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Cytological effects of the herbicide racer "flurochloridone" on *Allium cepa*

DENIZ YÜZBAŞIOĞLU[1], FATMA ÜNAL[1, *], CENGIZ SANCAK[2] and REŞAT KASAP[3]

[1] Gazi Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Teknikokullar, Ankara, Turkey.
[2] Ankara Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Ankara, Turkey.
[3] Gazi Üniversitesi, Fen-Edebiyat Fakültesi, İstatistik Bölümü, Teknikokullar, Ankara, Turkey.

Abstract - The present investigation was carried out to study the cytological effects of the herbicide racer "flurochloridone" (3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)= phenyl]-2-pyrrolidinone) on *Allium cepa* with respect to the cell response, mitotic index, mitotic abnormalities and chromosome aberrations. The roots of bulbs were treated with the following concentrations depending on the root growth inhibition test: 80 ppm (LD_{50}), 40 ppm ($LD_{50/2}$) and 20 ppm ($LD_{50/4}$). The results indicated that flurochloridone herbicide reduced the mitotic division in *A. cepa* as compared to their control groups. The reduction in mitotic activity was more significant when the concentration of the herbicide increased and the exposure time was prolonged. The treatment conducted with flurochloridone induced a significant percentage of abnormal mitosis. Seven types of abnormalities were recorded: C-metaphase, laggards, stickiness, bridges, fragments, multipolarity and poliploidy. Micronucleated cells were also observed at interphase. In additon, in pretreated root tips, chromosome breaks, fragments and sister union were detected.

Key words: *Allium cepa*, cytological effects, flurochloridone, herbicide, pesticide, racer.

INTRODUCTION

Pesticides are used in the modern agricultural practices and represent a very large input of chemicals in to our environment (CROSBY 1981). Though the use of these chemicals has become of necessity, their ingredients have induced acute toxic effects (AHMET and GRANT 1972; AMER and FARAH 1974; BADR and ELKINGTON 1982). The danger of pesticides is not necessarily a result of direct application, some pesticides accumulate in the food to a toxic level and consequently affect the public health. In this context, DRYANOVSKA (1987) and CANTOR *et al.* (1992) showed that the frequency of cancer increases among people who have been exposed directly or indirectly to pesticides.

The herbicides, one of the pesticides, used in agriculture in controlling weeds, unwanted plant species that compete with cultivated plants for nutrient, light and water. However, their ingredients inhibit cell division and induce chromosomal abnormalities in different experimental systems. Among more than 200 known bioassays in the literature (WATERS *et al.* 1990), plant bioassays are in general more sensitive than most of the other systems to detect the genotoxic effect of environmental pollutants (U.S. EPA PUBLICATION 1980). No studies have been carried out, to our knowledge, on the cytological effects of flurochloridone in plant systems. This study has been undertaken to investigate cytological effects induced by flurochloridone in the root tips of *Allium cepa*.

MATERIAL AND METHODS

The herbicide used in this research is flurochloridone in unclassified herbicide group. It is marketted under the name "Racer". Its chemical composition is

