TOXICOLOGICAL STUDIES OF PESTICIDE FORMULATIONS IMIDACLOPRID AND MALATHION ON ALBINO MICE

 $\mathbf{B}\mathbf{y}$

WALID SAID HELMY

B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Cairo Univ., 2005

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences (Pesticides)

Department of Economic Entomology and Pesticides
Faculty of Agriculture
Cairo University
EGYPT

2014

APPROVAL SHEET

TOXICOLOGICAL STUDIES OF PESTICIDE FORMULATIONS IMIDACLOPRID AND MALATHION ON ALBINO MICE

M.Sc. Thesis
In
Agric. Sci. (Pesticides)

By

WALID SAID HELMY

B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Cairo Univ., 2005

APPROVAL COMMITTEE

Dr. ABDALLA ELHUSSEIN ABDEL MONEM Professor of Pesticides, Fac. Agric., Al-Azhar University.
Dr.MOHAMMAD ABD-ELHADY KANDIL
Professor of Pesticides, Fac. Agric., Cairo University.
Dr. ESSAM ELDIN ABDEL-RAOUF EWEIS
Professor of Pesticides, Fac. Agric., Cairo University.
Dr. AMAL YOUSEF SALEH
Professor of Pesticides, Fac. Agric., Cairo University.

SUPERVISION SHEET

TOXICOLOGICAL STUDIES OF PESTICIDE FORMULATIONS IMIDACLOPRID AND MALATHION ON ALBINO MICE

M.Sc. Thesis
In
Agric. Sci. (Pesticides)

By

WALID SAID HELMY

B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Cairo Univ., 2005

SUPERVISION COMMITTEE

Dr. AMAL YOUSEF SALEH

Professor of Pesticides, Fac. Agric., Cairo University

Dr. ESSAM ELDIN ABDEL-RAOUF EWEIS

Professor of Pesticides, Fac. Agric., Cairo University

Name of Candidate: Walid Said Helmy Degree: M.Sc.

Title of Thesis: Toxicological Studies of Pesticide Formulations Imidacloprid and

Malathion on Albino Mice.

Supervisors: Dr. Amal Yousef Saleh

Dr. Essam Eldin Abdel-Raouf Eweis

Department: Economic Entomology and Pesticides **Branch:** Pesticides

Approval /

ABSTRACT

The toxicity of imidacloprid (in two formulations Gaucho 70% WP and Confidet 35% SC and malathion El Nasr and Kafr El Zayat 57 % EC) was investigated on male albino mice using 1/8, 1/4 and 2/5 oral sub-lethal doses of LD₅₀ in plasma, brain, liver and kidney for 24 and 48 hrs. Confidet caused a decrease in total protein content in plasma, brain, liver and kidney at of 1/8, 1/4 and 2/5 LD₅₀ doses versus an increase in total protein when using the same concentrations of Gaucho after 24 and 48 hrs. Confidet further inhibited the cholinesterase in plasma at 1/8, 1/4 and 2/5 of LD₅₀ doses after 24 and 48 hrs while treatment with Gaucho increased the activity of the same enzyme. The activity of aspartate aminotransferees in plasma increased after treatment with 1/8, 1/4 and 2/5 LD₅₀ in both Confidet and Gaucho but the rate of increase of activity was greater in Gaucho than Confidet, meanwhile enzyme activity decreased in liver in the cases of treatment with both formulations but the rate of decrease activity for Confidet was greater than it in the case of Gaucho. The activity of Alanine aminotransfers decreased in plasma after treatment with 2/5 LD₅₀ of both Confidet and Gaucho formulations after 24 and 48 hrs versus an increase in the activity of the same enzyme in liver in the case of treatment with Gaucho. Urea content in plasma increased after treatment with both Confidet and Gaucho after 24 and 48 hrs for all treatments. For all considered doses, Treatment with malathion 57% EC (EL Nasr Company) decreased the total protein content in plasma, liver, brain and kidney after 24 and 48 hrs. cholinesterase inhibited after the treatment of both malathion compounds. Aspartate aminotransfers level increased in plasma by the all treatments of both malathion formulations, but in liver it decreased in all treatment of malathion compounds after 24 and 48 hrs. Alanine aminotransfers level increased in plasma for all treatments of malathion, while in liver the same enzyme decreased as a result of using 1/8 and 1/4 LD₅₀ of malathion from Kafr El Zayat company. Urea in plasma and kidney increased in treatment with malathion (El Nasr and Kafr El Zayat) after 24 and 48 hrs, and increased in kidney by using 2/5 LD₅₀ of malathion (El Nasr) after 24 and 48 hrs.

Key words: Imidacloprid, malathion, Total protein, cholinesterase, aspartate aminotransferase, alanine aminotransferase.

