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Long Term Toxicity of Chlorpyrifos and Diuron to Chickens and Rabbits

السمية طويلة الأمد لمبيد الكلوروبيروفوس ومبيد الديرون على الدجاج و الأرانب

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إقـــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Long Term Toxicity of Chlorpyrifos and Diuron to Chickens and Rabbits

السمية طويلة الأمد لمبيد الكلوروبيروفوس ومبيد الديرون على الدجاج و الأرانب

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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/ رائد ابراهيم موسى لبد لنيل درجة الماجستير في كلية العلوم قسم علوم بيئية – الإدارة والمراقبة البيئية وموضوعها:

السمية طويلة الأمد لمبيدي الكلوروبيروفوس الديرون على الدجاج والأرانب

Long term toxicity of Chlorpyrifos and Diuron to Chickens and Rabbets

وبعد المناقشة العلنية التي تمت اليوم الأربعاء 25 شعبان 1437هـ، الموافق 2016/06/01 الساعة الثالثة والنصف بمبنى طيبة، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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واللجنة إذ تمنحه هذه الدرجة فإنها توصيه بتقوى الله ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

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Abstract

Background and Problem: Application of pesticide for agriculture and public health purposes, leaves low concentration of residues that may be harmful for the non target organism.

Objectives and aims: The study characterize biochemical changes associated with long term toxicity of Chlorpyrifos and Diuron in chickens and rabbits.

Methodology: Chlorpyrifos and Diuron were dissolved in corn oil and given to chicken and rabbit**s** at rate of 0.1mg/ kg/ day, to 2 weeks period. The behavior daily activity and weight were monitored daily at the 8 weeks. The animals were authenticated, blood samples were collected in heborinzed tubes and kept under cold condition until analysis .Liver, kidney, heart and brain samples were collected for enzymes analysis. Evaluation of toxicity was determined by analyzing the activities of the following enzymes in the collected blood serum, liver, brain, kidney and heart tissue: 1) Acetylcholine esterase (AChE), 2) Alkaline phosphates (ALP), 3) Aspartate aminotransferase, AST, 4) Alanine aminotransferase (ALT), 5) Total protein, kidney factions, crecetine, uric acid, and urea.

Results: Results showed reduced weights of rabbits and chicken due to repeated low does application. The effect of mixture in reducing rabbits weight was more pronounced than in Diuron or Chlorpyrifos. However the effect in chicken was more pronounced in Diuron treatments than Chlorpyrifos or mixture. Diuron treatments reduced the weight of liver in rabbits and increased it in chicken. Similar observation was in the heart weight in rabbit and chicken. All treatments elevated the levels of ALT in rabbit above the control sample whereas in chicken the ALT remained below the control sample. The treatment of mixture inhibited AChE in brain and serum in rabbets; whereas in chicken Diuron was the most toxic one. Mixture treatment elevated the level of uric acid and total protein above the control sample in rabbits; whereas Diuron treatments in chicken was more potent than others; and elevated urea, uric acid and total protein above the control.

Conclusions: It can be concluded that rabbit and chicken have different responses to low repeated does application of Chlorpyrifos, Diuron, and mixture. The enzyme reacted differently in all case.

Key words: rabbit , chicken , ACHE ,ALT , ALP ,uric acid, urea , Toxicity.

Abstract in Arabic

الملخص

خلفية و مشكلة البحث: إن استخدام المبيدات لأغراض الزراعة و الصحة العامة يمكن ان يترك تركيزا منخفضا من البقايا التي يمكن ان تكون ضارة للكائن الغير مستهدف .

أهداف البحث: وصف التغيرات البيوكميائية المصاحبة للسمية طويلة الأمد لمبيد الكلوروبيروفوس ومبيد الديرون في الدجاج والأرانب.

منهجية البحث : تم إذابة الكلوربيريفوس والديرون في زيت الذرة وتم إعطائها إلى الدجاج والأرانب بمعدل 0.1ملغم/**كجم**/ يوما، لمدة أسبوعين، وتم مراقبة نشاط السلوك اليومي، والوزن يوميا لمدة 8 أسابيع. تم التحقق من صحة مصدر الحيوانات، وجمع عينة الدم في أنابيب تحتوي على مادة الهيبارين وتم حفظها في حالة التبريد حتى موعد التحليل, وتم جمع عينات لكل من الكبد والكلى والقلب والدماغ وذلك لتحليل الإنزيمات. تم تحديد تقييم السمية من خلال تحليل أنشطة الإنزيمات التالية في جمع مصل الدم وأنسجة كل من الكبد والدماغ والكلى والقلب الأنسجة: 1) الأستيل كولين استريز (ACHE)، 2) إنزيم الفوسفاتاز القلوي (ALP)، 3) انزيم اسبارتات ترانس اميناز (AST)، 4) انزيم الانين ترانس اميناز (ALT)، 5) البروتين الكلي ووظائف الكلى، والكرياتينين، وحمض اليوريك، واليوريا.

نتائج البحث :النتائج أظهرت أنه يوجد انخفاض في أوزان الأرانب والدجاج، بسبب تكرار إعطاء جرعات قليلة. وكان تأثير الخليط في خفض أوزان الأرنب أكثر وضوحا مما كانت عليه في الديرون أو الكلوربيريفوس. أيضا كان التأثير عند إعطاء الديرون للدجاج أكثر وضوحا من إعطاءهم الكلوربيريفوس أو الخليط. وخفضت المعالجة بالديرون وزن الكبد في الأرانب وزادته في الدجاج. وثمة ملاحظة مماثلة في وزن القلب في الأرانب والدجاج. ورفعت جميع العلاجات مستويات ALT في الأرانب فوق العينة الضابطة، في حين بقي كبد الدجاج تحت العينة الضابطة في أنسجة الكبد. وأيضا المعالجة بالخليط ثبطت الأستيل كولين استريز في الدماغ ومصل الدم في الأرانب، بينما في الدجاج كانت المعاملة بالديرون هي الأكثر سمية، وكانت المعالجة بالخليط ترفع مستوى حمض اليوريك والبروتين الكلي أعلى من العينة الضابطة في الأرانب، بينما كانت معالجة الدجاج بالديرون أكثر فعالية من غيرها، وترفع اليوريا وحمض اليوريك والبروتين الكلي أكثر من العينة الضابطة.

خلاصة البحث: نستخلص من النتائج أن الأرانب والدجاج لهم استجابات مختلفة عند التعرض لجرعات قليلة متكررة من الديرون والكلوربيريفوس والخليط. وأن الانزيم يتفاعل بشكل مختلف في كل الحالات.

كلمات مفتاحيه : الأرانب, الدجاج, السمية ,الأستيل كولين استريز, حمض اليوريك، اليوريا, إنزيم الفوسفاتاز القلوي , انزيم الانين ترانس اميناز.

Epigraph Page


بسم الله الرحمن الرحيم


"ظَهَرَ الْفَسَادُ فِي الْبَرِّ وَالْبَحْرِ بِمَا كَسَبَتْ أَيْدِي


النَّاسِ لِيُذِيقَهُمْ بَعْضَ الَّذِي عَمِلُوا لَعَلَّهُمْ يَرْجِعُونَ"


صدق الله العظيم

{سورة الروم ،41}

Dedication

To all the spirit of all Palestinian martyrs who sacrificed themselves to enlighten the way of freedom for us.

To my parents who have always supporting me.

To my beloved sister Eman.

To my brother Ahmad who helped me to accomplish this thesis.

To my brothers, sister and sons of my brothers.

To my university Islamic University-Gaza which is continuously improving the research.

To all of them I dedicate this work.

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Chapter (1)
Introduction

1.1 Overview:

The population in Gaza is about 1,820,000 people living and working in an area of 365 km2. Gaza Strip suffers from the lack of grazing area, shallow fishing area, and low quality of animal production. Gaza Strip is a semi-arid region with an annual rainfall ranged from 180-450 mm. Deterioration of water quality results in a steady decrease in animal chicken production. Over 80% of the population lives below the poverty line and some 50% are unemployed. Agriculture in the Gaza Strip is highly intensive and it relies on frequent application of pesticides(PCBS, 2016). However, frequent application of pesticides resulted in contamination of food samples and agricultural commodities in many countries with pesticide residues (El-Nahhal, 2004). A lot of health hazards have been associated with pesticides application. For instance cancer cases have been reported in Gaza (Safi et.al., 1993, Safi 2002). Health problems and biochemical changes among farm workers (Yassin et.al., 2002, Safi et.al., 2006) health disabilities (El-Nahhal and Radwan 2013).

Pesticides are widely used worldwide in agriculture to increase crop yields by killing pests or eliminating diseases. The worldwide consumption of pesticides is about two million tonnes per year (45% of which is used in Europe alone) (Meffe and de Bustamante, 2014). Pesticides, including herbicides, insecticides, fungicides, bactericides and rodenticides, are widely used to control pests and pest-induced diseases (Damalas and Eleftherohorinos, 2011). The use of pesticides is an effective method to protect crops from being damaged and to improve yields (Mansour, 2004). Although pesticides have been developed to function with reasonable certainty and minimal risk to human health and the environment. The published results are not always in agreement with this fact. Even though the development of toxicity reference levels for pesticides incorporates uncertainty factors that serve to achieve this regulatory standard; in reality, we may never know whether a pesticide is safe under all circumstances, nor can we predict with certainty its performance in hypothetical situations. Scientific investigation is bound by the tools and the techniques that are available and therefore new developments continually redefine our capabilities.

Despite many studies on the fate and toxicity of pesticides, there are research gaps causing uncertainty in the predictions of their long-term health and environmental effects. On the basis of these contradictory results of the literature, discussions among scientists and the public focused on the real, predicted, and perceived risks that pesticides pose to human health (worker exposure during pesticide use and consumer exposure to pesticide residues found in fresh fruit, vegetables and drinking water) and the environment (water and air contamination, toxic effects on non-target organisms) are fully justified (Damalas, 2004; Pimentel, 2005; Burger et.al, 2008). It is crucial that the use of pesticides is assessed to ensure that it does not harm humans or nature. Therefore the use of pesticides in developing countries should be further investigated and clarified, to provide guidance for governments and international organizations in making appropriate policies (Konradsen et.al, 2003). Environmental pollution represents a serious hazard for terrestrial and aquatic ecosystems and may result in significant ecotoxicological effects. Over the last decades, diverse substances have been released into marine environment as a consequence of agricultural production, manufacturing processes and their by-products. These chemicals include herbicides, pesticides, fungicides, plasticizers, antifoulants and others (Wurl and Obbard, 2004)

Their excessive use causes serious damage to the ecosystem, terrestrial as well as aquatic, and consequently to the flora and fauna of the surroundings (Paliwal et.al., 2009).

Agricultural practices are among the main activities responsible for the release of hazardous chemicals into the environment, Among these chemicals, are pesticides (fungicides, herbicides and insecticides) have been used for decades without any control, resulting in a strong contamination of water, air, and foods as well as in the development of pesticide resistant organisms. This problem became more serious during the last years resulting in high risks to human health (Harms et.al, 2011). These pesticides affect the Agriculture community and neighborhoods . Therefore, considering that human health risk is a function of pesticide toxicity and exposure, a greater risk is expected to arise from high exposure to a moderately toxic pesticide than from little exposure to a highly toxic pesticide. However, whether or not dietary exposure of the general population to pesticide residues found on food and drinking

water consists of a potential threat to human health, is still the subject of great scientific controversy (Magkos et.al, 2006).

1.2 Significance:

The Palestinian soil is exposed to many of the human, agricultural and industrial activities, which have negative effects on the fertility of the soil and land. The most prominent issues facing the soil in Palestine is the excessive use of fertilizers and pesticides. Because of the large increase in population and the narrow of agricultural area, people have to use fertilizers and pesticides to increase the productivity of agricultural land (Heinze et.al, 2014).

Agricultural pesticide poisoning is a major public health problem in the developing world, killing at least 250,000–370,000 people each year (Sekiyama et.al, 2007). Targeted pesticide restrictions in Gaza Strip over the last 20 years have a significant increase in chronic diseases. However, regulatory decisions have thus far not been based on the human toxicity of formulated agricultural pesticides but on the surrogate of rat toxicity using pure formulated pesticides(El-Nahhal and Radwan, 2013).

Pesticide exposure causes harmful effects to farm workers to suffer more chemical-related injuries and illnesses than any other work force in Gaza Strip. Workers who mix, load or apply pesticides can be exposed to toxic pesticides due to spills, splashes, defective, missing or inadequate protective equipment, direct spray, or drift. Workers who perform hand labor tasks in areas that have been treated with pesticides face exposure from direct spray, drift or contact with pesticide residues on the crop or soil. Farm worker families can also be injured by pesticides when farm worker children play in treated fields; when workers inadvertently take home pesticide residues on their hair, skin or clothing; or when pesticides drift into residences, schools and other areas located near fields (Safi et.al., 2005).

Few studies (El-Nahhal et.al.,2016 ; El-Nahhal and Hamdona .,2016 El-Nahhal et.al.,2015 a,b) investigated the toxicity of Diuron , Bromecd and thiobendzol to fish and cyarobactean mats in Gaza Strip (Hams ,2015) .

So for, deep investigation of pesticides and their mixture to rabbits and chicken have not been carried out in Gaza strip. accordingly this study was initiated to deeply investigate the toxicity of pesticide and their mixture with different mode of action.

The results of this study will advance our understanding on the human toxicology and will provide data on mixture toxicity profiles of pesticides residues for the first time in Gaza. Moreover, the results of the project may connect the chronic disease in Gaza (heart disease and/or cancer) with pesticide use. In addition, the results will be a useful database for ministry of health/agriculture for future monitoring program. In General, the project will be a critical addition in the field of toxicology.

1.3 Objectives:

1.3.1 General Objectives:

The general objective of the study is to evaluate the long term toxicity of Chlorpyrifos, Diuron and their mixture to chicken and rabbit as farm animal models and to provide toxicological data that can be useful for human health.

1.3.2 The Specific objectives of the research include:

1. To characterize the long term toxicity of Chlorpyrifos , Diuron and their mixture on the weight of rabbit and chickens.
2. To evaluate the toxic effects of Chlorpyrifos , Diuron and their mixture on liver and kidney functions on rabbits and chickens.
3. To characterize the synergistic and /or the antagonistic effects of the tested pesticides.
4. To determine the toxic effects on enzyme activities on liver , kidney and brain tissues .

