

Evaluation of Cytological Effects of Fusilade and Sencor Herbicides on Somatic Cells of *Glycine max* (L.) Merrill

Magda I. Soliman^{1*}, Zakaria A. Baka², Ashraf A. El-sayed¹ and Lamyaa E. Mansour¹

¹Botany Department, Faculty of Science, Mansoura University, EGYPT

²Botany Department, Faculty of Science, Damietta University, EGYPT

*Correspondance: E-mail: magdaisoliman@yahoo.com

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ABSTRACT: The present study has been conducted to investigate the mutagenic effects of two herbicides: fusilade and sencor on somatic cells of *Glycine max* with respect to the mitotic index, phase index, mitotic abnormalities and chromosome aberrations. For evaluation of cytogenetic effects, root meristem cells of *G. max* were treated with (4.2, 12.5, 20.8 ml/L) fusilade and (1.6, 2.5, 3.3 g/L) sencor for three exposure times (6, 12, 24h). The obtained results indicated that fusilade had a marked mitodepressive action while sencor had a mitopromotor action on mitosis. However, both induced a large number of chromosomal abnormalities. Metaphase aberrations represent the most conspicuous type of abnormalities. The types of abnormalities produced were disturbed, non-congression, oblique, stickiness, polyploidy, two groups, late separation, diagonal, bridge and laggard chromosome.

Keywords: *Glycine max* (L.); Merrill- herbicides; mitotic index; phase index and chromosomal aberrations.

INTRODUCTION: Soybean is considered one of the most important food and industrial crops in the world (El Agroudy et al., 2011). It is a species of legume native to East Asia (China and Manchuria) (Markley, 1950). Cultivated soybean, *Glycine max* (L.) Merrill, is a diploidized tetraploid (2n=40), in the family Leguminosae (Bernard and Weiss, 1973). It has been cultivated all over the world since ancient times for its high protein and lipid content (Yoshiki et al., 2013). It is considered to be a cash crop due to its extraordinary qualities: it contains about 37-42% good quality protein, 6% ash, 29% carbohydrate and 17-24% oil, comprising 85% poly-unsaturated fatty acid with two essential fatty acids (lenoleic and linolenic acid), which are not synthesized by the human body (Antalina, 2000; Balasubramaniyan and Palaniappan, 2003).

In soybean, weed infestation is considered a persistent and complex constraint in many regions of production world-wide as it influences soybean growth and development (Vollmann et al., 2010). Strategies of weed management can vary, but now mainly rely on the use of synthetic herbicides (Thill et al., 1991; Cordeau et al., 2016). The widespread use of the herbicides for weed control and crop productivity in modern agriculture exerts a threat on economically important crops by way of cytological damage to the cells of crop plant or any side effects induced by the herbicides (Kumar, 2010). Many cytological investigations have been carried out to detect the harmful effects of herbicides on various crop plants (Inceer et al., 2004; Yuz-

basioglu et al., 2009; Rad et al., 2011; Kumar and Jagannath, 2015; Mustafa and Adham 2016).

Fusilade (fluazifop-p-butyl) is a worldwide well-known grass-selective herbicide for post-emergence application in numerous broad-leaved crops (Keul et al., 1990; Hussein et al., 2013). Fusilade is one of the systemic herbicides belonging to the aryloxyphenoxypropionates (AOPP) family of herbicides which is a class of graminicides (Fayez et al., 2014). The chemical structure of fluazifop-p-butyl as shown in Figure 1 is (R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy] phenoxy] propanate.

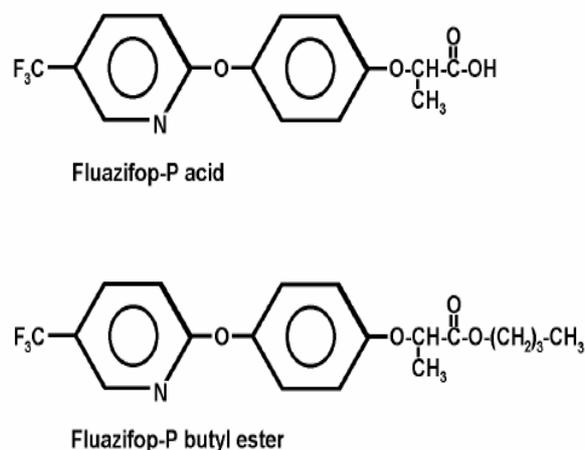


Figure 1: Chemical structure of fusilade (fluazifop-p-butyl).

The main target site of fusilade is the inhibition of acetyl-CoA carboxylase (ACCase) activity (Fayez et al., 2014). This enzyme catalyzes the carboxylation of acetyl-CoA to malonyl-CoA. It is required for the biosynthesis of fatty acids and secondary metabolites (Harwood, 1996; Nikolskaya et al., 1999, Yu et al., 2004), thereby inhibiting the biosynthesis of fatty acids necessary for membrane building and function (Harwood, 1988).

