The Islamic University-Gaza
Researches and Graduate Studies Affairs
Faculty of Science
Department of Environment and Earth Science

الجامعة الإسلامية – غزة
شئون البحث العلمي والدراسات العليا
كلية العلوم
قسم البيئة وعلوم الارض

Phytotoxicity of the Herbicides; Alachlor, Bromacil and Diuron and their Mixtures to Wheat, Watermelon, and Molokhia Plants in Gaza Strip, Palestine

السمية النباتية لمبيدات الأعشاب (الالاكلور، البروماسيل والديارون) وخلائطها على النباتات (القمح، البطيخ و الملوخية) في قطاع غزة- فلسطين

Prepared by:

Nisreen Mohamed Hamdona

Supervisor:

Dr. Yaser EL-Nahhal

Faculty of Science
The Islamic university - Gaza

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master in Environmental Management and Monitor/Department of Environment and Earth Science Faculty of Science

2013م /1435 ه

إقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Phytotoxicity of the Herbicides; Alachlor, Bromacil and Diuron and their Mixtures to Wheat, Watermelon, and Molokhia Plants in Gaza Strip, Palestine

السمية النباتية لمبيدات الأعشاب (الالاكلور، بروماسيل و ديارون) وخلائطها على النباتات (القمح، البطيخ و الملوخية)، غزة- فلسطين

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وإن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

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: Nisreen Mohamed Hamdona	اسم الطالب:
Signature: Nisreen Hamdona	التوقيع:
Date:30-3-2014	التاريخ:

بسم الله الرحمن الرحيم

الجامعة الإسلامية – غزة
The Islamic University - Gaza

مكتب نائب الرئيس للبحث العلمي والدراسات العليا هاتف داخلي 1150

الرقم ج س غ/35/ Ref

التاريخ 2013/12/30م Date

نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ **نسرين محمد رشيد حمدونة** لنيل درجة الماجستير في **كلية *العلوم*** قسم **علوم بيئية – الإدارة والمراقبة البيئية** وموضوعها:

(Phytotoxicity of the Herbicides; Alachlor, Bromacil and Diuron and their Mixtures to Wheat, Watermelon and Molokhia Plants in Gaza Strip, Palestine)

وبعد المناقشة العلنية التي تمت اليوم الاثنين 27 صفر 1435هـ، الموافق 2013/12/30م الساعة الحادية عشرة صباحاً بمبنى طيبة، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

د. ياسر زيدان النحال	**مشرفاً ورئيساً**	....
أ.د. سمير عبد الفتاح عفيفي	**مناقشاً داخلياً**	....
أ.د. عبد الرحيم عبد الله عاشور	**مناقشاً خارجيًا**	....

وبعد المداولة أوصت اللجنة بمنح الباحثة درجة الماجستير في **كلية *العلوم*/ قسم علوم بيئية– الإدارة والمراقبة البيئية.**

واللجنة إذ تمنحها هذه الدرجة فإنها توصيها بتقوى الله ولزوم طاعته وأن تسخر علمها في خدمة دينها ووطنها.

والله ولي التوفيق ،،،

مساعد نائب الرئيس للبحث العلمي و الدراسات العليا

أ.د. فـؤاد علي العاجز

Dedication

To my mother who I owe everything since I was born.

******

To my husband who supported and encouraged me at all stages of my study.

******

To my beloved son Majd, and beloved daughter Yasmeen who have been a great source of motivation and encouragement to me.

******

To my acquaintances, and all those who believe in the richness of learning.

Acknowledgment

First of all, I praise Allah for blessings and guidance in fulfilling this goal. I would like to thank all those who have assisted, guided and supported me in my studies leading to this thesis. I also would like to express my sincere gratitude to my supervisors **Dr.Yasser EL-Nahhal**, Assist Prof. of Environmental Chemistry of Pesticides, Environmental and Earth Science Department- Faculty of Science, Islamic University - Gaza Strip for his encouragement, guidance, constructive advices, support and great help. Special appreciation goes to teaching members committee in Environmental Science Program.

I would like to extend my thanks and appreciation to all stuff of Environment and Earth Science Laboratory in the Islamic University for providing all means of support needed in my experimental work. I also would like to extended my thanks to the stuff of general management of plant protection and Agriculture quarantine.

Abstract

This study aimed at investigating phytotoxicity of the herbicides: alachlor, bromacil, diuron, and their mixtures to wheat (*Triticum spp*), watermelon*(Citrulus lanatus)* and molokhia (*Corchorus Olitorius L.*) plants in Gaza strip. The study indicates that phytotoxicity for herbicides on plants decreases the growth of the plants that were exposed to a different concentration of herbicides.

This experiment was conducted in pots under controlled conditions for two weeks and then they were harvested. The biomass was used as an indicator for determination of growth inhibition. The phytotoxicity of selected herbicides was determined through the percentage of growth inhibition and was estimated by the toxic units of these used herbicides. These herbicides were used either individually or in a mixture of binary or tertiary. It has been found that the effect of the phytotoxicity of these herbicides and their mixtures on plants is varied from one herbicide to another. However, these variations may be referred to the difference concentration and EC50 for each herbicide.

It was found that EC_{50} for alachlor, bromacil and diuron were: 11.37, 4.77, 1.64 respectively on melon, 0.33, 0.29, 0.15 respectively on molokhia, and 0.11, 0.08, 0.24 respectively on wheat. In the binary mixture tests, The EC_{50} of (alachlor and bromacil), (alachlor and diuron), and (bromacil and diuron) was 12.21, 5.84, 10.22 for melon, 0.982, 925.4, 38.1 for molokhia, and 0.673, 1.34, 0.644 for wheat. In tertiary mixture tests EC_{50} of (alachlor, bromacil and diuron) the result was 633.9 for melon, 3.02 for molokhia and 32.174 for wheat.

EC_{50} was estimated by using the linear regression equation and it was found that the effect of the remaining herbicides on the plants growth for the individual experiments is more clearly compared with the experiment of the herbicides mixture. The individual herbicides test proved that the diuron is more toxic than both alachlor and bromacil. Other effects may be caused by interaction between two herbicides or more and it may be additive, synergistic or antagonistic.

Key words: antagonistic, additive, herbicides , EC_{50}, phytotoxicity, synergistic

ملخص الدراسة باللغة العربية

تبحث هذه الدراسة السمية النباتية لـ الألاكلور و البروماسيل و دايورون علي نباتات الملوخية والبطيخ والقمح، سواء كانت هذه المبيدات بشكل آحادي أو على شكل مخاليط . وقد اشيرت الدراسة أن السمية النباتية لـ مبيدات الأعشاب تعمل على تثبيط نمو النباتات المُعرضة لتركيزات مختلفة من مبيدات الأعشاب.

أجريت تجربة أصص تحت ظروف خاضعة للرقابة و أبقيت لمدة أسبوعين ومن ثم تم جمع الكتلة الحيوية واستخدامها كمؤشر لتحديد تثبيط النمو. ولقد تم تحديد السمية النباتية لمبيدات الأعشاب المختارة من خلال نسبة تثبيط النمو و كذلك تم تقدير وحدات التسمم للمبيدات المستخدمة سواء بشكل أحادي أو مخاليط ثنائية أو ثلاثية و قد وجد أن السمية النباتية لهذه المبيدات ومخاليطها تختلف في تأثيرها على نمو النبات من مبيد لأخر، ويرجع ذلك إلي اختلاف التركيزات المستخدمة وقيمة EC_{50}.

لقد وجد أن قيمة EC_{50} لـ الألاكلور ، البروماسيل والديورون 1.64 ,4.77 ,11.37 على البطيخ على التوالي، وكانت القيمة 15 .0 ,0.29 ,0.33 على ملوخية على الترتيب وكانت 0.11 ,0.08 ,0.24على القمح على الترتيب. أما في اختبارات الخليط ثنائي ، كانت قيمة EC_{50} من (الألاكلور و البروماسيل)، (الألاكلور و الدايورون)، و (البروماسيل و الدايورون) 12.21 ,5.84 ,10.22 لـ البطيخ على التوالي ، 925.2 ,38.1 ,0.982 لـ الملوخية على التوالي و0.673 ,1.34 ,0.644 لـ القمح على التوالي. وفي اختبارات الخليط الثلاثي بلغت قيمة EC_{50} للخليط المكون من (الألاكلورو البروماسيل والدايورون) كان 633.9 لـ البطيخ، 3.02 لـ الملوخية و32.174 لـ القمح على التوالي.

تم تقدير EC_{50} باستخدام معادلة الانحدار الخطي. وكانت تأثير المبيدات العشبية المتبقية على نمو النبات في اختبارات كل مبيد منفردا أكثر وضوحا بالمقارنة مع اختبارات مخاليط مبيدات الأعشاب، وأثبت نتائج اختبارات المبيدات كل على حدا أن الدايورون كان أكثر سمية من الألاكلور والبروماسيل. هذا وقد تنتج تأثيرات أخري نتيجة للتفاعل بين اثنين أو أكثر من مبيدات الأعشاب، قد تعمل على زيادة السمية أو تقليلها أو لا تحدث تأثير .

من خلال هذه البحث أصبح لدينا الفهم الواضح عن السمية النباتية من خليط مبيدات الأعشاب في غزة وفلسطين، والمساهمة في تحسين تقييم نمو النباتات تجاه تركيزات مبيدات الأعشاب المختلفة. أظهرت النتائج أن نمو المحاصيل يقل نتيجة لتركيزات متبقيات المبيدات المرتفعة؛ حيث تتأثر معايير نمو النباتات في وجود المبيدات بشكل أحادي مقارنة بوجودها عل شكل مخاليط.

Table of contents

Content **Page**

CHAPTER 1: INTRODUCTION

CHAPTER 2: LITERATURE ERVIEW

CHAPTER 3: MATERIALS AND METHODS

CHAPTER 4: RESULTS

CHAPTER 6:CONCLUSIONS AND RECOMMENDATIONS

List of tables

Content **Page**

List of figures

Content **Page**

List of abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
Al	Alachlor
BM_c	biomass of test plant in the control
BM_t	biomass of test plant in the treated samples
Br	Bromacil
Di	Diuron
EC_{50}	Effective Concentration on Fifty percent of tested organisms
G	Gram
GI	Growth Inhibition
IARC	International Agency for Research on Cancer
ICARDA	International Center for Agricultural Research in the Dry Areas caravan
km^2	Square kilometer
Kow	Adsorption Confection
LC_{50}	lethal concentration on Fifty percent of tested organisms
MCPA	2-methyl-4-chlorophenoxyacetic acid
mg ai/ kg	Milligrams Active ingredient per kilogram
mg/l	Milligrams per Liter
Ml	Milliliter
Mm	Millimeter
MOA	Ministry of Agriculture
MTI	Mixture Toxicity Index
OHS	Occupational Health Services
PCBS	Palestinian Central Bureau of Statistics
Ppb	***parts per billion***
Ppm	***parts per million***
R^2	Regression
SSRIs	Selective serotonin reuptake inhibitors
TOXNET	Extension Toxicology Network

TU	Toxic Unit
U.S.EPA	***United States Environmental Protection Agency***
USA	United States Agency
UV	Ultra violet
v/v	volume per volume
WSSA	Weed Science Society of America

Chapter 1

Introduction

1.1 Background information

Herbicides have widely variable toxicity. In addition to acute toxicity from high exposure levels, there is a concern of a possible carcinogenicity (Howard *et al*., 1992). The increasing of application led to the pollution of aquatic system which has raised concerns from the public (Wang & Freemark, 1995). Miller, (2009) wrote about the harmful of pesticides on non target organisms like human and wildlife. A few studies have been conducted to determine the harm of these pollutants to living organisms in the aquatic systems (Kasai, *et al*., 1993; Ma, 2005). However, an application of various herbicide formulations has resulted in contamination of groundwater in USA, Canada, Europe, and the Middle East (Thurman *et al*., 1996) and damaged the plants growing in the next crop cycle. Researchers tried harder to develop less hazardous and environmentally safe formulations for the herbicide to human and environment (El-Nahhal *et al*., 2001; Nir *et al*., 2000; Lagaly, 2001 and Rytwo, 2005).

For instance, an application of herbicides can affect the structure and the function of the aquatic communities by changing the species composition of an algal community (Ma *et al*., 2003). In addition, contamination of surface water with herbicides has been reported to have direct toxic effects on populations of phytoplankton (Ma *et al*., 2002). Herbicide mixture have different effect other than the individuals of these changes synergistic or antagonistic effects and other (Hatzios & Penner, 1985). Hussein *et al*., (1996) have reported that some of the clinical signs such as rapid respiration, increased the rate of gill cover movements, slow-down or reflex the swimming movement and the reduction of fish activities which were exposed to herbicides.

Gaza strip is considered as one of the most densely populated areas in the world. Gaza Governorate has a population of approximately 1,701,437 people (PCBS, 2013). Two thirds of Gaza strip area (total 365 km^2) is an agricultural areas. Recently, several pesticides were detected in the major vegetables consumed in Gaza. Safi *et al*., (2002) detected α and β-endosulfan, chlorpyrifos, carbofuran, chlorfluazuron, triadimenol I and II, penconazole, coptafolmetabolite, pyrimethanil and iprodione in some samples of cucumber, tomatoes and strawberries of Gaza. However, the residue levels were below the maximum residue limits (0.5 mg/Kg).

Wheat, Watermelon and Molokhia plants are common plants cultivated seasonally in Gaza. The local people have used them as alternatives and traditional plants. In addition, the cultivation of these plants has provided an income to the farmers. Furthermore, alachlor, bromacil and diuron were extensively applied in Gaza soil in the past few years. The phytotoxicity of each herbicide has been extensively studied, but the phytotoxicity of herbicides mixtures have never been studied in Gaza and very few information are available (MOA Palestine, 2012a).

1.2 Identification of the problem

Application of pesticides has resulted in contamination of food samples and agricultural commodities in many countries in the Middle East (El-Nahhal, 2004). Herbicides may enter freshwater ecosystems by spray drift, leaching, run-off, or accidental spills and present potential risks for several aquatic organisms. This situation may be associated also with health disabilities and chronic diseases (Safi et al., 1993; Safi, 2002). On the other hand, sustainable agriculture cannot give high yielded materials within reasonable prices without partial use of pesticides (El-Nahhal, 2004).

Multiple pesticides often used so, residues are present as mixtures in the environment. Moreover, pesticides are applied in different forms consisting of active and inert ingredients (e.g., solvents and carrier compounds). Their toxic effects may differ from a single compound. Specifically, they may undergo additive, synergistic or antagonistic effects resulting of the interaction between two or more herbicide (Hatzios & Penner, 1985). In addition, they become toxic to the target crops in the next growing season, create weed resistance, this makes the weed control very complicated, costly and create toxicity to the ecosystem (Thurman et al., 1996).

1.3 Objectives

- **The overall objective is:**

❖ To evaluate the environmental effects of herbicides.

- **Specific objectives:**

❖ To characterize the phytotoxicity of alachlor, bromacil and diuron to wheat, watermelon and molokhia.

- To examine the responses of the three tested plants to herbicides as binary and\or tertiary mixtures.
- To characterize the synergistic or antagonistic effects of these herbicides.

Chapter 2

Literature review

This chapter describes the toxicity of herbicides to different plants during the last 15 years.

