Journal of Toxicology and Environmental Health Sciences Vol. 4(10), pp. 175-184, December 2012
Available online at http://www.academicjournals.org/JTEHS
DOI: 10.5897/JTEHS12.052
ISSN 2006-9820 ©2012 Academic Journals

Full Length Research Paper

Cytotoxicity and genotoxicity of some agropesticides used in Southern Africa

Asita, A. O.* and Hatane, B. H

Department of Biology, National University of Lesotho, P. O. Roma 180 Maseru, Lesotho, Southern Africa.

Accepted 22 November, 2012

Pesticides use boosts agricultural yield by reducing crop losses. However, some pesticides are mutagens and active ingredients could produce different effects in different formulations. Wipe-out (WO) (Glyphosate 360 g/l), K-O Gard® (KOG) (Deltamethrin 10 g/l), Cutworm bait (CWB) (Sodium Fluosilicate, 100 g/kg), Snail ban (SB) (Metaldehyde, 30 g/kg and Carbaryl, 20 g/kg) and Coopex (CPX) (Permethrin 250 g/kg) were tested for cytotoxicity and genotoxicity using the onion (*Allium cepa*) test. Onion seeds were germinated and exposed (mg/l) to below EC_{50} values of WO (78.1, 156.3, 312.5), KOG (156.3, 312.5, 625.0), CWB (78.1, 156.3, 312.5), SB (156.3, 312.5, 625.0), and CPX (312.5, 625.0, 1250.0) for 24 h. Root tips were cut, fixed, hydrolyzed in 1 N hydrochloric acid, stained, squashed and observed microscopically. Cytotoxicity and genotoxicity induction at each treatment was compared with the negative control using t-test. WO (78.1, 156.3, and 312.5), KOG (312.5, 625.0) and CWB (312.5) were cytotoxic, ($P < 0.05$). WO (78.1, 312.5), CWB (156.3, 312.5), SB (625.0) and CPX (1250.0) induced genotoxicity ($P < 0.05$), mostly, sticky chromosomes. The genotoxic pesticides have potential to cause adverse environmental and health effects, because most adverse effects by genotoxins result from genetic damage and genotoxicity of chemicals is likely to result from cell division abnormalities.

Key words: *Allium cepa*, agropesticides, Southern Africa, mitotic index, chromosome aberration.

INTRODUCTION

Among crops, the actual loss due to agricultural pests (weeds, animal pests, pathogens and viruses) worldwide were estimated at 26 to 30% for sugar beet, barley, soybean, wheat and cotton, and 35, 39 and 40% for maize, potatoes and rice, respectively (Oerke and Dehne, 2004). Mohan and Fields (2002) stated that annual post-harvest losses resulting from insect damage and other bio-agents are estimated to be 10 to 40% of world agricultural production. Grain losses caused by insect pests in Africa are quite high and vary from country to country and from region to region. However, annual grain losses of over 50% in cereals (Abraham and Firdissa, 1991) have been reported, although the average stands at 20% (Youdeowi and Service, 1986; Philips and Throne, 2010). In order to cut down losses in agriculture, pesticides have been integrated into modern agricultural process to control weeds, diseases and insect pests that can markedly reduce the amount of harvestable produce and thereby increase outputs and productivity (Aktar et al., 2009). Pimentel et al. (1993) argue that on average, the economic benefits from pesticide use are about four times their direct cost to the users. Without pesticide application, the loss of fruits, vegetables and cereals from pest injury would reach 78, 54 and 32%, respectively (Cai, 2008).

Pesticides are different from other agricultural inputs in that they do not directly boost yields in the way that fertilizers do; instead they reduce crop losses caused by pests (Jha and Regmi, 2009). Though pesticides use reduces crop losses and so increases agricultural output, pesticides are known to produce a wide spectrum of adverse health and environmental effects. According to a report of World Health Organization (WHO) and United Nations Environment Programme (UNEP), worldwide, there are more than 26 million human pesticide poisonings with about 220,000 deaths per year (Richter, 2002).

*Corresponding author. E-mail: aoasita@yahoo.co.uk, ao.asita@nul.ls. Tel: +266 52213292, + 26663060354. Fax: +266 22 340000.

In a review of the literature on the health effects of pesticides, Mansour (2004) concluded that there is strong scientific evidence that pesticides, as a whole, can induce severe effects to human health ranging from myelotoxicity to cytogenetic damage and carcinogenicity. Other health effects of pesticides include acute and persistent damage in the nervous system (Kamel et al., 2007), lung and respiratory disorders (Hoppin et al., 2008), alterations in the reproductive organs (Hileman, 1994) birth defects (Rojas et al., 2000). Other causes of worry, according to Anwar (1997) are other side effects associated with agricultural chemicals such as the carcinogenic and genotoxic effects, which are considered as being among the most important of the effects. Most of the adverse effects to health, according to Norppa (2004) are the result of the genetic damage induced by genotoxic agents in somatic as well as in germinal cells and any genetic activity of chemicals, it has been suggested, is most likely to result from cell division abnormalities (Parry et al., 1999). Pesticides residues are known to persist in soil (Subbarao, 1999), water (Medina et al., 1999) and in fruits and vegetables (Ahouangninou et al., 2012; Osman et al., 2010) and represent a risk for human health. Genotoxicity and mutagenicity of pesticides for non target organisms and their influence on ecosystems are of worldwide concern (Pimentel et al., 1998).

According to Zhang et al. (2011), worldwide, there are currently about 500 pesticides used widely and in large quantities. In China alone, more than 400 companies are manufacturers of over 300 varieties of original pesticides and 3,000 formulations or commercial names. These pesticides are manufactured as insecticides, fungicides, herbicides, rodenticides and antimicrobials to protect crops. Pesticides of different types are used in agriculture to protect crops and stored foods in order to increase agricultural productivity. In the southern African subregion, commonly used agropesticides include Wipe-out (Glyphosate 360 g/l), K-O Gard® (Deltamethrin 10 g/l), Cutworm bait (Sodium Fluosilicate, 100 g/kg), Snail ban (Metaldehyde, 30 g/kg and Carbaryl, 20 g/kg) and Coopex (Permethrin 250 g/kg).