* Corresponding author: fax ++90-312-2122279; e-mail: funal@gazi.edu.tr

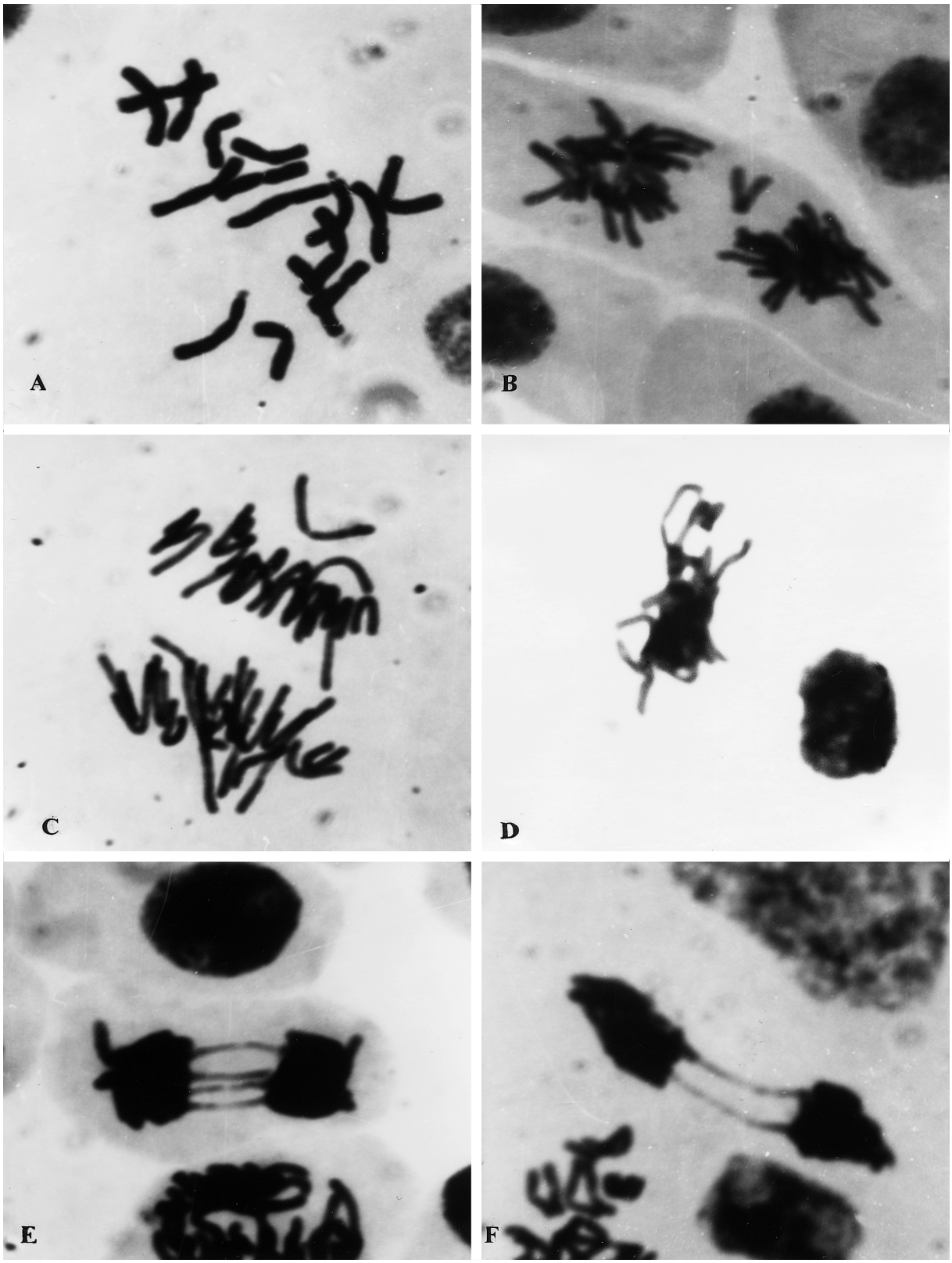
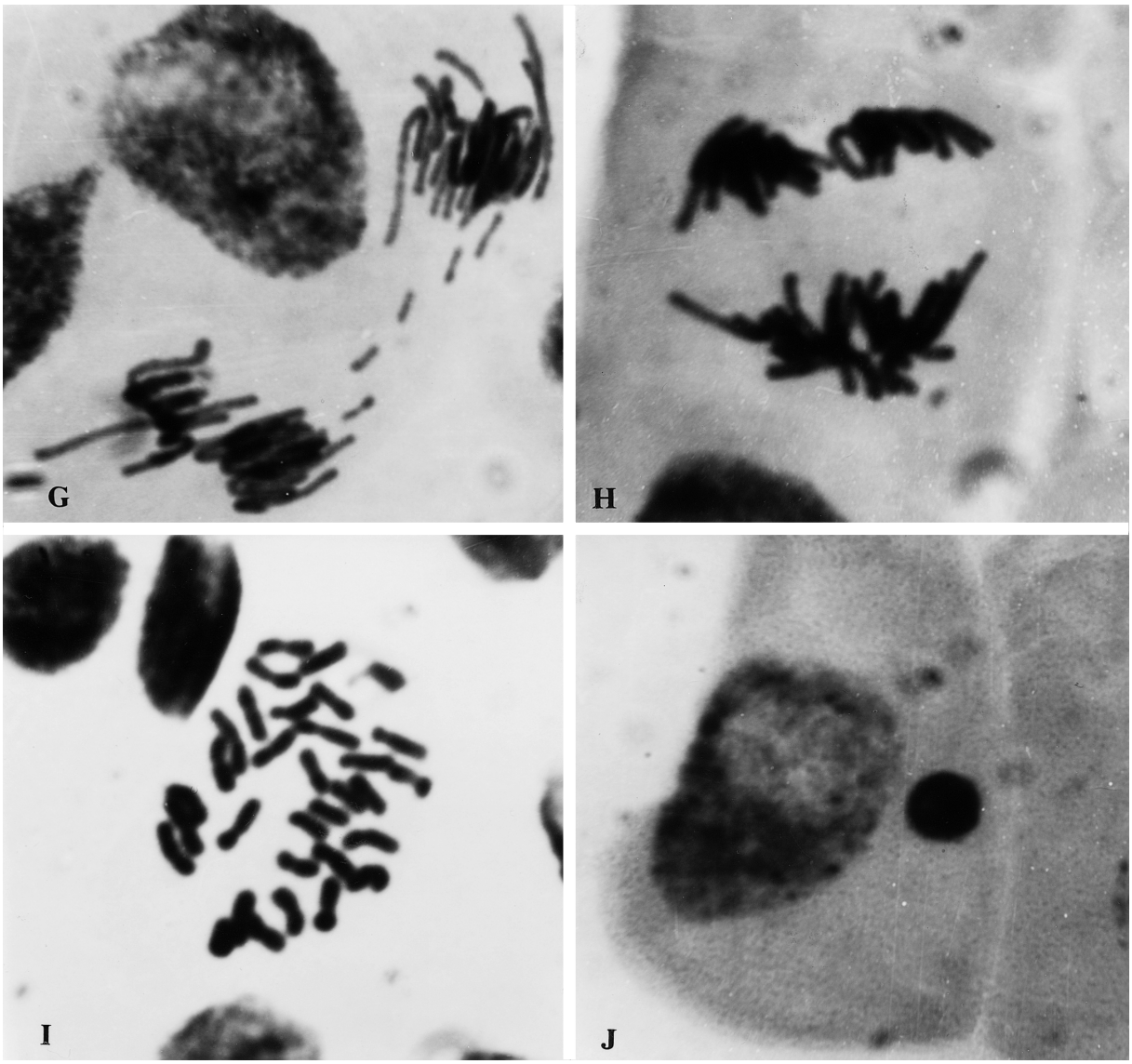
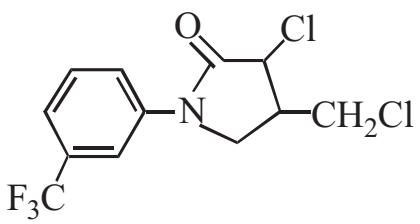