2.1 Toxicity of herbicide

Herbicides are unique in that they are designed to kill unwanted plants or control it in agricultural activities. Sufficiently high doses of herbicide will kill both crop and weed, while a small doses have no an effect upon crop and weed (Kellogg et al., 2000). However, herbicides may cause phytotoxicity effects on the plants that are not within the area over which the herbicide is applied, for example, as a result of wind-blown spray drift or from the use of herbicide-contaminated material (such as straw or manure) being applied to the soil (Stefan & Keith, 1998). The biological test requires to the choice of an indicator organism or species that is, very often, a terrestrial plant. After the herbicide treatment, one or more biological parameters of the plants that were affected was assessed. The toxicity factor was used in the risk assessment is usually the EC50 (Sandín-España et al., 2011).

Herbicide contamination of the water systems has recently been concerned. The pollution of aquatic environments with herbicides is affected through their use in the control of aquatic weeds, leaching and runoff from agricultural fields (Ying & Williams, 2000). Several studies have determined the harmful effects of pollutants on aquatic organisms (Fairchild *et al*., 1997 and Waldhoff *et al.,* 2002). Among these aquatic organisms, phytoplankton such as unicellular algae, which are important components of the aquatic food chain. Contamination of surface water with herbicides has been reported to have a direct toxic effect on the populations of phytoplankton (Ma *et al*., 2002).

Ma et al., (2001) examined the effects of 33 herbicides on green algae Chorella pyrenoidase. Different mechanism operates at different molecular sites of action. These mechanisms include; blocking the denovo synthesis of fatty acid (e.g. Diclofop-p), inhibition the activity of acetyle-CoA carboxylase (e.g. Tribenuron), Inhibition of the activity of acetolactate synthase (e.g. Tribenuron), inhibition of the activity of protoporphy-rinogen oxidase (e.g. Oxyfluorfen), inhibiting microtubule formation in mitosis (e.g. Cinmethylin), stimulating ethylene biosynthesis, which in turn lead to growth retardation and senescence (e.g. Auxin) and inhibiting photosynthetic process (e.g. duiron). Shaw et al., (2012) demonstrated that the runoff from the land based application of herbicides may reduce photosynthetic efficiency in corals of inshore reefs in the Great Barrier Reef.

2.1.1 Alachlor

Alachlor is a herbicide from the chloroacetanilide family. Its mode of action is to inhibit elongase and to inhibit geranylgeranyl pyrophosphate cyclisation enzymes, that is a part of the gibberellins pathway (Appleby *et al.*, 2002). Alachlor persists in soil from two weeks to a month, depending on soil type and climate (Kidd & James, 1991). About half of the compound remains in soil after eight days (Food and Drug Administration, 1986). The main means of alachlor degradation is by soil microbes (Beste & Chairman, 1983). It has a moderate mobility in sandy and silty soils and thus it can migrate to groundwater (U.S. EPA, 1987).

Alachlor is only moderately toxic to aquatic invertebrates and fish. The LC_{50} for alachlor is 2.4 mg/l in rainbow trout and 4.3 mg/l bluegill sunfish (Johnson & Finley, 1980). Other 96-hour LC_{50} values for the pesticide are: 6.5 mg/l (catfish), 4.6 mg/l (carp), and 19.5 mg/l (crayfish) (U.S.National Library of Medicine, 1992). The bioaccumulation factor of alachlor in the channel catfish is 5.8 times greater than that of the ambient water concentration, indicating that alachlor is not expected to accumulate appreciably in aquatic organisms (U.S. National Library of Medicine, 1995) and its slightly toxic to practically non-toxic to wildfowl. Alachlor has a five-day LC_{50} of greater than 5,000 ppm in young mallards and bobwhite quail (Beste & Chairman, 1983). Pheasants have an LC_{50} of greater than 10,000 ppm (U.S. National Library of Medicine, 1992).

UV-treatment of alachlor and metolachlor increased their toxicity compared to the parent compounds while an equal toxicity was found in photolysis products of acetochlor. This suggests that the toxic photodegradation products are generated from chloroacetamides under UV-treatment (Souissi *et al.*, 2013). In other studies, Jin-Clark et al., (2008) studied the effect of alachlor and metolachlor on the toxicity of chlorpyrifos and major detoxification enzymes in the aquatic midge, *Chironomus* *tentans* (Diptera: Chironomidae). They found that the coexistence of chlorpyrifos and these herbicides, particularly metolachlor, in surface waters may pose increased risks to midges in aquatic environments.

Absorption of alachlor is primarily by germinating shoots (Johnson & Finley, 1980), and it is readily translocated throughout the plant. Higher concentrations appear in the vegetative parts than in the reproductive parts of the plant. In plants, alachlor is almost completely metabolized within ten days (U.S. National Library of Medicine, 1992).

The use of commercially available formulations of alachlor has resulted in a serious environmental problems due to its leaching and migration to ground water sources (El-Nahhal, 1998).

Alachlor used to control annual grasses and certain broadleaf weeds in fields of corn, soybeans and peanuts. It is a selective systemic herbicide, absorbed by germinating shoots and by the roots. The compound works by interfering with a plant's ability to produce (synthesize) protein and by interfering with root elongation (U.S. National Library of Medicine, 1992 and Walker & Keith, 1992). The solubility of alachlor in water is 240 mg/l at 25 degrees C^0 (Meister, 1992).

2.1.2 Bromacil

Bromacil is a herbicide used for brush control on non-cropland areas. It is especially useful against perennial grasses. It is also used for selective weed control in pineapple and citrus crops. It works by interfering with photosynthesis, the process by which plants use sunlight to produce energy. Because bromacil has a low vapor pressure, it is unlikely to produce vapors which can be inhaled. No deaths occurred when rats were exposed to approximately 4.8 milligrams of bromacil per liter of air for four-hours (ACGIH, 1986).

In plants, bromacil is taken up rapidly by the roots and slightly absorbed through the leaves (Menzie, 1974 and WSSA, 1989). When it is applied at 10 ppm, some types of algae have slowed growth, but most strains are unaffected (Mar, 1975). Improper application of bromacil will destroy shade trees and other desirable vegetation. Label instructions should be followed carefully. Equipment and containers should not be emptied or rinsed out near desirable trees or shrubs (VanDriesche, 1985).

The median tolerance limit, or the concentration of bromacil that will kill 50% of the exposed fish after 48 hours of exposure, varies from 40 ppm to 164 ppm, depending on the type of fish tested (Clayton *et al*., 1981). The 96-hour LC_{50} in fathead minnows is 182 mg/l (DuPont Agricultural Products, 1990). In addition to that bromacil is available in granular, liquid, water soluble liquid, and wettable powder formulations (Meister, 1992 and Gosselin *et al*., 1984).

2.1.3 Diuron

Diuron, one of the most commonly used herbicides, belongs to derivatives that represents an important class of contact herbicides applied in pre-emergence and post-emergence to control broadleaf weeds in a wide variety of annual and perennial broadleaf and grass weeds (Field *et al*., 1997 and Gooddy *et al*., 2002).

Diuron is relatively persistent in the environment (with a half-life of over 300 days). Its chemical name is 3-(3,4-dichlorophenyl)-l,l-dimethylurea. It has a water solubility of about 42 ppm (mg/l) at 25ºC (Extoxnet, 1996). Diuron has been found to be highly toxic to some nontarget organisms (Nebeker & Schuytema, 1998 and Teisseire *et al*., 1999). Its potential toxicity at cellular and subcellular levels has also been demonstrated (Chauhan *et al*., 1998).

Diuron is slightly toxic to birds. In bobwhite quail, the dietary LC50 is 1730 ppm. In Japanese quail and ring-necked pheasant, the LC_{50} greater than 5000 ppm. The LC_{50} is approximately 5000 ppm in mallard ducks. The LC_{50} (48 hour) values for diuron range from 4.3 - 42 mg/L in fish, and range from 1-2.5 mg/L for aquatic invertebrates. The LC_{50} (96-hour) is 3.5 mg/L in rainbow trout (WSSA, 1994 and U.S. National Library of Medicine, 1995). Thus, diuron is moderately toxic to fish and highly toxic to aquatic invertebrates.

2.2 Extent of pesticide usage in Gaza governorates

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). 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 pesticide 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).

Palestinian Central Bureau of Statistics (PCBS) reported in 2009 that the annual rate of use of pesticides reached 893.3 tons, consisting of about 160 types. Nineteen of them is internationally banned for health reasons. The Palestinian National Committee identified in 2007 about 242 active ingredients that are adequate for use and permitted for application in the agricultural land and public health sector (MOA Palestin, 2008). More than 250 metric tons of formulated pesticides are used annually in the Gaza strip, about 90% of which is imported and 10% manufactured locally (Safi, 2002). 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).

In Gaza strip, several reports have reported misuse of pesticides by shop owners, farmers and agricultural workers (Issa, 2000 and Safi *et al*., 2002). Similarly, several extremely toxic pesticides that are banned or restricted in many countries are still used in Gaza (Safi, 2002). Poor medical records, the absence of health surveillance and monitoring systems, absence of legislation and control systems for pesticides have resulted due to a lack of awareness of potential hazards associated with pesticide handling and use among shop owners, farmers and the public (Safi *et al*., 2002). 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). Since there are no restrictions on the sale and use of pesticides in Gaza, farmers have easy access to all pesticides including banned, highly toxic and restricted types. Additionally, no permit or special training is required before buying pesticides (Issa, 2000).

Quantities of pesticides imported in 2011 are shown in Table 2.1. It is obvious that herbicides quantities reached to 27054 kg in the year, which occupy the 3rd of the scale after insecticides and fungicides used in Gaza. These data indicate that the use of herbicides is very high compared to the agricultural area of Gaza Strip (MOA Palestine, 2011a) .

Table 2.1 Quantities of pesticides entering in 2011 during the whole year

Type of pesticides	Quantity/kg
Herbicide	27,054
Sterilize	93,035
Insecticide	220, 169
Fungicides	136, 477
Total	476,734

(MOA Palestine, 2011a)

Furthermore, the restricted herbicides that are used in Gaza is shown in Table 2.2. It can be seen that 9 herbicides are restricted from general use probably due to their possibility to contaminate ground water. It could be noticed that Alachlor, bromacil and diuron are among the banned use. More banned herbicides are shown in Table 2.2.

Tabl.2.2 Herbicide banned use in Gaza in 2011

Scientific the name	Traditional name
Alachlor	Alanex
Atrazine	Atranex 50
Bromacil	Hyver X
Bromoxynil Octanoate	Bromotryl
Ethdimuron	Ostolin
Fluazifop-p-butyl	Degnol F
Metolachlor-s	Dual S
Terbutryne	Tropotex
Diuron	Karmex

(MOA Palestine, 2012)

2.3 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 a substrate (Streibig, 1988). Bioassays have been used widely in weed science to evaluate the dose response, herbicidal selectivity, species specificity, soil persistence, product formulations, adjuvants, herbicide resistance, and other effects (Santelmann, 1977).

Usually herbicide bioavailability has been evaluated using bioassays with either root extension or the whole plant (Streibig 1988; Vasilakoglou *et al*., 2001 and Pannacci *et al*., 2006). Bioassays directly determine the biological activity of a soil active herbicide and this is intrinsically related to bioavailability (Günther *et al*., 1989). The results of these bioassays are now used to quantify potential injury to the rotational crop (Pestemer *et al*., 1980).

A bioassay can detect if herbicides or chemical residues are present in the soil at concentrations high enough to adversely affect crop growth, yield and quality (Abdur *et al*., 2001). The bioassay technique is a useful tool that complements the analytical methods and provides information regarding herbicide bioavailability for the plant and its possible phytotoxicity (Kotoula-Syka *et al*., 1993 and Stork & Hannah, 1996).

In spite of bosses being non-specific, the effect of all residual herbicides present in soil is measured by bioassays (Johnson *et al*., 2005). Parameters that are frequently assessed in plant bioassays are root or shoot length, fresh or dry weight of roots or shoots, leaf area or plant height, visual estimation of plant injury, physiological and morphological effects such as photosynthetic activity, water consumption, or chlorosis (Horowitz, 1976). These measurements are assessed relatively to a control sample which is needed because of the variation in plant growth in soils of different properties.

El-Nahhal (2003 and 2004) developed a bioassay technique that allowed to determine the relative concentration of herbicides in soil. A bioassays offered several advantages, i.e. detection of low-phytotoxic residues in soil and detection of bioavailability of herbicide residues (Rahman, 1993 and pestemr *et al*., 1980). However, the disadvantages of the bioassay techniques are represented in that, the examined plant may not give a true response at a high concentration. In addition, the adsorbed fraction of herbicides may not be available to the examined plant. However, at a low adsorbed fraction of herbicides, the herbicides may not be bio-available to the examine plant (El-Nahhal, 2004).

El-Nahhal *et al*., (2013a) reported that *Corochorus Olitorius* (Molokhia) is the best growth response at the various levels of diuron.

2.4 Toxicity of mixtures of herbicide

In mixture toxicity, concentration-effect data is often used to generate conclusions on combined effect of toxicants. Studies examining the toxicity of chemical mixtures have focused on neutral organic substances (Broderius *et al*., 1995 and Niederlehner *et al*., 1998), and pesticides (Anderson & Lydy, 2002 and Trimble & Lydy, 2006).

In more recent studies, Christensen et al., (2007) studied the mixture and single-substance toxicity of selective serotonin reuptake inhibitors (SSRIs) toward algae and crustaceans. They demonstrated that the mixture toxicity of the SSRIs in the two bioassays is predictable by the model of concentration addition. Phenylurea herbicides are considered to be moderately to highly toxic to aquatic and these herbicides can inhibit photosynthesis by blocking electron transfer organisms (Breugelmans *et al*., 2007).

Koutsaftis &Aoyama, (2007) evaluated the toxicity of four antifouling biocides and their mixtures on brine shrimp. They observed synergistic interactions suggesting that water quality guidelines based on individual compounds may underestimating the adverse effects of combination of these chemicals. Andreozzi *et al*., (2008) evaluated the toxicity of ethyl parathion and cumene hydroperoxide by means of algal bioassays. Interactions between companion herbicides may significantly modify the biological behavior of each and every single herbicide in the mixture. These interactions often result in a reduction or an increase of the activity of the combined herbicides compared with activities when each one of them is applied alone.

Practically, the optimum herbicide combinations would be those that exhibit enhanced activity on target weed species and decreased toxicity on crops (increased selectivity). This, however, is difficult to predict since the behavior of each single herbicide in the mixture is often affected by the presence of the other(s) and the activity of the mixture may also vary considerably depending on plant species, growth stage, and environmental conditions (Damalas, 2004).

Aquatic ecosystems are frequently contaminated by mixtures of pesticides, often with differing modes of action (e.g. an insecticide and a herbicide) (Lydy et al., 2004 and Gilliom et al., 2006) resulting in the potential for community-level effects (Relyea & Hoverman, 2006).

❖ Types interactions of herbicide mixtures

The result of an interaction between two or more herbicides after their application in the mixture may be additive, synergistic, or antagonistic. In the first case, the activity of the mixture is equal to the sum of the activities of all herbicides in the mixture when these herbicides are applied separately. In the second and the third cases, however, the activity of the mixture is greater or lower, respectively, than the sum of the activities of all herbicides in the mixture when these herbicides are applied separately (Hatzios & Penner, 1985). It is obvious that in the case of antagonism, where the activity of the mixture is reduced, greater application rates of the affected herbicides are required, whereas in the case of synergism, where the activity of the mixture is enhanced, application rates can be reduced.