Sencor (Metribuzin) is a selective herbicide used for pre-and post-emergence control of grass and broad-leaved weed in specific crops like potatoes, soybeans, and tomatoes among others (Henriksen et al., 2002). Metribuzin is considered as a general use herbicide belonging to triazinone family of herbicides (Yahiaoui et al., 2011). The chemical structure of metribuzin as shown in Figure 2 is 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one.

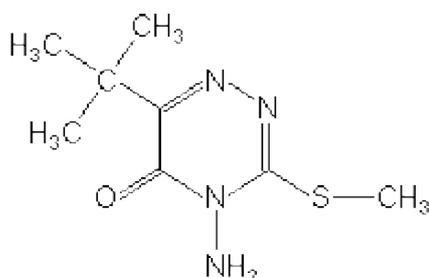


Figure 2: Chemical structures of metribuzin.

The mode of action of metribuzin is the inhibition of photosynthesis. Metribuzin inhibits photosynthesis by displacing plastoquinone at the QB-binding site on the D1 protein of the photosystem II complex in chloroplast thylakoid membranes. Herbicide binding at this protein location blocks electron transport from QA to QB and stops CO₂ fixation and production of ATP and NADPH₂ which are all needed for plant growth (Lambrevia et al., 2014; Roach and Krieger-Liszka, 2014).

Higher plants provide excellent models for the genotoxicity assessment of environmental chemicals because plant chromosomes are easy to analyze in terms of size, morphology and number, and respond to treatment with toxins in a similar way to mammals and other eukaryotes (Turkoglu, 2009). In addition, they are simple, cost effective and can be applied to detect wide range of genetic damage (Ozakca and Silah, 2013).

The aim of the present study was to investigate the potential cytogenetic effects of fusilade and sencor herbicides on the root meristem cells of *Glycine max* using the variations in mitotic index, phase index, mitotic abnormalities and chromosomal aberrations.

MATERIALS AND METHODS:

Materials: Seeds of pure strain of *Glycine max* (L.) Merrill (cv. Crawford) were obtained from the Crop Research Institute, Agriculture Research Center, Sakha, Kafr El-Sheikh, Egypt. The two used herbicides were commercially available and frequently used for weed control.

Methods: a. Preparation of herbicides: Three different concentrations (recommended dose-lower than recommended dose- higher than recommended dose) were prepared from each herbicide. The solutions were prepared by dissolving the appropriate amount of the herbicide in water.

b. Seed germination and herbicide treatment: Healthy seeds of *Glycine max* were selected, surface sterilized by dipping in mercuric chloride solution (0.001M) for 5 min then washed with tap water for 5 min. The seeds were soaked for about 6 h in water and then allowed to germinate at 28±2 °C. After the emergence of root tips, the seeds were treated with different concentrations of fusilade (4.2-12.5-20.8 ml/L) and sencor (1.6-2.5-3.3 g/L) herbicides for three exposure times (6, 12 and 24h). One set of germinated seeds was kept as control with water only. After completion of exposure time, a maximum of ten primary roots were picked up randomly from each group then the root tips excised from the seeds, and immediately fixed in freshly prepared Carnoy's solution (a mixture of 3 parts 96% ethanol and one part glacial acetic acid) and stored in refrigerator for at least 48 h.

c. Cytological analysis: For cytological preparations, the fixed root tips were hydrolyzed with 1N HCl at room temperature for 40-45 min. or at 60°C for 10 min. in water bath, washed thoroughly with water, and then stained by a double staining method using the modified Carbol Fuchsin reaction (Koa, 1975 a and b), where the root tips were put in Carbol Fuchsin overnight then transferred to a watch glass containing 2 % aceto-Orcein stain for 2-4 h (Chattopadhyay and Sharma, 1988). Root caps were removed and 1 mm of meristematic zones were immersed in a drop of 45% acetic acid on a clean slide and squashed under a cover glass in order to spread the cells on the surface of the slide. The slides were viewed under the electric microscope (Olympus CX 40) using the 100 x objective lens with oil immersion. At least 2000 cells from about 5 slides of each treatment were examined. The cells were recorded as normal or aberrant in different stages of mitotic division namely: interphase, prophase, metaphase, anaphase and telophase. All cells with aberrations were recorded and the most representative one for each abnormality were photographed using digital camera 5.0 Megapixel.

d. Data analysis: For each concentration and exposure time, the mitotic index, phase index, chromosome aberrations were determined. The mitotic index was calculated as the number of dividing cells/total number of cells \times 100.

e. Statistical analysis: Data were analyzed using SPSS, statistically significant differences between control and treatments were compared using T-test. The data are displayed as means \pm standard deviation (SD) and *p*-values less than 0.05 are considered statistically significant. All statistical analyses were based on 0.05 significance level (Snedecor and Cochran, 1976).

