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RESIDUE ANALYSIS AND ANTIACETYLCHOLINESTERASE PROPERTIES OF PROFENOFOS AND DIMETHOATE

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ABSTRACT

Profenofos and dimethoate at half, equal and twice recommended rate were applied onto cucumber plants. The residues were determined using gas chromatograph equipped with flame photometric detection. The residue levels of profenofos on cucumber fruits at harvesting time were 0.54, 1.32, and 1.90 mg/kg for half, equal, and twice recommended rate, respectively. The corresponding values of dimethoate were 1.13, 3.85, and 4.60 mg/kg, respectively. Profenofos and dimethoate showed inhibitory effects on rat brain AChE activity with I_{50} values equal to 16 and 370 μ M, respectively. The predicted K_i values for profenofos and dimethoate are 1.4×10^3 and $0.06 \times 10^3 \text{ M}^{-1}\text{min}^{-1}$, respectively. The twice-recommended field rate of dimethoate and profenofos caused 54.1 and 36.4 as a percent inhibition of control *in vivo* of rat brain AChE, respectively. However, profenofos was more potent inhibitor than dimethoate (*In vitro*).

INTRODUCTION

Profenofos (selecron), O-(4-bromo-2-chlorophenyl)-O-ethyl-S-propyl phosphorothioate and dimethoate (cygon), O,O-dimethyl-S-(N-methyl carbamoyl methyl)phosphorodithioate are organophosphorus insecticides widely used to control various caterpillars, white fly, and mites on vegetables in Egypt (Anonymous, 1985). Residues on vegetable crops have been subjected to major concern. Various regulatory bodies to ensure safe use of pesticides and to protect consumers from adverse side effects have imposed numerous restrictions. Most papers have focused on various aspects of organophosphorus toxicology, Ryhanen *et al.*, (1988), Guhathakurta and Bhattacharya, (1989), Bastos *et al.*, (1991), and Maxwell and Brecht (1992). Poisoning signs in chicks administered by profenofos correlated with *In vivo* inhibition of brain Acetylcholinesterase (AChE) activity (Glickman *et al.*, 1984). Parathion, fonofos, DEF, ethoprop and profenofos were oxidized by the reconstituted monooxygenase system to form AChE inhibitors on mice liver (Levi and Hodgson, 1985). Also, reduction of AChE activity was occurred due to the inhibition by the oxidized metabolite of chlorpyrifos (Harold and Ottea, 2000).

The present work was designed to evaluate the residue of profenofos and dimethoate in cucumber fruits at harvesting time and their effects on brain AChE activity in male white Norway rats.

MATERIALS AND METHODS

- Residue analysis :-

A field trial of cucumber (*Cucumis sativus* L.) var. California (U.S.A.) was carried out in randomized block design during May, 1995 at Rasheed area, Behera Governorate, Egypt. Separate test plots of cucumber were designed with three replicates. Common cultural and fertilization practices for cucumber production were followed. Cucumber plants were sprayed with profenofos 72% or dimethoate 40% emulsifiable concentrate, each at half, equal, and twice recommended field rate. Samples of cucumber fruits (100gm.each,) were collected randomly in three replicates from each treatment at harvesting time (eight days after insecticide application). The samples were brought to the laboratory in plastic bags and were frozen at -18°C until insecticide residue analysis. Treated fruits were extracted according to the method of Bowman (1980) and cleaned up using the procedure of Bowman and Leuck (1971). Gas Chromatograph Shimadzu-4CM equipped with Flame Photometric detector (FPD) with phosphorus mode was used for the determination of residues with an analytical glass column (2m x 3mm i.d) packed with 1.5% OV-17 on 80/100 Shimelite W. The operating temperatures (°C) for both insecticides tested were maintained as follows: Column 220 isothermal, injector and detector 270 and gas flow rate (ml/min) were: Nitrogen 40, hydrogen 80 and air 100; the limits of detection under these conditions were 2.8 and 1.5 ng for profenofos and dimethoate respectively. Identification of each tested insecticide residues was accomplished by retention time (t_R) and compared with known standard at the same conditions. The quantities were calculated on peak height basis. Using these conditions, the retention times of profenofos and dimethoate were 5.8 and 1.9 minutes, respectively.

- Antiacetylcholinesterase properties: -

To study the antiacetylcholinesterase properties of both tested insecticides, the white Norway rats (*Rattus norvegicus* var. *albus*) were weighed and individually caged with adequate water supply. Cucumber was divided into pieces nearly equal to 10% of rat body weight. The cucumber pieces were saturated with concentrations of each tested insecticide equal to the found residue at harvesting time (Table, 1) by dipping for 5 minutes. The tested rats were exposed to no-choice test by placing one cup containing the treated cucumber piece only inside the cage. However, after the rat had eaten the tested piece, normal food was supplied. Daily fresh treated cucumber was supplied to rats for one week.