Antagonistic interactions in herbicide mixtures often cause significant problems in weed control. For example, the application of pyrithiobac in mixture with fluazifop-P has been reported to reduce the efficacy of fluazifop-P on large crabgrass (Digitaria anguinalis) (Ferreira et al., 1995).Similarly, the application of tribenuron in mixture with diclofop has been reported to reduce the efficacy of diclofopon wild oat (Avena fatua) (Baerg et al., 1996). It is obvious that such herbicide combinations should be avoided. Antagonistic interactions, however, may be considered beneficial when they reduce herbicide activity on crops. For example, according to (Deschamps et al., 1990), mixtures of fenoxaprop with 4-chloro-2-methylphenoxy acetic acid showed reduced toxicity of fenoxaprop on wheat and barley compared with fenoxaprop, applied alone.

Furthermore, mixtures of thifensulfuron with bentazon showed reduced toxicity of thifensulfuron on soybean compared with thifensulfuron applied alone (Hart & Roskamp, 1998 and Lycan & Hart, 1999). Therefore, such herbicide combinations appear desirable unless antagonism on weeds also occurs. Synergistic interactions may be particularly beneficial when they result in more effective control of troublesome weeds. For example, (Scott *et al.*, 1998) found that mixtures of sethoxydim with dimethenamid were more effective on johnsongrass (*Sorghum halepense*) compared with separate applications. It is obvious that such herbicide combinations are particularly useful for more effective weed control.

Synergistic interactions, however, may cause significant problems when they result in increased herbicide activity on crops. For example, mixtures of ethametsulfuron with haloxyfop, fluazifop, fluazifop-P, quizalofop, and quizalofop-P may cause phytotoxicity and yield losses in *Brassica napus* and *Brassica rapa* (Harker *et al*., 1995). Furthermore, mixtures of thifensulfuron (sulfonylurea) with imazethapyr (imidazolinone) may cause phytotoxicity in soybean resistant to sulfonylureas (Simpson & Stoller, 1996).

2.5 Tested plants crops

Agriculture plays an important role in the Palestinian economy contributing to food requirements and providing jobs to more than 50% of the population (ICARDA, 2003). The total area of agricultural land in occupied Palestinian territories approximately 1.207.061 donums, 1.105,146 donums of which in west bank and 101,915 donums in Gaza governorates (MOA Palestine, 2011b).

2.5.1 Wheat

Wheat (*Triticum spp*) is the most important cereal crops in the world that is adapted to the Mediterranean region; it is the second most produced food among the cereal crops (PCBS, 2009).

wheat is the leading source of vegetable protein in human food, having a higher protein content than other major cereals, maize (corn) or rice. It is currently second to rice as the main human food crop (Aguiar and Santana, 2002).

Wheat kernel contains 2 to 4% germ and most nutrients with the exception of starch are concentrated in the germ, the germ is the richest known natural source of tocopherol, abundant in B-group vitamins and protein of high biological value (Tsen, 1985) and its oil of favourable fatty acid pattern (Paul et al., 1987), because of their beneficial nutritional values.

Wheat grain is a staple food used to make flour for leavened, flat and steamed breads, biscuits, cookies, cakes, breakfast cereal, pasta, noodles, couscous (Rapsomanikis *et al*., 2003). The number of wheat crops holdings in the Palestinian territories about 13.677 donum (MOA Palestine, 2012).

2.5.2 Molokhia

Molokhia (*Corchorus Olitorius L.*) is an essential green, leafy edible vegetable, consumed in many countries. Leaves of Molokhia are used for human consumption. The leaves are highly nutritious rich in proteins, vitamins A, C and E, beta-carotene, iron, calcium, thiamin, riboflavin, niacin, folate, dietary fiber, and most essential amino acids (Tulio *et al*., 2002 and Ogunrinde & Fasinmirin, 2011). Molokhia infusion and extracts with different polarities, and for their polyphenolic contents and antioxidant activities, were studied. It was concluded that higher antioxidant activity was seen in the samples with higher phenolic content (Öztürk & Favaroğlu, 2011). Different characteristics of Molokhia, e.g. morphological, biochemical, and molecular analysis of genome along with pest management, retting procedures, and tissue culture, were reviewed (Maity *et al*., 2012). The heavy metals presented in different vegetables were investigated in several areas, to check the level of these metals in the vegetables. Jew's mallow contained high levels of heavy metals in polluted areas, and this affect the residents' health (Ali & Al-Qahtani, 2012 and Doherty *et al*., 2012).

Despite that the Jew's mallow (Molokhia) is a leading leafy vegetable cultivated and traded in many countries, few statistical data on the production and marketing tendency of the crop are available (MOA Palestine, 2012). In Palestine, the total cultivated area of Molokhia in 2010 was 2458.5 donum. Among them, 1602.1 donums grown in open irrigated lands and 762.7 donum in plastic houses (PCBS, 2011), however the total cultivated area of Molokhia in 2011 in Gaza 7988 donums (MOA Palestine, 2012).

2.5.3 Watermelon

Watermelon (*Citrulus lanatus*) is native to tropical Africa and it is a popular thirst-quencher during hot summer weather (Perkins-Veazie & Collins, 2004). Watermelon juice is proven to be a very concentrated source of carotenoid, namely lycopene. The lycopene content of watermelon is found to be higher than many other fruits and vegetables. Also, it contains about 6% sugar and 91% water by weight (Holden, *et al*., 1999). As with many other fruits, it is a source of vitamin C and vitamin A (Edwards *et al*., 2003).

The amino-acid citrulline was first extracted from watermelon and analyzed (Wada, 1930). Watermelons contain a significant amount of citrulline and after consumption of several kilograms, an elevated concentration is measured in the blood plasma; this could be mistaken for citrullinaemia or other urea cycle disorders (Mandel, *et al*., 2005). The total cultivated area of watermelon in 2011 in Gaza strip 14846 donums (MOA Palestine, 2012b).

Chapter 3

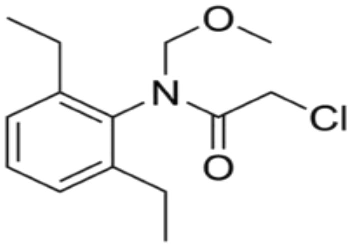
Materials and Methods

3.1 Materials and Instruments used in research

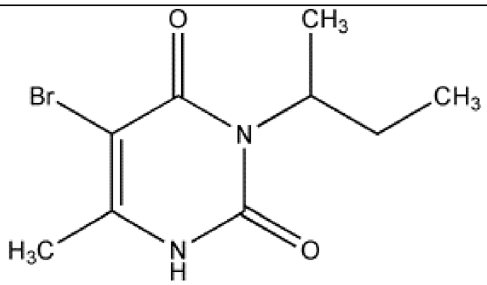
- Alachlor was purchased from Monsanto USA, however bromacil, diuron were purchased from SIGMA, Germany. These chemicals were used as standard materials for the study. The three selected herbicides. Some physical properties and the chemical structures of alachlor, bromacil and diuron were presented in Table (3.1) and Figure (3.1).
- Molokhia, watermelon and wheat seeds and plastic pots were purchased from a local certified shop for agricultural products in Gaza.
- Pure sandy soil, free from herbicides or any pesticides were obtained from Bait Lahia.
- A balance to weight soil and an analytical balance for sensitive weight of pesticides.
- Volumetric flask (1000 ml) was used for the preparation of stock solution.
- Distilled water to dilute the solution and potable tap water for watering plants.
- A graduated pipette and a slender glass tube were used to measure and dispense liquid with a very high degree of accuracy and precision.
- A plastic bags were used for homogenizing soil with the herbicides solution.

Table 3.1 physical properties of herbicide (Wauchope *et al*., 1992; Kidd &James, 1991)

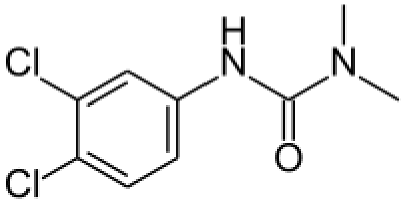
Herbicide	**Chemical name**	**in Solubility water(mg/l)**	**Adsorption coefficient (K_{ow})**
Alachlor	2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetamide	240	170
Bromacil	5-bromo-3-(butan-2-yl)-6-methylpyrimidine-2,4(1H,3H)-dione or 5-bromo-3-sec-butyl-6-methyluracil	813	32
Diuron	N-(3,4-dichlorophenyl)-N,N-dimethyl urea	42	480



Alachlor



Bromacil



Diuron

Figure 3.1 Chemical structures of Alachlor, Bromacil and Diuron (Kidd & James,1991**)**

3.2 Methods

3.2.1 Soil Collection

Used soil was collected from agricultural land in Bait Lahia, North of Gaza. The farm has not used alachlor or bromacil or diuron for 4 years. Soil sample was air-dried before storing for any extended length of time. When dry, crush clods to pea-sized particles soil and sieved through a 2-mm. A plastic experimental pots (3 replicates) were used.

3.2.2 Preparation of herbicide stock solutions

For a stock solution preparation, the required amount of the herbicides was weighted as following: alachlor 0.1055g, diuron 0.0206g and bromacil 0.02g. After that,the amount of herbicide was put in a clean volumetric flask 1000ml. Then, distilled water was added in the flask on many times until 1000 ml and shaking should be continued to dissolve the herbicide. In addition to that, the flask was added into a shaking machine for 12 hours with the addition of magnetic stirrer to help in dissolving the herbicide in a solution of water. After that, close the pipe tape to prevent volatilization material, or pollution. Finally, stored them in a place far from the sun to prevent photolysis.

3.2.3 Individual phytotoxicity tests of herbicides

In this procedure:

- Gradient concentrations of herbicides were prepared as the following: (0.0, 0.055, 0.11, 0.22, 0.44, 0.88 and 1.76 mg was mixed with 1kg of soil in a plastic bag, and distributed in pots. Ten seeds of molokhia, wheat and watermelon were planting in pots as shown in (Figure 3.1). The pots were kept under controlled conditions (green house) for 2 weeks and irrigated with tap water(El-Nahhal, 1998).

Figure 3.2 Cultivation of watermelon seeds in the soil (emergence of seeding)

- Two weeks after germination the biomass was collected and used as an indicator of growth inhibition by measuring the length of the stems as shown in (figure 3.3). Growth inhibition was made according to El-Nahhal et al. (1998), growth inhibition of the tested plant, was evaluated by plotting % (GI) = 100* (BM_c-BM_t) /BM_c versus herbicide concentrations.

Figure 3.3 Measurement of the stem length

Where BM_c and BM_t are the biomass of test plant in the control and the treated samples, respectively.

Due to the high sensitivity of molokhia plant (El-Nahhal et al., 2013) a very diluted solution was used . The concentrations of herbicides were as the following: (0.0; 0.005; 0.01; 0.02; 0.075; 0.1 and 0.15 mg ai/kg soil).

3.2.4 Phytotoxicity of herbicides as mixture

3.2.4.1 Binary mixtures toxicity

Binary mixtures toxicity of 3 herbicides of alachlor, bromacil and diuron which represent different chemical classes were mixed together, the mixtures with the ratios of 1:1 (v/v) were prepared from stock solutions to measure the effect of the mixture on growth as shown in (Table 3.2).

Plotting % growth data versus the concentration of the herbicide was analyzed by linear regression to calculate the EC_{50}.

The same procedure used in the other mixture of bromacil + diuron and alachlor +bromacil.

Table 3.2 Binary mixture toxicity of herbicide.

	Concentration of Herbicide as binary mixture(mg/l)		
	Alachlor	Bromacil	Diuron
CO	0	0	0
C1	220	813	42
C2	198	81.3	4.2
C3	154	243.9	8.4
C4	110	406.5	12.6
C5	66	569.1	21
C6	22	731.7	29.4

3.2.4.2 Tertiary mixtures of herbicide

Tertiary mixture toxicity of 3 herbicides, alachlor, bromacil and diuron which represent different chemical classes and they were mixed together. The mixtures with the ratios of 1:1:1 (v/v/v) were prepared from stock solutions to measure the effect of the mixture of growth as shown in Table 3.3. Plotting % growth data versus the concentration of the herbicide was analyzed by linear regression to calculate the EC_{50}.

Table 3.3 Tertiary mixture toxicity of herbicide alachlor, bromacil and diuron

	Concentration of Herbicide as tertiary mixture(mg/l)		
	Alachlor	Bromacil	Diuron
CO	0	0	0
C1	73.26	271.5	14
C2	55	203.25	21
C3	55	406.5	10.5
C4	110	203.25	10.5
C5	33	569.1	6.3
C6	33	121.95	29.4

3.2.5 Calculation of herbicides toxicity

a. Growth inhibition

According to El-Nahhal *et al.*, (1998), the % growth inhibition (GI) as toxicity indicator was calculated as the following: $\% (GI) = 100 * (BM_c - BM_t) / BM_c$. The toxicity (% GI) at a variant concentration of herbicides is evalued by plotting % growth inhibition.

b. Toxic units (TUS) in mixtures

According to Sprague & Ram say (1965), toxic units were calculated as the following:

Toxic units = actual concentration in solution / lethal threshold concentration Furthermore, Ishaque et al., (2006) defined toxic units as the concentration of a chemical in the toxic mixture divided by its single toxic concentration to the end point measured.

c. Mixture toxicity index in mixtures

To estimate the synergestic and / or antagonistic effects of herbicides mixtures we calculated the mixture toxicity index (MTI) proposed by Konemann, (1981) and Hermens *et al.*, (1985). MTI = 1- (Log M / Log n), where M = sum of the TUs in a mixture which produced the given response in Konemann's work. n = total number of compounds in the mixture. Table (3.5) shows the scale of mixture toxicity.

Table (3.4) : Mixture toxicity scale (Konemann, 1981).

MTI	**Classification for toxicity of mixture**
MTI < 0	**Antagonism effect**
MTI = 0	**No addition effect**
0 < MTI < 1	**Partial addition effect**
MTI = 1	**Concentration addition effect**
MTI >1	**Supra addition (synergistic)**

All samples were kept in the same conditions . The 0.0 concentration always means control sample (El-Nahhal *et al.*, 1998). EC50 was estimated by the same way described in bioassay test (Bonnet *et al.*, 2007; El-Nahhal *et al.*, 1998).

3.3 Statistical analysis

All experiments were performed in three replicates. Averages and standard deviation of the growth inhibition were calculated the regression analysis. The averages of growth inhibition were compared by Tukeys test and P-values were determined to evaluate the differences among treatments as the following, averages and standard deviation of the relative concentrations was calculated the regression analysis, averages may be compared by Tukey’s test at = 0.05, following one-way ANOVA and plotting % growth data versus the concentration of the herbicide was analyzed by linear regression to calculate the EC_{50}.

Chapter 4

Results

The results of the study shows the phytotoxic effect of a single and mixtures of the three herbicides (alachlor, bromacil and diuron) on three plants (watermelon, molokhia and wheat) in different concentrations and estimating of EC_{50} values of herbicide mixture.

