Bentonite-Clay for Controlled Release Formulation of Chlorpyrifos in Soil Environment

Aqra' babi ma' ashtumailti shila' haliyasa inna hii nitaj jehidi al-hak, bas atii wa mtet
alisthora bi zihi mawa, inna haliyasa kakk, oyi ayi jeem ena li mteem en qul foral driga o
lqeb alami o biyati ladi ayi muqassim taliilima o biqatah akhi.

DECLARATION

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **Bayan M. A. Wheidi**

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Date: 26/10/2014
Bentonite-Clay for Controlled Release Formulation of Chlorpyrifos in Soil Environment

By

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحث/ بيان محمود عبد الله الوحيدي لنيل درجة الماجستير في كلية العلوم قسم علوم بيئة - صحة بيئة وموضوعها:

Bentonite-Clay for Controlled Release Formulation of Chlorpyrifos in Soil Environment

وبعد المناقشة العلمية التي تمت اليوم الأربعاء 27 شعبان 1435 هـ، الموافق 25/06/2014 الساعه الثامنة والنصف صباحاً، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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وقد أوصت اللجنة بمنح الباحث درجة الماجستير في كلية العلوم قسم علوم بيئة - صحة بيئة.

واللائحة إذ تمنح هذه الدرجة فإنها توصي بتقوى الله ولزوم طاعته وأن يذكر الله في خدمة نبينا ووطنه.

والفتوى

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ظهَر الفَسَادُ في الْبَرَّ وَالْبَحْرِ يَا كُسَبَتْ أَيْدِي النَّاسِ لِيُذْنِيَهُمْ بِبَعْضٍ
الذي عملوا لَعَلَّهُمْ يَرْجِعُونَ
صدق الله العظيم
سورة الروم آية (41)
Dedication

To
MY PARENTS WHO DEVOTED THEIR LIFE TO ME

To
MY BROTHERS AND SISTER WITH LOVE AND RESPECT

To
MY WIFE WHO ENCOURAGED AND HELPED ME TO ACHIEVE THIS DEGREE

To
MY BELOVED SONS IYAS AND TAMIM, AND BELOVED DAUGHTER AREN WHO HAVE BEEN A GREAT SOURCE OF MOTIVATION TO ME.

Bayan Wheidi
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Bayan Wheidi
Abstract

The excessive usage of pesticides in general and insecticides in particular has shown a significant consequences not only on environment but also on human health. Our study focuses on one of organophosphorous insecticides; the chlorpyrifos. In spite of its efficacy as an insecticides chlorpyrifos has created an environmental problems due to residual presence in soil, aquifer and plants also dangerous health consequences such as carcinogenicity and acetylcholine esterase enzyme inhibition.

This study aims to get a controlled released of chlorpyrifos and to minimize of its leaching towards the aquifer.

Column methods accompanied with bioassay was the method of choice. The process has been run in an isotherm conditions to study the chlorpyrifos adsorption on bentonite clay and organo-clay. The non adsorbed concentrations of the chlorpyrifos were determined using HPLC techniques. The bioassay using mosquito larvae were used. Also the IR spectroscopy has been run showing which functionality in organic cations might show better binding with chlorpyrifos.

Results have shown the chlorpyrifos was adsorbed with different ratios, since little adsorption has been noticed on bentonite clay while on organo-clay was in higher amounts according to the organic cations compound added to bentonite. The results have shown the height adsorbed amount of chlorpyrifos was noticed on (Clay-BTBA0.8). However, the results show that there is directly proportional between adsorption and alkyl functionality in the organic cations compound.

IR spectroscopy was used to confirm the above result through binding with the chlorpyrifos through its alkyl functionality. Since the (Clay-BTBA0.8) has shown the dramatic decreasing in its wave length 1549 cm\(^{-1}\) to 1471cm\(^{-1}\) as a clear cut evidence of its stronger binding with chlorpyrifos.

The mobility of chlorpyrifos study using columns method revealed that both agricultural soil as well bentonite clay have a significant release to all layers and the lowest concentration has been found in the first layer (0-5cm) while the results revealed that (Clay-BTBA0.8) has shown lowest release of chlorpyrifos and the highest concentration
of chlorpyrifos was found in the first layer (0-5cm) while the layers (6-20cm) didn’t show any release towards them. The results have revealed that the chlorpyrifos is concentrated at the top soil layer and bentonite clay which indicated that the organic matter in agriculture soil, surface area and high cations exchange capacity (CEC) for bentonite clay as well as the modified surface character of bentonite clay through its conversion from hydrophilic to the corresponding hydrophobic feature.

The bioassay using mosquito larvae has shown dead larvae in all layers of agricultural soil and bentonite clay while in organo-clay lower dead larvae were detected and the most distinguished organo-clay among all types the (Clay-BTBA0.8) that was accompanied with highest ratio of dead larvae in the top soil layer (0-5cm) while in the layers (6-20cm). For that the bioassay can be considered as good route for detecting the chlorpyrifos in all layers.

**Keywords:** Bentonite-clay, Chlorpyrifos, Adsorption, Organo-clay complexes, Mobility, Controlled release, Gaza, Palestine
Abstract in Arabic
ملخص الدراسة

الاستخدام المفرط للمبيدات بشكل عام والمبيدات الحشرية بشكل خاص له الكثير من التداعيات البيئية والتاثيرات الضارة على صحة الإنسان والبيئة. تركز الدراسة على المبيد الحشري كلوربيريفوس فهو مبيد عضوي هيكليًا واسع المجال واستخدم بكثرة في قطاع غزة لغة تأليفي النحتة، إلا أنه تسبب في الكثير من المشاكل البيئية بضعف وجود بالمعن في التربة والمياه الجوفية والنباتات وأيضا الصحافة كمسام

للسرطان ومنتف لأنزيم أسيتيل كولين بترير.

أجريت هذه الدراسة لهدف التحكم في انسباب مبيد كلوربيريفوس والترميم من ضرره في الوصول إلىigliex أن المبيدات البيئية تستعمل استخدام طريقة isotherm و-radiusها، حيث تم دراسة سلوك إمتصاص مبيد كلوربيريفوس باستخدام طريقة experiments على سطح طين البيتونيت والطين العضوي. وتم تمصير التراكيز المتبقي يت الذي المبيد

HPLC، وتم دراسة التحكم في الأنساب المبيد بالاستخدام الأعمدة والتقسيم البيولوجي باستخدام برقات البعوضة، ودراسة طيف الأشعة تحت الحمراء لأكثر المجموعات الوراثية في المركب العضوي الكاتيوني قالت بالرتبط مع المبيد باستخدام جهاز FTIR.

أظهرت نتائج الامتصاص أن مبيد كلوربيريفوس حدد له إمتصاص قليل على سطح طين البيتونيت بينما

الإمتصاص على سطح الطين العضوي كان أكثر بكثيرات مختلفة حسب المركب العضوي الكاتيوني المضاد إلى طين البيتونيت حيث كان أكثر مركب حدد على سطحه إمتصاص 0.8

أثبت أن الإمتصاص يزيد بزيادة مجموعات الألكل في المركب العضوي الكاتيوني.

للتاكيد مما سبق فقد استخدم طيف الأشعة تحت الحمراء لدراسة طيف أكثر مركب عضوي قام بالرتبط مع المبيد من خلال المجموعات الوراثية كان Clay-BTBA0.8, و هذا ثبت أنه أكثر مركب عضوي كاتيوني حدث على

الإمتصاص وتم الربط مع الكلوربيريفوس بشكل قوي.

أظهرت نتائج التحكم في انسباب مبيد كلوربيريفوس باستخدام طريقة الإعمدة أن التربة الزراعية وطين البيتونيت أظهر أن انسباب للعيد إلى جميع طبقات التربة حيث أن أكثر تركز وجد على سطح الطبقة الأولى (Clay-BTBA0.8) وأقل مركب حدد منه انسباب المبيد 0.5cm. وقد بدت النتائج أن الطين العضوي 0.5cm ولم يحدث أنسباب إلى الطبقات الأخرى.

أظهرت الدراسة أيضاً أن المبيد يركز على طبقات السطحية الأولى للتنبأة وطين البيتونيت و هذا

يقل عن أن المادة العضوية في التربة الزراعية، والمساحة الكبرى والمساحة الكثيرة في التبادل الكاتيوني

لطن البيتونيت والümüzات الذي حدث على الخصائص السطحية لطن البيتونيت تحول من قطبي إلى

غير قطبي كان هو السبب الرئيسي في هذه الإضافة الجيدة من مواصفات مرجعة.

