FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (28) No. 3 pp 393 -407, 2008

CHOLINESTERASE AND GLUTATHIONE S-TRANSFERASE ACTIVITIES IN FIELD COLLECTIONS OF THE EARTHWORM, *Allolobophora caliginosa*, IN RELATION TO THEIR SENSITIVITY TOWARD THE OP-INSECTICIDE, PROFENOFOS

M. M. Azab
Plant Protection Dept., Faculty of Agric., Banha University, Banha, Egypt.

Received 19 May 2008 Accepted 6 July 2008

ABSTRACT

The sensitivity toward the OP-insecticide, profenofos, as well as acetylcholine esterase (AChE) and glutathione-S-transferase (GST) activities were compared in samples of earthworm, *Allolobophora caliginosa*, collected from six insecticide-contaminated locations of four Governorates in Egypt [Rashide and Shabrachit (Behera), Tanta and Zafta (Ghrbia), Kafr Sakr (Sharkia) and Moshtohor (Kalubyia)]. Other collections from Moshtohor gardens (insecticide-uncontaminated locations) were served as control treatments for biological and biochemical assays. The bioassay data revealed significant variations in the sensitivity of the field collected samples to profenofos (Based on the non-overlapping between the confidence limits). The order of sensitivity toward profenofos was Rashide > Kafr Sakr > Shabrachit > Moshtohor > Tanta = Zafta = Control.

In an attempt to link bioassay data with biochemical data, the lowest AChE and the greatest GST activities were measured in Rashide collection which exhibited the highest sensitivity to profenofos. In contrast, the greatest AChE and the lowest GST activities were measured in the control collection were associated with the highest tolerant to profenofos. For more explanation, the order of AChE activities (based on the least significant difference) was

Control > Zafta > Tanta = Moshtohor > Shabrachit > Kafr Sakr > Rashide. Contrast order was obtained for GST activities (Rashide > Kafr Sakr > Shabrachit > Moshtohor > Tanta > Zafta > control). The biologically active concentrations of profenofos (24 h LC_5, LC_{25}, LC_{50}, and LC_{75}) that established with Moshtohor gardens collection were tested for their effects on AChE and GST activities. Acetylcholine esterase inhibition and GST stimulation was profenofos concentration dependent. As profenofos concentration increased from LC_5 to LC_{75}, AChE inhibited by 22.7 to 76.5% and GST increased by 103.4 to 123.8%.

INTRODUCTION

There is concern that the widespread use of pesticides and other agrochemicals may be damaging for agro-ecosystems and wildlife resources. The use of biomarkers for monitoring environmental contamination with agrochemicals including pesticides is becoming a routine method (Booth *et al.* 2000).

Although AChE inhibition has been measured in the tissues of a variety of invertebrate species following organophosphate exposure, however, additional work is needed in invertebrates to better explain the relationship between AChE inhibition and mortality (Fulton and Key 2001).

Enzyme assays in the earthworms are being developed and can be used as biomarkers of toxicity (Booth *et al.* 1998). Organophosphate and carbamate pesticides act by inhibiting cholinesterases. The activity of cholinesterase is widely used as a biomarker for the two insecticidal groups. Stenersen *et al.* (1992) and Stenersen (1980 a, b) have characterized this enzyme in earthworms and have determined its responses to insecticides. Edwards and Fisher (1991) suggested that cholinesterase activity may be a sensitive biomarker to assess environmental contamination with cholinesterase-inhibiting pesticides.

Glutathione S-transferase (GST) is involved in the detoxification of various xenobiotic chemicals (Lamoureux and Rusness 1987; Motoyama 1980), including the degradation of some organophosphorus compounds (Yang 1976). Therefore, this enzyme could be useful as an indicator of pesticide exposure.

Sensitivity of earth worm enzymes to op-insecticide profenofos

Earthworms have a number of characteristics (large size, behaviour and high biomass) which make them model organisms for testing the contamination of soil with chemicals (Callahan, 1988; Goats and Edwards, 1988; Bouche1992). Consequently, they have been adopted as standard organisms for ecotoxicological testing by the European Union (EEC, 1984) and the OECD (1984). Biomarkers can be used as early warning indicators of environmental contamination and potential adverse effects on populations (Peakall 1994; Day and Scott 1990) and can provide a link between the presence of a chemical and its toxic effect.