4.1 Effect of herbicides as a single test

Effects of Alachlor, Bromacil and Diuron as administered separately on watermelon, molokhia and wheat growth are shown in Figures 4.1-4.3 respectively.

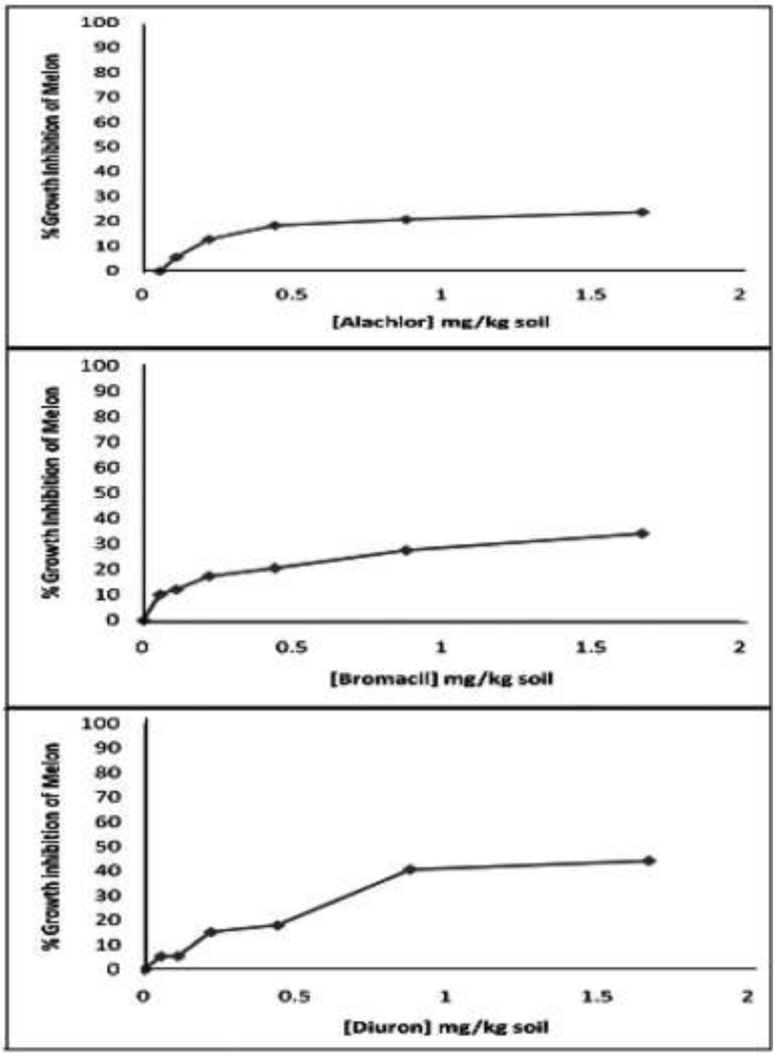


Figure 4.1. Effect of alachlor, bromacil and diuron as a single test on watermelon growth.

Figure 4.1 shows toxicity of alachlor, bromacil and diuron on watermelon growth. It is clear that there are increase growth inhibition of watermelon as concentrations of the tested compound increased in soil.

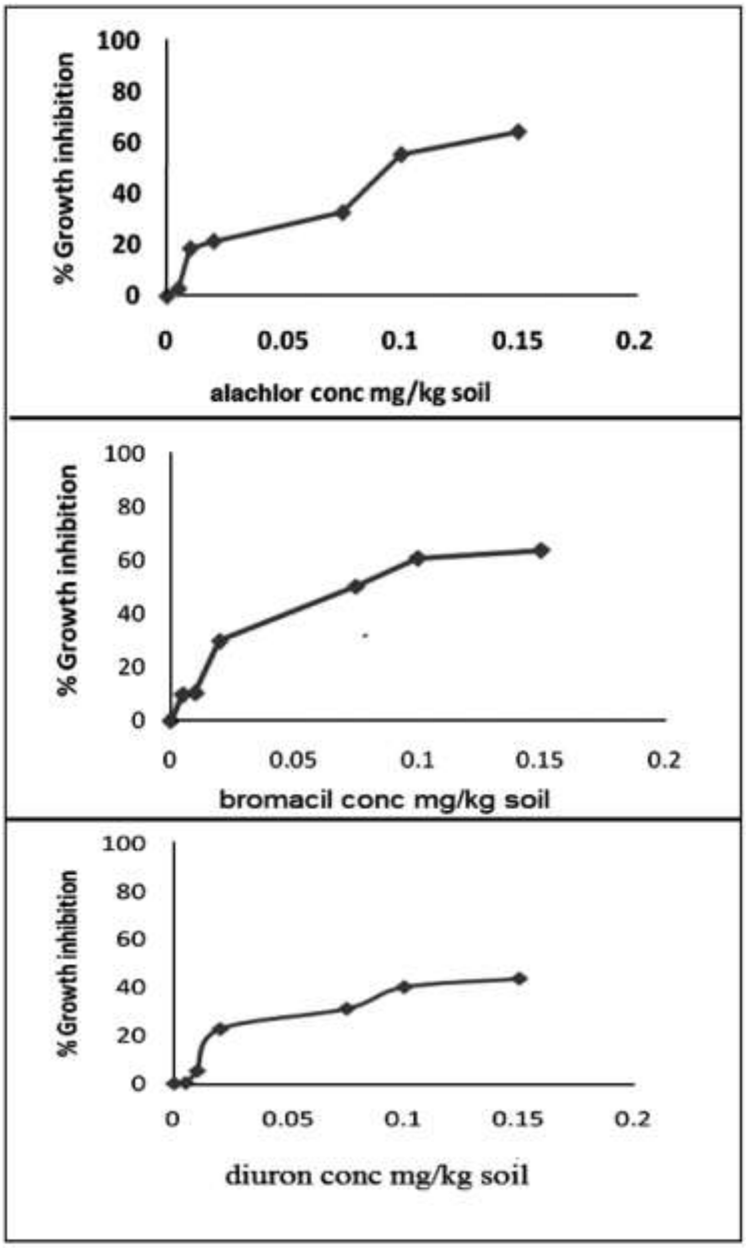


Figure 4.2. Effect of alachlor, bromacil and diuron as a single test on molokhia growth

Figure 4.2 shows the toxicity of alachlor, bromacil and diuron on molokhia growth. It is clear that there are increasing growth inhibition of molokhia at concentrations of the tested compound increased in soil.

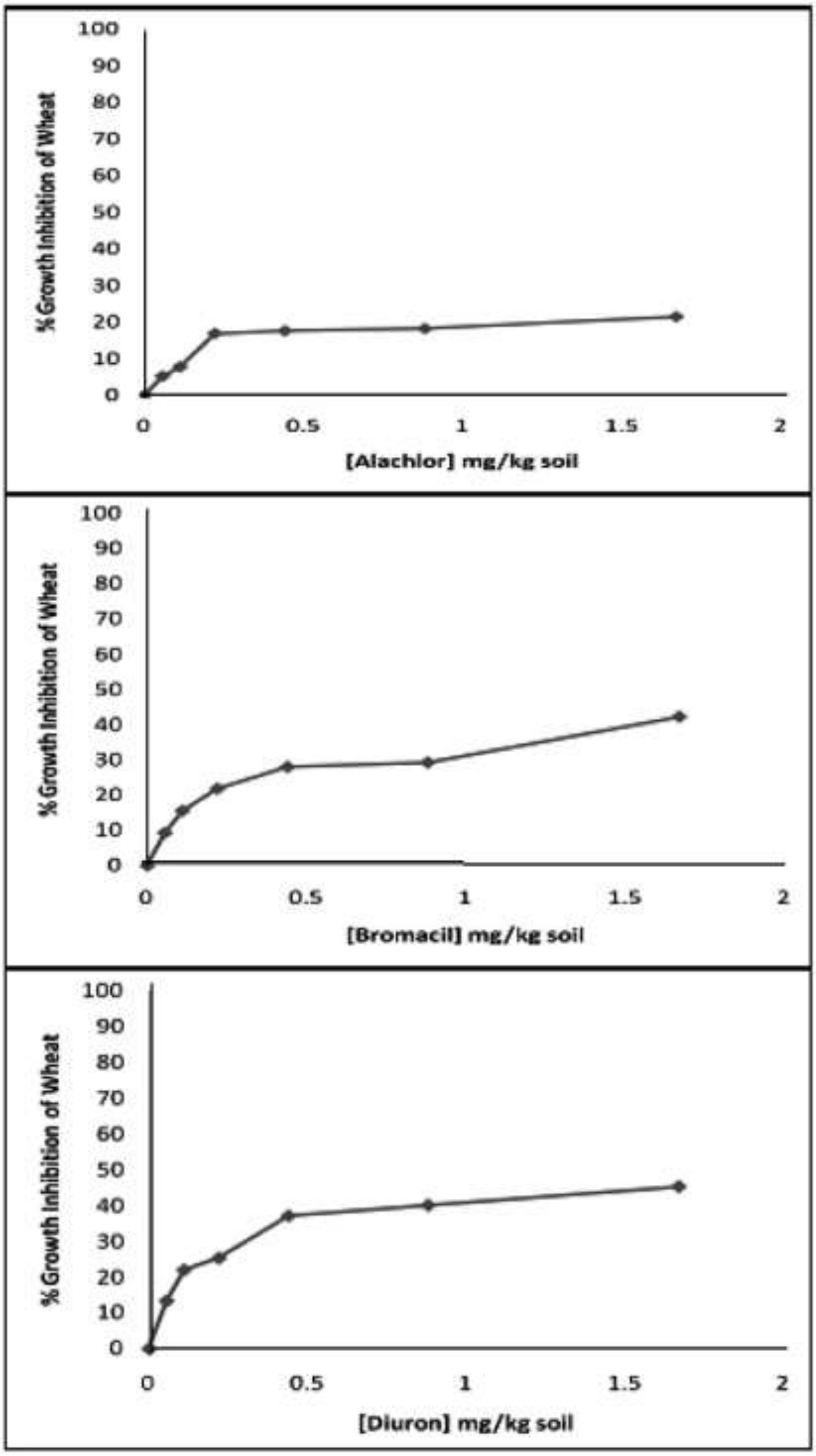


Figure 4.3 Effect of alachlor, bromacil and diuron as a single test on Wheat growth

Figure 4.3 shows the toxicity of alachlor, bromacil and diuron on watermelon growth. It is clear that there are increasing growth inhibition of watermelon at concentrations of the tested compound increased in soil.

A comparison of the phytotoxicity parameters of the tested herbicides among watermelon, molokhia and wheat is shown in Table 4.1.

Table 4.1 Phytotoxicity parameters of Alachlor, Bromacil and Diuron on Watermelon, Molokhia and Wheat.

Melon			
Herbicide	EC_{50} mg/kg soil	Equation	R^2
Alachlor	11.37	Y= 0.2798X+ 1.4045	0.958
Bromacil	4.77	Y=0.3615X+1.4538	0.993
Diuron	1.64	Y=0.6904X+1.55	0.946
Molokhia			
Alachlor	0.11	Y= 0.4598X+ 2.1444	0.909
Bromacil	0.08	Y=0.6077X+2.3673	0.939
Diuron	0.24	Y=0.3291X+1.9066	0.947
Wheat			
Alachlor	3.91	Y= 0.4282X+ 1.2922	0.946
Bromacil	3.08	Y=0.3342X+1.5355	0.953
Diuron	1.83	Y=0.3381X+1.61	0.957

Furthermore, the statistical analysis of the tested herbicides among the test plants is shown in Table 4.2.

Table 4.2 P-values of the tested compounds among plants

	Molokhia	Watermelon	Wheat
Al X Di	0.10	0.09	0.00
Al X Br	0.00	0.00	0.13
Br X Di	0.00	0.00	0.00

Furthermore, the effects of alachlor concentrations on molokhia growth can be visualized in Figure 4.4. It shows a reduction of the growth of watermelon as alachlor increased in soil.

Figure 4.4. Effects of alachlor concentrations (individual test) on Molokhia growth

CO, C1-C6 represents the control sample and the tested concentration representivly. More detail are shown in Tables 3.2 and 3.3.

4.2 Effect of herbicides as Binary Mixtures

Effects of binary mixtures (50% diuron and 50% alachlor), (50% diuron and 50% bromacil) and (50% bromacil and 50% alachlor) on watermelon, molokhia and wheat are shown in Figures 4.5-4.7.

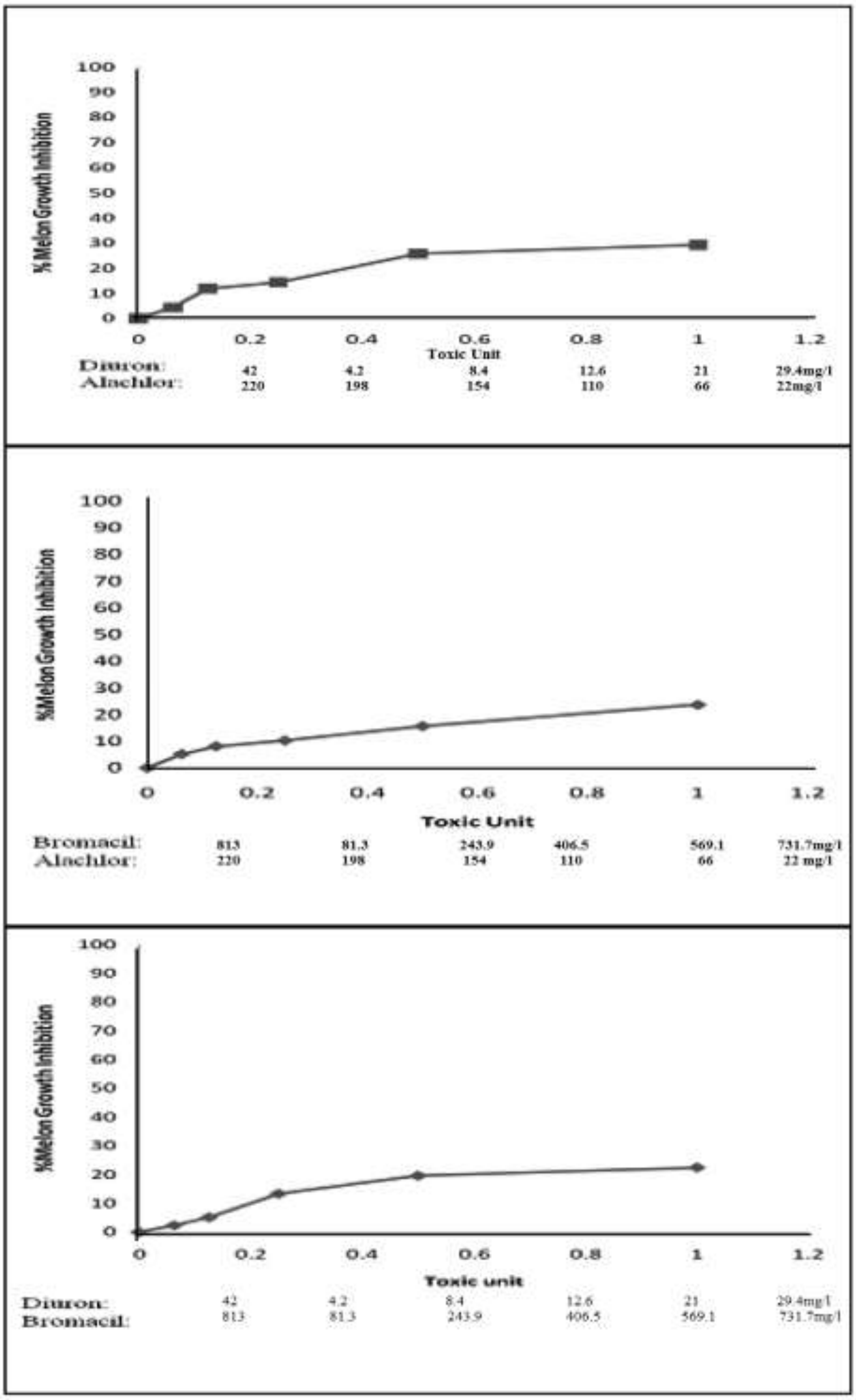


Figure 4.5. Effect of binary mixture on watermelon growth.