RESULTS AND DISCUSSION: The results in Table (1) demonstrated that the majority of treatments with fusilade induced considerable decreasing effects on mitotic indices than that of control of exposure time 6h, 12h and 24h (4.97% , 4.97% and 5.23%) respectively, whereas treatment of *Glycine max* root tips with 4.2 ml/L for 24h , 12.5 ml/L and 20.8 ml/L fusilade for 12h caused an increase in MI% (5.46% , 5.20% and 5.08%) respectively but this increase was non-significant when compared with control.

Using T- test at 0.05 level of significance, the mitotic index effect was found statistically significant for all treatments of fusilade (4.2, 12.5 and 20.8 ml/L) at exposure time 6 hours. Also, for fusilade 20.8 ml/L for 24h compared with control.

Almost all treatments affected the frequency of mitotic phases. There is an increase or decrease in percentages of prophase in treated roots with a minimum value was observed after treating *Glycine max* root tips with 12.5 ml/L fusilade for 24h as (0.61%) and a maximum value obtained in samples treated with 4.2 ml/L fusilade for 12h as (4.84%). The frequency of metaphase was increased in all treatments until it reached a maximum value of 92.68% after 24h from treatment with 20.8 ml/L fusilade. Conversely, the frequency of anaphase was gradually decreased in all treatment with a minimum value of 1.16% achieved in 12.5 ml/L fusilade for 6h. Also, the frequency of telophase was decreased in all treatments with a minimum percentage was attained in 20.8 ml/L fusilade for 24h as (2.44%).

According to fusilade, the recorded values showed that there were no abnormalities observed in prophase stage for three exposure times (6, 12 and 24 hours). On the contrary, it was noted that there were abnormalities at metaphase stage in fusilade treated root tips and their percentage was increased in all treatments when compared with control. In case of anaphase, the percentage of abnormalities was increased in all

treatments except in the following two treatments which are 4.2 ml/L fusilade (2.02%) and 12.5 ml/L fusilade (0.81%) for 24h the percentage was decreased than that of control. At telophase stage, the percentage of abnormalities was increased in all treatments except in one treatment with fusilade that is 20.8 ml/L for 24h the percentage was decreased.

From the results tabulated in Table 1, it is obvious that fusilade considerably increased the percentage of total abnormalities in *Glycine max* root tip cells in all treatments.

The results indicate that the treatment of *Glycine max* with fusilade induced different types of abnormalities in dividing cell (mitotic stages) such as disturbed, non-congression, oblique, stickiness, polyploidy and two groups at metaphase, late separation, diagonal, bridge and disturbed at anaphase and diagonal and bridge chromosomes at telophase stage (Plate 1, A-O). The proportions of these abnormalities are given in Table (2).

Concerning the effect of sencor on mitotic division of *Glycine max* root tips, it is obvious that the majority of treatments induced considerable increasing effects on mitotic indices than that of control of exposure time 6h, 12h and 24h (4.87%, 4.53% and 3.82%) respectively, whereas treatment of *Glycine max* root tips with sencor concentration 1.6 g/L as well as 3.3 g/L for 6h showed a slight reduction in MI% (4.06% and 4.84%) respectively. At exposure time 24h, the mitotic frequency was increased as concentration was increased when we compared with control.

Also the significance of the data was determined by statistical analysis and showed that the mitotic index effect was statistically significant for all treatments of sencor (1.6, 2.5 and 3.3 g/L) at three exposure time 6, 12 and 24 hours except for sencor concentration 3.3 g/L at exposure time 6 as well as 12 hours (4.84% and 6.62%) respectively the mitotic index effect was found to be non-significant when compared with control.

The analysis of the mitotic index data showed that the relative frequency of mitotic phases after sencor treatment differed from control. The frequency of prophase showed fluctuations in its values with a minimum value obtained after treatment for 6h with 3.3 g/L sencor as (0.41%), while the maximum value attained in samples treated with 1.6 g/L sencor for 24h as (4.40%). The frequency of metaphase was higher than control in all treatments with sencor except one treatment that is 2.5 g/L for 24h the frequency was decreased than that of control. Conversely, the frequency of anaphase was decreased in all treatment until it reached a minimum value of 1.50% after 24h

from treatment with 1.6 g/L sencor. In the same manner, the frequency of telophase was decreased in all treatment except in the following two treatments

which are 2.5 g/L sencor for 24h (38.13%) and 3.3 g/L sencor for 6h (28.30%) the frequency was increased.

Table 1: Mitotic index , normal and abnormal phase indices , total abnormalities in dividing cells after treating *Glycine max* root tips with fusilade herbicide; ET= Exposure time.

