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# The Mutagenic Potentialities of Some Herbicides Using *Vicia faba* as a Biological System

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**Abstract:** The present study has been carried out to investigate the mutagenic effects of three herbicides: metribuzin, chlorimuron-ethyl and brominal on mitosis, total nucleic acids (DNA and RNA) and protein banding pattern of *Vicia faba*. One concentration for each herbicide was applied for different treatment times: 12, 24 and 48 h. The obtained results indicate that the herbicides had the ability to cause different mitotic changes varying from reduction in mitotic index, phase distribution to the production of a large number of mitotic abnormalities. These changes appeared in varying degrees depending on the duration of treatment. The types of abnormalities produced were laggards, bridges, stickiness, C-metaphase and disturbed phases as well as micronuclei and multinucleate interphase cells. The amounts of both DNA and RNA were generally decreased with increasing the treatment time for each herbicide. At electrophoretic level, these herbicides induced alterations in the protein banding patterns of *Vicia faba* roots as compared with untreated samples. These alterations may be disappearance of some characteristic bands, the appearance of new bands, changes in band intensity, changes in band relative mobility or changes in some fractionation of some bands.

**Key words:** *Vicia faba*, herbicides, chromosome aberration, nucleic acid content, protein banding pattern

## INTRODUCTION

Many investigators have noticed that the use of chemical pesticides for controlling plant diseases in the modern agriculture causes deleterious effects on hereditary materials in both mitotic and meiotic cell division and causes genetic damage to man, domestic animals and economical plants[1-3].

Higher plants, particularly *Allium cepa* and *Vicia faba* possess many advantages that make them ideal for use by scientists in the field of environmental mutagenesis for screening and monitoring of genotoxic agents according to the standard protocol for the plant assays established by the International Program on Chemical Safety (IPCS) and the World Health Organization. The advantages of using higher plant genetic bioassays for testing, monitoring and screening chemicals or pollutants are; that higher plants are eukaryotes and have a chromosome structure similar to man and other animals. Plant cells also undergo mitosis and meiosis and can mutate in a manner similar to human and animal cells[4]. Many authors investigated the potentialities of higher plant genetic systems for monitoring and screening chemical mutagens[5-7].

Constantin and Owens[8] stated that mutagenicity and clastogenicity assays that employ higher plants offer several advantages in comparison to other assays to detect and/or monitor genotoxins in the environment. The use of plant root tips, particularly those of *Allium cepa* and *Vicia faba*, as a bioassay test system for the genotoxicity of pesticides has shown extremely good correlation with the bacterial and mammalian systems[9]. Ma[10] used *Vicia faba* pollen mother cells (PMC) in cytogenetic tests for environmental mutagens. Grant[4] showed that *Vicia faba* is an excellent model for the detection of environmental mutagens.

Number of investigators had studied the side effect of the herbicides on the heredity material of different plant cells. They found that these chemicals had genotoxic effects on both mitotic and meiotic cells when treated with high doses[11]. From these herbicides are gespax[12], tribunil[13,14], gramoxone[15], pendimethalin[16] and topogard[3].

On the other hand, several investigations were carried out to indicate the relation between changes in mitotic and meiotic activities with changes in nucleic acid contents as a result of treatment with pesticides[17]. Chromosomal damage produced by chemicals may be due to an action on DNA[18]. Some reports have also suggested that inhibition of cell division may cause an inhibitory effect on DNA and RNA synthesis[19,20]. Bell *et al*.[2] studied the effect of the herbicide paraquate on cell cycle and DNA synthesis of *Vicia faba*. They found a significant inhibition of DNA synthesis and statistically effect on the percentage of cell division. Moreover, Badr[22] found that treatment of *Vicia faba* with terbutryn resulted in reductions in DNA and RNA contents. The same results were obtained by Badr and

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Ibrahim[23] who reported that chlorsulfuron caused a depression in DNA and RNA content in *Vicia faba* and *Allium cepa*.

Electrophoretic techniques of protein have been used as a successful tool to estimate the possible mutagenic potentialities produced due to continuous and accumulative pollution by chemicals and pesticides and correlate the produced variation with chromosomal aberrations caused by these environmental pollutants[15,24,25].

Ghareeb[3] found that biochemical analysis of $M_2$ plants of *Vicia faba* after treatment with topogard showed several changes in protein banding patterns as compared with control pattern. Furthermore, El-Nahas[26] noticed that imazethapyr and its combination with urea has a great ability to induce changes in the protein banding patterns of *Vicia faba* seed storage protein as compared with untreated samples being more pronounced in treatment with the herbicide singly. Similar results were obtained by a number of authors after treating plant cell with pesticides as other chemical mutagens[24,25,27-30].

Thus, the aim of the present work was to ascertain the cytotoxic effects and the mutagenic potentialities of three herbicides belonging to different groups having varied modes of action (metribuzin, chlorimuron-ethyl and brominal) using *Vicia faba* as a biological system. The first herbicide belongs to S-triazine herbicides; the second belongs to sulfonylurea group while the third is a benzonitrile herbicide.

Cytological studies have been carried out to detect the genotoxic effects of the different herbicides on *Vicia faba* including mitotic index, phase index, chromosome aberrations, nucleic acids (DNA and RNA) as well as protein profiles.

## MATERIALS AND METHODS

Of the different groups of herbicides, three main categories were chosen for the present investigation, S-triazine represented by metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazine-5 (4H)-one], sulfonylurea represented by chlorimuron-ethyl [ethyl 2-[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl] sulfonyl]benzoate] and nitrile group represented by bromoxynil (brominal) [3,5-dibromo-4-hydroxy benzonitrile].

Seeds of pure strain of *Vicia faba* (cv. Giza 3) were obtained from the Crop Research Institute, Agricultural Research Center (ARC), Giza, Egypt. Dry seeds were soaked for 24 h in a tap water, and germination was carried out at 20°C in the dark. After 2 days, the primary roots of about 1.5-2 cm length were transferred to plastic containers (18x12x5 cm) with perforated covers. The containers were divided into four groups. One was left as a control in which distilled water was used; the other three groups were used for treatment with the different herbicides. The concentrations of metribuzin, chlorimuron-ethyl and brominal were $2.5x10^{-6}$, $5x10^{-8}$ and $10^{-4}$ M, respectively. In each group, the germinated seeds were passed through the bores of the covers till the root tips reach the solution of the herbicide. The root tip samples were collected after 12, 24 and 48 h after treatment.