Figure 4.5 shows toxicity of alachlor, bromacil and diuron on watermelon growth as toxic units. It is clear that there are increasing growth inhibition of watermelon as toxic units increased in soil.

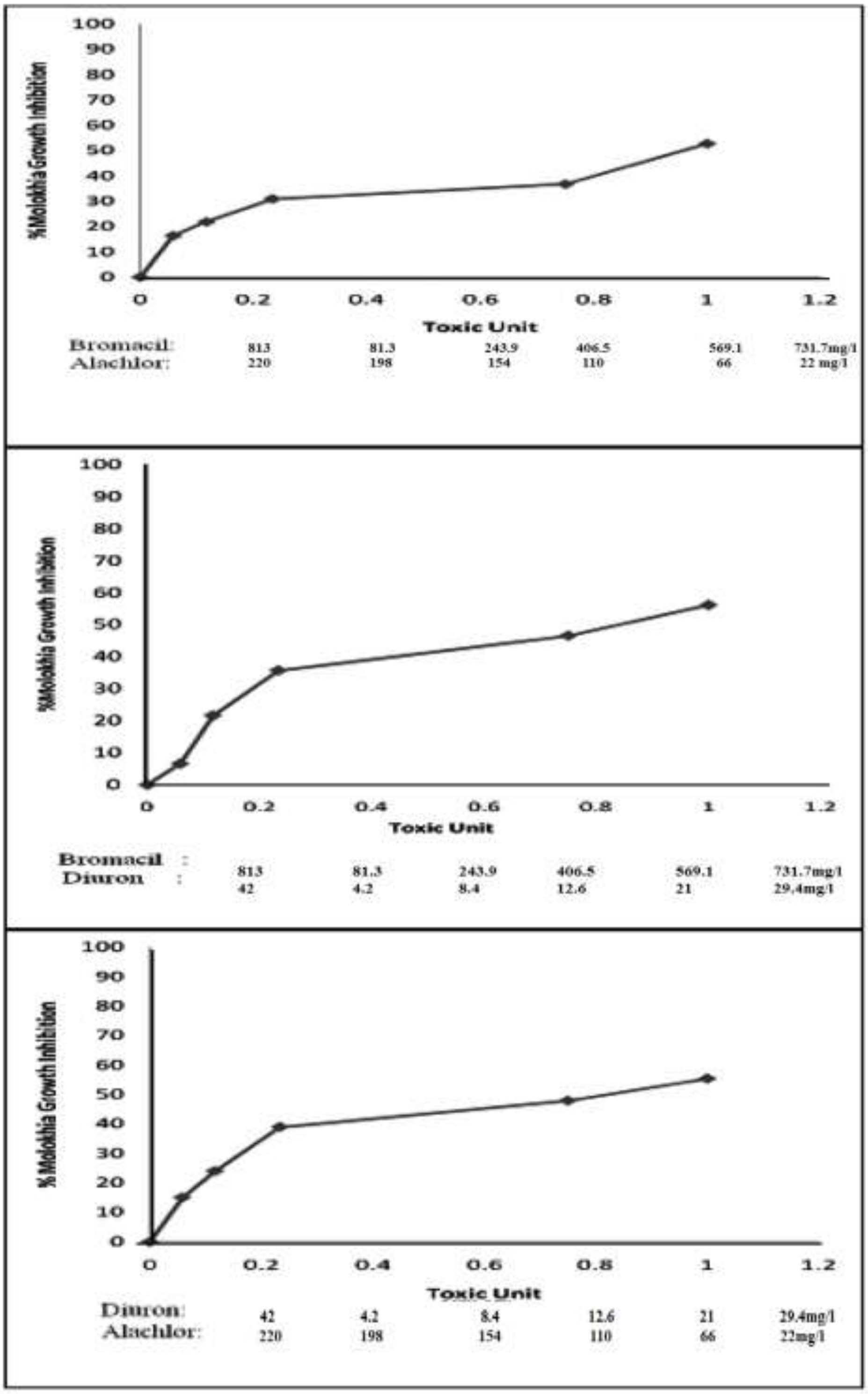


Figure 4.6. Effect of binary mixture on molokhia growth.

Figure 4.6 shows toxicity of alachlor, bromacil and diuron on molokhia growth as toxic units. It is clear that there are increasing growth inhibition of watermelon as toxic units increased in soil.

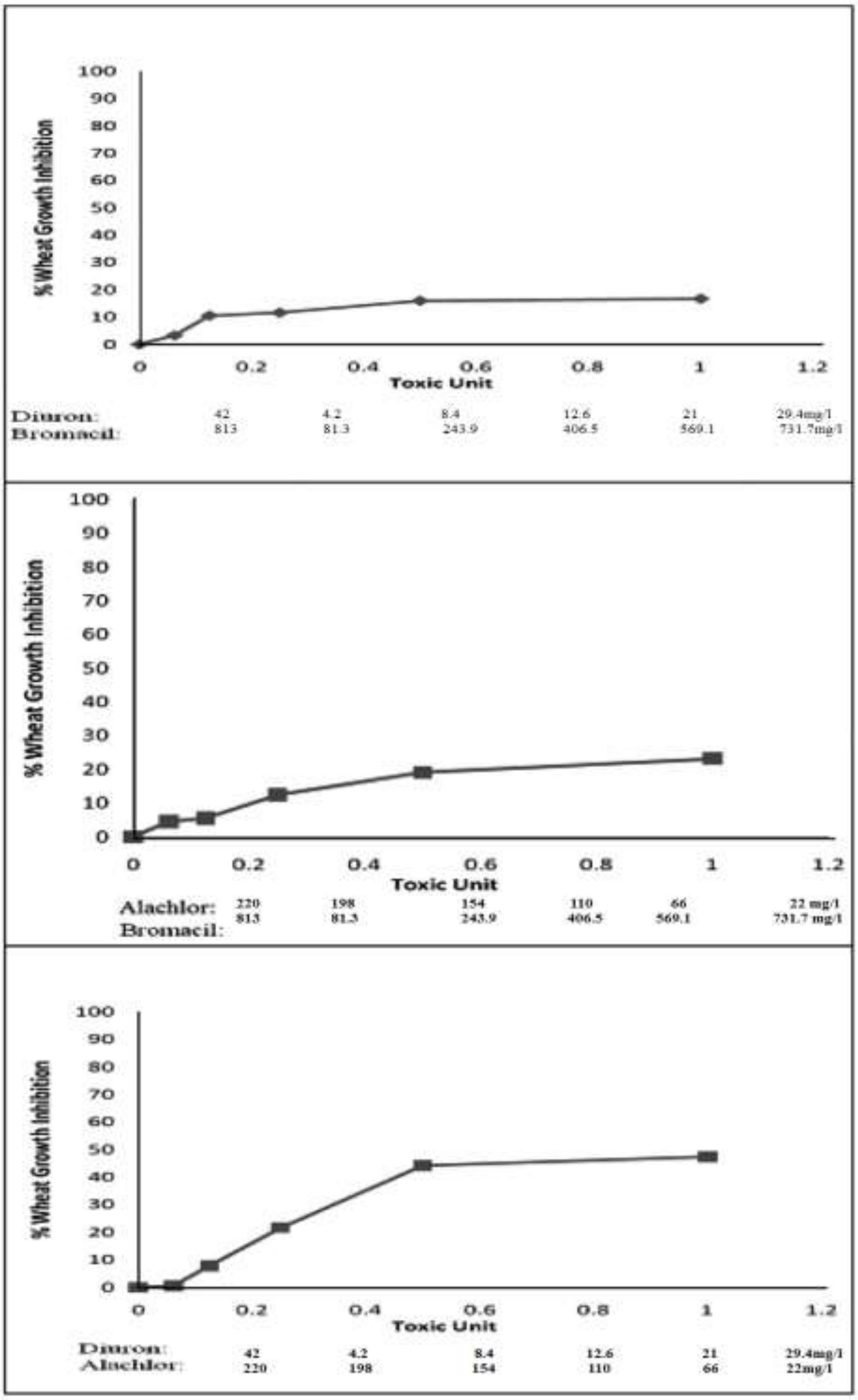


Figure 4.7 Effect of binary mixture on wheat growth

Figure 4.7 shows the toxicity of alachlor, bromacil and diuron on molokhia growth as toxic units. It is clear that there are increasing growth inhibition of molokhia as toxic units increased in soil.

Table 4.3. Phytotoxicity parameters of binary mixtures of alachlor, bromacil and diuron on Watermelon, Molokhia and Wheat.

Watermelon			
Mixture	**EC_{50} (TU/kg soil)**	**Equation**	**R^2**
Al+Di	8.92	Y=21.142X + 29.912	0.967
Al+ Br	83.51	y+14.819X + 21.522	0.933
Br +Di	28.52	y=18.176X + 23.55	0.972
Molokhia			
Al + Di	0.72	y=31.288X + 54.512	0.975
Al + Br	1.35	y= 25.529X + 46.639	0.894
Br + Di	0.73	y=37.062X + 55.003	0.973
Wheat			
Al + Di	0.982	y=43.142 X+50.33	0.955
Al+Br	38.1	y=16.956X + 23.194	0.964
Br +Di	925.4	Y=10.751X + 18.109	0.902

Furthermore, statistical analysis of binary effects on watermelon, molokhia and wheat growth is shown in Table 4.4.

Table 4.4: P-values of the tested compounds among plants as binary mixture

Mixture	**Molokhia**	**Watermelon**	**wheat**
(Al+Di)X(Br+Di)	0.33	0.00	0.37
(Al +Br)X(Br+Di)	0.29	0.24	0.06
(Al+ Br)X(Al+Di)	0.15	0.00	0.10

Furthermore, effects of binary mixtures on the test plants can be visualized in Figure 4.8

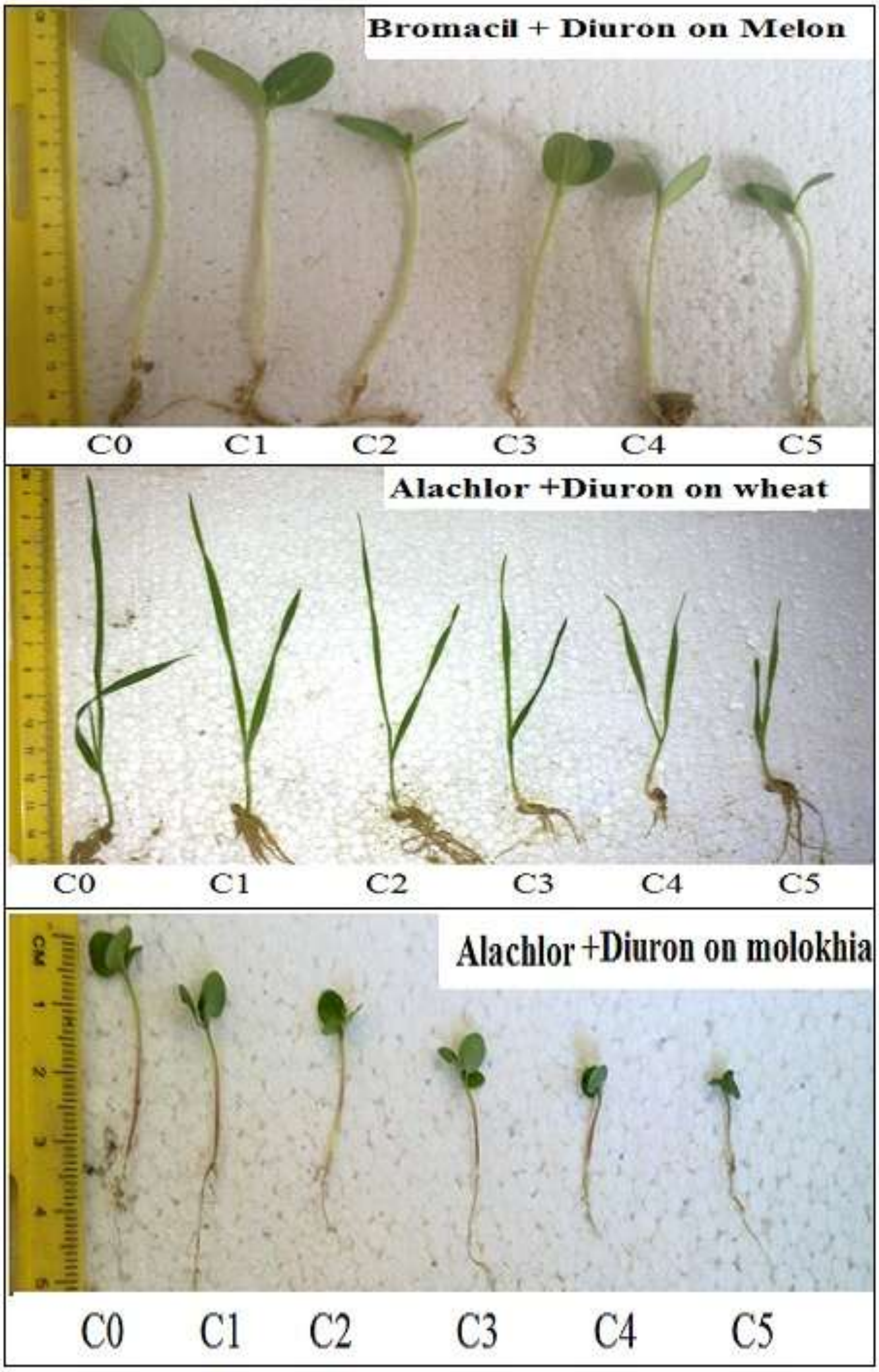


Figure 4.8. Phytotoxicity of binary mixture on watermelon, wheat and molokhia

CO, C1-C6 represents the control sample and the tested concentration representivly. More detail are shown in Tables 3.2 and 3.3.

Figure 4.8 shows the effect of different mixture on watermelon, wheat and watermelon growth. It's clear that the growth, decrease when the concentration of the herbicide mixture increased in soil.

4.3 Effects of Tertiary mixtures of herbicides on plant growth

The effect of Alachlor, Bromaci and Diuron as tertiary mixtures at (33.3: 33.3: 33.4%) on melon, molokhia and wheat growth is shown in Figures 4.9.