أظهرت نتائج التقييم البيولوجي على برقات البعوضة أن هناك نسبة من اليرقات الميتة كانت موجودة

في جميع طبقات التربة الزراعية وطن البيتونيت، أما في الطين العضوي كانت نسبة اليرقات الميتة قليلة وقليلة وقليلة. وأظهرت نتائج برقات ميتة كانت في الطين العضوي (Clay-BTBA0.8) في الطبقة السطحية الأولى (0-6cm) لباختصار وجود برقات ميتة، لهذا أن التقييم البيولوجي كان أداة

جيدة لتحديد تركيز المبيد في طبقات مختلفة للترية.
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<td>Mobility of chlorpyrifos from Clay-BTBA0.8 formulation in soil layers as measured by bioassay technique</td>
<td></td>
</tr>
</tbody>
</table>

Page No.: 40, 41, 41, 42, 42
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE</td>
<td>Acetyl Choline Esterase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BTBA</td>
<td>Benzyl Tri Butyl Ammonium</td>
</tr>
<tr>
<td>BTEA</td>
<td>Benzyl Tri Ethyl Ammonium</td>
</tr>
<tr>
<td>BTMA</td>
<td>Benzyl Tri Methyl Ammonium</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>CEC</td>
<td>Cations Exchange Capacity</td>
</tr>
<tr>
<td>CHID</td>
<td>Chlorpyrifos Hazard Identification Document</td>
</tr>
<tr>
<td>CPF</td>
<td>Chlorpyrifos</td>
</tr>
<tr>
<td>CR</td>
<td>Controlled Release</td>
</tr>
<tr>
<td>DAD</td>
<td>Diode Array Detector</td>
</tr>
<tr>
<td>DT50</td>
<td>Degradation Time 50</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical Conductivity</td>
</tr>
<tr>
<td>EP</td>
<td>Environmental Program</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>ESFA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infra-Red</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HSDB</td>
<td>Hazardous Substances Data Bank</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>KBr</td>
<td>Potassium Bromide</td>
</tr>
<tr>
<td>Koc</td>
<td>Soil Sorption Coefficient</td>
</tr>
<tr>
<td>Kow</td>
<td>Octanol - Water Partition Coefficient</td>
</tr>
<tr>
<td>LD50</td>
<td>Lethal Dose 50</td>
</tr>
<tr>
<td>MOA</td>
<td>Ministry of Agriculture</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MRLs</td>
<td>Maximum Residue Limits</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>Definition</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
</tr>
<tr>
<td>NCAP</td>
<td>Northwest Coalition for Alternatives to Pesticides</td>
</tr>
<tr>
<td>OC</td>
<td>Organic Carbon</td>
</tr>
<tr>
<td>OM</td>
<td>Organic Matter</td>
</tr>
<tr>
<td>OP</td>
<td>Organo-Phosphorus</td>
</tr>
<tr>
<td>pH</td>
<td>Hydrogen Power</td>
</tr>
<tr>
<td>PHI</td>
<td>Preharvest Interval</td>
</tr>
<tr>
<td>PMRA</td>
<td>Pesticide Management Regulatory Agency</td>
</tr>
<tr>
<td>PTMA</td>
<td>Phenyl Tri Methyl Ammonium</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>Half-Life Time</td>
</tr>
<tr>
<td>TCPy</td>
<td>TrichloroPyridinol</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Chapter (1)

Introduction

1.1 Overview

Pesticides are substances or mixtures of substances used for preventing, destroying, repelling, or mitigating plant or animal pests and may include herbicides, insecticides, fungicides, and rodenticides. More than a billion pounds of pesticides are used in the U.S. each year to control weeds, insects, and other organisms that threaten or undermine human activities (USEPA, 2009a). Such type of substances was introduced to agriculture to fulfill the increased food needs of the growing global population. By now, use of pesticides has become a necessary evil although this practice is playing chaos with human and other life forms. Residues of applied pesticides stay in the environment (air, soil, ground and surface water) for variable periods of time (Gavrilescu, 2005 and Tariq, et al., 2007). They are of great concern for their potential adverse human health effects also because of their extensive worldwide application leading to potentially widespread exposure through residues in food and living environment (Barr, et al., 2005). An environmental contamination from pesticides is a raising concern for the public and regulatory authorities. Their fate in soil is also of main concern, as they bring a great threat to ground water (Rice, et al., 2007). In U.S. pesticide expenditures in 2007 accounted for 32% of the world’s total pesticide spending and 39% for insecticides, specifically, with agriculture and home/garden (USEPA, 2011a). More than 55% of the land used for agricultural production in developing countries uses about 26% of the total pesticides produced in the world. However the rate of increase in the use of pesticides in developing countries is considerably higher than that of the developed countries. Pesticides are necessary to protect crops and losses that may amount to about 45% of total food production worldwide (Murugesan, et al., 2010). The problem of agricultural pesticides in the Arab countries is not only an issue of uncontrolled use, but also pertains to the handling, misuse and disposal of unwanted pesticides. This is exacerbated by undeveloped national laws and regulations in regard to potential fate and residuals impacts of pesticides on groundwater, food safety and public health. Extensive use of pesticides with residual contents exceeding the maximum residue limits, caused many European countries to ban certain agricultural exports from several Arab countries (Bashour, 2008).
1.2 Environmental Fate of Pesticides

The fate of pesticides in soil is controlled by chemical, biological and physical dynamics of this matrix. Pesticides may be transformed by degradation processes or transported from the site of application by several processes (Wanner, et al., 2005). These processes can be grouped into those that affect persistence, including chemical and microbial degradation, and those that affect mobility, involving sorption, plant uptake, volatilization, wind erosion, run-off and leaching through the soil to groundwater as shown in (Figure 1.1).

![Pesticide fate processes](image)

Source: (Baskin, 2011)

**Figure 1.1:** Fate of pesticide residues in soil

Pesticides are degraded by chemical and microbiological processes. Chemical degradation occurs through reactions such as photolysis, hydrolysis, oxidation and reduction (Bavcon, et al., 2003 and Kodaka, et al., 2003). Biological degradation take places when soil microorganisms consume or break down pesticides (Ghadiri, 2001, Nawab, et al., 2003 and Sassman, et al., 2004). These microorganisms are mainly
distributed in the top centimeters of the surface layer of the soil, where the organic matter acts as food supply (Navarro, et al., 2003).

1.3 Pesticides in Palestine

Pesticides are being used in all parts of the Palestinian districts for various purposes. They are used in households, public health, the veterinary sector, and in the agricultural sector. Plant diseases and pests are considered one of the most common factors that obstacle and reduce both quantity and quality of agricultural products. Therefore, in order to produce high products with suitable quality, it is necessary to control the pests in the region of Palestinian Authority (MOA, 1995). More than 242 types of pesticides, i.e., herbicides, fungicides, insecticides, soil disinfecting and others are used in the Palestinian territories. The consumption of active ingredients from different types of pesticides were annually estimated in the west bank governorates as 979 tons of pesticides during the year 2006 (MOA, 2008). However in recent years, Gaza strip applied more than 400 tons of pesticides annually shown in (Table 1.1).

Table 1.1: Quantities (ton) of pesticides used in the past years in Gaza Strip

<table>
<thead>
<tr>
<th>Year</th>
<th>Insecticide</th>
<th>Herbicide</th>
<th>Fungicide</th>
<th>Soil sterilants</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>56.714</td>
<td>20.440</td>
<td>74.336</td>
<td>300.700</td>
<td>0.98</td>
<td>453.170</td>
</tr>
<tr>
<td>2006</td>
<td>55.270</td>
<td>24.940</td>
<td>55.650</td>
<td>111.600</td>
<td>0.855</td>
<td>248.315</td>
</tr>
<tr>
<td>2007</td>
<td>35.580</td>
<td>18.800</td>
<td>34.270</td>
<td>93.800</td>
<td>3.500</td>
<td>185.950</td>
</tr>
<tr>
<td>2008</td>
<td>49.650</td>
<td>18.200</td>
<td>42.200</td>
<td>193.600</td>
<td>60.828</td>
<td>364.478</td>
</tr>
<tr>
<td>2009</td>
<td>139.337</td>
<td>39.432</td>
<td>123.694</td>
<td>394.392</td>
<td>10.771</td>
<td>711.802</td>
</tr>
<tr>
<td>2010</td>
<td>144.682</td>
<td>18.780</td>
<td>99.630</td>
<td>162.400</td>
<td>61.327</td>
<td>486.819</td>
</tr>
<tr>
<td>2011</td>
<td>220.169</td>
<td>27.054</td>
<td>136.477</td>
<td>93.035</td>
<td>7.429</td>
<td>484.164</td>
</tr>
<tr>
<td>2012</td>
<td>232.488</td>
<td>25.609</td>
<td>137.911</td>
<td>143.210</td>
<td>5.209</td>
<td>544.427</td>
</tr>
<tr>
<td>2013</td>
<td>180.664</td>
<td>24.251</td>
<td>104.705</td>
<td>125.690</td>
<td>8.577</td>
<td>443.887</td>
</tr>
</tbody>
</table>

Source: (MOA, 2013)
Palestinian agriculture is becoming increasingly dependent on pesticides and fertilizers. Pesticides that have been banned or restricted in many countries continue to be marketed and used in Gaza (IARC, 1999). Level of soil and water contamination with increased across Gaza in recent years (Issa, 2000). Palestine, like other Arab countries, is plagued by uncontrolled use, unsafe handling and misuse of pesticides in a proliferating range. The increasing shortage of reliable data has alerted the scientific communities and to some extent the general public to a need for facts on potential health hazards of pesticides through their indiscriminate use (Samhan, 2008).

The fate and impact of pesticides application in the Palestinian National Authority is likely to remain unknown to the public because the scientific communities lack experience in this field of work (WHO, 1993 and UNEP, 2003). Concern about the environmental impact of repeated pesticide use has prompted research into the environmental fate of these agents, which can emigrate from treated fields to air, soil and water bodies (Arias-Estevez, et al., 2008). Adsorption is one of the most important factors that affects fate of pesticide in soils and determines their distribution in the soil and water environment (Kah and Brown, 2007).
1.4 Chlorpyrifos

Chlorpyrifos is a non-systemic organophosphate (OP) insecticide with contact, stomach and respiratory action. Chlorpyrifos is due to the inhibition of acetyl-cholinesterase (AChE inhibitor) in the pest nerves and subsequent accumulation acetylcholine in the nerve endings (EFSA, 2012).

