academicJournals

Vol. 12(41), pp. 6000-6006, 9 October, 2013
DOI: 10.5897/AJB2013.12802
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Cytogenotoxic effects of cypermethrin, deltamethrin, lambdacyhalothrin and endosulfan pesticides on *Allium cepa* root cells

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Accepted 26 August, 2013

Increased pesticides application in agriculture and public health has contributed to the pollution of the environment. This study evaluates the cytogenotoxic effects of emulsifiable concentrate of cypermethrin, deltamethrin, lambdacyhalothrin and endosulfan on *Allium cepa* root cells. Five concentrations (1.0, 5.0, 10.0, 20.0 and 40.0 ppm) of each pesticide were used for microscopic (48 h) and macroscopic (72 h) evaluations with distilled water as the control. Data were analyzed by Student's t-test. A dose dependent reduction in *A. cepa* root length was observed for the pesticides. Significant reduction in treated root length was observed at 10.0 ppm of deltamethrin, cypermethrin and lambdacyhalothrin, and at 20.0 and 40.0 ppm of all the pesticides compared to the control ($P<0.05$). The EC_{50} values showed growth inhibition in the order of lambdacyhalothrin > cypermethrin > deltamethrin > endosulfan, while that of total aberrant cells was cypermethrin > lambdacyhalothrin > deltamethrin > endosulfan. Microscopic aberrations observed in the pesticide-treated onions include sticky chromosomes, disturbed spindle and chromosome bridges. Dose dependent reduction was observed in the total mitotic dividing cells and mitotic index of the pesticide-treated *A. cepa,* except for 5.0 ppm of endosulfan. The pesticides induced growth inhibition and caused cytogenotoxic effects on the meristematic cells of *Allium cepa.* The data herein provide more information on the pesticides of which exposure to substantial concentration might constitute health risk to non-target organisms.

Key words: Pesticides, mitotic aberration, pyrethroid, organochlorine, growth.

INTRODUCTION

Pesticides are used to exterminate pests in order to increase yield and improve the shelf life of agricultural products. Besides, they are used in public health to reduce morbidity and mortality from pest related diseases. In recent years, there has been a tremendous increase in the use of these chemicals without paying much attention to the adverse effects they may have due to the toxic ingredients (Badr and Ibrahim, 1987; Anis et al., 1998). Reports have shown organochlorine pesticides like endosulfan to be toxic and have potential to be bioaccumulated in the environment and run off from field application of endosulfan leads to aquatic pollution. Animals that live in endosulfan-contaminated waters can bioaccumulate endosulfan in their bodies, the amount of which may be several times greater than in the surrounding water (ATSDR, 2008). Endosulfan has been reported to alter haematological profile in animals (Gimeno et al., 1994; Das et al., 2010; Modaresi and Seif, 2011; Yekeen and Fawole, 2011). Its accumulation in the environment led to its ban in most developed countries. However, it is still being used in most of the developing countries. Endosulfan is highly toxic and due to its persistence in the environment, its harmful effects are expected to manifest even in future generation of exposed population (Kumar and Chaudhary, 2012).

Bioaccumulative effects of organochlorine and high

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toxic effects of organophosphates especially on non-target organisms led to the increase use of pyrethroids as a potential alternative. Lambdacyhalothrin, deltamethrin and cypermethrin are type II pyrethroids extensively used in agriculture. Pyrethroids are also used in public health to reduce malaria morbidity and mortality (Zaim et al., 2000).

Although technical grades of pyrethroids were reported to have less to no toxic effects on non-target organisms, emulsifiable concentrate formulations of pyrethroids were two to nine times more toxic compared to the technical grades (Sanchez-Fortun and Barahona, 2005). Evaluations of some pyrethroids through different biological endpoints in animals show that they cause alteration in the haematological profile of exposed animals (Gimeno et al., 1994; Yekeen et al., 2007; Khan et al., 2012; Yekeen et al., 2013, Muthuviveganandave et al., 2013). Cypermethrin caused significant increase in chromosome aberration and in micronucleated erythrocytes frequency in farm workers (Carbonell et al., 1995; Lander et al., 2000). DNA damage was detected in tissue of workers involved in the production of cypermethrin (Grover et al., 2003).