This research attempts to use *A. caliginosa* earthworms, cholinesterase and glutathione S-transferase activities and profenofos inhibitor, to establish: 1] A relationship between inhibition or activity, mortality and exposure, 2] Examination the cholinesterase inhibitions and glutathione S-transferase activities in six locations of four Governorates in Egypt through two years 2006 and 2007 and 3] The earthworm sensitivity to profenofos in the same tested locations.

MATERIALS AND METHODS

Earthworms:

Allolobophora caliginosa earthworms were collected during January 2006 and 2007 from six soil locations at four Governorates, Rashide and Shabrachit (Behera), Tanta and Zafta (Ghrbia), Kafr Sakr (Sharkia) and Moshtohor (Kalubyia). In each location, the earthworms were randomly collected from three positions, transported to the laboratory in plastic bags containing some soil. For control treatment, earthworms were collected from some gardens in moshtohor, which isolated from pesticide applications. The selected worms weighing between 1.3 and 1.5 g, were used for all tests.

Pesticide:

Profenofos, O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate [72% EC] offered by Kafer El-Zayat Chemical and Pesticide Co., Egypt.

Toxicity assessment:

The toxicity tests were done by using dipping methods suggested by Stenersen (1979), three replicates of 5 earthworms were dipped for 1 minute in each concentration (at least 5 progressive concentrations and water used for control replicates) of the tested pesticide. To each replicate 250 ml jar, 250 g soil at 25% moisture content was prepared (Booth *et al.* 2000). The worms were added to the surface of the soil to allow the organisms to burrow at 19-25 °C. Worm mortality was recorded at 24 hours after treatment. LC-p line values were calculated using Probit analysis (Finney, 1971).

Enzyme biomarker assays:

Four earthworms (from each position) were frozen for subsequent ChE and GST activity analysis (two earthworms for each analysis). The enzyme activity for the two earthworms was averaged to give a "replicate" activity.

Frozen samples of earthworms were defrosted on ice. The samples for determination of ChE activity were homogenised in phosphate buffer (0.02M; pH 7.5). Samples were maintained on ice for 60 minutes and the crude homogenate was used for analysis. For GST activity, earthworm samples were homogenized at 4 °C in a ratio of 1:4 (w/v) with 0.1 M phosphate buffer, pH 7.0. Analysis was conducted using the supernatant following centrifugation of the homogenate at 15,000 rpm for 5 minutes (Booth *et al.* 1998).

The method for the determination of ChE activity was adapted from Ellman *et al.* (1961). The substrate acetylthiocholine iodide was added to the reaction mixture containing the homogenate and 5,5',-Dithio-bis-2-nitrobenzoic acid (DTNB). Activity was expressed as nmol acetylthiocholine hydrolysed/min/mg protein. The GST activity was determined spectrophotometrically with 1-chloro- 2,4-dinitrobenzene (CDNB) as a substrate by monitoring the change in absorbance at 340 nm due to thioether formation at 25°C as described by Habig *et al.* (1974).

Samples were analysed for total protein content using the Bradford method (Bradford 1976). Bovine serum albumin was used as a standard.

Sensitivity of earth worm enzymes to op-insecticide profenofos

Data were subjected to analysis of variance (ANOVA) followed by least significant difference (CoStat Statistical Software, 1990).

Determination of enzyme activity after profenofos exposure:

Five Groups of earthworms (20 worms of each group) of Moshtohor gardens were treated using four concentrations of profenofos (LC_5, LC_{25}, LC_{50} and LC_{75}) as the same in the toxicity tests worms dipped in water were served as a control treatment. After 24 hours of treatment, the alived worms were used for AChE and GST activity assay as described above.

