

Full Length Research Paper

Effects of Glyphosate herbicide on chromosomes aberration, mitotic index in the root meristem cells

*¹Mustafa U. I and ²Adham K. Amin¹Department of Biology, College of Science, Missan University.²Department of Plant, College of Agriculture, Missan University.

Accepted 10 April, 2016

This study aims to evaluate the cytogenetic effects of Glyphosate (phomac) herbicides, which were evaluated in the root tip meristem cells of *Vicia faba*. In the *V. faba* L. root growth test, the herbicides with a control for each group Mitotic index decreased with increasing herbicide concentration at each exposure time. In anaphase - telophase cells, the total percentages of different chromosomal aberrations like stickiness, broken chromosomes, vagrant chromosomes, c-mitosis, D trubed metaphase and anaphase, laggards and micronuclei at high concentration (1 ppm) were calculated as 86%, the total number of chromosome aberration increased as Glyphosate herbicide concentration increased. Micro nucleated cells were observed at different stages of cell cycle.

Key words: Glyphosate, herbicides, mitotic index.

INTRODUCTION

Cytogenetic effects of synthetic chemicals used for plants protection have been well documented and previously investigated by many authors (Bader et al., 1985; Vyvyan, 2002; Mekki, 2008). All most all studies confirm the harmful effects of synthetic chemicals used in agriculture purposes (Jose et al., 2008). The indiscriminate use of herbicides in agriculture justifies the evaluation of toxicity of various chemical agents. Several studies have shown that chronic exposure to low to high level of herbicides can cause birth defects and that prenatal exposure is associated with carcinogenicity. Chromosomal aberrations can be accepted as an indicator of genotoxis damage induced by herbicides (Srivastava and Mishra, 2009). Plant test system is widely used for monitoring genotoxicity of chemicals

because of many advantages such as low cost, easily available throughout the year, good chromosomes condition for the study of chromosomes damage (Abraham and Johan 1989; Upadhyya et al., 1996). The higher plants like *Vicia faba*, *Allium cepa* and *Trudescantia paludosa* have large monocentric chromosomes in reduced numbers and are accepted as suitable test organisms for the study of environmental mutagenesis (Patra and Sharma, 2002). The mitotic index (MI), characterized by the total number of dividing cell cycle, has been used as 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 (Lubini et al., 2008). Glyphosate is a weak organic acid consisting of a glycine and a phosphonomethyl moiety. The empirical formula is C₃H₈NO₅P. Glyphosate is usually formulated as a salt of the acid of glyphosate and a cation, e.g., isopropyl amine or trimet generally above 90%. Glyphosate is a phosphonomethyl glycine according to IUPAC nomenclature herbicide compound, commonly used in agriculture in controlling weeds. The indiscriminate use of herbicides in agriculture and the increase of pollution in

*Corresponding author. E-mail: mustafa758@yahoo.com

Abbreviations: **BK**, chromosomal break(s); **Cm**, c-mitosis; **Dc**, delayed anaphase; **Sc**, Sticky chromosome; **ppm**, part per million.

ecosystems (Elefsiniotis et al., 2007; Mustafa and Arikan, 2008). The aim of this study was to investigate the effects of Glyphosate herbicide on chromosomes aberration, mitotic index in the root meristem cells of *V. faba* (L.).

MATERIALS AND METHODS

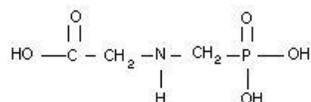
Cytogenetic parameters

Different concentration (0.1, 0.5, 0.75 and 1 ppm) of Phomac (glyphosate), a treated with a range of Glyphosate were used in the treatment of *V. faba* root tip, Healthy seeds of *V. faba* were selected and surface sterilized by dipping in 5% calcium hypochlorite solution for 5 min, washed with tap water for 10 min and soaked in tap water for about 12 to 24 h. The seeds were germinated between moist partite for 3 days at $24\pm 1^\circ\text{C}$. Seedling of *V. faba* (seed coat removed) with roots about 2 to 5 cm in length were cut carefully and fixed in fixative (ethanol:glacial acetic acid (3:1 part of 1N HCl for 1 min and squashed in camsa stain and 1N HCl (9:1) after intermittent heating for 3 to 5 min. After removing the root caps from well-stained root tips, they were immersed in a drop of 45% acetic acid on a clean slide, squashed under a cover slip with match stick and sealed with nail polish and examined microscopically (Olympus Cx21) and camera sony245. Ten root tip squashes were prepared for each treatment and a minimum 500 cells were examined for each concentration. Chromosomes aberrations in each treatment were recorded and the mitotic index was calculated using the method of (Mousa, 1982).

