

Comparative Toxicity Study between Some Organophosphorus and Abamectin Insecticides Using Earthworm (*Lumbricus Terrestirs*) as A Biomarker

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ABSTRACT

Organophosphorus insecticides are widely used in industry, agriculture, horticulture, domestic, and for public health applications. The consumption of insecticides in the household has almost risen exponentially. Over this half century there has been a significant increase in developmental disorders observed in humans which are thought to have an association with the exposure to pesticides in home environment causing family stress. In addition, abamectin as biocide is recently used as an insecticide. Very little information is available with regard to the sub-lethal effects of abamectin; therefore, the purpose of this work is to compare the toxicity of abamectin as a biocide with malathion and dimethoate as organophosphorus insecticides using the earthworm (*Lumbricus terrestris*) as a biomarker. The earthworm was exposed to the tested compound in artificial soil for 7 days. The LC₅₀ of these insecticides was determined. The effects of two concentrations (LC₅₀ & LC₂₅) of these insecticides were studied on the activity of acetylcholinesterase (AChE), glutathione-S-transferase (GST), acid and alkaline phosphatases (AcP & AIP), and the levels of the two neurotransmitters; gamma amino butyric acid (GABA) and L-glutamic acid. The data showed that the LC₅₀ of abamectin, dimethoate and malathion was 2.99, 13.7, and 39.69 mg/ kg, respectively. This means that abamectin was the most toxic, while malathion was the least toxic. Both LC₅₀ & LC₂₅ concentrations of dimethoate and malathion had significant inhibitory effect on the AChE activity, while abamectin had significant excitatory effects. AcP and AIP activities were significantly increased with the LC₅₀ and LC₂₅ of all the tested insecticides. GST induction activity was significant by the treatment of dimethoate and malathion, while there was significant decrease after treatment with LC₅₀ of abamectin. But there was a non-significant difference with LC₂₅ of abamectin. The lethal and sub-lethal concentrations of the tested insecticides caused a significant induction in GABA and L-glutamic acid concentrations. The data showed that abamectin has the highest effect on the concentration of GABA and L-glutamic acid while dimethoate had the lowest effect. From these results, it could be concluded that abamectin had toxic effect compared to the tested organophosphorus insecticides, also the earthworm can be

considered a good sensitive biomarker to test the toxicity of different compounds.

Keywords: household insecticides; Malathion; Dimethoate; abamectin, earthworm

Abbreviations: AChE, acetylcholinesterase; GST, glutathione-S-transferase; GABA, gamma amino butyric acid; AcP, acid phosphatases; AIP, alkaline phosphatases; LC₅₀, lethal concentration; CNS, Central Nervous System.

INTRODUCTION

Several epidemiological studies have suggested that health concerns may arise from the chronic exposure of young children to pesticides in their home environment (Charlotte et al 2006). Bryan Ballantyne and Salem (2006) showed that organophosphate (OP) and carbamate (CM) anticholinesterases (anti-ChEs) have a wide spectrum of applications that include pesticides in agriculture and horticulture, insecticides in domestic and public health applications, use in general commerce, use in therapeutic medicine, and as chemical warfare and terrorist agents. It can be used safely with the appropriate recommended protective and precautionary measures. However, because of their widespread and sometimes uncontrolled usage, but there is a potential for misuse. Although the principal mechanism of action that underlies the practical use of OPs is namely inhibition of the cholinesterase group of enzymes, it can also be responsible for some of their known human toxicity, because of the wide range of chemical structure involved the potential for numerous and different toxic effects, that are mediated by other mechanisms that can exist e.g. inflammation, immunotoxicity, myopathy, genetic toxicity, oncogenicity and developmental and reproductive toxicity.

Unwise use of insecticides can cause human health hazards which place a stress on family budgets and reduce individual physical and mental capacity as well as efficiency in his work.

Abamectin, a recently biocide used as a commercialized insecticide in the region, besides other

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Received August 27, 2008, Accepted September 14, 2008

insecticides available in the market, thus, there is a need for tools to monitor the impact of such compound on non-target organisms.

Biomarkers are biological responses that can be related to the toxic effect of one or several pollutants (Peakall 1994). Therefore many biochemical markers of exposure in the earthworms have been recommended as useful sub-lethal criteria (Saint-Denis *et al.*, 1998; Paoletti, 1999).

