

*Deanship of Graduate Studies*  
Al-Quds University



**Toxicities of Several Insecticides to the House Fly  
*Musca domestica* L. from Different Locations in  
the West Bank**

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**M.Sc. Thesis**

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*Musca domestica* L. From Different Locations in  
the West Bank**

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Al-Quds University  
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## Thesis Approval

### **Toxicities of Several Insecticides to the House Fly *Musca domestica* L. from Different Locations in the West Bank**

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Jerusalem - Palestine

1428/2007

## **DEDICATION**

*I present this work to my family, husband and dear daughter*

*Reem, for their patience, support and full understanding ...*

*To all of these, I wish them to accept my modest dedication*

## **DECLARATION**

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed: .....

Dima “Mohammad Jaber” Saleh Halawani

Date:

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Before all and above I would like to express my endless thanks to “ALLAH” the merciful for conciliation, and providing patience.

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## **ABSTRACT**

The common house fly *Musca domestica* L. causes nuisance to people all over the world. It was proven that it had shown the greatest ability to develop resistance against several insecticides. Since many years, certain insecticides had been used for fly control in the West Bank without being tested for their affectivity. Taking this into consideration, it is important to test if the house fly had developed resistance to the insecticides commonly used in the West Bank.

In this study house fly strains were collected from cow or poultry farms from five locations in the West Bank namely: Hebron, Bethany, Ramalla, Tulkarem and Jericho using a net to collect flies, or a jar of jam to attract them. Adult females, 3-5 days old, were used for the experiments. F1 adult flies tested in this work were the progeny of flies obtained at the intervals during June, 2004 to September, 2005

The LC<sub>50</sub> values of four insecticides most commonly used for fly control in the West Bank, namely: Diazinon, Malathion, Parathion and Lambdacyhalothrin, were measured using adult 3-5 days old house flies. A minimum of 6 different concentrations of each insecticide were tested on each house fly strain. Three replicates were made for each concentration. Fifteen females of the tested population of flies were exposed to the impregnated paper for 24 h. During this time good access of flies to water was ensured, but the impregnated filter paper remained dry.

Results from the five locations indicated that the pyrethroid insecticide; lambdacyhalothrin was the most effective insecticide with all fly strains tested, with LC<sub>50</sub> values as follows: Hebron (0.28 mg/L), Bethany (0.52 mg/L),

Ramalla (1.95mg/L) and Tulkarem (3.89 mg/L) respectively except for Jericho which showed considerably varied result with LC<sub>50</sub> value of 136.48mg/L. These relatively small LC<sub>50</sub> values for the Lambdacyhalothrin indicate that the house fly was susceptible to the insecticide. These results endorsed when the R/S ratios were calculated and compared to the most susceptible strain (Bethany) which were as follows: Tulkarem (7.48X), Ramalla (3.75X) and Hebron (0.54X) while Jericho had a very high R/S ratio (262.47X) and this result implies that house fly population from Jericho seems to be resistant to lambdacyhalothrin.

Diazinon was less effective in Tulkarem, Hebron and Ramalla with LC<sub>50</sub> values 15.14 , 14.06 and 13.39 mg/L and R/S ratios 12.11X, 11.25X and 10.71X, while it was more effective in Bethany and Jericho with LC<sub>50</sub> values of 1.25 and 8.79mg/L and R/S ratio 7.03X for Jericho.

Tulkarem results showed that flies were highly tolerant to parathion with LC<sub>50</sub> 321.87mg/L and R/S ratio 1609.37X, while in the other four locations flies were susceptible with LC<sub>50</sub> values: Ramalla (7.59mg/L), Jericho (1.99mg/L), Hebron (1.66mg/L) and Bethany (0.20mg/L). R/S ratios showed that there are degrees of tolerance among the house fly population from the different locations as it was Ramalla (37.95X), Jericho (9.95X) and Hebron (8.30X).

Bethany and Hebron strains of house fly were susceptible to Malathion with LC<sub>50</sub> 0.60 and 2.85mg/L and an R/S ratio 4.68X for Hebron, while in the rest of the three locations house flies were tolerant to Malathion with LC<sub>50</sub> values: Tulkarem (86.28mg/L), Jericho (11.48mg/L) and Ramalla (5.89mg/L) and R/S ratios 141.45X, 18.82X and 9.65X, for the tree locations respectively.



The present study revealed that house flies from different locations in the West Bank showed varying levels of tolerance to the insecticides tested. Therefore, it is important to monitor insecticides performance from time to time, in order to make necessary shifts and modifications.

## ملخص

دراسة سمية عدة مبيدات حشرية على الذبابة المنزلية *Musca domestica L.* من عدة مناطق في الضفة الغربية

تعتبر الذبابة المنزلية *Musca domestica L.* مشكلة صحية وبيئية في جميع أنحاء العالم. وقد أثبتت الحشرة قدرة عالية على تطوير مقاومتها للمبيدات المستخدمة في مكافحتها. وحيث أنه لم يكن هناك أي نوع من المراقبة لتلك المبيدات المستخدمة منذ سنين عديدة في الضفة الغربية برزت أهمية هذا البحث بهدف فحص فاعلية أهم المبيدات الحشرية المستخدمة في مكافحة ذباب المنزل في الضفة الغربية.

تم جمع الحشرات البالغة للذبابة المنزلية من مزارع الأبقار أو الدجاج من خمس مناطق في الضفة الغربية وهي: الخليل، العيزرية، رام الله، طولكرم وأريحا. وذلك باستخدام مصيدة شبكية أو عن طريق استخدام علبة تحتوي على مربي لجذب الذباب. وقد استعملت اناث الذباب البالغات والتي تتراوح أعمارهن ما بين 3-5 أيام في البحث. حيث تم نقل تلك الحشرات الى المختبر لتربيتها والحصول منها على الجيل الأول (F1) الذي استخدم في هذا البحث. يجدر الإشارة الى أن تجميع الحشرات تم خلال الفترة الواقعة ما بين حزيران 2004-أيلول 2005.

تم دراسة سمية أربع مبيدات حشرية، والتي تعتبر أهم المبيدات المستخدمة عادة في مكافحة الذباب المنزلي في الضفة الغربية. حيث عوملت اناث الذباب التي تتراوح أعمارهن ما بين 3-5 أيام بمحاليل المبيدات وذلك باستخدام طريقة ملامسة أطراف الذبابة لورق ترشيح يحتوي على تراكيز معروفة من المبيد الحشري. سجلت نتائج سمية المبيدات بواسطة حساب التركيز القاتل لخسمن بالمائة من الحشرات ( $LC_{50}$ ). وقد تم استخدام المبيدات التالية في البحث: الديازينون، المالثيون، البارثيون ولامبدا- سايهالوثرين، حيث تم استخدام ستة تراكيز مختلفة من كل مبيد على كل مجموعة ذباب من كل منطقة. في كل مرة تم استخدام خمسة عشر ذبابة حيث تم تعريضها لورق الترشيح لمدة أربع وعشرين ساعة، كان فيها وصول الذباب للماء مؤمن، وفي نفس الوقت تم الحفاظ على جفاف الورقة .

أشارت النتائج على الذباب الى ان المبيد البايثروديدي لامبدا- سايهالوثرين كان الأكثر فاعلية على الذباب من جميع المناطق، باستثناء الذباب من أريحا حيث كانت قيم (LC<sub>50</sub>) لهذا المبيد في المناطق المختلفة كما يلي: 0.28، 0.52، 1.95 و 3.89 ميلليغرام/ليتر (الخليل، العيزرية، رام الله وطولكرم) على التوالي. أما منطقة أريحا فقد أظهرت نتائج هذا المبيد صورة مختلفة تماما حيث بلغت قيمة (LC<sub>50</sub>) ١٣٦,٤٨ ميلليغرام/ليتر مما يدل على أن الذباب في منطقة أريحا قد اكتسب صفة المقاومة لهذا المبيد. وقد تم تأكيد هذه النتائج عن طريق حساب عامل المقاومة (R/S) مقارنة بذباب منطقة العيزرية (الأكثر حساسية). حيث كان عامل المقاومة بترتيب تنازلي كما يلي: طولكرم (7.48X)، رام الله (3.75X) والخليل (0.54X) بينما كانت نتائج أريحا كما يلي: (262.47X) حيث تثبت هذه النتيجة ان الذباب من منطقة أريحا مقاوم للامبدا- سايهالوثرين.

كما أظهرت نتائج الدراسة بأن الدايزنون كان أقل فاعلية في مناطق طولكرم، الخليل ورام الله حيث كانت قيم (LC<sub>50</sub>) 15.14، 14.06، 13.39 ميلليغرام/ليتر على التوالي. وقد كانت نتائج عامل المقاومة (R/S) كما يلي: 12.11X، 11.25X، 10.71 X و بينما أظهرت النتائج بأن هذا المبيد كان ذا فاعلية أكبر في منطقتي العيزرية وأريحا حيث كانت نتائج قيم (LC<sub>50</sub>) 1,25 و ٨,٧٩ ميلليغرام/ليتر على التوالي، وكانت نتائج عامل المقاومة (R/S) للذباب من منطقة أريحا هي: 7.03X.

أظهرت نتائج طولكرم بأن الذباب المنزلي من المنطقة اكتسب مقاومة عالية للباراثيون حيث كانت قيمة (LC<sub>50</sub>) 321.87 ميلليغرام/ليتر وكانت نتائج عامل المقاومة (R/S) هي 1609.37X. بينما كانت نتائج استجابة الذباب من المناطق الأربعة الأخرى تدل على أن الذباب حساس لهذا المبيد، وقد كانت نتائج قيم (LC<sub>50</sub>) كما يلي: رام الله (٧,٥٩ ميلليغرام/ليتر)، أريحا (١,٩٩ ميلليغرام/ليتر) والعيزرية (٠,٢٠ ميلليغرام/ليتر). وقد أظهرت نتائج عامل المقاومة (R/S) بأن هناك درجات من المقاومة تكونت لدى الذباب من المناطق المختلفة حيث كانت النتائج كالتالي: رام الله (37.95X)، أريحا (9.95X) والخليل (8.30X).

أظهرت نتائج العيزرية والخليل بأن الذباب حساس للمبيد مالاثيون حيث كانت نتائج قيم (LC<sub>50</sub>) هي: ٠,٦٠ و ٢,٨٥ ميلليغرام/ليتر على التوالي. وقد كان عامل المقاومة (R/S) لهذا المبيد على الذباب من الخليل هي 4.68 X، بينما أظهرت النتائج في المناطق الأخرى بأن الذباب مقاوم للمبيد حيث كانت قيم (LC<sub>50</sub>) لمنطقة طولكرم (86.28 ميلليغرام/ليتر)، أريحا (11.48 ميلليغرام/ليتر)، رام الله (5.89 ميلليغرام/ليتر) وكانت نتائج عامل المقاومة (R/S) 141.45X، 18.82X، 9.65X للمناطق الثلاث على التوالي.

أشارت نتائج البحث الى أن الذباب من المناطق المختلفة في الضفة الغربية قد طور درجات متفاوتة من المقاومة للمبيدات المستخدمة، وعليه يوصى بضرورة القيام بالمراقبة المنتظمة والمنهجية لفاعلية المبيدات المستخدمة لمكافحة الذباب وحشرات الصحة العامة بشكل عام، وذلك من أجل تمكين الجهات المعنية من اختيار اكثر المبيدات فاعلية في مكافحة بشكل مستدام.

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## List of Abbreviations

Abbreviation	Title
R/S	Resistant/Susceptible
LD <sub>50</sub>	Lethal dose that kills 50% of the tested individuals
LD <sub>90</sub>	Lethal dose that kills 90% of the tested individuals
LC <sub>50</sub>	Lethal concentration that kills 50% of the tested individuals
CSMA	Chemical Specialities Manufacturers Association
WHO	World Health Organization
IPM	Integrated Pest Management
OP	Organophosphorus
F1	First generation of the certain individuals
EPA	Environmental protection Agency

# **CHAPTER I**

## **INTRODUCTION**

## 1.1 INTRODUCTION

The common house fly *Musca domestica* L. is a cosmopolitan and domesticated insect and is present in nearly every habitation in the world. The fact that the house fly can transmit many diseases such as diarrhea, dysentery, food poisoning, cholera and many others created its importance as a public health pest (Mallis, 1969). Nowadays the house fly is considered a great problem especially in intensive farming regions where natural fertilizers including sheep, cattle and poultry manure are being extensively used.

Sanitation practices are the most important methods to control house fly, but these practices rarely prevent the breeding of flies and supplementary control with insecticides is usually necessary (Metcalf and Flint, 1967). It is well known that insecticides have many serious drawbacks in terms of environmental contamination, health hazards for human and non-target organisms and development of insecticide resistance. House fly resistance to chlorinated hydrocarbon, organic phosphate, carbamate, and pyrethroid insecticides is an international problem. Examples of such cases have been reported in many countries around the world, i.e., Canada, USA, Australia, Africa, Japan, Germany, Hungary, and Denmark (Chapman and Morgan, 1992). In Palestine, control of the house fly is mainly done individually and to certain extent by municipalities as well as some governmental bodies, e.g. Ministry of Health.

The total cultivated area of the West bank is around two million dunums. Of this, only hundred thousand dunums are under irrigation while 1.6 million dunums are rain fed and three hundred thousand dunums are fallow lands (ARIJ, 1994). It is estimated that 96.6% of irrigated land and 87% of rain fed land is treated with insecticides (Saleh, Neiroukh, Ayyash and Gasteyer, 1995). As reported by Farah, 2007 a total of 177 insecticides are currently being used in the West Bank, among them fourteen insecticides are internationally suspended, cancelled or banned, seven of these

insecticides are named under the dirty dozen; these are Aldicarb, Parathion, Chlordan, DDT, Lindane, Paraquate and Pentachlorophenol. According to a survey done by Saleh, Neiroukh, Ayyash and Gasteyer in 1995 the results reveal an overuse of insecticide in the West Bank particularly in irrigated areas in Tulkarem, Jenin and Jericho. The average seasonal consumption was found to be around 4Kg/dunum in open irrigated areas and 6.5Kg/dunum under plastic houses. The total quantity of insecticides used in the West Bank is around 493.82 tons per year (Saleh, Neiroukh, Ayyash. and Gasteyer, 1995). Figure [1.1] shows the average insecticide consumption according to district. Many tons of public health related insecticides are imported every year, many of them are newly introduced and more expensive insecticides are imported in the country each year which leads to increasing cost and environmental pollution.