Table 1.2: Selected characteristics of chlorpyrifos

<table>
<thead>
<tr>
<th>Identity</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common name</td>
<td>Chlorpyrifos</td>
</tr>
<tr>
<td>Trade name</td>
<td>Dursban</td>
</tr>
<tr>
<td>IUPAC name</td>
<td>((O,O\text{-diethyl} \ O-(3,5,6\text{-trichloro}-2\text{-pyridyl}) \text{phosphorothioate}))</td>
</tr>
<tr>
<td>Chemical Structure</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>(C_9H_{11}Cl_3NO_3PS)</td>
</tr>
<tr>
<td>Chemical type</td>
<td>Organophosphate</td>
</tr>
<tr>
<td>EPA toxicity</td>
<td>Class II (Moderately hazardous)</td>
</tr>
<tr>
<td>Application</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Mode of action</td>
<td>Cholinesterase inhibitor</td>
</tr>
</tbody>
</table>

Source: (Poorni, et al., 2008).
Table 1.3: Physical and chemical properties of chlorpyrifos.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Description</td>
<td>White crystalline</td>
<td>USEPA, 2006a</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>350.6 g/mol</td>
<td>USEPA, 2006a</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>$1.87 \times 10^{-5}$ mm Hg at 25°C</td>
<td>USEPA, 1999</td>
</tr>
<tr>
<td>Water Solubility</td>
<td>1.4mg/L (25°C)</td>
<td>Tomlin, 2006</td>
</tr>
<tr>
<td>Octanol – Water Partition Coefficient ($K_{ow}$)</td>
<td>4.70</td>
<td>Tomlin, 2006</td>
</tr>
<tr>
<td>Soil Sorption Coefficient ($K_{oc}$)</td>
<td>360 to 31.000</td>
<td>Smegal, 2000</td>
</tr>
<tr>
<td>Lethal dose (LD$_{50}$)</td>
<td>133 mg/kg</td>
<td>Tomlin, 2000</td>
</tr>
</tbody>
</table>

Chlorpyrifos ($O, O$-diethyl $O$-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is one of the most widely used organophosphorus insecticides. It is effective against a broad spectrum of pest insects to economically important crops ([Anwar, *et al.*, 2009]), and for pest control in agriculture since the 1960s ([Li, *et al.*, 2007]). It is registered for use in over 100 countries, for agricultural used. Public health uses include aerial and ground-based fogger treatments to control mosquitoes ([USEPA, 2011b]). Commercially, it is available under different brand names such as; Dursban, Lorsban, Agromil, Dhanwan, Dorson and Omexan ([Bhagobaty, *et al.*, 2007]). It is available as granules, wettable powder, dustable powder and emulsifiable concentrate. Chlorpyrifos is soluble in most organic solvents such as benzene, acetone, etc. It is slightly soluble in water and stable in air (nonvolatile) and it is not sensitive to UV radiation. It is stable to neutral and weakly acidic aqueous solution, however, stability decreases with increasing pH, but it is hydrolyzed by strong bases ([Amjad, *et al.*, 2010]).

Some properties of chlorpyrifos are shown in (Table 1.2). Extensive use of chlorpyrifos contaminates air, ground water, rivers, lakes, rainwater and fog water. The
contamination has been found up to about 24 kilometers from the site of application. Symptoms of acute poisoning include headache, nausea, muscle twitching and convulsions and in some extreme cases even death (NCAP, 2000). For instance, chlorpyrifos may affect the central nervous system, the cardiovascular system, as well as the respiratory system due to its high acute toxicity (Oliver, et al., 2000). Specifically, chlorpyrifos has a soil half-life ($t_{1/2}$) that ranges between 11 and 141 day, depending on the type of soil and other environmental conditions also is moderately persistence in temperate soil. Sterilization of soil decreased the degradation rate of chlorpyrifos, indicating that microorganisms played a significant role in the degradation of these compounds (Liang, et al., 2011). The persistent use of chlorpyrifos has led to widespread contamination of water and soils, resulting in serious damage to non-target species (Xiaohui, et al., 2008 and Gangming, et al., 2008).

Furthermore, the frequent use of chlorpyrifos in agriculture increased the public concern on potential human health risks that may result from acute or chronic dietary exposure to chlorpyrifos residues on food (Cochran, et al., 1995 and Yu, et al., 2006).

Additionally announced ban on the sale of the insecticide chlorpyrifos for householder use, in June 2000. This regulatory action was intended to phase out nearly all indoor and householder uses of this organophosphate, one of the most commonly used pest control substances in the United States. Before the ban, householder use of chlorpyrifos was particularly heavy in New York City (USEPA, 2000).

In the last ten years the quantity of chlorpyrifos used in agriculture in Gaza Governorate reached to 184,819 Liter. Historical applications of chlorpyrifos in Gaza are shown in (Table 1.4).
Table 1.4: Quantities (Liter) of Chlorpyrifos used in the last ten years in Gaza strip

<table>
<thead>
<tr>
<th>Year</th>
<th>Amount (L/Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>14025</td>
</tr>
<tr>
<td>2004</td>
<td>15273</td>
</tr>
<tr>
<td>2005</td>
<td>19672</td>
</tr>
<tr>
<td>2006</td>
<td>17352</td>
</tr>
<tr>
<td>2007</td>
<td>18125</td>
</tr>
<tr>
<td>2008</td>
<td>16300</td>
</tr>
<tr>
<td>2009</td>
<td>15256</td>
</tr>
<tr>
<td>2010</td>
<td>13298</td>
</tr>
<tr>
<td>2011</td>
<td>19247</td>
</tr>
<tr>
<td>2012</td>
<td>18146</td>
</tr>
<tr>
<td>2013</td>
<td>18125</td>
</tr>
</tbody>
</table>

Source: (MOA, 2013).

It is obvious that the applied quantity of chlorpyrifos in increased annually and reached about 19247 Liter/Year, the high applied rate of chlorpyrifos annually, indicates the potential risk of chlorpyrifos use in Gaza strip.

Therefore, it is necessary to develop remediation methods to degrade and reduce this pollutant from environments.

1.5 Problem Identification

The use of chlorpyrifos may create a lot of environmental problems. For instance, residues of chlorpyrifos were detected in vegetables in Gaza Strip, Palestine (Safi, et al., 2001) and in soil, water and consequently in food chain (El-Nahhal, 2004a). Moreover, an intensive spray action (Figure 1.2), may add more contamination to the environment.
Pollution of the ground water in Gaza is a major problem, not only are there numerous sources of pollution, but also the aquifer is highly vulnerable to pollution (UNEP, 2003). In Gaza governorates leaching of pesticides from soil can lead to the contamination of groundwater (Shomer, et al., 2006).

Accordingly, we try to control the release of chlorpyrifos to the soil environment in order to reduce this ecological damage.

1.6 Objectives:

1.6.1 General Objective:

To develop a controlled release formulations of chlorpyrifos to reduce environmental hazards.

1.6.2 Specific Objectives:

1-To evaluate the persistence of the new formulations under selected environmental conditions.
2-To evaluate the mobility of the new formulation in soil environment under laboratory conditions.

1.7 Significance

The Study may:

1- Reduce human and environmental health hazards.
2- Generate information that allow to better understanding the adsorption behavior of chlorpyrifos on modified clays and the mobility behavior of chlorpyrifos in soil
3- Generate data to allow us to analyze the activity of chlorpyrifos

1.8 Justification

The main justification of this study is attributed to the indiscriminate and extensive abuse of pesticides in general and insecticides in particular. The absence of enough knowledge might lead to an obvious harmful not only on human health but also on environmental health. More attention was paid on the Organophosphorous (OP) insecticide; Chlorpyrifos which is characterized by broad spectrum and availability in our life in both houses and agricultural fields.

In spite of being used as an effective insecticide; Chlorpyrifos has harmful role on our health through its ability to inhibit the acetylcholine esterase enzyme (AChE) that is responsible for the proper function of the neuron system (EFSA, 2012).

The fact that Chlorpyrifos has a strong tendency to favor the absorbance rather than the dissolved state, as a result of its non-polar nature, makes it easy for Chlorpyrifos to get into depth of soil penetrating from surface soil layer towards ground aquifer causing water contamination (HSDB, 2008).