Fig. 1 – Different types of aberrations induced by the flurochloridone herbicide in *Allium cepa* root tips. A) C-metaphase; B) Lagging chromosome at ana-telophase; C) Lagging chromosome at anaphas;e D) Sticky metaphase and fragment; E) Multi-bridges at telophase; F) Double-bridges at telophase; G) Fragments at anaphase; H) Multipolar anaphase; I) Polyploidy J) Micronucleus at interphase.



3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)=phenyl]-2-pyrrolidinone. CAS registry number: 61213-25-0. Chemical formula is $C_{12}H_{10}NOF_3Cl_2$. Molecular weight is 312.12 g/mol. The structural formula is as the following:



Racer is a novel pyrrolidone herbicide and its active ingredient is flurochloridone. It is a pre-emergence soil herbicide for potatoes, sunflower, carrots, winter wheat, winter rye, maize, sugar cane and fruit trees.

The plant material used for the genotoxicity was *Allium cepa*. Bulbs were obtained commercially. Equal sized bulbs were chosen and grown in distilled water for the first 24 h. Twelve onions were set up at each concentration and treated with 20, 40 and 80 ppm of flurochloridone for 12, 24 and 48 hours. The same amount of onions were used as control in all applications. Following all the treatments, most of the roots were detached, washed with water and fixed directly in 3:1 alcohol:acetic acid for 24 h and stored in 70% alcohol in refrigerator until use. The rest of the roots were treated with α–monobromonaphthalene for 3 hrs and then fixed in acetic ethanol (1:3). Cytological preparations were made from ten different onions using Feulgen schedule and made permanent by mounting in depex.