Chapter (2)

Literature Review

2.1 Introduction:

The effects of pesticides in the non-target organisms was reported elsewhere (Miller, 2009) damage to aquatic eco-systems (Wang and Freemark, 1995). Pesticide residues are present as mixtures in the environment. They may undergo synergistic or antagonistic effects that can alter the balance of human beings and/or an ecosystems (Wendt, et.al., 2004).

Number of studies investigated the toxicity of herbicides including Diuron to cyanobacteria and highlighted their ability to survive and degrade herbicides (Abed et.al., 2002, Safi 2004, El-Nahhal et.al., 2013; Safi et.al., 2014; El-Nahhal et.al 2015; Ma et.al., 2010), Toxicity on fish was also investigated (El-Nahhal et.al., 2014; El-Nahhal and Al-Dahduh 2015). Considerable concentrations of pesticide residues have been reported in Egyptian fruits and vegetables (Dogheim et.al., 2001), Jordan (Al-Nasir et.al., 2001). Palestine (Safi et.al., 2002, Safi 2002) and Kuwait (Sawaya et.al., 1999). Application of pesticides may result in high risks, not only to agricultural workers, but also to the general population (Safi et.al., 1993; El-Sebae and Safi, 1998; Safi, 1998; Yassin et.al., 2002; Safi et.al., 2006, El-Nahhal and Radwan, 2013).

The serious damage to non-target organisms from application of pesticides was extensively discussed (Rambabu and Rao, 1994; Soliman et al., 1997; Amr et al., 1997; El-Sebae and Safi, 1998; Kerkez 2013; El-Najjar 2013). It has been reported that pesticides can cause health risks to many organisms including human beings. It is necessary to understand behavior of pesticides to be able to minimize and/or control the possible health hazards associated with pesticide application.

Contamination of water resources by Chlorpyrifos has been established for instance low concentration of Chlorpyrifos have been detected in drinking water in USA (Rambabu et.al., 1994) Europe (Meffe et.al., 2014).

So far this low concentration of Chlorpyrifos can induct toxicity to different organism in the eco-system for instance toxicity to aquifer organism have been determined recently (EL- Nahhal et.al., 2015).

Furthermore Chlorpyrifos has been shown to create toxicity to animals (Yuan et.al., 2014) birds (Kammon et.al., 2006) and human body **(**Kaur et.al., 2000**)** moreover, Chlorpyrifos and accumulate in the food chain and may from one level to an other. It has been shown that Chlorpyrifos is persisted in the environment**.**

Application of Diuron in Gaza strip is progressively increased (MOA 2015) due to application for weed control. It application has been associated with residues in soil that harm the plant in the next growing season (EL- Nahhal et.al., 2014) and creat phyto toxicity to cyarobactean (EL- Nahhal et.al., 2015). Combination of Diuron and Chlorpyrifos may result in antagonistic or synergistic effects. Mixture toxicity of Chlorpyrifos and Diuron has not been established but other mixture were investigated.

2.2 Pesticides in Palestine:

The Gaza Strip is an elongated area located in a semi-arid region. It is bordered by Egypt from the south, the Negev Desert from the east, and the Mediterranean Sea from the west. The total surface area of the Gaza Strip is 365 km^2 and its population is estimated to be more than 1,750,000 people(PCBS, 2009). The main crops grown include citrus fruits, olives, almonds, grapes, other subtropical fruits, vegetables, and flowers (Safi, 1995). Pesticides are considered the main pollutants in Gaza governorates and with the expanding use of greenhouses, Palestinian agriculture is becoming increasingly dependent on chemical pesticides and fertilizers (Safi, 2002). 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 333 types of pesticides, i.e., herbicides, fungicides, insecticides, soil disinfecting and

others are used in the Palestinian territories. In Gaza governorates, the annual rate of use of agricultural fertilizers reached 12,000 tons of chemical fertilizers (PCBS, 2009), Annual tonnage used in agriculture in the Gaza governorates ranges from 500-700 tons/year, which leads to an annual average of 3.84 kg/donums of pesticides used in the target areas. Sixty eight different types of pesticides are commonly used in the agricultural sector in Gaza strip (Al-Saed et.al., 2011). Of the total pesticides used in the Palestinian territories, insecticides contribute to 49.4%, fungicides 33.7%, herbicides 12.8% and others 4.1% (Batta, 2003). Apparently, misuse of pesticides by the general public increased the level of soil and water contamination across Gaza (Issa, 2000). However in recent years, Gaza strip applied more than 1000 tons of pesticides annually shown in (Table 2.1).

Table 2.1: Quantities (ton) of pesticides used in the past years in Gaza Strip.

Year	Insecticide	Herbicide	Fungicide	Soil sterilants	Other	Total
2005	56.714	20.440	74.336	300.70	0.98	453.170
2006	55.270	24.940	55.650	111.600	0.855	248.315
2007	35.580	18.800	34.270	93.800	3.500	185.950
2008	49.650	18.200	42.200	193.600	60.828	364.478
2009	139.337	39.432	123.694	394.392	10.771	711.802
2010	144.682	18.780	99.630	162.400	61.327	486.819
2011	220.169	27.054	136.477	93.035	7.429	484.164
2012	232.488	25.609	137.911	143.210	5.209	544.427
2013	180.664	24.251	104.705	125.690	8.577	443.887
2014	192.740	41.046	131.074	383.880	10.874	759.614
2015	245.662	48.159	117.886	656.960	13.561	1082.228

Source :(MOA, 2015)

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). Consequently, farmers continue to use pesticides excessively without being aware of the hazards that many causes of their own health, that of the consumers and the environment (Issa, 2000). Moreover, there are no protocols to monitor pesticide residues in agricultural crops that might endanger the health of the whole population in Gaza (Safi et.al., 2002).

It can be seen that a gradient increase in the total consumption of pesticide (Table2.1) up to year 2014 . The a dramatic increase was observed in year 2015 . This is due to the fat that farmer used Metham sodium (soil fumigant) in large quantity as alternative to Methyl Bromide .

2.3 Chlorpyrifos:

(Vidal et.al., 1998). CPO represented 2–14% of total CPS exposure in air samples (Armstrong et.al., 2013).

Chickens are more commonly affected with pesticide toxicity because poultry houses are frequently dusted with pesticides. Chemical pesticide causes health consequences to the birds culminating in great economic loss. It is also posing a potential threat to public health due to the presence of pesticide residues in poultry meat and egg. Ample evidence exists to suggest that the use of pesticides on crops, in storehouses, in poultry houses plus the nonjudicious application for spraying animals or in dipping solutions to prevent ectoparasites leaves behind its residue causing serious health effects (Pal , Kushwah et.al., 1998).

studied the effect of chlorpyrifos on the morphology and the function of the rabbit heart by echocardiography and they detected functional heart disorders induced by chlorpyrifos (Slotkin et.al., 2005).

Organophosphates are anticholinesterase compounds, commonly used in veterinary medicine (Milatovic et.al., 2006). Chlorpyrifos is an organophosphorus insecticide that is widely used to control pests. It inhibits the acetylcholinesterase (AChE) enzyme, in the central and peripheral nervous systems (Pope et.al 1999, Barr et.al., 2006). Reduced CHE activity is a reliable indicator of organophosphorus (OP) poisoning and a biomarker of absorption of OP insecticides(Mohammad et.al 2008). Significant inhibition of CHE activity was reported in CPF intoxicated chicken(Kammon et.al., 2010) CPF act through their active oxon metabolites and inhibits the target CHE. Plasma and other tissue CHE are important for assessing the extent of poisoning induced by organophosphates. Plasma CHE inhibition by 20–30% usually indicates exposure to organophosphate, whereas 50% inhibition or more is associated with serious poisoning and adverse effects(Wilson,1998)

Aspartate aminotransferases (AST) and alanine aminotransferases (ALT) are intracellular aminotransferase enzymes, present in liver cells. After cell death or damage in liver cells, they are released into the circulation. Increased serum transaminases translate a susceptibility to liver damage (Andreoli et.al., 1995). The

increased ALT, AST, and ALP values might be attributed to the liver damage in the toxicant fed birds. The results suggest that administration of CPF caused necrotic changes in the liver, as seen in histopathological study, thus causing leakage of the enzyme into the blood. Significant increase in AST and ALT was reported in goats fed with CPF(Kaur et.al., 2000). AST is found in liver, skeletal muscle, heart, kidney, and brain in the variable amount between species. It is the last enzyme to rise after muscle or liver damage(Forbes et.al., 2001). Increased levels of these enzymes have also been reported in the serum of birds in CPF toxicity (Kammon et.al., 2010).

2.3 Diuron:

Herbicides are the main class of pesticides used extensively in home gardens and farms all over the world (Coelho-Moreira et.al., 2013).

Herbicides benefit food production by reducing weed pressure and improving the quality of crop products (Gianessi and Reigner, 2007). However, concerns over their potential adverse effect on the environment and human health are leading consumers to desire agricultural crops produced with greener technologies (Solomon and Schettler, 2000; Stillerman et.al., 2008).

The presence of pesticides in the environment is a matter of particular concern for the conservation of ecosystems and for human health. The natural process of transformation of such substances in the environment, as well as their elimination is called bioremediation. Therefore, the understanding of the biochemical reactions involved in their metabolism is the basis for identifying the time of persistence of such compounds in nature. Diuron (IUPAC name: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CAS number: 330-54-1) is a systemic herbicide, largely used in agriculture, belonging to the phenylamide family and the subclass of phenylurea. This substituted urea herbicide inhibits photosynthesis by preventing oxygen production (Wessels et.al., 1956).

Due to continuous use of pesticides, appreciable quantities of them and their degradation products may accumulate in the ecosystem. Prevailing data showed that only 2–3% of the applied chemical pesticides reach their targets, while the rest remains in the soil (US-EPA, 2005).

The compound acts in photosynthetic organisms by blocking electron transport in photosystem II, thus inhibiting photosynthesis. In the environment diuron can be transformed abiotically via hydrolysis and photodegradation reactions, but under natural conditions these reactions occur at very low rates (Giacomazzi et al., 2004).

Diuron is a phenylurea herbicide and it is one of the most often employed agrochemicals for controlling weeds in sugarcane, citrus and coffee crops. It has high

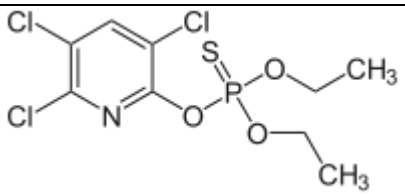
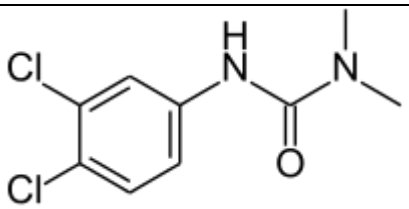
mobility in soils, low susceptibility to natural attenuation and strong toxicity. Besides, more toxic metabolites with genotoxic and teratogenic actions such as 3,4-dichloroanilines, N-3,4-dichlorophenylurea and N-(3,4-dichlorophenyl)-N-methylurea can be generated from diuron by biotic and abiotic reactions (Tixier et.al., 2000; Dellamatrice and Monteiro, 2004; Giacomazzi and Cochet, 2004). Diuron is highly persistent in the aquatic environment due to its photochemical stability. As a consequence, it has been detected in wastewater effluents and in surface waters at low concentrations up to the μg dm^{-3} level. As a potentially carcinogenic substance, its occurrence in such reservoirs poses serious threat to human health and it is also toxic to microorganisms. The European Water Framework Directive classifies Diuron as one of the priority substances, being a hazardous pollutant (European Commission Decision no 2001) These metabolites accumulate in the environment and some of them are more toxic than diuron (Tixier et.al., 2001).

Table 2.2 : Chemical properties of Diuron and Chlorpyrifos

2.2.1 Name and registry numbers.

Common name:	Chlorpyrifos	Diuron
IUPAC name:	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
CAS number:	2921-88-2	330-54-1
Trade names include	Lorsban, Dursban, Suscon Green, Empire, Equity	Karmex, Diuron, Direx
Molecular formula	$C_9H_{11}C_{l3}NO_3PS$	$C_9H_{10}C_{l2}N_2O$
Molecular weight	350.6	233.09

2.2.2 Structure

	Chlorpyrifos	Diuron
Chemical structure:		

Source: (Tomilin, 2000).

Chapter (3)

Materials and Methods

3.1 Specimen collection :

The tested animals (rabbits and chicken) were purchased from a local certified farm and brought to the laboratory for 2 weeks acclimatization according to US-EPA 2004. The animal health care was maintained in accordance with the animal welfare guidelines established at Ministry of Agriculture in Gaza and US-EPA 2004. Recent report (Aly and El-Gendy, 2015) recommended the use of rabbits as an experimental model to provide experimental results valid for human beings. Moreover chicken are also sensitive organism to low concentration of pesticides US-EPA 1995. Accordingly, about 16 Male white rabbits, four-six months old, 2-3 kg weight and another 16 female chicken , four-six months old, 1.5-2 kg weight , were purchased from a local certified production farm in Gaza. The animals were individually housed in stainless steel cages at 22–26^{o}C temperature, 40–70% humidity and controlled environment with a 12 h light/ dark cycle. Food and water were given ad libitum.

3.2. Chemicals

Technical amounts purity 99% of Chlorpyrifos and Diuron were purchased from Sigma Aldrich Co., Germany.

Figure 3.1 : Stainless steel animal house according to EPA stander.

3.3 preparation of pesticide solution :

appropriate amount of Chlorpyrifos 20mg and Diuron 250mg were dissolved in 125ml corn oil for 2 day under magnetic steering to ensure complete solubility of pesticide . This was visualized by clean solution of oil .

3.4 treatments of animals :

One ml pure corn oil was given orally administered to the control group of rabbits or chicken group 1 received 1ml corn oil containing the required concentration of pesticide this step was repeated for two weeks .Then treated animals were left six weeks monitoring period .