Treatment

The organic Herbicidal Glyphosate is the primary name of a weak organic acid that consists of a glycine moiety and a phosphonomethyl moiety. The determine Glyphosate is the primary name of a weak organic acid that consists of a glycine moiety and a phosphonomethyl moiety. The chemical name is N-(phosphonomethyl) glycine according to IUPAC nomenclature. The CAS name is glycine, N-(phosphonomethyl)-, and its CAS registry number is 1071-83-6. The empirical formula is $\text{C}_3\text{H}_8\text{NO}_5\text{P}$, and the structural formula is as follow;

- Chemical name is N-(phosphonomethyl) glycine according to IUPAC
- Nomenclature. The CAS name is glycine, N-(phosphonomethyl)-, and
- Its CAS registry number is 1071-83-6. The empirical formula is $\text{C}_3\text{H}_8\text{NO}_5\text{P}$ and the structural formula is as follows:



The effective concentration is 1 L of the Glyphosate to 200 L of water (Prog. Pest Cont. 2001 lasted.), that is 0.5 ml of the Herbicidal Glyphosate to 1000 ml of water (0.5 ppm). 0.1 ml of the Herbicidal Glyphosate to 1000 ml of water (0.1 ppm) 0.75 ml of the Herbicidal Glyphosate to 1000 ml of water (0.75 ppm)

Statistical analysis

Mitotic index (MI) was calculated by scoring dividing cells. The experimental data is presented as mean of triplicate experiments.

RESULTS AND DISCUSSION

In the present study, analyzing the control untreated roots it was revealed the normal feature of the chromosomes and also normal cell division behaviour with a mitotic index (MI). The mutagenic effects observed in cells treated with the herbicides included c-mitosis, stickiness, chromosomal breaks at anaphase and metaphase. These results are in agreement with Acepa root tip with different herbicides (Haliem, 1990; Mousa, 1982). Inhibition of mitotic activities was often used for tracing cytotoxic substances (Abderrhman, 1998). Herbicidal applied either to soil or crop plants are subjected to volatilization, leaching, which induce chromosomal aberration, chemical modification and microbial degradation, which have higher risk of bringing about environmental pollution (Pandey, 2007). According to Leme and Marin-Morales (2009), MI significantly lowers than the negative control can indicate alteration, deriving from the chemical action in the growth and development of exposed organisms. on the other hand , MI higher than the negative 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 (Campos et al., 2008).

In our study, the cytological investigation indicated that a significant decreasing of MI when Glyphosate (0.1, 0.5, 0.75 and 1 ppm). The numerous types of chromosomal aberrations were recorded in anaphase – telophase cells. The reduction of the mitotic index in our study can be explained by the arrest of the division of the interphasic nucleus, as well as by death of interphasic nucleus, hindering the onset of prophase and, thus, the division of the cells. In agreement of the second hypothesis, we observed various cells with cytoplasm shrinkage, nuclear condensation [Figures 1, 2 and 3; Table 1] (Elena et al., 2010).

These aspects were observed mainly in the concentration (0.1, 0.5, 0.75 and 1 ppm) of the Glyphosate treatment. Where the mitotic index showed a significant decrease in relation to the control (tip water), in the present may be due to the mito depressive action of chemicals indicating thereby the herbicide used