Mitotic index (MI), the frequency of mitotic phases and the number and type of mitotic abnormalities were found by observing 10000 cells. For each slide prepared from different onions, 1000 cells were screened and with ten onions this reached to 10000 cells per concentration and duration of time. We also recorded chromosomal aberrations per 100 metaphase cells for each concentration and period of time from 5 different slides prepared from different onions. The data obtained for the mitotic index statistically analysed using z-test and, for the frequency of mitotic phases, mitotic and chromosomal abnormalities analysed using χ^2 test.

RESULTS

Flurochloridone induced evidently dose-dependent root growth inhibition and LD_{50} value registired as 80 ppm. With the application of 80, 40 and 20 ppm of flurochloridone, the percentage of mitotic index (MI) (the number of dividing cells / total number of cells X 100) decreased significantly with the increase of concentration and duration of time as compared to the control (Table 1). MI was not significantly different in only 80 ppm at 12 h treatment. Recorded MI value was particularly low in roots treated with high concentration for prolonged exposure time. The reduction rate was about 55% in 80 ppm concentration at 48 h, as compared to the control.

The treatment conducted with flurochloridone showed no significant effect on the percentage of the mitotic stages. However, the frequency of cells in the metaphase stage increased with the increasing of concentration at 48 h treatment. In anaphase, a reverse was observed. Here, the number of cells decreased with the increasing of concentration at the same treatment time. In prophase and telophase stage, flurochloridone had no effect in the frequency of cells.

Flurochloridone treatments induced a wide range of mitotic abnormalities as compared to the control in the root tips of *A. cepa*. The types and percentage of these abnormalities are given in Table 1 and their photographs are in Fig. 1. The most common abnormality (42.67%) was colchicine type metaphase figures (C-metaphase) which was observed in all the treated groups as well as control, though it was very low. C-metaphase especially increased in 80 ppm concentration at 48 h. The second prominent abnormality was laggards (28.66%) which was also observed in all the treated groups but control. Again, 80 ppm at 48 h duration produced more laggards as compared to the others. Stickiness was the third dominant abnormality (14.33%) observed in all the treatment groups

Table 1 – Type and percentage of mitotic abnormalities in the root tips of *A. cepa* exposed to the flurochloridone.

Conc. (ppm)	Total cells scored	No. of dividing cells	MI±SE	% Abnormalities							% Total abnormalities	% Abnormal interphase cells
				C-metaphase	Laggards	Stickiness	Bridges	Fragments	Multipolarity	Poliploidy		
12h											-	
Control	10000	563	5.63±0.23	0.89	-	-	-	-	-	-	0.89	-
20	10000	420	4.20±0.20a³	2.62	3.10	2.14	0.71	1.67	0.24	-	10.48**	-
40	10000	410	4.10±0.20a³	10.00	3.17	2.44	0.73	0.49	0.24	-	17.07***	0.04
80	10000	501	5.01±0.22	6.78	4.99	1.60	1.40	0.60	-	-	15.37***	0.13
24h												
Control	10000	613	6.13±0.24	0.16	-	-	-	-	-	-	0.16	-
20	10000	539	5.39±0.22a¹	1.49	2.60	-	0.74	1.11	0.74	-	6.68*	0.01
40	10000	428	4.28±0.20a³	6.07	6.31	2.57	1.40	0.70	1.40	0.47	18.92***	0.07
80	10000	468	4.68±0.21a²	8.54	4.06	2.99	1.07	-	1.07	0.43	18.16***	0.24
48h												
Control	10000	680	6.80±0.25	0.29	-	-	-	-	-	-	0.29	-
20	10000	505	5.05±0.22a³	3.56	3.96	0.39	0.20	0.20	0.20	-	8.51**	0.03
40	10000	405	4.05±0.19a³	9.88	5.18	3.95	-	0.25	-	-	19.26***	0.03
80	10000	308	3.08±0.17a³	11.69	7.79	5.85	1.62	1.30	1.62	-	29.87***	0.14

a¹ Significant from the control P< 0.05 (z test)
a² Significant from the control P< 0.01 (z test)
a³ Significant from the control P< 0.001(z test)
* Significant from the control P< 0.05 (χ^2test)
** Significant from the control P< 0.01 (χ^2test)
*** Significant from the control P< 0.001(χ^2test)

except 20 ppm concentration for 24 h. In addition, a low number of bridges (5.54%), fragments (4.40%), multipolarity (3.75%) and poliploidy (0.65%) was also observed. Percentage of total abnormalities increased significantly as the concentration of the herbicide increased especially at 48 h (Fig. 2). At interphase, a low percentage of cells with micronuclei was also observed in all concentrations of flurochloridone at all treatment times except 20 ppm at 12 h (Table 1 and Fig. 1J). However, the percentage of micronuclei were not significant from the control.