Data gap exists in the information available on the genotoxicity of some of the active ingredients and/or these formulations of pesticides on *A. cepa*, because synergistic interaction or potentiation between or among pesticides that are mixed together is a well known phenomenon (Cloyd et al., 2007). For instance, we are not aware of information on the genotoxicity of sodium hexafluorosilicate and metaldehyde on *A. cepa*. The analysis of chromosomal alterations serves as a mutagenicity test and is one of the few direct methods to measure damages in systems exposed to possible mutagens or carcinogens (Tedesco and Laughinghouse (IV), 2012). Plants are direct recipients of agrotoxins and plant genotoxicity assays are relatively fast, inexpensive and give reliable results (Grant, 1978) and the *Allium* test is simple and just as reliable as the method where chromosome aberrations were recorded in all types of mitotic cells (Rank and Nielsen, 1997).

This study sought to investigate the cytotoxicity and genotoxicity of different concentrations of Wipe-out, K-O Gard®, Cutworm bait, Snail ban and Coopex, using the anaphase-telophase chromosome aberration assay with root tip meristem cells of onion (*A. cepa).* This study is a continuation (Asita and MatebesI, 2010) of our efforts to test the pesticides that are sold in garden centres in Maseru, Lesotho, Southern Africa, for cytotoxicity and genotoxicity in view of the known adverse health and environmental effects of pesticides.

MATERIALS AND METHODS

Test organism and pesticides

Seeds of *A. cepa* variety, Texas Grano 502 P.R.R., obtained from Sakata seeds, Lanseria 1748, South Africa were used for the study. Test pesticides included Wipe-out (WO), (Glyphosate 360 g/l), a product of Kombat (Pty) Ltd, South Africa; K-O Gard® (KOG) (Deltamethrin 10 g/l) and Coopex (CPX) (Permethrin 250 g/kg), products of Bayer (Pty) Ltd, South Africa; Cutworm bait (CWB) [Sodium Fluosilicate, 100 g/kg], Snail ban (SB) [Metaldehyde, 30 g/kg and Carbaryl, 20 g/kg], products of AGRO-SERVE (Pty) Ltd., South Africa.

Selection of pesticide dosages

A preliminary dose selection experiment was conducted to determine the doses to be used in the actual experiments. A total of 100 onion seeds were spread on filter paper moistened with different concentrations of each pesticide or with water (negative control) for 72 h at room temperature (22 ± 2°C). The effective concentration (EC_{50}) (mg/ml) was the concentration that inhibited the germination of 50% of the seeds or the effective concentration for 50% growth inhibition for relative reduction of root length when onion bulbs were used (Asita and Matabesi, 2012; Yildiz and Arikan, 2008). The effective concentration (EC_{50}) (mg/l) values for the five pesticides were approximately: WO (1250.0), KOG (> 20000), CWB (1500), SB (5000) and CPX (5000). Thus KOG did not inhibit germination even at the limit of solubility. In trial experiments, the EC_{50s} of the test compounds were too toxic therefore not enough cells in division stages could be observed. In the actual genotoxicity experiments therefore, the highest concentration of each pesticides tested was a fraction of each of their EC_{50} as follows, WO (1/4 EC_{50}), CWB (1/4 EC_{50}), SB (1/8 EC_{50}) and CPX (1/4 EC_{50}). The highest concentration of 6.25 mg/ml for KOG was an arbitrary value determined by solubility consideration. On the basis of the EC_{50} values, the results of trial experiments and the need for sufficient number of dividing cells on each slide for scoring, the following three concentrations (mg/l) of the pesticides were used in the actual cytotoxicity and genotoxicity experiments; WO (78.1, 156.3, 312.5), KOG (156.3, 312.5, 625.0), CWB (78.1, 156.3, 312.5), SB (156.3, 312.5, 625.0), and CPX (312.5, 625.0, 1250.0).

Experimental procedure

Genotoxicity assay

The method used was similar to the method of Matsumoto et al.

(2006). *A. cepa* (onion) seeds were spread on water moistened filter paper in a petri dish, at room temperature (22 ± 2°C), until they germinated (about 72 h) and the radicles reached a length of about 2 cm. For each treatment, ten germinated seeds were transferred onto filter paper moistened with the pesticide in a Petridish and kept for 24 h at room temperature. Distilled water was used as a negative control.

Root harvest and slide preparation

After the 24 h exposure, three root tips from three seedlings per treatment were collected at random and assessed. Root tips 1 to 2 cm long were cut from the treated germinated seeds and fixed in alcohol-acetic acid (ethanol: glacial acetic acid in 3:1 ratio) for 24 h in a fridge at 4 to 6°C. The root tips were washed twice with ice cold water for 10 min each and allowed to dry in a watch glass. A solution of 1 N HCl pre-heated to 60°C was added to the root tips for 10 min. The HCl treatment was repeated a second time. Three root tips from each treatment were transferred singly to clean microscope slides and cut 2 mm from the growing tip. The tips were kept and the rest was discarded. Aceto-carmine stain was added to each slide to cover the root tip for about 10 min. A glass cover slip was placed on the root tip on each slide and tapped gently with a pencil eraser to spread the cells evenly into a monolayer to facilitate the scoring process for normal and aberrant cells in the different stages of the cell cycle. The slides were viewed under the light microscope (Olympus CX 21) and the cells were scored under oil immersion at 1000× magnification. The slides were coded and scored blind. The most representative ones for each structural aberration class were photographed using a Zeiss PrimoStar Microscope mounted with a Canon Camera, model, Power Shot A640.

Scoring of slides and data analysis

Cytotoxicity: On each of three slides (n = 3) for each concentration, a total of 2,000 cells, classified into interphase or dividing cells (Prophase, Metaphase, Anaphase or telophase) were scored that is, a total of 6,000 cells each for the control and treatment groups. The mitotic index (MI) was expressed as the number of dividing cells per 1,000 cells scored. The mitotic index of each treatment was compared with that of the negative control group using t-test at a probability level of 0.05. Analysis was performed using the SPSS for windows version 11.0 software.