The animals were decapitated and the brains were removed from the skull using a scissor. Brains were rinsed with 0.9% (w/v) NaCl solution blotted to remove excess liquid, weighed and stored in liquid nitrogen at -80°C until AChE activity measurement.

Preparation of rat brain AChE

Membranes were prepared from rat brain as described by Eldefrawi *et al.* (1978). The brain was homogenized using a Polytron in 2-fold volume (w/v) of ice cold 0.05 M phosphate buffer (pH 7.4). The homogenate was

filtered through two layers of cheese-cloth and centrifuged at 8000 xg for 20 minutes. The supernatant was recentrifuged at 30000 xg for 60 minutes. The pellet was homogenized in the same phosphate buffer using glass homogenizer and used as a source for AChE. All operations were carried out at 4°C., and centrifugations was accomplished by MSE Scientific Instrument Europa 65 Ultracentrifuge with fixed angle roter. The protein concentration was determined by the method of Lowery *et al.* (1951).

Determination of AChE activity

The spectrophotometric method of Ellman *et al.* (1961) which was modified by Brownson and Watts (1973) was used for assaying AChE activity. The enzymatic activity was assessed by monitoring the rate of hydrolysis of substrate (10^{-3} M) acetyl thiocholine iodide (ATChI) into thiocholine and acetic acid. Subsequently, the thiocholine reacts with DTNB (5,5'-dithio-bis(2-nitrobenzoic acid) (10^{-3} M) in 50 nM sodium dibasic phosphate buffer (pH 7.2) and produces a yellow coloured anion of 5-thio-2-nitrobenzoic acid which was measured spectrophotometrically at 412 nm.

The *In vivo* procedure involved the addition of 1.5 ml aliquots of DTNB to each of two cuvettes containing 1.5 ml of ATChI with direct mixing and used as blanks in the adjustment of the double beam spectrophotometer (Pye Unicam SP-8100) to zero. A volume of 0.1 ml of enzyme preparation was pipetted to one of the cuvettes and the activity was recorded. The specific activity was expressed as absorbancy ($\Delta OD \cdot \lambda_{412}/\text{mg protein}/\text{min.}$).

In vitro inhibition of rat brain AChE activity

The effect of tested insecticides on AChE activity was studied *In vitro*. The enzyme preparation (1ml) was preincubated with 10 μl aliquots of insecticides (10^{-2} M stock in alcohol) at room temperature. After different time intervals, 0.1 ml from enzyme-inhibitor complex was measured as previously described and compared with standard preparations without inhibitor to determine the percent of *In vitro* inhibition using the following formula:

$$\% \text{ In vitro inhibition} = \frac{V - V_i}{V} \times 100$$

Where:

V = is the specific activity with no inhibitor.

V_i = is the specific activity in presence of inhibitor.

RESULTS AND DISCUSSION

Residue analysis:-

Residues of profenofos or dimethoate detected in cucumber fruits at harvesting time are shown in Table (1). The detected amounts of profenofos and dimethoate were more than MRL's (0.5 mg/kg of profenofos and 2 mg/kg

of dimethoate) recommended by FAO/WHO, (Anonymous, 1999) except the half recommended rate of dimethoate which was lower than MRL's.

Table (1): Profenofos and dimethoate residues in cucumber fruits.

Insecticide & rates	Residues* (mg/kg)
Profenofos:	
Half recommended rate	0.537 ± 0.050
Recommended rate (750 cm ³)	1.320 ± 0.042
Twice recommended rate	1.900 ± 0.044
Dimethoate:	
Half recommended rate	1.130 ± 0.041
Recommended rate (300 cm ³)	3.850 ± 0.180
Twice recommended rate	4.570 ± 0.550

Data are expressed as means ± SO for three determinations per each rate.

*Residues detected at the harvesting time (eight days after application).

Antiacetylcholinesterase properties: -

(a) AChE activities in rat brain fractions:

The acetylcholinesterase (AChE) specific activities ($\Delta OD_{412}/mg$ protein /min.) in rat brain fractions are presented in Table(2). The results indicated that the pellet, which obtained at 30000 xg centrifugation (P₃₀) contained the highest AChE activity compared with other fractions. The highest AChE activity in fraction (P₃₀) may be due to the partial purification steps, which was used by different speed of centrifugation.

Table (2): Specific activities of rat brain AChE from different fraction preparations

Fraction preparation	Specific activity ($\Delta OD_{412}/mg$ protein /min.)
Crude homogenate	2.40 ± 0.56
Pellet at 8000 xg (P ₈)	3.20 ± 0.82
Supernatant at 8000 xg (S ₈)	4.60 ± 0.65
Pellet at 30000 xg (P ₃₀)	6.50 ± 0.75
Supernatant at 30000 xg (S ₃₀)	4.50 ± 0.78

(b) *In vitro* inhibition of rat brain AChE activity:

The widespread use of anticholinesterase insecticides in agricultural pest control with potential hazards to both wild life and man has necessitated the development of a rapid method for detection of *In vivo* and *In vitro* inhibition of AChE. Hence, in the present study we used rat brain as a source of AChE and tested the anticholinesterase properties of profenofos and dimethoate insecticides.