After treatment, the root tips were fixed immediately in aceto-alcohol (1:3). After fixation, the root tips were hydrolyzed in 1N HCl at 60°C for 3-5 min. followed by staining in carbol fuchsin stain[31,32]. Root tips were then squashed in 2% aceto-Orcein stain in 45% acetic acid. Slides were made permanent mounted in Canada balsam, examined and photographed. The mitotic index, phase index and chromosome aberrations were determined by examination of at least 1000 cells. The mitotic index was calculated as the percentage of dividing cells to the total number of cells examined. The frequency of each mitotic phase was calculated as the percentage of cells in that stage to the total number of dividing cells examined. The same slides were analysed for the percentage and types of the chromosomal abnormalities in cells at each mitotic phase as well as nondividing cells. The significance of differences between treatments and control on both mitotic index and the frequency of chromosomal aberrations was evaluated with t-test[33].

For nucleic acids extraction, the method based on that of Shibko *et al.*[34]. DNA was estimated by diphenylamine colour reaction described by Burton[35]. RNA was determined followed the method of Dische[36].

For protein electrophoretic analysis, the method for discontinuous SDS-PAGE techniques was based on that of Laemmli[37]. For the determination of the molecular weight a mixture of the marker protein are used. The banding profile in gel was photographed. The number of bands for each sample was scored. The analysis percentage of the bands were carried out using BIO-RAD Video densitometer, Model Gel Doc 2000.

## RESULTS

Table 1 shows that treating the primary roots of *Vicia faba* with metribuzin for different times did not affect the mitotic index. In spite of the 12 h treatment induced slightly lower mitotic index than that of the control (5.58 and 6.00, respectively). Also, the significance of the data was determined by statistical analysis and showed that all treatments have no significant effect. Although there are a great change in the percentages of

Table 1: Effect of metribuzin on mitotic index, phase index, abnormal mitotic phases and percentage of total abnormalities in mitosis and interphase cells in *Vicia faba* roots

| | | Phase index | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Prophase (%) | | Metaphase (%) | | Anaphase (%) | | Telophase (%) | | | Mean % of total abn. | |
| Time of treatment | Total mitoses (%) | Total | Abn. | Total | Abn. | Total | Abn. | Total | Abn. | Mean mitotic index±S.E | Interphase±S.E | Mitoses±S.E |
| 12 h | 558.36 | 44.63 | 1.03 | 28.53 | 22.04 | 11.19 | 24.66 | 15.64 | 24.51 | 5.58±0.30N | 1.07±0.01 | 13.34±0.11* |
| 24 h | 571.59 | 47.54 | 4.38 | 26.73 | 26.64 | 10.95 | 40.00 | 14.79 | 52.59 | 5.72±0.36N | 1.50±0.03 | 21.36±0.10* |
| 48 h | 599.61 | 43.12 | 13.95 | 30.80 | 32.09 | 11.89 | 86.75 | 14.18 | 77.78 | 6.00±0.34N | 5.48±0.12 | 37.25±0.16* |
| control | 600.39 | 42.29 | | 25.57 | | 11.80 | | 20.33 | | 6.00±0.61 | 0.21±0.05 | 0.00 |

* Significant at 0.05 level from control, N: Not significant at 0.05 level from control, Total examined cells = 10000

Table 2: Types of chromosome abnormalities and its percent in each phase in *Vicia faba* cells treated with metribuzin at different exposure time

| | Abnormalities frequency (%) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Interphase | | | | Prophase | | | Metaphase | | | | | | |
| Time of treatment | Micro-nucleus | Large micronucleus | 2 micro-nuclei | Multi-nucleat | Micro-nucleus | Stick-iness | 2 micro-nuclei | Non-congression | Micro-nucleus | Stick-iness | Two groups | Chromo-some ring | Dis-turbed | C-meta-phase |
| 12 h | 0.98 | 0.09 | 0.0 | 0.0 | 0.34 | 0.69 | 0.0 | 8.06 | 1.07 | 3.76 | 3.23 | 0.54 | 4.84 | 0.54 |
| 24 h | 1.26 | 0.15 | 0.0 | 0.09 | 3.92 | 0.46 | 0.0 | 6.97 | 5.33 | 2.05 | 1.64 | 0.41 | 9.43 | 0.81 |
| 48 h | 3.67 | 0.53 | 0.47 | 0.81 | 11.96 | 0.33 | 1.66 | 9.30 | 8.37 | 6.05 | 1.86 | 0.47 | 4.65 | 1.39 |
| Control | 0.16 | 0.05 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | Anaphase | | | | | Telophase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Laggard | Bridge | Diagonal | Micronucleus | Late separation | Laggard | Bridge | Micronucleus | Chromosome ring | Disturbed |
| 12 h | 12.33 | 2.74 | 2.74 | 0.0 | 6.85 | 20.59 | 1.96 | 1.96 | 0.0 | 0.0 |
| 24 h | 15.00 | 10.00 | 1.00 | 10.00 | 4.00 | 34.81 | 5.19 | 2.22 | 0.0 | 10.37 |
| 48 h | 38.55 | 18.08 | 0.0 | 20.48 | 9.64 | 34.35 | 13.13 | 13.13 | 5.05 | 12.12 |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Different types of abnormalities were expressed as a percentage of the number of cells in each phase from interphase cells and total dividing cells, respectively.

Table 3: Effect of chlorimuron-ethyl on mitotic index, phase index, abnormal mitotic phases and percentage of total abnormalities in mitosis and interphase cells in *Vicia faba* roots

| | | Phase index | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Prophase (%) | | Metaphase (%) | | Anaphase (%) | | Telophase (%) | | | Mean % of total abn. | |
| Time of treatment | Total mitoses (%) | Total | Abn. | Total | Abn. | Total | Abn. | Total | Abn. | Mean mitotic index±S.E | Interphase±S.E | Mitoses±S.E |
| 12 h | 296.51 | 37.75 | 0.00 | 28.43 | 13.79 | 17.65 | 33.33 | 16.18 | 60.61 | 2.97±0.33* | 1.41±0.13 | 19.61±0.08* |
| 24 h | 137.46 | 25.00 | 66.67 | 16.67 | 33.42 | 25.00 | 41.67 | 33.33 | 16.56 | 1.38±0.17* | 1.51±0.37 | 83.64±0.28* |
| 48 h | 179.53 | 20.37 | 22.22 | 29.81 | 20.00 | 23.39 | 98.26 | 26.42 | 82.57 | 1.79±0.34* | 2.29±0.21 | 45.00±0.35* |
| control | 600.39 | 42.29 | | 25.57 | | 11.80 | | 20.33 | | 6.00±0.61 | 0.21±0.05 | 0.00 |

