EFFECT OF SOME INSECTICIDES ON ACETYLCHOLINESTERASE FROM BENEFICIAL INSECTS: *COCCINELLA SEPTEMPUNCTATA, CHRYSOPERLA CARNEA* AND *FORFICULA AURICULARIA*

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ABSTRACT

In vitro enzyme activity of head homogenates from adults of *Coccinella septempunctata*, *Chrysoperla carnea* and *Forficula auricularia* originated from different habitats in Belgium (wheat, barley, rye, set-aside fields and experimental orchard, uncultivated area) were investigated in presence of insecticide active ingredients. Using the procedure of Ellman, I_{50} (M) and Ki (M^{-1} min^{-1}) values were established. The beneficial insects showed the least susceptibility to diazinon and the differences between their measured values were not remarkable. Paraoxon was extremely toxic to the AChE of *F. auricularia* but *Ch. carnea* and *C. septempunctata* were similarly more tolerant to this organophosphate. In the case of malaoxon earwig and green lacewing AChEs were much more sensitive than AChE of the ladybird beetle. Measuring the carbaryl inhibition, *F. auricularia* was the least tolerant. The susceptibility of ladybird AChE differed highly from that of both species. According to the measured values, the green lacewing was less tolerant than the ladybird beetle but more tolerant compared with the common earwig. Summarizing our biochemical results, the order of susceptibility of beneficial insects to insecticides investigated was the following *F. auricularia* > *Ch. carnea* > *C. septempunctata*.

INTRODUCTION

The reintroduction or release of beneficial arthropods and the preservation of their local populations are important tools within integrated pest management (IPM) as ecologically safe techniques. However pesticides are also used in modern agriculture as biological control alone most often does not solve all pest and disease problems. The most crucial requirement for pesticides is that they must be compatible with biological control. Therefore only those pesticides should be used, which are most selective and which have acceptable adverse effects on beneficial organisms (Hassan 1989, Cross and Dickler, 1994; Francis *et al.*, 2001). It may also be helpful to find and select tolerant or resistant strains of natural enemies (Hoy, 1985; Grafton-Cardwell and Hoy, 1985). In this case, we have to be aware that only the selected strain of a species will tolerate the pesticide application and that other populations of that species still can be harmed. A prerequisite for an environmentally safe plant protection is the extensive study of the effects of pesticides on beneficial species.

Organophosphorous and carbamates are widely used in agriculture to control insect pests. Directly released into the environment, these compounds are known to be potential inhibitors of acetylcholinesterase (AChE) activities in beneficial insects (Boszik *et al.*, 1996). AChE is an important regulatory enzyme involved in the nerve impulse transmission across cholinergic synapses. It is responsible for the hydrolysis of acetylcholine (chemical transmitter of nerve impulses at synaptic nerve endings) to choline and acetic acid (Smallman and Mansingh, 1969; Matsumura, 1985).

The aim of the present study was to analyze the detrimental effects of some organophosphorous and carbamate insecticides on acetylcholinesterase(s) (AChE) of the adults of some useful species: seven-spot ladybird beetle, *Coccinella septempunctata* L., common green lacewing, *Chrysoperla carnea* Stephens and common earwig, *Forficula auricularia* L.. In addition, this analysis may contribute to the better knowledge of the AChEs of the above mentioned beneficials, that have been little or not studied.

MATERIALS AND METHODS

Insects

C. septempunctata adults were collected in Gödöllo (Hungary) and Gembloux (Belgium). One Hungarian (from a rye field) and six Belgian strains (from a rye, a barley, two different wheat, a set-aside fields and from the botanical garden of the Gembloux Agricultural University) population samples were used. The adults of *Ch. carnea* were caught in Gembloux (wheat field, park, experimental orchard of the University, natural reserve) in 1993 and 1995, and in Gödöllo (uncultivated area) in 1995. *F. auricularia* adults were collected in Gembloux (experimental orchard of the University) in 1995. Captures were obtained using a sweeping net. Insects were used for analyses immediately after capture.

Chemicals

All insecticide active ingredients were commercially purchased: paraoxon, malaoxon, diazinon, carbaryl (Riedel-de Haën AG, Germany). We obtained 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and acetylthiocholine chloride (ACh) from Sigma Chemical Co. Bovin serum albumin (BSA) used for the protein quantification was purchased from Bio Rad®.

Inhibition experiments

Preparation of homogenates

Thirty ladybird individuals were decapitated and their heads were homogenized with 2 ml cold HST buffer (Tris HCl 1 mM, pH 8; NaCl 1 M; Triton X-100 1% wt/vol; $MnCl_2$ 1 mM; $CaCl_2$ 1mM) in a motor-driven, Teflon pestle and glass tube tissue grinder. This homogenate was filtered through a thin layer of glass wool and centrifuged 15 min at 15300 g and 4 °C. Immediately after centrifugation the supernatant was used for AChE assays. This procedure was also made with the other two species using 40 and 10 individuals for lacewing and earwig respectively. A new homogenate was used for each tested pesticide.