3.5 Experimental design

The rabbits/chicken were divided into four groups (4 animals per each) G1, G2, G3 and G4. G1 group represents the control sample (did not receive any toxicant), G2, G3, and G4 represent the treatment in the experiment. Groups G2 and G3 received 1/40 of LD_{50}/kg/day for 2 weeks of Chlorpyrifos and Diuron. Group G4 receive mixture of the Diuron and Chlorpyrifos: 1/40 of LD_{50} of each (toxic unit) for the same period mentioned above.

The chicken groups received the same treatments as mentioned above. Chicken and rabbits were monitored during the 6 weeks of study period (2 weeks of treatment+ another 4 weeks without treatment). The body weights of control and treated animals were recorded weekly. At the end of the experiment the animals (Rabbits and Chicken) were sacrificed and dissected, then the brain, liver, kidney, and heart were removed, rinsed in saline solution (0.9% NaCl), dried on filter paper and weighted individually in all rabbits and chicken and the relative organ weight was calculated (organ weight: body weight).

Figure 3.2 : photo for show the oral injection of pesticides.

3.6 Collection of Blood, Heart , liver , brain and kidney Samples

The chicken and rabbits were subjected to an overnight fast after which they were anaesthetized and blood collected by cardiac puncture into sterile containers without anticoagulant.

Heart , liver , brain and kidney tissues were collected washed with saline solution0.9% . The weighted parts below 0.5g of each organ was mined in 10 ml saline solution then homogenized with the blander (Tekmar tissumizer) and confused of 3000g for 15 minute at 4c . The supernatants were separated in new open door tube and kept under cold conditions until determination of Acetyl colon esterase activity .

3.7 Biochemical analysis:

The blood of the tested organisms were collected in hebetinized tube, kept in ice and centrifuged to collect the serum. For determination of AChE and ALT, AST, ALP, Total protein, crecetine, uric acid, and urea enzyme activities. Moreover, Each organ was minced and homogenized separately in ice cold saline solution (10% w/v) in a polytron homogenizer (Tekmartissumizer). The homogenate was centrifuged at 10,000·g for 30 min at 4^{o}C using a cooling centrifuge. The resultant supernatant was used for different enzymes assay.

Kidney test focused on determining the concentrations of uric acid, urea and crecetine in the blood serum of the tested animal.

3.8 Determination of Liver enzymes

3.8.1 Determination of alanine aminotransferase

Serum alanine aminotransferase (ALT) activity was measured by using optimized UV-test according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), according to Guder method (Guder et al., 2001) using DiaSys reagent kits.

Principle

L-Glutamate + Pyruvate $\xleftrightarrow{ALT}$ L-Alanine + 2-Oxoglutarate

Pyruvate + NADH + H^{+} $\xleftrightarrow{LDH}$ D-Lactate + NAD^{+}

Reagents

Components	Concentration
Reagent 1	
TRIS pH 7.15	140 mmol/l
L-Alanine	700 mmol/l
LDH (Lactate dehydrogenase)	≥ 2300 U/l
Reagent 2	
2-Oxoglutarate	85 mmol/l
NADH	1 mmol/l

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(E.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

Sample	
Monoreagent	1000 µl
Sample	100 µl

Mix, read absorbance after 1 minute and start stop watch. Read absorbance again 1, 2 and 3 min thereafter at 340 nm.

Calculation

From absorbance reading calculates ΔA /min and multiply by the corresponding factor:

ΔA /min X factor (1745) = ALT activity [U/l]

3.8.2 Determination of aspartate aminotransferase

Serum aspartate aminotransferase (AST) activity was measured by using optimized UV-test according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), according to Thomas (Thomas, 1998) using DiaSys reagent kits.

Principle

L-Aspartate + 2-Oxoglutarate $\xleftrightarrow{AST}$ L-Glutamate + Oxaloacetate

Oxaloacetate + NADH + H^+ $\xleftrightarrow{MDH}$ L-Malate + NAD^+

Reagents

Components	**Concentration**
Reagent 1	
TRIS pH 7.65	80 mmol/l
L-Aspartate	240 mmol/l
MDH (Malate dehydrogenase)	≥ 600 U/l
LDH (Lactate dehydrogenase)	≥ 900 U/l
Reagent 2	
2-Oxoglutarate	12 mmol/l
NADH	0.18 mmol/l

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(E.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

Sample	
Monoreagent	1000 µl
Sample	100 µl

Mix, read absorbance was read after 1 min and start stopwatch. Absorbance was read again 1, 2 and 3 min thereafter at 340 nm.

Calculation

From absorbance reading calculates ΔA /min was calculated and multiply by the corresponding factor:

ΔA /min X factor (1745) = AST activity [U/l]

3.8.3 Determination of alkaline phosphatase

Serum alkaline phosphatase (ALP) activity was measured by kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), according to the method described by Soldin and his colleagues (Soldin et al., 2007) using DiaSys reagent kits.

Principle

p-Nitrophenylphosphate + $H_2O \xleftrightarrow{ALP}$ phosphate + p-nitroph

Reagents

Components	Concentration
Reagent 1 2-Amino-2-methyl-1-propanol pH10.4 Magnisium acetate Zinc sulphate HEDTA	 1.1 mmol/l 2 mmol /l 0.5 mmol/l 2.5mmol/l
Reagent 2 p-Nitrophenylphosphate	 80 mmol/l

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(E.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

	Blank	**Sample**
Monoreagent	1000 µl	1000 µl
Sample	-	20 µl
Dist. water	20 µl	-

Mix, read absorbance after 1 min and start stopwatch. Read absorbance again 1, 2 and 3 min at 405 nm.

Calculation

From absorbance reading calculates ΔA /min and multiplies by the corresponding factor:

ΔA /min X factor (2757) = ALP activity [U/l]

3.8.4 Determination of cholinesterase activity

Serum cholinesterase (ChE) activity was measured by kinetic photometric test, according to the recommendation of German Society of Clinical Chemistry (DGKC), the method described by Ellman and his colleagues (Ellman et al., 1961) using DiaSys reagent kits.

Principle

Cholinesterase hydrolyses butyrylthiocholine under release of butyric acid and thiocholine. Thiocholine reduces yellow potassium hexacyanoferrate (III) to colorless potassium hexacyanoferrate (II). The decrease of absorbance is measured at 405 nm.

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{cholinesterase}} \text{Thiocholine} + \text{Butytate}$$

$$2\text{Thiocholine} + 2(Fe(CN)_6)^{3-} + H_2O \longrightarrow \text{Choline} + 2(Fe(CN)_6)^{4-} + H_2O$$

Reagents

Components	**Concentration**
Reagent 1	
Pyrophosphate pH 7.6	75 mmol/l
Potassium hexacyanoferrate(III)	2 mmol/l
Reagent 2	
Butyrylthiocholine	15 mmol/l

Procedure

	Reagent /blank	**sample**
Sample	-	20 µl
Dist. Water	20 µl	-
Reagent 1	1000 µl	1000 µl
Mix, incubate approx.3 min, and then add:		
	Reagent /blank	**Sample**
Reagent 2	250 µl	250 µl

Mix, read absorbance after 2 min and start stop watch. Read absorbance again after 1, 2 and 3 minutes at 405 nm.

ΔA/min = [ΔA/min Sample] – [ΔA/min Blank]

Calculation

Calculate ΔA/min and multiply with 68500 =cholinesterase activity U/l.

3.9 Non- protein nitrogen constituents

3.9.1 Determination of urea

Serum urea was determined by using "Urease-GLDH": enzymatic UV test, according to Thomas method (Gutmann and Bergmeyer, 1974) using DiaSys reagent kits.

Principle

Urea + $2H_2O$ $\xrightarrow{\text{Urease}}$ $2NH^{4+} + 2HCO^{3-}$

2-Oxaloglutarate + NH_{4+} + NADH $\xrightarrow{\text{GlDH}}$ L-Glutamate +NAD_{+} +H_2O

GLDH: Glutamate dehydrogenase.

Reagents

Component	**Concentration**
Reagent 1: TRIS pH 7.8 2-Oxaloglutarate ADP Urease GLDH	 150 mmol/l 9 mmol/l 0.75 mmol/l ≥ 7 KU/l ≥1 KU/l
Reagent 2: NADH	 1.3 mmol/l
Standard	50 mg /dl (8.33 mmol/l)

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(E.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

	Blank	**Sample or standard**
Sample or standard	-	10 µl
Monoreagent	1000 µl	1000 µl

Mix and incubate for 60 sec. at 25 C**,** then read absorbance A1. After exactly further 60 sec. read absorbance A2 at 340 nm.

A= (A1-A2) sample or standered

Calculation

$$\text{Urea [mg/dl]} = \frac{\Delta \text{ A sample X conc. Std}}{\Delta \text{ A std /cal}} \text{ /Cal [mg/dl]}$$

3.9.2 Determination of Uric Acid

Serum Uric Acid was determined by using "Urease-GLDH": enzymatic UV test, according to Barham method (Barham and Trinder 1972) using DiaSys reagent kits.

Principle

The enzymatic reaction sequence employed in the assay of uric acid is as follows:

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{DHBS} \xrightarrow{\text{Peroxidase}} \text{chromogen} + 4\ H_2O$$

Reagents

Component	**Concentration**
Reagent 1 Pipes Buffer (pH 7.0) DHBS Uricase Peroxidase 4-Aminoantipyrin	 50 mmol/l 0.50 mmol/l ≥ 0.32 kU/l ≥1.0 kU/l 0.31 mmol/l
Standard	(5 mg/dl)

Procedure

	Reagent blank	**Standard (Calibr.)**	**Sample**
Reagent 1	**1.00** µl	**1.00** µl	**1.00** µl
Sample	**-**	**-**	**0.025** µl
Standard (Calibr.)	**-**	**0.025** µl	**-**
Distilled water	**0.025** µl	**-**	**-**

Calculation

Uric Acid (mg/dl)= $\Delta A_{sam} \times C_{st} \over \Delta A_{st}$ Cst = standard (calibrator) concentration

3.9.3 Determination of creatinine

Serum creatinine was determined by using kinetic test without deproteinization according to Newman and Price method (Newman and Price, 1999) using DiaSys reagent kits.

Principle

Creatinine forms a colored orange-red complex in an alkaline picrate solution. The different in absorbance at fixed time during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + picric acid ⟶ Creatinine picrate complex

Reagents

Component	**Concentration**
Reagent 1 **Sodium hydroxide**	0.16 mmol/l
Reagent 2 Picric acid	4.0 mmol/l
Standard	2 mg/dl (177 mmol /l)

Monoreagent preparation

Four parts of R1 were mixed with 1 part of R2

(E.g.20 ml R1+ 5 ml R2)= Monoreagent

Procedure

	Blank	Std./Cal.	Sample
Monoreagent	1000 µl	1000 µl	1000 µl
Sample	-	-	50 µl
Std./Cal.	-	50 µl	-
Dist. water	50 µl	-	-

Mix and read absorbance A1 after 60 sec against reagent blank at 492 nm, read absorbance A2 after further 120 sec.

Calculation

$$\text{Creatinine concentration [mg/dl]} = \frac{(\Delta \text{ A sample}) \text{ X Conc. Std [mg/dl]}}{(\Delta \text{ A standard})}$$

$\Delta A = [(A2 – A1) \text{ sample or standard}] – [(A2 –A1) \text{ Blank}]$

3.10 Protein profile

3.10.1 Determination of total protein

Serum total protein was determined by photometric test according to Thomas method (Thomas, 1998) using DiaSys reagent kits.

Principle

Protein together with copper ions forms a violet blue color complex in alkaline solution. The absorbance of color is directly proportional to concentration.

Reagents

Components	Concentrations
Reagent 1: Sodium hydroxide Potassium sodium tartrate	 80 mmol/l 12.8 mmol/l
Reagent 2: Sodium hydroxide Potassium sodium tartrate Potassium iodide Copper sulfate	 100 mmol/l 16 mmol/l 15 mmol/l 6 mmol/l
Standard	5 g/dl

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(e.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

	Blank	Sample
Monoreagent	1000 µl	1000 µl
Sample	-	20 µl
Dist. water	20 µ l	-

Mix, incubate for 5 min at 25°C and read absorbance against the reagent blank within 60 min at 540 nm.

Calculation

The protein concentration in the sample is calculated using the following general formula:

$$\text{Total protein [g/dl]} = \frac{(\Delta \text{ A sample}) \text{ X Conc. Std [g/dl]}}{(\Delta \text{ A standard})}$$

3.11 Statistical Analysis:

Average and standard deviation were Calculated. Analysis of Variances (ANOVA) was employed to detect significant differences among treatments at p-value 0.05. p-value below 0.05 indicate significant differences among treatments whereas values above 0.05 were not significant .

Chapter (4)

Results and Discussion

4.1 Results:

4.1.1 Rabbits:

Dynamic effect of Diuron, Chlorpyrifos and their mixture on rabbet are shown in Figure **4.2** and Table **4.1**. It can be seen that there is gradual increase in body weight at rabbits all treatments up to 2nd week in the treatment. then steady state growth were seen in all treatment. The growth in the control samples continues to increase with a slight slowdown. The body weight increased to the maximum point after 6 weeks and reached to 2.94±0.43Kg . The lowest weight 2.06±0.37 Kg were seen in the mixture treatment after 6 weeks of treatments.