Treatment		%MI (Mean± SE)	Phase index								% Total Mitotic abnormalities (Mean± SE)
Conc.	ET		% prophase		% Metaphase		% Anaphase		% Telophase		
			mitotic	Abn	mitotic	Abn	mitotic	Abn	mitotic	Abn.	
Control	6hrs	4.97±0.44	0.94	0.00	56.60	27.67	25.79	0.00	16.67	0.00	27.67±5.92
	12hrs	4.97±0.36	0.00	0.00	47.91	14.45	20.95	4.47	31.14	0.00	18.92±4.00
	24hrs	5.23±0.33	1.01	0.00	50.59	11.04	17.27	3.16	30.82	1.06	15.25±3.50
4.2 ml/L	6hrs	3.54±0.22*	4.43	0.00	76.04	54.43	7.03	0.78	12.50	1.56	56.77±5.99*
	12hrs	4.49±0.26 ns	4.84	0.00	57.53	46.64	16.80	8.47	20.83	3.36	58.47±5.32*
	24hrs	5.46±0.45 ns	0.00	0.00	66.52	51.66	5.76	2.02	27.72	5.14	58.82±4.28*
12.5 ml/L	6hrs	2.97±0.17*	1.16	0.00	92.25	70.54	1.16	0.00	5.43	0.00	70.54±6.54*
	12hrs	5.20±0.34 ns	3.30	0.00	71.79	55.34	8.94	5.77	14.90	2.14	63.25±3.94*
	24hrs	4.87±0.41 ns	0.61	0.00	74.39	52.03	2.85	0.81	22.15	2.44	55.28±5.47*
20.8 ml/L	6hrs	3.18±0.20*	1.16	0.00	80.23	43.02	0.00	0.00	18.60	2.33	45.35±7.41*
	12hrs	5.08±0.45 ns	1.61	0.00	63.20	55.65	8.44	5.73	25.96	5.78	67.16±4.40*
	24hrs	2.80±0.21*	0.00	0.00	92.68	60.98	4.88	4.88	2.44	0.00	65.85±6.86*

Total number of examined cells = 2000, ns = not significant at 0.05 level from control, * = the two means are significantly different at the 0.05 level.

Table 2: Mitotic index , normal and abnormal phase indices , total abnormalities in dividing cells after treating *Glycine max* root tips with sencor herbicide; ET= Exposure time.

Treatment		%MI (Mean± SE)	Phase index								% Total Mitotic abnormalities (Mean± SE)
Conc.	ET		% prophase		% Metaphase		% Anaphase		% Telophase		
			mitotic	Abn	mitotic	Abn	mitotic	Abn	mitotic	Abn.	
Control	6hrs	4.87±0.43	0.00	0.00	55.61	6.12	17.01	0.00	27.38	0.00	6.12±3.46
	12hrs	4.53±0.27	1.08	0.00	59.09	3.65	11.81	0.00	28.03	0.00	3.65±1.53
	24hrs	3.82±0.35	6.60	0.00	61.60	6.25	10.07	2.08	21.74	2.08	10.42±4.46
1.6 g/L	6hrs	4.06±0.30*	0.00	0.00	85.68	76.3	0.00	0.00	13.28	6.25	82.55±5.70*
	12hrs	6.40±0.35*	0.00	0.00	71.29	56.1	5.19	1.36	23.52	2.22	59.69±3.53*
	24hrs	6.21±0.68*	4.40	0.00	78.16	64.4	1.50	0.00	15.94	0.51	64.96±5.51*
2.5 g/L	6hrs	6.52±0.52*	0.96	0.00	65.54	42.1	14.97	8.65	18.53	3.53	54.31±4.33*
	12hrs	6.14±0.39*	3.22	0.00	75.12	57.3	0.00	0.00	21.67	5.12	62.49±4.11*
	24hrs	6.25±0.68*	4.24	0.00	48.16	36.9	9.47	7.32	38.13	19.27	63.54±8.82*
3.3 g/L	6hrs	4.84±0.40	0.41	0.00	63.20	44.0	8.10	2.86	28.30	6.84	53.74±6.21*
	12hrs	6.62±1.09	0.00	0.00	76.08	64.5	2.02	0.76	19.01	1.52	66.79±4.31*
	24hrs	6.92±0.67*	3.05	0.00	73.68	54.0	2.50	0.00	20.77	1.75	55.84±5.24*

Total number of examined cells = 2000, ns = not significant at 0.05 level from control, * = the two means are significantly different at the 0.05 level.

