4-chlorophenyl] amino] carbonyl]-2,6-difluorobenzamide). Each chemical except diflubenzuron was diluted in reagent grade acetone. Diflubenzuron was diluted in reagent grade dimethyl sulfoxide (DMSO). New chemical stock solutions were prepared for each replicate.

Dog food medium and incorporation of chemicals: The dog food/agar medium developed by Hara et al. (8) was used in all tests. After autoclaving, the medium was cooled to ca. 60 C. The appropriate concentration of the chemical was added to the medium, thoroughly mixed, and 25-ml aliquots poured into sterile petri dishes (100 × 15 mm). Dilutions of the chemicals were made to final concentrations of 0.001, 0.01, 0.1, and 1.0 mg/ml in the dog food medium. Untreated and solvent treated medium (at 1% v/v) served as controls. There were six dishes per treatment; treatments were replicated three times.

Nematode inoculation: After cooling to ca. 25 C, each dish was inoculated with ca. 100 infective juveniles of the A11 strain of *N. carpocapsae* in 0.3 ml of sterile water. The nematodes were obtained from monoxenic stock cultures maintained on pork-kidney peptone agar medium in 15 × 150-mm culture tubes (3,8). Dishes were sealed with Parafilm and incubated at 25 ± 1 C for 14 Days.

Bioassay: At 14 days postinoculation, 10 ml of physiological saline (0.65% NaCl) were added to the culture, swirled, and the nematode suspension decanted; counts of juveniles and female and male adults were made. Cultures were scored from 1 to 7 based on the number of live juveniles and adults (Table 1). A score of 1 indicated no nematode development in the presence of inoculated infective juveniles, or if adults were present, there were fewer than 10. A score of 2 indicated more than 10 but fewer than 50 infective juveniles, and more than 10 but fewer than 100 adult nematodes were observed on the plates, etc. A score of 7 indicated maximum nematode development and reproduction. Population scores were analyzed by nonparametric statistics using Kruskal-Wallis test (23).

Table 1. Population scores for *Neoaplectana carpocapsae* based on number of live juveniles and adults, at 14 days postinoculation.

Scores	Number of nematodes*	
	Juveniles	Adults
1	≧0†	≧0
2	$>1.0 \times 10^1$	$>1.0 \times 10^1$
3	$>5.0 \times 10^1$	$>1.0 \times 10^2$
4	$>1.0 \times 10^3$	$>1.0 \times 10^3$
5	$>1.0 \times 10^4$	$>1.0 \times 10^4$
6	$>1.0 \times 10^5$	$>5.0 \times 10^4$
7	$>1.0 \times 10^6$	$>5.0 \times 10^4$

*Number of live juveniles and adults in 10 ml saline samples of dog food culture.

†Inoculated infective juveniles only.

Further tests on the effects on nematode development of phenamiphos (0.005–0.05 mg/ml) and methomyl (0.01–0.10 mg/ml) were conducted. Counts of live juveniles and female and male adults were made as previously described. If nematode progeny occurred in methomyl and phenamiphos treated medium, the infective stages were tested for infectivity by a modification of Dutky's method (3,12). A fifth instar *Spodoptera exigua* was placed in a 60 × 15-mm petri dish containing ca. 200 infective juveniles on a 5.5-mm-d filter paper (Whatman No. 1) disc. Larvae of *S. exigua* were exposed to the nematodes for 48 h at 25 ± 1 C, washed in 0.5% sodium hypochlorite, and placed on another moist filter paper in a dish. Five days after exposure to the nematodes, each larva was dissected and examined for presence of nematodes. There were three replicates with 10 insects per replicate.

RESULTS

All organophosphates and carbamates significantly ($P < 0.05$) affected the development and reproduction of *N. carpocapsae* at $\leq$ 1.0 mg/ml, while acetone and DMSO at 1% (v/v) showed no significant effects (Table 2). Phenamiphos was the most toxic, with significant effects on development and reproduction at 0.01 mg/ml. Trichlorfon, mevinphos, methomyl, and carbofuran were moderately toxic, with significant effects at 0.1 mg/ml. Oxamyl was the least toxic, with significant effects at 1.0 mg/ml.