RESULTS AND DISCUSSION

Sensitivity of Earthworm Samples from Different Locations to Profenofos:

Probit data that shown in Tables 1a and 1b, reveal that earthworms of Rashide location were the most sensitive to profenofos followed by Kafr Sakr, Shabrachit, Moshtohor, Tanta and Zafta locations.

Table 1a: Sensitivity of earthworm samples from different locations to profenofos in 2006.

Location	*Lethal Concentrations (ppm) and Their 95% Confidence Limits*		*Slope±SD*	*R*
	LC_{50}	LC_{90}		
Rashide	$0.91 \times 10^{3\,e}$ (898.6-992.9)	$1.1 \times 10^{3\,e}$ (1062.7-1375.3)	14.6±0.07	0.999
Kafr Sakr	$2.3 \times 10^{3\,d}$ (2367.5-2633.1)	$3.1 \times 10^{3\,d}$ (2934.2-3551.5)	13.7±0.04	0.997
Shabrachit	$2.9 \times 10^{3\,c}$ (2766.6-3471.5)	$4.8 \times 10^{3\,c}$ (3951.4-6212.3)	7.6±0.03	0.991
Moshtohor	$5.8 \times 10^{3\,b}$ (4873.9-7385.8)	$12.1 \times 10^{3\,b}$ (9121.3-16331.4)	5.3±0.09	0.980
Tanta	$15.1 \times 10^{3\,a}$ (13251.4-16801.2)	$24.9 \times 10^{3\,a}$ (19711.5-31508.7)	7.5±0.04	0.989
Zafta	$15.9 \times 10^{3\,a}$ (14420.7-16234.1)	$25.7 \times 10^{3\,a}$ (15953.1-26410.6)	12.9±0.07	0.994
Control	$17.2 \times 10^{3\,a}$ (14510.1-16442.9)	$29.8 \times 10^{3\,a}$ (15881.8-31515.9)	15.2±0.05	0.997

SD: Standard deviation of mortality regression line.

R: Correlation coefficient of regression line.

Values within the same column having the same letters are non-overlapping of confidence limits.

Based on the overlapping between upper and lower confidence limits, collections from Zafta, Tanta and the Control collection were the least sensitive worms toward profenfos with no significant differences between them in this respect.

During the study in 2006 and 2007, the order of sensitivity was Rashide > Kafr Sakr > Shabrachit > Moshtohor > Tanta = Zafta = Control.

Table 1b: Sensitivity of earthworm samples from different locations to profenofos in 2007.

Location	*Lethal Concentrations (ppm) and Their 95% Confidence Limits*		*Slope±SD*	*R*
	LC_{50}	LC_{90}		
Rashide	$0.83 \times 10^{3\,e}$ (854.2-971.1)	$0.95 \times 10^{3\,e}$ (1041.7-1335.3)	15.0±0.04	0.989
Kafr Sakr	$1.95 \times 10^{3\,d}$ (2311.3-2601.4)	$2.7 \times 10^{3\,d}$ (2921.9-3509.1)	14.2±0.02	0.991
Shabrachit	$2.7 \times 10^{3\,c}$ (2730.5-3455.9)	$4.5 \times 10^{3\,c}$ (3913.4-6184.7)	7.9±0.09	0.997
Moshtohor	$5.4 \times 10^{3\,b}$ (4745.1-7122.3)	$11.5 \times 10^{3\,b}$ (8974.6-16114.5)	6.1±0.03	0.999
Tanta	$15.7 \times 10^{3\,a}$ (13313.9-16987.7)	$26.1 \times 10^{3\,a}$ (19711.5-31508.7)	7.8±0.08	0.993
Zafta	$16.3 \times 10^{3\,a}$ (14667.3-16578.4)	$28.4 \times 10^{3\,a}$ (16302.5-29758.8)	13.7±0.03	0.997
Control	$16.9 \times 10^{3\,a}$ (14498.3-16514.7)	$30.5 \times 10^{3\,a}$ (15733.8-31011.1)	15.8±0.09	0.999

SD: Standard deviation of mortality regression line.

R: Correlation coefficient of regression line.

Values within the same column having the same letters are non-overlapping of confidence limits.