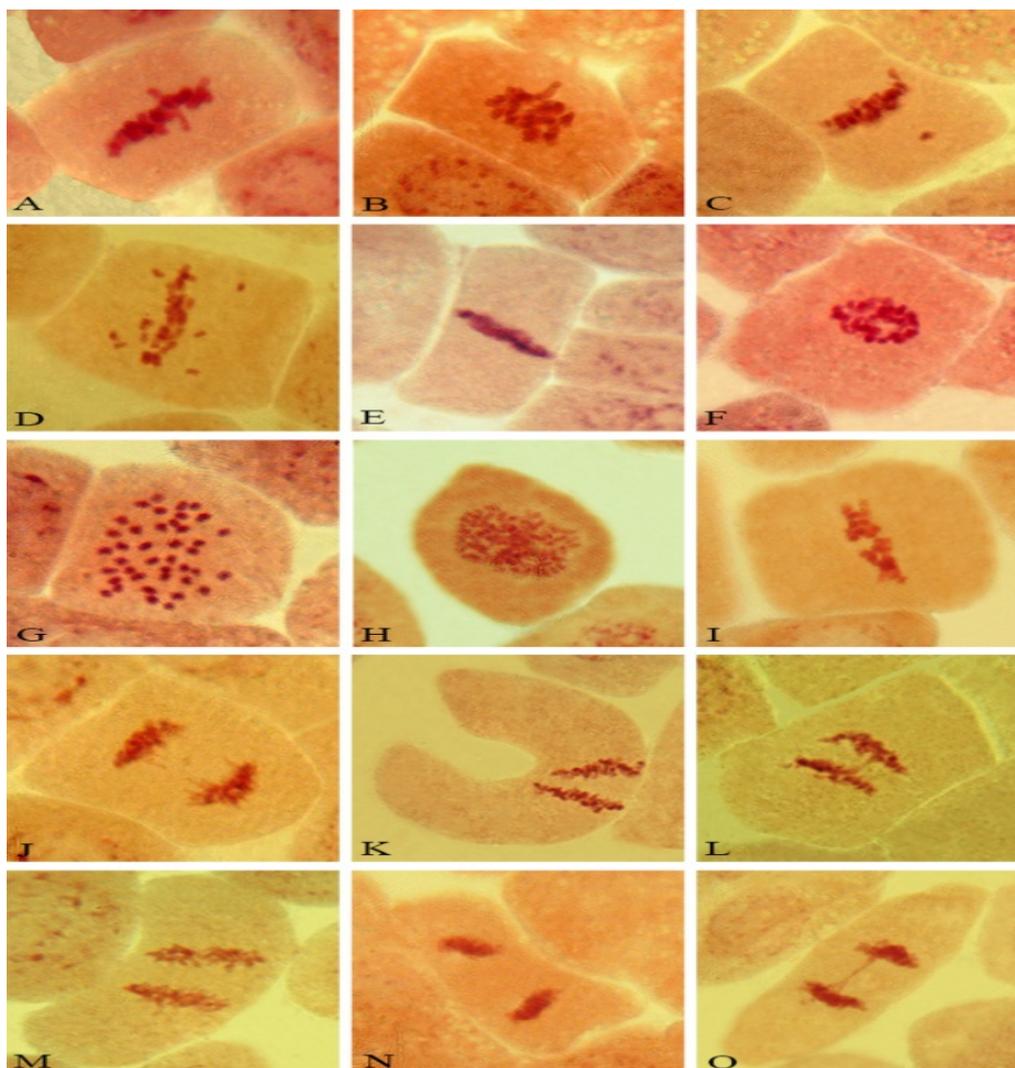


Plate 1: Types of mitotic abnormalities induced by treatment of *Glycine max* root tips with different concentrations of fusilade herbicide. (A) Disturbed at metaphase (20.8 ml/L-12h). (B) Disturbed at metaphase (12.5ml/L-24h). (C) Non-congression at metaphase (20.8 ml/L-12h). (D) Non-congression at metaphase (12.5ml/L-12h). (E) Oblique at metaphase (20.8 ml/L-12h). (F) Stickiness at metaphase (4.2ml/L-24h). (G) Diploid cell (20.8 ml/ L-6h). (H) Polyploid cell (4.2ml/L -12h). (I) Two groups at metaphase (4.2ml/L-6h).(J) Late separation at anaphase (20.8 ml/L-12h). (K) Diagonal at anaphase (4.2 ml/L-12h). (L) Bridge at anaphase (4.2 ml/L-12h). (M) Disturbed at anaphase (12.5ml/L-24h). (N) Diagonal at telophase (4.2 ml/L-12h). (O) Bridge at telophase (20.8 ml/L-12h). (X=1000).

Similar to fusilade, the recorded values showed that there were no abnormalities observed in prophase stage for three exposure times (6, 12 and 24 hours). At metaphase, the percentage of abnormalities was increased in all treatments with a maximum value (76.30%) at 1.6 g/L sencor for 6h. In case of anaphase the percentages of abnormalities showed fluctuation in their values when compared with control. At telophase stage, the percentage of abnormalities was increased in all treatments except in two treatments which are 1.6 g/L sencor for 24h (0.51%) and 3.3 g/L sencor for 24h (1.75%) the percentage was lower than that of control (2.08%) at 24h.