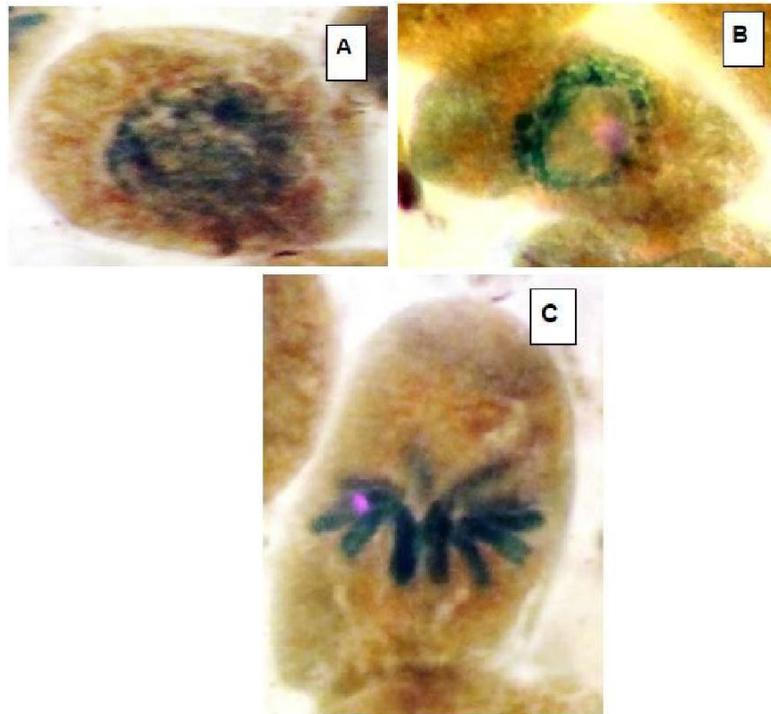


Figure 1. Control stage (A, B and C): (A , B) Prophase (C) Metaphase.

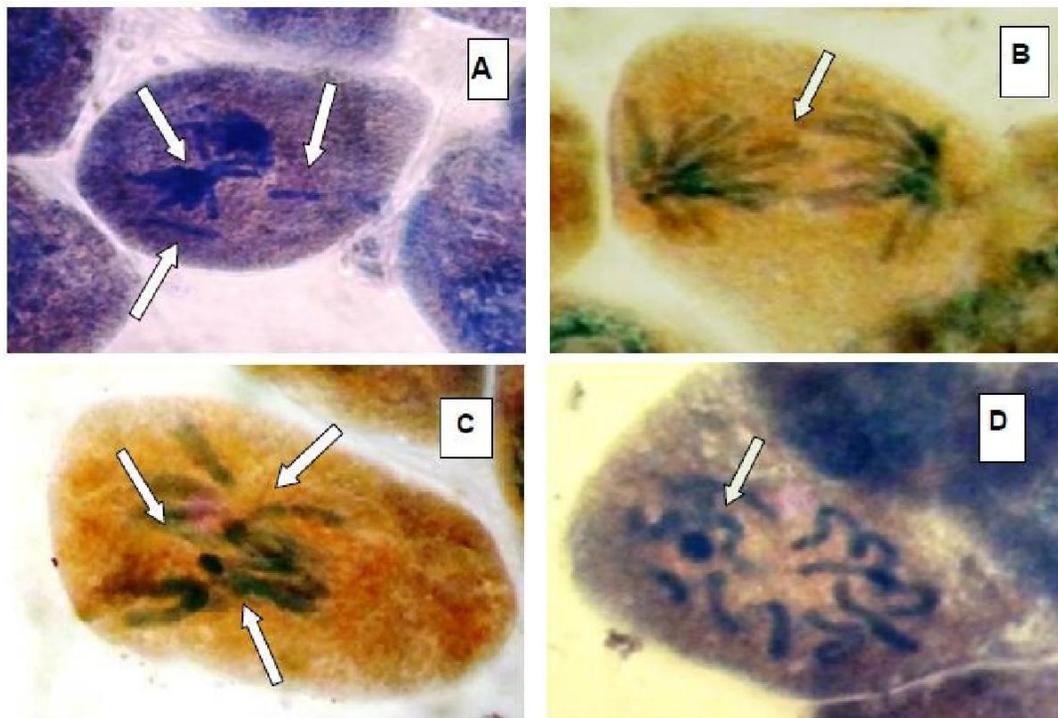


Figure 2. Chromosomes aberration observed in meristematic cells of *Vicia faba*. (A) Can be observed long cells disposed in lines , cells are plasmolytic , during prophase and in which an early chloroplasts formatting 600x. (B) Can be observed abnormal disposed cells without content mixed with plasmolytic cells (600x), (C) Anaphase with laggard chromosomes (arrow), (D) Stickiness metaphase with chromosomal breaks (arrow). (E) Can be observed micronuclei at metaphase (arrow),(I) C-mitosis and Micronuclei (arrow).