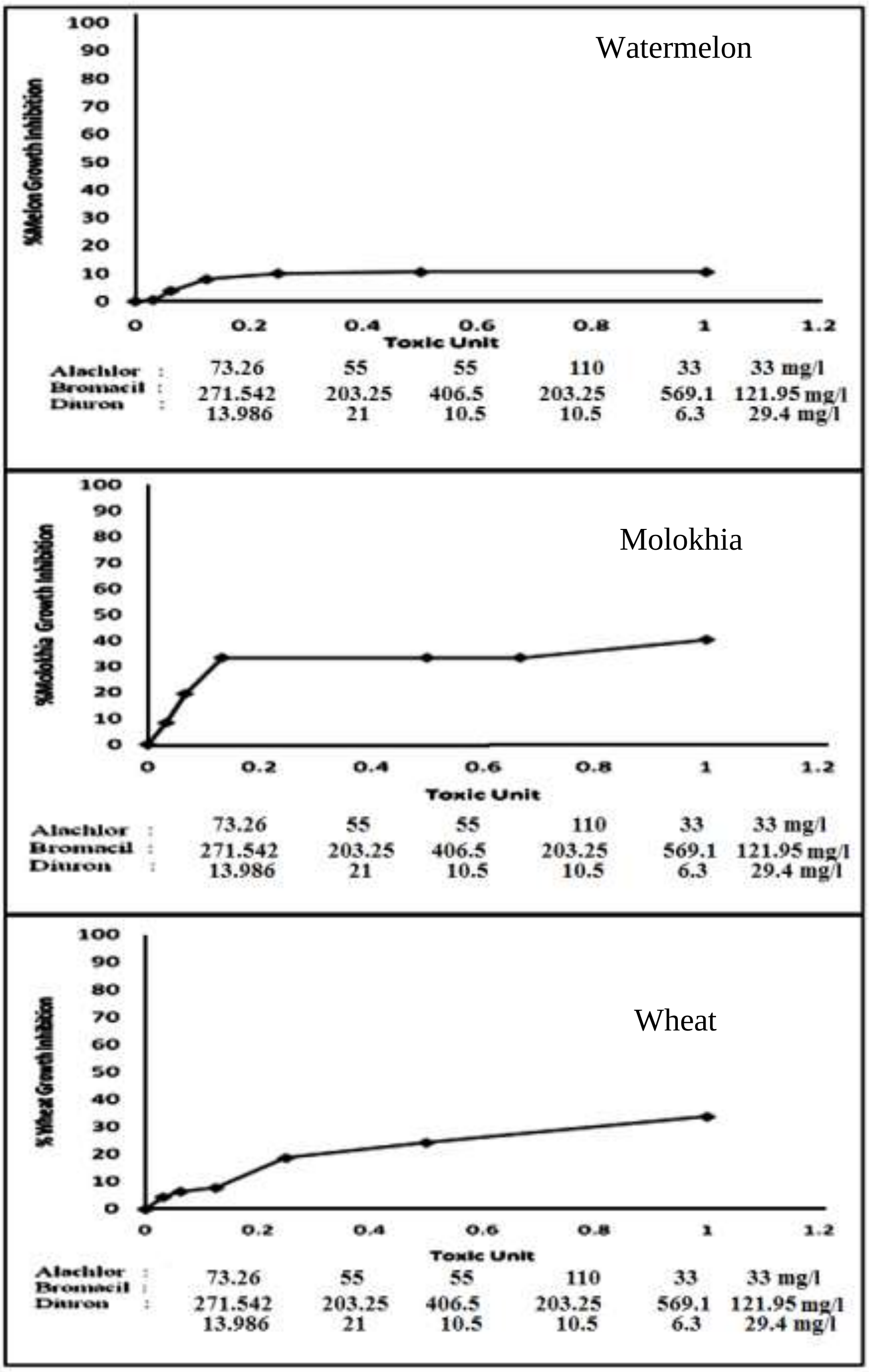


Figure 4.9. Effect of tertiary mixture on Watermelon, Molokhia and Wheat growth.

Figure 4.9 shows the toxicity of alachlor, bromacil and diuron on three plants as toxic units. It is clear that there are increase of growth inhibition of plant as toxic units increased in the soil and see that the watermelon in least effect from wheat as a tertiary mixture of herbicide.

A comparison of the phytotoxicity effect as a tertiary mixture on plant is shown in (Table 4.5).

Table 4.5. Phytotoxicity parameters of tertiary mixture compounds on plant.

Mixture	**Plant**	**EC$_{50}$ (TU/ kg oil)**	**Equation**	**R^2**
Al+Br+Di	Wheat	9	Y=19.996X + 30.918	0.93
Al+Br+Di	Watermelon	11060.65	y=8.771X + 14.532	0.93
Al+Br+Di	Molokhia	1.93	Y=21.221X + 43.927	0.77

Furthermore, statistical analysis of tertiary mixture show the effects on watermelon, molokhia and wheat growth in (Table 4.6). Vesiual effects of tertiary mixture on watermelon, Molokhia and wheat growth are shownin Figure 4.10.

Table 4.6 P-values of the tertiary mixture among plants

Crop	Al+Br+Di
Wheat X Watermelon	0.1
Watermelon X Molokhia	0.002
Molokhia X Wheat	0.03

Figure (4.10): Phytotoxicity of tertiary herbicide mixture on watermelon, wheat and molokhia

CO, C1-C6 represents the control sample and the tested concentration representivly. More detail are shown in Tables 3,2 and 3.3.

Figure 4.10 shows the effect of the tertiary mixture on watermelon, wheat and molokhia growth. It's clear that the growth, decrease when the concentration of the herbicide mixture increased in soil.

The mixtures Toxicity Index (MTI) is shown in Table (4.7). Its show the result of an interaction between two or more herbicides after their application in the mixture may be additive, synergistic, or antagonistic.

Table 4.7 Mixtures Toxicity Index (MTI) of mixture.

Plant	**Mixture**	**MTI**
Watermelon	Al+Br	-9.57
	Al + Di	-6.3
	Br+Di	-8.02
	Al+Br+Di	-6.96
Molokhia	Al+Br	-7.08
	Al + Di	-6.16
	Br+Di	-6.2
	Al+Br+Di	-1.33
Wheat	Al+Br	-8.43
	Al + Di	-3.12
	Br+Di	-13.04
	Al+Br+Di	-0.49

Chapter 5

Discussion

The tested compounds are herbicides and used widely in Gaza strip for Weed control (MOA Palestine, 2012).The data presented in Figures 4.1-4.3 clearly demonstrated the effects of alachlor, diuron and bromacil on watermelon, molokhia and wheat plants. It is obvious that % growth inhibition (Effect of herbicide) on watermelon, Molokhia and wheat increased linearly as the concentration of alachlor, bromacil or diuron increased in the soil up to 0.5 mg/kg soil. Furthermore, above 0.5 mg/kg soil of each herbicide nearly a steady increase of growth inhibition was observed in all cases. However, % growth inhibition did not exceed 50% in watermelon and wheat, whereas it did in molokhia.

The explanation of these results is that, at low concentration of herbicide, the compounds are available in the soil solution for plant uptake and accordingly considerable growth inhibition of the tested plants was observed. Whereas at high concentrations above 0.5 mg/kg soil, the herbicides tend to be distributed homoginized in the soil or may leached down the root zone, consequently, a reduction of growth inhibition may be observed. This suggestion is supported by the results of El-Nahhal *et al*., 1998, 1999, where they found reductions of growth inhibition due to leaching of herbicide concentration in deeper soil depths.

Furthermore, it has been shown that these plants may be resistant to the tested herbicides due to their enzymatic system. Beyer *et al*., (1988) and Kudsk & Streibing, (2003) found resistant of some plant due herbicide application. In addition, El-Nahhal *et al*., (1998) found different effects of alachlor on different tested plants. Recently, Awad, (2012) and Abadas, (2013) found different growth inhibition to the same herbicide. Moreover, the calculated EC50 value of each herbicide, included the phytotoxicity to the test plants. It is noticed that Diuron has the lowest EC_{50}value (1.64 mg/kg), the most toxic one, whereas alachlor has a value of 11.37 mg/kg which is nearly 7 times higher than that for diuron and 2.4 times higher than that for bromacil. These results indicated that Alachlor is to be used safely as a herbicide among the tested compounds. These variations are probably due to different modes of action of the tested herbicides given that each of them represents a different chemical class, beside the fact that the growth pattern of the tested plants are also different.

Furthermore, the regression equation (Table 4.1) indicating a linear mode of interaction and R^2 values close to 1 indicating a strong positive association between growth inhibition data (y) and herbicide concentration in soil (x).

In the case of molokhia, it appears that Bromacil has the lowest EC_{50} value (0.08 mg/kg), indicating that bromacil is most toxic one while diuron has the highest EC_{50} value (0.24 mg/kg) indicating its less phytotoxicity. Alachlor has nearly a half value of EC_{50} of diuron. Regardless of these variations of the EC_{50}, the three herbicides are still very toxic to Molokhia. In the case of Wheat, The EC_{50} values have the same trend of that of watermelon, Diuron is the most toxic one to wheat (EC_{50}= 1.83 mg/kg soil) and Alachlor has the highest record of safely one (EC_{50} 3.91 mg/kg soil). Regression equations and R^2 values are supporting our discussion.

Nevertheless, comparing EC_{50} values for the tested herbicide Molokhia with those on watermelon and wheat, one can realize that EC_{50} values on molokhia are the smallest among all indicating that Molokhia are the sensitive plant to the tested herbicides. The sensitivity of Molokhia plant may come from the fact that it has a shorter period of growth than watermelon or wheat, accordingly, it may not be able to develop resistant genotype for herbicides (Nahhal *et al*., 2013a). In addition, the regression values (R^2) of the linear relationships of all tested compounds ranged from 0.909 to 0.993, indicating strong positive associations between %growth inhibition (y) and herbicide concentration (x) in all cases of single toxicity tests. These results agree with Chen *et* al., 2003 and found similar trend for other cases. Furthermore, the variation of EC_{50} values of the tested compounds may also be adsorption coefficient (K_{Ow}) of each herbicide (Table 2.1), Diuron has a highest adsorption coefficient in soil (El-Nahhal et al., 2013a) that enabled diuron to stay for a longer time in the top soil.

Statistical analysis of the effects of the tested herbicides on different plants is shown in Table 4.2. It can be concluded that the effects of alachlor and diuron on molokhia and wheat are nearly similar with p-value ranges between 0.09-0.1 indicating no significant differences, whereas the effects on wheat are significantly different p-value less than 0.01. It can concluded the alachlor and bromacil have similar effect on wheat whereas the effect on molokhia total effect. Additionally the effects of bromacil and diuron in the 3 tested plants are significantly different with p-values that are less than 0.01. These values agree with the presented results shown in Figure 4.1-4.3.

Toxicity of binary mixtures:

Toxicity of binary mixtures of herbicides to the tested plants are shown in Figures 4.5– 4.7. The Figures clearly demonstrate an increase in growth inhibition as the concentration of the herbicide mixture increased in soil. However, the toxicity tests have similar trend, but different magnitude of plant response. To quantitatively evaluate the toxicity of binary mixtures, we calculated the EC_{50} of each mixture and present the results in Table 4.3. The EC_{50} values of the binary mixtures on watermelon (Table 4.3) clearly demonstrated that, alachlor diuron mixture is the most toxic one (EC_{50} = 8.92 TU/kg soil) followed by bromacil diuron mixture (EC_{50}=28.52 TU/kg soil), and alachlor bromacil was the most safer one among the mixture (EC_{50}= 83.51 TU/kg soil). These results indicate that mixing diuron with alachlor or bromacil produced high toxicity which can be referred to partial synergistic whereas mixing bromacil with alachlor produced high value of (EC_{50} (83.51 TU/kg soil) which can be referred to antagonistic effect.

As for the case on Molokhia, mixing diuron with alachlor or bromacil nearly produced similar phytotoxicity and can be referred to as a partial synergistic effect . The nearly high value of EC_{50} of alachlor with bromacil (1.35 TU kg/soil) suggesting an antagonistic effect occurs when mixing alachlor and bromacil. This suggests that mixtures contained diuron are more toxic than a mixture free of diuron. The explanation of these results is similar to that given above for individual toxicity of diuron.

As for the case of wheat, the trend is not similar. However, mixing diuron with alachlor produced a partially synergistic effect (EC_{50} =0.982 TU/kg soil) whereas mixing diuron with bromacil produced an antagonistic effect (EC_{50}=925.4 TU/kg soil). In contrast to the above cases, mixing bromacil with alachlor produced partial synergistic effects. Our results agree with Kerkez, (2013) who found that diuron and its mixtures were very toxic to cyanobacteria that have chlorophyll, as in the case with higher plants. Moreover, the EC_{50} values of the binary mixtures of wheat (Table 4.3) clearly demonstrated that, a mixture contained alachlor were the most toxic ones and have the lowest TU, whereas mixture did not contain alachlor has the highest EC_{50} which in some cases several hundred times higher than other cases, indicating that alachlor is responsible for the toxicity of mixtures against the wheat. These results agree with previous report (El-Nahhal *et al*. 1998 and 2013b) who found that wheat was sensitive to alachlor and acetochlor respectively.

Furthermore, the toxic effects of mixture can be visualized in Figure 4.8. It is obvious that there are gradual decreases in plant length as the concentrations of the tested herbicide mixtures increased in soil Statistical analysis of the phytotoxic effects of the mixtures on the test plant (Table 4.4) clearly detected significant differences on watermelon growth p-values were less than 0.01. Furthermore, no significant differences were detected on the effect of binary mixtures on Molokhia and wheat, indicating similar effects. The explanation of these variations is given above.

Toxicity of tertiary mixtures:

Toxicity of tertiary mixtures (Figure 4.9) clearly demonstrated that tertiary mixture containing alachlor, bromacil and diuron was very toxic to wheat and molokhia and less toxic to watermelon. These results suggest that watermelon can be a tolerant plant and can metabolize the toxic effects of herbicides to less toxic one. Furthermore, when mixing the 3 herbicides together evenly, this may enhance the interaction of the molecules to form a one a large molecule that can penetrate the plant root and make the needed toxicity. This suggestion is supported by the results of El-Nahhal & Safi (2004). They found that the addition of a naphthalene molecule to water containing soluble organic molecules, enhanced its solubility and both molecules reacted together to produce one molecule on the clay surfaces.

Nevertheless, comparing the EC_{50} values (Table 4.5), one can realize that the value in molokhia (1.93 TU/kg) is the lowest among all, the value of wheat is 9 TU/kg soil and the value on watermelon was the highest among all and reach to 11060.65 TU/kg soil.

By comparison, the effects of a single, binary and/or tertiary mixtures of the tested herbicides showed variations in the EC_{50} values. Analysis of these data and calculating the MTI showed negative values of binary and tertiary mixture (Table 4.7). These values indicate antagonistic effects, according to Konemann, (1981) and Hermens *et al*., (1985). However, comparing the MTI values in watermelon showed extreme negative values in comparison with those of Molokhia or wheat.

Furthermore, it may be possible to consider the MTI values of the mixtures that have a value close to zero as partially synergistic effects. Accordingly, the tertiary mixture (Al+Br+Di) on Molokhia and wheat that have MTI values equal to -1.33 and -0.49 and binary mixture (Al + Di) in wheat has a value equals to -3.12 are in the synergistic effects. Moreover, the mixtures that have MTI values less than -3.22 can be categorized as antagonistic mixtures. These results agree with the data presented in Figures 4.5-4.10. These results support by the result of Damalas, (2004) in another herbicide.