Data in Table (3) clearly indicated that profenofos and dimethoate reduced the rat brain AChE activity. However, profenofos was more potent inhibitor than dimethoate by 23 fold. The differences in their potencies were due to I₅₀ values, which represent 16.0 and 370 μ M for profenofos and dimethoate, respectively. These results are in accordance with that reported

by Wang and Murphy (1982) who revealed that the differences in I_{50} values were due to both different affinities and phosphorylation rates of AChE.

Also, Table (3) represents approximate predicted K_i values (bimolecular rate constant) of the reaction of these compounds for brain AChE. This could be calculated using the equation: $K_i = 0.695 / t.I_{50}$, which was proposed by O'Brien (1960). The predicted K_i values for the reaction of profenofos and dimethoate were 1.4×10^3 and $0.06 \times 10^3 \text{ M}^{-1} \text{ min}^{-1}$, respectively.

Both I_{50} and K_i values of profenofos and dimethoate indicated that profenofos is greater in potency than dimethoate as anticholinesterase inhibitor. Maxwell and Brecht (1992) made a comparison of the bimolecular rate constant (K_i) for inhibition of electric eel AChE by the oxono (i.e. P=O) and thiono (i.e. P=S) analogs of parathion, methyl parathion, leptophos, fonofos, sarin and soman. They revealed that the oxono/ thiono ratio of K_i values varied from 14 for soman to 1240 for parathion.

Maxwell and Brecht (1992) suggested that differences in hydrophobicity of oxono and thiono analogs of organophosphorus compounds may be as important as their electronic differences in determining their effectiveness as AChE inhibitors. These observations could explain the presence finding where the oxono analog (profenofos) was more potent as AChE inhibitor than the thiono analog (dimethoate).

Table (3): *In vitro* inhibition of rat brain AChE activity by profenofos and dimethoate Insecticide.

Insecticide	Empirical formula	I_{50}	$K_i \text{ M}^{-1} \text{ Min}^{-1}$
Profenofos	$\text{C}_{11}\text{H}_{15}\text{Br Cl O}_3 \text{ PS}$	16	1.44×10^3
Dimethoate	$\text{C}_5 \text{H}_{12} \text{N O}_3 \text{ PS}_2$	370	0.06×10^3

(c) *In vivo* inhibition of rat brain AChE activity:

Table (4) represent the *In vivo* effect of profenofos and dimethoate on the rat brain AChE activity. Data showed that increasing the concentration rate of profenofos or dimethoate leads to more inhibition of rat brain AChE activity.

In general, the results showed that dimethoate was more potent inhibitor than profenofos at all of the tested rates. The high potency of dimethoate as inhibitor for AChE may be due to its activation to phosphates (P=O) which are potent antiacetylcholinesterase, and activity of these compounds *In vivo* may be a function of several factors.

Finally, the application of twice the recommended rate of the two tested insecticides will be very harmful to mammals because the twice recommended rate of dimethoate caused 54.1% inhibition *In vivo* and profenofos caused 36.4% inhibition *In vitro* of rat brain AChE.

Table (4): *In vivo* inhibition of rat brain AChE activity by profenofos and dimethoate insecticides

Insecticide & rates	% Inhibition of control
Profenofos	
Half recommended rate	23.00
Recommended rate	33.60
Twice recommended rate	36.47
Dimethoate	
Half recommended rate	28.20
Recommended rate	39.17
Twice recommended rate	54.17

CONCLUSIONS

Rat brain AChE specific activity was high in pellet fraction, which obtained at 30000 xg centrifugation. The I_{50} values for profenofos and dimethoate were 16 and 370 μM , respectively. The predicted K_i values for profenofos and dimethoate were 1.4×10^3 and $0.062 \times 10^3 \text{ M}^{-1} \cdot \text{min}^{-1}$, respectively.

The I_{50} and K_i values of profenofos and dimethoate indicated that profenofos was more potent AChE inhibitor than dimethoate as *in vitro* effect and vice versa as *in vivo* effect. The *in vivo* effect of profenofos and dimethoate is concentration depending.

Finally, the detected amounts of dimethoate and profenofos residues in the samples after application of the twice recommended rate caused 54.1% and 36.4% inhibition *in vivo* of rat brain AChE, respectively.