The effects of organophosphates and carbamates on the development and reproduction of *N. carpocapsae* varied with the particular chemical and concentration. For example, with 0.01 mg phenamiphos/ml or 0.1 mg trichlorfon/ml, some of the infective juveniles developed to adults but they did not reproduce. At 1.0 mg/ml of phenamiphos or trichlorfon, only dead inoculated infective juveniles were present. Mevinphos and carbofuran significantly reduced reproduction at 0.1 mg/ml, and only inoculated infective juveniles (< 10% survival) and few adults (< 10% survival) occurred at 1.0 mg/ml.

Table 2. Toxicity of organophosphate and carbamate insecticides and nematicides on in vitro rearing of *Neoaplectana carpocapsae*.

Concentration (mg/ml)	Mean population scores*		
	Organophosphates		
	Phenamiphos	Trichlorfon	Mevinphos
0.0 (U.C.)†	6.4a	6.1a	6.1a
0.0 (S.C.)‡	6.4a	5.8a	6.2a
0.001	6.7a	6.1a	5.6a
0.01	1.7b	5.5a	5.9a
0.1	1.0b	1.6b	4.6b
1.0	1.0b	1.0b	1.0c
	Carbamates		
	Methomyl	Carbofuran	Oxamyl
0.0 (U.C.)	6.7a	6.2a	4.9a
0.0 (S.C.)	6.3a	5.6a	5.2a
0.001	6.6a	5.4a	4.9a
0.01	6.1a	5.4a	5.7a
0.1	1.0b	3.8b	4.6a
1.0	1.0b	1.4c	1.2b

*Each population score is the mean of 18 dog food nematode cultures per treatment (1 = least development and reproduction to 7 = maximum development and reproduction). All scores followed by different letters in a column are significantly different ($P < 0.05$) according to nonparametric multiple comparisons.

†U.C. = untreated control.

‡S.C. = solvent control (1% v/v).

Infectivity, development, and reproduction of nematodes from cultures treated with 0.005 mg/ml phenamiphos or 0.01 mg/ml methomyl were not adversely affected (Table 3). At 0.01 mg phenamiphos/ml or 0.03 mg methomyl/ml, only a few live female and male adults were found on the surface of the medium. The female-to-male sex ratio also increased with methomyl and phenamiphos. At moderate concentrations (0.03 mg/ml for phenamiphos and 0.05 mg/ml for methomyl), a few live females were observed, while all males were dead. At higher concentrations (0.5 mg/ml for phenamiphos and 0.1 mg/ml for methomyl), only dead inoculated infective juveniles were present.

Chlordimeform significantly reduced nematode development and reproduction at 1.0 mg/ml (Table 4). However, the chlorinated hydrocarbon (methoxychlor), the synthetic pyrethroid (fenvalerate), and the insect growth regulator (diflubenzuron) had no significant effect ($P > 0.05$) at concentrations as high as 1.0 mg/ml.

No visual difference was observed in bacterial growth on the medium of the various treatments. In cultures with no nematode development, the surface was covered with bacteria and contained only live and dead inoculated infective juveniles.

DISCUSSION

This study showed that the tested organophosphates and carbamates have sublethal and lethal effects on *N. carpocapsae,* depending on the concentration of the chemical. After 14 days, reduced reproduction, and development to adults but no reproduction, occurred at lower tested con-

Table 3. Number of live juveniles and female and male adults of *Neoaplectana carpocapsae* observed in cultures treated with phenamiphos or methomyl at different concentrations.

Chemical	Concentration	Mean (range)*			Sex ratio†
		Juveniles	Females	Males	
Phenamiphos	0.005‡	$1.8(1.6\text{-}2.4)\times10^{6}$	$6.2(3.8\text{-}8.0)\times10^{4}$	$3.9(3.1\text{-}5.1)\times10^{4}$	1.6:1
	0.01	0	10.3(4-12)	0.9(0-3)	10.9:1
	0.03	0	0.7(0- 3)	0	...
	0.05	0	0	0	...
Methomyl	0.01‡	$1.8(1.4\text{-}2.4)\times10^{6}$	$6.5(4.9\text{-}8.5)\times10^{4}$	$4.2(2.9\text{-}6.0)\times10^{4}$	1.6:1
	0.03	0	11.3(7-17)	1.8(0-5)	6.2:1
	0.05	0	7.6(2-15)	0	...
	0.10	0	0	0	...

*Mean of 18 culture dishes per treatment.
†Based on mean number of female to male adults.
‡No difference from control cultures.