With no exception, the percentage of total abnormalities was higher than control in all treatments. The highest percentages of abnormal mitosis was (82.55%) at 1.6 g/L sencor for 6h that decreased to reach (53.74%) at 3.3 g/L sencor for 6h.

Like fusilade, sencor induced a wide range of chromosomal abnormalities covering all stages of mitosis such as disturbed, non-congression, oblique, stickiness, polyploidy and two groups at metaphase, late separation, diagonal, bridge, laggard and disturbed at anaphase and late separation, diagonal, laggard and bridges at telophase stage (Plate 2, A-O).

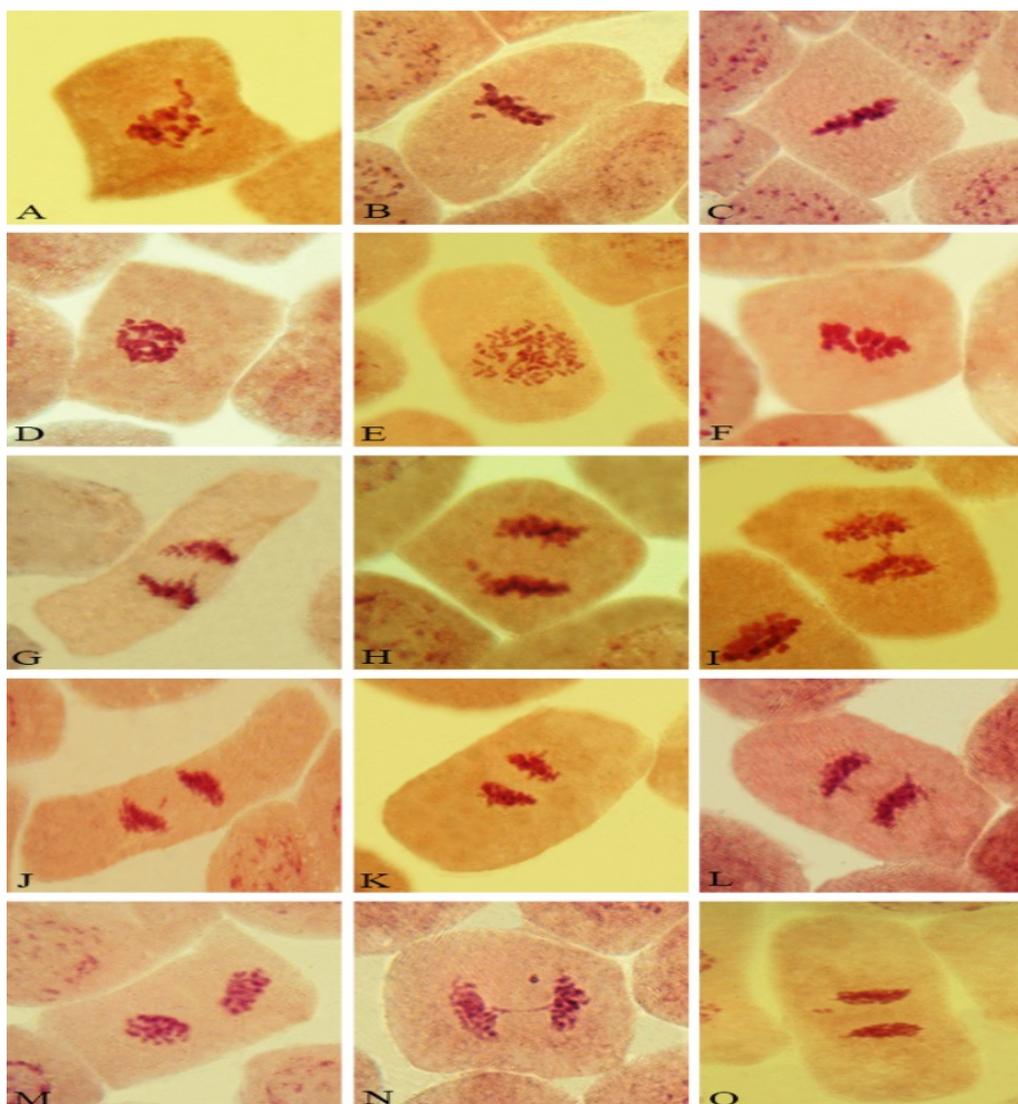


Plate 2: Types of mitotic abnormalities induced by treatment of *Glycine max* root tips with different concentrations of sencor herbicide. (A) Disturbed at metaphase (1.6 g/L-12h). (B) Non-congression at metaphase (2.5 g/L-24h). (C) Oblique metaphase (3.3 g/L-12h). (D) Stickiness at metaphase (3.3 g/L-24hrs). (E) Polyploid cell (2.5 g/L -24h). (F) Two groups at metaphase (3.3 g/L -24h). (G) Late separation at anaphase (2.5 g/L-24h). (H) Diagonal at anaphase (1.6 g/L-12h). (I) Bridge at anaphase (1.6 g/ L-12h). (J) Laggard chromosome at anaphase (2.5 g/L-24h). (K) Disturbed at anaphase (3.3 g/L-12h). (L) Late separation at telophase (3.3 g/L-24h). (M) Diagonal at telophase (1.6 g/L-24h). (N) Bridge at telophase (3.3 g/L-6h). (O) Laggard chromosome at telophase (2.5 g/L-24h). (X=1000).

