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EFFECTS OF TWO PESTICIDES (DURSBAN AND CUBEX) ON CHROMOSOME CELL DIVISION AND DNA IMAGE DURING CELL CYCLE IN MITOTIC CELLS OF *ALLIUM CEPA* ROOTS

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ABSTRACT

The effect of the two pesticides Dursban and Cubex on mitotic activity, mitotic abnormalities and changes in the DNA content has been investigated using *Allium cepa* root tip assay. Treatment of *Allium cepa* root tips with the different doses of each of the two pesticides resulted in a marked effect on the frequency of mitotic phases depending on the concentration used. Each of the used pesticides induced a marked decrease in the mitotic index and gradual increase in the percentage of chromosomal abnormalities as the concentrations of each of the two pesticide increased. Chromosomal abnormalities associated with stickiness of chromosomes were the dominant abnormalities induced by these chemicals. Some of the chromosomal abnormalities, produced by these pesticides, particularly breaks, bridges and micronuclei indicate its genotoxic potential. Image cytometric measurements demonstrated a dose-dependent effect of of the pesticide treatments on the components of mitotic cell cycle in *Allium cepa* root tip cells. The most evident effect appears to be the accumulation of cells in the Go/G_1 phase with resultant reduction in the proportion of cells in other phases of the cycle (S phase , G_2 phase and mitosis). Frequent use of these chemicals has a potential mutagenic effect in the environment. The results obtained in the present study indicate that the cytological examination of induced chromosomal aberrations can be used to monitor genetic damage caused by pesticides.

INTRODUCTION

Pesticides are part of modern agriculture but their use must be carefully managed. Management of pesticides application includes the study of their damage to non-target organisms. A number of bioassay test systems have been developed to monitor the action of pesticides and other environmental hazards on living organisms. One serious damage that has been caused by several pesticides is the effects on the chromosomes. A root tip assay using *Vicia faba* (Kihlman, 1975) or *Allium cepa* (Grant, 1982) has been widely used as a valid cytogenetic procedure to monitor the chromosome damage of environmental hazards (Grant, 1994). Using these systems cytotoxic effects and damage to chromosomes of numerous pesticides have been reported by several authors (Badr, 1983; 1988; Badr *et al.*, 1985; Adam *et al.*, 1990; Pandey *et al.*, 1994; Barakat and Hassan, 1997; Ghareeb and George, 1997; Sabir *et al.*, 1998). The chromosome damage induced by these and other agents has been considered as indication of their genotoxic potential (Grant, 1982; 1994).

Flow and image cytometry has been progressively used as a rapid and accurate method to measure nuclear DNA image throughout cell cycle (Hammatt *et al.*, 1991; Baranyi and Greilhuber, 1996). With this method it is possible to perform cell cycle analysis and study its regulation in plant cells (Zhang *et al.*, 1992; Gilab *et al.*, 1994). A possible application of this approach may be its use for evaluating the cytotoxicity of environmental hazards which certainly include pesticides. This procedure can be used to examine the effect of aflatoxin B on cell cycle progression in *Vicia faba* root cells (El-Shazly and El-Sheikh, 1999). The results clearly demonstrated that aflatoxin B_2 acts as an inhibitor of cell cycle at the G_1 phase. The present study aims to investigate the chromotoxic effect of two widely used pesticides and their influence on cell division as well as on a number of cell cycle parameters in meristematic cells of root tips of *Allium cepa* in order to screen the genotoxic potential of these compounds and report their action on DNA at different stages of cell cycle. The used herbicides are Dursban and Cubex which are frequently used in agriculture and in home gardens for better yield of different vegetables and better performance of several ornamentals.

MATERIAL AND METHODS

The material used throughout the present work was *Allium cepa*. Bulbs of *A. cepa* variety Giza 2 were used in this investigation. The pesticides tested in the current study are Dursban (O,O-Diethyl-**O-**3,5,6 trichloro-2 pyridyl) with 40% active ingredient and Cubex (N,N-Diethyl-2,4-dinitro-6-trifluro-methyl-N-phenylene diamine) With 25% active ingredient. Concering the first pesticide (Dursban), three concentrations (1, 10, 100 ppm) were used. For the second pesticide (Cubex), two concentrations (2.1, 6.7 ppm) were used. The concentrations were calculated on a weight basis of the active ingredient for each pesticide dissolved in 100 ml of distilled water.