The solution for these hazardous consequences can be solved through reducing the leaching towards ground water by forcing the Chlorpyrifos to stay at the soil surface rather than being migrated down and deeply. The main idea of reducing the leaching of chlorpyrifos is to adsorb it to clays whose surfaces are modified from hydrophilic to
hydrophobic by adsorption of suitable organic cations. This can be accomplished dealing with its chemical character as a non-polar organic compound. For that, it was suggested to mix an organic solvent with inorganic clay providing suitable conditions that allow the Chlorpyrifos to accommodate on soil surface. As a result, the Chlorpyrifos will not be available to contaminate the ground water (EL-Nahhal, 2002).
Chapter (2)

Literature Review

2.1 Introduction

Dispersion and transformation of Chlorpyrifos from the sources affect uptake into the environment and may be influenced by transport, climate and the characteristics of the area where they are being applied. The use of Chlorpyrifos for agricultural and gardening purposes will lead to accumulation in soil, water and on food such as vegetables and fruit as well as atmospheric dispersal. However, residential use is considered to be the main source of contamination in the majority of the population, alongside contaminated food consumption (Van den Hazel, 2005 and Becker, 2006). Moreover, El-Nahhal, et al., (2000) have provided a study about Montmorillonite–Phenyltrimethylammonium yields environmentally improved formulations of hydrophobic herbicides, and showed improved weed control in comparison with formulations based on other organo-clay. These formulations maintained herbicidal activity in the topsoil and yielded the most significant reduction in herbicide leaching. In addition El-Nahhal, et al., (2001a) conducted another study about Organo-clay formulation of Acetochlor for reduced movement in soil, and showed improved weed control in field and greenhouse experiments in comparison with the commercial formulation. The phenyltrimethylammonium (PTMA)-clay formulation of Acetochlor maintained herbicidal activity in the topsoil and yielded the most significant reduction in herbicide leaching and persistence under field condition. The application of this formulation can minimize the risk to groundwater and can reduce the applied rates conditions. Furthermore El-Nahhal, et al., (2001b) focused on another study about organo-clay formulations of pesticides: reduced leaching and photo degradation. New experimental results are presented for alachlor formulation which significantly reduces herbicide leaching under conditions of heavy irrigation. The application of this method for herbicide formulation would produce ecologically acceptable herbicide formulation that can significantly minimize the risk to groundwater.
Brewer, et al., (2003) determined chlorpyrifos residue levels in avian food and found chlorpyrifos residues in all avian food sampled 10 days following the application under field experiment. Furthermore, Phillips and Bode, (2004) studied Pesticides in surface water runoff in New York State, and found chlorpyrifos among other insecticides in water samples from the Kisco River, New York, and the concentrations exceeded that of water quality criteria for the protection of aquatic life.

Randhawa, et al., (2007) determined residues of CPF in fresh and processed vegetables. They showed that Chlorpyrifos residues were above maximum residue limits (MRLs). In addition, Rahmanikhah, et al., (2010) determined chlorpyrifos residues in the surface and ground water in the Southern Coast Watershed of Caspian Sea. They showed the average concentrations of diazinon, chlorpyrifos, ethion and edifenphos in the surface water samples were 0.041, 0.007, 0.009 and 0.005µg/l, respectively while their concentrations in the ground waters were 0.019, 0.016, 0.012 and 0.012µg/l, respectively.

Yunus and Anwar, (2011) investigated chlorpyrifos transport in soil and groundwater. They showed that chlorpyrifos, could reach groundwater because of their percentage remaining in the groundwater in high level. Also Angioni, et al., (2011) determined chlorpyrifos residues in fruits and vegetables after field treatment. Chlorpyrifos was applied on oranges, peaches, tomatoes, wine and table grapes. They showed that chlorpyrifos can accumulate leading to residue levels at the preharvest interval (PHI) over the maximum residue level (MRL), especially on oranges and peaches.

Alamgir, et al., (2012) studied occurrence of organophosphorus and carbamate pesticide residues in Surface Water Samples, and found chlorpyrifos, carbofuran and carbaryl residues in 24 surface water samples and five ground water samples from Pirgacha Thana, Rangpur district, Bangladesh.
2.2 Environmental Fate of Chlorpyrifos

2.2.1 Chlorpyrifos Fate in Soil

Both biotic (e.g.: Microbial degradation) and abiotic (e.g.: Hydrolysis and photolysis) processes contribute to the degradation of chlorpyrifos. In separate study chlorpyrifos may undergoes photolytic degradation in sunlight (Gebremariam, et al., 2012). Accordingly sterilization of soil decreased the degradation rate, indicating that microorganisms played a significant role in the degradation of these compounds (Liang, 2011). However, the main route of degradation appears to be via aerobic and anaerobic microbial metabolism. Chlorpyrifos degrades slowly in soil under both aerobic and anaerobic conditions. The major degradation of chlorpyrifos in the environment, under most conditions, is 3,5,6-trichloro-2-pyridinol (TCPy) (US EPA, 2006b). The degradation pathway of chlorpyrifos is shown in (Figure 2.1).

Source: (Eaton, et al., 2008).

**Figure 2.1:** Environmental degradation of chlorpyrifos
Hydrolysis of chlorpyrifos is a more important transformation route under alkaline conditions than under slightly acidic or neutral conditions (PMRA, 2004). The main metabolites following hydrolytic degradation are (TCPy) and O-ethyl-O-(3,5,6-trichloro-2- pyridoyl) phosphorothioic acid (phosphorothioate) (EFSA, 2005). Soil acidity (pH) has been shown to affect the degradation rate of chlorpyrifos. Low pH the degradation rate was slower than of high pH (EFSA, 2005).

Chlorpyrifos is persistent in soils under some conditions. There is a wide range of degradation time (DT50) reported in the literature for soil persistence, ranging from a few days to 4 years, depending on application rate, ecosystem type, and various environmental factors. The persistence of chlorpyrifos on soil, vegetation, and fruit has also been found to vary with different formulations containing different considerably inert ingredients, with a microencapsulated formulation being the most persistent (Montemurro, et al., 2002).

Singh, et al., (2002) studied Persistence of Chlorpyrifos, Fenamiphos, Chlorothalonil, and Pendimethalin in soil and their effects on soil microbial characteristics. Results of the study revealed that the half-life of chlorpyrifos was about 36 and 46 days for first and second treatments respectively. Furthermore leaching of chlorpyrifos process depends on soil, weather and hydrological conditions, as well as on the application procedure (Reichenberger, et al., 2007). Therefore leaching of chlorpyrifos to groundwater creates a threat to human and environment (Dabrowski, et al., 2002).

2.2.1.1 Controlled Release

Controlled-release (CR) technology for pesticides has received increasing attention due to a growing awareness of the undesirable environmental effects of pesticides as observed from their application (Kydonieus, 1980). The use of CR formulations regulates the supply of the active ingredient (ai) at the required rate for pest control, thus reducing the amount of chemical introduced in the environment and protecting it from its deleterious effects (Fleming, et al., 1992).
2.2.2 Chlorpyrifos Fate in Water

Chlorpyrifos breaks down in water by microbial action, hydrolysis, and photolysis (Racke, 1993). Chlorpyrifos degradation is significantly slower in seawater than it is in fresh water. One study in California found a DT$_{50}$ for seawater of 49.4 days at 10°C, compared with 18.7 days in freshwater at 21°C, the DT$_{50}$ for seawater was 15.2 days, so temperature has a significant effect on sea water degradation (Bondarenko and Gan, 2004). On the other hand, they states that the persistence of chlorpyrifos in sediment from a San Diego creek was found to increase significantly under anaerobic conditions: the DT$_{50}$ for aerobic conditions was 20.3 days but for anaerobic conditions it was 223 days, although only 57.6 days in sediment from Bonita Creek.

2.2.3 Chlorpyrifos Fate in Air

Harnly, et al., (2005), determined concentrations of chlorpyrifos in outdoor air following ground application of chlorpyrifos in an agricultural setting. They found considerable concentration of chlorpyrifos in air sample. Moreover chlorpyrifos was found in all oceanic air samples taken over the Sea of Japan, the East China Sea, and the Bering and Chukchi seas, the concentration decreasing from Asia to the Arctic (Zhong, et al., 2012).

In addition high concentrations of chlorpyrifos (70-80 ng/L) were determined in snow collected from sea ice at 3 sites in northwest Alaskan Arctic estuaries in the Chukchi and Beaufort Seas during 1995-96 (Garbarino, et al., 2002).

Moreover several studies detected chlorpyrifos in dust, air, carpets, and on surfaces within indoor environments (Hore, et al., 2005 and Morgan, et al., 2005). Chlorpyrifos residues were measured in samples of air and from non-target surfaces including plush toys, smooth surfaces, furniture, windowsills and flooring after the homes were treated with a water emulsion crack and crevice formulation containing 0.25 to 0.50% chlorpyrifos.

Chlorpyrifos was detected in all homes within the treated areas throughout the two week post-application period. The highest concentrations of chlorpyrifos detected were 816
ng/m$^3$ in air, 24.6 ng/m$^3$ on non-target surfaces, and 1949 ng per ton on plush toys (Hore, et al., 2005).

### 2.2.4 Chlorpyrifos Fate in Plants

Chlorpyrifos is not expected to be taken up from soil through the roots of plants (Tomlin, 2006). Chlorpyrifos may be taken up by plants through leaf surfaces, much of the applied chlorpyrifos is usually lost due to volatilization, and very little is translocate throughout the plant (Roberts and Hutson, 1999). However, Kamrin, (1997) reported chlorpyrifos residues to remain on plant surfaces for 10 to 14 days after application.

Zhang, et al., (2011) studied Phytotoxicity and uptake of chlorpyrifos in cabbage in southern China. The results showed that chlorpyrifos can be taken up by roots and cabbage from water and consequently translocated as a function of time.

### 2.3 Eco-toxicity of Chlorpyrifos

USEPA, (2009b) reassessment of chlorpyrifos identified concerns about acute and chronic risks to birds, mammals, terrestrial invertebrates, fish, and aquatic invertebrates. It stated there were 278 reported ecological incidents between 1974 and 2005 associated with the use of chlorpyrifos. It was the “probable” or “highly probable” causative agent of 108 out of 121 reported adverse aquatic incidents such as fish kills, and for 70 out of 107 terrestrial incidents mainly bird and bee kills.