4.1.1.1 Effects on body weight:

Average weight of rabbits are shown in Figure **4.1**

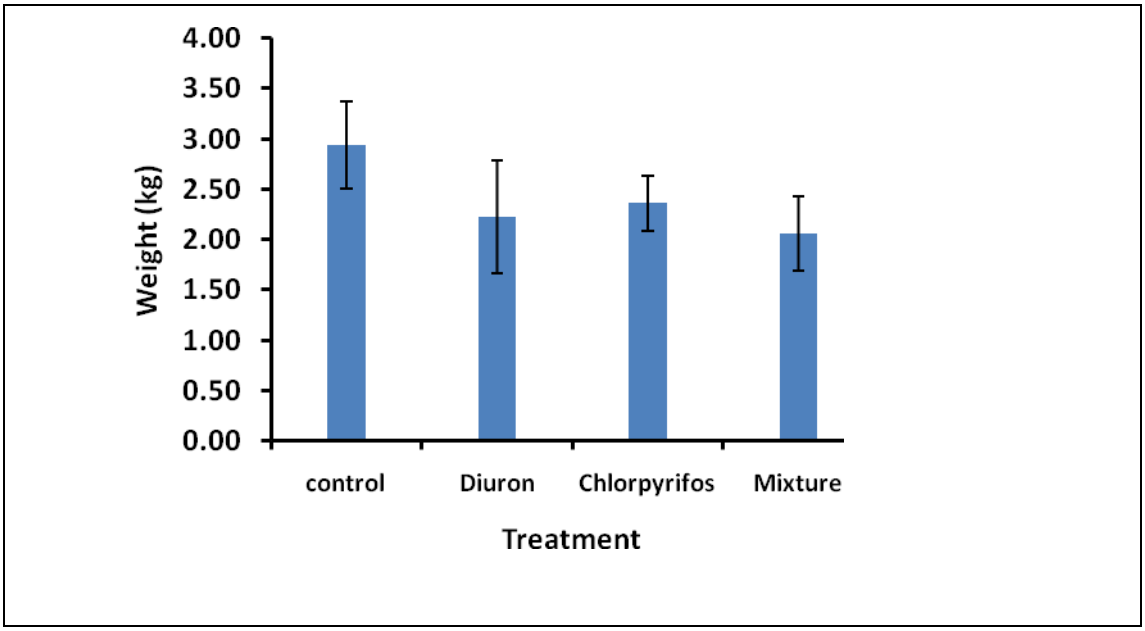


Figure 4.1. Average weight of rabbits at the end point of the experiments (7th week). Error bars indicate standard deviations.

It can be seen that a considerable decrease in rabbit body weight due to exposure to Diuron, Chlorpyrifos and their mixture. The decrease is more pronounced in the mixture treatments.

Table 4.1. Effects of toxic substances and their mixture on rabbits weight overtime . (average ± standard deviation)

Time	control	Diuron	Chlorpyrifos	Mixture	p-values
1st *week	1.67±0.07	1.75±0.02	1.85±0.18	1.73±0.09	0.056-0.18
2nd** week	2.23±0.31	2.24±0.35	2.32±0.24	2.30±0.21	0.33-0.47
3rd **week	2.38±0.35	2.37±0.35	2.44±0.30	2.24±0.36	0.31-0.47
4th*** week	2.80±0.31	2.41±0.25	2.28±0.55	2.42±0.05	0.053-0.34
5th*** week	2.79±0.36	2.37±0.34	2.23±0.69	*2.32±0.11	0.049-0.14
6th*** week	2.80±0.40	2.28±0.38	2.39±0.89	*2.21±0.19	0.039-0.25
7th*** week	2.94±0.43	2.23±0.56	2.36±1.27	*2.06±0.37	0.029-0.26

(* week acclimatization ,** weeks of treatment ,*** weeks without treatment)

*p-values less than 0.05

p-value among treatments in the same week of expect indicates no differences between the control and each treatments except those who have star * in Table 4.1

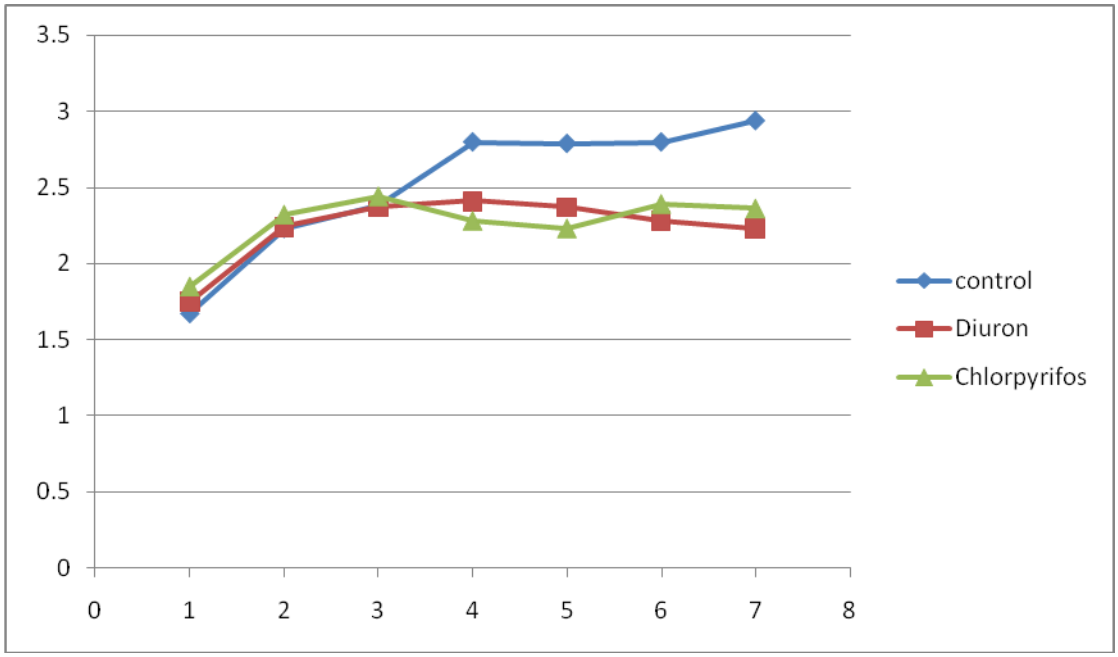


Figure 4.2. Long term effects of Diuron, Chlorpyrifos and their mixture at a concentration of 0.1mg/kg body/day on rabbit body weight.

The data in Figure 4.2 shows the long term effects of a repeated does of toxic substance on the growth of rabbits over time.

Also the effects of the tested compounds on % reduction of rabbits weight are shown in Figure 4.3.

It can be seen that mixture treatment has the strongest effect in weight reduction followed by Diuron whereas Chlorpyrifos has the lowest effect in body reduction.

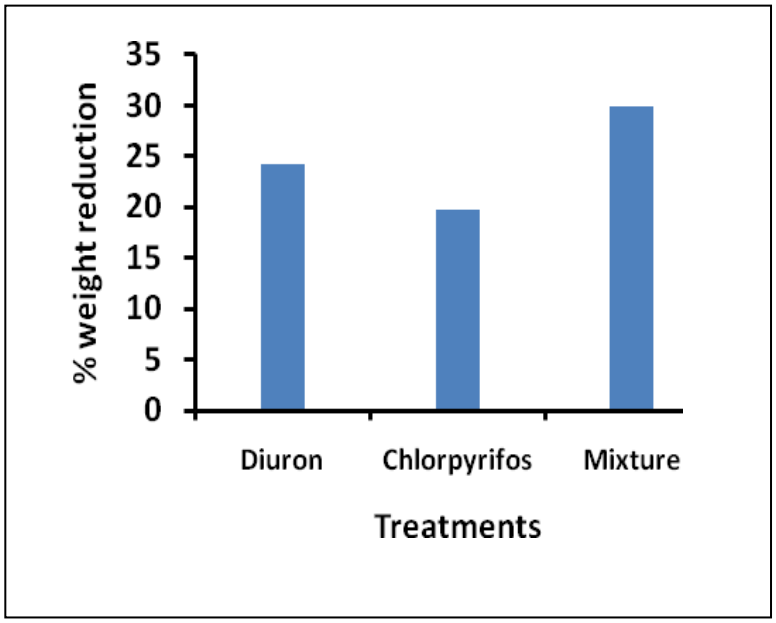


Figure 4.3 Weight reduction % on rabbits treated with 0.1mg/kg body/day of toxic substances.

4.1.1.2 Effect on body organ:

Effects of the tested compounds on the liver, heart and kidney weight are shown in Figure 4.4. The corresponding data are presented in Table 4.2.

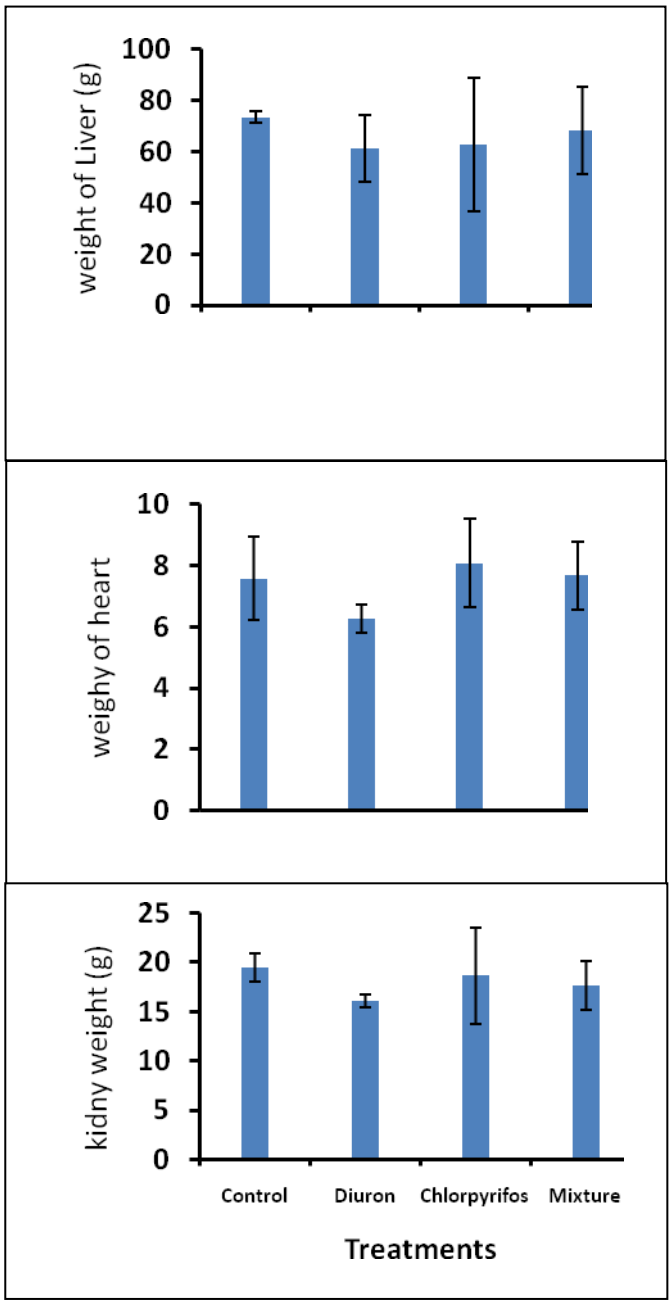


Figure 4.4. Effects of Diuron, Chlorpyrifos and their mixture at a concentration 0.1mg/kg body/day on the weight of liver, heart and kidney on rabbits.

Table 4.2 Weight of body organs of rabbits treated with different toxic substances and their mixtures.

Treatment	Average weight ± standard deviation		
	Liver	Kidney	Heart
Control	73.45±2.37	19.42±1.42	7.57±1.36
Diuron	*61.21±13.04	*16.02±0.64	*6.28±0.45
Chlorpyrifos	62.67±26.22	18.62±4.85	8.07±1.45
Mixture	68.41±17.05	17.67±2.48	7.67±1.10
p-values	0.08-0.32	0.01-0.18	0.05-0.387

* indicate significant differences at p-values 0.05

It can be seen that the liver weight is the highest in the control sample . Decreases in the liver weight were shown in Diuron and Chlorpyrifos treatments. The mixture treatment has nearly elevated weight of liver. The trend in kidney and heart weights are similar to the liver.

So far, statistical analysis did not detect significant differences on liver weight in all treatment P-value ranged between 0.08-0.42; significant difference was found only with Diuron and control on kidney, p –value =0.01; p-values for heart treatments ranged between 0.055-0.39.

It can be seen that the average weight of liver, hearts and kidney are the height in the control sample; so far the lowest weight were observed in rabbits treated with Diuron, followed by mixture treatment. Rabbits received Chlorpyrifos have a heart weight similar to that of control sample.

4.1.1.3 Hepatosomatic factors:

Effects of the tested compound on hepatosomatic ration are shown in Figure 4.5.

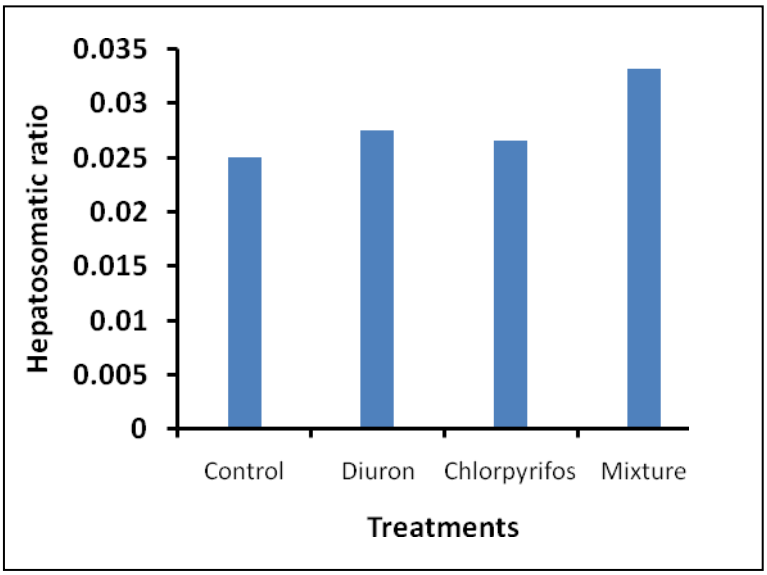


Figure 4.5 Hepatosomatic factors on rabbits treated with Diuron, Chlorpyrifos and their mixture at a concentration 0.1mg/kg body/day.

It can be seen the all tested compound increased the hepatosomatic factor indicating of toxic the effect.

So far mixture application has the highest hepatosomatic factor whereas the hepatosomatic factor of the control, diuron and chlorpyrifos are close to each other indication similar effects.

Figure 4.6 : Photo shows lever shape and color of the tested compounds

4.1.1.3 Biochemical effects on Rabbits:

4.1.1.3.A Effects on liver enzymes:

Effect of the tested compound on the activity of ALT,AST and ALP extracted from liver, hearts , Brain and kidney are shown in Figures 4.7-4.10 and Table 4.3.

It can be seen that ALT activity is the lowest among treatments , whereas ALP is the highest in the control sample. AST has a higher level only in the mixture treatments .

In the hearts tissue (Figure 4.8) the treatments are different . the enzymes activities are higher in the treatments than in the control samples. the activity of enzymes in brain tissues (Figure 4.9) is similar to those in liver tissues (Figure 4.7).