Organophosphate and carbamate pesticides act by inhibiting cholinesterase's (ChE), which are a group of enzymes in the central nervous system (Edwards and Fisher 1991). Glutathione-S-transferase (GST) is involved in the detoxification of various xenobiotic chemicals. It is a very important enzyme to earthworm because of their role as general scavengers for lipophilic xenobiotics or endogenous formed waste products in earthworms (Stenersen *et al.*, 1979).

Enzymes assay in earthworm are being developed to give a measure of pesticide exposure and as biomarkers of toxicity. Therefore, the aim of the present study is (i) to investigate the effect of the lethal and sub-lethal concentrations of the abamectin, malathion and dimethoate on some biochemical markers: acetylcholinesterase (AChE), Glutathione-S-transferase (GST), acid phosphatases (AcP) and alkaline phosphatases (AIP); (ii) to study the level of two neurotransmitters gamma amino butyric acid (GABA) and L-glutamic acid in the earthworm, and (iii) study the comparative toxicity of the abamectin which was used as safer alternative compounds with some conventional OP's insecticides available commercially for agriculture and home uses specially, malathion and dimethoate as insecticides, by using earthworms as a biomarker.

MATERIALS AND METHODS

2.1. Experimental animal

Earthworms (*Lumbricus terrestris*) were collected from the garden of the Agriculture Research Center, Sabahia, Alexandria. The worms were acclimatized at the laboratory conditions (at room temperature and 12 hours light/12 hours dark) in the artificial soil (using an evenly blended dry weight mixture of 20% kaolin clay, 70% silica sand, 10% sphagnum peat and 0.3% calcium carbonate according to OECD (1984) guideline 207, before testing.

2.2. Insecticides

Organophosphorus insecticides; malathion (egythion, 57% EC) and dimethoate (egythoate, 40% EC) was obtained from the Egyptian Center for Agriculture Services, Biocide; abamectin (Cam-mec, 1.8% EC) was obtained from Trading Company for Agriculture Requirements. All other chemicals used in this study

were obtained from Sigma, Aldrich and BDH companies with high purity.

2.3. Experimental organism

Earthworms were divided into two groups; the first group was used to determine the toxicity of the tested insecticides (lethal concentrations; LC₅₀). The second one was to study the impact of the lethal and sub-lethal concentrations (LC₅₀ and LC₂₅) of these insecticides on the activity of some enzymes in tissues of earthworm. The experiments were performed with adult worms approximately 7.13 ± 1.5 cm in length and 0.523 ± 0.08 gm in weight with clitellum's.

2.4. Determination of the lethal pesticide concentrations

The toxicity was evaluated according to OECD (1984) guideline 207 by the artificial soil test. The insecticide was mixed into artificial soil as an aqueous solution to give the desired working concentration (six concentrations for each insecticide) with three replicates each including control tests soil contained water without pesticide. Each concentration was prepared in 450 grams of the artificial soil and was divided into three quantities in plastic cups.

Five worms were added to each cup and covered with cloth netting secured with rubber band to prevent worms from escaping. Mortality was assessed after 7 days and the LC₅₀ values were calculated according to Finney, (1971) and expressed as mg/kg soil.

2.5. Assessment of enzymes activity

Earthworms were exposed to the estimated concentrations from the initial bioassay as the median lethal concentration (LC₅₀) and sub-lethal concentration (LC₂₅) of the three insecticides for 7 days. The survival worms were homogenized in saline solution (1:10 W/V) and centrifuged for 30 minutes at 8000 rpm. The supernatants were used as sources of enzymes. AChE assay was performed spectrophotometrically utilizing the method of Ellman *et al.* (1961); the activity was expressed as μ moles acetylthiocholine hydrolyzed/ mg protein/minute. GST activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate according to Vessey and Boyer (1984). Acid and alkaline phosphatases were estimated by the method of Bessey *et al.* (1946). The GABA and glutamic acid contents were determined according to Maynert *et al.* (1962) and Pepeu *et al.* (1970). Samples were analyzed for protein content according to Lowry *et al.* (1951).

2.6. Statistical analysis

Regression lines of the different pesticides (LC₅₀'s) and confidence limits were calculated by probit analysis computer program according to Finney, (1971). Data of the toxicological study were analyzed by one-way

ANOVA to determine differences between treatments (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSIONS

3.1. Toxicity Test

Mortality has been the most frequently used parameter to evaluate the chemicals toxicity in earthworms (Liang and Zhou, 2003, Lydy and Linck, 2003). The toxicity of the tested insecticides against *Lumbricus terrestris* was illustrated in Table (1).