Genotoxicity: On each of three slides for each dose, 100 anaphase and telophase cells were examined for chromosome aberration, that is, 300 anaphase and telophase cells per pesticide treatment. The following aberrations, adapted from Parry et al. (1985) were observed and scored:

1. Chromosome fragments (F): Pieces of chromosome broken from whole chromosome as a result of pesticide treatment and lacking centromere;
2. Anaphase or Telophase bridge (AB): Dicentric chromosomes that form a bridge between both poles at anaphase or telophase. Often it indicates paracentric inversions or other possibilities that include breakage and fusion of chromosomes and sister chromatid reunion;
3. Laggard (L): Whole chromosomes that fail to migrate to either pole at anaphase because of damage to the centromere and/or kinetochore;
4. C-Mitosis (C-Mit): Mitotic cells that lack spindle fibres so that the chromosomes lie scattered throughout the cell. The effect is usually produced in cells treated with the spindle poisons, colchicines or colcemid, hence C-Mitosis;
5. Multipolar anaphases and telophases (Multipolar): Anaphase and telophase cells with three or more spindle poles instead of the normal two. It is caused by damage to the centrosome;
6. Sticky chromosomes (S): Sticky chromosomes fail to condense completely so that at metaphase, the chromosomes are still long like prophase chromosomes and remain entangled with each other.

In extreme cases, chromatin masses, undistinguishable as chromosome are seen as clumps. The cells lack spindle fibres. The percentage of anaphase and telophase cells with aberrations in each treatment group of three slides at each concentration of pesticide was compared with that of the negative control group using t-test.

RESULTS

Cytotoxicity of the pesticides

The results of the effects on the mitotic index, of the treatment of onion root tip meristem cells with different concentrations of the pesticides are presented in Table 1. WO (78.1, 156.3, 312.5 mg/l), KOG (312.5, 625.0 mg/l) and CWB (312.5 mg/l) had mitotic indices significantly lower than that of the control ($P < 0.05$) and were adjudged cytotoxic. None of the three treatment concentrations of SB and CPX that were tested induced significantly depressed mitotic index when compared to the control and were accordingly adjudged not cytotoxic. The highest concentrations of the pesticides relative to their EC_{50}s were: WO (1/4 EC_{50}), CWB (1/4 EC_{50}), SB (1/8 EC_{50}) and CPX (1/4 EC_{50}). The highest concentration of 625.0 mg/l for KOG was an arbitrary value determined by solubility consideration.

Genotoxicity of the pesticides

Photographs of the most representative pictures of normal mitotic cells and cells containing the different types of chromosome aberrations that were observed and scored are presented in Figure 1. Six main types of chromosome aberrations were recorded in anaphase-telophase cells: F = fragment; AB = Anaphase or Telophase bridge; L = Laggard; C-Mit = C-Mitosis; S = Sticky chromosomes. The result of the determination of genotoxic effects of the pesticides on onion root tip meristem cells are presented in Table 2. WO (78.1, 312.5 mg/l), CWB (156.3, 312.5 mg/l), SB (625.0 mg/l), and CPX (1250.0 mg/l) induced genotoxicity ($P < 0.05$). No concentration of KOG induced genotoxicity. WO induced L, C-Mit, Multipolar and S types. CWB induced F, Multipolar and the S types. SB induced the S types only. CPX induced multipolar and the S type of aberrations. Sticky chromosomes were induced by all the pesticides that induced genotoxicity. This type of aberration was more prevalent and contributed upwards of 60% of the aberrations induction in each case. In Table 3 are presented the averages of prevalence of the six types of aberrations. The order was; S (65% average) > F (5% average) > C-mit (3% average) = multipolar > AB (1%

Table 1. The Cytotoxic effects of pesticides treatments on the different cell cycle stages of *Allium cepa*.

TC (mg/l)		Statistics	Cells in interph	Cells in division stages					Total cells scored	Mitotic index	
				Proph	Metaph	Anaph	Teloph	Total in mitose		MI	As a ratio of control
Water	Control	Mean	1797	105	43	22	34	203	2000	101.5	1
		SD	45	37	8	2	1	45	0	22.25	
WO	78.1	Mean	1900	42	23	13	22	100	2000	49.83	0.49‡
		SD	24	10	6	2	12	24	0	11.93	
	156.3	Mean	1908	64	7	5	10	85	2000	42.67	0.42‡
		SD	35	44	1	3	3	43	0	21.39	
	312.5	Mean	1924	38	18	8	12	76	2000	38	0.37‡
		SD	9	4	9	3	1	9	0	4.58	
KOG	156.3	Mean	1823	101	31	17	29	177	2000	88.67	0.87
		SD	10	9	6	3	12	10	0	5.13	
	312.5	Mean	1891	64	18	14	13	109	2000	54.67	0.54‡
		SD	34	12	10	9	8	34	0	17.1	
	625.0	Mean	1904	62	15	8	12	96	2000	48.17	0.47‡
		SD	28	15	4	5	6	28	0	14.05	
CWB	78.1	Mean	1872	73	37	7	12	128	2000	64	0.63
		SD	28	20	12	3	3	28	0	13.81	
	156.3	Mean	1849	89	33	12	17	151	2000	75.67	0.75
		SD	38	24	14	4	5	38	0	19.2	
	312.5	Mean	1883	60	29	12	16	117	2000	58.5	0.58‡
		SD	18	10	13	2	4	18	0	9.04	
SB	156.3	Mean	1855	77	32	12	24	145	2000	72.67	0.72
		SD	7	5	4	2	10	7	0	3.62	
	312.5	Mean	1860	83	24	16	17	140	2000	70.17	0.69
		SD	30	19	1	9	5	30	0	15.14	
	625.0	Mean	1853	86	22	14	25	147	2000	73.67	0.73
		SD	25	17	3	3	10	25	0	12.57	
CPX	312.5	Mean	1820	94	37	20	29	180	2000	90	0.89
		SD	8	6	4	4	7	8	0	3.77	
	625.0	Mean	1875	59	29	11	25	125	2000	62.67	0.62
		SD	54	18	17	8	13	54	0	27.02	
	1250.0	Mean	1846	73	26	20	35	154	2000	77.17	0.76
		SD	7	16	2	7	3	7	0	3.55	

TC = Test Compound; SD = Standard deviation; WO = Wipe-out; KOG = K-O. Gard; CWB = Cutworm Bait; SB = Snail Ban; CPX = COOPEX; MI = Mitotic Index; Interph = Interphase; Proph = Prophase; Metaph = Metaphase; Anaph = Anaphase; Teloph = Telophase; ‡ = Significant difference from negative control at $P < 0.05$ in the t-test, n = 3.

average) = L. The sticky chromosomes, C-mitosis and multipolar aberrations together constituted between 85 to 100% of the aberrations induced by the individual pesticides.