The activity of enzymes in kidney tissue (Figure 4.10) are higher than all.

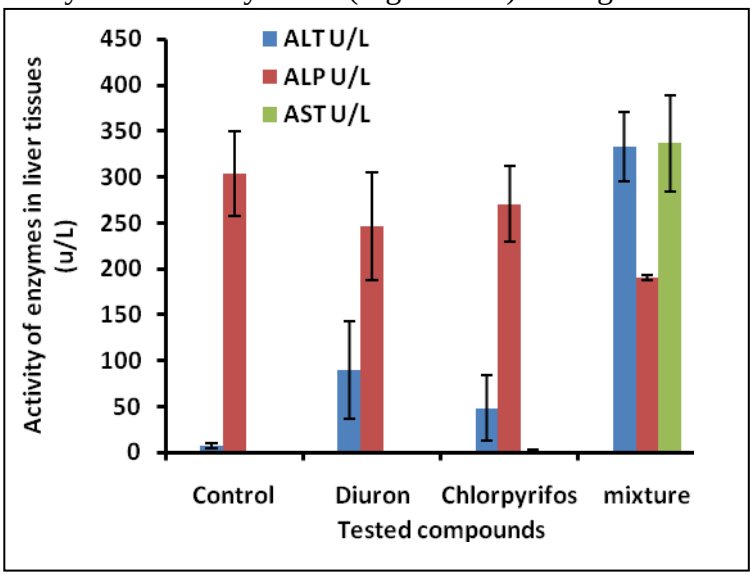


Figure 4.7 Activity of ALT. ALP and AST enzymes in liver tissue of rabbets. Error bars represent standard deviation.

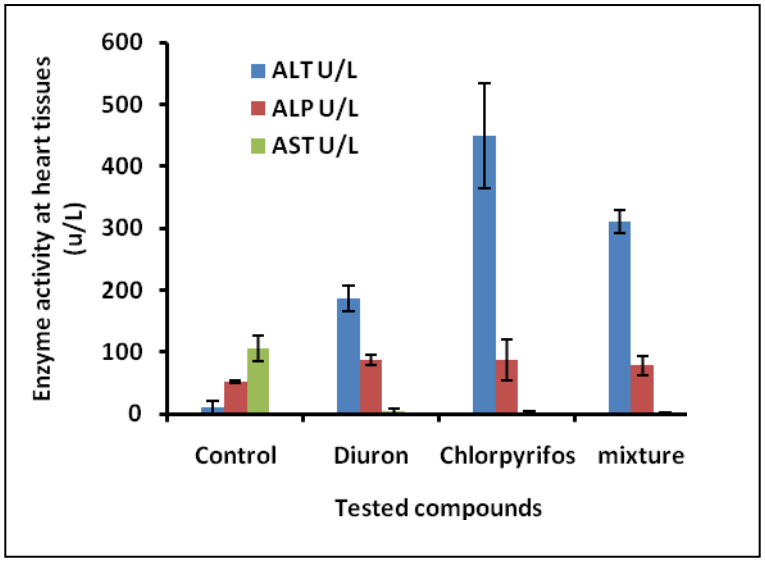


Figure 4.8 Activity of ALT. ALP and AST enzymes in heart tissue. Error bars represent standard deviation.

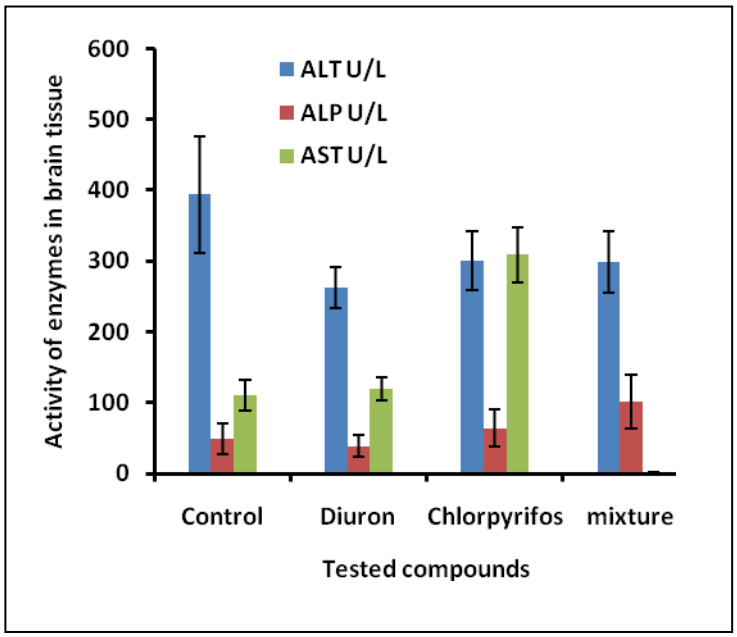


Figure 4.9 Activity of ALT. ALP and AST enzymes in brain tissue. Error bars represent standard deviation.

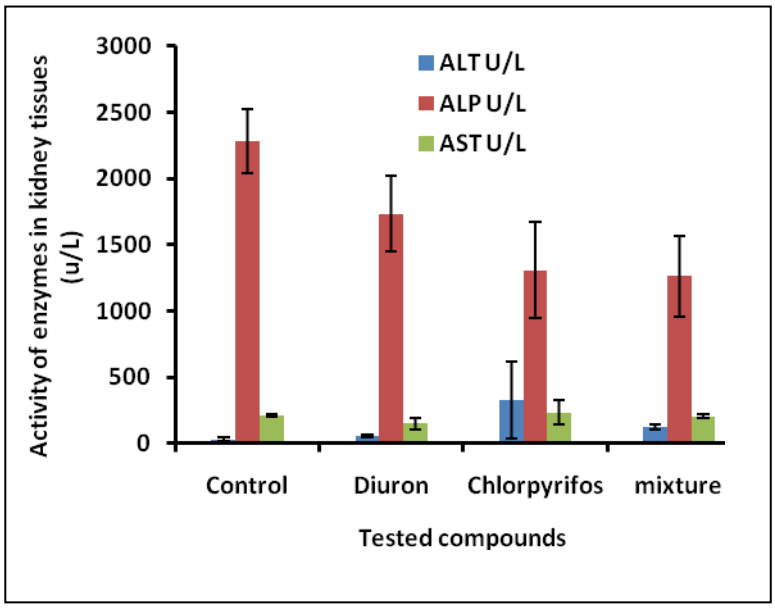


Figure 4.10 Activity of ALT. ALP and AST enzymes in kidney tissue. Error bars represent standard deviation.

It can be seen that , the activity of ALT and AST reached the highest values 333.5±38 and 337±52.33 u/ L in liver tissue (Table 4.3).

Moreover the value of ALT was higher in Diuron than in chloropyrofos or in control samples, whereas ALP value was highest in mixture samples .

For the brain tissue, ALT was the highest in the control samples . ALP activity in the treatments of chloropyrofos and mixture was higher than the activity in the Diuron.

AST activity in liver tissues was very low and close to 0.0 and become vary high and reach 337±52.33 in treatment of mixture . In the heart tissues AST activities were the highest in the control sample and the level reduced in the treatments the activity was the lowest in the mixture treatment . In brain tissues AST was very high in the control sample and reduced to 110.5 ± 22.33 the level increased in Diuron and chloropyrofos treated and reduced 119.5 ± 17 and 309 ± 38 respectively . In the kidney tissues the activates were in the range of 209.00±12.73– 234.25±89.85 in the control and the treatments except Diuron treatment the activity was 148.5±43.32 .

Table 4.3. Activity of ALT. ALP and AST enzymes in liver, brain, heart, and kidney tissue. Average ± standard deviation.

Treatment	**Activity of enzymes u/l**		
Liver			
Treatment	ALT	ALP	AST
Control	7.67±2.89	303.00±46.12	0.00±0.00
Diuron	*89.50±52.91	246.75±58.50	0.00±0.00
Chlorpyrifos	*67.5±17.68	270.75±41.04	1.00±2.00
mixture	*333.50±27.83	*190.67±2.89	*337.00±52.33
p-values	0.00-0.004	0.006-0.18	0.00-0.5
Brain			
Control	393.75±82.66	49.50±21.56	110.50±4.57
Diuron	*262.5±29.65	38.75±15.3	119.5±10.21
Chlorpyrifos	300.75±42	64.25±25.86	*261.00±50
mixture	298.00±43.5	*101.33±38.28	1.00±1.41
p-values	0.01-0.06	0.03-0.22	0.00-0.089
Heart			
Control	11±10.58	52±2.16	105.5±10.8
Diuron	*186.3±20.3	*87±8.485281	3.75±3.86
Chlorpyrifos	*449.67±85	*87±33.50	*1.75±3.50
mixture	*310.3±19	*78.3±15.53	*1±1
p-values	0.00-0.004	0.00-0.04	0.00-0.089
kidney			
Control	28.00±14.93	2281.00±240.17	209.00±9.73
Diuron	*55±11.2	*1734.25±287.62	*148.5±43.32
Chlorpyrifos	*327.25±292.22	*1308.50±359.31	234.25±89.85
mixture	*120.00±18.36	*1260.67±303.79	212.5±10.61
p-values	0.001-0.01	0.001-0.002	0.00-0.089

* indicate significant differences at p-values 0.05 .

It can be seen that ALT activity is the highest in brain tissues of the control samples, and the lowest in liver tissue treatments with Diuron, Chlorpyrifos. Mixture treatment increased ALT levels in liver, brain and heart and tissue and reduced it in the kidney tissue. ALP activities were reduced in liver and kidney tissues treated with Diuron, Chlorpyrifos and / or Mixture .

Whereas the activities are increased in the brain and heart tissues treated with tested compounds except Diuron on brain tissues .

Statistical analysis of the data in Table 4.3 indicated significant different between ALT activities and that of treatments (Diuron, Chlorpyrifos and Mixture).

P-value of ALT in liver were below 0.02 , similarly low values were were obtained in Brain , Heart and kidney tissues.

These data suggest that ALT is targeted by the tested compounds.

Statistical difference of ALP less pranced than those of ALP S

4.1.1.4B. Effects on the kidney functions:

Effects of the Diuron and Chlorpyrifos and the mixture on the kidney functions (urea, uric acid, creatinine and total protein) are shown in Figure 4.11 and Table 4.4.

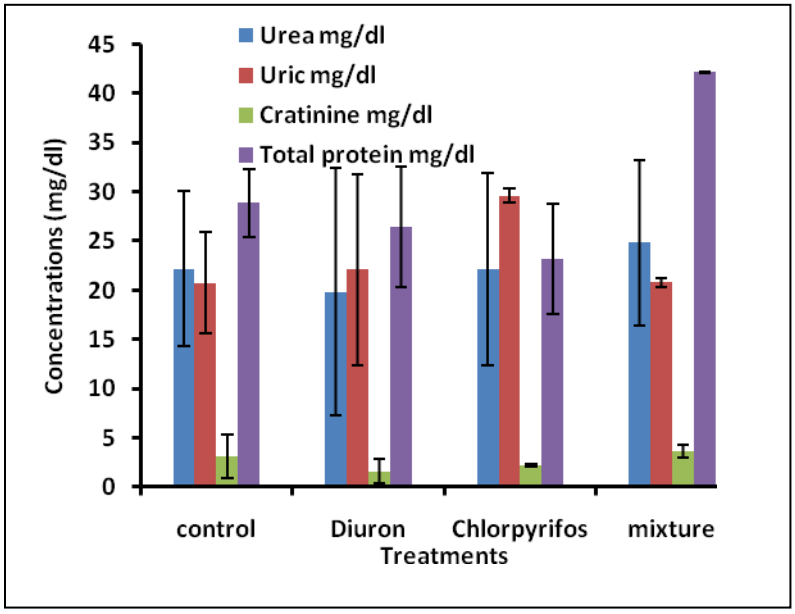


Figure 4.11 Concentrations of urea, uric acid, creatinine and total protein in rabbet treated with Diuron, chlorpyrifos, and Their mixture at 0.1 mg/kg body /day. Error bars represent standard deviation.

Table 4.4. Urea, uric acid, cratinine and total protein in rabbet. (Average ± standard deviation).

Treatment	Urea mg/dl	Uric acid mg/dl	Cratinine mg/dl	Total protein mg/dl
Control	22.17±7.86	20.76±5.20	3.17±2.21	28.87±3.47
Diuron	19.83±12.61	22.13±9.71	1.60±0.21	26.45±6.15
Chlorpyrifos	22.15±9.80	*29.61±0.70	2.20±0.14	23.15±5.59
Mixture	24.85±8.38	20.79±0.49	3.65±0.64	42.15±0.07
p-values	0.187-0.424	0.359-0.4	0.17-0.44	0.1-0.17

* indicate significant differences at p-values 0.05 .

It can be seen that urea concentration in blood serum is elevated in rabbits treated with mixture of Diuron and Chlorpyrifos and reached 24.85±8.38, whereas the concentration in rabbits treated with Diuron and Chlorpyrifos are similar to the control. The level of uric acid in the control rabbits is nearly similar to that in Chlorpyrifos, indicating no effect. The concentration of cratinine in rabbits treated with mixture is the highest among all treatments, whereas the concentration in rabbits treated with Diuron or Chlorpyrifos is lower than those of the control samples indicating of nepheotoxicity.

Similar trend was observed for the concentration of total protein.

These results suggest that mixing Diuron with Chlorpyrifos has strong effect an protein synthesis.

p-value of urea were in the range of 0.187-0.424 indicating no significant different.

p-value of uric acid were in the range of 0.359-0.4 except that of Chlorpyrifos was 0.047 indicating significant different .

4.1.1.5 Effects on the nervous system:

Effects of the tested compound on the activity of Acetyl cholinesterase collected from brain tissue and blood serum are shown in Figure 4.12-4.13 and Table 4.5.