Deltamethrin as a synthetic dibromo-pyrethroid insecticide and acaricide has been known to be three times more powerful than some other pyrethroids (Bradbury and Coats, 1989), which enhances its usage both indoor and outdoor. Cabral et al. (1990) reported that deltamethrin does not appear to be carcinogenic in mice or rats, while a very low dose of deltamethrin dis-plays harmful effects by disrupting hepatic and renal function and cause DNA damages in pubescent female rats (Chargui et al., 2012).

A non-significant induction of sperm cell aberra-ions in mice was reported for emulsifiable concentrate form of deltamethrin (Yekeen et al., 2007). Lambdacyhalothrin is used in public and animal health applications where it effectively controls a broad spectrum of insects and ectoparasites (Davies et al., 2000). The cytogenetic effects of lambdacyhalothrin were investigated in humans and various animal species using different endpoints such as micronucleus (MN) formation, induction of chromosomal aberrations and sister chromatid exchange (Fahmy and Abdalla, 2001; Celik et al., 2005), while studies on plant assay are limited.

The present study sought to evaluate the cytotoxic effects of cypermethrin, deltamethrin, lambdacyhalothrin and endosulfan in *Allium cepa*. This plant assay was selected because it is cost effective and as reliable as other methods for evaluation of chromosome aberrations (Rank and Nielsen, 1997) and can be easily used to assess toxicity via effective concentration determination (Yildiz and Arikan, 2008).

MATERIALS AND METHODS

Test chemicals

All pesticides were procured in the form (emulsifiable concentrate) commonly available in the market and widely used: Thionex® 35 EC (350 g/L) for endosulfan, Karate ® 2.5 EC for lambda-cyhalothrin, Deltaforce ® 2.5% EC for deltamethrin, and 10% EC for cypermethrin. Carmine salt was purchased from Zayo Sigma Chemicals Limited, Nigeria. All other chemicals used were of analytical grade.

Allium cepa assay

The onion bulbs (*Allium cepa* L.) used for experiment were sun-dried for three weeks, and the outer scales and brownish bottom plates were carefully removed, leaving the root ring primordial intact. Five concentrations (1.0, 5.0, 10.0, 20.0 and 40.0 ppm) of each pesticide were prepared with distilled water used as diluents as well as the control.

Twelve (12) onion bulbs were planted per concentration with each bulb placed on 50 ml capacity beaker filled separately with the prepared concentrations of the pesticides. Onion roots were grown at room temperature (25±1°C) in a dark cupboard. The contents of the beaker were replaced with freshly prepared pesticide solution at every 24 h.

The root tips used for microscopic evaluation were harvested from five onion bulbs per concentration at 48 h, and fixed in ethanol-ethanoic acid (3:1 v/v) before been transferred to 70% ethanol. The root tips were then hydrolyzed in 1 N HCl at 65°C for 3 min. Two root tips were squashed on slides, and then stained with acetocarmine for 15 min.

One thousand (1,000) cells per slide and a total of 5000 cells per concentration were scored for the frequency and occurrence of different types of chromosomal aberrations in the dividing cells at 1000x as previously described (Fiskesjo, 1985; Bakare et al. 2000; Lateef et al. 2007). The photomicrographs were taken with the Ocular VGA adapted Bresser Erudit DLX microscope (Germany). The mitotic index and mitotic inhibition were determined from the scores obtained for dividing cells based on these formulae:

$$\text{Mitotic Index (MI)} = \frac{\text{number of dividing cell in the treatment} \times 100}{\text{Total number of cell}}$$

$$\text{Mitotic Inhibition} = \frac{\text{Mitotic index of control} - \text{Mitotic index of treatment} \times 100}{\text{Mitotic index of control}}$$

The length of each root from the 5 onion bulbs per concentration and the control were measured at 72 h for macroscopic evaluation, and growth inhibition was evaluated. The EC_{50} was extrapolated from the graph of percentage root growth relative to control against pesticides concentrations.