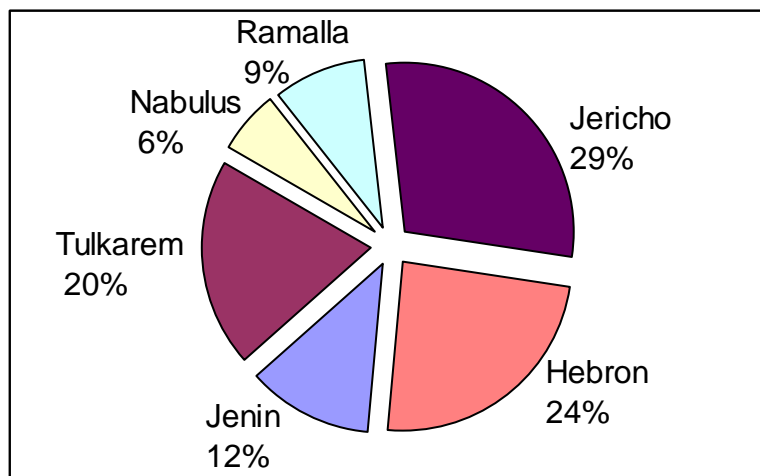


Figure [1.1]: Average insecticide consumption according to district in the West Bank (source; Saleh, Neiroukh, Ayyash and Gasteyer, 1995)

### **1.1.1: Importance of the study**

Many studies done by several investigators from different parts of the world indicated that the house fly had shown the greatest ability to develop resistance against used insecticides. Since many years certain insecticides had been used for fly control in Palestine without being tested for their affectivity. This raises the question if the local flies have or are to develop resistance to the insecticides in use. Taking this into consideration, it is necessary from time to time to monitor the resistance of the local flies to the insecticides in use.

### **2.1.1: Objectives of the study**

The goal of the present study is to determine the susceptibility of house fly populations collected from different locations in the West Bank for several insecticides commonly used in Palestine, namely Malathion, Parathion, Diazinon and Lambdacyhalothrin.

The results to be obtained from the present study will be of great importance to many official and unofficial institutions such as:

1. The Ministry of Agriculture.
2. The Ministry of Health.
3. Municipalities...etc.

These data will act as guidelines for these institutions in order to help choosing the most effective insecticides for fly control purpose and to confirm on monitoring the insecticides performance from time to time to make necessary shifts and modifications.



## **CHAPTER II**

### **LITERATURE REVIEW**

## 1.2 Literature review

Since the forties chlorinated hydrocarbons and organophosphates were used for many years as insecticides. These insecticides were applied to surfaces at which flies prefer to rest. DDT and Lindane, for instance, appeared to be highly effective against adults and larvae of house fly *Musca domestica* and other fly species (Lindquist, Madden, Wilson, Knipling, 1945). However, these insecticides are toxic to a large spectrum of animal species, also killing non-target organisms. A further problem is the development of resistance to the killing power of the insecticides (Pospischill, Szomm, Londershausen, Schroder, Turberg, Fuchs, 1996). In 1946 it was noticed that house flies were no more responding to DDT and to other many new insecticides as was reported by Brown in 1971. Another case of resistance was noticed in Denmark and USA in 1955 by Brown and Pal also in 1985, Chapman reported that a strain of house flies collected from a farm in England was resistant to 18 toxicants.

A study was made by Hansens, Benezet and Jr. in 1967 on the development of resistance of house flies in two countries of New Jersey using 0.5% and 1% residual treatments with Diazinon, Ronnenl and Dimethoate for four years. Results indicated that resistance increased in the summer, depending on sanitation, weather conditions and insecticide use and had decreased in the winter.

In 1973 a study carried out by Sacca in Jordan on house fly resistance to several insecticides collected from Amman area. He found that there was high resistance to DDT and commencement resistance to Lindane, Bromphos, Fenthion and Malathion. Negligible resistance was noticed to Pirimiphosmethyl and to Propxure while there was no resistance to Tetrachlorvinphos.

Rupes et al investigated the insecticide resistance in 1980 in 83 wild house fly population from farms in Czechoslovakia for four years. He discovered that the resistance was very high to Trichlorfon, and was considerable to Propoxur, Bediocab and Dioxicarb which are carbamate insecticides and also to the Fentherion. No significant resistance was found to the pyrethroids: Tetramethrin, Permethrin and Deltamethrin while very high resistance was found to DDT.

In 1979 a study done by Patil V., Shah P. and Guthrie F. considering the absorption of insecticides in resistant and susceptible house flies showed that absorption of DDT, dieldrin, diazinon, parathion and carbaryl in resistant (Rutgers and Fc) and susceptible (CSMA) strains of *M. domestica* L. was studied in adult females, larvae and puparia. Absorption of all insecticides (except DDT in the Fc strain) in adult flies of the resistant strains was slower than the susceptible strain. In larval and puparial stages no consistent trend of absorption related to resistance was found. Absorption in the CSMA strain was highest in the adult and lowest in the puparial stages

In 1983 Sisli et al carried out a study to determine the LD<sub>50</sub> and LD<sub>90</sub> of Malathion, Fenitrothion and Propoxur for the house fly, under laboratory conditions. The house fly populations were collected from the municipality garbage area of Ankara in September 1981, April and September, 1982. The resistance tests were applied on both sexes by means of topical application method. The highest resistance level for Malathion was observed in Ankara strains. The values of LD<sub>50</sub> were 261.1, 302.1, 312.6 micrograms/female, respectively. The populations were not showed high resistance ratio to Fenitrothion (R/S ratio 93.3, 126.6, 56.6 at LD<sub>50</sub>) and Propoxur (R/S ratio 34.2,

31.4, 21.4 at LD<sub>50</sub>). It is clear that Propoxur was more toxic to female and male flies than Fenitrothion.

1984, Al Azzeh evaluated the toxicity of several insecticides by topical application method to adult female house flies collected from two locations in Amman. He found that house flies had developed resistance to organophosphorus compounds; Dimethoate, Diazinon and Dichlorovus, also resistance was found to chlorinated hydrocarbon insecticides; DDT, Dieldrin and Lindane, where house flies were tolerant to pyrethroid insecticides; Deltamethrin and Permethrin. However, pyrethroid insecticides d-phenothrin showed high effectiveness to the tested insecticides in both locations.

(Funaki et al, 1986) studied the susceptibility to several insecticides of house fly populations collected from hog and chicken farms as well as garbage dumping land-fill sites in Chiba-ken, Tochigi-ken, Ibaraki-ken and Tokyo, it was determined by the topical application method and compared with that of CSMA, a susceptible strain. A significant level of Resmethrin resistance, i.e. 5.2 .mu.g/female in LD<sub>50</sub> and 179 fold in resistance factor, was detected only with the colony collected from the same hog farm in Mashiko. All other colonies were highly susceptible to Resmethrin except one from the garbage dumping land-fill site of Yachiyo, Chiba-ken, which showed a slight decrease in susceptibility to the pyrethroid. All the colonies except CSMA strain were highly resistant to DDT. The flies were also found retaining resistance reported in 1970s to organophosphorus insecticides such as Diazinon and Fenitrothion to varying degrees.

Levot et al in 1989 studied population of the house fly and the false stable fly, *Muscina stabulans* (Fallen), collected on poultry farms west of Sydney were tested against insecticides by topical application. House flies were resistant to the organophosphorus insecticides; Diazinon, Azamethiphos, Trichlorphon and Malathion and to the carbamate; Propoxur. No resistance was detected to the synthetic pyrethroid; Permethrin.

Studies made by Abu Nada 1990, on house flies collected from Central Jordan Valley showed that Propetamphos and Cypermethrin were more effective against the house fly than the other six insecticides he used, which was; Fenthion, Permethrin 25:75, d-tetramethrin, Propoxur, Permethrin 40:60, Cyphuthrin.

Pap and Farkas held an experiment in 1994 on samples of 24 house fly populations were collected from animal farms in Hungary and kept in the laboratory to determine their susceptibility to different types of insecticide: organochlorines, organophosphates, carbamates, pyrethroids, macrocyclic lactone and insect growth regulators. The adulticides were tested with topical bioassay in all 24 populations; the larvicides were studied with treated larval medium in 16 populations. The percentages of populations which had resistance ratios  $> 10$  at  $LD_{50}$  or  $LC_{50}$  were: 63 % to DDT, 50 % to Methoxychlor, 13 % to Lindane, 83 % to Malathion, 63 % to Ttrichlorfon, 4 % to Propetamphos, 96 % to Dioxacarb, 46 % to Propoxur, 4 % to Methomyl, 13 % to Pyrethrum, 96 % to Bioresmethrin, 63 % to Permethrin, 58 % to Cypermethrin, 79 % to SK-80, 79 % to Deltamethrin, 38 % to Iinvermectin, 0 % to Diflubenzuron, 0 % to Cyromazine

Saleh, 1996 proved through his work on toxicity of eight insecticides on house flies collected from different locations in Jordan that pyrethroid insecticides tested: Lambda-cyhalothrin, Deltamethrin, Cypermethrin and Cyfluthrin in addition to organophosphorus compound Chlorpyrifos were the most effective insecticides with LD<sub>50</sub> values 1.27, 4.22, 7.08, 7.37 and 10.69 μgm/gm respectively. Propoxur and Malathion were the least effective with LD<sub>50</sub> 4230.47 and 3493.30 μgm/gm.

In 1998 a study about the status of pesticide resistance in arthropod pests in Israel done by Horowitz A.R., Weintraub P.H. and Ishaay I revealed that a complex of events and factors, pertinent to a specific insect and insecticide, governs the development of resistance to insecticides. In Israel, resistance to conventional and novel insecticides occurred in insect pests such as *Bemisia tabaci* and *Spodoptera littoralis* (that damage agricultural crops), *Tribolium castaneum* and other flour beetles (that contaminate stored products), and *Pediculus humanus* spp., house flies and mosquitoes (that threaten public health). In the mid-1980s an insecticide resistance management (IRM) strategy was established for all cotton grown in Israel and is being adjusted on a yearly basis as needed. At present, insect pest management and IRM strategies are being developed and implemented area-wide for three regions in Israel: Bet She'an Valley, western Galilee, and western Negev.

In 1999 a study held in Egypt by Mostafa and Zayed on house fly collected from Gamasa city and a laboratory bred strain to do susceptibility tests. Their data suggested that the levels of resistance in Gamasa population against Malathion, Diazinon, Diamethoate (organophosphorus compounds) and Permethrin (pyrethroid) were developed while Deltamethrin and Cypermethrin were still effective.

Another study was done in 2002, Egypt by Shalaby, Mostafa and Allam to determine the susceptibility of field population of house fly to four organophosphorous and two pyrethroid insecticides. Field population flies collected from nine governorates, Giza, Faiyoum, Suez, Behaira, Menoufia, Sharkia, Kafr El-Sheikh, Assiut and Aswan. The results indicated that, all tested insecticides were very effective on flies collected from Sharkia, Kafr El-Sheikh and Aswan, but there was an evidence of increased tolerance among those collected from Kafr El-Sheikh for Bioresmethrin and tolerance for Diazinon in Aswan. In Assiut, flies exhibited high sensitivity to Diazinon, Deltamethrin and Bromophos while Malathion was the least effective adulticide. Resistance to Malathion, Diazinon, Fenthion and Bioresmethrin was noticed in varying degrees at all governorates. The highest average resistance ratio was recorded for Malathion in Behaira (55.3 folds) and in Suez (26 folds). Resistance was also more pronounced in Menoufia for Diazinon (23.3 folds). As for Bioresmethrin an apparent increase in the resistance ratio was detected in Suez (25 folds). In Giza, house flies were resistant to Fenthion (14.5 folds). Regarding the difference between the six insecticides used, Deltamethrin was the most potent insecticide in all governorates.

Lately, June 2006 a study was carried out in Turkey/Antalya by Akiner and Cagler on field strains of the house fly that were collected in April and September 2002 from cow farms (Antalya, Izmir) and garbage dumps (Adana, Ankara, Istanbul, Sanliurfa). The resistance levels of offspring were evaluated against six insecticides (Cypermethrin, Cyphenothrin, Deltamethrin, Permethrin, Resmethrin, Fenitrothion). Resistance levels for pyrethroid group insecticides ranged from 23.27 (Permethrin-Istanbul fall strain) to 633.09 (Cypermethrin-Izmir spring strain) and for Fenitrothion ranged from 5.78 (Istanbul fall strain) to 51.04 (Antalya spring strain). The results showed that pyrethroid

resistance was high and changed from spring to fall in relation to usage and application frequencies of these compounds at the study sites. Although Fenitrothion resistance levels were determined to be lower than pyrethroids, these levels were still high and led to control failure. Flies from cow farms were more resistant than those from garbage dumps, but resistance levels for Sanliurfa and Adana strains were also high in relation to usage of different insecticides for agricultural purposes.

In 2006 the Israel Ministry of Environmental Protection has decided to ban the use of pest control products containing the organophosphates chlorypyrifos and diazinon beginning on December 31, 2007. This is in light of their ban in the US and the growing body of evidence concerning the risk factors associated with these organophosphates.

In the past, these substances were permitted for use taking into account toxicity risks, largely tested on the basis of acetylcholinesterase inhibition. In recent years a growing body of evidence has accumulated regarding previously unknown risks from these substances. A risk assessment conducted by the US Environmental Protection Agency (EPA), which led to banning the use of these products in the U.S., and additional updated toxicological data, point to the rise of developmental neurotoxicity in embryos and infants associated with the exposure of pregnant women and babies to chlorypyrifos and diazinon.

## **2.2 Morphology and life cycle of house fly**

### **1.2.2: Overview**

The house fly is a non biting fly belonging to a group of flies known as filth flies. This name comes from the female flies' habit of laying their eggs in various types of moist,



decaying organic materials (Pickens, Schmidtman and Miller, 1994). The adult house fly is a medium sized, non metallic fly, it is about 4.6-7.5 mm in length and about 13-14 mm across the wings, and the females are usually larger than the males. The colour of adults varies from light to dark grey (Mallis, 1969), (Harwood and James, 1979), (Service, 1980), with four dark lengthwise stripes on the thorax, pale yellow sides on the abdomen, and reddish eyes (Pickens, Schmidtman and Miller, 1994). They have sponging mouthparts and eat solid food by first liquefying it with their saliva (Moon, Mullen and Durden, 2002).

The house fly is a well-known cosmopolitan pest of both farm and home. This species is always found in association with humans or activities of humans. This is the most common species found on hog and poultry farms, horse stables, and ranches. Not only are they a nuisance, but they also can transport disease-causing organisms. Excessive fly populations are obnoxious to farm workers, and when there are nearby human habitations a public health problem is possible (Amano, 1985), as it is a vector for many diseases such as bacillary dysentery, amoebic dysentery, diarrhea, typhoid, paratyphoid, food poisoning, cholera, helminths, polomyelitis, trachoma, cutaneous diphtheria, yaws and leprosy (Service, 1980), ( Keiding, 1986).