REFERENCES

- Anonymous, (1985). Pesticide dictionary. Farm Chemical Handbook. Meister Publishing Co., Willoughby, PP. 258.
- Anonymous (1999). List of Codex Maximum Residue Limits. August, 31, WHO/FAO.
- Bastos, V.L.F.; J. C. Bastos; J. S. Lima and M. V. Faria (1991). Brain acetylcholinesterase as an *In Vitro* detector of organophosphorus and carbamate insecticides in water. *Water Res.*, 25 (7): 835-840.
- Bowman, M. C. (1980). Analysis of organophosphorus pesticides in "Analysis of pesticide residues", Ed. By H. A. Moyer, John Wiley, Sons Inc., Chapter 7, PP. 263-332.
- Bowman, M. C. and D. B. Leuck (1971). Determination and persistence of phoxim and its oxygen analog in forage corn and grass. *J. Agric. Food Chem.*, 19(6): 1215-18.
- Brownson, J. D. and B. P. Watts (1973). A modification of cholinesterase activity by S,S-dithio-bis-(2-nitrobenzoic acid) included in the coupled spectrophotometric assay. *Biochem.*, J. 31: 369.
- Eldefrawi, M. E.; A. T. Eldefrawi; N. A. Mansour; J. W. Dely; B. Witkep and E. X. Albuquerque (1978). Acetylcholine receptor and ionic channel of

- Torpedo electroplax: Binding of perhydrohistrionicotoxin to membrane and solubilized preparations. *Biochemistry*, 17: 5474-5484.
- Ellman, G. L.; K. D. Courtney; V. J. Andres and R. M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, 7: 88-95.
- Glickman, A. H.; K. D. Wing and J. E. Casida (1984). Profenofos insecticide bioactivation in relation to antidote action and stereospecificity of acetylcholinesterase inhibition, reactivation, and aging. *Toxicol. Appl. Pharmacol.*, 73(1): 16-22.
- Guhathakurta, S. and S. Bhattacharya (1989). In vitro inhibition of goat brain acetylcholinesterase by pure and commercial anti cholinesterase pesticides. *Ecotoxicol. Environ. Safety*, 17: 16-20.
- Harold, J. A. and J. A. Ottea (2000). Characterization of esterases associated with profenofos resistance in the tobacco budworm, *Heliothis virescens* (F). *Arch. Insect. Biochem. Physiol.*, 45(2): 47-59.
- Levi, P. E. and E. Hodgson (1985). Oxidation of pesticides by purified cytochrome P-450 isozymes from mouse liver. *Toxicol. Lett.*, 24(2-3): 221-228.
- Lowery, O. H.; N. J. Rosebrough; A. L. Farr and R. J. Randall (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Maxwell, D. M. and K. M. Brecht (1992). Quantitative structure activity analysis of acetylcholinesterase inhibition by oxono and thiono analogues of organophosphorus compounds. *Chem. Res. Toxicol.*, 5(1): 66-71.
- O'Brien, R. D. (1960). Toxic phosphorus esters. Academic Press, New York, PP. 175-190.
- Ryhanen, R.; M. Kajovaara; M. Harri; E. Kaliste-Korhonen and O. Hanninen (1988). Physical exercise effects cholinesterases and organophosphate response. *Gen. Pharmacol.*, 19: 815-818.
- Wang, C. and S. D. Murphy (1982). Kinetic analysis of species differences in acetylcholinesterase sensitivity to organophosphate insecticides. *Toxicol. Appl. Pharmacol.*, 66: 409-419.

متبقيات البروفينوفوس والدايمثويت وتأثيرها كمثبطات لانزيم الاستيل كولين استيريز

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وجد أن نشاط إنزيم الاستيل كولين استيريز أعلى في المستخلص المنفصل على درجة 30.000 دورة / الدقيقة طرد مركزي. كذلك وجد أن قيم I_{50} (التركيز المثبط لنشاط الإنزيم) لكل من البروفينوفوس والدايمثويت كانت 11 و 370 ميكرومولس على التوالي. بينما كانت قيم K_i (ثابت التثبيط) المتوقعة 1.4×10^{-6} و 0.62×10^{-6} لكل مولر/ ثانية لكل المبيد على التوالي.

بمقارنة كل من قيم I_{50} و K_i لكلا المبيدات أتضح أن البروفينوفوس يملك قوة تثبيطية أعلى من الـدايمثويت وذلك عن طريق التثبيط الخارجي *In vitro* وبالنسبة للتثبيط الداخلي وجد أن التثبيط يعتمد على زيادة التركيز لكل المبيدات والدايمثويت له تأثير تثبيطي داخلي *In vivo* أكثر من البروفينوفوس. كذلك أعطت الكميات المقترحة باستخدام ضعف التركيز تثبيط داخلي للأنزيم المنفصل من مسخ الفسار يساوي 47% و 36% للبروفينوفوس و 17% و 4% للدايمثويت على التوالي.