Chlorpyrifos, diazinon and carbaryl reduced earthworm populations after the treatment (Cranshw 2007).

The use of species toxicity data to predict the effects of pollutants on communities is an important development in ecotoxicology, as well

as the toxicity tests on the invertebrates are needed for biomonitoring in the field and for chemical testing in the laboratory.

Although AChE and GST activities measured in the collections of Tanta and Zafta were significantly different, however, the two collections were statistically similar in their response to profenofos.

Effect of Profenofos on the Activity of AChE and GST in the Earthworm *A. caliginosa*:

The sensitivity of AChE and GST in earthworms collected from Moshtohor gardens toward profenfos is showon in Fig.1. Results revealed that LC_5, LC_{25}, LC_{50} and LC_{75} of profenofos to earthworms gave the inhibition of acetycholinesterase (AChE) compared to control by 22.7, 31.8, 51.4 and 76.5%, respectively, while the activity of glutathione S-transferase (GST) increased at the same time by 103.4, 110.1, 117.4 and 123.8%, respectively .

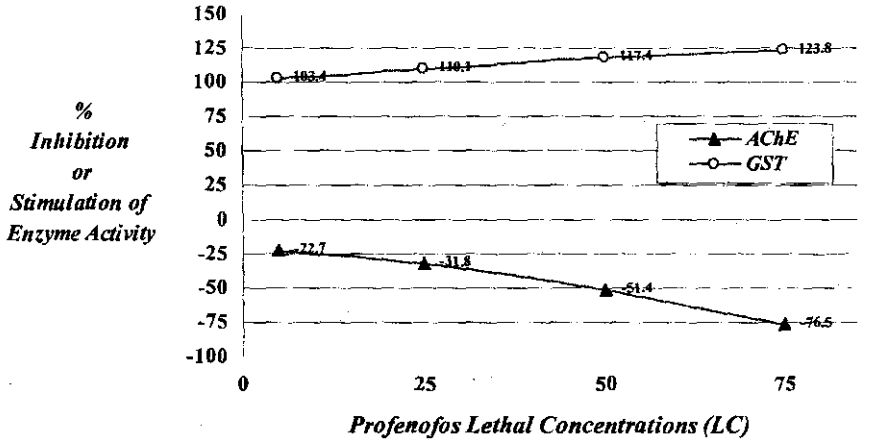

Fig. 1: Enzyme sensitivity in relation to profenfos concentrations.

Such findings are in agreement with those obtained by several researchers such as Kale and Krishnamoorthy (1982), who showed up to 80% inhibition of ChE by carbaryl in the earthworm *Pontoscolex corethrurus*. Stenersen (1979) subjected earthworms to a number of organophosphates and carbamates by dipping them in an aqueous

solution and demonstrated up to 85% inhibition of AChE levels, compared to controls. Booth *et al.* (1998) found the activity of ChE in earthworms was severely inhibited by diazinon and chlorpyrifos within 24 hours of exposure to sub-lethal levels of pesticides and this activity showed no return to pre-exposure levels even after 14 days. ChE activity was inhibited after a few days of insecticide exposure, indicative of a neurotoxic effect in earthworms. The significant increase in GST activity revealed the metabolisation of these products (Schreck *et al.* in press).

The neurotoxic potentiality of monocrotophos was assessed in a soil organism, *Eisenia foetida*, by using a marker enzyme, AChE in both in vitro and in vivo experiments. The progressive signs of morphological destruction are correlated with percentage inhibition of AChE in the in vivo experiments (Rao and Kavitha 2004).

GST activity was induced by profenofos and this is consistent with reports of induction of GST activity in worms by other pesticides (Hans *et al.* 1993) which showed a maximum induction of 250% by endosulphan.

AChE and GST Activity in Earthworm Samples:

Data presented in Table 2 indicate that the activity of AChE and GST in the earthworm *A. caliginosa* was significantly different in all tested locations (except AChE activity in the earthworms between Moshtohor and Tanta locations in 2006 and 2007 and between Shabrachit and Kafr Sakr locations in 2006 was not significantly different).