Bulbs of equal size were placed on top of vials filled with water for fibrous root initiation. Growing root apices of *A. cepa* of about 1-1.5 cm were dipped for 12 hrs in all the above mentioned concentrations of the two pesticides or water which served as control. After the termination of treatments, root tips were cut, washed thoroughly in tap water and fixed in aceto-alcohol (1:3 v/v) for 24 hrs then preserved in 70% ethyl alcohol at 4°C. For cytological preparation, root tips were hydrolysed for 8 min. at 60°C and stained in Feulgen reagent for two hours. At least sex slides for each treatment were examined. Mitotic index was caliberated as the percentage of dividing cells. The same slides were screened for chromosomal abnormlities.

For cytometric measurement of nuclear DNA content, roots of *A. cepa* (1.5-2 cm in length) which were treated with aqueous solutions of the different concentrations of each of Dursban and Cubex were hydrolysed in 1N HCl at 60°C for 12 minutes, washed in distilled water and stained in Schiff's reagent for 12 hrs at 14°C; and root tips squashed in 45% acetic acid. At least six slides were prepared for each treatment and the control. Feulgen-stained slides were analyzed with the CAS Image Analyzer; software (CAS, Inc. Version 3.0). Analysis screen followed the caliberation step. On average 150 consecutive cells per each slide were selected for DNA measurements.

Based on estimating the amount of DNA in the nucleus, DNA ploidy analysis, the fraction of cells undergoing different phases of cell cycle were calculated. These include; cells with DNA amount less than the 2C value, cells with 2C DNA (G0/G1), cells with 2C-4C DNA (S-phase), cells with 4C DNA (G2-phase), cells with DNA amount more than the 4C value and cells in mitotic phases.

RESULTS AND DISCUSSION

The treatment of *A. cepa* roots with either Dursban or Cubex had altered the mitotic profile favoring metaphase at the expense of prophase and ana-telophase stages especially at high concentrations (Table 1). The degree of mitotic inhibition is dose dependent, where mitotic index value was decreased as the concentrationc of the pesticide increased. The mitodepressive effect was more pronounced after treatments with Cubex than by Dursban. Recorded mitotic values reached a minimum values of 2.15% and 0.94% after treatment with highest concentrations of Dursban and Cubex, respectively as compared with a control value of 8.01% (Table 1).

Table 1: Frequency of mitotic phases and the mean value of mitotic index after treating *Allium Cepa* root tips with different concentrations of Dursban and Cubex.

Conc.	M.I.±SE	Frequency of mitotic phases		
		Prophase	Metaphase	Ana- teophase
Control	7.92±0.71	41.43	25.48	33.09
Dursban				
1.0 ppm	6.19±0.80	44.05	26.94	29.01
10.0 ppm	3.80±0.33**	40.67	32.12	27.21
100.0 ppm	2.21±0.21**	38.91	36.07	25.02
Cubex				
2.1 ppm	3.24±0.37**	49.12	31.39	19.49
6.7 ppm	0.96±0.09***	52.84	34.02	13.14

**** highly significant at 0.01. *** very highly significant at 0.001.**

A wide spectrum of mitotic chromosomal abnormalities were recorded in the treated roots after treatment with different concentrations of either Dursban or Cubex pesticides. In almost all treatments, increasing concentration led to a parallel increase in the percentages of abnormalities. The maximun values of the mean percentage of the total abnormalities 40.34% and 38.19% were recorded with highest concentration of Dursban and Cubex, respectively (Table 2). Treatment of *A. cepa* root tips with each of the pesticides used in this study induced different types of chromosomal abnormalities including stickiness, disturbed cells, bridges, laggards and teolphase cells with micronuclei (Table 2 and Fig.1). Stickiness (Fig. 1a) was noticed with a high percentage in all mitotic phases after all treatments with each of the two chemicals. Disturbance (Fig. 1b) was also observed in considerable percentages in both metaphase and ana-telophase after all treatments. Lagging chromosomes (Fig. 1c) and bridges (Fig. 1d) were seen

Figure 1: Photomicrographs of the mitotic abnormalities in root-tip cells of *Allium cepa* induced by different treatments of Dursban and Cubex.
a) Severe sticky-metaphase (Dursnan, 10 ppm).
b) Disturbed anaphase (Dursban, 10 ppm).
c) Lagging chromosome (Cubex, 6.7 ppm).
d) Double bridged anaphase (Dursban, 100 ppm).
e) Non-congression at metaphase (Dursban, 10ppm).
f) C-metaphase (Cubex, 2.1 ppm).
g) Micronuclei (Dursban, 1ppm).

in relatively high proportions at ana-telophase after all treatments with the two pesticides. Non-congression was observed at metaphase (Fig. 1e) Another abnormality that was observed in the current study was C-metaphase (Fig. 1f), its proportion was higher in cells treated with Dursban than with Cubex. Micronuclei (Fig. 1g) were also seen in ana-telophase following all treatments of the two pesticides.