Table 4. Effects of formamidine (F), chlorinated hydrocarbon (CH), synthetic pyrethroid (SP), and insect growth regulator (IGR) on in vitro development of *Neoaplectana carpocapsae.*

Concentration (mg/ml)	Mean population scores*			
	Chlordimeform (F)	Methoxychlor (CH)	Fenvalerate (SP)	Diflubenzuron (IGR)
0.0 (U.C.)†	5.1a	6.5a	5.7a	6.1a
0.0 (S.C.)‡	5.6a	6.1a	5.7a	6.2a
0.001	5.4a	5.7a	5.6a	6.2a
0.01	5.8a	5.6a	6.1a	6.0a
0.1	5.8a	6.4a	5.6a	6.1a
1.0	1.0b	5.8a	6.0a	5.8a

*See same footnote, Table 2.
†U.C. = untreated control.
‡S.C. = solvent control.

centrations. The increase in live female-to-male sex ratio observed in treated cultures (Table 3) was probably due to more male adults being killed than female adults. The mechanism for differential toxicity between sexes is unknown. At higher concentrations, inoculated infective juveniles were alive but failed to develop or died during the 14-day test period.

The effects of the tested organophosphates and carbamates to *N. carpocapsae* appear related to the mode of action of organophosphate and carbamate nematicides against plant-parasitic nematodes. The generally accepted mode of action of these nematicides is not the direct killing of the nematodes, but the adverse effects on various aspects of behavior and development. Some of the behavioral effects include inhibition of motility, dispersion, attraction of plant hosts, and attraction of males to females (7,14,15). Direct developmental effects include inhibition of egg production, hatching, and molting (10,16). Although it was beyond the scope of this study to determine exactly what aspects of *N. carpocapsae's* behavior and development might have been affected, our results indicate that some of the effects of organophosphates and carbamates are similar to plant-parasitic nematodes. Reduced reproduction, or reproductive failure, may be explained in part by the disruption of the nematode's mating behavior. Lack of juvenile development may be due to the inhibition of molting by the organophosphates and carbamates.

Because of the similar effects of organophosphates and carbamates on *N. carpocapsae* and plant-parasitic nematodes, some of the nematicides used for control of below-ground plant-parasitic species may be toxic to *N. carpocapsae* in the soil. For example, in this study, the organophosphate nematicide (phenamiphos) was the most toxic to *N. carpocapsae,* with inhibition of reproduction and development between 5 ppm (0.005 mg/ml) and 10 ppm (0.01 mg/ml). With plant-parasitic nematodes, phenamiphos was toxic between 0.3 ppm and 5 ppm in the laboratory (1,9,14,16). Furthermore, field application rates of nematicides (phenamiphos 5–10 ppm) are customarily slightly higher than the toxic concentration in the laboratory (9).

The three chemicals which exhibited no detectible toxicity to *N. carpocapsae* were the chlorinated hydrocarbon (methoxychlor), synthetic pyrethroid (fenvalerate), and the insect growth regulator (diflubenzuron). Diflubenzuron did not significantly reduce reproduction, development, or infectivity in *N. carpocapsae* at concentrations as high as 1.0 mg/ml. Veech (18,19, 20) reported that diflubenzuron inhibited reproduction in the root-knot nematode *Meloidogyne incognita* and the free-living nematodes *Pelodera* sp., *Acrobeloides* sp., and *Panagrellus redivivus* at concentrations greater than 0.01, 0.1, and 1.0 mg/ml, respectively. Veech concluded that diflubenzuron may inhibit reproduction by interfering with chitin synthesis in the egg shell. The reasons for the nonsensitivity of *N. carpocapsae* to diflubenzuron is unknown.

Neoaplectana carpocapsae has been successfully used against certain insect species in ecological situations that favor its survival and infectivity (13). At the present time, *N. carpocapsae* is most promising against boring or subterranean pestiferous insects whose life stages occur in moist habitats (6). If adequate control cannot be achieved with *N. carpocapsae* alone, other tactics that can control the same and/or other life stages may have to be integrated. As observed in this study, some chemicals, in particular the organophosphates and carbamates, are toxic to *N. carpocapsae,* and, therefore, compatibility tests between a particular chemical and *N. carpocapsae* are needed before both can be used in IPM programs. This is especially true if establishment of the nematode is desired in the environment where nematode reproduction in the pest species is of primary importance.

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