The lowest AChE activity and the highest GST activity were found in worms of Rashide location followed by Kafr Sakr, Shabrachit, Moshtohor, Tanta and Zafta locations. Samples collected from Moshtohor gardens (Control) exhibited the greatest AChE and the lowest GST activity.

Although the activity of AChE was lower in earthworms collected in 2007 from Rashide locations (28.30 nmol/min/mg protein), Kafr Sakr (33.90 nmol/min/mg protein) and Shabrachit (36.11nmol/min/mg protein) locations than in 2006, it was increased more in 2007 from Moshtohor (41.86 nmol/min/mg protein), Tanta (42.45 nmol/min/mg

protein) and Zafta (44.07 nmol/min/mg protein) locations than in 2006.

On the other hand, the activity of GST was higher in the earthworms collected in 2007 from Rashide (311.07 nmol/min/mg protein), Kafr Sakr (283.07 nmol/min/mg protein) and Shabrachit (274.45 nmol/min/mg protein) locations than in 2006, it was reduced more in 2007 in Moshtohor (246.97 nmol/min/mg protein), Tanta (237.72 nmol/min/mg protein) and Zafta (211.54 nmol/min/mg protein) locations than in 2006.

Table 2: Activity of AChE and GST in earthworm from different collections.

Location	*AChE Activity (nmole/min/mg protein)*		*GSTActivity (nmole/min/mg protein)*	
	2006	*2007*	*2006*	*2007*
Rashide	31.98 [e]	28.30 [f]	300.21 [a]	311.07 [a]
Kafr Sakr	36.85 [d]	33.90 [e]	277.99 [b]	283.07 [b]
Shabrachit	37.29 [d]	36.11 [d]	265.06 [c]	274.45 [c]
Moshtohor	40.39 [c]	41.86 [c]	251.16 [d]	246.97 [d]
Tanta	40.83 [c]	42.45 [c]	246.21 [e]	237.72 [e]
Zafta	41.86 [b]	44.07 [b]	224.76 [f]	211.54 [f]
Control	45.20 [a]	45.68 [a]	180.64 [g]	182.74 [g]
LSD 0.05	0.78	0.65	4.12	4.29

The presented values are means of three measurements.

Values within the same column having the same letters are not statistically different, $p<0.05$.

Molecular markers of the biological effects of contaminants on organisms (i.e. biomarkers) could be used as diagnostic and prognostic early-warning tests to detect and assess the effects of pollution, particularly low concentrations of complex mixtures of contaminants, on environmental quality (Livingstone, 1993).

Earthworms are common in a wide range of soils and may represent 60-80% of the total soil biomass (Bouche 1992; Rida, 1994).

This makes them one of the most suitable bioindicator organisms for testing chemicals in soils (Callahan, 1988; Goats and Edwards, 1988).

Cholinesterase activity is routinely used in vertebrate animals to diagnose exposure to organophosphates (Fairbrother *et al.* 1991). Edwards and Fisher (1991) showed that cholinesterase activity in several terrestrial and aquatic invertebrates can be significantly inhibited in cases of sublethal exposures to pesticides. They suggested that this enzyme may be a sensitive biomarker with which to assess environmental contamination by such pesticides. AChE activity has been shown to be strongly inhibited by organophosphates and carbamates in a number of invertebrate species (Day and Scott 1990).

Glutathione S-transferase (GST) plays an important role in the biotransformation of various insecticides (Lamoureux and Rusness 1987; Motoyama 1980), including the degradation of some organophosphorus compounds (Yang 1976). Therefore, this enzyme could be useful as an indicator of pesticide exposure.

CONCLUSION

From the results, it may be concluded that *A. caliginosa* was the most sensitive in Rashide location, while it was the least sensitive in Zafta location compared to the other tested locations. This can also allow avoidance of hazardous materials in areas where highly sensitive species occur.

It is proposed that *Allolobophora caliginosa* and a suite of its biochemical responses could be a sensitive tool for use in soil contamination surveys.

