

EFFECT OF ENDOXAN AND ETHYLE METHANE SULPHONATE ON CHROMOSOMAL BEHAVIOUR AND MITOTIC INDEX IN SOMATIC CELLS OF ONION (*Allium cepa* ,L .)

El - Adl, A. M. ; Kawther S. Kash ; K. A. Zaied and Mervat I. Kamal

Dept. of Genetics , Fac. of Agric. , Mans. Univ. , Egypt.

ABSTRACT

This study aimed to investigate abnormal chromosomal behaviour in mitosis resulted from Ethyle methane sulphonate (EMS) and Endoxan . Commercial onion bulbs were used as a material of mitotic cell division . In addition , this study compared between the effect of mutagenic agent (EMS) with anticancer compound (Endoxan) on mitotic index . Onion bulbs were germinated under the effect of EMS and Endoxan , in addition to control treated with tap water . The differences between treatments and control were statistically evaluated . The results achieved that mitotic index of control had the largest number of normally divided cells about 15% , while Endoxan about 10% and EMS about 4% . This indicated that EMS had a strong effect of inhibiting mitosis than of Endoxan. In general , Endoxan and EMS reduced mitotic index than control in addition to several types of chromosomal aberrations . These chemical agents included sticky chromosomes , anaphase bridge chromosomes , lagging chromosomes , disrupted chromosome segregation and star cluster chromosomes . with variable percentage of each type. This reflects the dangerous of these chemical compounds on mitotic cell division .

Keywords: *Allium cepa* , L . chromosomal aberrations , Cyclophosphamide, cytogenetic effects, Ethyle methane sulphonate , mitotic index .

INTRODUCTION

Mitosis normally occurs as usual way of cell reproduction which yield two identical cells . When water is pure , mitosis takes place at normal fashion and continue to produce new normal cells. There are some chemical materials have been found to exert an effect on mitosis, this effect exerted on cell division not only inhibit mitotic index but also causing different kinds of chromosomal aberrations . These chromosomal aberrations would include serious anomalies such as : large chromosomal deletion or losing a hole chromosome , sticky chromosomes , anaphase bridge chromosomes , lagging chromosomes , disrupted chromosome segregation , star cluster chromosomes and clumped chromosomes in metaphaseetc.[Khan *et al* . 2009 and Green *et al* . 2012]. In many causes these damages changed the cells from normal cells to carcinogenic cells .

Unfortunately , there are a quite few of chemical materials which are used as medicine but they have a side effects which damage cells and changed them to carcinogenic ones . Carcinogenesis or oncogenesis or tumorigenesis is literally the creation of cancer. It is a process by which normal cells are transformed into cancer cells. It is characterized by a progression of changes on cellular and genetic level that ultimately reprogram

a cell to undergo uncontrolled cell division, thus forming a malignant mass. Cell division is a physiological process that occurs in almost all tissues and under many circumstances. Under normal circumstances, the balance between proliferation and programmed cell death, usually in the form of apoptosis, is maintained by tightly regulating both processes to ensure the integrity of organs and tissues. Mutations in DNA that lead to cancer (only certain mutations can lead to cancer and the majority of potential mutations will have no bearing) disrupt these orderly processes by disrupting the programming regulating the processes. Carcinogenesis is caused by this mutation of the genetic material of normal cells, which upsets the normal balance between proliferation and cell death. This resulted to uncontrolled cell division and the evolution of those cells by natural selection in the body. The uncontrolled and often rapid proliferation of cells can lead to benign tumors; some types of these may turn into malignant tumors (cancer). More than one mutation is necessary for carcinogenesis. In fact, a series of several mutations to certain classes of genes is usually required before a normal cell will transform into a cancer cell. Only mutations in those certain types of genes that play vital roles in cell division, apoptosis (cell death), and DNA repair will cause a cell to lose control of its cell proliferation. (see From Wikipedia, the free encyclopedia website). Ethyle methane sulphonate (EMS) is one of these mutagenic and carcinogenic agents . It was very closed to the nitrogen base guanine Swann(1990) .Thus , it could produce unknown mutations as a result of the random nucleotide substitution of guanine . The dangerous effect of EMS appeared in mitosis resulted from its interference with DNA replication which appears as different chromosomal aberrations. (Sultan and Celik . 2009) .

Endoxan , exerts its anti - cancer affect by a process called alkylation where it damages the DNA of cells then prevents them from dividing and therefore, cause them to die . Since cancer cells , in general , divide faster than healthy cells , cancer cells are more sensitive to this damage Svenja *et al.* (2006) .