### **2.2.2: Life cycle**

The house fly has a complete metamorphosis with distinct egg, larva or maggot, pupal and adult stages Figure [1.2]. The house fly over winters in either the larval or pupal stage under manure piles or in other protected locations. Warm summer conditions are generally optimum for the development of the house fly, and it can complete its life cycle in as little as seven to ten days and as many as 10 to 12 generations may occur in one summer. Each female fly can lay up to 500 (in five batches of 100 eggs each).

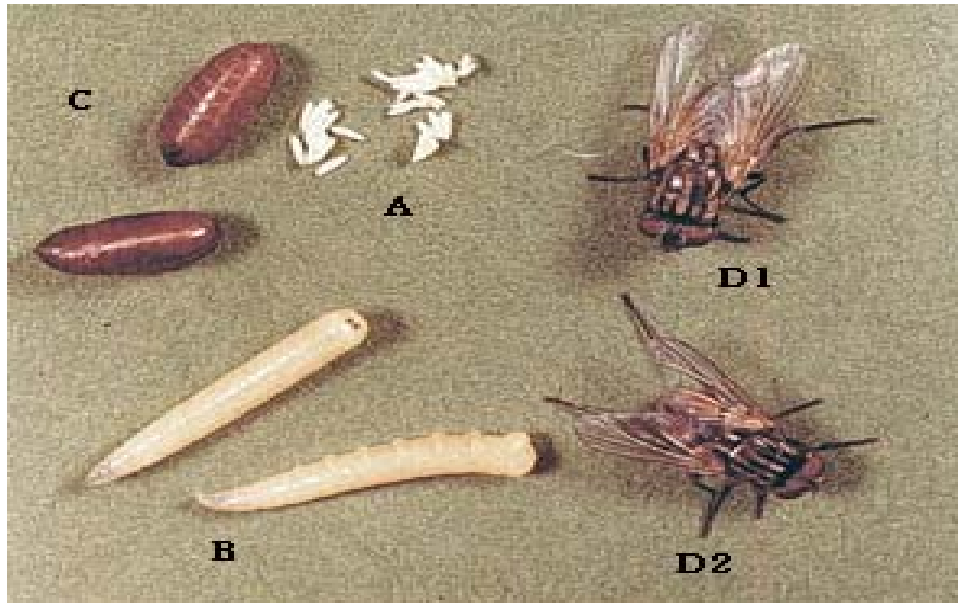


Figure [1.2] Stages in the life cycle of the house fly *Musca domestica* L., A: eggs, B: larvae, C: pupae, D1: adult female fly, D2: adult male fly

Eggs: The egg is white, elliptical, about 1mm long by 0.26 mm wide, with both ends bluntly rounded and the anterior slightly tapered. On the dorsal side there are two longitudinal curved ridges. Cellular cleavage in the egg begins soon after egg deposition (in about 8 minutes). Hatching of the larva is through a slit in the dorsal side of the egg. The slit extends posteriorly as the larva crawls out, anterior end foremost. After the emergence of the larva, the chorion collapses (Krafsur, Black, Church and Barnes, 1985).

Larva: The integument of the house fly larva consists of an outer acellular cuticle and an inner single layer of epithelium which rests on a basement membrane. The cuticle is covered with an epicuticle and has a stratified structure. The cuticle is 5 $\mu$ m thick in young larvae (36 hours old), 25 $\mu$ m in 60-hour-old larvae and 40 $\mu$ m in last (third) instar-larvae (Howard and Wall, 1996).

The larva is white and cylindrical, with the posterior end broad and flattened. It tapers anteriorly. There are no eyes or appendages, although there are some ventral spiny ridges which aid locomotion. The larvae have 13 segments, but the first two are

partially fused so that only 12 segments are apparent. Some of the internal organs may be seen through the cuticle. The spiracles are openings for air to enter the respiratory system of the larva. The posterior spiracles (on the broad blunt end) are distinctive. Within a day, the larvae (maggots) hatch from the eggs; they live and feed in (usually dead and decaying) organic material, such as garbage or feces. They are pale whitish, 3-9 mm long, thinner at the mouth end, and have no legs. At the end of their third instar, the maggots crawl to a dry cool place and transform into pupae (Graczyk, Knight, Gilman and Cranfield, 2001).

Although there are variations in the reported time of development of larvae at different temperatures the following are typical:

Table [1.2]: Durations of larval development of the house fly *Musca domestica L.* at different temperatures

°C	Days to pupation
16	11-26
18	10-14
20	8-10
25	7-8
30	5-6
35	3-4

Pupa: During pupation, the larva contracts within its own integument so that the integument becomes a cylindrical colored reddish or brown and puparium about 6.3

mm long. The puparium gradually darkens to a rich, dark brown color. Since the pupal case is formed by the larval skin, the pupa within is said to be coarctate. Locomotor pads persist on the ventral surface although the puparium is immobile. The adult flies then emerge from the pupae. Most of the basic features of an adult fly develop within the puparium in 48 hours. Full development requires 3–21 days depending on temperature:

Table [2.2]: Durations of pupal development of house fly *Musca domestica L.* at different temperatures

°C	Days to adult emergence
16	18– 21
18	12– 15
20	10– 11
25	7 – 9
30	4 – 5
35	3 – 4

(This whole cycle is known as complete metamorphosis.) Upon completion of adult development, the adult pushes off the anterior end of the puparium. A circular slit appears in segment six (fifth visible segment) of the puparium and the detached cap splits into two parts. This is done with the ptilinum, an inflated sac that protrudes from the frontal region of the head just dorsal to the base of the antennae of the adult fly. Eversion of the ptilinum is by changes in blood pressure and contraction is by muscles (Lancaster and Meisch, 1986) once its head is free, the fly crawls out of the puparium.

It crawls about while the wings unfold and the exoskeleton hardens and dries. When completely withdrawn, the ptilinum leaves only the crescent-shaped suture (frontal lunule) above the antennae adults live from half a month to a month. After having emerged from the pupae, the flies cease to grow; small flies are not young flies but the result of little food during the maggot stage. (Dübendorfe, Hediger, Burghardt, Bopp, 2002). Some 36 hours after having emerged from the pupa, the female is receptive for mating. The male mounts her from the back to inject sperm. Normally the female mates only once, store the sperm to use it repeatedly for several sets of eggs. Males are territorial: they defend a certain territory against other males and try to mount any females that enter that territory.

### **3.2 Breeding sites of the house fly**

House Flies may be found feeding and breeding in fresh manure, rotting fruits and vegetables, damp garbage and damp, decaying organic materials that are located outside of the structure (Amano, 1985). It is also known to breed in many types of organic material such as decaying plant material, spilled grain, and in all kinds of animal manure. In caged layer houses the manure is a very good location for breeding. In houses where sanitation is poor and where water spills keep the manure moist, fly breeding may especially be a problem. The house fly prefers sunlight and is a very active fly, which crawls over filth, people, and food (William, Brogdon and Janet, 1998). To rap up, the breeding sites of the house fly include the following:

1. Garbage and waste from processing: Garbage may include wastes from food from home, market wastes, various wastes from food processing and industry is also a source (Harwood and James, 1979).

2. Sewage: Under suitable conditions house flies may breed in sewage sludge and solid organic waste in open sewage drains, cesspools, seepage pits, etc..., or in sewage beds. Moreover, flies can breed in soil wetted by household dish water, where a sewage system is lacking (Keiding, 1986).
3. Decaying Organic Material: Human excrement is very attractive to house flies and open latrines are important breeding sources, also house flies may breed in accumulations of dung of most domestic animals and birds, provided it has the right moisture (not too wet) and texture (not too solid). Besides dung, such manure may include fish meal, blood, and bone meal, oil seed cakes, prawn dust, etc...(Keiding, 1986)
4. Accumulation of plant material: It was reported that decaying grass or garden heaps are very important sources of house flies in some urban and suburban areas (Keiding, 1986) .The insect can be managed to some extent by sanitation measures that reduce accumulation of waste materials that serve as breeding sites. For the most part, however, fly control is most commonly achieved with insecticides, but unfortunately, house flies have shown a remarkable ability to develop resistance to these.

## **4.2 Dispersal of the house flies**

House flies are good flyers but they are not migratory by nature (Keiding, 1986), they usually stay near their breeding places. Nevertheless records show that they can travel up to 45 Kilometres carried by wind currents. These flies can move 6-8 Kilometre within 24 hours. Flies prefer to rest on corners and edges of thin objects such as wire and strings. At night they usually rest near their food sources, 1.5-5 m off the ground. Studies using marked house flies showed that 60% to 80% were captured within a mile of their release point. Most of the rest, 85% to 95% of the total, were caught within

about 3 kilometre of the release site within the first 4 days after they were turned loose (Lee, 1997).

A few flies have been shown to travel 8 to 32 kilometre but these tend to be “record” individuals. In general, fly control efforts for a community problem are focused within 1.609 Km of the source. Passive transport of house flies on vehicles, from the garbage truck to buses and private cars, etc... does play an important role in their dispersion (Keiding, 1986).

During the day, house flies tend to rest less than 1.5 m from the ground on walls, floors, and various objects. At night they rest primarily above 1.5 m on ceilings, walls, electric wires, dangling light cords, edges/corners of buildings, plants, etc... Their night resting places are usually near their daytime food sources.

## **5.2 Public health importance of house flies**

Flies are considered environmental pollutants just by their presence. They feed by using sponging type mouthparts. As the fly moves about from one food source to another, it samples and eats its food by regurgitating liquid and dropping it on the food to liquefy it. Light colures spots called fly specks are visible signs of this type of feeding. Darker fly specks associated with house flies are fecal spots (Institute of food and agricultural sciences, 1991).

More than 100 pathogens associated with the house fly may cause disease in humans and animals, including typhoid, cholera, bacillary dysentery, tuberculosis, anthrax ophthalmia and infantile diarrhea, as well as parasitic worms (Hewitt, 1914). Pathogenic organisms which are picked up by flies from garbage, sewage and other sources of filth are viruses, bacteria, protozoa, eggs and cysts of helminthes, etc... both

externally, on their mouthparts, the body and leg hairs, and sticky pads (pulvilli) of the feet, and internally in their crop and intestinal tract (Keiding, 1986).

## **6.2 Nuisance**

*M. domestica* is the most common nuisance around homes. It can move many miles in one day, especially if aided by the wind. Therefore, a nuisance fly problem on an individual place may impact neighbors and communities some distance away. Flies are attracted to human body odors, foods and waste, it tends to fly at or near head level. The rapid beating of their wings (about 1,000 times per second) is responsible for the buzzing noise that accompanies a close fly-by. Flies have a physiological effect not only as a nuisance but also as their presence is a sign of unhygienic conditions (Keiding, 1986).

## **7.2 House flies management strategies**

The most common control measures involved with the control of house flies are sanitation, use of traps, and insecticides, but in some instances integrated fly control has been implemented. The use of biological control in fly management is still at a relatively early stage (Rutz and Axtell, 1981).

Flies found inside a building have entered from the outside in almost all cases. Therefore; barriers preventing access to the building are the first line of defense. Cracks around windows and doors where flies may enter should be sealed. Well-fitted screens will also limit their access to buildings (Moon, 2002). It is important to find out where the breeding sources are located and how they are entering the buildings. In residential areas, manure which is not picked up regularly can be a breeding source for house flies. Unfortunately, house flies have shown a remarkable ability to evolve



resistance to insecticides. This trait, combined with loss of available insecticides through regulatory processes, has resulted in an urgent need for new house fly control agents (Daljit, Sarkaria and Jeffrey 2004).

### **1.7.2: Sanitation or cultural control**

The cultural control of flies is defined as the manipulation of abiotic factors (environmental conditions such as temperature, moisture of breeding habitat and humidity) that suppress fly numbers. Good sanitation is the basic step in all fly management. Foods and materials on which the flies can lay their eggs must be removed, destroyed as breeding medium, or isolated from the egg-laying adult. Since the house fly can complete its life cycle in as little as seven days, removal of wet manure at least twice a week is necessary to break the breeding cycle. Wet straw should not be allowed to pile up in or near buildings. Since straw is one of the best fly breeding materials, it is not recommended as bedding. Spilled feed should not be allowed to accumulate but should be cleaned up two times a week. Killing adult flies may reduce the infestation and, but elimination of breeding area is necessary for good management. Garbage cans and skips should have tight-fitting lids and be cleaned regularly. Dry and wet rubbish should be placed in plastic rubbish bags and sealed up. All waste receptacles should be located as far from building entrances as possible. For control at waste disposal sites, refuse should be deposited onto the same area as inorganic wastes to deteriorate the capacity of breeding resources, or the disposed refuse should be covered with soil or other inorganic wastes 15 cm thickness or every weekend (Axtel, 1970).

### **2.7.2: Fly traps**

Can capture large numbers of house flies but generally do not reduce their numbers significantly. Ultraviolet light traps, bottle traps, and fly sticky strips can be useful,

particularly in the milk room where insecticide applications are limited and fly numbers are low. Many insects are sensitive to UV light with a wavelength of approximately 350 nm (Deay and Taylor, 1962) showed that wavelengths between 320-380 nm were most attractive to *M. domestica*. However, Burkhard (1962) and McCann and Arnett (1972) found that there were two peaks in the visual systems of houseflies one of 350 nm and the other around 500 nm. Bellingham and Anderson (1993) even found three spectral peaks flies at 350, 450-550 and 630 nm. Nowadays light traps (lamps emitting attractive wavelengths) in combination with electric grids which kill the flies) are commonly used for capturing flies. Morgan and Pickens (1968) tested several types of lamps with spectra between 310 and 720 nm at temperatures between 19 and 32 °C for their attractiveness to house flies.

### **3.7.2: Biological control**

The objective of biological control is to encourage naturally-occurring populations of predators and parasites to survive. This includes the cultural step of making manure as dry and as hospitable as possible. With increasing incidence of insecticide resistant house fly populations, rising costs of insecticides and a growing public concern about actual or potential problems associated with insecticides, interest in alternative house fly control strategies has increased. The use of biological control agents in fly management programs is still at a relatively early stage. At present, parasitic wasps are the most widely used biological control agents for house flies (Pickens, Schmidtman and Miller. 1994).