Flurochloridone has also induced a significant increase of chromosomal aberrations at metaphase stage in the root tips treated with α-monobromonaphthalene at all concentrations and treatment periods as compared to control groups (Table 2, Fig. 3 and 4). All concentrations and treatment times induced chromosome breaks. The frequency of breaks was %77.5. Some numbers of fragments (%21.25) and only one cell with three sister unions (%1.25) was also observed in these root tips.

DISCUSSION

Allium test has been used widely for the monitoring of genetic effects of different chemicals, due to its relative simplicity (BADR 1983; FISKESJO 1985, 1988; HIDALGO *et al.* 1989; RANK and NIELSEN 1997). In this study, with the application of flurochloridone to the root meristem of *Allium cepa*, the mitotic index has decreased significantly in all treated groups (except one application) as compared to their controls. The reduction in mitotic activity was more significant when the concentration of the herbicide increased and the exposure time prolonged. The reduction in 80 ppm at 48 h was 55% as compared to control. If the LD_{50} value is chosen as the highest concentration and the others as $LD_{50/2}$, $LD_{50/4}$ for the genotoxicity test as in this study, the mitotic index will not deacreased well below 50% of the control (RANK and NIELSEN 1997). Several other herbicides have been reported to inhibit mitosis in different plants. This include some substituted urea compounds in *Hordeum* and *Tradescantia* (TOMKINS and GRANT 1972), some substituted phenols (CHAND and ROY 1981) in *Nigella sativa*, trifluralin (LIGNOWSKI and SCOTT 1972) and nitralin (BADR 1979) in *Allium cepa*, carbamates (AMER and FARAH 1976) in *Vicia faba*, terbutryn and atrazine (BADR 1986) in *Vicia faba*, gasegard and igran in barley (TOPAKTAŞ and RENCÜZOĞULLARI 1991). Significant decrease in mitotic index suggests mitodepressive action of the compound, indicating that pesticides interfere with the normal sequence of cell cycle to reduce the number of cells starting to divide at interphase