* Significant at 0.05 level from control, N: Not significant at 0.05 level from control, Total examined cells = 10000

Table 4: Types of chromosome abnormalities and its percent in each phase in *Vicia faba* cells treated with chlorimuron-ethyl at different exposure time

| | Abnormalities frequency (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Interphase | | | Prophase | Metaphase | | | Anaphase | | | | Telophase | | | |
| Time of treatment | Micro-nucleus | Large micr-onucleus | 2 micro-nuclei | Micro-nucleus | Non-con-gression | Two groups | Dis-turbed | Micro-nucleus | Lag-gard | Bridge | Dist-urbed | Laggard | Bridge | Dist urbed | Micro nucleus |
| 12 h | 1.29 | 0.09 | 0.03 | 0.0 | 6.89 | 3.45 | 3.45 | 0.0 | 13.89 | 13.89 | 5.55 | 39.39 | 12.13 | 9.09 | 0.0 |
| 24 h | 1.28 | 0.23 | 0.0 | 66.67 | 62.85 | 0.0 | 21.49 | 3.29 | 37.14 | 31.29 | 24.42 | 6.85 | 5.26 | 12.33 | 0.56 |
| 48 h | 2.01 | 0.28 | 0.0 | 22.22 | 17.26 | 0.0 | 0.0 | 2.74 | 30.56 | 22.18 | 0.0 | 21.43 | 17.79 | 29.06 | 2.11 |
| control | 0.16 | 0.05 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Different types of abnormalities were expressed as a percentage of the number of cells in each phase from interphase cells and total dividing cells, respectively.

different mitotic phases. There was a slight increase in the frequency of prophase in treated roots than that of the control. It reached a maximum frequency of 47.54% after 24 h of treatment compared with the control value of 42.29%. Also, the frequency of metaphase increased gradually until it reached a maximum value of 30.80% after 48 h from treatment compared with the control value of 25.57%. On the other hand, the frequency of anaphase all over treatment times has nearly the same value of the control (11.80%) and the frequency of telophase decreased gradually until it reached a minimum value of 14.18% after 48 h from treatment compared with the control value of 20.33%.

The percentage of aberrant dividing cells generally increased by increasing treatment times (Table 1). It increased from 13.34% after 12 h to 37.25% after 48 h from

Table 5: Effect of brominal on mitotic index, phase index, abnormal mitotic phases and percentage of total abnormalities in mitosis and interphase cells in *Vicia faba* roots

| | | Phase index | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Prophase (%) | | Metaphase (%) | | Anaphase (%) | | Telophase (%) | | | Mean % of total abn. | |
| Time of treatment | Total mitoses (%) | Total | Abn. | Total | Abn. | Total | Abn. | Total | Abn. | Mean mitotic index±S.E | Interphase±S.E | Mitoses±S.E |
| 12 h | 352.82 | 35.98 | 24.42 | 26.78 | 23.44 | 15.89 | 63.16 | 21.34 | 68.63 | 3.53±0.21* | 0.54±0.08 | 30.96±0.12* |
| 24 h | 292.05 | 40.43 | 21.05 | 21.28 | 46.67 | 11.35 | 81.25 | 26.95 | 65.79 | 2.92±0.24* | 4.88±0.31 | 37.59±0.10* |
| 48 h | 524.58 | 38.32 | 14.38 | 30.18 | 27.83 | 13.39 | 50.98 | 18.11 | 42.03 | 5.25±0.41 N | 3.67±0.16 | 30.96±0.15* |
| control | 600.39 | 42.29 | _ | 25.57 | _ | 11.80 | _ | 20.33 | _ | 6.00±0.61 | 0.21±0.05 | 0.00 |

* Significant at 0.05 level from control, N: Not significant at 0.05 level from control, Total examined cells = 10000

Table 6: Types of chromosome abnormalities and its percent in each phase in *Vicia faba* cells treated with brominal at different exposure time

| | Abnormalities frequency (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Interphase | | | Prophase | | Metaphase | | | | | |
| Time of treatment | Micro nucleus | Multin-ucleat | 2 micro-nuclei | Micron-ucleus | Distur bed | Non-con-gression | Two groups | C-met-aphase | Distur-bed | Micro-nucleus | Chromosome ring |
| 12 h | 0.33 | 0.06 | 0.15 | 24.42 | 0.0 | 12.50 | 4.69 | 4.69 | 1.56 | 0.0 | 0.0 |
| 24 h | 4.30 | 0.16 | 0.42 | 19.30 | 1.75 | 20.00 | 3.34 | 10.00 | 13.33 | 0.0 | 0.0 |
| 48 h | 3.40 | 0.10 | 0.17 | 2.05 | 12.33 | 5.22 | 6.09 | 2.61 | 4.35 | 8.69 | 0.87 |
| control | 0.16 | 0.05 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | Anaphase | | | | | Telophase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Laggard | Bridge | Micronucleus | Diagonal | Disturbed | Laggard | Bridge | Chromosome ring | Micronucleus | Disturbed |
| 12 h | 42.11 | 13.16 | 0.0 | 0.0 | 7.89 | 35.29 | 19.61 | 0.0 | 0.0 | 13.73 |
| 24 h | 62.50 | 12.50 | 0.0 | 0.0 | 6.25 | 31.58 | 18.42 | 0.0 | 0.0 | 15.79 |
| 48 h | 23.53 | 7.84 | 5.88 | 3.92 | 9.81 | 15.94 | 10.14 | 7.25 | 1.45 | 7.25 |
| control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Different types of abnormalities were expressed as a percentage of the number of cells in each phase from interphase cells and total dividing cells, respectively.