The house fly has many natural enemies and among the more important in poultry facilities are the wasps *Muscidiforax raptor* and *Spalangia cameroni*. Leaving a layer of old manure in the pits when manure is removed might enhance or stabilize the suppression of the house flies densities by parasitoids and predators. Periodic release of

parasitoids during winter and spring, and following manure removal, might effectively suppress densities in poultry facilities (Axtell, 1970).

Other agents could be used in house fly biological control which are bacteria, fungal agents (Ex. *Beauveria bauiana*) and some botanical agents. (Journal of Medical Entomology, 2005). Other agents are chemical agents like heavy metals: Mercury, Aluminum and Cadmium (Indian Experimental Journal of Biology, 2001).

In controlling the house fly by bioagent, a multi agent approach is needed, one should not seek a single agent as the ideal one, but a combination of parasites and predators should be used (Keiding, 1986).

#### **4.7.2: Chemical control**

After maximum effort to suppress fly numbers by proper cultural and biological methods, insecticides are often needed to achieve the desired degree of fly control. If fly suppression by cultural and biological methods is maximized, then the effectiveness of insecticide treatments will be enhanced, and the rate of development of fly resistance to the insecticide will be reduced. Insecticides are either adulticides (baits, spray-on, paint-on), or larvicides (spraying breeding sites and using feed additives).

*Adulticides:* Baits consist of an insecticide mixed with a substance that is attractive to flies, such as sugar. Fly sex pheromone is added to the formulation to increase the effectiveness of the bait.

*Larvicides:* Larviciding is an important component of an Integrated Pest Management strategy (IPM), alongside adulticiding, and cultural and biological control measures.

Larviciding of manure and other fly breeding sites is accomplished with the same types of equipment used for residual surface sprays. A coarse spray and high volume is required. The most satisfactory use of a spray-on larvicide is to apply it only to those areas in which an abundance of fly larvae is observed. Larviciding can also be accomplished by incorporating an insecticide into animal food.

### **5.7.2: Residual treatment**

Treatment should be directed against surfaces in and around animal shelters, fly-breeding sites and areas where flies congregate for feeding or resting. Night-time resting sites are particularly important, as houseflies prefer edges of objects, strings, wires and thatch material under the roofs of houses and animal shelters. When average temperatures are high, many house flies remain outside at night and rest on the exterior surfaces of buildings and on fences, trees and shrubs. Blowflies and flesh-flies normally rest outdoors.

A preliminary assessment of susceptibility to insecticides should be undertaken before one is selected for control of any medically important insect. This is particularly important in the case of house flies as resistance is so widespread. House fly populations have developed resistance to DDT and related compounds in all parts of the world. Organophosphate resistance is also common and appears to be increasing worldwide in terms of the level, distribution and compounds involved. Resistance to carbamates and to pyrethroids is also becoming widespread.

Hand compression or power-operated sprayers are used to apply the formulations. The spray volumes required vary with the nature of the surface to be treated, 40–80 ml/m might suffice for smooth, non-absorbent surfaces, but volumes up to 250 ml/m might be required for treatment of highly absorbent surfaces, such as refuse tips or refuse

collection areas. Contamination of food, food preparation surfaces and drinking-water should be avoided.

### **6.7.2: Fly cords**

Cotton fly cords impregnated with residual insecticides can be hung from ceilings of buildings. Cords are effective because they take advantage of the habit of the house fly to rest on vertical objects. These cords should be handled with care because they are usually formulated with high concentrations of relatively toxic insecticides. (Fay and Kilpatrick, 1958). Insecticides used in the preparation of the cords include Diazinon, Dichlorvos, Dimethoate, Fenchlorphos, Fenthion and Propoxur. Solutions or emulsions of these insecticides are used, often mixed with sugar and attractant with glue or oil for making durable film (WHO, 1986).

### **7.7.2: Space treatment**

Space treatment is the most effective method for rapidly reducing fly density inside and outside houses. Insecticides applied as aerosols at relatively low doses will kill adult flies that come into contact with spray droplets. There is, however, no residual effect of the insecticide, and larvae and pupae at breeding sites are unaffected; thus, areas often undergo rapid repopulation with new adults.

Space Sprays; mists and fogs, these sprays are designed for quick knockdown and kill of flies with no residual action. They are usually the most effective and economical method to control potentially heavy populations of adult flies. Because they do have very little residual activity, resistance to the insecticides recommended as space sprays is low, especially when using products containing synergized natural pyrethrins. There are many machines on the market designed to produce the small particle spray desired for this type of application treated. Space application should be made to the point of

“filling” the room with the spray mist. Treatments should be made as frequently as needed to keep fly numbers down below identified nuisance levels. This method of fly control is best achieved in the cooler early morning hours when flies are resting higher up in the house and ventilation fans can be safely turned off during the time of spraying without causing increased house temperatures. Insecticides used for this purpose include Diazinon, Dichlorvos, Dimethoate, Fenchlorphos, Fenthion, Malathion, Bioresmethrin, Cypermethrin, Deltamethrin and Permethrin (WHO, 1984).

#### **8.7.2: Toxic baits**

Baits are placed or applied on sites where adult flies congregate to feed, such as in and around livestock farms, dairies and food-handling establishments. Dry baits typically contain 0.5–2% in a carrier such as sugar or sugar plus sand, ground corncobs or crushed oyster shells. Liquid baits typically contain insecticide at 0.1–1.25% in ready-to-use spray solution and sugar at 100–112.5 g/l in water. Baits can contain special attractants such as fish-meal, fermenting yeast, cheese flavour or the house fly pheromone *z*-9-tricosene. Insecticides used in toxic baits are: Diazinon, Propoxur, Spinosed, Imidacloprid, Thiamethoxam, Azamethiphos, Dimethoate, Naled, Phoxim, Trichlorfon (WHO, 1984).

#### **9.7.2: Integrated fly control**

Integrated Pest Management (IPM) is an effective, economical approach to pest control. It involves identifying and correcting the problems that lead to pest problems and the use of nonchemical and least-hazardous control methods to address existing infestations (Bullen, 2000). IPM combines different techniques to prevent pest damage without harming the environment. Pests can include insects and mites, rodents and certain birds, plant diseases, and weeds. IPM practices include monitoring, modifying

pest habitat, protecting natural enemies, and, when needed, the use of insecticides (Hollingsworth, 1914).

## **8.2 House Fly resistance to insecticides**

Insecticides are natural or synthetic agents that are used to kill unwanted plant or animal pests. While the term *insecticide* is now often associated with synthetic chemical compounds, it was not until recently that synthetic insecticides came into use. Naturally occurring compounds or natural extracts have been used as insecticides since ancient times. The earliest insecticides were most likely salt, sulfurous rock, and extracts of tobacco, red pepper, and the like (Pimental and Levitan, 1986). Insecticides can be classified either by target pest or by chemical identity. Classification by target pest is the most familiar. Insecticides can also be organized by their chemical class (a group of compounds that share a common chemistry) (Jury, Focht and Farmer, 1987). For example, all insecticides in the class organophosphate (OP) are derivatives of phosphoric acid, and all insecticides in the class organo-chlorine are composed of carbon, hydrogen, and chlorine. When discussing an insecticide, it is possible to refer to the insecticide compound itself or to the insecticide product or formulation. The compound itself is also known by the active ingredient (the chemical responsible for killing the target pest) (Cheng, 1990).

Resistance is defined as a reduction in the sensitivity of a population, which is reflected in repeated failure of a product to achieve the expected level of control (Graves, 1994).

Tolerance of an individual is defined as the ability of the organism to show less response to a specific dose of a chemical than was shown on prior occasion from the same dose.

Insecticide resistance has been a problem in all insect groups that serve as vectors of emerging diseases. Although mechanisms by which insecticides become less effective are similar across all vector taxa, each resistance problem is potentially unique and may involve a complex pattern of resistance foci (Brogdon, 1989).

Resistance to insecticides was first documented in 1914 by Melander in the *Journal of Economic Entomology*. He described scale insects, still alive, under a "crust of dried spray" of an inorganic insecticide. Between 1914 and 1946, another 11 cases of resistance to inorganic insecticides were recorded. Then came development of organic insecticides, such as DDT, and the agricultural industry breathed a sigh of relief, believing that insecticide resistance was an issue of the past. Unfortunately, that feeling of relief quickly faded by 1947, house fly resistance to DDT was documented, with every new insecticide introduction; cyclodienes, carbamates, formamidines, organophosphates and pyrethroids table [3.2].



Table [3.2]: Group, common names, chemical names and structures for insecticides used in the test

Group	Common Name	Chemical Name	Chemical Structure
Organophosphates	Diazinon	Phosphorothioic acid 0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl) ester	
Organophosphates	Malathion	Diethyl [(dimethoxyphosphinothioyl)-thio]butanedioate	
Organophosphates	Parathion	O,O0-Dimethyl O-p-nitrophenyl phosphorothioate	
Pyrethroids	Lambdacyhalothrin	alpha-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane-carboxylate	

### **1.8.2: Factors determining the development of resistance**

How quickly resistance develops depends on several factors, as reported by Keiding 1986, these are:

- Previous use of related and unrelated insecticides and development of resistance to them.
- Use of the same or related insecticide over large area and for long periods.
- Short life cycle ( Many generations of housefly per year)
- High selection pressure, i.e. high proportion of the fly population exposed to insecticide dosages at which the resistant flies can survive and breed.
- Isolation of the treated population from contact with untreated population.
- Exposure of both larvae and adults to the same or related insecticides.

### **9.2 Behavioural resistance**

Resistant insects may detect or recognize a danger and avoid the toxin. This mechanism of resistance has been reported for several classes of insecticides, including organochlorines, organophosphates, carbamates and pyrethroids. Insects may simply quit feeding if they come across certain insecticides, or leave the area where spraying occurred, insects may stop short of consuming or eating enough toxin to kill them. Several (Anon, 1989), cases of behavioural resistance selection are known, generally known as increased irritability. The genetic bases of behavioural resistance have not been described (Lockwood, Sparks and Story, 1984).

### **10.2 Penetration resistance**

Resistant insects may absorb the toxin slower than susceptible insects. Penetration resistance occurs when insects can slow absorption of chemicals into their bodies because their outer cuticle has developed barriers against the products. The bad news is that this can protect insects from a wide range of insecticides. Penetration resistance is

usually present along with other forms of resistance, and reduced penetration intensifies the effects of those other mechanisms (Roush, Tabashnik, Chapman and Hall, 1990).

### **11.2 Metabolic resistance**

Resistant insects may naturally detoxify or destroy the toxin faster than susceptible insects, or quickly rid their bodies of the toxic molecules. Metabolic resistance is the most common mechanism and often presents the greatest challenge. Insects use their internal enzyme systems to break down insecticides. Resistant strains may possess greater levels or more efficient forms of these enzymes. In addition to being more efficient, these enzyme systems also may be broad spectrum, meaning they can degrade many different insecticides (Brown, 1971).

### **12.2 Organochlorine resistance**

Resistance to organochlorine compounds including DDT, HCH, and cyclodienes is still present everywhere (WHO, 1980). All DDT resistant housefly strains can detoxify DDT to DDE by the mean of DDT\_dehydrochlorinase. Resistance to cyclodienes does not involve detoxification, but it is a result of less sensitivity of the nerve. For HCH compounds it was observed that resistant strains showed an increase in activity in certain enzymes (Brown, 1971).

### **13.2 Organophosphorous resistance**

In the early 1950's, the resistance to organophosphorus insecticides was first noticed after failure to control certain pest species in the field (Motoyama and Dauteman, 1974). Organophosphorous resistance had increased on a global scale, as to distribution, levels and compounds involved. Resistance to many organophosphorous compounds occurs in China, the Mediterranean region and the Middle East. Organophosphate insecticide (Parathion/Diazinon) resistance in house fly is associated

with the change in carboxylesterase activity. The product of *Md*  $\alpha$ E7 gene is probably playing a role in detoxification of xenobiotic esters (Taskin and Kence, 2004). It is important to mention that the Israel Ministry of Environmental Protection has decided to ban the use of pest control products containing the organophosphates chlorpyrifos and diazinon beginning on December 31, 2007. This is in light of their ban in the US and the growing body of evidence concerning the risk factors associated with these organophosphates and this was due to the rise of developmental neurotoxicity in embryos and infants associated with the exposure of pregnant women and babies to chlorpyrifos and diazinon.

## **14.2 Carbamate resistance**

The use of carbamates as insecticides began in the 1950s; approximately 25 carbamate compounds are in use today as insecticides or pharmaceuticals. Carbamates are among the most popular insecticides for home use, both indoors and on gardens and lawns. The resistance mechanism of house fly against carbamate involves detoxification by microsomal monooxygenase (Hassal, 1982).

## **15.2 Pyrethroid resistance**

Pyrethroid resistance is emerging despite early optimism that because of its rapid toxicologic action this newest large class of insecticides would not produce resistance (William, Brogdon and McAllister, 1998).

Resistance to pyrethroids could be due to detoxification of individual insecticides at sites susceptible to enzymatic attack, and reduction of sensitivity at the active site (De Vires and Georghiou, 1980). The mechanism of resistance to house fly differs from one

strain to another, many pyrethrin –tolerant house flies have risen from strains resistant to DDT (Brown and Pal, 1971).

CYP6D1 is the cytochrome P450 responsible for pyrethroid resistance in the LPR strain of house fly, which was originally collected from New York and was recently implicated as a mechanism of resistance in house flies from Georgia. We sequenced CYP6D1 from the NG98 strain of house fly from Georgia and found that the CYP6D1 allele in this strain is identical to that found in the LPR strain (CYP6D1v1). This is in contrast to the other five alleles of CYP6D1 from pyrethroid-susceptible strains which were all unique (i.e., different from all other strains). These results indicate that CYP6D1-mediated resistance may have evolved once and then spread between these two states. This is unexpected as house flies are not documented to disperse over long distances. The finding of identical alleles in the pyrethroid-resistant NG98 and LPR strains supports the hypothesis that the different CYP6D1 protein in resistant strains contributes to their resistance. (Seifert J. and Scott J., 2002)

Another study was done on two house fly strains, ALHF and SeALHF, were collected from Alabama after control failures with pyrethroids. While pyrethroid resistance in ALHF partially conferred by P450 monooxygenase- and hydrolase- mediated metabolism has been reported, no studies have been conducted on resistance of SeALHF. The current study was carried out to investigate mechanisms of pyrethroid resistance in SeALHF and the possible role of target site insensitivity, due to *kdr* mutation, in pyrethroid resistance of ALHF. Resistance to permethrin in SeALHF was dramatically and partially suppressed by PBO and DEF, respectively, suggesting that P450 monooxygenase-mediated metabolism plays a major role in permethrin resistance in this strain, while hydrolytic metabolism has a minor contribution to resistance. Incomplete suppression of permethrin resistance by PBO and DEF suggests that one or

more additional minor mechanisms are involved in overall resistance of SeALHF. Injection did not decrease levels of resistance to permethrin in SeALHF, implying that a decreased rate of cuticular penetration (pen) does not play a role in permethrin resistance in this strain. A 392 bp para-type sodium channel gene fragment, where kdr (L1014F) and superkdr (M918T) mutations reside, was generated by RT-PCR from ALHF and SeALHF. The M918T mutation was not detected in ALHF or SeALHF, suggesting that the super-kdr mutation is not important in permethrin resistance of these two house fly strains even though ALHF possesses a much higher level of resistance than SeALHF. The L1014F mutation was present in ALHF, but not in SeALHF, suggesting that the kdr mutation is an important factor in pyrethroid resistance in ALHF. A leucine to histidine (L1014H) substitution at the position corresponding to kdr mutation was detected in SeALHF (Liu N. and Pridgeon J., 2002).