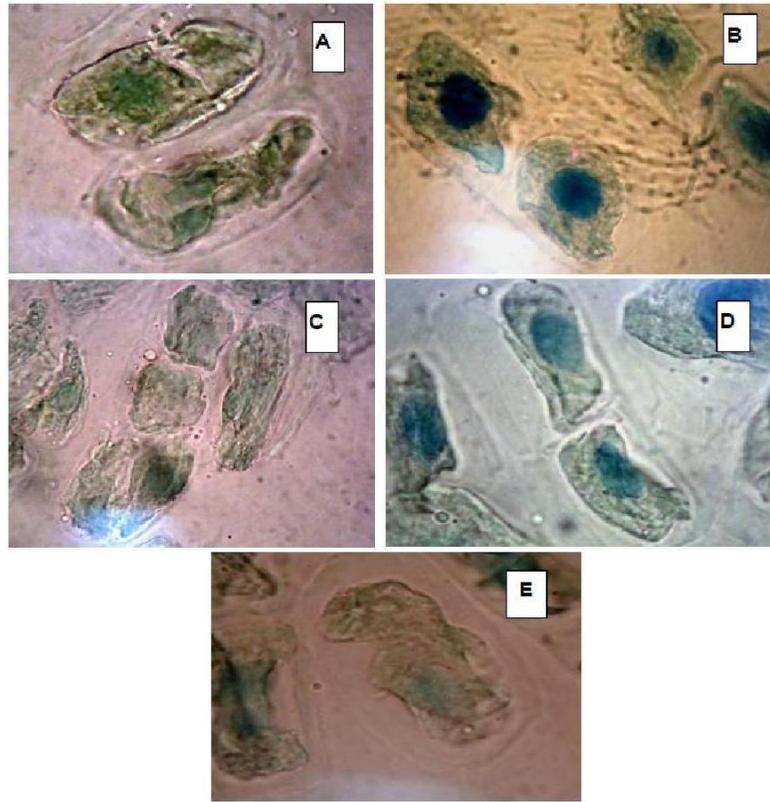


Figure 3A, B, C, D and E. Disturbed stages of mitosis in root tip cells in *Vicia faba* treated with Glyphosate for 24 h. It can be observed long cells deposited in lines; cells are strongly plasmolytic, during prophase and in which we can observed an early chloroplasts formatting (600x).

Table 1. Abnormalities chromosomes *in vitro* effects of different treatments of *Glyphosate on root tip of *Vicia faba* (L.), mitotic index and percentage.

Exposure	Concentration ppm	No. of cells examined	Mitotic index MI	Chromosomes aberration type			** %
				Disturbed Metaphase and anaphase mean	Broken Chromosomes mean	Stiky Chromosomes mean	
3h	Control/tip water	500	11.70	-	-	0.66	0.13
	0.1*	500	9.40	3.00	18	5.33	3.93
	0.5*	500	8.20	6.33	19	7.66	4.19
	0.75*	500	6.33	11.66	32	7.66	4.79
	1*	500	4.90	14.66	50	12.33	6.79
6h	Control/tip water	500	9.62	-	-	0.66	0.13
	0.1*	500	7.20	3.33	7.66	8.33	3.86
	0.5*	500	4.21	8.66	7.00	10.66	5.26
	0.75*	500	4.70	13.00	15.00	10.00	7.6
	1*	500	2.30	17.66	9.66	12.00	7.8
24h	Control/tip water	500	8.90	0.66	0.66	0.33	0.33
	0.1*	500	3.30	6.00	8.33	9.33	6.93
	0.5*	500	2.10	6.33	7.00	7.66	5.73
	0.75*	500	1.60	7.00	7.33	12.33	10.79
	1*	500	0.2	13.00	4.66	14.00	15.73

**Total No. of chromosomal aberration/Total No. of cells examined .

interfere in the normal cell cycle resulting in decrease in number of dividing cells. Similar results were observed in previous studies and cell death was the major depressor of the mitotic index (Campus et al., 2008; Sousa et al., 2010). Chromosomes aberration (CA), on the other hand are characterized by changes in either chromosomal structure or in the total number of chromosomes, which can occur both spontaneously and as a result from exposure to physical or chemical agents (Leme and Marin-Morales, 2009).