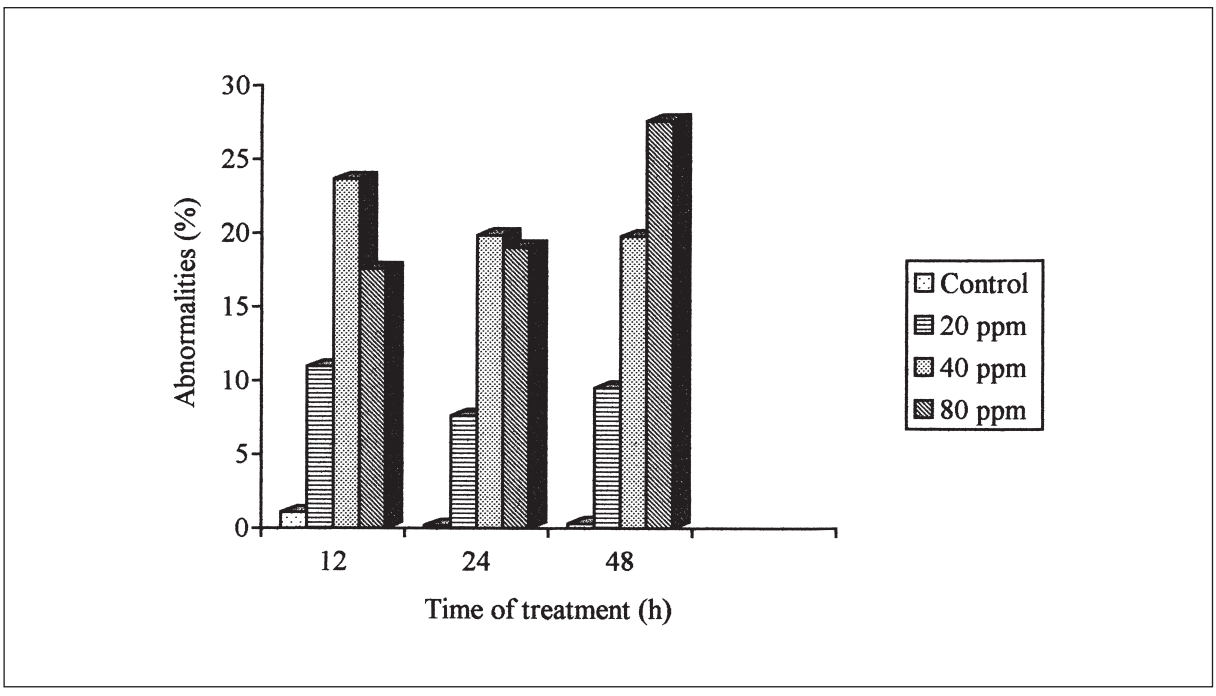


Fig. 2 – Frequency of mitotic abnormalities in the root tips of *A. cepa* exposed to the flurochloridone.

(BADR 1986). The reduction in mitotic activity could be due to the inhibition of the DNA sythesis which is one of the major prerequisites for a cell to divide (SADIA and VAHIDY 1994). In this context, BADR (1986) and EL-GHAMERY *et al.* (2000) have observed that different treatments of terbutryn and atrazine herbicide reduced the amounts of DNA and RNA in dividing cells. The decrease of the MI might be caused by the decreasing ATP level and pressure from the functioning of the energy production centre (JAIN and ANDSORBHOY 1988). On the other hand, the inhibition of certain cell cycle specific proteins remains as a possible herbicide target site. Thus, inhibition of the enzyme DNA polimerase, which is necessary for the synthesis of DNA precursors as well as other enzymes more directly involved with spindle production, assembly or orientation, could explain the reported antimitotic effect (HIDALGO *et al.* 1989).

The treatment conducted with flurochloridone showed no significant effect on the percentage of the different mitotic stages as with trichlorphon (AMER and ALI 1983) and with rotenone (AMER and MIKHAEL 1986). However, it caused the induction of a significant percentage of mitotic abnormalities when compared to their control groups. Seven types of abnormalities recorded were C-metaphase, laggards, stickiness, bridges, fragments, multipolarity and poliploidy. All treatment concentrations and period of time induced significant amount of abnormalities. The percentage of total abnormalities gradually increased as the concentration increased at 48 h treatment. These results are in aggrement with those obtained after treating the *A. cepa* root tips with different pesticides, such as sencorer (MOUSA 1982; HALIEM 1990), cyanazine (PAPES *et al.* 1989), gazegard (MOUSA 1982), carbetamex and paradone plus (BADR 1983) and after treating of root tips of *Vicia faba* with gespax (BADR *et al.* 1985), of barley with gazegard and igran (TOPAKTAŞ and RENCÜZOĞULLARI 1991) and some others.