REFERENCES

Booth, L. H.; V. Heppelthwaite and C. T. Eason (1998): Cholinesterase and glutathione S-transferase in the earthworm *Aporrectodea caliginosa* as biomarkers of organophosphate exposure. Proc. 51st NZ Plant Prot. Conf.: 138-142.

Booth, L. H; S. Hodge and K. O'Halloran (2000): The use of enzyme biomarkers in *Aporrectodea caliginosa* (Oligochaeta; Lumbricidae) to detect organophosphate

contamination: a comparison of laboratory tests, mesocosms and field studies. Environ. Toxicol. Chem. 19: 417-422.

Bouche, A. (1992): Earthworm species and ecotoxicological studies. In: Greig-Smith, P.W., Becker, H., Edwards, P.J., Heimbach, F. (Eds.), Ecotoxicology of Eearthworms. Intercept, Andover, pp. 20-35.

Bradford, M. M., (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.

Callahan, C. A. (1988): Earthworms as ecotoxicological assessment tools. In: Edwards, C. A. and E. F. Neuhauser (Eds.): Earthworms in Waste and Environmental Assessment. SPB Academic Publishing, The Hague, pp. 295-301.

CoStat Statistical Software, (1990): Microcomputer program analysis Version 4.20, CoHort Software, Berkeley, CA.

Cranshw, W. (2007): IPM of Midwest Landscapes: Tactics and Tools for IPM. University of Minnesota, Chapter (10), pp. 45-48.

Day, K. E. and I. M. Scott (1990): Use of acetylcholinesterase activity to detect sublethal toxicity in stream invertebrates exposed to low concentrations of organophosphate insecticides. Aquatic Toxicol. 18: 101-114.

Edwards, C. A. and S. W. Fisher (1991): The use of cholinesterase measurements in assessing the impacts of pesticides on terrestrial and aquatic invertebrates. Pp 256- 275 In: Cholinesterase-inhibiting Insecticides: their Impact on Wildlife and the Environment, P. Mineau (Ed); Elsevier, Amsterdam.

EEC (European Economic Community) (1984): Directive 79/831/EEC, Annex V, part C. Method for the determination of ecotoxicity. Level 1. Earthworms: artificial soil test. Commission of the European Communities, DGXI/128/82 Rev. 5, Brussels.

Ellman, G. L.; K.D. Courtenay; A. J. Valentino and E. M. Featherstone (1961): A new rapid colorimetric

determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7: 88-95.

Fairbrother, A.; B. T. Marden; J. K. Bennett and M. J. Hooperds (1991): Methods used in determination of cholinesterase activity. Pp 35-72 In: Cholinesterase-inhibiting Insecticides: their Impact on Wildlife and the Environment, P. Mineau (Ed), Elsevier, Amsterdam.

Finney, D. J. (1971): Probit analysis. 3rd edition. University Printing House, Cambridge, 318 pp.

Fulton, M. H. and P. B. Key (2001): Acetylcholinesterases inhibition in estuaine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ. Toxicol. and Chem., (20) 1: 37-45.

Goats, G. C. and C. A. Edwards (1988): The prediction of field toxicity of chemicals to earthworms by laboratory methods. In: Edwards, C.A., Neuhauser, E.F. (Eds.), Earthworms in Waste and Environmental Assessment. SPB Academic Publishing, The Hague, pp. 283-294.

Habig, W. H.; M. J. Pabst, and W. B. Jacoby (1974): Glutathione-S-transferases, the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 249 (22): 321-336.

Hans, R. K.; M. A.Khan; M. Farooq and M. U. Beg (1993): Glutathione-S-transferase activity in an earthworm (*Pheretima posthuma*) exposed to three insecticides. *Soil Biol. and Biochem.* 25(4): 509-511.

Kale, R. D. and R. V. Krishnamoorthy (1982): Residual effect of Sevin® on the acetyl cholinesterase activity of the nervous system of earthworm *Pontoscolex corethrurus*. Curr. Sci. 51(18): 885-886.