Statistical analysis

The means with the standard errors for each of the concentrations per pesticide were calculated. The data obtained for the root length of the treated groups and the control was compared using t-test and considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The mean root length of the treated *A. cepa* for the four pesticides in all concentrations was lower compared to the control (Table 1). A dose dependent reduction was observed in *A. cepa* root length for the pesticides except at 5.0 ppm of deltamethrin. Significant difference in root

Table 1. Macroscopic and microscopic evaluations of the pesticide treated *Allium cepa*.

Concentration (ppm)	Mean root length	% inhibition	Dividing cells	Mitotic index	Mitotic inhibition	Sticky chromosome	Disturbed spindle	Chr bridge	Chr fragment	Chr Laggard	C mitosis	Total aberration	% Frequency
Control													
0	2.88±0.08	-	162	3.24	-	-	-	-	-	-	-	-	-
Cypermethrin													
1.0	2.50±0.23	13.19	101	2.02	37.65	-	9	2	-	3	-	14	0.28
5.0	2.18±0.08	24.31	85	1.70	47.53	1	14	3	-	-	-	19	0.38
10.0	1.88±0.12*	34.72	65	1.30	59.88	3	10	5	-	2	-	20	0.40
20.0	1.54±1.12*	46.53	41	0.82	74.69	-	6	1	1	-	1	9	0.18
40.0	0.88±0.05*	69.44	26	0.52	83.95	-	6	3	-	-	-	9	0.18
Deltamethrin													
1.0	2.28±0.16	20.83	112	2.24	30.86	1	6	-	-	2	-	9	0.18
5.0	2.49±0.10	13.54	102	2.04	37.04	-	8	3	1	1	2	15	0.30
10.0	1.84±0.10*	36.11	78	1.56	38.27	-	-	2	-	-	-	2	0.04
20.0	1.58±0.12*	45.14	10	0.20	93.83	-	1	-	-	-	-	1	0.02
40.0	0.98±0.08*	65.97	0	0	100.0	-	-	-	-	-	-	-	-
Lambdacyhalothrin													
1.0	2.01±0.13	30.21	145	2.90	10.49	-	1	-	-	-		1	0.02
5.0	1.95±0.09	32.29	86	1.72	46.91	1	-	2	-	-		3	0.06
10.0	1.12±0.07*	57.99	73	1.46	54.94	-	15	-	-	1		16	0.48
20.0	1.04±0.08*	63.89	75	1.50	53.70	-	19	3	1	1		24	0.32
40.0	0.69±0.08*	76.04	32	0.64	80.25	-	3	1	-	1		5	0.10
Endosulfan													
1.0	2.84±0.10	1.39	102	2.04	37.04	-	-	-	-	-	-	-	-
5.0	2.53±0.08	12.15	136	2.72	16.06	-	5	1	-	1	-	7	0.14
10.0	2.12±0.06	26.39	79	1.58	51.23	-	2	-	-	1	-	3	0.06
20.0	1.78±0.05*	38.19	32	0.64	80.25	-	1	-	-	-	-	1	0.02
40.0	0.95±0.08*	67.01	17	0.34	89.51	-	2	-	-	-	-	2	0.04

5000 cells were scored per concentration; Chr: chromosome; *Student t-test show significant difference compared to control ($P \leq 0.05$).