### 2.4 Residues of Chlorpyrifos in the Human Body

Chlorpyrifos is absorbed by inhalation, ingestion and skin penetration. Researchers evaluated the absorption of chlorpyrifos by oral and dermal exposure in five human volunteers. Exposure to chlorpyrifos by inhalation results in the fastest appearance of toxicity symptoms, followed by oral and then dermal routes of exposure (Reigart and Roberts, 1999). It is distributed throughout the body following exposure (Blodgett, 2006). Although some chlorpyrifos may be stored in fat tissue, bioaccumulation to be significant due to an elimination half-life in humans of less than three days (Kamrin, 1997). It is completely metabolised to chlorpyrifos Oxon and then to 3,5,6-trichloro-2-
pyridinol (TCPy) in the liver by the cytochrome P450 enzyme system (CYP450) (Hodgson and Rose, 2008). Chlorpyrifos oxon is approximately 12 times more toxic than chlorpyrifos (US EPA, 2013). Cytochrome P450 replaces the sulfur of the P=S group with oxygen in a process known as oxidative desulfuration to form the oxon (Flaskos, 2012). The oxon is a more potent inhibitor of acetyl cholinesterase than chlorpyrifos itself (Verma, et al., 2009).

One study in Minnesota, USA, found that over a 5-day period metabolites of chlorpyrifos were detected in the urine of 98% of the urban children tested (Adgate, et al., 2001). Elimination of chlorpyrifos occurs mainly through the kidneys. It is excreted in the urine (CDC, 2005).

2.5 Human health impacts caused by chlorpyrifos

Age and genetic/acquired predisposition may determine health effects from the CPF exposure dose. Toxicity of chlorpyrifos is due to the inhibition of acetyl cholinesterase by the CPF-Oxo, preventing efficient degradation of acetylcholine and leading to accumulation of transmitter molecules in the nerve synapse. Elevated synaptic acetylcholine levels result in persistent receptor stimulation and the alteration of signaling pathways with functional changes at tissue organism level (Pope, et al., 2005).

(US EPA, 2011c) reported no evidence of carcinogenicity in animal studies, but there are a number of epidemiological studies indicating that chlorpyrifos may be carcinogenic in humans, the association being strongest is for lung and rectal cancers.

2.5.1 People at Heightened Risk

Newborn children can be 65-164 times more exposed than adults to the OPs chlorpyrifos, diazinon and parathion (Furlong, et al., 2006).

(US EPA, 2011d) states that pregnant women may be more sensitive to chlorpyrifos than non-pregnant women, because of a reduced capacity of the detoxifying enzymes.
(Virginia, et al., 2012) studied Brain anomalies in children exposed prenatally to a common organophosphate pesticide. They investigated associations between chlorpyrifos exposure and brain morphology using magnetic resonance imaging (MRI) in 40 children, 5.9–11.2 year, selected from a nonclinical, representative community-based cohort. Twenty high-exposure children and twenty low-exposure children, accordingly chlorpyrifos concentrations in umbilical cord blood. And they showed abnormalities, represented in atrophy or abnormal growth in different areas of the brain associated with behavior and emotion and intelligence, among children exposed to higher than normal rates of chlorpyrifos.
Chapter (3)
Materials and Methods

3.1 Materials

3.1.1 Bentonite Clay

The used bentonite clay was obtained from Clays Repository, Clay Minerals Society, and Columbia, Minnesota, USA.

3.1.2 Soil Collection

The soil samples were collected from depth of 0-25 cm of agricultural field before treatment in the soil with chlorpyrifos from zaitune area, Gaza city. The soil samples were air-dried and passed through a sieve of 2 mm mesh, then stored in well-closed plastic bags at room temperature until treated used.

3.1.3 Tested Chemicals

Chlorpyrifos, purity 99.9%, was purchased from Sigma Aldrich Co., Germany. Commercial emulsifiable formulation of chlorpyrifos, was purchased from Dow Agro Sciences Co., USA, and Methanol of HPLC grade, purity 99.9% , was purchased from Lab-Scan Co., Poland was purchased from Gaza.

3.1.4 Tested Organisms

The organisms used in this study were Mosquito Larvae, which were collected from pools of European hospital, Khanyounis. The organisms were brought in plastic bottles containing micro holes cover with 2mm mesh and remain 48 hour at lab. temperature before testing adaptable.

3.1.5 Soil Columns

Tin macro-columns,10X10 cm² surface area, 25 cm height, divided into two half's 5X10 cm² surface area. The column bottom contained several micro holes of 2 mm mesh, diameter and two litters sandy soil were transferred to each column (Figure 3.1).
3.1.6 Organo-clay Complexes

The following organo-clay complexes Clay-BTMA0.5, Clay-BTBA0.8, Clay-BTEA0.25 and Clay-PTMA0.5 have been supplied and prepared according to (EL-Nahhal, et al., 1998, 2000, 2001, and EL-Nahhal, 2003). In this procedure; dry organic salts equivalent to 0.25, 0.5 and 0.8 mmol/g clay were added to a 1% (w/v) aqueous suspension of bentonite under continuous stirring up to 3 days. The yielded organo-clay complex was separated after 30-min centrifugation (6000 g), the precipitate was washed three times with distilled water to have free chloride ion in the washing water. The complexes were freeze-dried, then grounded to < 50μm and kept in plastic bottles at room temperature.

3.2 Methods

3.2.1 HPLC-Measurement

As described previously (El-Nahhal, 2002), chlorpyrifos concentrations in the supernatant were determined by Chrom Tech with Diode Array Detector (DAD230°) equipped with manual-injection system. The column was a 200 mm X 4.6 mm (i.d.) reverse-phase. Packing ODS-BP5μm (C18), injection volume is 50μl and wave length
of detection was 280 nm, Mobile phase is water: methanol 1:9. The flow rate was maintained at 1.5 ml min\(^{-1}\). External calibration was used for quantification of chlorpyrifos.

### 3.2.2 Standard Curve of Chlorpyrifos

A dose response curve was prepared by a volume of the stock solution 2.5 ml, containing 1mg chlorpyrifos, was transferred to a 1 L volumetric flask and diluted in distilled water up to the mark to a concentration 1ppm as working standard. A series of chlorpyrifos standards of 0.0, 0.2, 0.4, 0.6, 0.8 and 1 mg/L. The adsorption was measured by HPLC at wavelength 280 nm and retention time 4.1 min.

### 3.2.3 Adsorption of Chlorpyrifos

#### 3.2.3.1 Adsorption of chlorpyrifos on organo-clay complexes

Adsorption of chlorpyrifos on Bentonite-clay exchanged with either organic quaternary ammonium cations was measured at room temperature. 10 mg of chlorpyrifos was dissolved in 25 ml methanol and acts as a stock solution. 2.5 ml of the stock solution, containing1mg chlorpyrifos, was transferred to a 1 litter volumetric flask and diluted with distilled water up to the mark and used to perform the adsorption experiments. Following the procedure described by El-Nahhal, (2002). To measure the adsorbed amounts, 50 ml of an aqueous solution of chlorpyrifos (0.05mg/l) was added to a 10 mg clay or organo-clay in a 30 ml centrifuge tube. The final concentration of the sorbent matrix was 0.2 g/l in all experiments. The samples were kept under continuous rotary shaking at 25± 1 °C during 48 hours. The supernatant was separated by centrifugation at 6,000g for 1 hours.

#### 3.2.3.2 Adsorption isotherm experiments

Adsorption isotherm of chlorpyrifos is conducted on clay, clay-BTMA0.5, clay-BTEA0.25, clay-BTBA0.8 and clay-PTMA0.5 complexes. The adsorption isotherms were measured in the range of 0-1000 \(\mu\)g chlorpyrifos/l, the maximum solubility limit. Appropriate aliquots of an aqueous solution of chlorpyrifos 1mg/L were diluted in 50 ml distilled water and added to a 10 mg clay or organo-clay in a 30 ml
centrifuge tube. The samples were kept under continuous rotary agitation for 48 hours at 25± 1 °C. As shown by El-Nahhal and Safi, (2004).

The supernatant was separated as described above. The adsorbed amounts were calculated using equation (1).

\[ Q_s = (Q_i - Q_e) \frac{V}{M} \quad \ldots \ldots (1) \]

Where \( Q_i \) and \( Q_e \) are the initial and equilibrium concentration \( \mu g/l \) of chlorpyrifos and \( Q_s \) is the concentration in the solid phase \( \mu g/g \) (the adsorbed amount). \( V \) is the volume (L) and \( M \) is the mass of adsorbent (g). Chlorpyrifos concentration in the supernatants was determined by a high performance liquid chromatography (HPLC) as described below.

3.2.3.3 FTIR-Spectroscopy

FTIR spectroscopy has been used to examine the interactions between chlorpyrifos and clay pre-adsorbed with the respective organic cations as previously described (El-Nahhal and Safi, 2004). The FTIR spectra were obtained using KBr pellets with Agilent Technologies, Cary 640 FTIR spectrometer. Spectra were recorded at room temperature in the range of 4000-1200 cm\(^{-1}\). Difference spectra were obtained by subtracting the spectra of corresponding organo-clay or homoionic clay from the spectra of organo-clay-chlorpyrifos complexes or clay-chlorpyrifos complexes. Absolute and difference spectra will be also recorded in the range of 1800-1200 cm\(^{-1}\).