Lamoureux, G. L. and D. G. Rusness (1987): Synergism of diazinon toxicity and inhibition of diazinon metabolism in the house fly by Tridiphane: inhibition of glutathione-S-transferase activity. *Pest. Biochem. Phys.* 27: 318-329.

Livingstone, D.R., (1993): Review Biotechnology and pollution monitoring: use of molecular biomarkers in the aquatic environment. J. of Chem. Tech. and Biotech. 57: 195-211.

Motoyama, N., (1980): Glutathione-S-transferases: Their role in the metabolism of organophosphorus insecticides. *Rev. Biochem. Toxicol.* 2: 49-69.

OECD (1984): Test 207: earthworm, acute toxicity tests. In: Organization for Economic Co-operation and Development (Ed.), OECD Guidelines for Testing of Chemicals, Paris.

Peakall, D. B. (1994): Biomarkers, the way forward in environmental assessment. Toxicol. Ecotoxicol. News 1: 55-60.

Rao, J.V. and P. Kavitha (2004): Toxicity of azodrin on the morphology and acetylcholinesterase activity of the earthworm *Eisenia foetida.* Environmental Research, 96 (3): 323-327.

Rida, A. M. A. (1994): Les vers de terre et l'environnement. La Recherche 25: 260-267.

Schreck, E.; F. Geret; L. Gontier and M. Treilhou (2008): Neurotoxic effect and metabolic responses induced by a mixture of six pesticides on the earthworm *Aporrectodea caliginosa nocturna.* Chemosphere in Press.

Stenersen, J. (1979): Action of pesticides on earthworms. Part 3: Inhibition and reactivation of cholinesterases in *Eisenia foetida* (Savigny) after treatment with cholinesterase-inhibiting insecticides. Pest. Sci. 10: 113-122.

Stenersen, J. (1980a): Esterases of earthworms. Part I: Characterisation of the cholinesterases in *Eisenia foetida* (Savigny) by substrates and inhibitors. Comp. Biochem. and Physiol., 66C: 37-44.

Stenersen, J. (1980b): Esterases of earthworms. Part II. Characterisation of the cholinesterases in the earthworm *Eisenia foetida* (Savigny) by ion exchange chromatography and electrophoresis. Comp. Biochem. and Physiol., 66C: 45-51.

Stenersen, J.; E. Brekke and F. Engelstad (1992): Earthworms for toxicity testing; species differences in response towards cholinesterase inhibiting insecticides. Soil Biol. & Biochem., 24: 1761-1764.

Yang, R. S. H. (1976): Enzyme conjugation and insecticide metabolism. Pp 177-187 *In:* Biochemistry and Physiology of Insecticides, C.F. Wilkinson (Ed); Plenum Press, New York.

نشاط إنزيمات الكولين إستيريز والجلوتاثيون إس ترانسفيريز فى ديدان الأرض مع الإهتمام بدراسة حساسية الإنزيمين مجال الدراسة للمبيد الفوسفورى العضوى البروفينوفوس

محمد محمد عزب

قسم وقاية النبات – كلية الزراعة – جامعة بنها

أجرى هذا البحث بهدف دراسة حساسية ديدان الأرض لمبيد البروفينوفوس فى ٦ مواقع تتبع أربع محافظات هى رشيد وشبراخيت (البحيرة) طنطا وزفتا (الغربية) كفر صقر (الشرقية) ومشتهر (القليوبية) وقد أوضحت النتائج أن ديدان الأرض لموقع رشيد كانت أكثرها حساسية له والتركيز القاتل ل 50% منها هو 0.83×10^3 جزء فى المليون (فى عام٢٠٠٧) يليها كفر صقر ثم شبراخيت ثم مشتهر ثم طنطا وكانت أقلها حسلسية للمبيد هى ديدان الأرض بالنسبة لموقع زفتا وكان التركيز القاتل ل 50% منها هو 16.3×10^3 جزء فى المليون فى نفس العام كما أن حساسية ديدان الأرض للمبيد زادت بالنسبة لمواقع رشيد و كفر صقر و شبراخيت فى عام ٢٠٠٧ عنها فى ٢٠٠٦ بينما إنخفضت الحساسية للمبيد بالنسبة لمواقع مشتهر و طنطا وزفتا خلال نفس الفترة الزمنية للدراسة.