This study aimed to identify mitotic index and chromosomal abnormalities caused by EMS as one of mutagenic agents compared with Endoxan as one of anti cancer compound using onion (*Allium cepa*, L .) roots meristem for bioassay .

MATERIALS AND METHODS

Materials :

Onion (*Allium cepa* , L .) roots were used for bioassay . Onion bulbs were purchased from the local market in Mansoura city through April 2010 to be used in this study. Root meristem raised in water were treated with three different concentrations of EMS and Endoxan .

About 28 cleaned onion bulbs were set up and allowed to produce roots in tap water for two days and the tap water was changed daly . In the second day , four bulbs were used as a control and the other 24 were transferred to be treated with Ethyl methane sulphonate (EMS) and Endoxan .

Mutagenic agents :

Ethyl methane sulfonate (EMS): The linear formula of EMS is $\text{CH}_3\text{SO}_3\text{C}_2\text{H}_5$

This formula was referred to .The free chemical database: (**ChemSpider ID: 5887**) .

Germinated bulbs were subjected to the following concentrations ; 200 , 300 and 400 ppm for two hours .

Cyclophosphamide (Endoxan) at the concentrations of 3.0 , 6.0 and 9.0 mg / ml

The linear formula of endoxan is $\text{C}_7\text{H}_{15}\text{Cl}_2\text{N}_2\text{O}_2\text{P}$

Fixation and storage solutions:

Root tips excised from treated and controlled materials were fixed in 1 : 3 acidic alcohol composed of a mixture of glacial acetic acid and ethanol respectively and then preserved in 70 % ethyle alcohol. (Iwegbue *et al* .2007)

Staining agent (orcein).

An orcein stain was prepared at the concentration of 2% by dissolving it in 45% acetic acid . Before adding the stain , root tips were put in Hcl for five minutes for loosening the tissue (Sehgal and Kumar 2006) .

Methods :

Root harvest and slide preparation

Onion bulbs were germinated at lab temperature using small bottles (100 ml) filled with enough tap water to top . Wait two or three days for root tips to grow .Bulbs subjected to treatments were transferred to each concentration of EMS and Endoxan after the length of the roots reached to 1-1.5 cm maximum . Roots were harvested at half past seven in the morning . Root tips excised from treated and controlled materials were fixed in 1: 3 acidic alcohol and preserved in 70% ethyle alcohol . Root tips squashed were conducted using 2 % orcein stain .

Mitotic index (MI) determination :

The slides were viewed under the light microscope (American microscope) using 100 x objective lens. On one slide for each treatment dividing cells (prophase , metaphase , anaphase and telophase) were counted to determine MI. MI was expressed as the number of dividing cells per 1000 cells scored (Ivanova *et al* . 2005) .

Chromosomal aberrations were characterized and classified in the following categories: large chromosomal deletion or loss of a chromosome , sticky chromosomes , anaphase bridge chromosomes , lagging chromosomes , disrupted chromosome segregation , star cluster chromosomes , clumped chromosomes in metaphaseetc. (Inceer *et al* . 2000) . These aberrations were saved in photographic pictures .

Statistical analysis :

The data were subjected to statistical analysis of variance using the system of SAS (2004) to comparing the means based on the value of least significant differences (LSD) at 0.05 and 0.01 levels of probability.

RESULTS AND DISCUSSION

Mitotic Index:

Means of mitotic index (MI %) resulted by Endoxan and EMS are shown in Table 1. The means of mitotic index at three levels of Endoxan were close to each other and the same trend was also obtained by EMS. These results appeared that the differences between different levels of each agent were insignificant.

The means of dividing cells treated with Endoxan were significantly higher than that of EMS. This indicated that Endoxan did not interfere with mitosis and did not prevent cell division if compared with EMS which decreased the mitotic index and interfered with mitosis to greater extent.

Therefore, it can be concluded that EMS was more inhibitor of cell division than Endoxan. This may be due to more damage resulted by EMS affected on DNA replication during mitosis. This agreed with Hassan and Ahmad (2000), who found that although all types of chemical mutagens were effective for the induction of chromosomal aberrations, but chromosomal aberrations were increased due to the effect of EMS in comparison to the other chemical mutagens.

Table 1 . Effect of EMS and Endoxan on mitotic index and chromosomal aberrations in the root meristem cells of *Allium cepa*, L .