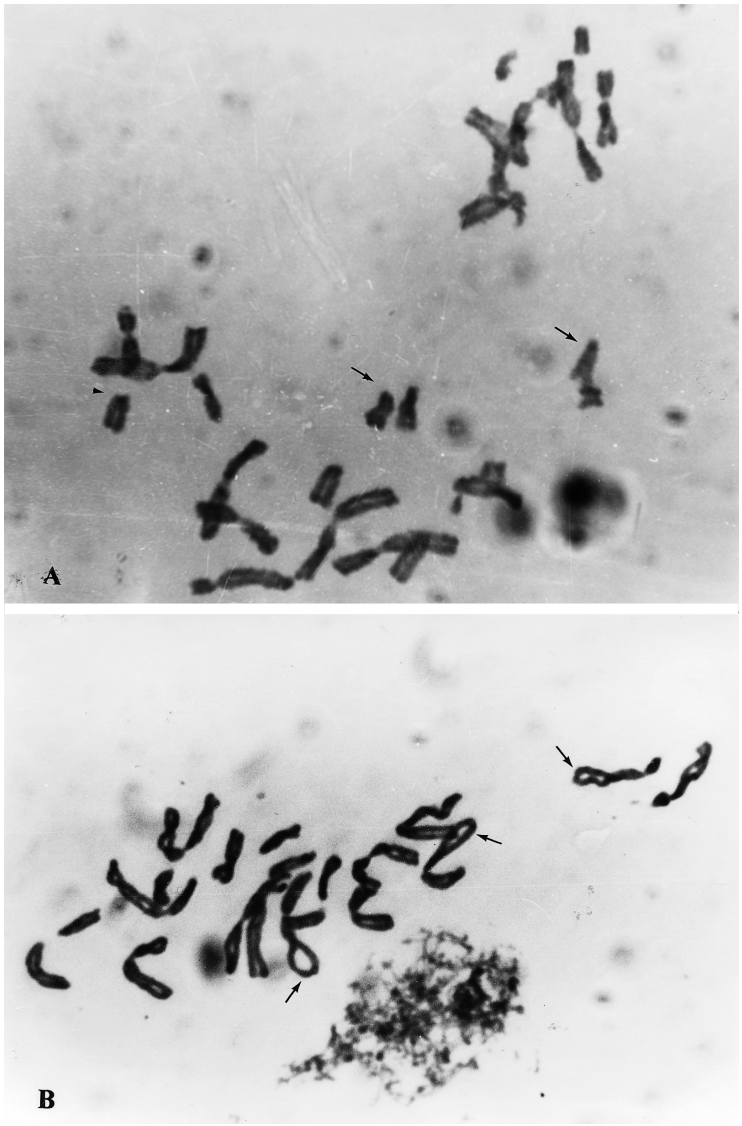


Fig. 3 – The chromosome aberrations in metaphase cells in α-monobromonaphthalene pretreated roots after treating with different concentration of flurochloridone in different exposure times A) Fragments (some were shown by arrow) and chromosome break (arrow head) B) Sister union (arrow).

The formation of C-mitosis, lagging chromosome(s), multipolarity and poliploidy may be due to the disturbance in the spindle formation which affected by the herbicide (BADR *et al.* 1985; HALIEM 1990). It might also be the result of lyphophylic chains of spindle proteins and causes the bending of polipeptides (EL-GHAMERY *et al.* 2000). Stickiness is considered to be a chromatid type aberration (KLASTERSKA *et al.* 1976; BADR 1986) and is induced by the effect of herbicide on chromosomal proteins attributed to the improper folding of chromosome fibers, which render the chromatids connected by means of subchromatid bridges (KLASTERSKA *et al.* 1976; BADR and IBRAHIM 1987). Stickiness may be produced by the action of the herbi-

Table 2 – The types and number of chromosome aberrations in metaphase cells in α-monobromonaphthalene pretreated root tips of *A. cepa* exposed to the flurochloridone.

Treatment	Total cells scored	Aberrations			% Total aberrations
		Chromosome breaks	Fragments	Sister union	
12h					
Control	100	-	-	-	0
20	100	4	2	-	6*
40	100	7	5	-	12***
80	100	2	3	-	5*
24h					
Control	100	-	-	-	0
20	100	4	-	-	4*
40	100	13	-	-	13***
80	100	10	1	-	11***
48h					
Control	100	-	-	-	0
20	100	5	-	1	6*
40	100	8	5	-	13***
80	100	9	1	-	10**

* Significant from the control $P<0.05$ (χ^2 test)
** Significant from the control $P<0.01$ (χ^2 test)
*** Significant from the control $P<0.001$ (χ^2 test)

cide on the polimerization process, or may be resulted in the fragmentation of chromosomes and bridges form at anapahase-telophase stages. The majority of multipolar anaphases involved chromosomal bridges that might be resulted from sticky chromosomes. It is well known that bridges and fragments lead to structural changes in chromosomes (EL-GHAMERY 2000).

In addition to the mitotic abnormalities, cells containing micronucleus were also observed at