ACKNOWLEDGEMENT

The author wishes to express his deep thanks to Dr. Amal Yousef Saleh Professor of Pesticides, Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University for her supervision, encouragement and valuable comments continuous throughout all phases of this work as well as for revising the manuscript. Thanks are also extended to Dr. Essam Eldin Abdel-Raouf Eweis, Prof. of Pesticides, Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, for his valuable suggestions, and sincere supervision, guidance and the unlimited help he gave during the whole course of this work. The help, backing and cooperation of all of the members of Economic Entomology and Pesticides Department, Faculty of Agriculture, Cairo University, is deeply acknowledged.

INTRODUCTION

In Egypt, the majority of pesticides are imported as readyto-use formulations. As a matter of fact, establishing the industry of pesticides formulations utilizing local basic ingredients would contribute much to the national income. Therefore, efforts should be directed towards using local raw materials and products for the formulation of different types of pesticides. Moreover, pesticide formulation at reasonable prices should be regarded as a consequent approach for decrease in the cost of pest control. Adjuvants are solvents, knockdown materials, sprayer materials, synergism materials ... etc that increases the toxicity of pesticide formulations (Tsuji and Fuyama 1982). The discovery of neonicotinoids as important novel pesticides may be regarded as a milestone in insecticide research throughout the past three decades (Nellore et al., 2013). It is related to nicotines in their structure and action at the nicotinic acetylcholine receptor. (Casida and **Quistad 1998).**

Imidacloprid, as a member of the neonicotinoid insecticides, has become an important pest control agent on many crops. The selective toxicity of imidacloprid to insects but not to mammals refers to differences in its binding affinity or potency at the nicotinic acetylcholine receptor (Chao & Casida, 1997 and Casida & Quistad, 1998). Such advantage made imidacloprid one of the fastest

sold insecticides across the world. (Soujanya *et. al.*, 2013). Neurophysiological studies confirmed that imidacloprid is an agonist at the postsynaptic nicotinic acetylcholine receptor of insects. It appears to act like acetylcholine, by exciting specific nerve cells (Nagata, 1998).

Malathion (diethyl mercaptosuccinate S-ester with 0.0phosphorodithioate) is of dimethyl one the least toxic organophosphorus insecticides to mammals. It is extensively used to control a wide range of sucking and chewing pests of field crops, fruits and vegetables (Wankhade et al., 2009). malathion, like the other members of the OP common mechanism group, inhibits ChE as a mode of toxic action. It blocks the acetylcholinesterase enzyme which decomposes acetylcholine. Immobilization of this enzyme results in accumulation of excessive amounts of acetylcholine in the nervous tissues and muscular motor plates, and reflects symptoms of poisoning. (Harlin and Dellinger, 1993). malathion is usually formulated as a technical preparation, a dust, EC, a ready-to-use, a pressurized liquid, and WP.

This study compare two different formulations of the same active ingredient (imidacloprid) and also two different formulations of the same percent of active ingredient of malathion, produced by two different Egyptian companies. The two tested formulations of imidacloprid were Confidet 35% SC and Gaucho 70% WP while the 2 tested formulations of malathion are malathion 57% EC from El Nasr Company and also malathion 57% EC from Kafr El Zayat Company.

Each company has its special additives that may enhance the formulation properties. Also every active ingredient could be formulate in a different form to use it against different kinds of organisms (insects, nematodes, fungi.....etc.). This work investigates how the various additives added by each company can change either pesticide efficacy or their side effects on non-target organisms.

The objectives of this study are:

- 1) Determination of the acute oral LD_{50} for albino mice of both tested imidacloprid and malathion insecticidal formulations
- 2) Comparative studies of the sublethal toxicity of two imidacloprid formulations (Confidet 35% SC and Gaucho 70% WP) on the activity of certain enzymes in albino mice at 24 and 48 hours after treatment.
- 3) A comparative study of the sub-lethal toxicity of malathion 57% EC emulsion concentrates produced by different companies (El-Nasr and Kafr El-Zayat companies) on the activity of different enzymes in albino mice after 24 and 48 hours after treatment.