## **16.2 Cross-resistance:**

When fly population is exposed to selective pressure with one insecticide they may develop resistance also to other insecticides. This phenomenon is called cross-resistance (WHO, 1976). Cross-resistance to insecticides can be within a class of insecticides or between classes with similar modes of action, it also becomes a major consideration, as resistance to older products has been found to confer resistance to newer products.

This can be because the chemicals share a mutual target site within the insect (e.g. DDT and synthetic pyrethroids), or because of broadly effective mechanisms (e.g. penetration resistance), or because specific biochemical mechanisms affect both molecules (e.g. elevated esterase levels) (Georghiou and Taylor, 1986). Examples (Keiding, 1986):

Table [4.2]: Examples on cross resistance in insecticides

Selector	Cross-resistant to:
<p>Chlorinated hydrocarbons</p> <p>DDT</p> <p>HCH( Lindane)</p>	<p>Methoxychlor, pyrethroids in some cases</p> <p>Dieldrin and chlordane</p>
<p>Organophosphorous compounds</p> <p>Diazinon</p> <p>Fenclorphos</p> <p>Dichlorvos</p> <p>Fenitrothion</p> <p>dimethoate</p>	<p>Parathion</p> <p>Promophos, jodfenphos</p> <p>Trichlorfon</p> <p>Diazinon</p> <p>Fenthion, trichlorfon</p>
<p>pyrethroids</p>	<p>DDT</p>

## 17.2 Preventing or reducing the development of resistance

Sanitation is the first measure of defense, even though there are various traps and sprays that are used to kill flies, it is necessary to eliminate the source in order to eliminate them.

As reported by Keiding, 1986 the key of reducing the development of resistance is the reduction of selection pressure with insecticides using the following steps:

- Restrict use of residual spray
- Restrict extent of treatment: treat only where necessary and use spot treatments.
- Restrict frequency of treatment and try to use non-chemical methods as far as possible.

The sequence of insecticides used in along term program is of importance for the rate and extent of development of resistance. Alternation between unrelated insecticides and between chemical and non-chemical control methods may postpone resistance development (but the use of mixtures of insecticides is not recommended).

Change to a new insecticide, as resistance increased to conventional insecticides, changing to new insecticides that have a noble mode of action had been used in many countries (Iseki, Georghiou, 1986).



## **CHAPTER III**

### **MATERIALS**

**&**

### **METHODS**

## 1.3 Materials and methods

### 1.1.3: House fly collection

The house flies were collected during the period June, 2004 to September, 2005 from cow farms, poultry farms from five different locations namely: Jericho, Hebron, Ramalla, Tulkarerm and Bethany (Figure 1.3).

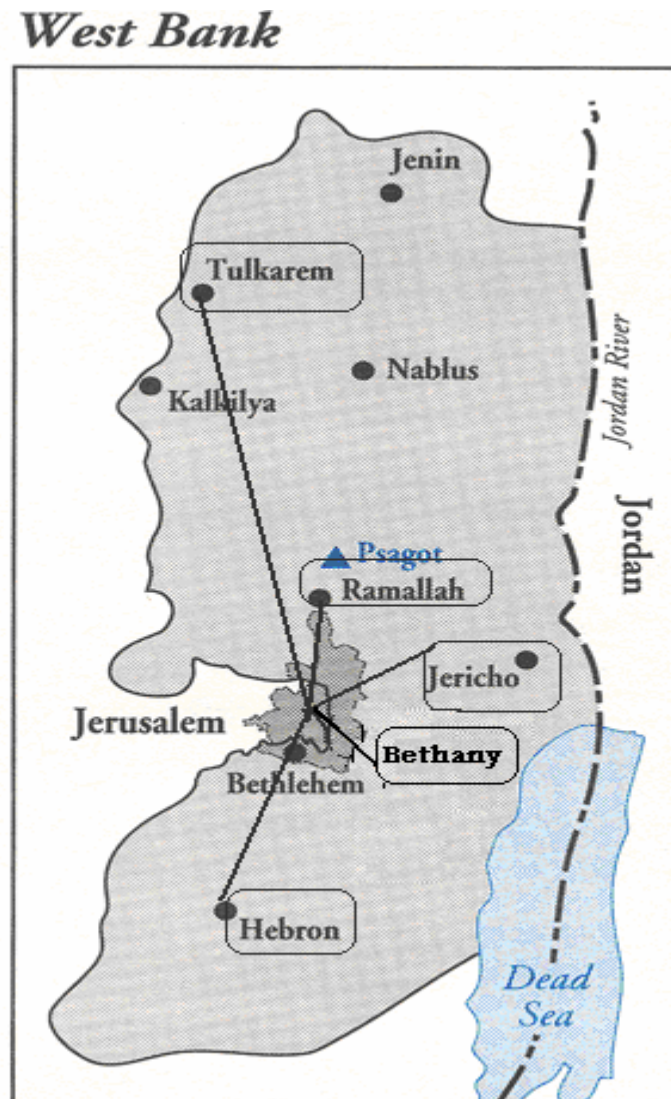


Figure [1.3]: House fly collecting locations in the West Bank

Collection of flies was made by two ways, using a collecting net and using a jar containing approximately 10 ml of fruit jam. The jar was placed in the location, and

after few flies were attracted, it was closed with cheese cloth and transferred to a rearing cage which will be described later. Collection was repeated several times until approximately 200 flies from each location were obtained. Flies collected were then taken to the laboratory for rearing to obtain F1 generation which was used for testing.

### **2.1.3: Rearing house flies**

House flies were reared at room temperature in the university's Ecology and Agriculture Research Lab. Five wooden frame cages of 40 cm long, 40 cm wide and 40 cm high were used for rearing the house fly, as shown in figure [2.3]. The cages were covered with mesh screen with cloth sleeve opening at the front which is closed by the means of removable rubber (Sawicki and Holbrook, 1961)



Figure [2.3]: House fly rearing cages in the university's Ecology and Agriculture Research Lab.

### **3.1.3: Obtaining F1 generation and collection of eggs**

Feeding the adults: Adult flies were fed on diet composed of two parts of defatted powdered milk and one part of sugar dissolved in water. A cotton pad was immersed in

the previous composition then placed on a Petri dish inside the rearing cage, water was also provided to the adult flies on a cotton pad using a beaker provided with a piece of polystyrene to keep the cotton pad floating on top of water surface . This cotton pad was changed regularly every three days. Eggs laid within approximately 20 hrs and these were then inoculated in the larval media to produce the adults that were used in the test (Anonymous, 1977)

Feeding the larvae: Eggs collected from the Petri dish were then inoculated in a medium prepared for rearing the larval stage. The larval rearing media was prepared as follows: 100 gm of wheat bran, 50 gm of chicken broiler diet, and 150 ml water placed in a 2.0 litre glass beaker. About 500 eggs were cultured in each beaker, and then beakers were covered with muslin cloth. Two days after eggs hatching larvae start to move and when these are about to pupate they move to a drier and cooler place (Anonymous, 1977). For that reason a 3-4 cm layer of sand was added to the top of the medium. Pupae were collected by using a mesh-sieve, and were then put in a Petri dish and transferred to another 2.0 litre-glass beaker for fly emergence; each beaker contained 15 flies for the test.

Feeding the newly emerged flies was done by supplying food composed of honey and water placed on a cotton pad which was placed on top of the muslin cloth covering the beaker (Kence and Kence, 1993).

#### **4.1.3: Insecticides**

Four insecticides were used in this test namely Malathion, Parathion, Diazinon which are all organophosphorous insecticides and Lambdacyhalothrin which represents the pyrethroid group (table 1.3).

For each insecticide, at least six concentrations were used. Appropriate concentrations for each insecticide were determined by testing two widely ranged concentrations for

each insecticide, the other concentrations were determined according to % mortality that has been shown by the first two concentrations.

The method of tarsal contact was used to determine the values of LC<sub>50</sub> (Rupe, Pivora, Rettich, 1975).

A minimum of 6 different concentrations of preparations were tested each time. Fifteen females of the tested population of flies were exposed to the impregnated paper for 24 h. During this time good access of flies to water was ensured, but the impregnated filter paper remained dry. The mortality of flies was determined after 24 h. Controls were carried out simultaneously by exposing flies to a filter paper impregnated with drinking water dry at the time of test. The criterion of mortality was the inability of the insect to show active locomotion (Saleh, 1996).

Table [1.3]: Trade names, common names and percent of active ingredient of used insecticides

Trade name	Common name	Percent of active ingredient/L
Dizictol	Diazinon	235gm/L
Malathion	Malathion	500gm/L
Parathion	Parathion	470gm/L
Karate	Lambdacyhalothrin	50gm/L

### 5.1.3: Statistical analysis

Estimating LC<sub>50</sub> (effective concentration that kills 50% of the tested individuals), its confidence limits, the slope and intercept of the log dose. probit line were calculated by a computer program (Probit) based on the method of Finney (1971). The software was developed by the American Environmental Protection Agency EPA (2001).

### **6.1.3: Resistance ratio**

The (R/S) (resistance/susceptible) ratio is used to determine the degree of susceptibility or resistance of a field collected strain compared to a susceptible strain. R/S ratio at  $LC_{50}$  can be calculated by dividing the  $LC_{50}$  for a field strain by the  $LC_{50}$  for the susceptible strain. (Keiding, 1976). As we were not able to obtain a laboratory susceptible strain because in Palestine they don't rear flies for research purposes and it was hard for us to get the flies from abroad due to political problems, therefore, the R/S ratio in the present study was calculated by dividing the  $LC_{50}$  of the less susceptible field strains on that of the most susceptible one, which was Bethany strain.

## **CHAPTER IV**

### **RESULTS**

**&**

### **DISCUSSION**

## 1.4 Results and discussion

A computer program (Probit) was used to analyze the data and get the parameters:  $LC_{50}$  gm/L, slope of log. conc. probit line and its standard error, confidence limit and the intercept points for insecticides tested on flies collected from Ramalla, Hebron, Jericho, Tulkarem and Bethany. Results are presented in tables [1.4]-[5.4] and figures [1.4]-[5.4]

$LC_{50}$  is the concentration of a chemical which kills 50% of a sample population, when it is high this means that the insecticide is not effective as it needed higher concentration from that insecticide to kill 50% of the house fly population, but this can't alone be an indication for effectiveness because there are other factors that should be taken into account, especially the homogeneity or the heterogeneity of the fly population to tested insecticides

Kensler and Streu , 1967 and Keiding, 1976 had concluded that when the R/S ratio is less than two this implies that the insect is considered to be susceptible and when R/S ratio is between 2-10 the insect population is considered to have various degrees of tolerance , and if the R/S ratio was higher than 10 so the insect population is said to have various degrees of resistance .

The slope of the log. conc. probit line is a measure of the diversity of the response or the heterogeneity of the insecticide towards the toxicant used. As the slope line becomes higher the insect population is considered heterogeneity, while when the slope decreases the insect population will show a wide range of homogeneity.

Ward and Tan, 1977 had explained the indication of the slope line as follows: When the slope line is greater than one, the insect population is said to have various degrees of



heterogeneity to the tested insecticides. On the other hand when the slope line is less than one, the insect population is said to be homogenous to that insecticide.

#### **1.1.4: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to Bethany strain of house flies**

The four insecticides, Diazinon, Malathion, Parathion and Lambdacyhalothrin were tested on flies from Bethany to find out whether the flies are susceptible to those insecticides or not. LC<sub>50</sub> and slope were determined (table 1.4).

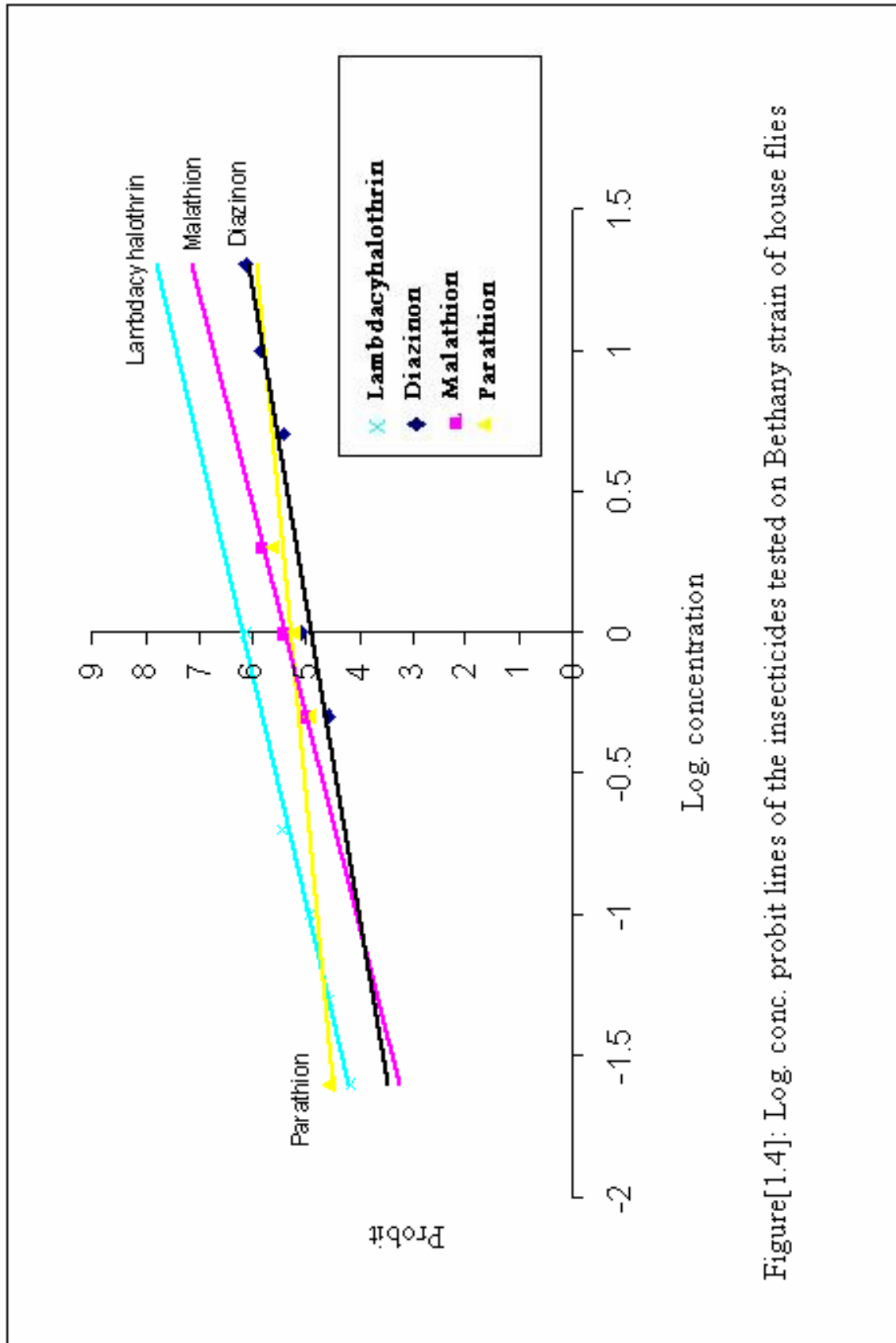
The results show that flies from Bethany are susceptible to most of the used insecticides with an LC<sub>50</sub> in a descending order Diazinon (1.25 mg/L), Malathion (0.60mg/L), Lambdacyhalothrin (0.52 mg/L) and parathion (0.20 mg/L).