Table 1. The LC₅₀ of the earthworm (*Lumbricus terrestris*) after 7 days of artificial soil exposure to abamectin, dimethoate and malathion

Insecticides	LC ₅₀ mg/kg	95 % Confidence limits*		Slope
		lower	upper	
Abamectin	2.99	2.54	3.51	2.24
Dimethoate	13.7	11.98	15.65	2.44
Malathion	39.69	34.69	38.49	6.01

*P<0.05.

The tested insecticides could be arranged according to the LC₅₀'s values (mg/kg) and the toxicity index as follows: abamectin > dimethoate > malathion. The data showed that abamectin was the most toxic (LC₅₀; 2.99 mg/kg) while malathion was the least toxic (LC₅₀; 39.69 mg/kg) on the earthworm.

The data are parallel to Mosleh *et al.* (2003) who reported that earthworm; *Aporrectodea caliginosa* is a good biomarker for soil contamination by endosulfan

Table 2: The Lethal effects of malation, abamectin and dimethoate on the activities of enzyme activities and neurotransmitters of the earthworm (*Lumbricus terrestris*) after 7 days of artificial soil exposure

Parameter	Control	Experimental group					
		Malathion		Abamectin		Dimethoate	
		LC50	LC25	LC50	LC25	LC50	LC25
AChE	0.018 ± 0.0005 ^c	0.013 ± 0.0013 ^b	0.01 ± 0.0014 ^b	0.035 ± 0.004 ^d	0.029 ± 0.0014 ^d	0.006 ± 0.0008 ^a	0.017 ± 0.0 ^c
AcP	0.107 ± 0.002 ^a	0.853 ± 0.006 ^g	0.622 ± 0.002 ^f	0.272 ± 0.006 ^c	0.213 ± 0.003 ^b	0.478 ± 0.0011 ^d	0.604 ± 0.019 ^e
AIP	0.08 ± 0.011 ^a	0.564 ± 0.38 ^f	0.407 ± 0.01 ^e	0.256 ± 0.0 ^c	0.211 ± 0.003 ^b	0.334 ± 0.016 ^c	0.333 ± 0.008 ^d
GST	0.063 ± 0.003 ^b	0.07 ± 0.002 ^c	0.072 ± 0.005 ^c	0.064 ± 0.001 ^b	0.053 ± 0.004 ^a	0.105 ± 0.001 ^e	0.084 ± 0.004 ^d
GABA	0.468 ± 0.007 ^a	0.743 ± 0.006 ^b	0.640 ± 0.025 ^{ab}	0.93 ± 0.015 ^c	0.72 ± 0.006 ^b	0.649 ± 0.203 ^b	0.552 ± 0.009 ^a
L-glutamic	0.365 ± 0.006 ^a	0.633 ± 0.02 ^d	0.51 ± 0.003 ^c	0.849 ± 0.01 ^e	0.630 ± 0.008 ^d	0.492 ± 0.005 ^b	0.483 ± 0.006 ^b

Values are expressed as means ± SE; n=5 for each treatment group Means values within a row not sharing a common superscript letter (a-g) were significantly different, p<0.05.

AChE ; μ mol /mg protein/ min , AcP; μ moles P-nitro-phenol/mg protein/min,

AIP (μ moles P-nitro-phenol/mg protein/min, GST OD/mg protein/min, GABA and L-glutamic acid

(μ g /mg wet tissue.)

and aldicarb. Also the results of Hallely *et al.* (1993) for abamectin were parallel to the present finding, although the worms were of different genus. Aly (2005) showed that the earthworm is quite sensitive, when introduced into the soil system and could be used as a biological marker (biomarkers) for soil system contaminated with the pesticides.

3.2. The toxicological studies

The earthworms exposed to the contaminated artificial soil with LC₅₀ and LC₂₅ of the tested insecticides for 7 days were used to measure the activities of acetylcholinesterase (AChE), Glutathione-S-transferase (GST), Acid phosphatase (AcP), Alkaline phosphatase (AIP) and the neurotransmitters (gamma amino butyric acid; GABA and L-glutamic acid). Cholinesterase activity is routinely used in vertebrate animals to diagnose the exposure to organophosphates (Fairbrother *et al.*, 1991; Edwards and Fisher 1991). In the present study, AChE activity of the treated and untreated earthworms is presented in Table (2).