The effect of Dursban and Cubex on the fraction of cell cycle phases is documented in Table 3. Inspection of the figures given in this table clearly demonstrates an increase of the percentage of cells with the 2C value (G_0/G_1) as the concentration of the applied pesticide increased. There was an increase of this percentage from 51.22 in control roots to 62.81 and 69.47 in cells treated with the highest concentration of Dursban and Cubex, respectively. On the other hand, the fraction of cells in the DNA synthesis peroid (S-phase), the G_2-phase and in mitotic phases progressively decreased with the increase of the applied dose of the two pesticides. The proportion of cells in the S-phase was reduced from 22.83 in control roots to 14.62 and 13.68 in root cells treated with highest concentrations of Dursban and Cubex, respectively. Also the proportion of G_2-phase cells was reduced from a control value of 16.25 to 8.07 and 7.17 in root cells treated with 100 ppm Dursban and 6.7 ppm Cubex respectively. Likewise the fraction of cells in mitosis was decreased from 8.01 to 2.15 and 0.94 in root tips treated with the highest concentrations of Dursban and Cubex, respectively. A fraction of cells with DNA content less than 2C value or more than the 4C value was observed. There was some increase in those with DNA < the 2C value with increased concentration of the applied pesticides but the change in cells with DNA content >the 4C value was dose independent (Table 3).

Table 3 : Effects of Dursban and Cubex treatments on the measured cell cycle parameters in root meristematic cells of *Allium cepa.*

Treat.	DNA±SE <2C	G0/G1±SE phase	S±SE phase	G2±SE phase	Mit±SE. phases	DNA±SE >4C
Control	0.67 ± 0.11	51.22± 5.93	22.83± 1.84	16.25± 1.23	8.01± 0.76	0.02± 0.003
Dursban						
1.00 ppm	6.17± 0.71***	53.12± 4.81	19.88± 1.32	11.72± 1.18*	6.24± 0.84	2.87± 0.19***
10.00 ppm	7.35± 0.93***	59.19± 6.31	16.71± 1.71*	9.93± 0.81**	3.73± 0.31**	3.09± 0.29***
100.00 ppm	7.94± 0.51***	62.81± 5.60	14.62± 1.37*	8.07± 0.78**	2.15+ 0.18**	4.41± 0.32***
Cubex						
2.10 ppm	5.91± 0.68***	62.14± 6.82	15.49± 1.25*	10.36± 0.91*	3.27± 0.38**	2.83± 0.11***
6.70 ppm	6.24± 0.57***	69.47± 6.10	13.68± 1.18**	7.17± 0.61**	0.94± 0.08***	2.50± 0.21***

*** significant at 0.05 **highly significant from control at 0.01**
*****very highly significant from control at 0.001**

DISCUSSION

The two presently tested chemicals were found to have a retarding effect on cell division and induced cytological disturbances during root tip mitosis . All the used concentrations were capable to induce different types of chromosomal abnormalities, the frequency of abnormalities increased in most cases, with the concentration of pesticides. The treatments with these pesticides also induced a considerable percentage of disturbed metaphase and ana-telophase. This phenomenon may be a result of partial effect of the two pesticides, on spindle apparatus (Amer and Mikhael 1986; Sobhi and Haliem, 1990). Similar effects have been reported in different plant materials by several other chemicals (Pandey *et al.* 1994; Ahmed and Yasmin 1992; Bellani *et al.* 1991; Kumar and Sinha 1991). Stickiness was the most common of the abnormalities induced by all treatments with Dursban and Cubex in all phases of mitotic cell divison. Klasterska *et al.*, (1976) attributed such anomaly to the enlargement of interchromosomal chromatin fibres which, leads to subchromatid connections between chromosomes. Recently, Patil and Bhat, 1992 suggested that, stickiness is a type of physical adhesion involving mainly the proteinacious matrix of chromatin material.

Another abnormality noticed in the present study is the appearance of C-metaphas. This type of abnormality was observed as a result of inhibition of spindle fiber formation (Levan, 1954). Pickett-Heaps *et al.* (1982) showed that, colchicine inhibited spindle formation due to interference with tubulin and / or polymerization of the microtubular sub-units. Bridges were observed in ana-telophase of cell division after all treatments with Dursban and Cubex. In such case, the bridge may be due to the general stickiness of the chromosomes and subsequent failure of anaphase separation (Abraham and Koshy, 1979) or it may be the result of chromosomal breakage and reunion (Tomkins and Grant, 1972).