The slope of log. conc. probit lines for the tested insecticides in a descending order were: Malathion (2.00), Lambdacyhalothrin (1.24), Diazinon (1.04) and Parathion (1.01), all were above (1.00) which indicates that Bethany fly strain is said to be heterogenous.

Table [1.4]: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to Bethany house flies

Insecticide	LC <sub>50</sub>	95% Confidence	Slope of Log.Conc ±SE
Diazinon	1.25 c	0.37 - 2.42	1.04±0.24
Malathion	0.60abc	0.22 - 0.99	2.00±0.54
Parathion	0.20 a	0.05 - 0.46	1.01±0.20
Lamdacyhalothrin	0.52 ab	0.19 - 1.02	1.24±0.22

\* Values in the same column followed by the same letter are not significantly different at 95% confidence level.



Figure[1.4]: Log. conc. probit lines of the insecticides tested on Bethany strain of house flies

#### **2.1.4: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to Ramalla strain of house flies**

The four insecticides, Diazinon, Malathion, Parathion and Lambdacyhalothrin were tested on flies from Ramalla to find out whether the flies are susceptible to those insecticides or not.  $LC_{50}$  and slope were determined (table 2.4) and compared with the house flies from Bethany (table 1.4)

The results show that flies from Ramalla are less susceptible to Diazinon with  $LC_{50}$  13.39mg/L. Meanwhile tolerance is developing in flies exposed to Malathion with  $LC_{50}$  5.89mg/L and with relatively high slope 1.42, which also indicates tolerance development.

Lamdacyhalothrin is the most effective to be used with the lowest  $LC_{50}$  1.95mg/L and the relatively high slope 1.11, which indicates that flies are susceptible to this insecticide. According to the Palestinian Ministry of Health (Samer Sawalha, Nov.2006, personal communication), they are not using Lambdacyhalothrin in Ramalla for controlling flies besides it is not also used in agriculture which explains why it was the most effective.

When comparing the  $LC_{50}$  of the tested insecticides to the  $LC_{50}$  of the house flies from Bethany, it was found that the fly population had showed various degrees of susceptibility towards Lambdacyhalothrin and Malathion with R/S ratio 3.7X and 9.65X, while it appeared to be resistant to Diazinon with R/S ratio 10.7X and to greater extent to Parathion with R/S ratio 37.95X.

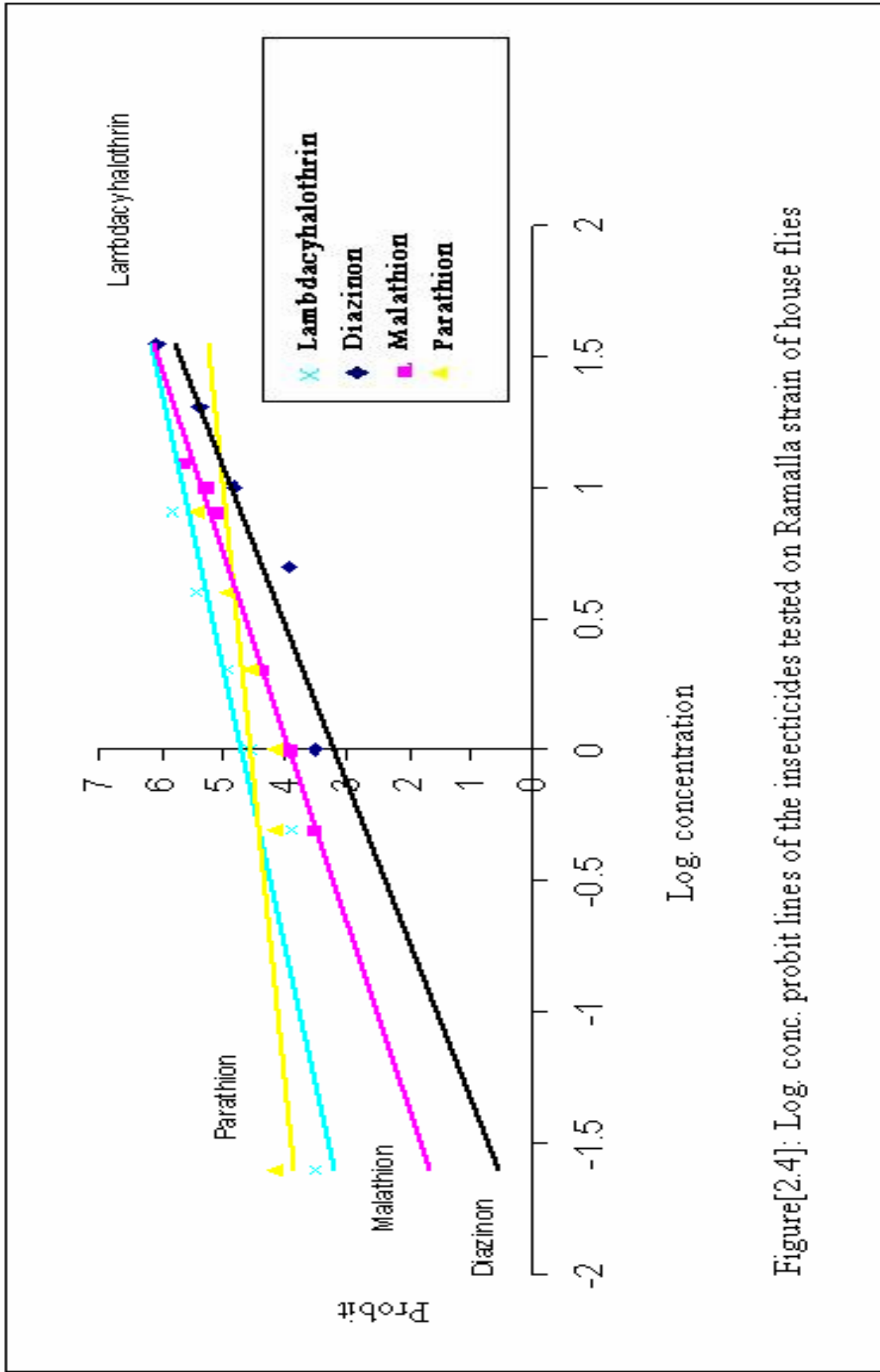
The slope of log. conc. probit lines for the tested insecticides in a descending order were: Diazinon (2.43), Malathion (1.42), Lambdacyhalothrin (1.11) and Parathion

(0.49). Table (2.4) implies that fly population shows homogeneity towards Parathion, and it said to be heterogenous to the other three insecticides.

Table [2.4]: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to house flies collected from Ramalla

Insecticide	LC <sub>50</sub>	95%Confidence	Slope of Log.Conc. ±SE	Resistance Ratio at LC <sub>50</sub> (R/S)
Diazinon	13.39 d	7.37 - 20.38	2.43±0.70	10.71
Malathion	5.89 bc	3.66 - 10.95	1.42±0.31	9.65
Parathion	7.59 b	1.97 - 7142.48	0.49±0.19	37.95
Lambdacyhalothrin	1.95 a	1.03 - 4.19	1.11±0.29	3.75

\* Values in the same column followed by the same letter are not significantly different at 95% confidence level.



Figure[2.4]: Log. conc. probit lines of the insecticides tested on Ramalla strain of house flies

### **3.1.4: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to Hebron strain of house flies**

The four insecticides, Diazinon, Malathion, Parathion and Lambdacyhalothrin were tested on flies from Hebron to find out whether the flies are susceptible to those insecticides or not.  $LC_{50}$  and slope were determined (table 3.4) and compared with the house flies from Bethany (table 1.4)

This data indicates that flies are highly tolerant to Diazinon with  $LC_{50}$  14.06 and the relatively small slope 1.17 and tolerance didn't reach its limit, while flies are susceptible to both Lambdacyhalothrin and Malathion with  $LC_{50}$  0.28mg/L and 2.86 mg/L. Flies exposed to Parathion showed certain homogeneity to the insecticide taking into consideration the low  $LC_{50}$  1.66mg/L and the very small slope, which also indicates tolerance of house flies to the Parathion.

Lamdacyhalothrin seems to be the most effective one of the insecticides used on this area. According to the Palestinian Ministry of Public Health (Samer Sawalha, Nov.2006, personal communication) there is no procedure for controlling house flies but meanwhile Diazinon is highly used in the agricultural sector which explains the high degree of resistance against the insecticide.

Lamdacyhalothrin was never used in the public health sector or in the agricultural sector.

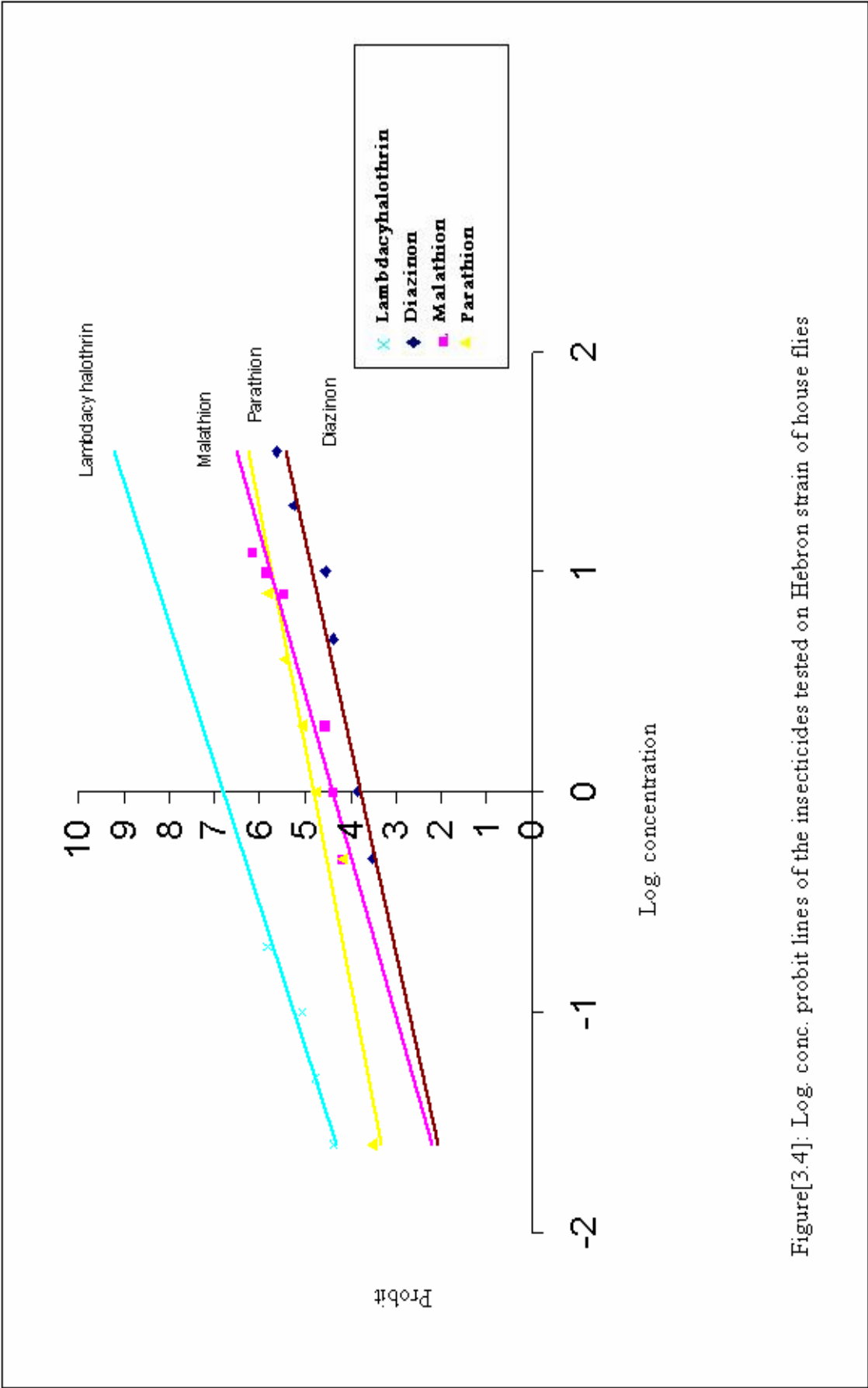
When comparing the  $LC_{50}$  of the tested insecticides to the  $LC_{50}$  of the house flies from Bethany it was found that the fly population had showed various degrees of tolerance towards Malathion and Parathion with R/S ratio 4.68X and 8.30X, while it appeared to be resistant to Diazinon with R/S ratio 11.25, while Lambdacyhalothrin was susceptible with R/S ratio 0.54X.