DISCUSSION

The results of this study have shown that WO, KOG and CWB significantly depressed the mitotic index (MI) in treated root tip meristem cells of *A. cepa*, when compared to the control at one or more concentrations ($P < 0.05$). SB and CPX were not cytotoxic. Rank and Nielsen (1997) showed that when the EC_{50} value was chosen as the highest concentration for the genotoxicity test, the mitotic index was never below 50% of the control. With the exception of KOG that the correct EC_{50} could not be

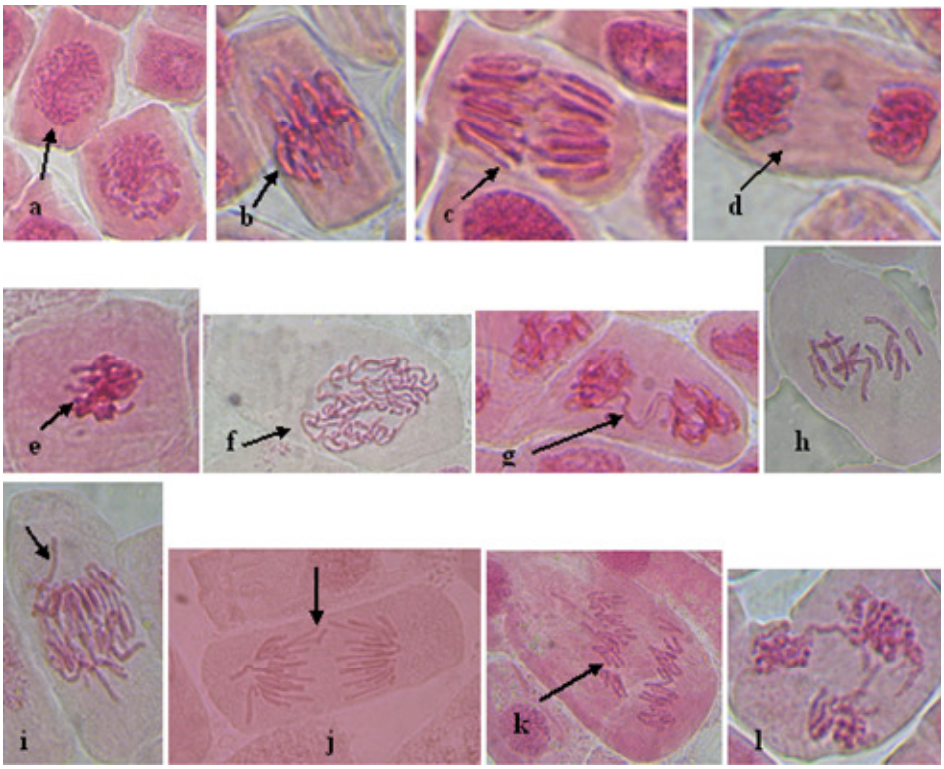

Figure 1. Photographs of cells of Allium cepa shwing untreated cells in normal division stages and pesticides treated cells with the different types of chromosomal abnormalities. Magnification (1000×). (a) Normal prophase cell, (b) normal metaphase, (c) normal anaphase, (d) normal telophase, (e) sticky metaphase, (f) sticky, (g) sticky telophase bridge, (h) c-mitosis, (i) vagrant or laggard chromosome, (j) fragment at anaphase, (k) multipolar anaphase, (i) sticky, multipolar and bridges at telophase.

determined even at the limit of its solubility, the highest concentrations of each pesticide that was tested was below the EC_{50}.

In the present study, the mitotic indices of meristem cells of *A. cepa* were thus reduced to below 50% of the control value when they were treated with concentrations of WO, KOG and CWB that were below their respective EC_{50}s. In each case, a lower concentration of the pesticide was required to achieve a reduction of the MI of treated cells to below 50% of control than to achieve the EC_{50}. The MI was thus a more sensitive end point than the EC_{50} which was the concentration of pesticide that inhibited the germination of 50% of seeds after 72 h treatment at room temperature. In trial experiments when the EC_{50}s of the pesticides were used as the highest concentration in a genotoxicity experiment, the MI was so depressed that not enough cells in division stages were available for observation and scoring.

The results of the present study showed that when the EC_{50} value was chosen as the highest concentration for the genotoxicity test, the mitotic index reduced to below 50% of the control, which was in contrast to the observations of Rank and Nielsen (1997). The difference in the results could be because while Rank and Nielsen tested pure compounds (*N*-methyl-*N*-nitrosourea, maleichydrazide, sodiumazide and ethylmethanesulfonate), the pesticides tested in the present study were mixtures, and one aspect of pesticide mixtures is the opportunity for complex interactions including synergism or antagonism (Cloyd et al., 2007). No concentration of WO tested was cytotoxic.

Another formulation that contained glyphosate called Roundup UltraMax (450 g/l glyphosate) was however shown to reduce MI at 100, 250 and 500 mg/L in the *A. cepa* test (Cavuşoğlu et al., 2011) and in tests using *Vicia faba* (El-Tayeb and Zaki, 2009). The results of the present study thus contradicted those of the studies cited above which could be due to the fact that the highest concentration of the WO tested was 312.5 mg/l which was ¼ of the EC_{50} and lower than the top concentration tested in the studies cited above. Another reason for the difference in the results of the present study and those of previous glyphosate in the formulation used in the present study was 360 g/l, lower than the 450 g/l glyphosate in Roundup UltraMax. The two highest concentrations of KOG (312.5, 625.0 mg/l) tested in this study significantly reduced the mitotic index ($P < 0.05$) and were cytotoxic. Reduction of the mitotic index

Table 2. Chromosome aberrations induced in root tip cells of *Allium cepa* following treatment with pesticides.