Table 7: Effect of herbicides on DNA and RNA amounts in the root tips of *Vicia faba*

| Treatments | Time of exposure | DNA (μg $g^{-1}$) | RNA (μg $g^{-1}$) |
|---|---|---|---|
| Control | 12 h | 221.52 | 641.52 |
| | 24 h | 252.71 | 672.57 |
| | 48 h | 256.34 | 673.21 |
| Metribuzin | 12 h | 213.54 | 653.71 |
| | 24 h | 225.84 | 637.19 |
| | 48 h | 209.39 | 623.26 |
| Chlorimuron-ethyl | 12 h | 192.68 | 634.65 |
| | 24 h | 187.56 | 627.84 |
| | 48 h | 172.91 | 615.34 |
| Brominal | 12 h | 207.41 | 698.59 |
| | 24 h | 201.52 | 580.63 |
| | 48 h | 197.67 | 553.84 |

treatment. Generally, the effect of metribuzin at all treatment times on the induction of aberrations in dividing cells was significant as well as there are an increase in the percentages of aberrant nondividing cells. It reached 5.48% after 48 h from treatment as compared with control value of (0.21%). Metribuzin induced different types of abnormalities. Their frequency depends on the duration of treatment. These abnormalities affected almost all the stages of mitosis. The proportions of different types of mitotic phases abnormalities produced by metribuzin after 12, 24 and 48 h of treatment are given in Table 2 and shown in Fig. 1.

Concerning the effect of chlorimuron-ethyl on mitotic division of *Vicia faba* root tips, it was obvious that the herbicide has a marked reducing effect on mitotic index values. Prolonged treatments resulted in a further inhibition of mitotic index (MI) values. It decreased from 2.97 after 12 h to 1.79 after 48 h from treatment compared with the control value of (6.0). Such decrease in the MI was found to be statistically significant at all treatment times (Table 3). The analysis of the mitotic index data showed that the relative frequency of mitotic phases after chlorimuron-ethyl treatment differed from the control. Nearly all treatments of chlorimuron-ethyl resulted in a smaller prophase population compared with the control value (42.29%). It reached a minimum value (20.37%) after 48 h of treatment. On the other hand, the frequency of metaphase increased especially after 48 h and reached 29.81% compared with control value (25.57%). Also, the frequency of anaphase generally increased in treated samples and reached a maximum value of 25.0% after 24 h of treatment while it was 11.8% in control. Telophase frequency was higher than control and reached a maximum value of 33.33% after 24 h from herbicide treatment.

Chlorimuron-ethyl increased considerably the percentage of total abnormalities in *Vicia faba* root tip cells compared with control (Table 3). The total percentage of abnormalities in dividing cells increased by increasing the time of exposure to the herbicide and reached a maximum value (83.64 %) after 24 h of treatment. From the microscopic observations and Table (3) it was

Table 8: Protein electrophoretic analysis of different herbicides treated *Vicia faba* samples

| Control | | | | Metribuzin | | | |
|---|---|---|---|---|---|---|---|
| Band number | Relative front | Mol.Wt.KDa | Average OD | Band number | Relative front | Mol.Wt.KDa | Average OD |
| 1 | 0.11 | 341.44 | 0.147 | 1 | 0.12 | 331.13 | 0.122 |
| 2 | 0.13 | 324.58 | 0.171 | 2 | 0.13 | 320.21 | 0.106 |
| 3 | 0.14 | 311.16 | 0.172 | 3 | 0.14 | 308.54 | 0.154 |
| 4 | 0.17 | 278.83 | 0.149 | 4 | 0.24 | 181.03 | 0.154 |
| 5 | 0.18 | 267.30 | 0.155 | 5 | 0.27 | 135.57 | 0.197 |
| 6 | 0.24 | 181.03 | 0.236 | 6 | 0.28 | 126.38 | 0.183 |
| 7 | 0.27 | 135.57 | 0.247 | 7 | 0.37 | 89.58 | 0.237 |
| 8 | 0.28 | 126.38 | 0.338 | 8 | 0.39 | 82.74 | 0.230 |
| 9 | 0.30 | 117.45 | 0.377 | 9 | 0.41 | 76.71 | 0.230 |
| 10 | 0.31 | 115.33 | 0.244 | 10 | 0.46 | 62.95 | 0.238 |
| 11 | 0.35 | 95.41 | 0.245 | 11 | 0.51 | 52.41 | 0.429 |
| 12 | 0.36 | 91.82 | 0.258 | 12 | 0.53 | 48.14 | 0.407 |
| 13 | 0.39 | 82.74 | 0.314 | 13 | 0.59 | 37.61 | 0.279 |
| 14 | 0.41 | 76.71 | 0.324 | 14 | 0.59 | 36.59 | 0.281 |
| 15 | 0.51 | 52.41 | 0.416 | 15 | 0.66 | 28.02 | 0.603 |
| 16 | 0.53 | 48.14 | 0.467 | 16 | 0.69 | 25.25 | 0.759 |
| 17 | 0.59 | 37.61 | 0.311 | 17 | 0.74 | 20.59 | 0.633 |
| 18 | 0.59 | 36.59 | 0.322 | 18 | 0.75 | 19.42 | 0.612 |
| 19 | 0.66 | 28.02 | 0.636 | 19 | 0.80 | 16.00 | 0.269 |
| 20 | 0.69 | 25.25 | 0.655 | 20 | 0.83 | 14.43 | 0.306 |
| 21 | 0.74 | 20.59 | 0.634 | 21 | 0.86 | 12.78 | 0.336 |
| 22 | 0.77 | 18.06 | 0.609 | 22 | 0.91 | 10.53 | 0.469 |
| 23 | 0.83 | 14.43 | 0.335 | 23 | 0.96 | 8.49 | 0.546 |
| 24 | 0.86 | 12.78 | 0.343 | - | - | - | - |
| 25 | 0.91 | 10.53 | 0.481 | - | - | - | - |
| 25 | 0.74 | 20.59 | 0.698 | - | - | - | - |
| 26 | 0.92 | 10.11 | 0.551 | - | - | - | - |
| 27 | 0.96 | 8.49 | 0.565 | - | - | - | - |
| - | - | - | - | - | - | - | - |
| - | - | - | - | - | - | - | - |
| - | - | - | - | - | - | - | - |
| - | - | - | - | - | - | - | - |