REFERENCES

- Abderrhman SM(1998).Cytogenetic effects of Peganum harmala extracts on Maize root tip. *Cytologia*, (63):283-291.
- Abraham S, John AT (1989). Clastogenic effects produced by black pepper in mitotic cells of *Vicia faba*. *Mut. Res.*, 224: 281-285.
- Badr A, Hamoud M, Haroun S (1985). Effects of herbicide Gespax on mitosis , mitotic chromosomes and nucleic acid in *Vicia faba* L. root meristems. *Proc. Saudi boil. Soc.*, (8):359-369.
- Campos JMS, Davide LC, Soares GLG, Vaccine LF (2008). Mutagenic effects due allelopathic action of fern(*Gleicheniaceae*) extracts. *Allelopath J.* 22: 143-152.
- Elefsiaioti I, Liatsos G, Stamelakis D, Moulakakiis (2007). Case report mixed cholestatic hepatocellular liver injury induced by the herbicide Quizalofop-p-ethyl. *Environmental Molicular*,115.1479-1481.
- Elena D, Maria M, Daciana S, Marioara T (2010). Cytogenetic effects induced by manganese and lead Microelements on germination at *Triticum aestivum* L. *Scine, Biology-Ecology Seri.*, (14),2010.
- Haliem,A. (1990).Cytological effects of the herbicide sencorer on mitosis of *A . cepa* Egypt . *J. Bot.* 33: 93-104.
- Jose M, David I, Viocini G (2008). Mitodepressive and clastogenic effects of aqueous extracts of the lichens *Myelocheoa lindmanii* and *Canoparmelia texana* (Lecanorales, Parmeliaceae) on Meristematic cells in plant bioassays. *Genet. Mol. Biol.*, 31(1);1-7.
- Leme DM, Marin-Morales MA (2009). *Alium cepa* test and environmental monitoring: A review on its application. *Mutat. Res.*, 682: 71-81.
- Lubini G, Fachinetto JM, Laughinghouse HD, Paranhos JT, Silva AC, Tedesco SB (2008). Extracts affecting mitotic division in root-tip meristematic cells. *Biologia* 63: 647-651.
- Marant F, Castellano A, Rosas F, Augier J, Barreira J (2003). Identification and quantification of allelochemicals from the lichen *Lethariella canariensis* . *Phytotoxicity and intoxicative activity .J.*, (29):2049-2074.
- Mekki LE (2008). The effect of three agricultural chemicals on mitotic division and seed protein banding profiles of *Vicia faba* . *Int. J. Agric. Biol.*, 10(5):499-504.
- Mousa M (1982). Mitoinhibition and chromosomal aberration induced by some herbicides in root tips of *A.cepa* ,*Egyptian J. Genet. Cytol.*, 11:193 – 207.
- Mustafa Y, Arikan E (2008). Genotoxicity testing of quizalofp-p-ethel herbicide using the *Allium cepa* anaphase – telophase chromosome aberration assay *Caryologia*, 61:45-51.,
- Pandey R (2007). Cytotoxic effects of pesticides in somatic oils of *Vicia faba* L. *Cytol Genet.*, 42,373-377.
- Patra M, Sharma A (2002). Relative efficacy of *Allium cepa* and *Allium sativa*umin anaphase telophase test screening metal genotoxiicity .*Biology*, 57:409- 414.
- Souza TS, Hencklein FA, Angelis DF, Goncalves RA, Fontanetti CS (2009). The *Allium cepa* bioassay to evaluate landfarming soil, before andafter the addition of rice hulls to accelerate organic pollutants biodegradation *Ecotoxicol. Environ. Saf.*, 72(5): pp. 1363-8.
- Srivastava K, Mishra K (2009). Cytogenetic effects of commercieally formulated atrazine on the somatic cells of *Allium cepa* and *Vicia faba* *Pesticide Biochemistry and Physiology* 93:8-12.
- Upadhyya MD, Tiwari SP, Chandra Ramesh (1986) Localized chromosome breakage inducedby sodium metabisulphide in *Vicia faba*. In: GK Manna and U Sinha (eds), “*Perspectives in Cytology and Genetics*. 5: 565-569.
- Vyvyan JR (2002). Allelochemicals as leads for new herbicides and agrochemicals, *Tetrahedron*, 58:1639-1646.