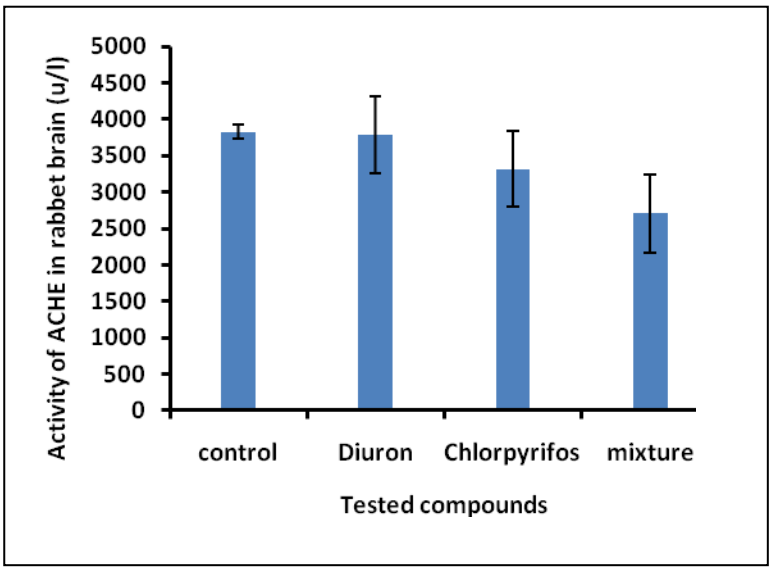


Figure 4.12 Activity of Acetylcholine esterase collected from rabbet brain treated with Diuron, chlorpyrifos, and Their mixture at 0.1 mg/kg body /day. Error bars represent standard deviation.

It can be seen that the activities of ACHE are higher in the control samples of brain and blood serum samples, than in the treatments .

The activities in rabbit treated with Diuron are slightly reduced whereas further reduction of ACHE activities are observed in rabbits treated with Chlorpyrifos. the interesting outcome is that the activities of the ACHE were sevenly reduced in rabbits treated with Diuron and Chlorpyrifos mixture.

Statistical analysis shown no significant different between control and rabbits treated with Diuron whereas the difference are not significant for the case of control and Chlorpyrifos in the brain enzymes p-value P- value 0.087.

Significant differences were detected in the mixture and control samples for both brain and serum.

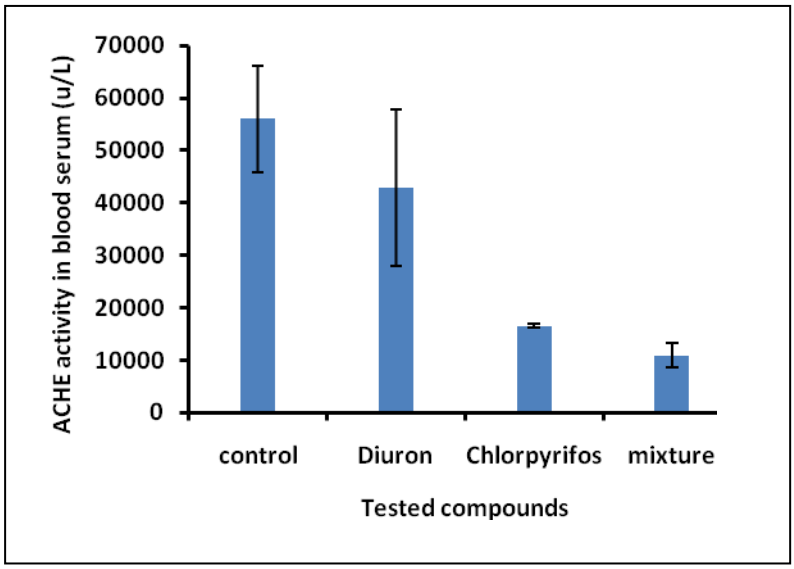


Figure 4.13 Activity of acetylcholine esterase in blood serum of rabbet treated with Diuron, chlorpyrifos, and Their mixture at 0.1 mg/kg body /day. Error bars represent standard deviation.

Table 4.5 Acetylcholinesterase activity in brain and serum and their corresponding p-values in rabbits treated with different toxic substances and their mixtures.

Treatment	ACHE activity u/ l		p-values	
	Brain	Serum	Brain	Serum
Control	3825±98	55997±10120	Control vs	Control vs
Diuron	3789±533	42779±14925	0.5	0.0917
Chlorpyrifos	3315±520	16496±344	0.087	0.0075
Mixture	2704±544	9765±2273	0.045	0.01

4.1.2 Chicken:

4.1.2.1 Effects on body weight:

Average weights of chicken at end points of treatments are shown in Figure 4.14. whenever the dynamic effect are shown in Figure 4.15.

Detailed data are presented in Table 4.6.

It can be seen that the weight of chicken in the control and mixture sample are nearly similar regardless to the high value of standard deviation in the mixture samples.

However, the Chicken weight of Diuron or Chlorpyrifos treatments are nearly higher than control and mixture.

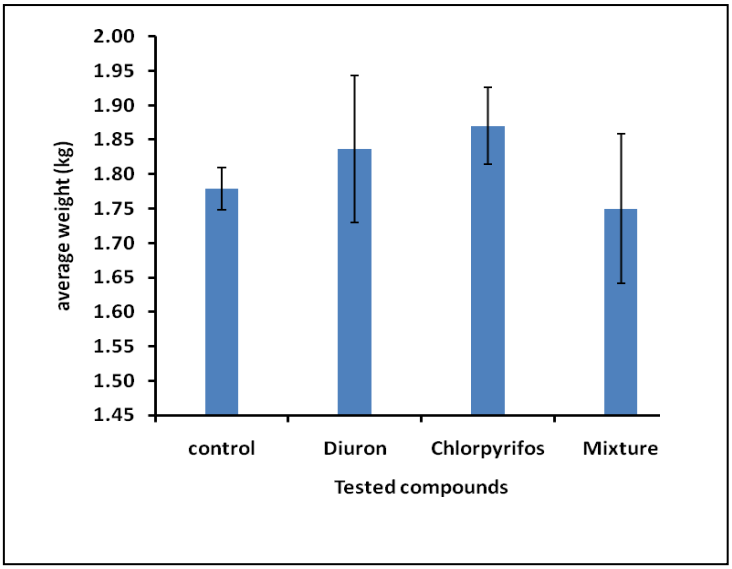


Figure 4.14 Average weight of chicken at the end of the experiments (7^{th} week). Error bars indicate standard deviations.

Hepatosomatic factors are shown in Figure 4.17.

It can be seen that Diuron has the highest Hepatosomatic factors followed by control and mixture whereas Chlorpyrifos has the lowest Hepatosomatic factor.

Moreover the dynamic effect (Figure 4.15) showed similar trend of dynamic effects.

Calculating growth factors shows that the % increase in body weight in the control samples in 10.67% whereas the growth factor for Diuron, Chlorpyrifos and mixture are: 7.6% , 10.16% and 8.75% respectively.

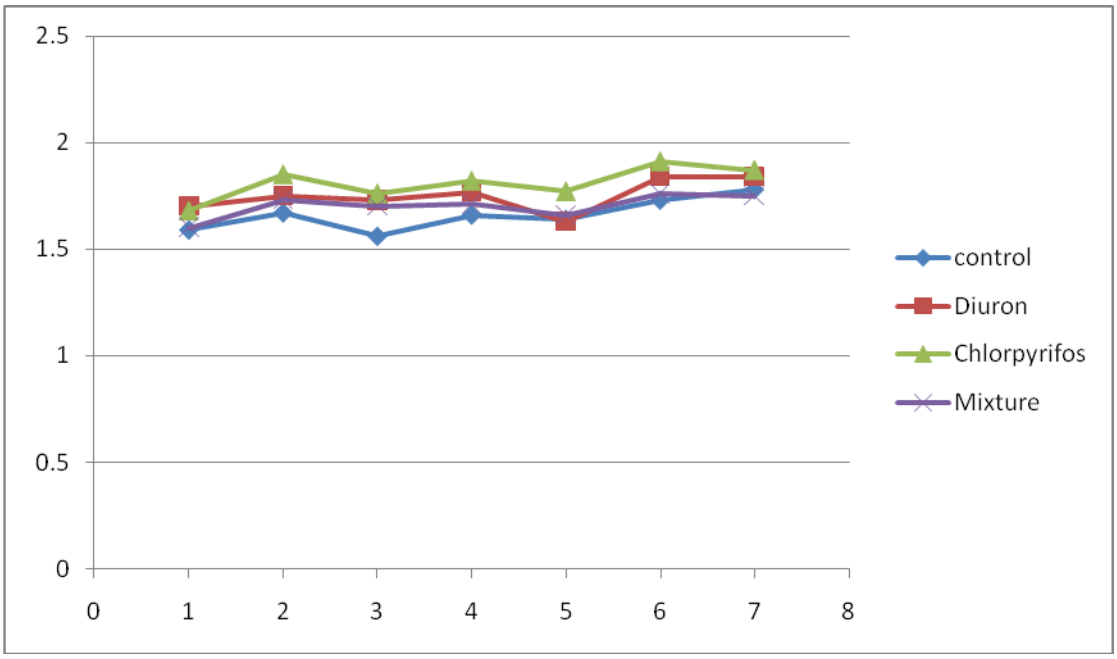


Figure 4.15 Long term effects of Diuron, Chlorpyrifos and their mixture at a concentration of 0.1mg/kg body/day on the body weight of chicken.

Tablen4.6. Average weight, growth factor and p-values between control and treatments.

Item	control	Diuron	Chlorpyrifos	Mixture
average± stdev	1.66±0.08	1.75±0.08	1.81±0.08	1.70±0.06
growth factor	10.82	7.69	10.03	8.79
p-value	-	0.024	0.002	0.042

Regardless to the average weight of chicken in all treatments the growth factor (Table 4.6) is highest in the control treatments than all statistical analysis showed significant differences among all treatments.

Table 4.7. Dynamic chicken weight (kg) after treatments.

Time	Treatments				
	control	Diuron	Chlorpyrifos	Mixture	p-values
1st* week	1.59±0.03	*1.70±0.08	*1.68±0.09	1.60±0.08	0.02-0.41
2nd **week	1.67±0.07	1.75±0.02	1.85±0.2	1.73±0.09	0.057-0.17
3rd** week	1.56±0.08	*1.73±0.11	*1.76±0.08	*1.70±0.1	0.004-0.037
4th ***week	1.66±0.11	1.77±0.09	*1.82±0.07	1.71±0.1	0.024-0.27
5th ***week	1.64±0.12	1.63±0.09	*1.77±0.04	1.66±0.1	0.038-0.45
6th ***week	1.73±0.12	1.84±0.11	*1.91±0.07	1.76±0.08	0.02-0.33
7th ***week	1.78±0.03	1.84±0.11	*1.87±0.06	1.75±0.11	0.014-0.31

(* week acclimatization ,** weeks of treatment ,*** weeks without treatment)

* indicate significant differences at p-values 0.05 .

4.1.2.2 Effect on the body organs:

Effect on liver and heart weight influence of the tasted compounds on liver, heart weight of chicken are shown in Figure 4.16.

Regardless to the standard deviation, it can be seen that liver weight is the highest on chicken treated with Diuron. the weight of other treatments is nearly similar. For the case of heart we can conclude that the weights in all treated are nearly similar.

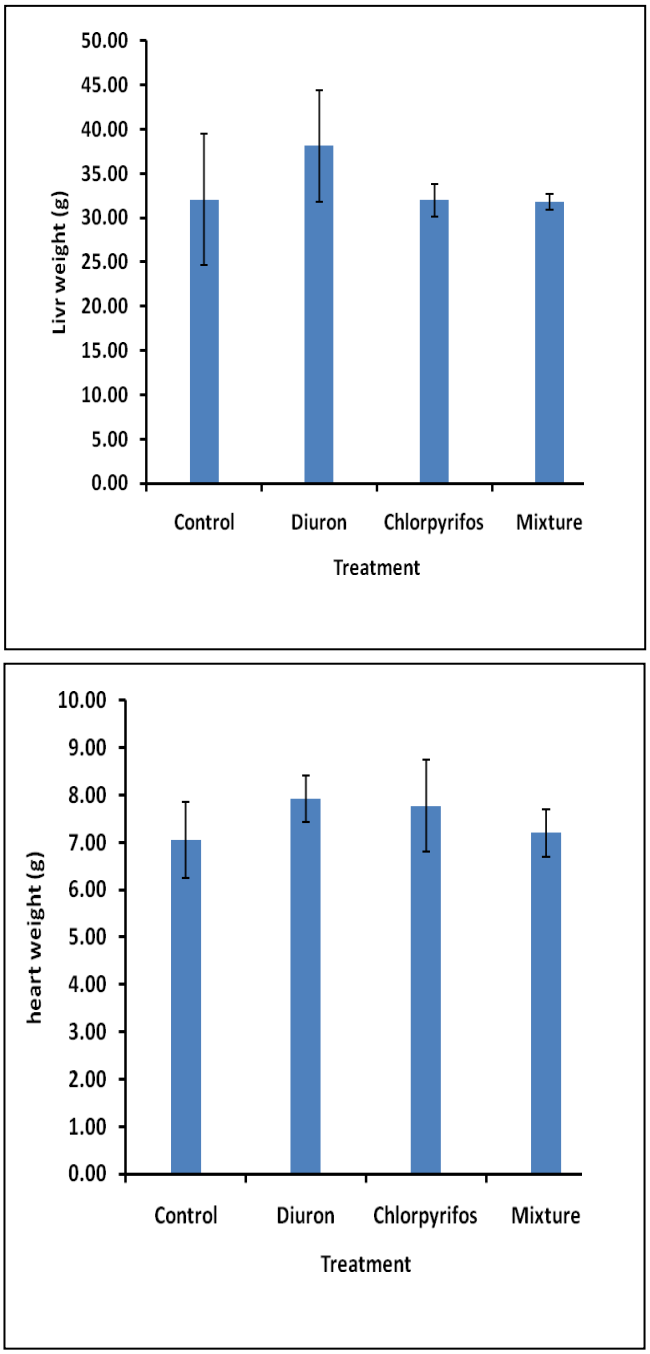


Figure 4.16 Effects of Diuron, Chlorpyrifos and their mixture at a concentration 0.1mg/kg body/day on the weight of liver and heart on chicken.