The slope of log. conc. probit lines for the tested insecticides in a descending order were: Malathion (1.36), Diazinon (1.11), Lambdacyhalothrin (1.02) and Parathion (0.93). This indicates that flies exposed to Malathion, Diazinon and Lambdacyhalothrin are heterogenous to the insecticides but the flies exposed to Parathion are homogenous to the insecticide.

Table [3.4]: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to house flies collected from Hebron

Insecticide	LC <sub>50</sub>	95% Confidence	Slope of Log. Conc. ±SE	Resistance Ratio at LC50(R/S)
Diazinon	14.06 d	7.61 - 34.69	1.11±0.26	11.25
Malathion	2.85 c	1.64 - 4.73	1.36±0.28	4.68
Parathion	1.66 b	0.81 - 3.54	0.93±0.25	8.30
Lambdacyhalothrin	0.28 a	0.00 - 1.46	1.02±0.34	0.54

\* Values in the same column followed by the same letter are not significantly different at 95% confidence level.



Figure[3.4]: Log. conc. probit lines of the insecticides tested on Hebron strain of house flies



#### **4.1.4: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to Jericho strain of house flies**

The four insecticides, Diazinon, Malathion, Parathion and Lambdacyhalothrin were tested on flies from Jericho to find out whether the flies are susceptible to those insecticides or not.  $LC_{50}$  and slope were determined (table 4.4) and compared with the house flies from Bethany (table 1.4)

The  $LC_{50}$  for insecticides tested on flies collected from Jericho indicate high degree of variability between the four insecticides tested. The table shows that organophosphate insecticides; Parathion and Diazinon, were effective with  $LC_{50}$  1.99, 8.79 mg/L and relatively high slopes; 1.25 and 1.25. Flies were not susceptible to Malathion that showed relatively high  $LC_{50}$  11.48 mg/L. Lambdacyhalothrin was the least effective with  $LC_{50}$  136.38mg/L and very low slope 0.28. This indicates that flies are highly tolerant to Lambdacyhalothrin and shows certain heterogeneity toward the other three insecticides.

According to the Palestinian Ministry of Public Health (Samer Sawalha, Nov.2006, personal communication), the most used insecticide for controlling sand fly and has great effect in minimizing house fly numbers in Jericho is Diazinon. Malathion was used four to five years ago but now they stopped using it. The obtained results in this thesis ensure that their controlling procedure is effective in minimizing house flies numbers.

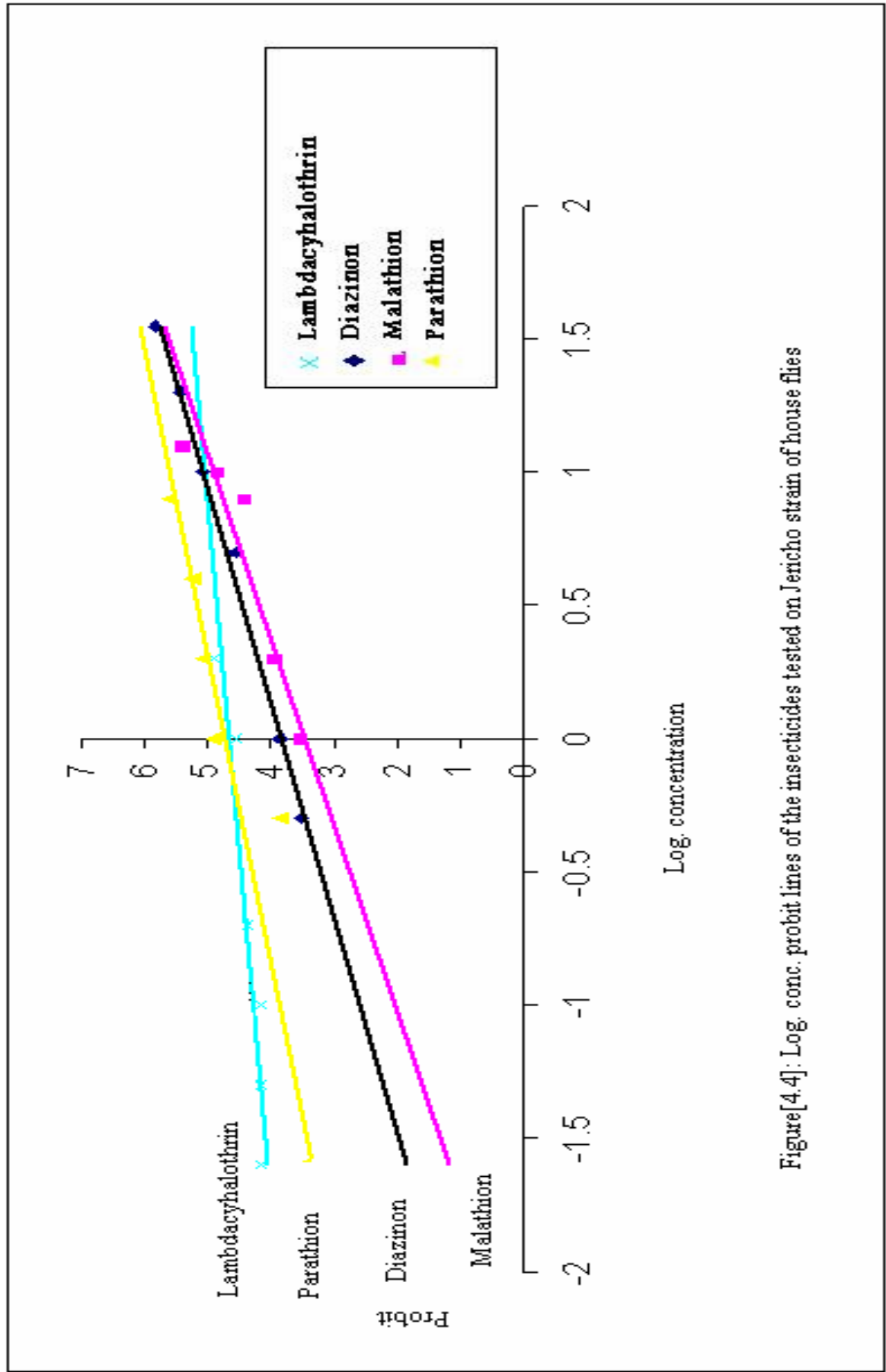
When comparing the  $LC_{50}$  of the tested insecticides to the  $LC_{50}$  of the house flies from Bethany we found out that the fly population had showed various degrees of tolerance towards Diazinon and Parathion with R/S ratio 7.03X and 9.95X, while it appeared to

be less susceptible to Malathion with R/S ratio 18.82X, and to a higher extent to Lambdacyhalothrin with R/S ratio 262.47X.

Table [4.4]: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to house flies collected from Jericho

Insecticide	LC <sub>50</sub>	95% Confidence	Slope of Log.Conc. ±SE	Resistance Ratio at LC50(R/S)
Diazinon	8.79 b	4.99 - 16.47	1.25±0.26	7.03
Malathion	11.48 bc	4.63 - 179.56	1.59±0.74	18.82
Parathion	1.99 a	0.95 - 4.911	1.25±0.24	9.95
Lamdacyhalothrin	136.48 d	65.3 – 340.21	0.28±0.19	262.47

\* Values in the same column followed by the same letter are not significantly different at 95% confidence level.



Figure[4.4]: Log. conc. probit lines of the insecticides tested on Jericho strain of house flies

#### **5.1.4: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to Tulkarem strain of house flies**

The four insecticides, Diazinon, Malathion, Parathion and Lambdacyhalothrin were tested on flies from Tulkarem to find out whether the flies are susceptible to those insecticides or not.  $LC_{50}$  and slope was determined (table 5.4) and compared with the house flies from Bethany (table 1.4).

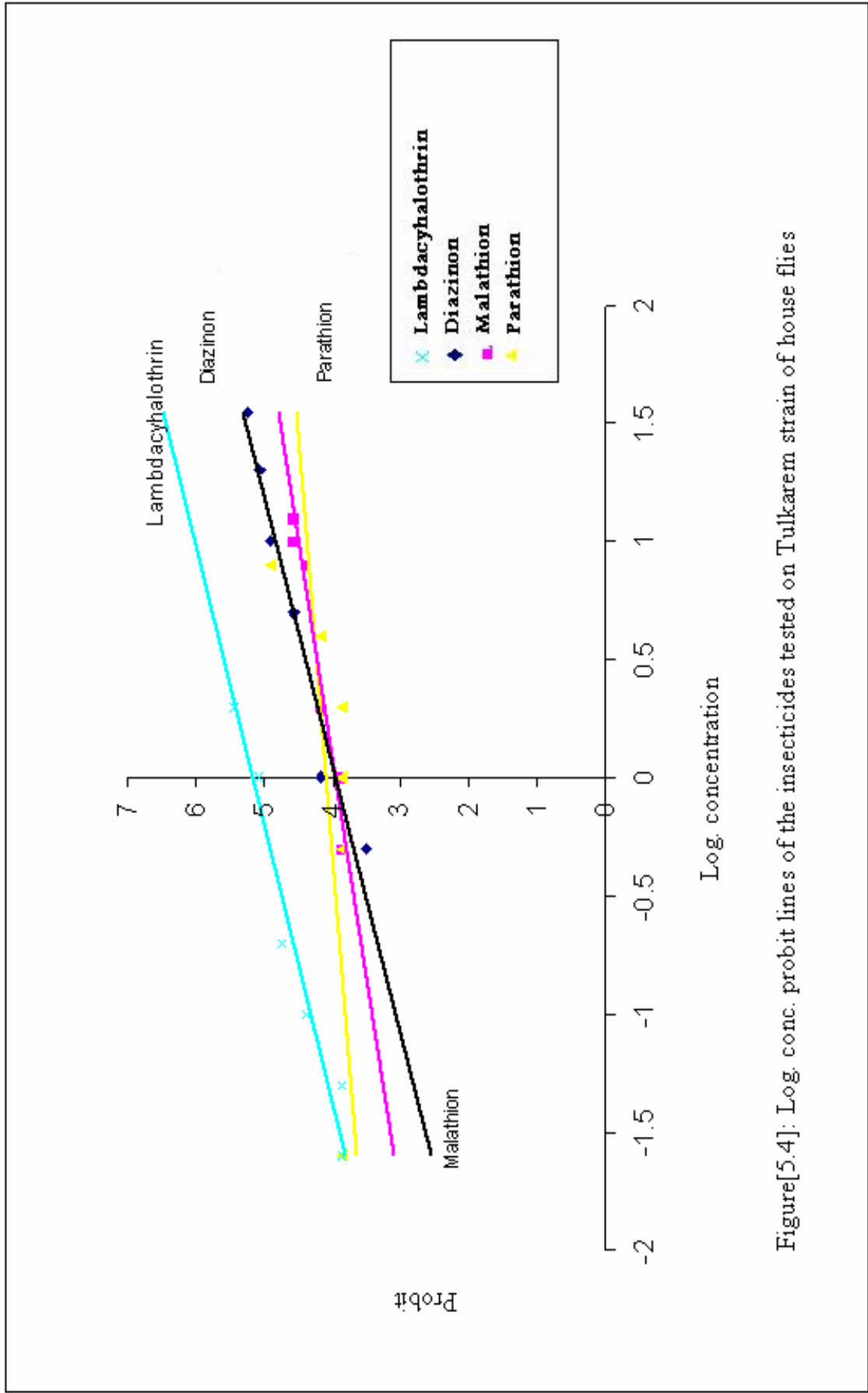
Data indicates that Lambdacyhalothrin is the most effective against flies with  $LC_{50}$  3.89mg/L followed by Diazinon with  $LC_{50}$  15.14mg/L. According to the Palestinian Ministry of Public Health (Samer Sawalha, Nov.2006, private communication), the most used insecticide for controlling sand fly and has great effect in minimizing house fly numbers in Tulkarem is Diazinon 60% which seems to be effective according to my results. Lambdacyhalothrin is not used in this procedure but we may explain my result that due to the excess use of Diazinon a cross resistant to Lambdacyhalothrin was developed. Meanwhile flies showed high tolerance to Parathion with  $LC_{50}$  321.873mg/L in spite of not using it four to five years ago, followed by Malathion with  $LC_{50}$  86.283mg/L and relatively low slopes for both Parathion and Malathion.

When comparing the  $LC_{50}$  of the tested insecticides to the  $LC_{50}$  of the house flies from Bethany we found out that the fly population had showed various degrees of tolerance towards Diazinon, Malathion and Parathion with R/S ratio 12.4X, 141.45X and 1609.37X, while it appeared to be susceptible to Lambdacyhalothrin with R/S ratio 7.48X.

Table [5.4]: Toxicity of Diazinon, Malathion, Parathion and Lambdacyhalothrin to house flies collected from Tulkarem

Insecticide	LC <sub>50</sub>	95% Confidence	Slope of Log. Conc. ±SE	Resistance Ratio at LC <sub>50</sub> (R/S)
Diazinon	15.14 ab	7.16 - 63.53	0.85±0.23	12.11
Malathion	86.28 abc	50.55 - 103.23	0.53±0.29	141.45
Parathion	321.87 c	300.90 -450.33	0.35±0.21	1609.37
Lambdacyhalothrin	3.89 a	1.55 - 39.74	0.85±0.22	7.48

\* Values in the same column followed by the same letter are not significantly different at 95% confidence level.



Figure[5.4]: Log. conc. probit lines of the insecticides tested on Tulkarem strain of house flies

## **2.4 Conclusions**

Laboratory tests of tarsal contact method of different insecticides to the house fly collected from five locations in the West Bank revealed the following:

- Field house flies from most of the tested locations showed varying levels of tolerance to the insecticides tested.
- Lambdacyhalothrin was the most effective against house flies from all the locations except in Jericho and this may be attributed to less intensive use of the insecticide in the four locations compared to Jericho.

## **3.4 Recommendations**

- Since the house flies population was found susceptible to Lambdacyhalothrin in most of the locations tested, it is recommended to use it for house fly control purpose.
- It is important to monitor insecticides performance from time to time, in order to make necessary shifts and modifications.
- Since house flies showed different degrees of tolerance to the tested insecticides, it is recommended to take this into consideration when selecting compounds to be used in controlling house flies.