The data showed that both concentrations of dimethoate and malathion had significant inhibitory effect on the AChE activity. In contrast abamectin had significant excitatory effects at the lethal and sub lethal concentrations. These data are parallel to many investigators, who showed that malathion exposure reduced AChE activity in the cerebral cortex and hippocampus. Brocardo, *et al* (2005) reported that like other OP compounds; malathion is known to inhibit acetylcholinesterase (AChE) activity, an effect that is thought to express the neurotoxicity elicited by these

compounds (Kwong, 2002). Dongren *et al.* (2002) found that the activity of AChE was significantly inhibited in a dose and time-dependent manner when cells were exposed to dimethoate for 2 h. AChE activity was significantly inhibited 14 and 24 h after application of dimethoate (Augustyniak, 2007). Oxidative stress due to dimethoate may be ascribed to the inhibition of AChE (Yukti Sharma, et al 2005). Also a research by NIH (2005) showed that farmers who used agricultural insecticides including OP's experienced increased neurological symptoms, even when they were no longer using the products (e.g. headaches, fatigue, insomnia, dizziness, nausea, hand tremors, numbness and other neurological symptoms).

GST is a very important enzyme to the earthworm because of its role as general scavenger for lipophilic xenobiotics or endogenous formed waste products in earthworms. Therefore it can be postulated that this enzyme activity can reduce the hazard of pollutants around the earthworm (Stenersen *et al.*, 1979). The effect of the lethal and sub-lethal concentrations of the tested insecticides on GST activity of earthworms after 7 days was summarized in Table (2). The data showed that there was a significant increase's in the activity of GST after the treatment of dimethoate and malathion. On the other hand, there was a significant decrease at the LC₅₀ of abamectin but there was a non-significant difference with LC₂₅ of abamectin. GST activity was significantly decreased 24 h after application of dimethoate (Augustyniak, 2007). Oxidative stress due to dimethoate may be ascribed to the disturbance in activities of GST enzymes (Yukti Sharma, 2005). Also, malathion exposure reduced the activity of GST (Brocardo, *et al.* 2005). Hazarika et al (2003) found that GST activity was decreased in the liver with malathion and its combination with anilofos.

Effects of the LC₅₀ and LC₂₅ of the tested insecticides on AcP activity of the earthworm after 7 days are summarized in Table (2). AcP activity was significantly increased with the LC₅₀ and LC₂₅ of all the tested insecticides. The data showed that malathion had the highest effect on AcP activity while abamectin had the least effect. Many pesticides and metals increase the level of acid phosphatases (Abou-Donia et al. 1986, El-Gendy et al. 1990)

The alteration in the activity of AIPase in whole body of the earthworm exposed for 7 days to LC₅₀ and LC₂₅ of the tested pesticides is presented in Table 2. The obtained results revealed that insecticides (malathion, abamectin and dimethoate) significantly increased the activity of AIPase of earthworms. The

highest activity of earthworm alkaline phosphatases was noticed by malathion.

3.3. L-glutamic acid and GABA concentrations

On the bases of neurophysiological studies, amino acids have been separated into general classes: excitatory amino acids (glutamic, aspartic, cysteic and homocysteic), which depolarize neurons in mammalian CNS and the inhibitory amino acids (GABA, glycine, taurine and β -alanine), which hyperpolarize mammalian neurons. Strictly from a quantitative standpoint, acetylcholine and the amino acids dopamine and 5-hydroxytryptamine are probably the major transmitters and probably account for transmission at only a small percentage of synaptic sites (Cooper *et al.*, 1978). The results indicated that the lethal and sub-lethal concentrations of tested insecticides caused a significant ($P < 0.05$) induction in GABA and L-glutamic acid concentrations. The data showed that abamectin had the highest effect on the concentration of GABA and L-glutamic acid while dimethoate had the lowest effect. These results are parallel to those by El-Sayed (2004) who reported that there was an increase in the concentration of GABA and L-glutamic acid. Yamazaki *et al.* (1989) found that ivermectin is an agonist for the GABA concentration. Abamectin acts on gamma amino butyric acid (GABA) and glutamate-gated chloride channels, leading to activation of the chloride ion channel at higher concentrations and paralysis of pests (Putter, 1981, Bloomquist, (2001).

