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THE EFFECT OF CARBOFURAN AND ENDOSULFAN ON THE AFRICAN CATFISH, *CLARIAS GARIEPINUS*

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By

ISAM ELDIN MOHAMED ELAMIN ABU ZEID

Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

February 2001

DEDICATION

To the memory of my late father, MOHAMED ELAMIN who left us alone while I am conducting this study, and to my beloved wife GHADA and sons MOHAMED and RAZI who were the source of inspiration and encouragement throughout the period of this study.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Assoc. Prof. Dr. Mohd. Arif Syed

Faculty: Science and Environmental Studies

This study was undertaken to determine the toxic effects of sub-lethal concentrations of carbofuran and endosulfan on some behavioural, morphological, biaccumulation, biochemical, histopathological, and molecular aspects of the freshwater African catfish, *Clarias gariepinus*. The toxicity of carbofuran and endsulfan was ascertained by estimating the LC_{50}.

The calculated 96-h LC_{50} values of carbofuran and endosulfan for juveniles of *Clarias gariepinus* were found to be 10.4 p.p.m and 21.6 p.p.b respectively, under tropical condition. The test fish swam erratically, struggled to breath, often swam to the surface, followed by loss of equilibrium. The color of the skin became progressively pale during the period of the test, The liver glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and acetycholinesterase (AchE) were determined photometrically. Within 16 days of treatment, the activity levels of GOT and GPT were significantly

($p<0.05$, $p<0.01$, $p<0.001$) increased by 203% and 121% for carbofuran and 167% and 195% for endosulfan respectively, whereas, AchE activity levels were inhibited following exposure to the test pesticides. With no exceptions, the *in vivo* and *in vitro* effects of carbofuran and enosulfan on GOT, GPT and AchE were qualitatively similar.

The pesticide concentration in the tissues was in the order of liver> intestine> gill> brain> muscle. The highest concentration of the test pesticides (353.47 μg/g carbofuran and 1409.35 ng/g endosulfan) were found in the liver 24 hours after treatment. Liver lesions were observed following exposure to both pesticides. The evidence of pesticide accumulation was elucidated by necrosis and damaged fish liver.

The depletion in the protein content was observed following exposure to carbofuran and endosulfan. The RNA concentrations were significantly decreased in *Clarias gariepinus* exposed to both pesticides, whereas, DNA concentrations tend to remain constant. The DNA molecular weight of the control fish was found to be about 16832 bp, whereas, the DNA molecular weights of carbofuran and endosulfan exposed fish were 14505 and 14505 bp respectively. The liver-somatic index decreased to 2.11 for carbofuran and 1.59 for endosulfan in comparison to control. The biochemical, molecular, bioaccumulation, histological, behavioural and morphological techniques employed in this study may be used to detect and assess any pesticidal pollution in the aquatic environment at an early stage of pollution.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

KESAN KETOKSIKAN OLEH ENDOSULFAN DAN KARBOFURAN KE ATAS IKAN KELI AFRIKA, *CLARIAS GARIEPINUS*

Oleh

ISAM ELDIN MOHAMED ELAMIN ABU ZEID

Februari 2001

Pengerusi: Prof. Madya Dr. Mohd. Arif Syed

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Kesan ketoksikan endosulfan dan karbofuran ke atas perlakuan, morfologi, bioakumulasi, biokimia, histopathologi dan aspek molekul telah dikaji pada ikan keli Afrika dengan memberikan rawatan sublethal. Ketoksikan endosulfan dan karbofuran telah ditentukan nilainya dengan LC_{50}.

Nilai LC_{50} bagi rawatan 96 jam karbofuran dan endosulfan bagi *Clarias gariepinus* didapati masing-masing adalah 10.4 ppm dan 21.6 ppb. Ikan didapati menyelam tidak tentu hala, menghadapi masalah pernafasan, kerap muncul ke permukaan dan hilang daya imbangan. Semasa ujikaji, warna kulit dilihat menjadi semakin pucat. Pada hati, enzim glutamat oxaloacetat transaminase (GOT), glutamat piruvat transaminase (GPT) dan asetilkolinesterase (AchE) ditentukan secara fotometrik. Semasa 16 hari rawatan, paras aktiviti GOT dan GPT adalah signifikan ($p<0.05$, $p<0.01$, $p<0.001$). Paras masing-masing telah meningkat sebanyak 203% dan 121% untuk karbofuran, 167% dan 195% untuk endosulfan. Aktiviti AchE pula didapati terencat akibat pendedahan kepada racun

perosak tersebut. Dari permerhatian *in vivo* dan *in vitro,* kesan karbofuran dan endosulfan ke atas GOT, GPT dan AchE adalah sama secara kualilitatif.

Kandungan racun perosak pada tisu adalah tinggi menurut turutan hati>usus>insang>otak>otot. Kandungan tertinggi racun perosak (353.47µg/g karbofuran dan 1409.35ng/g endosulfan) di dalam hati, selepas 24 jam rawatan. Pemerhatian kerosakan hati telah dilakukan selepas dirawat racun perosak. Kesan pengumpulan racun perosak telah berjaya dibuktikan melalui kerosakan dan nekrosis pada hati tersebut.

Kandungan protein didapati berkurang pada ikan yang telah dirawat dengan carbofuran dan endosulfan. Jumlah RNA juga didapati menurun pada *Clarias gariepinus* yang terdedah kepada kedua-dua racun perosak ini manakala kepekatan DNA didapati tidak ada sebarang perubahan. Berat molekul DNA bagi kawalan adalah 16832 bp dan ikan terdedah kepada endosulfan dan carbofuran masing-masing berat molekulnya adalah 14505bp. Nisbah berat badan ikan kepada berat hati jika dibandingkan dengan kawalan didapati telah menurun kepada 2.11 bagi carbofuran dan 1.59 bagi endosulfan.