TC (mg/l)		Statistics	MI	A and T cells Scored	Cells with aberrations in 100 anaphase and telophase cells scored						
					F%	AB%	L%	C-Mit%	Multipolar%	S%	Total CA %
Water	Control	Mean	101.5	100	0	0	0	0	0	0	0
		SD	22.25	0	0	0	0	0	0	0	0
WO	78.1	Mean	49.83	100	0	0	0	17.67	3.67	37.67	59*
		SD	11.93	0	0	0	0	4.51	2.08	2.52	7.94
	156.3	Mean	42.67	100	0	0	0.67	0	1.67	23	25.33
		SD	21.39	0	0	0	1.15	0	1.53	19.92	19.63
	312.5	Mean	38	100	0	0	3.67	3.67	1.67	15.33	24.33*
		SD	4.58	0	0	0	3.79	6.35	1.53	3.51	9.29
KOG	156.3	Mean	88.67	100	0	0	0	0	0	1	1
		SD	5.13	0	0	0	0	0	0	1	1
	312.5	Mean	54.67	100	0	0	0	0	0	0	0
		SD	17.1	0	0	0	0	0	0	0	0
	625.0	Mean	48.17	100	0	0	0	0	0	0	0
		SD	14.05	0	0	0	0	0	0	0	0
CWB	78.1	Mean	64	100	0	0	0	0	0	0	0
		SD	13.81	0	0	0	0	0	0	0	0
	156.3	Mean	75.67	100	11.7	0	0	0	3.33	25.33	28.67*
		SD	19.2	0	1.15	0	0	0	3.51	5.69	2.31
	312.5	Mean	58.5	100	17	0	0	0	2	39.67	41.67*
		SD	9.04	0	2	0	0	0	2.65	7.57	4.93
SB	156.3	Mean	72.67	100	0	0	0	0	0	1.33	1.33
		SD	3.62	0	0	0	0	0	0	2.31	2.31
	312.5	Mean	70.17	100	0	0	0	0	0	1.67	1.67
		SD	15.14	0	0	0	0	0	0	2.08	2.08
	625.0	Mean	73.67	100	0	0	0	0	0	15	15*
		SD	12.57	0	0	0	0	0	0	7.55	7.55
CPX	312.5	Mean	90	100	0	0	0	0	0	0	0
		SD	3.77	0	0	0	0	0	0	0	0
	625.0	Mean	62.67	100	0	0.33	0	0	0	2	2.33
		SD	27.02	0	0	0.58	0	0	0	3.46	3.21
	1250.0	Mean	77.17	100	0	0	0	0	0.33	6.67	7*
		SD	3.55	0	0	0	0	0	0.58	3.79	4.36

TC = Test Compound; SD = Standard deviation; WO = Wipe-out; KOG = K-O. Gard; CWB = Cutworm Bait; SB = Snail Ban; CPX = COOPEX; MI = Mitotic Index; A and T = Anaphase and Telophase cells scored;CA % = Cells with chromosome aberration as % of A&T cells examined; F = Fragment; A.B = Anaphase or Telophase bridge; L = Laggard; C-Mit = C-Mitosis; ;S = Sticky; *Significant difference at $P < 0.05$ in the t-test, n = 3.

following treatment of *A. cepa* roots with deltamethrin, the active ingredient in the KOG, has been demonstrated previously (Chauhan et al., 1986; Saxena et al., 2009).

Only the highest concentration of CWB significantly reduced the mitotic index ($P < 0.05$) and was cytotoxic. We are not aware of any study in which Sodium hexa-fluorosilicate, the active ingredient in CWB, was tested for the effect on mitotic index in a plant system. None of the three concentrations of Snail ban (SB) significantly reduced the mitotic index. Therefore, none of the concentrations of Snail ban tested was cytotoxic. None of the three concentrations of coopex (CPX) that was tested in the present study reduced the mitotic index. The present study provides information on the reduction of mitotic index in a plant genotoxicity test system following treatment with pesticide formulation containing permethrin.

Table 3. Comparative induction of different chromosome aberration types in root tip cells of *Allium cepa* treated with different pesticides.

TC (mg/l)		Total CA	F as % of total CA	AB as % of total CA	L as % of total CA	C-Mit as % of total CA	Multipolar as % of total CA	S as % of total	C-M + Multipolar + S as % of total CA	C-Mit + S as % of total CA
Water	Control	0	0	0	0	0	0	0	0	0
	78.1	59	0	0	0	30	6	64	100	94
WO	156.3	25.33	0	0	3	0	7	91	97	91
	312.5	24.33	0	0	15	15	7	63	85	78
	156.3	1	0	0	0	0	0	100	100	100
KOG	312.5	0	0	0	0	0	0	0	0	0
	625.0	0	0	0	0	0	0	0	0	0
	78.1	0	0	0	0	0	0	0	0	0
CWB	156.3	28.67	41	0	0	0	12	88	100	88
	312.5	41.67	41	0	0	0	5	95	100	95
	156.3	1.33	0	0	0	0	0	100	100	100
SB	312.5	1.67	0	0	0	0	0	100	100	100
	625.0	15	0	0	0	0	0	100	100	100
	312.5	0	0	0	0	0	0	0	0	0
CPX	625.0	2.33	0	14	0	0	0	86	86	86
	1250.0	7	0	0	0	0	5	95	100	95
	Average		5	1	1	3	3	65	71	68

TC = Test Compound; WO = Wipe-out; KOG = K-O. Gard; CWB = Cutworm Bait; SB = Snail Ban; CPX = COOPEX; CA % = Total cells with chromosome aberration as % of A and T cells examined; F = fragment; AB = Anaphase or Telophase bridge; L = laggard; C-Mit = C-mitosis; S = sticky.