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## 5.4: Appendix

### 1.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Diazinon-Ramalla

EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	1	7	0	-
0.5	-0.301	15	1	7	0	-
1	0	15	2	13	7	3.52
5	0.698	15	3	20	14	3.92
10	1	15	7	47	43	4.82
20	1.301	15	10	67	65	5.38
35	1.544	15	13	87	86	6.08

Mu = 0.969712  
Sigma = 2.270853

Parameter    Estimate    Std. Err.    95% Confidence Limits

-----  
Intercept    4.572975    0.283454    ( 4.017404, 5.128545)

Slope        0.440363    0.265103    ( -0.079240, 0.959966)

Spontaneous    0.066323    0.064124    ( -0.059360, 0.192007)

Response Rate

Y=0.44x+ 4.6

5=0.44x+ 4.6

X=0.909

LC<sub>50</sub>=Antilog( 0.909)= 8.11mg/L

2.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Diazinon-Hebron

EPA PROBIT ANALYSIS PROGRAM  
 USED FOR CALCULATING LC/EC VALUES  
 Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.5	-0.301	15	1	7	7	3.52
1	0	15	2	13	13	3.87
5	0.698	15	4	27	27	4.39
10	1	15	5	33	33	4.56
20	1.301	15	9	60	60	5.25
35	1.544	15	11	73	73	5.61

Mu = 1.144724  
 Sigma = 0.903507

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	3.733021	0.274484	( 3.195034, 4.271009)
Slope	1.106799	0.258100	( 0.600922, 1.612675)

Theoretical Spontaneous Response Rate = 0.0000

Y=1.106 x+3.73  
 5=1.106 x+3.73  
 X=1.148  
 LC<sub>50</sub>= 14.06mg/L



3.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Diazinon-Jericho

EPA PROBIT ANALYSIS PROGRAM  
 USED FOR CALCULATING LC/EC VALUES  
 Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.5	-0.301	15	1	7	7	3.52
1	0	15	2	13	13	3.87
5	0.698	15	5	33	33	4.56
10	1	15	8	53	53	5.08
20	1.301	15	10	67	67	5.44
35	1.544	15	12	80	80	5.84

Mu = 0.940793  
 Sigma = 0.799745

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	3.823633	0.266109	( 3.302060,	4.345207)
Slope	1.250399	0.257585	( 0.745532,	1.755265)

Theoretical Spontaneous Response Rate = 0.0000

$$Y = 1.25x + 3.82$$

$$5 = 1.25x + 3.82$$

$$X = 0.944$$

$$LC_{50} = 8.79 \text{ mg/L}$$

**4.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Diazinon-Suseptible House Flies**

**EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5**

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.5	-0.301	15	5	33	33	4.56
1	0	15	8	53	53	5.08
5	0.698	15	10	67	67	5.44
10	1	15	12	80	80	5.84
20	1.301	15	13	87	87	6.13
35	1.544	15	15	0	0	-

Mu = 0.087802  
Sigma = 0.959428

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.908485	0.198338	( 4.519742, 5.297228)
Slope	1.042288	0.237067	( 0.577637, 1.506939)

Theoretical Spontaneous Response Rate = 0.0000  
 $Y=1.04x +4.90$   
 $5=1.04x +4.90$   
 $X=0.096$   
 $LC_{50}= 1.25\text{mg/L}$

5.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Diazinon-Tulkarem

EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.5	-0.301	15	1	7	7	3.52
1	0	15	3	20	20	4.16
5	0.698	15	5	33	33	4.56
10	1	15	7	47	47	4.92
20	1.301	15	8	53	53	5.08
35	1.544	15	9	60	60	5.25

Mu = 1.185303  
Sigma = 1.179620

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	3.995182	0.240747	( 3.523318, 4.467046)
Slope	0.847731	0.232678	( 0.391682, 1.303779)

Theoretical Spontaneous Response Rate = 0.0000

Y=0.85x +4.00  
5=0.85x +4.00  
X= 1.18  
LC<sub>50</sub>=15.14mg/L

**6.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Malathion-Ramalla**

EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.5	-0.301	15	1	7	7	3.52
1	0	15	2	13	13	3.87
2	0.301	15	4	27	27	4.39
8	0.903	15	8	53	53	5.08
10	1	15	9	60	60	5.25
12.5	1.0969	15	11	73	73	5.61

Mu = 0.770332  
Sigma = 0.705208

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	3.907652	0.243102	( 3.431172, 4.384131)
Slope	1.418022	0.307047	( 0.816209, 2.019835)

Theoretical Spontaneous Response Rate = 0.0000

Y=1.41x +3.91  
5= 1.41x +3.91  
X=0.77  
LC<sub>50</sub>= 5.89mg/L

7.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Malathion-Hebron

EPA PROBIT ANALYSIS PROGRAM  
 USED FOR CALCULATING LC/EC VALUES  
 Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.5	-0.301	15	3	20	20	4.16
1	0	15	4	27	27	4.39
2	0.301	15	5	33	33	4.56
8	0.903	15	10	67	67	5.44
10	1	15	12	80	80	5.84
12.5	1.0969	15	13	87	87	6.13

Mu = 0.220007  
 Sigma = 0.981222

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.775783	0.153390	( 4.475138, 5.076427)
Slope	1.019137	0.249355	( 0.530401, 1.507873)

Theoretical Spontaneous Response Rate = 0.0000

Y=1.02x +4.78  
 5=1.02x +4.78  
 X=0.22  
 LC<sub>50</sub>= 1.66mg/L

**8.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Malathion-Jericho**

**EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5**

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	1	7	0	-
0.5	-0.301	15	1	7	0	-
1	0	15	2	13	7	3.52
2	0.301	15	3	20	14	3.92
8	0.903	15	5	33	28	4.42
10	1	15	7	47	43	4.82
12.5	1.0969	15	10	67	65	5.38

Mu = 1.059108  
Sigma = 0.629772

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	3.318267	0.684866	( 1.975930, 4.660604)
Slope	1.587876	0.743130	( 0.131341, 3.044412)

Spontaneous Response Rate 0.068491 0.056040 ( -0.041348, 0.178329)  
 $Y=1.59x + 3.32$   
 $5=1.59x + 3.32$   
 $X=1.06$   
 $LC_{50}=11.48 \text{ mg/L}$

**9.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Malathion-susceptible House Flies**

**EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5**

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	1	7	0	-
0.5	-0.301	15	8	53	50	5.00
1	0	15	10	67	65	5.38
2	0.301	15	12	80	79	5.81
8	0.903	15	15	100	100	-
10	1	15	15	100	100	-
12.5	1.0969	15	15	100	100	-

Mu = -0.217940  
Sigma = 0.499967

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.435908	0.216961	( 5.010665, 5.861151)
Slope	2.000131	0.536937	( 0.947735, 3.052527)

Spontaneous Response Rate  
 $Y=2.00x +5.44$   
 $5=2.00x +5.44$   
 $X=-.22$   
 LC50=9.78 mg/L

**10.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Malathion-Tulkarem**

EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.5	-0.301	15	2	13	13	3.87
1	0	15	2	13	13	3.87
2	0.301	15	3	20	20	4.16
8	0.903	15	4	27	27	4.39
10	1	15	5	33	33	4.56
12.5	1.0969	15	5	33	33	4.56

Mu = 1.935923  
Sigma = 1.896677

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	3.979308	0.225094	( 3.538124,	4.420492)
Slope	0.527238	0.289324	( -0.039837,	1.094313)

Theoretical Spontaneous Response Rate = 0.0000

Y=0.53x+3.98

5=0.53x+3.98

X= 1.93

LC50=85.11 mg/L



**11.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Parathion-Ramalla**

EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.025	-1.602	15	3	20	20	4.16
0.5	-0.301	15	3	20	20	4.16
1	0	15	3	20	20	4.16
2	0.301	15	5	33	33	4.56
4	0.602	15	7	47	47	4.92
8	0.903	15	10	67	67	5.44

Mu = 0.872371  
Sigma = 2.049599

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.574370	0.141390	( 4.297246, 4.851494)
Slope	0.487900	0.194803	( 0.106086, 0.869714)

Theoretical Spontaneous Response Rate = 0.0000  
 $Y=0.49x+4.57$   
 $5=0.49x+4.57$   
 $X=0.88$   
 $LC50=7.59\text{mg/L}$

**12.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Parathion-Hebron**

EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.025	-1.602	15	1	7	7	3.52
0.5	-0.301	15	3	20	20	4.16
1	0	15	6	40	40	4.75
2	0.301	15	8	53	53	5.08
4	0.602	15	10	67	67	5.44
8	0.903	15	12	80	80	5.84

Mu = 0.220007  
Sigma = 0.981222

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.775783	0.153390	( 4.475138, 5.076427)
Slope	1.019137	0.249355	( 0.530401, 1.507873)

Theoretical Spontaneous Response Rate = 0.0000

Y=1.02x+ 4.78

5=1.02x+ 4.78

X=0.22

LC50=1.66mg/L

**13.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Parathion-Jericho**

**EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5**

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.025	-1.602	15	1	7	7	3.52
0.5	-0.301	15	2	13	13	3.87
1	0	15	7	47	47	4.92
2	0.301	15	8	53	53	5.08
4	0.602	15	9	60	60	5.25
8	0.903	15	11	73	73	5.61

Mu = 0.298846  
Sigma = 1.063654

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.719038	0.151763	( 4.421583, 5.016494)
Slope	0.940156	0.240951	( 0.467891, 1.412420)

Theoretical Spontaneous Response Rate = 0.0000

$Y=0.94x +4.72$

$5=0.94x +4.72$

$X=0.30$

LC50=2.00 mg/L

**14.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Parathion-susceptible House Flies**

**EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5**

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.025	-1.602	15	5	33	33	4.56
0.5	-0.301	15	7	47	47	4.92
1	0	15	9	60	60	5.25
2	0.301	15	11	73	73	5.61
4	0.602	15	15	100	100	-
8	0.903	15	15	100	100	-

Mu = -0.691079  
Sigma = 1.142187

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.605049	0.154690	( 5.301857, 5.908242)
Slope	0.875514	0.195639	( 0.492062, 1.258966)

Theoretical Spontaneous Response Rate = 0.0000  
 $Y=0.88x+5.61$   
 $5=0.88x+5.61$   
 $X=-0.69$   
 LC50=9.31 mg/L

15.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Parathion-Tulkarem

EPA PROBIT ANALYSIS PROGRAM  
 USED FOR CALCULATING LC/EC VALUES  
 Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.025	-1.602	15	2	13	13	3.87
0.5	-0.301	15	2	13	13	3.87
1	0	15	2	13	13	3.87
2	0.301	15	2	13	13	3.87
4	0.602	15	3	20	20	4.16
8	0.903	15	7	47	47	4.92

Mu = 2.507685  
 Sigma = 2.888958

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.131976	0.155750	( 3.826706, 4.437246)
Slope	0.346146	0.214398	( -0.074075, 0.766366)

Theoretical Spontaneous Response Rate = 0.0000  
 $Y=0.35x+ 4.13$   
 $5=0.35x+ 4.13$   
 $X=2.49$   
 LC50=309.03 mg/L

**16.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Lambdacyhalothrin-Ramalla**

**EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5**

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	0	-
0.025	-1.602	15	1	7	7	3.52
0.5	-0.301	15	2	13	13	3.87
1	0	15	5	33	33	4.56
2	0.301	15	7	47	47	4.92
4	0.602	15	10	67	67	5.44
6	0.778	15	12	80	80	5.84

Mu = 0.291290  
Sigma = 0.900346

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.676469	0.159469	( 4.363911, 4.989028)
Slope	1.110684	0.286447	( 0.549247, 1.672121)

Theoretical Spontaneous Response Rate = 0.0000

Y=1.11x +4.68

5=1.11x +4.68

X=0.29

LC50=1.95mg/L

17.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Lambdacyhalothrin-Hebron

EPA PROBIT ANALYSIS PROGRAM  
 USED FOR CALCULATING LC/EC VALUES  
 Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	-	-
0.025	-1.602	15	4	27	27	4.39
0.5	-0.301	15	6	40	40	4.75
1	0	15	8	53	53	5.08
2	0.301	15	12	80	80	5.84
4	0.602	15	15	100	100	-
6	0.778	15	15	100	100	-

Mu = -0.550717  
 Sigma = 0.982834

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.560335	0.249428	( 4.867924, 6.252747)
Slope	1.017465	0.340317	( 0.072746, 1.962185)

Theoretical Spontaneous Response Rate = 0.0000  
 $Y=1.02x+ 5.56$   
 $5=1.02x+ 5.56$   
 $X=-0.55$   
 LC50=9.45mg/L

**18.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Lambdacyhalothrin-Jericho**

EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	-	-
0.025	-1.602	15	3	20	20	4.16
0.5	-0.301	15	3	20	20	4.16
1	0	15	3	20	20	4.16
2	0.301	15	4	27	27	4.39
4	0.602	15	5	33	33	4.56
6	0.778	15	7	47	47	4.92

Mu = 2.135079  
Sigma = 3.597495

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.406509	0.142400	( 4.127405, 4.685614)
Slope	0.277971	0.194198	( -0.102657, 0.658600)

Theoretical Spontaneous Response Rate = 0.000

Y=0.28x+ 4.41

5=0.28x+ 4.41

X=2.11

LC50=128.83mg/L



**19.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Lambdacyhalothrin-susceptible House Flies**

EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	-	-
0.025	-1.602	15	3	20	20	4.16
0.5	-0.301	15	5	33	33	4.56
1	0	15	7	47	47	4.92
2	0.301	15	10	67	67	5.44
4	0.602	15	13	87	87	6.13
6	0.778	15	15	100	100	-

Mu = -0.284789  
Sigma = 1.014828

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	5.280628	0.147713	( 4.991109, 5.570146)
Slope	0.985388	0.218103	( 0.557907, 1.412870)

Theoretical Spontaneous Response Rate = 0.0000

$Y = 0.99x + 5.28$

$5 = 0.99x + 5.28$

$X = -0.28$

LC<sub>50</sub> = 9.72mg/L

**20.5.4: Mathematical Procedure in determining LC<sub>50</sub> to Lambdacyhalothrin-Tulkarem**

**EPA PROBIT ANALYSIS PROGRAM  
USED FOR CALCULATING LC/EC VALUES  
Version 1.5**

Conc. (mg/L)	Log10 Conc.	Total No.	No. Dead	% Mortality	Corrected% Mortality	Probit
0.00	-	15	0	0	-	-
0.025	-1.602	15	2	13	13	3.87
0.5	-0.301	15	2	13	13	3.87
1	0	15	4	27	27	4.39
2	0.301	15	6	40	40	4.75
4	0.602	15	8	53	53	5.08
6	0.778	15	10	67	67	5.44

Mu = 0.592751

Sigma = 1.443080

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.589246	0.145551	( 4.303966, 4.874526)
Slope	0.692962	0.224290	( 0.253354, 1.132570)

Theoretical Spontaneous Response Rate = 0.0000

$Y=0.69x+ 4.59$

$5=0.69x+ 4.59$

$X=0.59$

LC50=3.89mg/L