Lagging chromosomes were also observed in ana-telophases after all treatments with the two used pesticides. The presence of lagging chromosomes may be attributed to hindrance of the prometaphase movement accompanied by the adhesion of the centrromers of one or more chromosomes to the inner surface of the plasma membrane (Barthelmess, 1957). Patil and Bhat (1992) stated that laggards are irregular orientation of chromosomes. The induction of laggards may lead to micronuclei formation. In the current study the presence of micronuclei in ana-telophases of all treatments of both pesticides support this view.

The applied treatments of the two pesticides clearly demonstrated a dose-dependent effect of these toxins on the compnents of mitotic cycle of meristematic cells of *A. cepa* roots. The most evident effect appears to be the accumulation of cells in the G_0/G_1 phase, at the expense of other phases which is evident by the frequent increase in the percentage of cells in this phase associated with reductions in the proportion of other cell cycle phases. These results show the inhibitory effects of these chemicals on cell cycle at the G_1 transition point. In this respect the action of these chemicals resembles that of olomoucine when applied to *Petunia* mesophyll cells in culture (Gilab *et al.*, 1994) and also the action of AFB_2 on meristematic cells

of *V. faba* roots (El-Shazly and El-Sheikh, 1999).Inhibition of mitosis by Dursban and Cubex was accompanied by a reduction in the amount of DNA, as repoted by other authors using different chemicals (Schlemper *et al.*, 1991; Choy, 1993; Badr *et al.*, 1995, El-Shazly and El-Sheikh, 1999). The reduction in the fraction of cells in the S-phase, which was recorded in the cells treated by the two pesticides used, is consistent with the results of other investigators (Schlemper *et al.*, 1991; Choy, 1993).

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تأثير المبيدين دورسبان و كيوبكس على الكروموسومات وإنقسام الخلية وصورة دنا خلال دورة الخلية بجذور البصل.

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****قسم العلوم البيولوجية والجيولوجيا-كلية التربية-حامعة عين شمس-روكسي-القاهرة.**

تم في هذا البحث دراسة تأثير المبيد الحشري دورسبان والمبيد العشبي كيوبكس علي الانقسام الميتوزي والتغيرات الطارئة علي كمية دنا في جذور نبات البصل بالجرعات 1و10و100 جزء في المليون في حالة الدورسبان وبالجرعات 2.1 و 6.7 جزء في المليون في حالة الكيوبكس. وقد وجد ان المبيدان يسببان انخفاضا في معدل الانقسام الميتوزي و يحدثان تأثير واضح على نسب أطوار الانقسام ,كما ينشأ عن معاملاتهما العديد من التشوهات الكروموسومية بالانقسام الميتوزي تشمل لزوجة الكروموسومات ـ خلل في خيوط المغزل-تلكؤ بعض الكروموسومات-الكروموسومات التائهة والخلايا متعددة الأقطاب. وقد إستحثت معاملات المبيدين تغيرات جوهرية في الأطوار المختلفة لدورة الخلية حيث تزايدت خلايا المرحلة الفاصلة الاولى بينما تناقصت الخلايا فى مرحلة تخليق الدنا والمرحلة الفاصلة الثانية فضلا عن تناقص نسبة الخلايا فى اطوار الانقسام المختلفة للمبيدين. وتشير التغيرات الكروموسومية التي تم تسجيلها وبصفة خاصة الكسور والقناطر الكروموسومية والتغيرات في كمية دنا الي السمية الوراثية للمبيدين دورسبان وكيوبكس.

Table 2: Frequency of total abnormalities and different types of chromosomal abnormalities in mitotic phases after treating *Allium Cepa* root tips with different concentrations of Dursban and Cubex.

Conc.	% 0f Total Abno.	% of prophase abnormalities		% of metaphase abnormalities					% of ana-telophase abnormalities					
		Stick.	Irreg.	Stick.	Dist.	C-m.	Non-or.	Break	Stick.	Dist.	Brid.	Lag.	M.pol.	Mic.n.
Control	1.94	-	-	-	-	-	-	-	-	-	-	-	-	-
Dursban														
1.0 ppm	28.11		1	30.27	41.65	15.28	10.24	2.56	27.15	19.37	22.47	20.63	7.79	2.59
10.0 ppm	34.69	5.77		25.94	43.19	19.68	8.07	3.12	30.49	14.62	23.92	18.15	10.79	2.03
100.0 ppm	40.34			24.16	43.98	20.35	6.73	4.78	34.90	11.17	26.09	17.69	8.70	1.45
		10.83												
		93.60	6.40											
Cubex														
2.1 ppm	30.74			21.55	29.93	42.61	5.91	0.00	44.21	10.13	17.83	11.09	15.79	0.95
6.7 ppm	38.19	19.29		22.51	31.01	44.15	2.33	0.00	39.80	15.15	13.12	13.60	17.84	0.49
		89.27	10.73											