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DETECTION OF SOME ORGANOPHOSPHROUS COMPOUNDS RESIDUES IN NILE FISH MARKETS OF SOME VILLAGES OF KALIOBIA GOVERNORATE

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ABSTRACT

A total sixty samples Niletilapia (Qreochromis niloticus) and clarias garpinus (Thirty of each) collected from fish Markets of some villages of kaliobia governorate and subjected to fat Extraction and determination of organophosphrous residue by gas chromatography.

Organophosphrous residues including diazinon, chlorpyrifos – ethyl, Malathion, Methyl – parathion, ethyl parathyion and Ethion with an incidence from muscles of tilapia nilotica were (33% , 6.7%, 20%, 12%,12% and 3% respectively) and from clarias garpinus were(50%, 6.7%, 16%, 10%, 12% and 3% respectively).

The obtained result revealed that the residues of diazinon was came in the first rank followed other organophosphrous residue. Most residues detected within action limit for fish, however, residues of these pesticide were not detected in collected samples may be due to their percent in very trace amount or gave indicter for safety environment from different types of pollution.

INTRODUCTION

Organophosphrous pesticides is a one encompassing a wide variety of substance used in food production to control undesirable plant, insect and other animal populations.

Many factors influence the persistence pesticide residues in or on food. Residues may very depending on the time between application and harvest **(Bhoopendra and Dograc 2009)**. organophosphrous pesticides are more persistent in environment (one of persistent organic pollutants), for this reason some insects develop resistance to this chemical compound, they are efficiently absorbed by gastrointestinal tract (GIT) and intact skin. Although the mechanism of action is not yet fully understood, in acute subacute and chronic poisoning from these insecticides it would be of interest to know the effect of organophosphrous cholinesterase inhibitor and its relation ship to clinical situation **(WHO, 1990).**

Organophosphrous pesticides classifier as highly toxic participles including (chlorpyrifos, diazinon, dimethoate, Malathion and Parathien)

Moderately toxic including (coumaphs, phorate and phosalone) and relatively non toxic as(chlorthalonil) dazomet and cyanazine **(Watson et al., 2000) .**

In general, the effects of high exposure to organophosphrous can pounds are salivation, sweating , rhinorrhea and tearing also in severe cases causes respirtory depressions , loss of consciousness , seizures and cholinesterase inhibition **(FAO, 2004).**

The food and drug administration **(FDA, 1994b)** has established action limits for dilation chlorpyrifos, Malathion and parathion were 0.02, 0.05 , 0.05 and 0.05 ppm respectively indelible portion of fish .

The object of this study was to Colette and analyze the inedible portion of fatty fish such as "clarias garpinus" and other public Egyptian fish as tilapia nilotica ".

MATERIALS AND METHODS

1- Fish Samples

Sixty random samples are collected from different muscles of fish sample from markets of some villages of kaliobia Governorate (30 sample of tilapias nilotica and 30 sample of clarias garpinus). Samples were kept in an insulated ice box and transferred to Food Hygiene Department in animal health institute.

2- Apparatus

(a) Refrigerated centrifuge , able to rotate at 3000rpm at – 15C^{o}

(b) Rotetory evaporator: with vacum device and cooler.

(c) Gas chromate graph system: with injection device and electron capture detector .

(d) Capillary column 30 X 0.25

(e) Volumetric pippetis

3- Reagents

(a) Pesticide standard (Sigma Aldrich) solution in hexane: phorate, dimethate, chlorpyripos, parathion, diazinon, and ethion, all at weight 10ng/ml of D. W.

(b) Acetone

(c) Petroleum ether

(d) N-hexane

(e) Methanol

(f) Sodium sulfateAnhydrous

(g) Filter paper.

(2) Method of organophasphorus resuies in fish samples: according to AOAC (2002)

5 gram of fish sample was collected, weighed and placed in beaker mixed with 75 gram of anhydrous sodium sulfate (Fisher scientific). Samples were then placed in a digestion at room temperature for at least 12 hr. the mixture was transfused into a pre-rinsed glass (size 22) thimble containing a plug of glass wool. Samples were extracted with soxhlet apparatus for at least 7hr. with 300/ml hexane (analar) at turn over rate of 4-5 times per h. the extract was evaporated to lease than 5ml by rotary hypobaric evaporation at 40C^{o} and transferred into screw-top tube (16 mm X125mm) . The extracted lipid was solved in 5 ml petroleum ether and this solution was partition a times with 30ml acetonitrile, saturated with petroleum ether by shaking vigorously for 2 minutes each tine Each tine, the separated acetonitrile layer was transferred into separating funnel containing 650 ml deionized water, 40 ml saturated Nacl solution and 100/ml petroleum ether. The petroleum ether was then evaporated under asteamol nitrogen. The extract was transferred to gas

chromatographic column – containing 20 gram aestivated florisil, was aestivated at 130 C^o for 24 hours and cooled at room temperature. The florisil was topped with 1 cm layer anhydrous sodium sulfate, rinsed with 50 ml petroleum ether. An adequate of each extract was transferred to 2 ml injection vial for analysis by electron capture chromatography – fraction.