AChE assay

Protein determination was performed using the original Lowry method (Lowry *et al.* 1951). The content was then mixed and after 15 min of reaction the absorbance was read at 750 nm. AChE activity determination was carried out using the method of Ellman (Ellman *et al.* 1961). The supernatant (50 μl) was added to a 1.5ml-tube containing 930 μl of 0.1 M phosphate buffer (pH 7.5), 10 μl of 100 mM DTNB and 10 μl of 0.1 M acetylthiocholine chloride (AChE). The content was then mixed and the absorbance read continuously for 1 min at 412nm using a Shimadzu 160A UV spectrophotometer. Enzyme activity unit was reported as mmol of product formed per mg protein per minute. Percentages of AChE inhibition was derived by expressing the activity levels of exposed animals as a percentage of the activity in controls. At least duplicate analyses at each of 5-10 concentrations of pesticides were carried out. Statistical significance of I_{50} and K_i values was evaluated by t-test (Sváb, 1981).

RESULTS

When measuring AChE susceptibility in ladybird head homogenates (populations sampled in 1993) to paraoxon, no anomaly was observed until 10^{-4} M concentration. Increasing paraoxon concentration increased also the inhibition of AChE. After the latter concentration value the AChE inhibition decreased for each tested seven-spot population. The repetition of the assay with paraoxon resulted in variable inhibition values. Relationship between paraoxon concentrations and AChE inhibition was determined using linear regression. All data pairs, the ones until 10^{-4} M (4 data) and the ones from 10^{-4} M (6 data) were separately plotted and analyzed. Three different shapes of relationship with strong and very strong positive correlation were found. Correlation coefficient was higher when considering separated data, and inversely, much higher standard error was characteristic for the unseparated data (14.83 compared to 0.99 and 0.84 related to separated data). Also the regression coefficients (slope) and the intercepts differed remarkably. This indicates that *C. septempunctata* must have two different forms of AChE (a highly sensitive and a more tolerant) according to their response to paraoxon. Although the number of repetitions did not allow statistical analysis of the inhibition parameters of insects from different habitats, the BarleyGe population seems to be more tolerant toward paraoxon than the other ones (Table 1).

Table 1 - Susceptibility of AChE to Paraoxon and Malaoxon of *Coccinella septempunctata* Adults Originated from Different Habitats at Gödöllo (Hungary) and Gembloux (Belgium)

Ladybird Strains	Agricultural habitats	Paraoxon		Malaoxon	
		I_{50} (M)	K_i ($M^{-1}min^{-1}$)	I_{50} (M)	K_i (M^{-1} min^{-1})
Gödöllö	Rye	2.3×10^{-4}	3075.8	7.0×10^{-5}	9854.9
Gembloux	Rye	4.9×10^{-4}	1414.3	8.4×10^{-5}	8283.9
	Barley	1.8×10^{-3}	373.9	8.6×10^{-5}	8086.0
	Wheat	3.0×10^{-4}	2277.7	7.8×10^{-5}	8950.2
	Wheat	5.2×10^{-4}	1347.8	5.3×10^{-5}	13037.9
	Set-asides	2.7×10^{-4}	2563.0	8.9×10^{-5}	7832.4

In brackets standard deviation. * indicates statistical significance at $P < 0.0001$ level. $K_i = 0.695/I_{50}\ t$

As the malaoxon inhibition, no anomaly was found with paraoxon. The susceptibility of ladybird AChE(s) to paraoxon and malaoxon differed. The latter was more effective than paraoxon. Comparing the mean values of their I_{50} and K_i parameters with two tailed t-test, the I_{50} values did not significantly differ from each other, but K_i values did. The inhibition parameters of studied seven-spot ladybird populations to malaoxon did not reveal any difference (Table 1). Diazinon and carbaryl were tested only on one ladybird population of Gembloux (from botanical garden) in 1995. Carbaryl highly inhibited the ladybird AChE but diazinon proved to be much weaker inhibitor (Table 2). The pesticide efficiency against the ladybird AChE(s) was the following: carbaryl > malaoxon > paraoxon > diazinon.
Only five populations of the common green lacewing were tested. Diazinon as before proved to be the weakest inhibitor. Malaoxon and carbaryl were the most active pesticides and paraoxon was only moderately harmful to lacewing AChE.

Table 2 - Susceptibility of AChE to Paraoxon and Malaoxon of *Chrysoperla carnea* Adults Originated from Different Habitats at Gödöllo (Hungary) and Gembloux (Belgium).