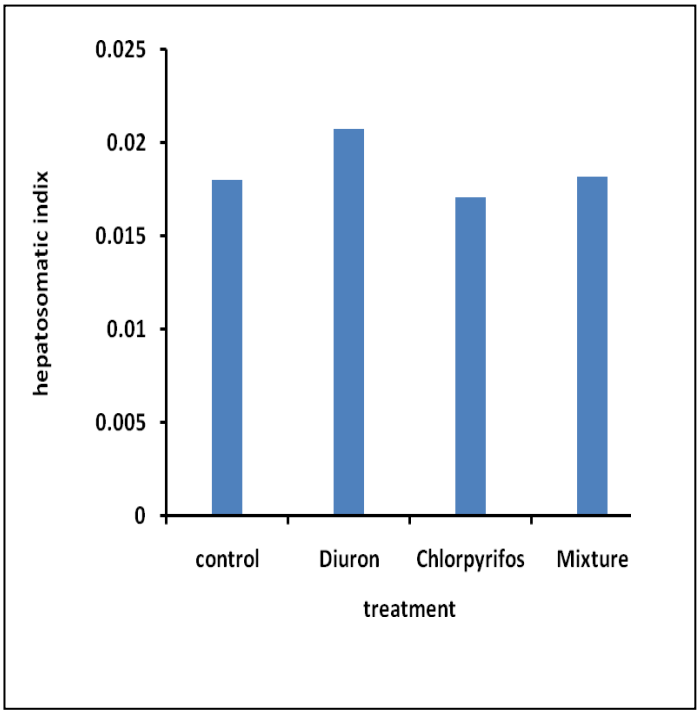


Figure 4.17 Hepatosomatic factors on chicken treated with Diuron, Chlorpyrifos and their mixture at a concentration 0.1mg/kg body/day.

Table 4.8 liver and heart weight in chicken treated with diuron, chlorpyrifos and their mixture.

Treatment	Average weight ± standard deviation	
	Liver	Heart
Control	32.05±7.38	7.05±0.81
Diuron	38.11±6.31	7.92±0.50
Chlorpyrifos	31.98±1.82	7.77±0.96
Mixture	31.80±0.87	7.20±0.49
p-values	0.129-0.47	0.059-0.386

* indicate significant differences at p-values 0.05 .

p-values for liver range between 0.129-0.47, whereas for the heart range from 0.059-0.39, indicating no significant differences.

4.1.2.3 Biochemical effects in chicken:

Effect of the activities of ALT, ALP and AST extracted from liver, heart and brain are presented in Figure 4.18 respectively and Table 4.9 .

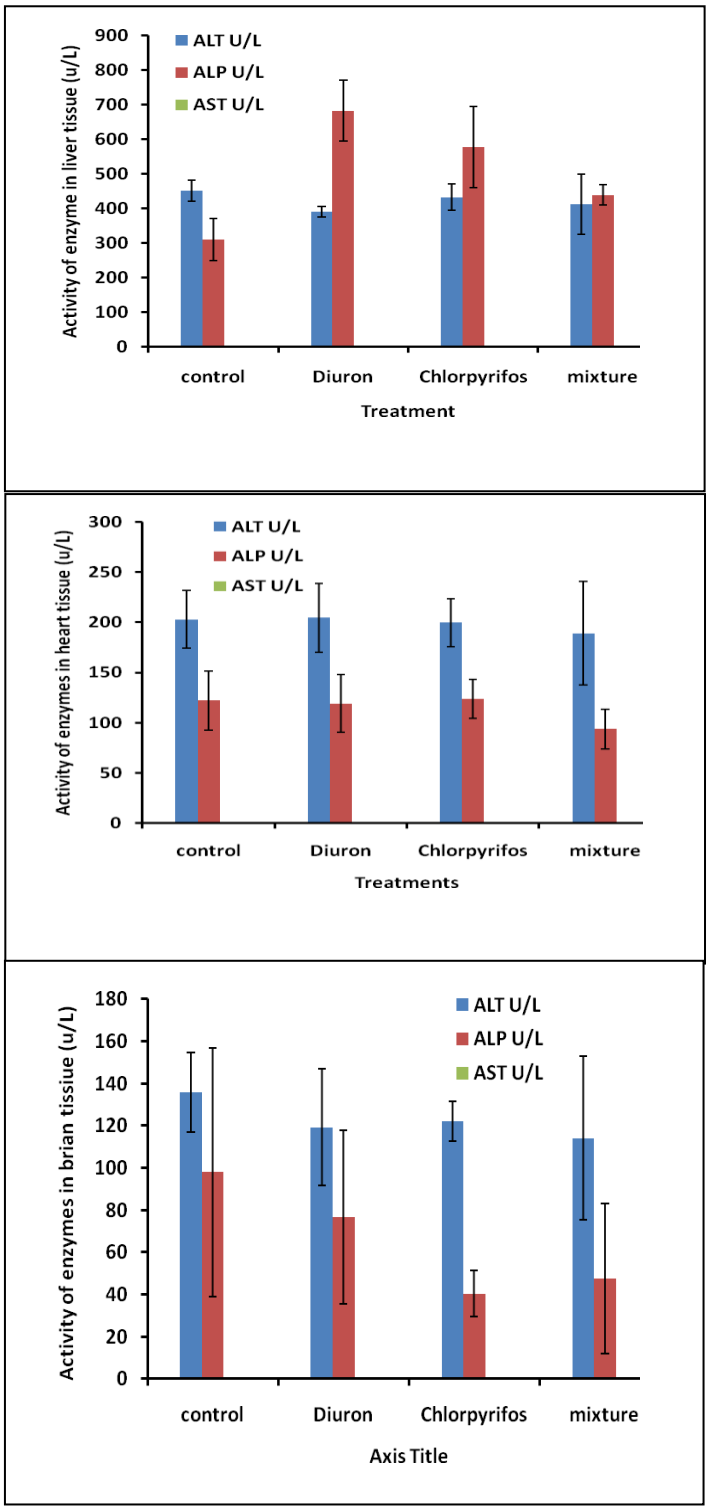


Figure 4.18 Activity of the activities of ALT, ALP and AST extracted from liver, heart and brain are presented.

Table 4.9 Activity of ALT, ALP and AST in different tissues of chicken.

Treatment	Liver		
	ALT U/L	ALP U/L	AST U/L
control	452±30	311±61	1±0.8
Diuron	*391±15	*683±87	0.75±1.5
Chlorpyrifos	433±37	*578±117	1±0.81
mixture	412±87	*440±29	0.13±0.05
p-values	0.005-0.22	0.0002-0.005	0.06-0.389
	Brain tissues		
control	136±19	98±59	0.25±0.50
Diuron	119±28	77±41	0.25±0.50
Chlorpyrifos	122±10	40±11	0±0.00
mixture	114±39	47±36	0.33±0.58
p-values	0.12-0.227	0.072-0.287	0.195-0.5
	heart tissues		
control	203±29	122±29	0.13±0.05
Diuron	205±34	119±29	0.13±0.05
Chlorpyrifos	200±24	124±20	0.75±0.5
mixture	189±52	94±20	0.13±0.05
p-values	0.287-0.42	0.135-0.485	0.03-0.5

* indicate significant differences at p-values 0.05 .

It can be seen that ALT activities in liver tissues were the highest in the control sample and the lowest in the treatment of Diuron . The trend is similar in Brain and Heart tissues . ALP activities increased in the liver tissues of all treatments and reached to the highest level 683±87 in the Diuron treatment . In contrast the ALP levels are reduced in the Brain tissues with the treatments of Diuron , Chlorpyrifos and mixture but the reduction in the Diuron treatments is less than others . The trend in Heart tissues is similar to that in the Brain tissues . The levels of AST were very close to each other.

Influence on ACHE activities in blood serum and brain tissues are shown in Figure 18 receptivity.

It can be seen that the activity of AChE is the highest in the control samples and lowest at Diuron treatments.

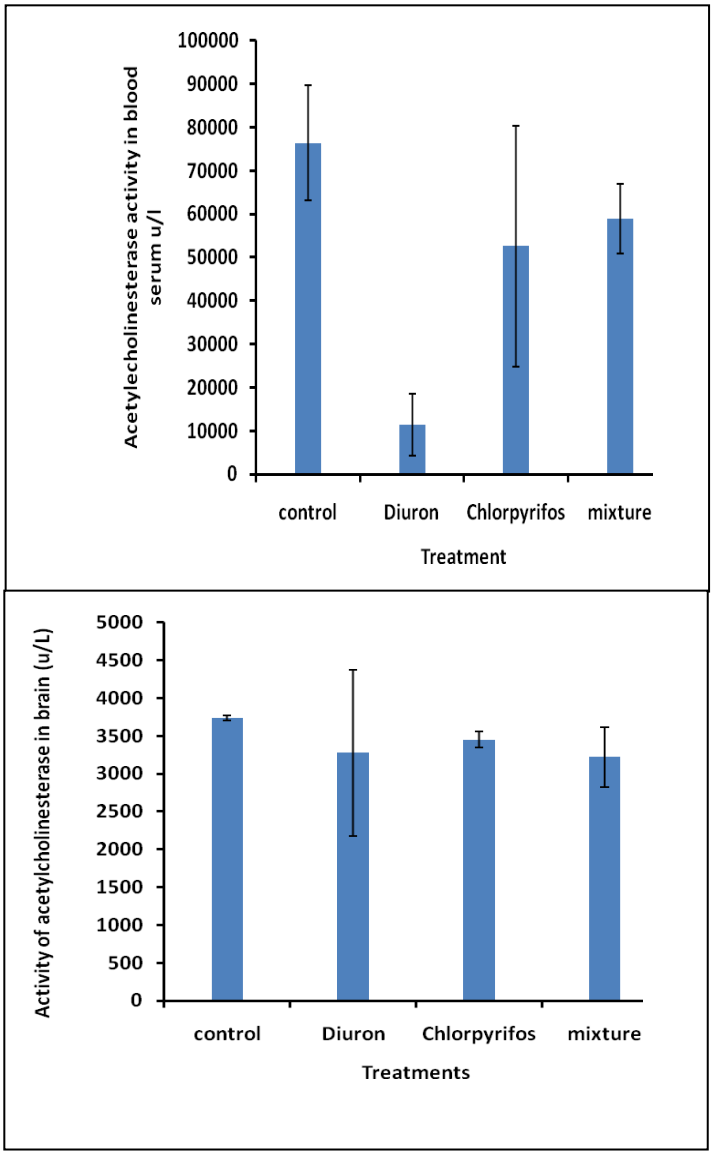


Figure 4.19 Activity of Acetylcholinesterase in blood serum and brain tissue of chicken treated with Diuron, Chlorpyrifos and their mixture at 0.1 mg/kg body weight/day. Error bars represent standard deviation.

Table 4.10. ACHE activities in serum and brain tissue.

Treatment	ACHE activity u/l		p-values	
	serum	Brain	serum	Brain
Control	76311±13280	3742±33		
Diuron	14189±7195	3277±1098	0.002	0.22
Chlorpyrifos	52571±27720	3450±105	0.006	0.024
Mixture	58909±8067	3222±396	0.069	0.002

ACHE activities were higher in the control sample of blood serum and in the Brain tissues . sever reduction in the ACHE activities were observed in the all treatments indicating sever inhabitation of ACHE .

Statistical analysis showed significant differences between control and Diuron treatments p-value 0.002 for blood serum whereas of the differences are not significant for other treatments, p-value (0.06-0.07), for brain tissues, the differences were significantly differences in all treatments except for Diuron .

Effects on kidney function.

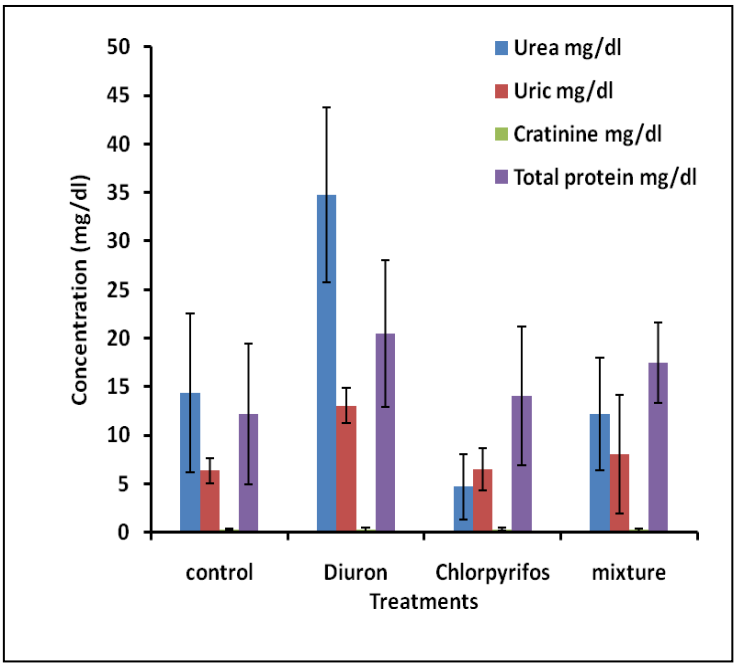


Figure 4.20 Concentrations of urea, uric acid, creatinine and total protein (mg/dl) in blood serum of chicken treated with Diuron, Chlorpyrifos and their mixture at 0.1 mg/kg body weight/day. Error bars represent standard deviation.

Table 4.11. Effects of the tested compounds on kidney functions.

Treatments	Urea mg/dl	Uric acid mg/dl	Cratinine mg/dl	Total protein mg/dl
Control	14±8.16	6±1.28	0.30±0.10	12±7.21
Diuron	27.2±14.54	11.32±3.76	0.38±0.28	18.13±7.76
Chlorpyrifos	5±3.40	6.42±1.79	0.30±0.17	11.25±8.11
Mixture	11.2±5.13	6.54±5.85	0.2±0.14	15.48±5.23
p-values	0.057-0.27	0.04-0.48	0.17-0.338	0.17-0.43

* indicate significant differences at p-values 0.05 .

It can be seen that urea concentration in blood surem of the control sample is 14±8.16 mg/dl whereas the level increased in Diuron treated and reached to 27.2±14.54 mg/dl . So far , the urea level in Chlorpyrifos and Mixture treatments remain below the level of control sample . Uric acid , Cratinine and Total protein

followed the same trend . these result suggest that Diuron has strong effects on kidney function .