| Chlorimuron-ethyl | | | | Brominal | | | |
|---|---|---|---|---|---|---|---|
| Band number | Relative front | Mol.Wt.KDa | Average OD | Band number | Relative front | Mol.Wt.KDa | Average OD |
| 1 | 0.09 | 375.78 | 0.182 | 1 | 0.14 | 308.54 | 0.122 |
| 2 | 0.12 | 334.25 | 0.289 | 2 | 0.15 | 299.82 | 0.104 |
| 3 | 0.13 | 317.89 | 0.239 | 3 | 0.19 | 260.08 | 0.097 |
| 4 | 0.15 | 295.84 | 0.184 | 4 | 0.24 | 186.81 | 0.175 |
| 5 | 0.19 | 260.08 | 0.211 | 5 | 0.27 | 135.57 | 0.119 |
| 6 | 0.24 | 181.03 | 0.241 | 6 | 0.28 | 126.38 | 0.162 |
| 7 | 0.27 | 135.57 | 0.266 | 7 | 0.35 | 98.22 | 0.219 |
| 8 | 0.28 | 126.38 | 0.318 | 8 | 0.36 | 93.88 | 0.217 |
| 9 | 0.31 | 115.33 | 0.286 | 9 | 0.41 | 79.71 | 0.249 |
| 10 | 0.32 | 107.51 | 0.285 | 10 | 0.44 | 66.89 | 0.199 |
| 11 | 0.35 | 95.41 | 0.384 | 11 | 0.46 | 62.95 | 0.216 |
| 12 | 0.37 | 89.58 | 0.335 | 12 | 0.50 | 54.09 | 0.408 |
| 13 | 0.39 | 82.74 | 0.323 | 13 | 0.53 | 48.14 | 0.461 |
| 14 | 0.41 | 76.71 | 0.283 | 14 | 0.59 | 37.61 | 0.305 |
| 15 | 0.42 | 72.24 | 0.276 | 15 | 0.59 | 36.59 | 0.319 |
| 16 | 0.44 | 66.89 | 0.316 | 16 | 0.66 | 28.02 | 0.615 |
| 17 | 0.48 | 58.68 | 0.324 | 17 | 0.69 | 25.25 | 0.618 |
| 18 | 0.51 | 52.41 | 0.642 | 18 | 0.73 | 21.52 | 0.559 |
| 19 | 0.53 | 48.14 | 0.576 | 19 | 0.74 | 20.59 | 0.536 |
| 20 | 0.55 | 43.95 | 0.480 | 20 | 0.77 | 18.06 | 0.199 |
| 21 | 0.59 | 37.61 | 0.408 | 21 | 0.83 | 14.43 | 0.207 |
| 22 | 0.59 | 36.59 | 0.402 | 22 | 0.89 | 11.34 | 0.420 |
| 23 | 0.66 | 28.02 | 0.719 | 23 | 0.91 | 10.53 | 0.401 |
| 24 | 0.69 | 25.25 | 0.814 | 24 | 0.96 | 8.48 | 0.493 |
| 26 | 0.75 | 19.42 | 0.687 | - | - | - | - |
| 27 | 0.81 | 15.83 | 0.264 | - | - | - | - |
| 28 | 0.83 | 14.43 | 0.309 | - | - | - | - |
| 29 | 0.89 | 11.34 | 0.521 | - | - | - | - |
| 30 | 0.91 | 10.53 | 0.524 | - | - | - | - |
| 31 | 0.96 | 8.49 | 0.478 | - | - | - | - |

Table 9: Comparative analysis of relative concentration (band %) and molecular weight (M.Wt.) of the different types of protein bands of root tips of *Vicia faba* treated with different herbicides. These bands were separated using SDS-PAGE technique

| | Band % | | | | |
|---|---|---|---|---|---|
| Band No. | Contol | Metri-buzin | Chlorim-uron-ethyl | Brom inal | M.Wt. (KDa) |
| 1 | _ | _ | 0.68 | _ | 375.78 |
| 2 | 0.52 | _ | _ | _ | 341.44 |
| 3 | _ | _ | 0.89 | _ | 334.25 |
| 4 | _ | 0.29 | _ | _ | 331.13 |
| 5 | 0.59 | _ | _ | _ | 324.58 |
| 6 | _ | 2.02 | _ | _ | 320.21 |
| 7 | _ | _ | 0.89 | _ | 317.89 |
| 8 | 2.66 | _ | _ | _ | 311.16 |
| 9 | _ | 0.37 | _ | 2.98 | 308.54 |
| 10 | _ | _ | _ | 0.32 | 299.82 |
| 11 | _ | _ | 0.69 | _ | 295.84 |
| 12 | 0.31 | _ | _ | _ | 278.83 |
| 13 | 1.64 | _ | _ | _ | 267.30 |
| 14 | _ | _ | 0.66 | 0.59 | 260.08 |
| 15 | _ | _ | _ | 0.72 | 186.81 |
| 16 | 0.99 | 0.73 | 0.75 | _ | 181.03 |
| 17 | 1.39 | 0.78 | 0.50 | 0.76 | 135.57 |
| 18 | 1.19 | 0.73 | 0.79 | 0.56 | 126.38 |
| 19 | 1.59 | _ | _ | _ | 117.45 |
| 20 | 1.03 | _ | 1.42 | _ | 115.33 |
| 21 | _ | _ | 1.24 | _ | 107.51 |
| 22 | _ | _ | _ | 1.14 | 98.22 |
| 23 | 0.52 | _ | 1.43 | _ | 95.41 |
| 24 | _ | _ | _ | 0.56 | 93.88 |
| 25 | 0.73 | _ | _ | _ | 91.82 |
| 26 | _ | 1.13 | 1.04 | _ | 89.58 |
| 27 | 1.76 | 1.28 | 0.80 | _ | 82.74 |
| 28 | 1.36 | 1.09 | 1.41 | 0.86 | 76.71 |
| 29 | _ | _ | 0.69 | _ | 72.24 |
| 30 | _ | _ | 1.57 | 0.69 | 66.89 |
| 31 | _ | 1.13 | _ | 0.93 | 62.95 |
| 32 | _ | _ | 1.21 | _ | 58.68 |
| 33 | _ | _ | _ | 1.76 | 54.09 |
| 34 | 1.75 | 2.38 | 3.19 | _ | 52.41 |
| 35 | 1.96 | 2.58 | 2.51 | 2.39 | 48.14 |
| 36 | _ | _ | 1.49 | _ | 43.95 |
| 37 | 0.87 | 1.99 | 3.50 | 1.58 | 37.61 |
| 38 | 0.68 | 3.79 | 0.76 | 1.38 | 36.59 |
| 39 | 3.12 | 2.87 | 4.47 | 6.91 | 28.02 |
| 40 | 3.68 | 3.61 | 4.05 | 7.47 | 25.25 |
| 41 | _ | _ | _ | 5.31 | 21.52 |
| 42 | 4.00 | 3.01 | 2.60 | 4.63 | 20.59 |
| 43 | _ | 3.40 | 3.41 | _ | 19.42 |
| 44 | 4.28 | _ | _ | 1.20 | 18.06 |
| 45 | _ | 1.07 | _ | _ | 16.00 |
| 46 | _ | _ | 1.31 | _ | 15.83 |
| 47 | 1.88 | 1.21 | 1.35 | 0.89 | 14.43 |
| 48 | 1.20 | 1.60 | _ | _ | 12.78 |
| 49 | _ | _ | 2.91 | 5.81 | 11.34 |
| 50 | 2.03 | 2.98 | 1.96 | 3.12 | 10.53 |
| 51 | 2.70 | _ | _ | _ | 10.11 |
| 52 | 1.59 | 3.03 | 1.49 | 2.98 | 8.49 |
| Total bands | 27 | 23 | 31 | 24 | |