**Preparation of a KBr disk:**

KBr disk has been prepared as follow:

(a) A spatula full of KBr was added into an agate mortar and grind it to fine powder.
(b) The powder was added to the 7mm collar and Put the die together with the powder into the Hand Press assembly.
(c) For 1 minute press to form a good thin and transparent pellet.
3.2.4 Mobility of Chlorpyrifos

3.2.4.1 Mobility of chlorpyrifos in soil columns

The controlled release of chlorpyrifos was prepared by dissolving appropriate amounts, 60 mg, of chlorpyrifos in 50 ml methanol. Ten ml of the aqueous solution of chlorpyrifos were added to 300 mg powdered clay, or organo-clay in 50 ml glass beaker then 10 ml methanol solvent was added. The mixture was mixed thoroughly with magnetic stirrer. The solvent was evaporated under reduced pressure as described (El-Nahhal, et al., 1999). Several loads of chlorpyrifos on the clay-complex were examined.

The experiment leads to leaching assessment of chlorpyrifos in laboratory

Tin macro-columns, 10X10 cm surface area, 25 cm height were filled with air-dried sandy soil sieved through 2 mm screen. The macro-column surface was sprayed with various formulations of chlorpyrifos at field rate using an atomizer.

The columns were carefully irrigated with 500 ml water (the saturation capacity), applied in portions during 3-5 hours with 20 min intervals. The macro-columns were left for 48 hours for equilibration and then were sliced along their lengths, hence forming two pots 10X5X25 cm each. One pot was used for chemo assay by HPLC and the second was for bioassay as described by El-Nahhal, (2004b) with a slight modification.

3.2.4.2 Bioassay Technique

Bioassay makes possible the measurement of a biological response by a living organism to determine the presence and/or concentration of a chemical in substrate (Streibig, 1988). As applicable step towards confirming this concept; mosquito larvae were used to indicate the presence of chlorpyrifos in soil layers. However a significant chlorpyrifos presence in soil might lead to mosquito larvae death while the absence of such insecticide is expected to show mosquito larvae not being affected.
In this procedure, the soil column was divided into 4 layers of 5 cm long. The soil layers were transferred to a 1L glass beaker and mixed thoroughly with 1000 ml of distilled water. Ten misquote larvae were transferred to each beaker. Relative concentration of chlorpyrifos could be determined at various soil layers by a percent death insect using equation (2).

\[
\% \, \text{Death} = 100\times (L_c - L_t) / L_c \quad \ldots \ldots \quad (2)
\]

Where \(L_t\) and \(L_c\) are the live cases in the treated and control samples, respectively.

### 3.2.5 Soil Analysis

Soil pH, electrical conductivity, Calcium carbonate, organic matter, organic carbon and soil texture, were determined in the central laboratory for water and soil in Ministry of Agriculture, Gaza. The methods of analysis used are described below.

**a- Soil pH:**

Soil pH value was measured potentiometrically in a 1: 2.5 soil – water suspension, as described by (Dhyan, et al., 1999).

**b- Electrical Conductivity:**

Electrical Conductivity of soil was measured potentiometrically in a 1: 2.5 soil – water suspension, according to method developed previously (van-Reeuwijk, 1992).

**c- Calcium Carbonate (CaCO\(_3\)):**

The water-insoluble calcium carbonate salt (CaCO\(_3\)) is present in the solid form. One of the most well-known methods for calcium carbonate analysis is described by Drouineau, (1942) through taking 1g. of dry soil which then transfer to 250 ml of Erlenmeyer flask followed by adding 10 ml of 1N HCl to the mixture with stirring and being left overnight. Then the mixture was treated with 50-100 ml of distilled water and 2-3 drops of phenol phathaline indicator. Finally the solution was titrated with NaOH(1N) until light pink color is observed.
To calculate the percentage of CaCO$_3$ in soil; the following equation:

$$\% \text{ CaCO}_3 = \left[ (10 \times N_{HCl}) - (R \times N_{NaOH}) \right] \times 0.05 \times \frac{100}{\text{wt}}$$

Where:

$N_{HCl}$: Normality of HCl
$N_{NaOH}$: Normality of NaOH
$R$: Volume of NaOH (ml)
Wt: Weight of dry soil (g)

**d- Organic matter**

The organic carbon content in the soil used was analyzed by using Walkley-Black method. The organic carbon in the sample is oxidized with potassium dichromate and sulphuric acid. The excess potassium dichromate is titrated against ferrous ammonium sulphate. Taking 1g soil and transferred into 500 ml conical flask. Adding 10 ml of 1N K$_2$Cr$_2$O$_7$ and 20 ml of concentration H$_2$SO$_4$, swirled carefully then left to stand for 30 minutes. Adding 200 ml of distilled water and 10 ml H$_3$PO$_4$ slowly. Then 1 ml of diphenylamine indicator was added and the resulted suspension was titrated against 0.5 N ferrous ammonium sulphate solution until green color appeared indicating the end point. Blank must run simultaneously (Walkely and Black, 1934).

The carbon content was calculated using the following equation:

$$\text{Organic Carbon} = \frac{10 \times (B - S) \times 0.39 \times \text{mcf}}{(B \times W)}$$

Where:

B: Blank of Ferrous ammonium sulphate (ml)
S: Sample of Ferrous ammonium sulphate (ml)

mcf: Moisture correction factor
W: Weight of sample (g)
0.39: Conversion factor (including a correction factor for a supposed 70% oxidation of organic carbon.

\[ \text{% Organic Matter} = 1.72 \times \text{% Organic Carbon} \]

### 3.2.6 Statistical Analysis

The adsorption experiment conducted in three replicates with a blank sample average and standard deviation were determined from each concentration. The percent deaths data were subjected to analysis of variance, and main effects and interactions were tested for significance using repeated measures ANOVA. Means of effects of different formulations on insecticide activity were compared by Tukey’s test at \( \alpha = 0.05 \). A semi logarithmic dose response curve was fitted to percent of death for each replicate in each treatment.
Chapter (4)
Results and Discussion

4.1 Results

4.1.1 Characteristics of Used Soil

Physico-chemical properties of used soil in this study are shown in (Table 4.1) and the results were obtained through analysis in central laboratory for water and soil in the Ministry of Agriculture in Gaza city. It can be seen that pH value is (7.0), which indicates soil was a neutral. Furthermore the EC value is (0.95) ms/cm, the CaCO₃ as solid phase of soil, value is (0.05%) . The clay fraction of soil is (3%), the organic matter value is (1.73%). This indicates that soil is poor in organic matter which acts as a non-polar surface.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>pH</th>
<th>EC ms/cm</th>
<th>OC %</th>
<th>OM %</th>
<th>CaCO₃ %</th>
<th>Clay %</th>
<th>Silt %</th>
<th>Sand %</th>
<th>Soil texture mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>7.0</td>
<td>0.95</td>
<td>1.0</td>
<td>1.73</td>
<td>0.05</td>
<td>3</td>
<td>13</td>
<td>84</td>
<td>Loamy sand</td>
</tr>
</tbody>
</table>

Table 4.1: Physico-chemical properties of used Agricultural soil.
4.1.2 HPLC-Chromatogram of Chlorpyrifos

The HPLC chromatogram of standard chlorpyrifos is shown in (Figure 4.1). It is obvious that a sharp peak with considerable peak area were obtained at 4.1 min. This indicates the accuracy of the used method for determination.

![HPLC-Chromatogram of chlorpyrifos](image)

**Figure 4.1: HPLC-Chromatogram of chlorpyrifos**

4.1.3 Standard Curve of Chlorpyrifos

The relationship between peak area and gradient concentration of chlorpyrifos are shown in (Figure 4.2). It is obvious that the peak area increased linearly as the concentration of chlorpyrifos increased in the solution. These findings indicate a linear relationship. Regression analysis showed a correlation coefficient ($R^2$) of 0.998 indicating a strong positive association. This linearity indicates the validity and the suitability of the used method and allows direct measurements of chlorpyrifos in the supernatants by HPLC.
**Figure 4.2:** Relationship between peak area and standard concentration of chlorpyrifos

### 4.1.4 Adsorption of Chlorpyrifos

Adsorption of chlorpyrifos on row bentonite and bentonite modified with PTMA0.5, BTMA0.5, BTEA0.25 and BTBA0.8 are shown in (Figures 4.3 - 4.7).

(Figure 4.3) shows a low concentration of chlorpyrifos was adsorbed on clay alone without surface modification.

**Figure 4.3:** Adsorption of chlorpyrifos on clay alone.
Figure 4.4) Shows a slightly increase of chlorpyrifos adsorbed on clay-PTMA 0.5.

(Figure 4.5) shows a considerable increase of chlorpyrifos adsorbed on clay-BTMA 0.5.

**Figure 4.4:** Adsorption of chlorpyrifos on clay-PTMA0.5

**Figure 4.5:** Adsorption of chlorpyrifos on clay-BTMA0.5
(Figure 4.6) shows the high increase of chlorpyrifos adsorbed on clay- BTEA 0.25.

**Figure 4.6:** Adsorption of chlorpyrifos on clay- BTEA0.25

(Figure 4.7) shows extreme increase of chlorpyrifos adsorbed on clay- BTBA 0.8.