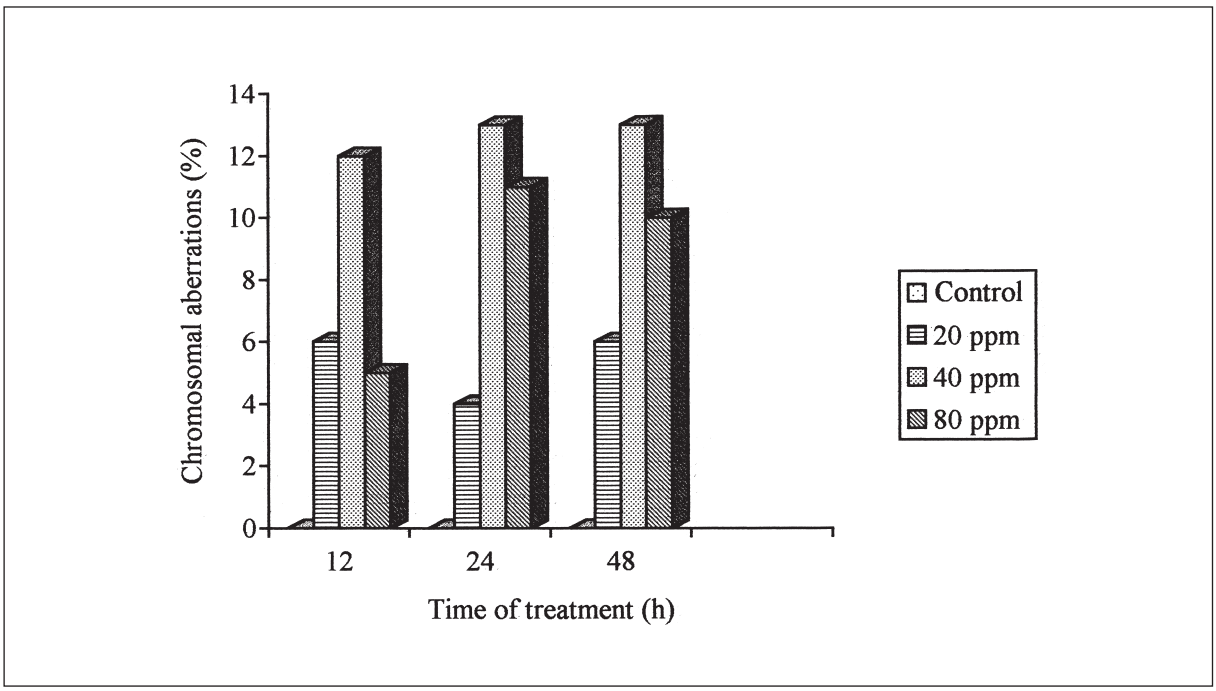


Fig. 4 – Frequency of chromosomal abnormalities in α-monobromonaphthalene pretreated methaphase cells in the root tips of *A. cepa* exposed to the flurochloridone.

interphase stage in all tretaments except 20 ppm at 12 h treatment. The most effective concentration was 80 ppm at 24 h. Micronuclei are the result of acentric chromosome fragment as well as failure of normal spindle function (CHAUHAN *et al.* 1986). According to ROMAGNA (1993), the systems of micronuclei have an important influence on the explanation of the potential of genotoxic agents in vivo and in vitro. CHAUHAN and SUNDARARAMAN (1990) reported that micronucleated cells may lead to aneuploid and poliploid cells in subsequent mitotic division and thus cause mutation.

When metaphase cells were investigated for each concentration and for each treatment time after pretreatment of roots in α-monobromonaphthalene, chromosome breaks, fragments and sister union type of chromosomal abnormalities were also found. The percentage of chromosome abnormality in all treatment concentrations and period of times were significant as compared to their controls. In this respect, flurochloridone resembles some other herbicides including substituted ureas, substituted phenols and s-triazine compounds (AHMET and GRANT 1972; BADR and ELKINGTON 1982; BADR 1986). Chemicals which induce chromosome breakage are known to exert a clastogen action on chromosomes which is generally regarded to involve an action on DNA (BADR and ELKINGTON 1982; CHAUHAN and SUNDARARAMAN 1990). Thus, the present study is consistent with previous investigations in demonstrating flurochloridone as potent clastogen.

The types of abnormalities produced by flurochloridone may be grouped in three classes (BADR 1986): The first is often referred to as mitotic abnormalities and is the result of an action on the mitotic apparatus. These include C-metaphase, multipolarity, poliploidy and chromosome lagging. The other class of anomalies observed is chromosomal stickness which is generally regarded as a physiological effect on chromosomes during division (SAVAGE 1975) and has been considered as a category of chromatid aberration (KLASTERSKA *et al*. 1976). The third class is chromosome aberrations including chromose breaks, bridges, fragments, sister union and micronuclei (BADR 1986; EL-GHAMERY 2000). In this respect, the chromosomal aberrations (clastogenic type) are concidered as reliable indicators of mutational change that are used as reliable evidence for screening the mutational activity (KIHLMAN 1975). RANK and NIELSEN (1997) reported that if a chemical is able to cause damage to the chromosomes in a reliable plant assay, then the chemical should be concidered as having the potency of damaging the chromosomes of other organisms in the environment. In this study, clastogenic type of aberrations were observed indicating that flurochloridone should be regarded as an agent that has mutational activity.

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