Sample were analyzed at 3600 electron capture gas chromatograph. Extracts were injected into single inlets that was split into the dual columns, which settings were as follows: injector and detector temperatures, 230/C^o and 300/C^o, respectively , hydrogen cannier gas, nitrogen Make –up gas temperate program – start at 150/C^o held 5 min, ramped at 5C^o/ min to 170/C^o , held 10 min ramped at 10C^o / min to 220C^o, total running time 44 min .

Chromatographic analysis

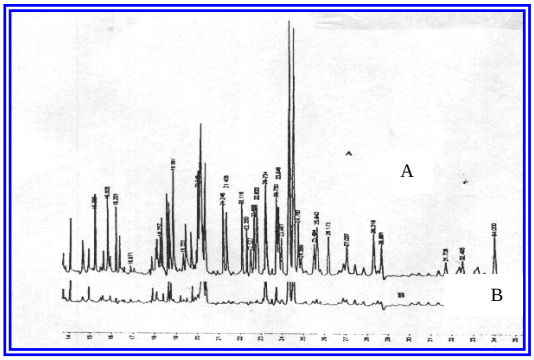
The final stage of pesticide analysis is the separation and instrumental determination of residues in the restively (B extractive – free extranet produced of the sample clean-up).

The instrument technique used depends on the structure of pesticide of interest and the sensitivity required.

Gas chromatography:

The majority of regicides are relatively volatile .

G.C has proved to be and excellent technique for pesticide determinations and is by for the most widely used. The analyst is of ten required to determine pesticide levels as analyst is often required to determine pesticide levels as low as 10μg /mg and to attain such sensitivity, G.C with selective detectors is essential (Hayes and Lawes 1991).



A examined sample retention times (min) (23) 40μg/mg A clarias garpinus) B standard curve

RESULTS

Table (1) showing the incidence of positive samples of organophosphrous residues of muscles of tilapia nilotica and clarias garpinus samples.

Detectable organosphorus residue	No of examine sample		No of Positive sample		Incidence	
	tilapia nilotica	claris geriepinus	tilapia nilotica	claris geriepinus	tilapia nilotica	claris geriepinus
Diazinon	30	30	10	15	33%	50%
Chlorpyrifos-ethly	30	30	2	2	6.7%	6.7%
Malthion	30	30	6	5	20%	16%
Methyl-Parathion	30	30	4	3	12%	10%
Ethyl - Parathion	30	30	4	4	12%	12%
Ethion	30	30	1	1	3%	3%

Table (2) Number of examined samples containing . organophosphrous residues , Average total detected residues:

Detectable organophosphrous residue	**FDA Aetion limit ppm**	**LOQ (ppm)**	**HLQ (ppm)**	**Incidence Positive samples with in hazard effect**		FDA's Action
				Tilapia	Claris	
Diazinon	(2 **ppm)**	0.02	0.37	20%	50%	0.19
Chlorpyrifos-ethly	(5 **ppm)**	0.01	0.37	2%	6%	0.18
Malthion	(5 **ppm)**	0.02	0.5	15%	16%	0.26
Methyl-Parathion	(2 **ppm)**	0.02	0.092	10%	10%	0.06
Ethyl - Parathion	(2 **ppm)**	0.02	0.072	10%	12%	0.046
Ethion	(2 **ppm)**	0.02	0.32	3%	3%	0.1

LOQ= lower limit of quanitation .
HLQ= High limit of quanitation
*Average for each pesticide wop calculated from positives sample only

DISCUSSION

Diazinon was detected in 33% and 50% of tilapia nilotica and clarias garpinus (table 1). The lower limit of quantitation for each was 002 ppm. The predominate organophosphrus compounds residues was Diazinon with average range 0.19 ppm Diazinon was considerd as high toxic (1st group) of organophosphrous compounds. Which reported by food and Drug Administration (FDA, 2000) in the total Diet study and in infant foods sampled during 1998. Average diazinon residues in fish have been reported as high as 10/ppm for wild fish collected in national survey , however typical levels are demonstrated by other reports that indicate average levels in largemouth bass and channel cat fish from lake

providence loaisiana at 8.96 and 2.27 ppm (Niethammer et al, 1995).