The results obtained in this investigation showed that the used herbicides induced different mitotic alterations on root tips of *Glycine max*. Such alterations vary from changes in mitotic and phase indices of meristematic cells and the production of a large number of chromosomal aberrations.

The mitotic index (MI), which reflects the frequency of cell division, has been used as a parameter to assess the cytotoxicity of several agents. The cytotoxicity levels of an agent can be determined by the increase or decrease in the MI (Fernandes et al., 2007). According to Hoshina et al., 2002, MIs significantly

lower than control can indicate alterations, deriving from the chemical action in the growth and development of exposed organisms. On the other hand, MIs higher than control are results of an increase in cell division, which can be harmful to the cells, leading to a disordered cell proliferation and even to the formation of tumor tissues.

It is evident from the results that fusilade induced a considerable decrease in mitotic index and it was found statistically significant at the majority of treatments. Inhibition of mitotic index was also reported by many researchers following the treatment of *Allium*

cepa roots with pentachlorophenol, 2, 4-D, butachlor (Ateeq et al., 2002), racer (Yüzbaşıoğlu et al., 2003), arsenal (Grisolia et al., 2004), maleic hydrazide (Marcano et al., 2004), atrazine (Bolle et al., 2004; Sharma and Vig, 2012), avenoxan and diuron (Sharma and Vig, 2012), diclofop-methyl (Mesi and Koplikua, 2013) and quizalofop-P-ethyl (Mustafa and Arikan, 2008; Sharma and Vig, 2012) herbicides.

There are some possible mechanisms for the chemically decreased mitotic index in plant cells. The first is that a decrease in MI could be due to a blocking of G1, thus suppressing DNA synthesis (Schneiderman et al., 1971). The second possible mechanism is a blocking of G2, thereby preventing the cell from entering mitosis (Van't Hof, 1968). The lowering of the mitotic index might have been achieved by the inhibition of DNA synthesis at the S-phase (Sudhakar et al., 2001).

From the percentages of different mitotic phases, it could be observed that there is an increase in percentages of prophase and metaphase but there is a decrease in percentages of anaphase and telophase. Similar action on the mitotic stages has been observed after treatment of *Allium cepa* roots with marshal insecticide (Topaktas and Rencüzogulları, 1996). These results demonstrated an obvious accumulation of prophase, metaphase stage, and probably an inhibition of mitosis or extension of cell cycles. The accumulation of these dividing cells in these stages indicates that fusilade herbicide may influence the sequence of mitotic division and reduce the number of cells entering mitotic division by blocking the process at the end of prophase. It may also be due to interaction with the spindle which can cause an arrest of the cell division at metaphase (Mukherjee et al., 1990; Bessonova, 1991; Duan and Wang, 1995; Liu et al., 1996). Arrested metaphase indicated that, fusilade herbicide caused inhibition of spindle formation. In this context, this herbicide may be considered as post metaphase inhibitors. Many workers referred to the effectivity of certain pesticides in inducing spindle inhibition (Mousa, 1982a; Soliman and Al-Najjar, 1984; Amer and Mikhael, 1986; El-Khodary et al., 1987, 1989, 1990a).

Thus, significant reduction in MI, noted in the present study may be due to the mitodepressive action of the chemicals indicating thereby the herbicide used (fusilade) interfere with normal sequence of cell division resulting in decrease in number of dividing cells. Similar results had also been reported by (Badr, 1986; Sadia and Vahidy, 1994; Inceer et al., 2003; Yuzbasioglu et al., 2003; Sharma and Vig, 2012) on various crop plants. The inhibition of certain specific proteins of cell cycle remains as a possible herbicide target site that inhibit DNA polymerase which is nec-

essary for the synthesis of DNA and other enzymes directly involved with spindle production, assembly or orientation, resulting in antimitotic effect (Hidalgo et al., 1989).

In case of sencor, the results demonstrated that the majority of treatments induced considerable increasing effect on mitotic indices than that of control but the increase effect did not normally correlate with the herbicide concentration. At exposure time 24h, the mitotic frequency was increased as concentration was increased when compared with control.

The increase in mitotic index may be due to shorting the duration of mitotic cycle and enhancing the interphase cells to enter the subsequent division stages (Haroun and Al shehri, 2001). Also it may be due to inducing the synthesis of DNA in dividing cells (Chand and Roy, 1981; Badr et al., 1985). Considering the percentage of the different mitotic stages, it could be observed that, there is an increase or decrease in percentages of prophase, metaphase and telophase but there is a decrease in percentages of anaphase.