CONCLUSION

The present results showed that earthworm is quite sensitive to the toxicity and, could be used as biomarkers for the different groups of insecticides which are introduced into the soil system. . Also, the abamectin is more toxic than dimethoate and malathion. Accordingly, care must be taken into account to avoid the farmers and housewives' exposure to these insecticides and attention should be paid to traces of it at home environment. Also some precautions must be considered against the toxicity of these insecticides. However, although actual exposure of residents were not determined, additional studies which better estimate the residents' knowledge ,attitudes and practice as well as the potential for spray and surface contamination by insecticides.

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الملخص العربي

دراسة مقارنة بين مدى سمية بعض مبيدات الفوسفور العضوية والأبامكتين باستخدام

دودة الأرض *Lumbricus terrestris* كمؤشر حيوي

عنتر قناوى، منى شرف

- استهدفت هذه الدراسة مقارنة تأثير سمية مبيد الابامكتين أحد افراد مجموعة الأفرمكتين وهى مجموعة من المنتجات المتخمرة من سلالة الاستربتومييسيس أفرميتيليس من نتاج تطبيقات البيوتكنولوجى الحديثة وأحد صور المبيدات الحيوية-والذى انتشر استخدامه على النطاق التجارى كمبيد حيوى حديث ومقارنته بنوعين من المبيدات التقليدية من مجموعة الفسفور العضوية وهما الدايمثويت والملاثيون وذلك باستخدام ديدان الارض كمؤشر حيوي لتقييم سمية المبيدات. حيث تم تعريض ديدان الارض لتربة صناعية معاملة بالمبيدات لمدة 7 ايام لتقدير قيمة التركيز اللازم لقتل 50% من الافراد المعاملة. وكذلك دراسة تأثير هذا التركيز و نصفه على نشاط بعض النظم الانزيمية المتمثلة فى الاسيتيل كولين استيريز والفوسفاتيز الحمضى والقاعدى والجلوتاثيون-أس- ترانسفيريز بالاضافة الى تقدير تركيز مستوى اثنين من الناقلات العصبية هما الجاما امينو بيوتريك اسيد وال L جلوتاميك اسيد.
- أظهرت النتائج ان:
- التركيز اللازم لقتل 50% من ديدان الارض لكل من الابامكتين والداى ميثويت والملاثيون هو 2.99 - 13.7 - 39.69 مجم/كجم كحجم على الترتيب مما يوضح أن الابامكتين كان اكثرهم سمية على ديدان الارض.
- حدوث تثبيط معنويا لنشاط انزيم الاسيتيل كولين استيريز فى الديدان المعاملة بالتركيزات المميته وتحت المميته لكلا من الدايمثويت والملاثيون بينما حدث زيادة معنوية فى نشاط هذا الانزيم فى الديدان المعاملة بالأبامكتين.
- زيادة معنوية فى نشاط انزيمى الفوسفاتيز الحمضى والقاعدى بالتركيزات المستخدمه لكل المبيدات.
- زيادة نشاط انزيم الجلوتاثيون-اس- ترانسفيريز زيادة معنوية فى الديدان المعاملة بالدايمثويت والملاثيون بينما حدث انخفاضا معنويا فى الديدان المعاملة بالابامكتين مقارنة بالعينة الضابطة.
- حدوث زيادة واضحة للجاما امينو بيوتريك اسيد و ال L جلوتاميك اسيد فى الديدان المعاملة بكل المبيدات.
- ومن هذه النتائج يتضح أن الأبامكتين كمبيد حيوى كان أكثر سمية من المبيدات التقليدية من مجموعة الفوسفور العضوية.
- وكما هو معروف أن عملية شراء واستهلاك السلع الاستهلاكية تعتبر أهم عامل مؤثر على صحة أفراد الأسرة والذى ينعكس بدوره على التنمية البشرية المجتمعية، وقد انتشر استخدام المبيدات وتزايد فى العقد الاخير من القرن الماضى وحتى اليوم وظهر له مخاطر صحية على الانسان مما يريك ميزانية الأسرة فى العلاج وكذلك يقلل من قدرة الفرد الجسميه والعقلية وكفاءته فى العمل.