length was observed at 10.0, 20.0 and 40.0 ppm of deltamethrin, cypermethrin and lambda-cyhalothrin, while endosulfan showed difference at 20.0 and 40.0 ppm ($P<0.05$). Highest percent-tage root inhibition was observed at 40 ppm of each of the pesticides. Figure 1 shows the percentage root length relative to control where the EC_{50} values of 9.0, 21.5, 23.5 and 29.00 ppm were obtained for lambdacyhalothrin, cyperme-thrin, deltamethrin and endosulfan, respectively, which indicate the decreasing order of their inhibitory effects on *A. cepa* root growth. The growth inhibitory effect of the pesticides is indicated by the significant reduction of root length compared to the control. Table 1 also shows the microscopic evaluation of the pesticides. A dose dependent reduction in the total mitotic dividing cells and mitotic index was observed in *A. cepa* treated with the pesticides, except for 5.0 ppm of endosulfan. However, complete cell arrest was observed only in deltamethrin at 40.0 ppm. The values of mitotic

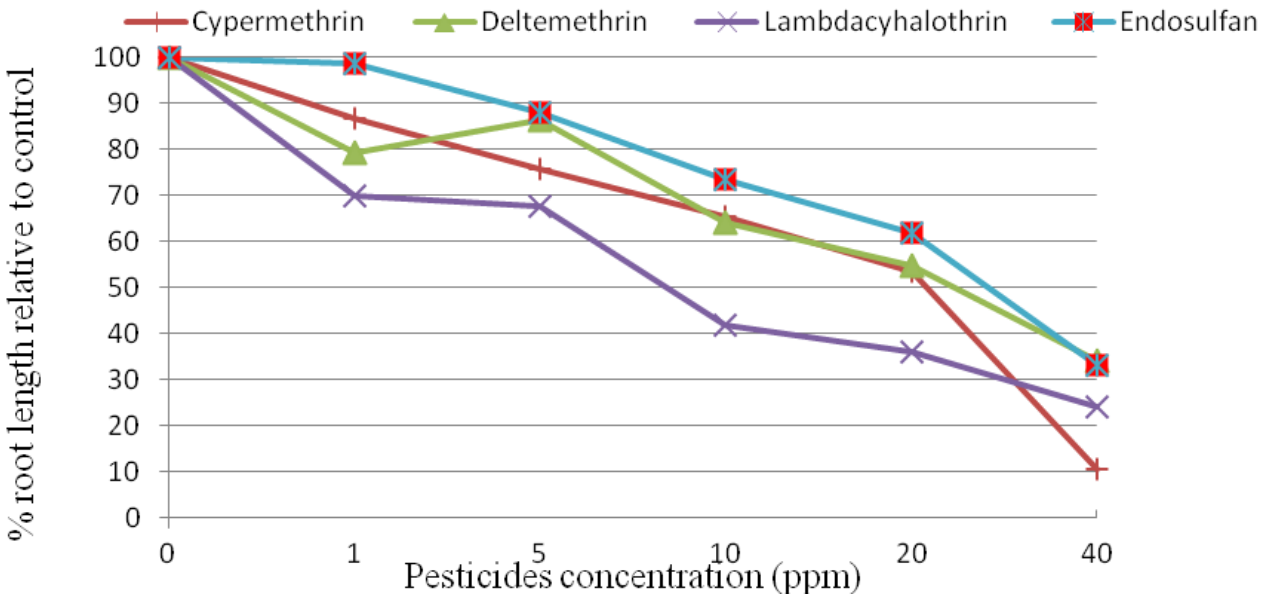

Figure 1. Growth inhibitions of pesticides treated *A. cepa* root.

index obtained for all pesticides at 10.0 (except in endosulfan), 20.0 and 40.0 ppm were lower than half of the negative control, which reflect their cytotoxicity. Similar observation was reported in *A. cepa* treated with different pesticides (Asita and Matebesi, 2010; Sibhghatulla et al., 2012). The total chromosomal aberrations induced were in the order: Cypermethrin > lambdacyhalothrin > deltamethrin > endosulfan.