clear that the percentage of abnormal cells was lower in interphase than in mitotic phases. It gradually increased by increasing time and reached a maximum value of 2.29% after 48 h of herbicide treatment while it was 0.21% in the control. The results showed that chlorimuron-ethyl has the ability to induce a wide range of chromosomal abnormalities in all mitotic stages. The types and percentages of abnormal cells are given in Table 4 and shown in Fig. 2.

Table 5 shows that brominal caused a decrease in the mitotic indices in all treated faba roots as being compared with that of the control. The results showed that prophase frequency was generally less than that of the control allover treatment times. It decreased from a range of 40.43% after 24 h to 35.98% after 12 h while it was 42.29% in control. This reduction in the prophase frequency was accompanied with an increase in the percentages of metaphase, anaphase and telophase stages. The frequency of metaphase reached a maximum value of 30.18% after 48 h as compared with control value of 25.57%. Also, the frequency of anaphase was slightly increased and reached a maximum value of 15.89% after 12 h from treatment while it was 11.8% in control.

The frequency of the total abnormal dividing cells increased with the increase of the herbicide treatment duration with an exception after 48 h. The lowest percentage of the mitotic abnormalities was 30.96% and recorded in faba roots treated with brominal for 12 and 48 h. The highest value (37.59%) was scored after 24 h from treatment with the herbicide. It induced a wide range of abnormalities covering all stages of mitosis. The types and frequencies of such abnormalities are given in Table 6 and illustrated in Fig. 3.

The amounts of both DNA and RNA in root tips of *Vicia faba* after treatment with various herbicides are given in Table 7. It can be noticed that there was a sharp decrease in DNA content after treatment with herbicides and the results clearly indicated that the DNA content was generally decreased with increasing the treatment time for each herbicide. This is particularly evident from a comparison of the values of these compounds in treated roots to their values in control. The highest diminution was found for chlorimuron-ethyl (172.91 $\mu g\ g^{-1}$) followed by brominal (197.67 $\mu g\ g^{-1}$) and finally by metribuzin (209.39 $\mu g\ g^{-1}$) after 48 h of treatment.

Also, RNA content in faba bean decreased after treatment with herbicides as compared with control. The minimum value (553.84 $\mu g\ g^{-1}$) was recorded after treatment with brominal and the maximum value (623.26 $\mu g\ g^{-1}$) was observed after treatment with metribuzin while the value of RNA after treatment with chlorimuron-ethyl was (615.34 $\mu g\ g^{-1}$) at the end of experiment as compared with that of the untreated roots (673.21 $\mu g\ g^{-1}$).

The electrophoretic analysis of protein extracts from treated and untreated samples after 24 h from treatment using discontinuous SDS-PAGE gel are illustrated in

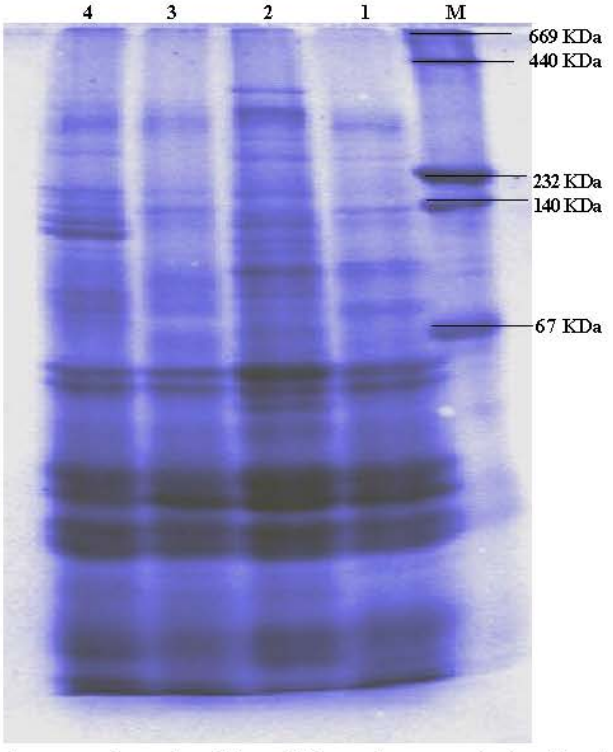


Fig. 4: Polyacrylamide gel illustrating protein bands of Vicia faba roots trated with different herbicides
Lane 1: Control Lane 2: Metribuzin
Lane 3: Chlorimuron-ethyl Lane 4: Brominal

Fig. 4. The scanning of SDS-PAGE gel of the different samples are shown in Fig. 5, their molecular weights (MW), relative front (Rf), number of bands and average optical density (OD) are given in Table 8.

Electrophoretic analysis of protein extracts showed variations among the studied samples. Each sample exhibited a distinctive electrophoretic pattern. The observed changes were both qualitative and quantitative and may be illustrated by the appearance of new bands, disappearance of some bands, changes in band intensity, changes in band relative mobility or changes in some fractionation of some bands.

From Table 9 it is obvious that the total number of recorded bands was 52 with molecular weights ranging between 375.78 and 8.49 KDa. Such bands are not always expressed in all samples. Thus the number of bands in the studied samples ranges between 31 bands in treated samples with chlorimuron-ethyl and 23 in samples treated with metribuzin while the number of bands in case of brominal was 24 bands.