Mitotic index is considered a parameter that allows estimates to be made of the frequency of cellular division (Marcano et al., 2004) and inhibition of mitotic activities is often used for tracing cytotoxic substances (Linnainmaa et al., 1978; Smaka-Kincl et al., 1996). A depression of the mitotic index has been recorded by many investigators as a result of treatment with pesticides (Panda and Sahu, 1985; Amer and Farah, 1974). Treatment of the root-tip meristem cells of *A. cepa* with WO, CWB, SB or CPX caused cytological abnormalities at one or two concentrations of the pesticide screened in this work. The sticky chromosomes, multipolar and C-mitosis aberrations together constituted between 96 to 100% of the aberrations in all cases. Wipe-out induced genotoxicity at 78.1 and 312.5 mg/l in *A. cepa* root tip cells. Treatment of *A. cepa* root meristem cells with another pesticide formulation, Roundup UltraMax that contained 450 g/l glyphosate, induced chromosomal aberrations at 100, 250 and 500 mg/L in the *A. cepa* test (Cavusoglu et al., 2011).

Roundup treatment also induced chromosome aberrations in *Vicia faba* (El-Tayeb and Zaki, 2009). However results of mutagenicity and genotoxicity assays of glyphosate itself have been

been negative (Weed Science Society of America (WSSA), 1994). No concentration of K-O Gard® tested in the present study induced genotoxicity in *A. cepa* root tip cells. KOG was not toxic in preliminary toxicity tests even at the limit of solubility. However, treat-ment of garlic cloves/onion bulbs (Saxena et al., 2009) or root meristems of *A. cepa* (Chauhan et al., 1986; Bhanu et al., 2011) with pure deltamethrin induced mitotic and chromosomal aberrations. The studies of Chauhan et al. (1986) employed deltamethrin concentrations of between 0.05 to 2 ppm that is, 0.05 to 2 mg/l. Only the top two doses (156.3 and 312.5 mg/l) of CWB induced genotoxic effects in the present study with *A. cepa* root tip cells. However the pure compound, sodium hexafluorosilicate was negative in the Salmonella/microsome test, micro-nucleus test on mouse bone marrow and sex-linked recessive lethal mutations in *Drosophila* (Gocke et al., 1981; IARC, 1987a). The induction of genotoxicity by a pesticide formulation containing sodium hexafluorosilicate in the present plant assay system is therefore informative.

Snail ban induced mitotic and chromosomal aberrations at the highest concentration (625.0 mg/l) tested in the present study with *A. cepa* root tip cells. No mutagenic effect was observed in tests of 98% pure metaldehyde in several strains of *Salmonella typhimurium*, with or without metabolic activation (IPCS, 1996). According to the EPA (2006), the mutagenicity data for metaldehyde are deficient and new data are required, but examination of the old data suggests that metaldehyde is not a mutagen. For carbaryl however, a joint meeting of the Food and Agriculture Organization (FAO) panel of experts on pesticide residues in food and the environment and the WHO core assessment group in 1996 noted that, carbaryl has been evaluated for mutagenicity in a number of tests *in vitro* and *vivo* and been found to induce disturbances in the spindle fibre mechanism in plant and mammalian cells *in vitro* and chromosomal damage in human, rat and hamster cells and in plants treated *in vitro* with high doses of carbaryl (FAO and WHO, 1996).

Coopex induced mitotic and chromosomal aberrations at the highest concentration of 1250.0 mg/l tested in *A. cepa* root tip cells. The result of the present study with a plant assay system agreed with another study with an insect assay system where it was found that exposure to Ambush (a permethrin-containing insecticide) during larval development increased sex-linked lethal mutations in fruit flies (Kale et al., 1995).

According to All et al. (1977), Comins (1986) and Cloyd et al. (2007), one aspect of pesticide mixtures is the opportunity for complex interactions including synergism or antagonism. One well known example of synergism or potentiation (enhanced efficacy) is glyphosate, which alone rarely caused genetic damage in laboratory tests and Roundup, a glyphosate product, which demonstrated mutagenicity (Rank et al., 1993). It is therefore not unlikely that some of the differences in the results of the present study compared with results of some of the studies cited above have been caused by synergistic and/or antagonistic interactions in the pesticides mixtures used in the different studies.

Stickiness has been shown to be as a result of DNA condensation (Österberg et al., 1984) and entanglement of inter-chromosomal chromatin fibers which lead to subchromatid connections between chromosomes (Patil and Bhat, 1992). The results of the present study suggested that treatment of *A. cepa* root tip meristem cells with WO, CWB, SB and CPX induced DNA conden-sation, chromosome coiling and entanglement of inter-chromosomal chromatin fibers. Levan (1938) described colchicine mitosis (c-metaphase or c-anaphase) as an in-activation of the spindle followed by a random scattering of the condensed chromosomes in the cell. According to Yildiz and Arikan (2008), a large number of laggard chro-mosomes and c-anaphases indicate a test compound acted as a potent spindle inhibitor. The induction of va-grant chromosomes according to Elghamery et al. (2003), leads to the separation of unequal number of chromosomes in the daughter nuclei and subsequently formation of daughter cells with unequal sized or irregularly shaped nuclei at interphase.

In the present study, only the treatment with WO induced C-mitosis in the onion root tip meristem cells. The induction of anaphase/telophase bridges has been attributed to chromosome breaks, stickiness and brea-kage and reunion of the broken ends (Parry et al., 1985; Badr et al., 1992). The presence of the anaphase/ telophase bridges suggested a clastogenic effect of CPX.