The aberrations observed in the three pesticides included sticky chromosome, disturbed spindle, c-mitosis, chro-mosome-bridge and laggard chromosomes (Table 1 and Figure 2). Stickiness observed in the pyrethroid-treated onion roots may be due to physical adhesion of the proteins of the chromosome (Patil and Bhat, 1992). The occurrence of c-mitosis indicates that spindle formation was adversely affected (El-Ghamery et al., 2003). Disturbed spindle resulted in inability of chromosomes to move to the poles.

Chromosome bridge is formed by breakage and fusion of chromosomes and chromatids, the stickiness of chromosome and subse-quent failure of free anaphase separation, and unequal translocation or inversion of chromosome segments (Gomórgen, 2005). Permjit and Grover (1985) attributed laggard chromosomes to the delayed terminalization, stickiness of chromo-some ends or the failure of chromosomal movement.

Aberrations of mitotic cycle, change of mitotic index and chromosomal abnormalities observed after exposure to toxic metals, metalloids or organic pollutants were attributed to the disorganization and depolymerization of microtubules, which underlie these processes in higher plant cells (Liu et al., 2009; Xu et al., 2009; Dho et al., 2010; Eleftheriou et al., 2012, 2013; Adamakis et al., 2013). Cypermethrin among other pesticides tested in this study has the highest total chromosomal aberration. Seehy et al. (1983) reported that in mice, both technical and formulated products of alpha cypermethrin showed a dose dependent sister chromatid exchanges in dividing cells at all dose levels but the highest doses inhibited mitotic division.

Cypermethrin and alphamethrin were reported to elicit varying degrees of cytotoxic, turbagenic (toxicity to spindle) and clastogenic effects but generally more turbagenic and weak clastogenic (Rao et al., 2005). However, Asita and Makhalemele (2008) reported that alpha-thrin (active ingredient of alpha-cypermethrin) was only cytotoxic but not genotoxic at various concentrations in treated *A. cepa*. Cypermethrin has been classified as a possible human carcinogen (EPA, 2002).

The pesticides used induced significant growth inhibition at 10.0, 20.0 and 40.0 ppm. Also, at these concentrations, the mitotic index was lower than half of the values obtained for the control which indicate their cytotoxic effects. Induction of chromosomal aberrations at different concentrations shows the genotoxic effects on the meristematic cells of *A. cepa.* The aberrations observed were however not dose dependent, which may be due to fewer number of dividing cells at higher concentration of the pesticide and complete cell arrest observed at 40.0 ppm of deltamethrin.

Our results are in accord with the previous reports, where mitotic inhibition and genotoxicity of pesticides were demonstrated (Mosuro et al 1999; Chauhan et al., 1999; Kumar and Chaudhary, 2012). Reduction in mitotic activity could be due to the inhibition of DNA synthesis (Schneiderman et al., 1971; Sudhakar et al., 2001) or due to a block in the G2-phase of the cell cycle, thus preventing the cell from entering mitosis (Van't Hof, 1968).