The most visible changes in protein profiles of faba bean samples treated with herbicides were the appearance of three new bands with molecular weights of 331.13, 320.21 and 16.00 KDa in case of metribuzin while these

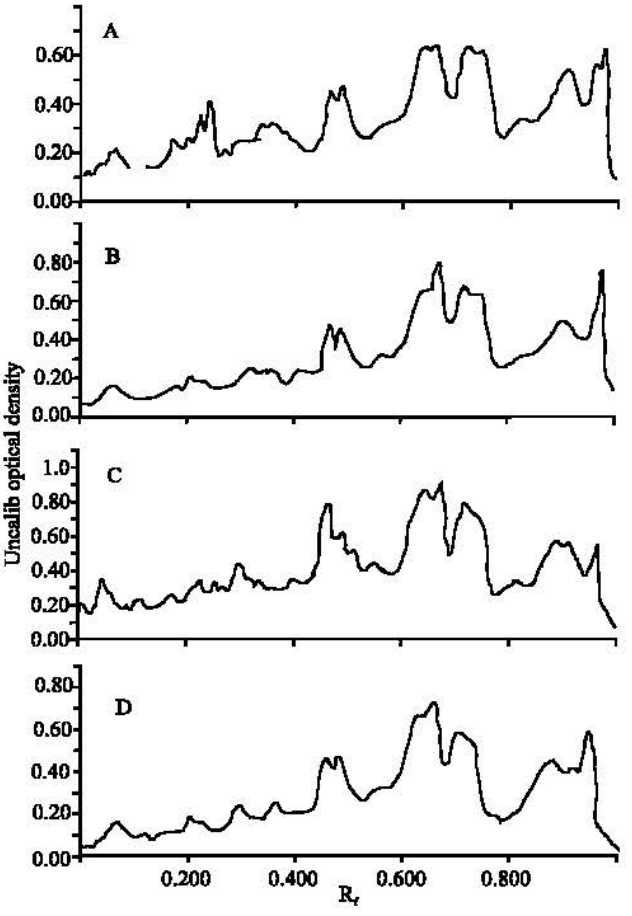


Fig. 5: The densitometer scanning pattern of protein of Vicia faba roots treated with different herbicides: Control (A), Metribuzin (B), Chlorimuron-ethyl (C) and Brominal (D)

bands are not observed in untreated samples and other nine bands with molecular weights of 375.78, 334.25, 317.89, 295.84, 107.51, 72.24, 58.68, 43.95 and 15.83 KDa after treatment with chlorimuron-ethyl. Also, treatment with brominal resulted in appearance of six new bands at 299.82, 186.81, 98.22, 93.88, 54.09 and 21.52 KDa. On the other hand, some other bands appeared in control while disappeared in treated samples, such bands are of molecular weights 341.44, 324.58, 311.16, 278.83, 267.30, 117.45, 91.82 and 10.11 KDa.

## DISCUSSION

The results obtained in this investigation showed that the used herbicides induced different mitotic changes on root tips of *Vicia faba*. Such changes vary from the reduction of mitotic index of meristematic cells, changes in phase index and the production of a large number of

chromosomal aberrations. These changes appeared in varying degrees depending on the duration of the treatment.

The reduction of mitotic activity seems to be a common effect of most herbicides tested for their action on mitosis[19,28,29,14,26]. In fact, Haliem [40] reported that metribuzin has the ability to cause a great reduction in mitotic activity of meristematic cells of *Allium cepa*. Also, Kumar *et al.*[41] found that atrazine caused a reduction in mitotic index in maize and this reduction was directly proportional to dose and duration of treatment.

Inhibition of mitotic division in plants has been attributed to a number of factors[42,29]. The inhibition of mitotic index may be due to the interference of the herbicide in the normal process of mitosis by reducing the number of the dividing cells[39,43,40,44]. Many other investigators have attributed the depression in mitotic index values to the inhibition of protein synthesis[45]. Basyczynki *et al.*[46] showed that cycloheximide induced a reduction in mitotic index. They attributed such reduction to the inhibition of certain types of nuclear proteins essential in the mitotic cycle. Similar results were obtained by El-Nahas[26] where the inhibition of cell division was accompanied with many changes in the protein banding patterns of *Vicia faba* M2 seeds whose parents were previously treated with imazethapyr herbicide.

Such mitotic inhibition could also be due to the inhibition of DNA synthesis which is considered as one of the major perquisites for a cell to divide[39,43]. Support for this view is the present results on DNA and RNA content with the results obtained by Badr *et al.*[12] who reported that the reduction in mitotic activity of *Vicia faba* after treatment with gespax herbicide was also associated with a reduction in the amounts of DNA and RNA.

Metribuzin caused accumulation of prophase in *Vicia faba* on the expense of frequencies of both anaphase and telophase which were recorded at low level. This prophase accumulation was similar to that observed by El-Khodary *et al.*[47] after treatment of *Allium cepa* roots with anti-inflammatory drug (hydrocortisone sodium succinate).

In other treatments, the prophase frequency was generally less than that of the respective controls. This reduction in the prophase frequency was accompanied with an increase in the percentages of metaphase, anaphase and telophase stages. These results may indicate that the herbicides are active in obstructing the onset of mitosis by preventing the interphase cells to enter prophase as proposed by Miltwoch and Wilkei[48] and Kabarity *et al.*[49] . On the other hand, the increase in the metaphase and ana-telophase frequencies may be due to the action of the herbicides on the spindle which resulted in the arrest of division at these stages. Similar results were obtained following treatments of *Allium cepa*, *Hordeum vulgare* and *Vicia faba* with nitralin[38,20]; glean on *A. cepa* and *V. faba*[23] and tribunil on *V. faba* and *A.cepa*[13,40].

Different types of chromosomal abnormalities such as laggards, bridges, stickiness and c-metaphase were observed in faba bean after treatment with the three herbicides used in the present investigation. These results indicate the potentiality of the investigated herbicides to induce mitotic irregularities, which agree with the findings of many authors after studying the genotoxic effects of pesticides or other agents[27,50].

The most common type of aberrations in the present investigation was the appearance of lagging chromosomes at metaphase (non-congression), anaphase and telophase. The induction of laggard could be attributed to the failure of the spindle apparatus to organize and function in a normal way rather than inhibition of these spindle fibers and this may lead to irregular orientation of chromosomes[18,13,51].