Conclusion

The exposure of *A. cepa* root-tip meristem cells to concentrations below their respective EC_{50}s of WO (78.1, 156.3, 312.5 mg/l); KOG (312.5, 625.0 mg/l) and CWB (312.5 mg/l) resulted in a significant reduction ($P < 0.05$) in the mitotic index compared with the negative control, which was indicative of mitodepressive effect of the pesticides. The exposure of *A. cepa* root-tip meristem cells to concentrations of WO (78.1, 312.5 mg/l); CWB (156.3, 312.5 mg/l); SB (625.0) and CPX (1250.0 mg/l) caused significant increase ($P < 0.05$) in cytological ab-normalities at one or two concentrations of the pesticides. The following types of chromosome aberrations were recorded in anaphase-telophase cells: S (65% average) > F (5% average) > C-mit (3% average) = multipolar > A.B (1% average) = L. The sticky chromosomes, multipolar and C-mitosis aberrations together constituted between 85 to 100% of the aberrations in each case. The most prevalent type of aberration was the sticky chromosome type which was induced by WO, CWB, SB and CPX. Be-cause most adverse health effects by genotoxic agents are the result of genetic damage and any genetic activity of chemicals is most likely to result from cell division abnormalities, the genotoxic pesticides have potential to

cause adverse environmental and health effects.

REFERENCES

Abraham T, Firdissa E (1991). Insect pests of farm-stored maize and their management practices in Ethiopia. International Organization for Biological and Integrated Control of Noxious Animals and Plants. West Palaearctic Reg. Sect. Bull. 23:45-57.

Ahouangninou C, Martin T, Edorh P, Bio-Bangana S, Samuel O, St-Laurent L, Dion S, Fayomi B (2012). Characterization of health and environmental risks of pesticide use in market-gardening in the rural city of Tori-Bossito in Benin, West Africa. J. Environ. Prot. 3:241-248.

Aktar W, Sengupta D, Chowdhury A (2009). Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. 2(1):1-12.

All JN, Ali M, Hornyak EP, Weaver JB (1977). Joint action of two pyrethroids with methyl-parathion, methomyl, and chlorpyrifos on *Heliothis zea* and *Heliothis virescens* in the laboratory and in cotton and sweetcorn. J. Econ. Entomol. 70:813-817.

Amer SM, Farah OR (1974). Cytological Effects of Pesticides. VI. Effect of the Insecticide Roger on the Mitosis of *Vicia faba* and *Gossypium barbadense*. Cytologia 39:507-514.

Anwar WA (1997). Biomarkers of human exposure to pesticides. Environ. Health Perspect. 105(4):801-806.

Asita AO, Matebesi LP (2010). Genotoxicity of Hormoban and seven other pesticides to onion root tip meristematic cells. Afr. J. Biotechnol. 9(27):4225-4232.

Badr A, Ghareeb A, El-din HM (1992). Cytotoxicity of some pesticides in mitotic cells of *Vicia faba* roots. Egypt. J. Appl. Sci. 7:457-468.

Bhanu S, Archana S, Ajay K, Bhatt JL, Bajpai SP, Singh PS, Vandana B (2011). Impact of Deltamethrin on Environment, use as an Insecticide and its Bacterial degradation – A preliminary study. Int. J. Environ. Sci. 1(5):977-985.

Cai DW (2008). Understand the role of chemical pesticides and prevent misuses of pesticides. Bull. Agric. Sci. Technol. 1:36-38.

Cavuşoğlu K, Yalçin E, Türkmen Z, Yapar K, Cavuşoğlu K, Çiçek F (2011). Investigation of Toxic Effects of the Glyphosate on *Allium cepa*. J. Agric. Sci. 17:131-142.

Chauhan LKS, Dikshith TSS, Sundararaman V (1986). Effect of deltamethrin on plant cells I. Cytological effects on the root meristems of *Allium cepa*. Mutat. Res. 171(1):25-30.

Cloyd RA, Galle CL, Keith SR (2007). Greenhouse pesticide mixtures for control of silverleaf whitefly (Homoptera: Aleyrodidae) and twospotted spider mite (Acari: Tetranychidae). J. Entomol. Sci. 42: 375-382.

Comins HN (1986). Tactics for resistance management using multiple pesticides. Agric. Ecosyst. Environ. 16: 129-148.

Elghamery AA, Elkholy MA, Elyousser A (2003). Evaluation of cytological effects of Zn^{2-} in relation to germination and root growth of *Nigella sativa L.* and *Triticum aestivum L.* Mutat. Res. 537:29-41.

El-Tayeb MA, Zaki H (2009). Cytophysiological response of *Vicia faba* to a glyphosate-based herbicide. Am. Eurasian J. Agron. 2(3):168-175.

FAO, WHO (1996). Pesticide residues in Food - Carbaryl (008). FAO Plant Production and Protection Paper - 140) http://www.fao.org/docrep/W3727E/W3727E00.htm.

Gocke E, King MT, Eckhardt K, Wild D (1981). Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat. Res. 90:91-109.

Grant WF (1978). Chromosome Aberration in plants as monitoring system. Environ. Health Perspect. 27: 37-43.

Hileman B (1994). Environmental estrogens linked to reproductive abnormalities and cancer. Chem. Eng. News 72:19-23.

Hoppin JA, Umbach DM, London SJ, Henneberger P-K, Kullman GJ, Sandler DP (2008). Pesticides and atopic and nonatopic asthma among farm women in the agricultural health study. Am. J. Respir. Crit. Care Med. 177(1):11-18.

IARC (International Agency for Research on Cancer) (1987a). Overall evaluations of carcinogenicity: An updating of IARC monographs, Lyon. 1-42:208-210).

International Program on Chemical Safety (IPCS) (1996). "WHO/FAO DATA SHEETS ON PESTICIDES No. 93 METALDEHYDE". July. http://www.inchem.org/documents/pds/pds/pest93_e.htm#2.1.7.

Jha RK, Regmi AP (2009). 'Productivity of Pesticide Used for Vegetable Production in Nepal'. The South Asian Network for Development and Environmental Economics (SANDEE) working paper pp. 37-09. http://www.sandeeonline.org/uploads/documents/publication/869_PUB_Policy_Brief_37.pdf accessed on 04/09/12.

Kale PG, Petty Jr BT, Walker S, Ford JB, Dehkordi N, Tarasia S, Tasie BO, Kale R, Sohni YR (1995). Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. Environ. Mol. Mutagen 25(2):148-153.

Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MCR, Sandler DP (2007). Neurologic symptoms in licensed pesticide applicators in the agricultural health study. Hum. Exp. Toxicol. 26(3):243-250.

Levan A (1938). The effect of colchicine on root mitoses in *Allium*. Hereditas 24:471-486.

Linnainmaa K, Meretoja T, Sorsa M, Vainto H (1978). Cytogenetic effects of styrene and styrene oxide. Mutat. Res. 58:277-286.

Mansour S (2004). Pesticide exposure-Egyptian scene. Toxicology 198(1-3):91-115.

Marcano L, Carruyo I, Del campo A, Montiel X (2004). Cytotoxicity and mode of action of maleic hydrazide in root tips of *Allium cepa* L. Environ. Res. 94:221-226.

Matsumoto ST, Mantovani MS, Malaguttii MIA, Dias AL, Fonseca IC, Marin-Morales MA (2006). Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis* and chromosome aberrations in onion root-tips. Genet. Mol. Biol. 29(1):148-158.

Medina D, Prieto A, Ettiene G, Buscema I, Abreu de V A (1999) Persistence of Organophosphorus Pesticide Residues in Limón River Waters. Bull. Environ. Contam. Toxicol. 63:39-44.

Mohan S, Fields PG (2002). A simple technique to assess compounds that are repellents or attractive to stored products insects. J. Stored Prod. Res. 33:289-298.

Norppa H (2004). Cytogenetic biomarkers and genetic polymorphisms. Toxicol. Lett. 149(1-3):309-334.

Oerke EC, Dehne HW (2004). Safeguarding production—losses in major crops and the role of crop protection. Crop Prot. 23:275-285.

Osman KA, Al-Humaid AM, Al-Rehiayani SM, Al-Redhaiman KN (2010). Monitoring of pesticide residues in vegetables marketed in Al-Qassim region, Saudi Arabia. Ecotoxicol. Environ. Saf. 73:1433-1439.

Österberg R, Persson D, Bjursell G (1984). The condensation of DNA by chromium (III) ions. J. Biomol. Struct. Dyn. 2:285-290.

Panda BB, Sahu UK (1985). Induction of Abnormal Spindle Function and Cytokinesis Inhibition in Mitotic Cells of *Allium cepa* by the Organophosphorus Insecticide Fensulfothion. Cytobios 42:147-155.

Parry EM, Mumba LE, Asita A, Parry JM (1999). Mechanisms of Action of Aneuploidy Inducing Chemicals with Particular Reference to Spindle Inhibitors and Neurotoxins. In: Sobti RC, Obe G, Quillardet P (eds.), Trends in environmental mutagenesis. Tausco Book distributors, New Delhi, India. pp. 101-110.

Parry EM, Sharp DC, Parry JM (1985). The observation of mitotic division aberrations in mammalian cells exposed to chemical and radiation treatments. Mutat. Res. 150(1-2):369-81.

Patil BC, Bhat TGI (1992). A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in *Clitoria termata L.* Cytologia 57: 259-264.

Philips TW, Throne JE (2010). Biorational approaches to managing stored-product insects. Ann. Rev. Entomol. 55, 375-397.

Pimentel D, Acquay H, Biltonen M, Rice P, Silva M, Nelson J, Lipner V, Giordano S, Horowitz A, D'amore M (1993). 'Assessment of Environmental and Economic Impacts of Pesticide Use.' In: David P, Hugh L (eds.), The Pesticide Question. Chapman & Hall. Chapter 3, pp. 47-84.

Pimentel D, Greiner A, Bashore T (1998). Economic and Environmental Costs of Pesticides use. In: Environmental Toxicology: current Developments. Gordon and Breach Science Publisher, London UK. pp. 121-187.

Rank J, Nielsen MH (1997). *Allium cepa* anaphase-telophase root tip chromosome aberration assay on N-methyl-N-nitrosourea, maleic hydrazide, sodium azide and ethyl methanesulfonate. Mutat. Res.

390:121-127.
Rank J, Jensen AG, Skov B, Pedersen LH, Jensen K (1993). Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test, and *Allium* anaphase-telophase test. Mutat. Res. 300:29-36.
Richter ED (2002). Acute human poisonings. In: Pimentel D (Ed.), Encyclopedia of Pest Management. Dekker, New York. pp. 3-6.
Rojas A, Ojeda M, Barraza X (2000). Congenital malformations and pesticide exposure. Rev. Med. Chil. 128(4):399-404.
Saxena PN, Murthy RC, Gupta SK (2009). Evaluation of cytogenetic effects of deltamethrin in root meristem cells of *Allium sativum* and *Allium cepa*: A possible mechanism of chromosome damage. Toxicol. Environ. Chem. 91(3):577-594.
Smaka-Kincl V, Stegnar P, Lovka M, Toman MJ (1996). The evaluation of waste, surface and ground water quality using the Allium test procedure. Mutat. Res. 368:171-179.
Subbarao NS (1999). Soil Microbiology, 4th Edition. Science Publishers, Inc. pp. 303-324.
Tedesco SB, Laughinghouse IV HD (2012). Bioindicator of Genotoxicity: The *Allium cepa* test. http://cdn.intechopen.com/pdfs/29315/InTech-Bioindicator_of_genotoxicity_the_allium_cepa_test.pdf.
EPA (2006). Reregistration Eligibility Decision for Metaldehyde. http://www.epa.gov/opp00001/reregistration/REDs/metaldehyde_red.pdf
Weed Science Society of America (WSSA) (1994). Herbicide Handbook, Seventh Edition. Champaign, IL. pp. 10-59.
Yildiz M, Arikan ES (2008). Genotoxicity testing of quizalofop-P-ethyl herbicide using the *Allium cepa* anaphase-telophase chromosome aberration assay. Caryologia 61(1):45-52.
Youdeowi A, Service MW (1986). Pest and vector management in the tropics. English Language Book Society/Longman, Singapore.
Zhang W, Jiang F, Ou J (2011). Global pesticide consumption and pollution: with China as a focus. Proc. Int. Acad. Ecol. Environ. Sci. 1(2):125-144.