4.2 Discussion:

4.2.1 Rabbets:

Diuron and Chlorpyrifos are herbicides and insecticides widely used in Gaza. the LD_{50} of Diuron for rabbits > 2000 mg/kg whereas Chlorpyrifos has an LD_{50} 1000-2000 mg/kg for rabbits (Tomiln, 2000, Milatovic et.al., 2006, Albers et.al., 2004).

The tested compounds have difference mode of action on animal.

The repeated dose of low concentration (1/40 of LD_{50}) for 2 weeks followed by 6 weeks monitoring period may have different toxic effects.

Beside the fact that the tested animal may have developed a metabolic reaction that result in detoxification of the tested compounds. Moreover, mixing two compounds with diffident mode of action may have synergistic or antagonistic difference on the tested animal (Boomathi, et.al., 2005). So far the data presented in (Figure 4.1), clearly showed that Diuron inhibited the growth dynamics of rabbits due to exposure to the repeated dose. So far the growth rate of the treated rabbits is reduced compared to the control sample.

Statistical analysis showed significant different between the control and the treated rabbits as shown from p-value (Table 4.1).

The explanation of the results is that the tested compound may reduce appetites of the tested animals accordingly a reduction of food consumption occurred consequently reduction of growth rate was observed. Our results agree with Aly and El-Gendy, (2015).

Furthermore it can be suggested that the tested compound may interfere with the metabolic path way of food contents accordingly redaction of body weight was obtained. Our results agree with Aly and El-Gendy, (2015) ,who reported the effects of carbryl on rabbits and provide similar explanation .Further support our discussion comes from the experimental worth of (Mokdad, et.al., 2001) who revealed the interaction of pesticide in body weight redaction.

Moreover the weight reduction (Figure 4.2) clearly show the magnitude of weight, whereas Figure 4.3 shows variation in percentage weight reduction which ranged between 17-28% for all case.

Weight reduction indicate different mechanism. Our result agree with recent toxicological studies Aly and El-Gendy, (2015) ,who revealed reduction of body weight of rabbits due to repeated dose of parathion.

It is well known that Diuron has different mode of action in the toxic effect from Chlorpyrifos which is considerate as cholinesterase inhibitor (Tomilin 2000).

However mixing Diuron and Chlorpyrifos (concentration at a ration 1:1) showed more reduction on body weight (Figure 4.2-4.3). These results indicate significant effect of mixtures. Our results agree with Aly and El-Gendy, (2015) , who found synergistic effect when mixing Diuron with Chlorpyrifos.

The explanation of these result is that mixing tow compounds with different mode of action may increase the toxic effect as shown in our case.

This can be refer to as significant effect.

Our results agree with Aly and El-Gendy, (2015) who found significant effect when mixing Diuron with nemucor on fish (Al-Nahhal 2016) un published results.

Effect on body organ (Figure 4.4) clearly shown similar trend regardless to the high value of the standard deviation of some case.

weight reduction of body organs (liver, heart and kidney) are similar to that body reduction (Figure 4.1- 4.3) .

The explanation of these results is similar to that given above for the general body weight reduction.

Moreover, the hepatosomatic factor (Figure 4.5) is the highest in the mixture treatment.

The explanation is that the weight of liver is significantly reduced in Diuron and Chlorpyrifos treatments (Table 4.2) and small redaction in the mixture treatments the effect is also visualized in Figure 4.6 , which shows different liver size .

Furthermore, the redaction in the body weight in the treatments enlarge Hepatosomatic factor which is a mathematical ratio between the liver weight of body weight . A reduction in body weight would increase the Hepatosomatic factor.

4.2.1.1 Biochemical changes:

The presented data in Figure 4.7- 4.10, clearly show the activities of ALT, ALP and AST in liver, brain, heart and kidney tissues.

In all tissues except brain, ALP levels in the control sample are the lowest among all treatments. For the case of brain tissue the level is the highest in the control sample ,

followed by Chlorpyrifos and mixture treatments . lower level was found in Diuron treatments . Moreover the levels of ALT in liver tissue in the mixture treatment is the highest in all tissue.

In general, treatments of rabbit with Diuron, Chlorpyrifos and their mixture significantly increased ALT in all tissue except brain this indicates hyper activity of ALT in liver, heart and kidney issues. This also suggest oxidation stress in the organs. For the case of ALP the activities decreased from 303±46.2 in the control sample to 190.67±2.84 in the mixture treatments. Similar trend was observed in kidney tissues. These results indicate the inhibition in enzymes of the liver and kidney tissues. So far the levels increased from 49.5±21.5 in the control sample to 101.33±38.28 in brain of the mixture treatment and from 52±2.16 in the control of Heart to 78.3±15.53 in mixture treatment in heart tissues. This indicate increased activity of the enzyme in brain and heart tissues. Furthermore AST in brain and heart tissue reduced from high levels 110.5±22.32 to lower levels 1.0001±1.4. This indicates inhibition of the enzymes activity in brain and heart . Chlorpyrifos is reported to induce oxidative stress by inhibiting mammalian acetylcholine esterase. In addition it also disrupts the endocrine actions of androgenic, estrogenic, thyroid and parathyroid hormones (Tripathi et.al., 2013)

Our results agree with Aly and El-Gendy, (2015) who found similar effect on rabbit treated with carbaryl.

4.2.1.2 Effect on kidney function:

The data presented in Figure 4.11 and Table 4.4, clearly demonstrate the concentration of urea, uric acid, creatinim and total protein.

Statistical analysis of the data did not detect significant difference between the control and the treatments regardless to the variations in the values.

This indicates that the treatments did not affect the kidney function. However, the value at total protein in the mixture treatments is significantly higher from all treatments indicating synergistic effects.

Our result agree with (Laetz et.al., 2009) who found synergistic effects in treatments containing Diuron and diazinon. More supports to our results come from the experimented work of Hermens et.al., (1985).

4.2.1.3 AChE activity:

The presented results in Figure 4.12 and 4.13 and Table 4.5 clearly demonstrate the levels of AChE on brain tissues and blood serum. It is obvious that AChE in the brain tissues is less inhibited than AChE serum.

This indicates the sensitivity of AChE in the blood serum, moreover the levels of AChE in control sample are the highest among all treatments whereas the levels are reduced in all case. However, the inhibition was more prorated in the serum than in brain tissues.

So for, the levels are reduced from 3825±98 brain tissues, 55997±1012 in serum to 2704±544 brain tissue and 9765±2273 serum respectively. Moreover it can be seen that a serum enzyme reductions were observed in Chlorpyrifos and mixture treatments. Statistical analysis did not detect significant difference between the control and Diuron treatments in the brain and serum.

Whereas significant difference were detected with Chlorpyrifos and mixture treatments in the serum. The significant difference among treatments are presented in Table 4.5.

The explanation of the results is that Diuron is not cholinesterase inhibitor (Tomlin 2000) as well known in the literate whereas Chlorpyrifos is considered as AChE inhibitors (Tomlin 2000). More supports to our results come from recent work (Yen et al 2011). Moreover mixing Diuron with Chlorpyrifos results in further inhibition of AChE in brain and serum indicating synergistic effects. Our results agree with (El-Nahhal et.al 2015) who found synergistic effects on fish when mixing carbaryl with Diuron. Chlorpyrifos is a neurotoxicant that inhibits neuronal and blood cholinesterase leading to overstimulation of cholinergic neurotransmission (Geller et al., 1998). Acetylcholinesterase (AChE) inhibition, indicating that the action of compound chlorpyrifos (CPS) and chlorpyrifos oxon (CPO) on developing brain may be through different pathways than inhibition of AChE enzymatic activity. It has also been suggested that CPO, with a much higher potency than CPS, may act directly on the morphogenic capability of AChE and on targets such as cell signaling molecules or cytoskeleton proteins (Flaskos, 2012). The classic mechanism of CPS toxicity is through inhibition of acetylcholinesterase (AChE) by its active metabolite (CPO) although many other serine hydrolases.

The higher toxicity of Diuron and Chlorpyrifos on AChE blood serum maybe due to possible metabolism of Chlorpyrifos to the oxone radical which is more toxic than Chlorpyrifos but maybe oxidized rapidly to non toxic fragments due to the peruse of oxinase in huge amount in resistant spection as in rabbits . our results agree with Flaskos (2012) and Armstrong et al 2013 , who provide similar explanation for other results .

4.2.2 Chicken:

The data in Table 4.7-4.8 and Figures (4.14-4.15) clearly show the progressive weight of chicken exposed the tested compound for eight weaks. It is obvious that chicken gaining and losing weight as a cyclic process (a cycle of gaining weight followed by a cycle of losing weight).

Regardless to the above variation of results, it is obvious in Table 4.6 that the growth factor of the control sample is the highest among all treatments followed by that of Chlorpyrifos and mixture indicating more toxicity.(Table 4.6)

The growth factor for Diuron treatment is the lowest among all.

The explanation of these results is that chicken are not sensitive to Chlorpyrifos, regardless to its high toxicity (Tomlin 2000).

Moreover it can be suggested that Chlorpyrifos is rapidly metabolized in chicken to nontoxic fragments.

This suggests is supported by the result of (Kammon et.al., 2010) who found resistant developed in chicken exposed to oregano phosphorus compound. In addition Malik et.al.,(2001) found that oreganophosophrus compound did not affect the weight on chicken more technical supports can be obtained from the findings of (Krishnamoorthy et.al., 2007) who found similar observation in hen.

In contracts Diuron mixture treatment significantly reduced the weight (Table 4.7), P-value ranged from 0.002-0.0042

These data suggest that Diuron or its mixture effect the feeding habits of chicken or partly damaging the feeding control system in chicken, accordingly a reduction in the weight was observed. Our results agree with Shaw et.al., (1994) who found reduction

of chicken weight due to expose to urea derivatives more support to our results can be obtained from the results of Jortner and Ehrich,(1987), who revealed changes in feeding behavior of chicken due to expose to toxic compounds.

4.2.2.1 Effects on Liver and Heart :

The data presented in Figure 4.16 clearly shows that Diuron increase the weight of liver and heart whereas Chlorpyrifos and mixture did not affect liver weight . The explanation of these results is that Diuron enhance the cordiotoxicity these results agree with Domingues et.al.,(2011) who revealed cordiotoxicity among chicken treated with Diuron. Moreover liver toxicity was also reported with Diuron (Fernandes et.al., 2007).

Hepatostomic factor are shown in Figure 4.17. It can be seen that Diuron and mixture treatments increased the hepatostomic factor whereas the hepatostomic factor of the control and Chlorpyrifos treatments are quite similar. The explanation of their results is that Diuron and its mixture reduced the weight of the total body accordingly the hepatostomic factor is increased.

4.2.2.2 Biochemical changes:

The data presented in Figure 4.18 and Table 4.9, clearly demonstrated the activity of ALT, ALP and AST in liver brain and heart tissues. It is obvious that, actives of the enzymes are different in the three tissues.

So far, ALT has the highest activity of the enzymes in the control sample whereas the activity are reduced in the treatments. This inhibition indicates toxicity to liver. Furthermore the activity of ALT in liver tissues are higher than brain and heart tissues. This suggest cell damage and release of ALT from liver tissues.

Moreover, the activity of ALP is higher in control treatment of liver tissues than in brain or heart tissues. Moreover, ALP activities increased the liver tissues above that of the control sample due to administration of repeated low dose whereas a reduction was observed in the treatments in brain and heart tissues.

AST are very low in all cases. This indicates suppression of AST activity by indirect ways. our results agree with Kaur et.al., (2000) who found similar results.

The data presented in Figure 4.19 and Table 4.10,clearly shows the effects of the treatments on serum and brain tissues acetylcholine esterase activity.

It can be seen that the effects is more pronounced in serum than in brain tissues.

The explanation of these results is that serum AChE is more sensitive that brain AChE, beside the fact that exposing animals to the toxic substrates expose serum AChE to direct inhabitation due to direct contact with the toxic substance whereas brain AChE is nearly protected by blood brain barrier which make the direct contact with brain AChE more different accordingly the enzymes activity was less effected in brain . Our results agree with Yassin et.al.,(2005)who found similar results for other causes.

Moreover, further supports to our results come from El Nahhal (2016) who revealed the inhibition of AChE on fish due to pesticide application .

In addition, Diuron treatments show different AChE inhibition in blood serum than in brain tissues. For AChE in serum, statistical analysis shows significant difference between control and Diuron, P-value = 0.002 whereas no significant difference were detected between control and Chlorpyrifos, or mixture.

For brain AChE , In the way around, significant difference was not detected between the control and Diuron whereas significant different were detected between control and Chlorpyrifos, or mixture, P-value were in the range 0.024 - 0.002 .

The data presented in Figure 4.20, and Table 4.11, clearly shows the effects of the tested compounds on kidney function.

It can be seen that Diuron treatment increased urea, uric acid and Total protein above that of control or other treatments , similar treated was observed with the mixture.The explanation of these results is similar to that given for rabbits.

Chapter (5)

Conclusion and Recommendations

5.1 Conclusion:

- The rational of this work emerged from the facts that long term exposure to a low concentration of pesticide residues may enhance the biochemical degradation and elimination of toxic metabolite as shown for the reduced toxicity of Chlorpyrifos application of repeated does of low concentration of Diuron, Chlorpyrifos and there mixture significantly affected the weights on rabbits and chicken .
- The tested compound dramatically affected the weight of Heart , liver and kidney.
- The biochemical changes should sever inhabitation of ALT ,ALP and AST, Diuron should stronger effect than Chlorpyrifos .
- The effect on ACHE was more pronounced on blood serum than brain tissues .
- An inhaling out come is that Diuron showed stronger inhibition on ACHE in chicken than rabbit .
- Diuron effected urea , uric acid and Total protein in chicken .
- Mixture toxicity shows reduced toxicity in rabbits.
- Enhanced toxicity in chicken was observed in addition the effects of liver enzymes activity were more pronounced in rabbit than chicken.

5.2 Recommendation:

- Further studies for long term exposure and toxicities are recommend.
- Development of awareness program which cover all health and environmental impact of chemical and their judicious and safe use.
- Establishment of poison control centers with sufficient clinical and analytical capacities in addition to functions of treatment and prevention.
- Establishment of sound chemicals management system involving all concerned parties from government, agricultural workers, industry, research institutes, non-governmental organizations and academia through multi-stakeholder committee.
- Careful attention may be given to the application Diuron in the eco-system.

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