Induction of chromosomal and chromatin bridges at anaphase and telophase stages was also observed after treatment with metribuzin, chlorimuron-ethyl and brominal herbicides. These bridges may result from chromosome stickiness[52,39]. Due to such stickiness the separation of the daughter chromosomes becomes incomplete even in the presence of spindle fibers and thus they remain connected by chromatin bridges[49]. Similar results were obtained by several authors[11,53]. Bridges may also result from breakage of chromosomes followed by proximal chromatid reunion, which evidently results in dicentric chromosomes and form characteristic anaphase bridges[18,19].

In the present investigation, a remarkable correlation exists between stickiness and bridges. This supports the hypothesis that the occurrence of bridges whether chromosomal or chromatidal is most likely due to the general stickiness rather than chromosome breakage and reunion. These results were in accordance with those obtained by Badr[38] and Mansour[13].

Among aberrations that appeared frequently in faba bean after treatment with the three herbicides was stickiness. Such stickiness covers the whole chromosome complement leading to the appearance of chromatin masses where the general appearance of chromosomes is lost. Mc Gill *et al.*[54] and Klasterska *et al.*[55] interpreted stickiness as a result of the improper folding of chromosome fibers into chromatids and thus there is an intermingling of the fibers and chromosomes become

attached to each other by means of subchromatid bridges.

Disturbed mitotic configuration comprised considerable percentages of abnormalities was induced by treatments with the three herbicides. These were indicated by chromosome spreading irregularly all over the spindle apparatus[30]. Such type of irregularity was also reported by[56]. Disturbed phases may be due to disturbance in the function of the mechanism of chromosomes movement and the orientation of these chromosomes at the equatorial plate[29]. It may also be induced as a result of an effect on the spindle apparatus[3].

C-metaphase where chromosomes appear scattered in the cytoplasm was found in treatments of metribuzin and brominal. In this type of aberration, the metaphase chromosomes are shorter, thick and show no equator orientation. In the normal mitosis, chromosome movement is the result of the interaction between centromere and spindle fibers, but in c-mitosis there is no such interaction and the few movements that do occur appear to be a result of the stronger tendency of the chromosomes to straighten[57]. Such type of anomalies is an indication of the action of the herbicide on the inhibition of spindle fiber formation by their action on microtubules, which play the major role in the formation of spindle fibers[29] .

In addition to the types of chromosomal anomalies induced in dividing cells micronuclei were observed in the interphase cells as well as in different mitotic stages. Micronuclei have been reported by Clowes[58] to be formed as a result of exclusion of acentric fragment of chromosomes out of the nuclear envelope during the completion of mitosis. Micronuclei may also originate from lagging chromosomes or chromosome fragments in a preceding mitosis[59].

Multinucleated cells were recorded in few percentages after treatment with metribuzin and brominal. The formation of multinucleated cells may be the result of a preceding multipolar mitosis or the failure of cell plate formation following mitosis[18].

The present study showed that the DNA and RNA contents in *Vicia faba* were sensitive to the applied herbicides; it decreased than those of the control. The reduction in nucleic acid contents could be due to inhibition of DNA synthesis. These results are in accordance with the results obtained by Badr[22]. However, the reduction in DNA content might be due to the interference with energy metabolism, since Gruenhagen and Moreland[60] found a close correlation between inhibition of nucleic acid synthesis and reduced ATP levels tissue.

The inhibition of DNA and RNA synthesis by the herbicides may be due to the inhibition of DNA replication as suggested by Scott[61] or due to the reduction of oxidative phosphorylation resulting in lowering ATP level in the cell as suggested by Chand and Roy[62]. The same results were obtained by Badr *et al.*[12]. Also, Ashton and Crafts[63] suggested that herbicides that reduce ATP are strong inhibitors of RNA synthesis. The present results indicated that the decrease in the rate of the cell division (low MI) is also accompanied by a decrease in the DNA content of cells. In other words, there should be a positive correlation between the rate of cell division and DNA synthesis as has been found by Ibrahim[64].

Electrophoretic SDS-protein profiles were successfully used by some authors to establish biochemical genetic finger prints of many plants[65,3]. Hussein and Salam[66] stated that each band in the protein banding pattern of an organism reflects a separate transcriptional event. Furthermore, electrophoretic analysis of the protein provides information concerning the structural genes and their regulatory systems that control the biosynthetic pathways of that protein[30]. The results obtained in this study showed that the used herbicides caused many changes in the protein banding pattern of *Vicia faba*. Similar results were obtained by other investigators following different treatments[56,27,29,50].

The used herbicides caused disappearance of some bands in faba bean. The disappearance of electrophoretic bands could be attributed to the loss of the genetic materials due to fragmentation, laggards and micronuclei as found in the present study. This conclusion is in agreement with many investigators[3,50,26]. Also, Hassan[30] attributed the absence of some bands to the deletion of their corresponding genes. Treatment with metribuzin, chlorimuron-ethyl and brominal caused appearance of new bands which were absent in untreated faba bean. The appearance of new characteristic bands could be explained on the base of mutational event at the regulatory system of unexpected gene (s) that activated it[56]. Also, herbicides used in this investigation caused changes in band intensity, these changes in band intensity could be explained on the basis of induction of gene mutation at the regulatory system which modulates, attenuate or enhances transcription rate of a particular structural gene[67]. This leads to the production of faint or over expressed protein bands[16]. Also, the recorded changes in band intensity could be attributed to the cytological abnormalities induced by herbicides. This conclusion is in accordance with Salam *et al.*[16] who concluded that the increase in band intensity could be interpreted on the base of gene duplication which is a result of cytological abnormalities. Changes in bands relative mobilities could be attributed to the occurrence of point mutation in the concerned structural genes that

create stop codon prior or post the original. They gave rise to the production of shorter or longer polypeptide chains[16,3,26].

The above-mentioned variations in the protein patterns of the differently herbicides treated plants might be assumed to result from changes in the gene expression. This assumption excluded that protein variability might be attributed to seed heterogeneity since pure lines were used and the experimental conditions were thoroughly controlled. Thus, the different herbicides treatments applied in the present work are presumed to affect the protein patterns at one ore more of the above mentioned control points.

It may concluded from the present results that the three herbicides under investigation caused cytotoxicity to *Vicia faba*, however the effect of metribuzin and brominal is more pronounced in this regard than chlorimuron-ethyl.

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