**Figure 4.7:** Adsorption of chlorpyrifos on clay-BTBA0.8
Table 4.2: P-value of adsorption results

<table>
<thead>
<tr>
<th>Items</th>
<th>P-Value</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay : Clay- BTMA0.5</td>
<td>0.0385</td>
<td>2</td>
</tr>
<tr>
<td>Clay - BTMA0.5 : Clay- PTMA0.5</td>
<td>0.0622</td>
<td>4</td>
</tr>
<tr>
<td>Clay - PTMA0.5 : Clay - BTBA0.8</td>
<td>0.0124</td>
<td>1</td>
</tr>
<tr>
<td>Clay - BTBA0.8 : Clay - BTEA0.25</td>
<td>0.4000</td>
<td>5</td>
</tr>
<tr>
<td>Clay - BTEA0.25 : Clay- BTMA0.5</td>
<td>0.0425</td>
<td>3</td>
</tr>
</tbody>
</table>

Statistical analysis of the adsorption results are shown in (Table 4.2). It is obvious that there are significant differences between Clay – PTMA 0.5: Clay - BTBA0.8, Clay: Clay- BTMA0.5 and Clay – BTEA 0.25: Clay- BTMA0.5 whereas in Clay – BTMA 0.5: Clay- PTMA0.5 and Clay – BTBA 0.8: Clay - BTEA0.25 no significant differences were obtained. P-value below 0.05 indicates significant difference.
4.1.5 FTIR-Interaction of Chlorpyrifos with Clay and Organo-clays

Spectrum of chlorpyrifos in its free form adsorbed to raw bentonite or bentonite modified with different organic cations are shown in (Figures 4.8 – 4.12).

(Figure 4.8) shows a spectrum of free chlorpyrifos before adsorption on surface bentonite clay and organo-clay. Two peaks at 1549 cm\(^{-1}\) and 1410 cm\(^{-1}\) are observed clearly.

(Figure 4.9) shows a slightly shifted in the spectrum from 1549 cm\(^{-1}\) to 1541 cm\(^{-1}\).

(Figure 4.8): FTIR spectrum of free molecule of chlorpyrifos

(Figure 4.9): FTIR spectrum of chlorpyrifos adsorbed on raw bentonite clay.
(Figure 4.10) shows a highly shifted peak at 1489 cm$^{-1}$ compared with 1549 cm$^{-1}$.

**Figure 4.10:** FTIR spectrum of chlorpyrifos adsorbed on clay-PTMA0.5

(Figure 4.11) shows a highly shifted peak at 1483 cm$^{-1}$ compared with 1549 cm$^{-1}$.

**Figure 4.11:** FTIR spectrum of chlorpyrifos adsorbed on clay-BTMA0.5
(Figure 4.12) shows a very highly shifted peak at 1471 cm\(^{-1}\) compared with 1549 cm\(^{-1}\).

Figure 4.12: FTIR spectrum of chlorpyrifos adsorbed on clay-BTBA0.8

4.1.6 Mobility of Chlorpyrifos

4.1.6.1 Chemo assay of Chlorpyrifos in Soil Columns

Mobility of chlorpyrifos in soil profile as determined by HPLC are shown in (Figures 4.13 - 4.17).

In (Figure 4.13) it is obvious that the percentage of commercial formulation released was 17.5\% in the top soil layer (0-5cm), then reduction of chlorpyrifos release is observed in the other layers.

Figure 4.13: Percentage of commercial chlorpyrifos released in soil layers as determined by HPLC.
In (Figure 4.14) it is obvious that the percentage of chlorpyrifos released was \(16.3\%\) in the top soil layer (0-5cm), then reduction of chlorpyrifos release is observed in the other layers.

**Figure 4.14:** Percentage of chlorpyrifos released from bentonite clay in soil layers as determined by HPLC.

In (Figure 4.15) it is obvious that the percentage of chlorpyrifos released was \(14.5\%\) in the top soil layer (0-5cm), then reduction of chlorpyrifos release is observed in the other layers.

**Figure 4.15:** Percentage of chlorpyrifos released from clay-PTMA0.5 in soil layers determined by HPLC.
In (Figure 4.16) it is obvious that the percentage of chlorpyrifos released was **13.6%** in the top soil layer (0-5cm), then reduction of chlorpyrifos release is observed in the other layers.

**Figure 4.16:** Percentage of chlorpyrifos released from clay-BTMA 0.5 in soil layers as determined by HPLC.

In (Figure 4.17) it is obvious that the Percentage of chlorpyrifos released was **11%** in the top soil layer (0-5cm), then reduction of chlorpyrifos release is observed in the other layers.

**Figure 4.17:** Percentage of chlorpyrifos released from Clay-BTBA0.8 in soil layers as determined by HPLC.
4.1.6.2 Bioassay of Chlorpyrifos in Soil Columns

Mobility of chlorpyrifos in soil profile as measured by bioassay is shown in (Figures 4.18-4.24).

The relationship between number of dead mosquitoes larvae and standard concentration of chlorpyrifos are shown in (Figure 4.18). Series standards were 0.0, 0.05, 0.1, 0.15, 0.2, and 1 mg/L. It is observed that the number of dead mosquitoes larvae increased linearly as the concentration of chlorpyrifos increased in the solution. These findings indicate a linear relationship and strong positive correlation.

Figure 4.18: Toxic effects of different concentrations of chlorpyrifos to mosquitoes larvae as measured by percent death
Figure 4.19: Log scale of different concentration of chlorpyrifos to mosquitoes larvae.

In (Figure 4.20) it appears that the death percentage of mosquito larvae was **30%** in top soil layer (0-5cm), **20%** in layer (6-10cm) and **10%** last two layers (11-20cm).

**Figure 4.20:** Mobility of commercial chlorpyrifos formulation in soil layers as measured by bioassay technique.
In (Figure 4.21) it appears that the death percentage of mosquito larvae was 40% in the top soil layer (0-5cm), 20% two layers (6-15cm) and 10% layer (16-20cm).

Figure 4.21: Mobility of chlorpyrifos from bentonite clay formulation in soil layers as measured by bioassay technique.

In (Figure 4.22) it appears that the death percentage of mosquito larvae was 20% in top soil layer (0-5cm), 20% in soil layer (6-10cm) and last two layers (11-20cm) were 10%.

Figure 4.22: Mobility of chlorpyrifos from Clay-PTMA0.5 formulation in soil layer as measured by bioassay technique.
In (Figure 4.23) it appears that the death percentage of mosquito larvae was **20%** in top soil layer (0-5cm), **10%** in soil layer (6-10cm) and last layers (11-20cm) were **0%**.

![Figure 4.23: Mobility of chlorpyrifos from Clay-BTMA 0.5 formulation in soil layers as measured by bioassay technique.](image)

In (Figure 4.24) it appears that the death percentage of mosquito larvae was **10%** in top soil layer (0-5cm), and others layers (6-20cm) were **0%**.

![Figure 4.24: Mobility of chlorpyrifos from Clay-BTBA0.8 formulation in soil layers as measured by bioassay technique.](image)
Table 4.3: P-value of mobility results

<table>
<thead>
<tr>
<th>Items</th>
<th>P- Value</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial chlorpyrifos : Clay- BTMA 0.5</td>
<td>0.0302</td>
<td>2</td>
</tr>
<tr>
<td>Clay- BTMA 0.5 : Clay- PTMA 0.5</td>
<td>0.1835</td>
<td>4</td>
</tr>
<tr>
<td>Clay- PTMA 0.5 : Clay- BTBA 0.8</td>
<td>0.0339</td>
<td>3</td>
</tr>
<tr>
<td>Clay- BTBA 0.8 : Clay</td>
<td>0.00017</td>
<td>1</td>
</tr>
</tbody>
</table>

Statistical analysis of the mobility results are shown in (Table 4.3). It is obvious that there are significant differences in all complexes except Clay- BTMA 0.5: Clay- PTMA 0.5. It shown no significant difference. P-value below 0.05 indicates significant difference.
4.2 Discussion

4.2.1 Adsorption of chlorpyrifos on clay and organo-clay complexes

The differences in adsorption between the clay and organo-clays can be considered as the key point for this research.

As it is illustrated in (Figure 4.3), low concentration of chlorpyrifos adsorbed can be attributed to the hydrophilic nature of clay compared with the hydrophobicity of Chlorpyrifos. The weak interaction result between chlorpyrifos and clay is consistent with facts found by Tomlin, (2000) who states that the hydrated mineral surface of the clay and hydrophobic chlorpyrifos are most likely not to interact and $K_{ow} = 4.7$.

The research aims to improve the nature of the clay to afford circumstances that provide more adsorption of chlorpyrifos. That needs to implement sort of modification to change the hydrophilic system to the corresponding hydrophobic one.

From structure point of view, the clay holds a negative charge and once an organic cations has been added to the negative clay, the charge complementarily will lead to terminate the charges. The absence of charges in the hydrophilic compound means its conversion to the corresponding hydrophobic pattern. As a result organo-clays and chlorpyrifos might have similarity and consequently interaction or binding between chlorpyrifos and organo-clays is expected to be accomplished and the adsorption will increase structural network of three materials.

Our thoughts and ideas on what did (Mortland, 1970) who found that adsorption of chlorpyrifos will increase. As a result of organic cations being adsorbed on the surface of clay through neutralizing the charge making more broad network as well as other factors such hydrogen bonding, Vander Waals, and ion-dipole forces. The above theoretical principles supported the obtained results.