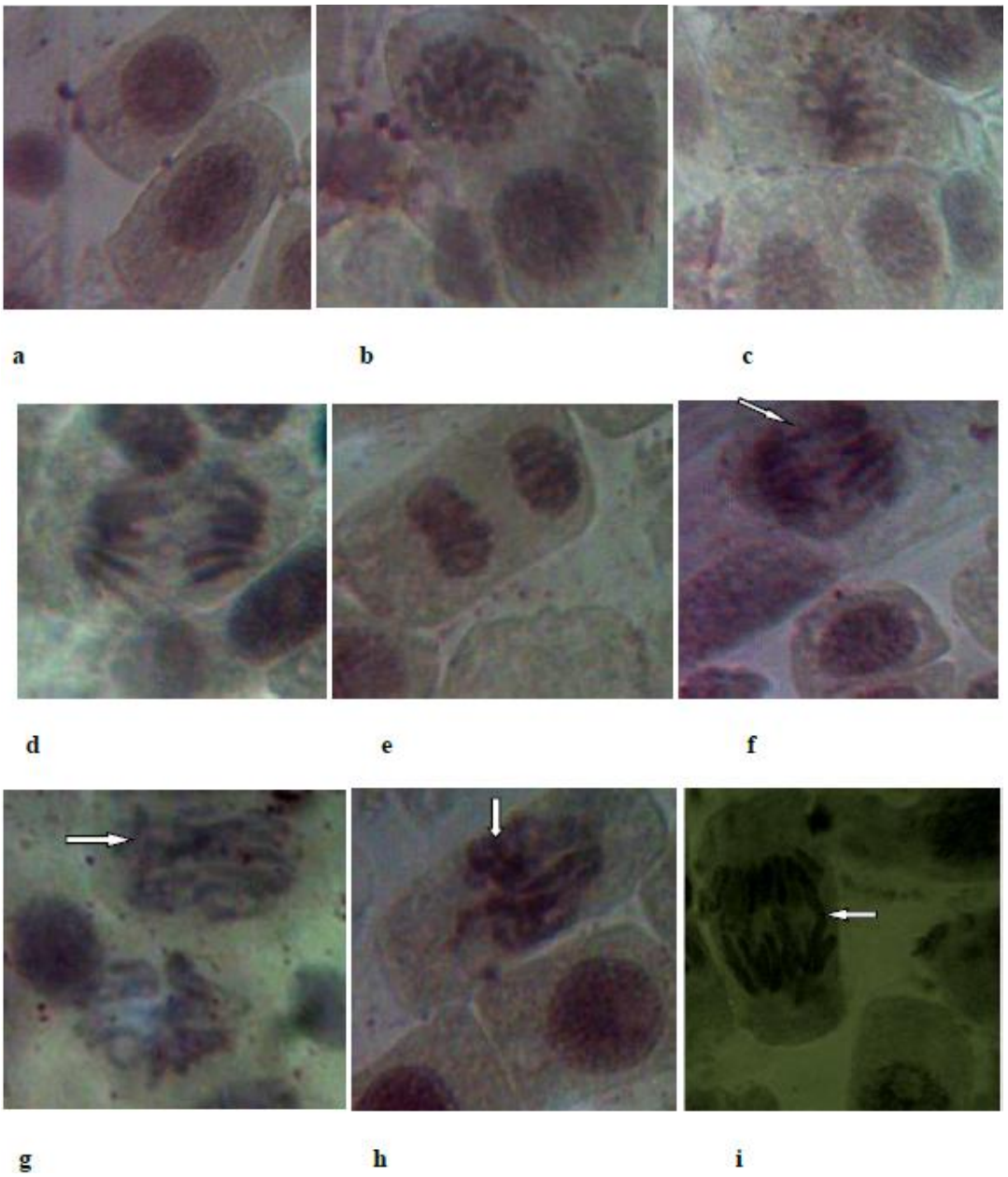

Figure 2. Normal and aberrant cells observed in *allium cepa* treated with pesticide. **a,** normal interphase; **b,** normal prophase; **c,** normal metaphase; **d,** normal anaphase; **e,** normal telophase; **f,** laggard chromosome; **g,** disturbed spindle and fragmentation; **h,** sticky chromosome; **I,** chromosome bridge.

Prior to occurrence of chromosome aberrations, there is always some growth restriction which is the cumulative response of all the damaging effects (Fiskejo, 1997).

Conclusion

The inhibition of growth and induction of chromosomal aberrations by the pesticides show their cytogenotoxic effects. This data provide more information on the cypermethrin, deltamethrin, lambdacyhalothrin and endo-sulfan of which exposure to substantial concentration may constitute health risk to non-target organisms and thus will assist in future ecotoxicological evaluations.

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