Chapter 6

Conclusion and Recommendations

6.1 Conclusion

This study showed variations of Watermelon, Molokhia and Wheat in their responses to Alachlor, bromacil and diuron. The single toxicity test indicated that Molokhia was the most sensitive plant.

The toxicity of these herbicides and their mixtures to plant growth varied from one herbicide to another due to the concentration, EC_{50}. The results of single tests clearly demonstrate that diuron is more toxic than alachlor and bromacil. Furthermore, the results of mixtures toxicity indicted the highest toxicity of the mixture that contained diuron.

The toxicity of herbicides to watermelon and wheat follow this sequence: Diuron > Bromaci >alachlor, whereas, the toxicity in Molokhia follows this sequence (Bromacil > Alachlor >Diuron).

Binary mixture toxicity on Watermelon and Molokhia follows the sequence of Al+Di > Br+Di > Al+Br, whereas toxicity on Wheat follows another sequence: Al+Di > Al+Br >Br+Di.

Tertiary mixture toxicity follows the following sequence in plants: Molokhia > Wheat > Watermelon. Furthermore, the antagonistic effect was shown in all mixtures due to the negative values of MTI but mixtures have MTI values closed to zero were rated as partially synergistic regardless to the negative value of MTI.

6.2 Recommendations

The following recommendations are proposed for further research and studies to form a complete picture of evaluation of the toxicity of herbicides on plant:

1. It is necessary to conduct similar research under field conditions to have a complete picture of the mixture toxicity of herbicides.
2. It is recommended to conduct similar research in different soils, plants., and climates.
3. It is necessary to include the effect of the mixture in weeds, especially the resistant types.
4. It is necessary to investigate the effect of the mixture on root growth, chlorophyll content, plant weight and other indices.
5. It is necessary to investigate the mode of action of the mixture toxicity on plant.

References

Abadsa, M. (2012): Adsorption and leaching potential of Diuron and linuron in Gaza Soil. MSc Thesis, The Islamic University-Gaza.

Abdur, R., Paul, S. and Ieuan, E. (2001): Plant Bioassay Techniques for Detecting and Identifying Herbicide Residues in Soil. Agdex 609-1.

ACGIH (1986): Documentation of the threshold limit values and biological exposure indices. Fifth edition. Cincinnati, OH: Publications Office, ACGIH.

Aguiar, DRD and Santana ,JA (2002): Asymmetry in farm to retail price Washington, DC.(http://assets.opencrs.com/rpts/RL34474_20080529.pdf). Price transmission and welfare impact in Africa. Vol.50.

Ahmed, Z. and Nizam, S.A. (2008): Jute - microbiological and biochemical research. Plant Tissue Culture and Biotechnology. Vol.18, P:197-220.

Ali, M.H. and Al-Qahtani, K.M. (2012): Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. The Egyptian Journal of Aquatic Research. Vol.38, No.1, P:31–37.

Alonso-Prados, J. L., Hernández –Sevillano, E., Llanos, S., Villarroya, M. and García-Baudín, J .M. (2001): Effects of sulfosulfuron soil residues on barley (Hordeum vulgare), sunflower (Helianthus annuus) and common vetch (vicia sativa). Crop Protection, Vol.21, P:1061-1066.

Al-saed, R., Ramlawi, A. and Salah, A. (2011): A national survey on the use of agricultural pesticides in Palestine. International Journal of Environmental Studies, Aquatic Insects, Vol. 68, No. 4, P: 519– 529.

Anderson, T.D. and Lydy, M.J. (2002): Increased toxicity to invertebrates associated with a mixture of atrazine and organophosphate insecticides. Environmental Toxicology and Chemistry, Vol.21, P: 1507–1514.

Andreozzi, R., Di Somma, I., Pollio, A., Pinto, G., and Sanchirico, R. (2008): Toxicity of unwanted intermediates and products formed during accidental thermal decomposition of chemicals. Journal of Hazardous Materials, Vol. 150, No.2 ,P:433-7.

Appleby, A., Müller, F. and Carpy, S. (2002): "Weed Control" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim.

Awad, Y. (2012): Biodegradation of Herbicides by Cyanobaterial mats In Gaza Soils. MSc Thesis, Al-Azhar University Gaza.

Baerg, R.J., Gronwald, J.W., Eberlein, C.V. and Stucker, R.E. (1996): Antagonism of diclofop control of wild oat (*Avena fatua*) by tribenuron. Weed science, Vol. 44, P: 461–468.

Batta, Y. (2003): Application and usage of pesticides in Palestine: Current and future outlook. AL-Najah University Journal for Research -Natural Sciences, VOL. 17, NO. 1, P: 089-098.

Beste, C.E. and Chairman (1983): Herbicide Handbook of the Weed Science Society of America. Weed Science Society of America, Champaign, IL.

Beyer, E.M.;. Duffy, M.J; Hay, J.V. and Schlueter, D.D. (1988): Sulfonylureas. Chapter 3 in Herbicides: Chemistry, Degradation, and mode of action, Volume 3 (edited by Kearney, P.C. & D.D.Kaufmann), Marcel Dekker, Inc. New York, USA, p:117–189.

Blacklow, W. M. and Pheloung, P.C. (1991): Sulfonylurea herbicides applied to acidic sandy soils: a bioassay for residues and factors affecting recoveries. Australian Journal of Agricultural Research, Vol. 42, P: 1205-1216.

Bonnet, J., Bonnemoy, F., Dusser, M. and Bohatier, J. (2007): Assessment of the potential toxicity of herbicides and their degradation products to non target cells using two microorganisms, the bacteria *Vibrio fischeri* and the ciliate Tetrahymena pyriformis. Environmental Toxicology., Vol. 22. No.1,P:78-91.

Breugelmans, P., P.J. D'Huys, R., De Mot and Springael, D. (2007): Characterization of novel linuron-mineralizing bacterial consortia enriched from longterm linuron-treated agricultural soils. FEMS Microbiology Ecology, Vol. 62,P: 74-385.

Broderius, S.J., Kahl, M.D. and Hoglund, M.D. (1995): Use of joint toxic response to define the primary mode of toxic action for diverse industrial organic chemicals. Environmental Toxicology and Chemistry, Vol. 14, P:1591–1605

Chauhan, L.K.S., Safena, P.N., Sundararaman, V. and Gupta, S.K. (1998): Diuron-induced cytological and ultrastructural alterations in the root meristem cells of Allium cepa. Pesticide Biochemistry and Physiology, Vol. 62, P: 152–163.

Chen, S.H., Zhou, Q.X., Sun, T.H., Li, P.J., (2003): Rapid ecotox-icological assessment of heavy metal combined polluted soil using canonical analysis. Journal of Environmental Sciences. 15, 854-858.

Christensen, A, M., Faaborg, A, S., Ingerslev, F., and Baun A., (2007): Mixture and single-substance toxicity of selective serotonin reuptake inhibitors toward algae and crustaceans. Vol. 26, Issue 1, P: 85–91.

Clayton, G.D. and Clayton, F.E. (1981): Patty's industrial hygiene and toxicology. Third edition. Vol. 2: Toxicology. NY: John Wiley and Sons

Damalas, C.A. (2004): Herbicide Tank Mixtures: Common Interactions. International Journal of Agriculture and Biology, Vol. 6, No.1.

Deschamps, R.J.A., Hsiao, A.I. and Quick, W.A. (1990): Antagonistic effect of MCPA on fenoxaprop activity. Weed Science, Vol. 38, P: 62–6.

Doherty, V.F., Sogbanmu, T.O., Kanife, U.C. and Wright, O. (2012): Heavy metals in vegetables collected from selected farm and market sites in Lagos, Nigeria. Journal of Environmental Science and Toxicology.Vol.6, No.1, P:137-142.

Duke, S.O. (1988): Glyphosate. In: Herbicides. Chemistry, Degradation, and Mode of Action,Vol.3.

DuPont Agricultural Products (1990): Material Safety Data Sheet for Bromacil Technical. Du Pont, Registration and Regulatory Affairs, Wilmington, DE.

Edwards, A.J, Vinyard, B.T, Wiley ER, E.D, Brown, Collins J.K, Perkins-Veazie , P, Baker, R.A, and Clevidence B.A (2003): Consumption of Watermelon Juice Increases Plasma Concentrations‖, J. Nutr., Vol.133, p: 1043.

El-Nahhal, Y. (2003): Adsorptive behavior of acetochlor on organoclay complexes. Bulletin of Environmental Contamination and Toxicology, Vol. 70, NO. 6, P: 1104-1111.

El-Nahhal Y. (2004): Contamination and safety status of plant food in Arab countries. Journal of Applied Science, Vol. 4, P:411-417.

El-Nahhal, Y., Nir, S., Polubesova, T., Margulies, L. and Rubin, B. (1998): Leaching, phytotoxicity and weed control of new formulations of alachlor. Journal of Agricultural. Food Chemistry,Vol. 46, P:3305-3313.

El-Nahhal, Y., Nir, S., Serban, S., Rabinowitz, O. and Rubin, B. (2001): Organoclay formulation of acetochlor for reduced movement in soil. Journal of Agricultural and Food Chemistry, Vol. 49, P: 5464-5371.

El-Nahhal, Y., Safi, J. and Awad, Y. (2012): Biodegradation of Acetochlor and Diuron Herbicides in Soil and Liquid Media by Cyanobacterial Mats Collected fromWadi Gaza. MSc Thesis, AL-Azhar University-Gaza.

El-Nahhal, Y., Abadsa, M., and Affifi, S. (2013a): Adsorption of Diuron and Linuron in Gaza Soils. American Journal of Analytical Chemistry, Vol 4,No 7, p:94-99.

El-Nahhal, Y., Awad, Y. and Safi, J. (2013b): Bioremediation of Acetochlor in Soil and Water Systems by Cyanobacterial Mat. International Journal of Geosciences, Vol 4, No. 6, pp 880-890.

El-Najjar, S. (2013): Eco-toxicity of Carbaryl, Chlorpyrifos, and Diuron, as Individual and in Mixtures on *Daphnia magna* and *Tilapia nilotica*. MSc Thesis, The Islamic University-Gaza.

Extoxnet. (1996): Extension Toxicology Network, Pesticide Information Profiles: Diuron. http://extoxnet.orst.edu/pips/diuron.htm.

Fairchild, J.F., Ruessler, D.S., Haverland, P .S. and Carlson, A.R. (1997): Comparative sensitivity of *Selenastrum capricornutum* and *Lemna minor* to sixteen herbicides. Archives of Environmental Contamination and Toxicology. Vol.32, P:353-357.

Ferreira, K.L., Burton, J.D. and Coble, H.D. (1995): Physiological basis of antagonism of fluazifop–P by DPX–PE350. Weed Science, 43: 184–91 Green, J.M., 1989. Herbicide antagonism in the whole plant level. Weed Technology, Vol.3, P: 217–26.

Field, J.A., Reed, R.L., Sawyer, T.E. and Martinez, M. (1997): Diuron and its metabolites in surface water and ground water by solid phase extraction and in-vial elution. Journal of Agricultural and Food Chemistry, Vol. 45, NO. 10, P: 3897–3902.

Food and Drug Administration (1986): The FDA Surveillance Index. Bureau of Foods, Department of Commerce, National Technical Information Service, Springfield, VA.

Gilliom, R.J., Barbash, J.E., Crawford, C.G., Hamilton, P.A., Martin, J.D., Nakagaki, N., Nowell, L.H., Scott, J.C., Stackelberg, P.E., Thelin, G.P. and Wolock, D.M. (2006): The Quality of Our Nation's Waters: Pesticides in the Nation's Streams and Ground Water, 1992-2001. United States Geological Survey, Virginia.

Gooddy, D.C., Chilton, P.J. and Harrison, I. (2002): A field study to assess the degradation and transport of diuron and its metabolites in a calcareous soil. Science of the Total Environment, Vol. 297, P: 67–83.

Gosselin, R.E., *et al*., (1984): Clinical toxicology of commercial products. Fifth edition. Baltimore, MD: Williams and Wilkins.

Günther, P., Rahman, A. and Pestemer, W. (1989): Quantitative bioassays for determining residues and availability to plants of sulphonylurea herbicides. Weed Research, Vol. 29, P: 141-146.

Hall, D.O. and Rao, K.K. (1995): Photosynthesis. Cambridge University press, Cambridge.

Harker, K.N., Blackshaw, R.E. and Kirkland, K.J. (1995): Ethametsulfuron interactions with grass herbicides on canola (*Brassica napus*, *B.rapa*). Weed Technology, Vol. 9, P: 91–98.

Hart, S.E. and Roskamp, G.K. (1998): Soybean (*Glycine max*) response to thifensulfuron and bentazon combinations. Weed Technology, Vol.12, P: 179–184.

Hatzios, K.K. and Penner, D. (1985): Interactions of herbicides with other agrochemicals in higher plants. review weed science, Vol.1, P: 1-63

Hermens, J., Leeuwngh, P., and Musch, A. (1985): Joint toxicity of mixture of group of organic aquatic pollution to the guppy (*Poecilla reliculata*). Ecotoxicology and Environmental Safety, Vol.9, P: 321-326.

Holden, J.M., Eldridge, A.L., Beecher, G.R., Buzzard, I.M., Bhagwat, S., Davis, C.S., Doughlass, L.W., Gebhardt, S., Haytowitz, D., Schakel, S. (1999): Carotenoids content of U.S. foods: an update of the database, J. Food Compos. Anal. P: 169–196.

Horowitz, M. (1976): Application of bioassay techniques to herbicide investigations. Weed 3607 Research, Vol.16, P: 209-215.

Howard, I. Morrison, Kathryn Wilkins, Robert Semenciw, Yang Mao and Don Wigle (1992): "Herbicides and Cancer". Journal of the National Cancer Institute, Vol.84, P: 1866–1874.

Hudson, R.H., Tucker, R.K., and Haegele, M.A. (1984): Handbook of Toxicity of Pesticides to Wildlife. U.S. Department of the Interior, Fish and Wildlife Service, Resource Publication 153.

Hussein, S. Y., El-Nasser, M. A. and Ahmed, S. M. (1996): Comparative Studies on the Effects of Herbicide Atrazine on Freshwater Fish *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt. Vol. 57, Issue 3, p:503-510

IARC (1999): Occupational Exposures in Insecticide Application, and Some Pesticides. Summary of Data Reported and Evaluation. Vol. 53, IARC Monographs. Lyon, France.

ICARD (2003): Supporting agricultural development in Palestine. Issue No.18/19.

http://www.icarda.org/Publications/Caravan/Caravan18- 19/Article26.htm

Ishaque, B.A., Jonson, L., Gerald, T., Boucaaud, O., Gkoh, J. and Tchounwou, B.P. (2006): Assessment of individual and combined toxicities of four non essential metals (As, Cd, Hg and Pb) in the micro toxic assay. International Journal of Environmental Research and Public Health, Vol.3., No.1, P: 118-120.

Issa, Y. (2000): Exposure to Pesticides, an occupational exposure survey on exposure to pesticides among farmers in Palestine, Dissertation for Master of International Community Health. Institute of General Practice and Community Medicine, the Faculty of Medicine, University of Oslo, Norway, P: 63.