The incidence of Diazinon from examined sample which in levels higher than the high limit of quanitation (HLQ) of **FDA (2000)** were 20% and 50%, From ***tilapia nilotica*** and ***clarias garpinus*** respectively Diazinon was considered most popular and usable in agriculture which used as insecticide, herbicide acricide and nematicide, also applied mostly as winter wash for frost trees **(Klassen , et al 1986).** With persistence, both in humans and in the environment , has caused public concern highly, in certain situations, they may pose health and environmental problems, **Bhoopendra and Dograc (2009)** , reported that the six different organophosphorus insecticides was 86.3% for phorale 78.3% for dunetgiate , , 82.3% for Malathion, 79.4% for chlorpyrifos, 80.2% for diazinon and 68.5 % for ethion at the μg/ml levels from serum samples of nine workers who had been occupationally exposed to Malathion in an insecticide Manufacturing factory, were analyzed and Malathion was found at low levels in all the samples .

From the point of our data, the result was similar to results recorded by **Niethammer (1995), stefanelli et al (2002); Santerre et al., 2000,** also lower results obtained by **James et al., (1989**) who found that diazinon was not detected in whole body fish collected from fresh water fish from lower Gile River, Arizona.

chlorpyrifos considered as colorless and odorless insecticide which use in large scale in governorate especially in kaliobia governorate chlorpyrifos was found in 2 samples of **tilapia nilotica** and clarias garpinus (6.7%) of each them. Due to the withdrawal of chlorpyrifos from selected river area so not detected in all other sample. The average and range for chlorpyrifos in Nile fish were 0.18ppm and 0.01 to 0.37ppm respectively (table 2).

Ueno et al (2002) reported that the body length normalize values of participles (organophosphrus) on a lipid weight baris which was adjusted form 100 cm body length were indicative of present state water pollution by organophasphate compound these resembles suggest that tuna fish is a suitable bio indicator for monitoring organophosphrous compounds (comaphos) can termination in the open sea.

Wander et al (1987) reported no change or an apparent increase in pesticide residue when lake trout and salmon packed, boiled or poached, however they did not account for changes in moisture on fat during cooking .

Malathion residues was found in 6 samples of tilapia nilotica 20% while found in 4 sample only of clarias garpinus (16%) within average 0.26 ppm (table 2) but only 5% of examined tilapia nilotica within normal detection limit reported by **(Eclipse 2009)** , also reported that there is no evidence that long term exposure to small amounts of Malathion and chlorpyrifos causes any harmful health effect in people.

Malathion was widely used to eradicate insect and it is also persistent in environment. The obtained data are nearly similar to the data obtained of **James et al., (1980),** who reported that the Malathion

residue in water fish . From lower Gila River varied with in .range 0.167.

Parathion compound either Methyl parathion or ethyl parathion were classified as slightly Extremely hazard organophosphrus compound by WHO (1990). Parathion used as insectside in large scale due to cheap and available for each.

Parathion residues were detected in 12% each examined sample of tilapia nilotiea and claris gariepinus (table 1). The average Methyl parathion was 0.06 ppm. While Ethyl parathion was 0.04 ppm . The range of Methyl and ethyl parathion were 0.02. to 0.092 and 0.02 to 0.072 ppm respectively.

From obtained data and coinciding with the results of **Aanlerre et al., (2001)** Who reported that the parathion residues could not detected in cat fish.

Also **Christiansen et al., 1991** reported that the Maximum parathion residues in channel cat fish in amounts varied from 0.09 to 0.01 ppm.

Our data obtained higher than that reported by **(James et al., 1989)** which reported that there is no parathion residues could be detected from fresh water fish coughed from lower Cila River Arizona.

Finally Ethion residue were detected also in only one sample of each (Tilapia nilotica and Claris gariepinus) with the same incidence (3%) of each. The average of Ethion residue 0.02 to 0.32 ppm with the range 0.1 ppm .

These obtained our result was higher than **WHO (1990)**. But nearly similar **(Eclipose 2009)** which reported that the normal detection level from water and fish were 0.05 and 0.02 ppm respectively.

By comparison our results between the two categories of fish (Tilapia nilotica and claris gariepinus) notice the all organophosphous residues more in levels detectable in all elaris graininess sample (97%) , while detected in lower than in tilapia nilatica (60%). That's may be due to claris gariepinus habit at is near sludge treatment and waste water treatment in which bioaccumulative toxic chemicals were deposed of this class of chemicals or emerging pollutants such as Azinphos, Acephate , Bromophos ethyl, chlorpyrifos and diazinon are transformed or detoxified during one sludge treatment process anaerobic digestion **(Donna et al., 2005).**

The scientific assessment found no-evidence that current levels of organophosphrous compounds in environment are harming human heal that moment. However the rapid increase level of organophosphrous compound in environment over the last several years is a matter or concern. Health effects have been observed in lab animals (neurotoxic) but only at level much greater than those to which people are exposed (FAO, 2004) .Conclusively , pollution of rivers and lake water by products of man continues to create public health problems. Also pollution of natural aquatic environment of fish should be controlled. The first step in control is to prevent access by untreated sewage or human faeces to streams or lakes.

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