Kesimpulannya, teknik biokimia, molekul, bioakumulasi, histologikal, perlakuan dan morfologikal dapat digunakan bagi mengesan dan mengenalpasti pencemaran racun perosak di dalam persekitaran akuatik terutama di peringkat awal pemcemaran berlaku.

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And above all to Almighty ALLAH, the Merciful and Benevolent.

I certify that an Examination Committee met on 2nd February 2001 to conduct the final examination of Isam El Din Mohamed El Amin Abu Zeid on his Doctor of Philosophy thesis entitled "The Effect of Carbofuran and Endosulfan on the African Catfish, *Clarias gariepinus*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for degree of Doctor of Philosophy.

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Date: 10 APR 2001

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Isam El Din Mohamed El Amin

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AchE	Acetylcholinesterase
ANOVA	Analysis of variance
Bp	Base-pair
DDVP	Dichlorvos
DO	Dissolved oxygen
EDB	Ethylene dibromide
EROD	Ethoxyresorufin O-deethylase
GOT	Glutamate oxaloacetate transaminase
GPT	Glutamate pyruvate transaminase
H & E	Hematoxylin and Eosin
H	Hepatic cell
HCH	Hexachlorocyclohexane
IAEA	International Atomic Energy Agency
LC_{50}	Lethal concentration that results in 50% death.
LSC	Liquid scintillation counter
LSI	Liver-somatic index
MATC	Maximum acceptable toxicant concentration
MFO	Mixed function oxygenase
N	Nucleus
NC	Necrotic cell

NRC	National Research Council
NRCC	National Research Council of Canada
NU	Nucleolus
PCB	Polychlorinated biphenyl
PCP	Pentachlorophenol
SAC	Safe application concentration
SD	Standard deviation
SDS	Sodium dodecylsulfate
SEM	Scanning electron microscope
TCDD	Tetrachlorodibenzo-p-dioxin
TEM	Transmission electron microscope
UPM	Universiti Putra Malaysia

CHAPTER I

INTRODUCTION

The growing demand for increased food productivity to meet the needs of the global population has led farmers to use sophisticated agricultural technology in which pesticides play a crucial role. Pesticide use has a positive and dramatic impact on agricultural production through protection of crops against insects, pests and diseases. The extensive use of pesticides in agriculture has given rise to criticisms in recent years, due to their persistent nature in the environment (Hernandez *et al.*, 1993), and accumulation in different tissues of plants (Kaplan, 1999), animals (Miao *et al.*, 2000) and human beings (Saleh *et al.*, 1998). Therefore, the use of pesticides is a mixed blessing, while their benefits for preventing crop losses (Kacew *et al.*, 1996) and saving human lives (Emerson *et al.*, 1999) are well recognised, they very often result in unwanted side effects. Therefore, the identification of pesticides which are effective against pests and at the same time relatively safe to human and non-target organism is of considerable importance.

Among the numerous environmental impact of the application of pesticides in agriculture are their undesirable effect on the aquatic fauna of freshwater courses (Barlas, 1999). Pesticides have the potential to enter the aquatic environment by direct spraying or broadcast of granular formulations,

drift deposition of sprayable formulations, and in runoff water from treated field (Sharma, 1990). Redeposition from the atmosphere is another route of entry, as some studies have shown the presence of pesticides in rain water (Richards *et al.*, 1987). The pollution of the aquatic environment by pesticides is known to pose a constant threat to fish by altering their habitat, behavioural pattern, growth, and reproduction (Jarvian *et al.*, 1977). Fish is extremely sensitive to pollutants and exhibit a very high bioaccumulation rate of dissolved chemicals relative to their concentration (Al-Yousuf *et al.*, 2000). Fish may accumulate pollutants and pass them to human beings through food causing chronic or acute diseases (Adeyeye *et al.*, 1996). The importance of fish as one of the major source of cheap and available protein-rich food for human being is recognised (Begum and Vijayaraghavan, 1996). The nutritional value of different fishes depends on their biochemical composition; protein, amino acids, vitamins, and mineral contents (Ganeson *et al.*, 1989). Pesticides affect these biochemical composition and may cause biochemical and physiological changes in different fish tissues (Ramaswamy *et al.*, 1999).

Due to increasing pesticide applications, it has become necessary to evaluate their hazards and develop biological indicators of aquatic contamination Various approaches have been used to evaluate or predict the effects of environmental stress on fish. The most common of these are laboratory tests of acute and chronic toxicity. Although these approaches are valuable for achieving such objectives as formal water quality criteria (Adams, 1990), however, they

lack ecological realism (Cairns, 1981). Recently, researchers used the biological markers approach to detect the effect of pollutants in the environment (Hoque, 1997). Biological markers permit the detection of stress-related factors that are biologically and ecologically variable. They provide early signals of adverse ecological effects as they use the lower levels of biological organisation, evaluate the specific response for each type of environmental stress as well as the overall integrated response, and can predict and evaluate the ecological significance and chronic stress (Adams, 1990). Changes in fish such as behavioural (Rice *et al.*, 1997), morphological (Richmond and Dutta, 1992), biochemical (Juzu *et al.*, 1998; Abu Zeid *et al.*, 1997), histological (Dhanapakiam and Premlatha, 1994), and molecular (Thomas, 1990) have been attributed to pesticides. These changes have the potential to be used as possible biological markers for the assessment of pollution in the aquatic environment.