The results indicated that both herbicides exert a marked increase in the percentage of total chromosomal abnormalities in *Glycine max* root tip cells. This increase was significant in all concentrations applied when compared to the control. Also, it can be recognized that sencor herbicide was more effective in induction of abnormal mitotic division than fusilade. Moreover, the recorded values showed that there were no abnormalities observed in prophase stage. Both herbicides produced similar types of chromosomal abnormalities except for lagging chromosome which appeared only in samples treated with sencor herbicide. These abnormalities were disturbed, non-congression, oblique, stickiness, polyploidy and two groups at metaphase; late separation, diagonal, bridge, laggard and disturbed at both anaphase and telophase.

Non congression chromosomes were the most prominent and frequent aberration observed at metaphase stage. Stickiness or sticky chromosomes was another most frequently observed aberration at metaphase stage. Mc Gill et al., (1974) reported that stickiness has been shown to be the result of entanglement of interchromosomal chromatin fibres and this leads to subchromatid connections between chromosomes. Stickiness may also be due to the action of herbicides on the polymerization process, resulting in the fragmentation of chromosomes and bridges formation at ana-telophase stage (El-ghamery et al., 2000). Chromosome stickiness have been reported by many investigators following treatment with some pesticides including endosulfan, dieldrin and aldrin on *Vicia faba* (Pandey, 2008), atrazine (Strivastava and Mishra,

2009), illoxa on *Allium cepa* (Yuzbasioglu et al., 2009), imazethapyr on wheat (Rad et al., 2011) and Butachlor 50 EC on wheat (Kumar and Jagannath, 2015). In the study of Marcano et al., (2004) it is indicated that stickiness is regarded as physiological effect exerted by herbicides in plants and the phenomenon has been reported as indicative of high toxicity. The stickiness of chromosomes makes their separation and free movement incomplete; thus, they remain connected by bridges (Jabee et al., 2008).

Disturbed meta and anaphases where the chromosomes spread irregularly over the cell were recorded in a considerable frequencies in all treatments. The irregular spreading of chromosomes may be attributed to the disturbance of the spindle apparatus (Amer and Ali, 1974). The impairment of mitotic spindles leads to the formation of mitotic aberrations which is a frequent effect of herbicidal chemicals on plant cells reported with several herbicides (Hess, 1983; Chauhan and Sundararaman, 1990). Disturbed phases may also due to disturbance in function of the mechanism of chromosomes movement and the orientation of these chromosomes at the equatorial plate (Shehata et al., 2000).

Chromosomal bridges represent a type of abnormalities observed at anaphase and telophase stages the treatment with both herbicides. These bridges could be due to the general stickiness of the chromosomes, accordingly the separation of chromosomes at anaphase stage becomes incomplete and the two chromosomal groups become attached together by chromatin bridges (Badr et al., 1992). The presence of stickiness after treatments with both herbicides supports this view. These results are in accordance with those obtained by Soliman and Ghoneam, (2004) using metribuzin, chlorimuron-ethyl and brominal herbicides, Mustafa and Arikan, (2008) using quizalofop-P-ethyl herbicide and Badr et al., (2013) using Metosulam herbicide.

At anaphase stage during the migration of the chromosomes to the opposite poles, one or more chromosomes may fail to reach any of the two poles and remain lagging. The formation of lagging chromosome(s) may be due to disturbance in the spindle fiber formation caused by the pesticides or herbicides (Badr et al., 1985; Haliem, 1990). Induction of laggards is considered an important aberration from genotoxicity point of view as it leads to aneuploidy (Srivastava and Mishra, 2009). The same results were obtained by several pesticides such as quizalofop-P-ethyl herbicide (Mustafa and Arikan, 2008) and diclofop-methyl (herbicide) and lindane (insecticide) (Mesi and Kopluku, 2013).

Diagonal was another type of abnormalities observed at anaphase and telophase in *Glycine max* root tip cells. In this type the two groups of chromosomes did not orient at the same axis in the cell, which could be due to the effect of herbicides on the spindle apparatus.

CONCLUSION: In conclusion, the obtained results showed that both herbicides have cytotoxic effects on *Glycine max* root tip cells but the cytotoxic activity of sencor was more as compared to fusilade. The two herbicides caused alterations in mitotic index and induced a number of chromosomal aberrations so they should be regarded as mutagenic agents for plants. Thus the use of these herbicides should be under control in agricultural fields. Regular application of herbicides in agricultural practices is a potential threat to genetic constitution of economically important plants like *Glycine max*. Therefore, judicious application of these chemicals is essential. Indiscriminate use of pesticides and herbicides should be discouraged as far as practicable.

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