كما تم إجراء مقارنة نشاط كلاً من إنزيم الأسيتايل كولين إستيريز والجلوتاثيون إس ترانسفيريز خلال عامى ٢٠٠٦ و ٢٠٠٧ فى دودة الأرض لنفس مواقع الإختبار وذلك لإمكانية مدى إستخدامها كدلائل حيوية فى المستقبل لتتبع مستوى تلوث التربة الزراعية بمثبطات الكولين إستيريز مثل المبيدات الفوسفورية العضوية والكرباماتية أو المعادن الثقيلة.

فأظهرت النتائج وجود إختلاف معنوى فى نشاط كلاً من إنزيم الأسيتايل كولين إستيريز والجلوتاثيون إس ترانسفيريز فى دودة الأرض بين كل المواقع التى تم دراستها

ماعدا نشاط إنزيم الأسيتايل كولين إستيريز بين كلاً من مشتهر (القليوبية) و طنطا (الغربية) فى عام ٢٠٠٦ و٢٠٠٧ وكذلك بين موقعى كفر صقر (الشرقية) وشبراخيت (البحيرة) فى عام ٢٠٠٦ وقدر أقل نشاط لإنزيم الأسيتايل كولين إستيريز وأعلى نشاط لإنزيم الجلوتاثيون إس ترانسفيريز فى ديدان الأرض لموقع رشيد يليها كفر صقر ثم شبراخيت ثم مشتهر ثم طنطا ثم زفتا.

وقد دلت النتائج كذلك على أنه بالرغم من إنخفاض نشاط إنزيم الأسيتايل كولين إستيريز فى ديدان الأرض لمواقع رشيد و كفر صقر و شبراخيت فى عام ٢٠٠٧ عنها فى عام ٢٠٠٦ فقد إزداد نشاط هذا الإنزيم فى ديدان الأرض لمواقع مشتهر و طنطا و زفتا فى عام ٢٠٠٧ عنها فى عام ٢٠٠٦ بينما كان على العكس من ذلك إنزيم الجلوتاثيون إس ترانسفيريز فى ديدان الأرض لمواقع رشيد و كفر صقر و شبراخيت حيث إزداد نشاطه فى عام ٢٠٠٧ عنها فى عام ٢٠٠٦ وإنخفض نشاطه فى ديدان الأرض لمواقع مشتهر و طنطا و زفتا فى عام ٢٠٠٧ عنها فى عام ٢٠٠٦ وقد إرتبطت حساسية ديدان الأرض موقع رشيد بأقل حساسية لإنزيم الأسيتايل كولين إستيريز وأعلى نشاط لإنزيم الجلوتاثيون إس ترانسفيريز.

كما تم دراسة العلاقة بين نشاط كلاً من إنزيم الأسيتايل كولين إستيريز والجلوتاثيون إس ترانسفيريز والتركيزات المميتة والتحت المميتة لمبيد البروفينوفوس مثل التركيزات القاتلة لكلاً من 5 و 25 و 50 و 75% لديدان الأرض فأوضحت النتائج أن هذه التركيزات أدت إلى تثبيط إنزيم الأسيتايل كولين إستيريز مقارنة بالكنترول بنسبة 22.7 و31.8 و 51.4 و 76.5 % على التوالى ، بينما أدت فى نفس الوقت إلى زيادة نشاط إنزيم الجلوتاثيون إس ترانسفيريز بنسبة 103.4 و 110.1 و 117.4 و 123.8 % على التوالى.

وقد يستنتج من ذلك أنه خلال العام الثانى للدراسة (٢٠٠٧) ربما يكون قد حدث تحسن فى التربة الزراعية لمواقع مشتهر و طنطا و زفتا بينما مواقع رشيد و كفر صقر و شبراخيت قد إزدادت تلوثاً. كما يجب الحذر من إستخدام المبيدات فى المواقع التى تظهر الكائنات الحية النافعة بها حساسية لتلك المبيدات.