Ladybird Strains	Agricultural habitats	Paraoxon		Malaoxon	
		I_{50} (M)	K_i ($M^{-1}min^{-1}$)	I_{50} (M)	K_i (M^{-1} min^{-1})
Gödöllö	Fallow Field	nd	nd	4.3×10^{-5}	162511.1
Gembloux	Wheat	1.9×10^{-4}	3531.5	4.8×10^{-5}	14363.6
	Set-asides	1.3×10^{-4}	5299.3	6.1×10^{-5}	11397.6
	Woodland	1.0×10^{-5}	67905.8	3.8×10^{-5}	18239.6

nd = not determined

Due to the weak repetition number, it was not possible to calculate significant differences between the values of the various chrysopid populations. Nevertheless, the Hungarian uncultivated area collected population seems to be more susceptible to malaoxon than the Belgian ones, and similarly the woodland population from Gembloux showed considerably higher susceptibility to paraoxon than the other ones (Table 3).

Table 3 - Susceptibility of AChE to Paraoxon and Malaoxon of *Forficula auricularia* Adults originated from Gembloux

Biochemical parameters	Insecticide	
	Paraoxon	Malaoxon
I_{50} (M)	3.6×10^{-6}	193188.3
K_i (M^{-1} min^{-1})	5.5×10^{-6}	126086.3

The pesticide efficacy against the lacewing AChE was the following: malaoxon > carbaryl > paraoxon > diazinon. Only one *F. auricularia* population sample was tested against the above mentioned chemicals. Diazinon showed the lowest efficiency. Its I_{50} and K_i values were very similar to those assessed on ladybird and lacewing. As to the other inhibitors, earwig's AChE was extremely susceptible to them. Their I_{50} and K_i values were very higher than the corresponding values on ladybird and lacewing (Table 4). Insecticide active ingredient efficiency against the earwig AChE can be classifed as following: paraoxon > carbaryl > malaoxon > diazinon.

Table 4 - Susceptibility of AChE to Diazinon and Carbaryl of *Coccinella septempunctata, Chrysoperla carnea, Forficula auricularia* Adults originated from Gembloux (Belgium)

Natural enemies	Diazinon		Carbaryl	
	I_{50} (M)	K_i (M^{-1} min^{-1})	I_{50} (M)	K_i (M^{-1} min^{-1})
C. septempunctata	3.1×10^{-3}	221.1	7.5×10^{-6}	92714.1
C. camea	5.4×10^{-3}	129.8	4.8×10^{-5}	14615.0
F. auricularia	3.4×10^{-3}	206.7	4.26×10^{-6}	163175.3

DISCUSSION

Considering the differences between the diverse populations of natural enemies, it seems that the ladybird populations responded quite alike to the inhibitors, except the sample from barley, that proved to be more tolerant to paraoxon. The common green lacewing populations exhibited greater dissimilarity between each other. Sample from Gembloux woodland was really more susceptible to paraoxon than the others. Moreover, the Hungarian uncultivated sample presented a very high susceptibility to malaoxon. According to the response of the inhibition process to paraoxon, the seven-spot ladybird seems to have at least two forms of AChE responding differently for the paraoxon inhibition. In order to confirm with certitude this hypothesis a more sophisticated analysis of AChE of *C. septempunctata* will be carried out in the near future.

All the three beneficial species showed the least sensitivity to diazinon and the measured values were very similar. *F. auricularia* was very susceptible to paraoxon while *Ch. carnea* and *C. septempunctata* tolerated it much better. As toward malaoxon, earwig and lacewing AChEs' reaction were much stronger, causing considerable inhibition. Carbaryl proved to be extremely toxic to *F. auricularia*. The susceptibility of ladybird and lacewing AChE(s) did not reach the ten part of that of earwig AChE. Summarizing the results, the order of tolerance of the considered natural enemies was: *C. septempunctata* > *Ch. carnea* > *F. auricularia*.

In general, the pesticide toxicity assessment on a natural enemy is made empirically, i.e. by standard tests (laboratory, semi-field, field tests) reporting recommended rates of commercially available pesticide formulations in order to estimate acceptable toxic effect (Hassan *et al.*, 1985, Hassan, 1988, Vogt, 1994; Colignon *et al.*, 2001). In most cases, this kind of tests cannot explain the nature of toxic processes. It can only give an answer on the direct toxicity of a tested pesticide rate on a beneficial species. This is of course a valuable information for the practical use of the pesticide as it indicates its compatibility or incompatibility with IPM. Nevertheless, there is another fascinating opportunity for the acetylcholinesterase (AChE) inhibiting insecticides: the determination of the interaction of the AChE of a natural enemy with insecticides (Boszik *et al.*, 1996). The determination of the AChE activity is a useful tool. Indeed, enhanced activity of detoxifying enzymes (monooxidases, esterases) also reduced susceptibility of AChE. Enzyme inhibition induced by an insecticide can be implicated in the tolerance or resistance of an organism. Analysis and characterisation of the natural enemies AChE and its response to inhibitors provides an effective method for measuring and comparing natural or changed tolerance of different species or different field populations of a species, for monitoring field applications with regard to potential adverse effects and for detecting the pesticide pressure in a habitat.

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