LIST OF ABBREVIATIONS

Abbreviation

3β HSDH 3β- hydroxysteroid dehydrogenase

AChE Acetylcholinesterase

ACP Acid phosphatase

AKP Alkaline phosphatase

ALT Alanine aminotansferase

AST Aspertate aminotransferase

BChE Butyrylcholinesterase

BUN blood urea nitrogen

CAT Catalase

ChE Cholinesterase

EC Emulsifiable concentrate

GGT Gamma-glutamyl transferase

GST Glutathione-S-transferase

LD₅₀ Median lethal dose

LDH Lactate dehydrogenase

MDA Malondialdehyde

NOEL No observed effect level

Ops Organophosprous insecticides

OS Oil solutions

SC Suspension concentrate

SChE Serum cholinesterase

SOD Superoxide dismutase

TLC Total leukocyte count

ULV Ultra low volume

VLDL Very low density lipoprotein

WBC White blood cell

WP Wettable powder

CONTENTS

INTRODUCTION
REVIEW OF LITERATURE
MATERIALS AND METHODS
1. Test Animal
2. Insecticides
a. imidacloprid
b. malathion
3. Methods used
a. Toxicity study
b. Estimation of body weight gain, internal organs
weight and preparation of samples
c. Methods of analysis
1. Plasma Total Protein
2. Determination of cholinestrase activity
3. Determination of liver function
a. Determination of aspartate aminotransferase (AST)
b. Determination of Alanine aminotransferase (ALT)
4. Determination of Enzymatic Urea
d. Statistical analysis
RESULTS AND DISCUSSION
1. imidacloprid
a. Acute oral toxicity of imidacloprid
b. Physiological studies
1. Effect of imidacloprid on mice body-weight
2. Effect of imidacloprid on organs weight
c. Biochemical aspects
1. Effect of imidacloprid on total protein
2. Effect of imidacloprid on cholinesterase
3. Effect of imidacloprid on (AST) values
4. Effect of imidacloprid on (ALT) values
5. Effect of imidacloprid on Urea
2. malathion
a. Acute oral toxicity of malathion
b. Physiological studies
1. Effect of on body-weight

i

2.	Effect of malathion the weight of internal organs
c. B	iochemical aspects
1.	Effect of malathion on total protein
2.	Effect of malathion on cholinesterase
3.	Effect of malathion on (AST) values
4.	Effect of malathion on ALT values
5.	Effect of malathion on urea
UMM	ARY
	RENCES
	CSUMMARY

LIST OF TABLES

No.	Title	Page
1.	LD ₅₀ (mg/kg) of Confidet in male albino mice	33
2.	LD ₅₀ (mg/kg) of Gaucho in male albino mice	33
3.	LD ₅₀ (mg/kg) of imidacloprid formulations (Gaucho and Confidet) at 1/4, 1/8 and 2/5 sub-lethal doses in male albino mice.	35
4.	Effect of administration of Confidet on the body weight gain in male albino mice	36
5.	Effect of administration of Gaucho on the body weight gain in male albino mice	36
6.	Effect of administration of Confidet on organs weight-gain in male albino mice	37
7.	Effect of administration of Gaucho on organs weight-gain in male albino mice	37
8.	Effect of administration of Confidet SC and Gaucho W.P on total protein in plasma, brain, liver and kidney after 24 and 48 hrs in male albino mice	46
9.	Activity of cholinesterase as affected by treatment with Confidet and Gaucho at 1/8, 1/4 and 2/5 LD ₅₀ in male albino mice after 24 and 48 hrs	48
10.	The effects of Confidet and Gaucho on aspartate aminotransaminase after treatment with $1/8$, $1/4$ and $2/5$ LD ₅₀ on albino mice after 24 and 48 hrs	54
11.	The effects of Confidet and Gaucho on alanine aminotransaminase after treatment with 1/8, 1/4 and 2/5 LD ₅₀ on male albino mice after 24 and 48 hrs	58

12.	Effect of administration of 1/8, 1/4 and 2/5 LD ₅₀ of Confidet 35% SC and Gaucho 70% W.P on Urea in plasma and kidney in male albino mice after 24 and 48 hrs	61
13.	LD ₅₀ (mg/kg) of malathion 57% EC (El Nasr Company) in male albino mice	62
14.	LD ₅₀ (mg/kg) of malathion 57 % EC (Kafr El Zayat Company) in male albino mice	62
15.	LD ₅₀ (mg/kg) of malathion produced by El Nasr and Kafr El Zayat Companies at 1/4, 1/8 and 2/5 sub-lethal doses in male albino mice.	63
16.	Effect of oral administration of malathion (El Nasr company) on the body weight gain in male albino mice	64
17.	Effect of oral administration of Malathion (Kafr El Zayat company) on the body weight gain in male albino mice	64
18.	Effect of oral administration of malathion (El Nasr Company) on organs weight gain in male albino mice	65
19.	Effect of oral administration of malathion (Kafr El Zayat Company) on organs weight gain in male albino mice	66
20.	Effect of oral administration of malathion (El Nasr and Kafr El Zayat companies) on the total protein content in plasma, brain, liver and kidney of male albino mice after 24 and 48 hrs	72
21.	Effect of oral administration of malathion (El Nasr and Kafr El Zayat companies) on Cholinesterase in plasma after 24 and 48 hrs in male albino mice	75
22.	Effect of oral administration of malathion (El Nasr and Kafr El Zayat companies) on AST in plasma and liver in male albino mice after 24 and 48 brs	81

23.	Effect of oral administration of malathion (El Nasr and Kafr El Zayat companies formulations) on ALT in plasma and liver in male albino mice after 24 and 48 hrs	85
24.	Effect of administration of malathion (El Nasr and Kafr El Zayat companies) on Urea in plasma and kidney after 24 and 48 hrs in male albino mice	89