Jin-Clark Y., Anderson T.D. and Zhu K.Y. (2008): Effect of Alachlor and Metolachlor on Toxicity of Chlorpyrifos and Major Detoxification Enzymes in the Aquatic Midge, *Chironomus tentans* (Diptera: Chironomidae). Archives of Environmental Contamination and Toxicology. Vol 54, p:645-652.

Johnson, E.N., Moyer, J.R., Thomas, A.G., Leeson, J.Y., Holm, F.A., Sapsford, K.L., Schoenau, J.J., Szmigielski, A.M., Hall, L.M., Kuchuran, M.E. and Hornford, R.G. (2005): Do repeated applications of residual herbicides result in herbicide stacking? In Soil Residual Herbicides: Science and Management. Topics in Canadian Weed Science, ed. R.C. Van Acker, Vol. 3, P: 53-70.

Johnson, W.W. and Finley, M.T. (1980): Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. U. S. Department of the Interior, Fish and Wildlife Service, Resource Publication 137.

Kasai, F., Takamura, N. and Hatakeyama, S. (1993): Effect of simetryn on growth of various freshwater algal taxa. Environmental Pollution.,Vol. 79, P: 77-83.

Kellogg, R.L., Nehring, R., Grube, A., Goss, D.W., and Plotkin, S. (2000): Environmental indicators of pesticide leaching and runoff from farm fields (http://www.nrcs.usda.gov/technical/NRI/pubs/eip_pap.html, accessed 11/2010) Washington, DC: U.S. Department of Agriculture, Natural Resources Conservation Service .

Kerkez, S. (2013) Potential Toxicity of the Herbicides; Diuron, Diquat, Terbutryn and their Mixtures to Cyanobacterial Mats, Wadi Gaza, Palestine. MSc Thesis, The Islamic University-Gaza.

Kidd, H. and James, D.R. (1991): The Agrochemicals Handbook. Royal Society of Chemistry Information Services, Cambridge, UK (as updated), P: 3-11.

Konemann, H. (1981): Fish toxicity tests with mixtures of more than two chemicals: A proposal for a quantitative approach and experimental results. Toxicology , Vol.9, P:229-238.

Kotoula S., E., Eleftherohorinos, I.G., Gagianas, A. and Sticas, A.G. (1993): Phytotoxicity and persistence of chlorsulfuron, metsulfuron-methyl, triasulfuron and tribenuronmethyl in three soils. Weed Research, Vol.33, P: 355-367.

Koutsaftis, A. and Aoyama, I. (2007): Toxicity of four antifouling biocides and their mixtures on the brine shrimp Artemia salina. Science of the Total Environment, Vol. 387, No. (1-3), P:166-174.

Kudsk, P. and J.C. Streibig (2003): Herbicides – a two-edged sword. Weed Res, 43:90-102.

Lagaly, G. (2001): Introduction: Pesticide-clay interactions and formulations. Applied Clay Science, Vol, 18, P: 205–209.

Lycan, D.W. and Hart S.E. (1999): Physiological response of soybean (*Glycine max*) and two weed species to thifensulfuron and bentazon combinations. Weed Science. Vol.47, P: 143–148.

Lydy, M., Belden, J., Wheelock, C., Hammock, B. and Denton, D. (2004): Challenges in regulating pesticide mixtures. Ecology and Society, Vol. 9, P: 1-15

Ma, J. (2005): Differential sensitivity of threecyanobacterial and five green algal species toorganotins and pyrethroids pesticides.., Science of the Total Environment, Vol. 341, P:109-117.

Ma, J., Liang, W., LX V.,Wang, S. and Wei, J.L. (2001): Acute Toxicity of 33 Herbicides to the Green Alga *Chlorella pyrenoidosa*. Bulletin of Environmental Contamination and Toxicology. Vol. 66, P:536-541.

Ma, J., Zheng, R., Xu, L. and Wang, S. (2002): Differential sensitivity of two green alga Scenedesmus obliqnus and Chlorella pyrenoidosa to 12 pesticides. Ecotoxicology and Environmental Safety. Vol. 52, P:57-61.

Ma, J., Lin, F., Wang, S. and Xu, L., (2003): Toxicity of 21 herbicides to the green alga Scenedesmus quadricauda Bulletin of Environmental Contamination and Toxicology. Vol. 71, P:594–601.

Maity, S., Chowdhury, S. and Datta, A.K. (2012): Jute biology, diversity, cultivation, pest control, fiber production and genetics. In: Lichtfouse, E, editors. Organic Fertilisation, Soil Quality and Human Health, Sustainable Agriculture Reviews. Vol.9, P:227-262.

Mandel, H.; Levy, N.; Izkovitch, S. and Korman, S. H. (2005). "Elevated plasma citrulline and arginine due to consumption of *Citrullus vulgaris* (watermelon)". Berichte der deutschen chemischen Gesellschaft .Vol.28, P: 467–472.

Mar, (1975): Initial scientific and mini economic review of bromacil. Office of Pesticide Programs. EPA-540/1-75-006. Washington, DC.

Meister, R.T. (1992): Farm Chemicals Handbook 1992. Meister Publishing Company, Willoughby, OH.

Menzie, C. M. (1974): Metabolism of pesticides. U.S. Department of the Interior. Fish and Wildlife Service Special Scientific Report: Wildlife. Washington, DC: U. S. Government Printing Office. Vol,381.

Miller, G. T. (2009): Living in the Environment. Scott Spolman, 16th edition, USA , P:295.

MOA Palestine (2011a): Quantities of pesticides entering in 2011 and previous years, registration and import department of pesticide.

MOA Palestine (2011b): Plant Protection and Quarantine.

MOA Palestine (2012a):Vegetables cultivated areas in the Gaza Strip for the agricultural season 2011 to 2012.

MOA Palestine (2012b): Statistical record on pesticides in Gaza Governorates, annual reports, Gaza-Palestine.

MOA Palestine (2008): Statistical records (2006/2007) on pesticides use in Palestine. Unpublished report, Ramallah, West Bank, Palestine.

Nebeker, A.V. and Schuytema, G.S. (1998): Chronic effects of the herbicide diuron on freshwater cladocerans, amphipods, midges, minnows, worms, and snails. Archives of Environmental Contamination and Toxicology, Vol. 35, P: 441–446.

Niederlehner B.R., Cairns J. Jr. and Smith E.P. (1998): Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to Ceriodaphnia dubia. Ecotox Environmental, Vol. 39 , P:136–146.

Nir, S., Undabeytia, T., Yaron-Marcovich, D., El-Nahhal, Y., Polubesova, T., Serban, C., Rytwo, G., Lagaly, G. and Rubin, B. (2000): Optimization of Adsorption of hydrophobic herbicides on montmorillonite preadsorbed by monovalent organic cations: Interaction between phenyl rings. Journal of Environmental Science and Technology, Vol. 34, P: 1269-1274.

Ogunrinde, A.T. and Fasinmirin, J.T. (2011): Soil moisture distribution pattern and yield of Jute mallow (*Corchorus olitorius* L.) under three different soil fertility management. Proceedings of the Environmental Management Conference, Federal University of Agriculture, Abeokuta, Nigeria. P:373-381.

Öztürk, N. and Favaroğlu, F. (2011): Antioxidant activities of Molokhia (*Corchorus olitorius* L.) extracts. In: Gökçekus H, Türker U, LaMoreaux JW, editors. Survival and Sustainability, Environmental concerns in the 21st Century. P:535-543.

Pannacci, E., Onofri, A. and Covarelli, G., (2006): Biological activity, availability and duration of phytotoxicity for imazamox in four different soils in Central Italy. Weed Research, Vol. 46, P: 243-250.

Paul, A.A., Southgate, D.A. and Russel, J., (1987): First supplement to Mccance and widowson's the component of foods. Her majesty's stationary office, London.

PCBS (2013): Population, housing and establishment census. Palestinian National Authority, Palestinian Central Bureau of Statistics, Ramallah.

PCBS (2009): Household environmental survey, Marine finding.

PCBS (2011): agricultural census, (2010) press conference on the final results, Palestine.

http://www.pcbs.gov.ps/Portals/_pcbs/Agriculture/tab10.htm

Perkins-Veazie ,P. and Collins, J.K. (2004): Flesh quality and lycopene stability in fresh cut watermelon, Postharvest Biol. Technol. P:159–166.

Pestemer, W., Stalder, L., and Eckert, B. (1980): Availability to plants of herbicide residues in soil." Part II: data for use in vegetable crop rotations, Weed Research, Vol. 20, P: 349-353.

Rahman, T., James, K. and Gunter, P. (1993): "Bioassays of soil applied herbicides." Proc. Int. Symp. Indian Society of Weed Science. NO.1, P: 95–106.

Rapsomanikis G, Hallam D, and Conforti, P. (2003): Market Integration and Price Transmission in Selected Food and Cash Crop Markets of Developing Countries: Review and Applications", in Commodity Market Review 2003-2004, FAO, Rome.

Rick, R and Jason, H (2006): Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecology Letters, Ecology Letters Vol. 9, P: 1157–1171.

Rytwo, G. (2005): Interaction of pendimethalin with organo-montmorillonite complexes. Applied Clay Science, Vol. 28, P: 67-77.

Safi, J.M. (2002): Association between chronic exposure to pesticides and recorded cases of human malignancy in Gaza strip (1990–1999). The Science of the Total Environment, Vol. 284, P: 75–84.

Safi J., El-Nahhal Y., Soliman S. A. and Elsebae A. H. (1993): Mutagenic and carcinogenic pesticides used in the Gaza strip Agricultural

Environmental. The Science of the Total Environment, Vol. 132, P: 371-380.

Safi, J., Abu Foul, N., El-Nahhal, Y., and El-Sebae, A. (2002): Monitoring of pesticide residues on cucumber, tomatoes and strawberries in Gaza Governorates, Palestine. *Nahrung/Food*, Vol.46, No.1, P :34-49.

Sandín-España, P., Loureiro, L., Escorial,C., Chueca,C. and Santín-Montanyá, I. (2011): The Bioassay Technique in the Study of the Herbicide Effects, Herbicides, Theory and Applications, P: 431-454.

Santlemann, P.W. (1977): Herbicide bioassay. In B. Truelove, ed. Research Methods in Weed Science. 2nd ed. Auburn, AL: Southern Weed Science Society, P: 79–87.

Stefan, B. and Keith, H. (1998): Pests, diseases and disorders of garden plants. Collins. P: 609.

Scott, R.C., Shaw, D.R. and Ratliff, R.L. (1998): Effect of SAN 582 on sethoxydim efficacy in johnsongrass (*Sorghum halepense*) and soybean (*Glycine max*). Weed Science., Vol.46, P: 2–7.

Shaw, C.M., Brodie, J. and Mueller, J..F (2012): Phytotoxicity induced in isolated zooxanthellae by herbicides extracted from Great Barrier Reef flood waters. Marine Pollution Bulletin. Vol. 65, P::355-62

Simpson, D.M. and Stoller, E.W. (1996): Physiological mechanisms in the synergism between thifensulfuron and imazethapyr in sulfonylurea–tolerant soybean (*Glycine max*). Weed Science, Vol.44, P: 209–14

Souissi, Y., Bouchonnet, S., Bourcier, S., Kusk, K.O., Sablier, M., and Andersen, H.R. (2013): Identification and ecotoxicity of degradation products of chloroacetamide herbicides from UV-treatment of water. Science of the Total Environment, P: 458-460.

Sprague J.B. and Ramsay B. (1965) – Lethallevels of mixed copper-zinc solutions for juvenile salmon. J. Fish Res Bd Lan. , Vol.22, P: 425-432.

Stork, P. and Hannah, M.C. (1996): A bioassay method for formulation testing and residue strudies of sulfonylurea and sulfonalide herbicides. Weed Research, Vol.36, P: 271-278.

Streibig, J.C. (1988): Herbicide bioassay. Weed Research, Vol. 28, P: 479-484.

Teisseire, H., Couderchet, M. and Vernet, G. (1999): Phytotoxicity of diuron alone and in combination with copper or folpet on duckweed (Lemna minor). Environmental Pollution, Vol. 106, P: 39–45.

Tsen, C.C ., (1985): Amino acid composition and biological value of cereal germs. Railed publishing co., Boston, P: 453-466.

Thurman, E.M., Goolsby, D.A., Aga, D.S., Pomes, M.L. and Meyer, M.T. (1996): Occurrence of alachlor and its sulfonated metabolite in rivers and reservoirs of the Midwestern United States: the importance of sulfonation in the transport of chloroacetanilide herbicides. Environmental Science and Technology, Vol. 30, P: 569–574.

Trimble, A.J. and Lydy, M.J. (2006): Effects of triazine herbicides on organophosphate toxicity in Hyalella azteca. Archives of Environmental Contamination and Toxicology, Vol. 51, P:29–34.

Tulio, A.Z., Ose, K., Chachin, K. and Ueda, Y. (2002): Effects of storage temperatures on the postharvest quality of jute leaves (*Corchorus olitorius* L.). Postharvest Biology and Technology. Vol.26, P:329-338.

U.S. National Library of Medicine (1992): Hazardous Substances Databank. Toxnet, Medlars Management Section, Bethesda, MD.

U.S. National Library of Medicine (1995): Hazardous Substances Databank. Bethesda, MD,9-9.

U.S.EPA (1987): Health Advisory, Office of Drinking Water.

VanDriesche, R.G. (1985): Pesticide profiles: Bromacil. Cooperative Extension Service. Department of Entomology, University of Massachusetts. Amherst, MA: Cooperative Extension Service.

Vasilakoglou, I.B., Eleftherohorinos, I.G. and Dhima, K.B. (2001): Activity, adsorption and mobility of three acetanilide and two new amide herbicides. Weed Research, Vol. 41, P: 535-546.

Wada, M. (1930): "Über Citrullin, eine neue Aminosäure im Presssaft der Wassermelone, Citrullus vulgaris Schrad". *Biochem. Zeit.* Vol.224, P: 420.

Waldhoff, D., Furch, B. and Junk, W.J. (2002): Fluorescence parameters, chlorophyll concentration, and anatomical features as indicatorsfor flood adaptation of an abundant tree species in centralAmazonia: Symmeria paniculata. Environmental and Experimental Botany. Vol.48, P: 225-235.

Walker, M.M. and Keith L.H. (1992): EPA's Pesticide Fact Sheet Database. Lewis Publishers. Chelsea, MI.

Wang, W. and Freemark, K. (1995): The use of plants for environmental monitoring and assessment. Vol. 30, P 289–301.

Wauchope, R.D., Buttler, T. M., Hornsby A.G., Augustijn-Beckers, P.W.M. and Burt, J. P(1992): SCS/ARS/CES Pesticide properties database for environmental decisionmaking.

WSSA (1989): Herbicide Handbook of the Weed Science Society of America, 6th Ed. WSSA, Champaign, IL, P:301.

WSSA (1994): Herbicide Handbook, Seventh Edition. Champaign, IL, P: 8-16

Ying, G.G. and Williams, B. (2000): Laboratory study on the interaction between herbicides and sediments in water systems, Department of Environmental Science and Management, The University of Adelaide, Roseworthy Campus, Roseworthy, SA 5371, Australia, Volume 107, P: 399–405.