As it is clear in (Figures 4.4 - 4.7), the adsorbed amount of chlorpyrifos on organo-clay was much more than on clay alone. In more details; it is remarkable that there is a gradual increasing of adsorbed amount of chlorpyrifos on clay; starting from clay-PTMA0.5, passing through clay-BTMA0.5 and ending with clay-BTEA0.25 and clay-
BTBA0.8. In regard; it was noticed that the lowest adsorption was on the clay-
PTMA0.5 while the highest adsorption was on the clay-BTBA0.8, this can be
interpreted as the difference in alkyl group being owned by the compound. Since the
PTMA compound has only three methyl groups and phenyl ring while the BTBA
compound has three butyl groups and benzyl ring, the relatively high adsorption of
Chlorpyrifos on clay-BTEA0.25 than clay-BTMA0.5 is attributed to alkyl groups
contents in each, as methyl versus ethyl and this is an evidence about the directly
proportional feature of adsorption and number of carbon atoms in the organo-clay. As
general we deduce that increasing the alkyl groups in the organo-clay will increase the
adsorbed amount of chlorpyrifos. This is supported by Lagaly, (1987) who confirmed transfer from hydrophilic to hydrophobic by coating clay with different types of alkyl
ammonium that make an increasing in adsorption due to more coverage of negatively
charged clay by long chain alkyl group. Another factors can be added to reasons causing
these results is the π-π interaction between pyridine ring in chlorpyrifos and phenyl ring
in the organo-clay. This result is supported by Stevens and Anderson, (1996) who
found that the orientation of the adsorbed molecules due to energy transfer through
molecules. The adsorption comparison shows that the arrangement of these complexes
can be given as following.

Clay- BTBA0.8 > Clay- BTMA0.5 > Clay- PTMA0.5 > Clay

4.2.2 FTIR spectrum of chlorpyrifos with clay and organo-clay complexes

FTIR spectroscopy has been used to confirm binding and interaction between functional
groups through monitoring the stretching peaks shifting. The comparative study for
chlorpyrifos functionality, clay-chlorpyrifos and organo-clays-chlorpyrifos are under
investigation.

The IR stretching will take into account the areas (1549cm⁻¹ and 1410 cm⁻¹). As expected
stretching for Chlorpyrifos functionality. The reference Chlorpyrifos alone has shown a
stretching peak at 1549cm⁻¹ while the IR evaluation for clay-chlorpyrifos has shown
stretching peak at 1541cm⁻¹ that means there is a weak interaction between Clay and
Chlorpyrifos. This is an indicative route to find out whether the binding does occur
weakly or strongly.
FTIR spectra are illustrated in (Figures 4.10- 4.12). It is clear that the effect of binding on stretching peak value of chlorpyrifos 1549 cm\(^{-1}\). As clay-PTMA0.5, clay-BTMA0.5 and clay-BTBA0.8 showing 1489 cm\(^{-1}\), 1483 cm\(^{-1}\) and 1471 cm\(^{-1}\). The above spectroscopic observation can be used as a clear evidence about proportional directly relationship between alkyl chain length and Chlorpyrifos adsorption.

4.2.3 Mobility of chlorpyrifos formulations in soil columns (Chemo assay)

We try to understand how chlorpyrifos does mobile or release towards lower layers, for that; columns method has been used as a lab condition scale as the 20 cm columns has been divided into two half’s vertical each half four layers; in which each layer height is 5 cm. Commercial chlorpyrifos that is used in this research, it is illustrated in (Figure 4.13) the top soil layer (0-5cm) was containing the highest concentration of chlorpyrifos compared with other layers. This can be justified as there soil has organic matter as clear in (Table 4.1), that shows the physico-chemical properties of such soil. That indicates organic matter in the soil reduces the release of chlorpyrifos. This is consistent with result obtained by (Afifi and Abu - Swareh, 1999) who revealed the organic matter in Gaza soils play an important role in the adsorption of chemicals.

Inspection to Figure (4.14), reveals that the chlorpyrifos has been adsorbed mostly in top soil layer (0-5cm). This can be attributed to high surface area and cations exchange capacity (CEC) of bentonite clay. In addition that the agriculture soil content of organic matter, helps in minimizing the release of chlorpyrifos. However; it is still less than the agriculture soil.

As it is illustrated in (Figures 4.15 - 4.17); the release of chlorpyrifos is increasing gradually from one complex to another. The highest release was from the clay-PTMA0.5, then the clay-BTMA0.5 and finally the lowest release was from the clay-BTBA0.8, in the top soil layer (0-5cm) compared with others. It was noticed the released amount of chlorpyrifos in the organo-clay complexes were less than the amount released in the case of raw bentonite clay alone and agriculture soil. This due to several factors such as the hydrophobicity harmony between organo-clay and chlorpyrifos; this might lead to strong interaction that affect positively on adsorption. Also the properties
of bentonite clay and agriculture soil, all these support the minimizing in chlorpyrifos release and consequently increase adsorption.

### 4.2.4 Mobility of chlorpyrifos formulations in soil columns (Bioassay)

Since chlorpyrifos is classified as an effective insecticide, it is good functionalize such as insecticides to make an indirect study of its effect. However its presence can be noticed through its effect. For that the comparative results were achieved using mosquito larvae as insects to be the indicator for such assay.

(Figure 4.20), indicate that the dead mosquitoes larvae are present in all layers of the column with different ratios. However, the highest ratio of dead mosquitoes larvae was in the top soil layer (0-5cm); this means chlorpyrifos concentration, as a result of more chlorpyrifos being adsorbed due to the organic matter in the soil compared with other layers. As a conclusion of such behavior ; the chlorpyrifos has ability to be release down reaching the ground water causing contamination. This result does agree with Bortoluzzi, (2007) when he found high concentration of chlorpyrifos in the ground water.

(Figure 4.21), show there are dead mosquitoes larvae in all layers with different percentages in the top soil layer (0-5cm), the percentage was the highest and more than the case in agriculture soil. In spite of chlorpyrifos release in agriculture soil in higher than the bentonite clay, but the main reason for this observation is the source of chlorpyrifos, since two kinds of chlorpyrifos ; one of them is liquid commercial while the other one is powder with 99.9% purity ratio, and was prepared in university laboratory. The commercial one may contain impurities and preservatives or not stored in the proper condition, all these point might add alter the reactivity. As a result purity has played role of efficacy that was observed through the number of dead mosquito larvae.

(Figures 4.22 - 4.24), illustrate the high percent death in top soil layer (0-5cm), while a reduction in death percentage is observed in layers (5-20cm), that indicates chlorpyrifos is adsorbed slightly on the surface of clay-PTMA0.5. In addition, clay-BTMA0.5 has shown high percent death in top soil layer (0-5cm), these results indicate that chlorpyrifos retained in top layer and then a reduction of death percentage is observed in layers (6-10cm) and zero death percentage in layers (11-20cm) as an indication about
the absence of chlorpyrifos concentration in this layer consequently the adsorbed amount of chlorpyrifos was high in organo-clay. Furthermore clay-BTBA0.8 also has shown low death percentage in top soil layer (0- 5cm), these data indicate that chlorpyrifos retained on surface top soil layer and zero death percentage in layers (6 - 20cm). These results indicate the absence of chlorpyrifos concentration in the layers(6 - 20cm) that means the adsorbed amount of chlorpyrifos was very high in organo-clay. Since increasing the number of dead mosquito in the top soil layer (0-5cm) indicates more concentration of chlorpyrifos and consequently more adsorption and less mobility towards lower layers. Finally, it is proven that adsorption of chlorpyrifos can be improved through providing a formulation that includes clay adsorbed on organic ammonium salt and to get more adsorption or to increase chlorpyrifos adsorption efficiency; it can be concluded that an increase of the aliphatic part in the organic ammonium salt which will lead to provide more hydrophobic character; consequently less mobility and more binding with chlorpyrifos resulting more adsorption. For more details; from experimentally death percentage results, the following arrangement has been observed:

Clay > Soil > Clay- PTMA0.5 > Clay- BTMA0.5 > Clay- BTBA0.8
5.1 Conclusion

This study revealed the adsorption behavior of chlorpyrifos on raw bentonite clay and on different organo-clay complexes. The main idea aimed to reduce the release of chlorpyrifos by adsorbing it to clays whose surfaces are modified from hydrophilic to hydrophobic by adsorption of suitable organic cations. The results showed that lower adsorbed amount was on bentonite clay before modification and higher adsorbed amount was obtained on clay modified with quaternary ammonium (BTBA, BTEA, BTMA, and PTMA), as results exchanging the clay with organic cations is important to enhance the adsorption process.

Studying the mobility of chlorpyrifos in agriculture soil from Gaza using columns technique was affected by physical and chemical properties of soil. The chlorpyrifos was strongly retained in the top soil layers. Then organic cations and organic matters in soil have strongly influences in adsorption consequently reducing in mobility of chlorpyrifos was achieved.

The adsorption and mobility process of chlorpyrifos and contamination of groundwater of chlorpyrifos depends on physical and chemical properties of soil and chlorpyrifos.

5.2 Recommendations

1- Chlorpyrifos of great danger to environment and human. For that both authorities in charge and researchers must carry out an active role by periodic sampling from wells, soil and harvests.

2- Addition of organic matter to soil may increase the soil adsorption capacity and biological activity reducing leaching of chlorpyrifos toward ground water.

3- Addition of organo-clays and an organic matter to soil increases adsorption process of chlorpyrifos.

4- Mitigation measurements and awareness programs for farmers should be arranged.
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