Compound	Concentration	Total cells	Dividing cells	MI%	Disrupted chromosome	Sticky chromosome	Bridge chromosome	Lagging chromosome	Star chromosome	% aberrant cells
EMS	200ppm	2512	117	4.66	38	24	0	0	0	2.47
	300ppm	1930	75	3.89	25	17	1	0	2	2.33
	400ppm	1750	66	3.77	24	2	22	1	3	2.97
Endoxan	3mg/ml	2554	258	10.10	13	0	7	0	8	1.10
	6mg/ml	2740	268	9.78	14	0	7	0	8	1.06
	9mg/ml	2889	279	9.66	14	0	8	0	8	1.04

The results appeared that magnitude of abnormal divided cells resulted from EMS were higher than that resulted from Endoxan. This indicated that EMS was dangerous than Endoxan via increasing the rate of abnormal divided cells and reducing the magnitude of dividing cells. Most of chromosomal aberrations resulted by endoxan were; disrupted chromosome, bridge chromosome and star chromosome. The percentage of these aberrations were: 1.10, 1.06, 1.04% at three levels; 3,6 and 9 mg / ml of Endoxan. Therefore it would be indicated that most chromosomal damage was caused by EMS than endoxan. The percentage of these aberrations resulted from EMS were: 2.47, 2.33, 2.97% at the following three levels 200, 300 and 400 ppm. The results agreed with Hahn and Kim (1979), who found that four alkylating agents (methyl methane sulphonate, ethyle methane sulphonate, dim-ethyl nitrosamine and diethyl nitro-samine), under various concentrations on mouse bone marrow erythrocytes, using

micronucleus test ,which were compared and discussed with respect to micronucleus production from chromosomal aberrations .

Table 2: Mitotic index in onion root tip cells treated with three concentrations of EMS and Endoxan .

Compound	Concentration	Interphase	Cells in division stages scored				Total	MI%	MI as % of control
			Prophase	Metaphase	Anaphase	Telophase			
H ₂ O	2616	219	50	51	80	400	132.62	100
EMS	200ppm	2395	31	14	6	4	55	21.89	16.50
	300ppm	1855	18	8	3	1	30	15.54	11.72
	400ppm	1684	9	6	0	0	15	8.57	6.46
Endoxan	3mg/ml	2296	126	32	27	45	230	90.05	67.90
	6mg/ml	2472	130	33	28	48	239	87.22	65.76
	9mg/ml	2610	135	34	29	51	249	86.18	64.98

Where : MI = Mitotic index (number of cells in division stages out of 1000 cells) .

It is very importance to determine the percentage of dividing cells with respect to control divided cells (Table 2) . The estimates showed the effect of different levels of the both chemical agents on cell division with respect to the control .

These results indicated that Endoxan exerted an effect on cell division via reducing the normal divided cells than the control .

EMS showed higher reduction in mitotic index with respect to the control. The results appeared that the increase of EMS dose decreased cell division. This indicated a dose – response . The results agreed with Kayraldiz *et al* . (2001) , who found that sodium metabisulfite (SMB) significantly decreased mitotic index (MI) at all concentrations and both treatment periods. Sultan and Celik (2006) found that lycopene reduced mitotic index (MI) in treatment groups in comparison with controls.

2. Types and magnitudes of chromosomal aberrations resulted by Endoxan and EMS.

It was indicated earlier that both endoxan and EMS showed variable percentage of abnormal dividing cells (Dube *et al* . 2011) . This abnormality varied from Endoxan to EMS and also varied among the levels of both mutagenic agents (Maraj and Ali 2011) . The percentage of abnormal divided cells are presented in Table 3 with respect to total number of divided cells in the control. The abnormal divided cells at mitosis would include different types of chromosomal aberrations . These chromosomal aberrations including : disrupted chromosome , sticky chromosome , bridge chromosome , lagging chromosome and star chromosome . Number of abnormal dividing cells with respect to the total number of divided cells indicated that Endoxan showed less effect than EMS. Endoxan induced about 10% of abnormal dividing cells with respect to the total number of divided cells . On the other hand , EMS induced from 50 to 70 percentage of abnormal divided cells with respect to total number of divided cells . This indicated that EMS caused a great damage during mitosis than Endoxan and most of the dividing cells were abnormal .

Table 3 : Mitotic index of abnormal cells in relation to divided cells .

Compound	Concentration	Divided cells		Mitotic index %		Abnormal % per divided cells A / N+ A
		Normal	Abnormal	Normal	Abnormal	
EMS	200ppm	55	62	2.19	2.47	52.99
	300ppm	30	45	1.55	2.33	60.00
	400ppm	15	51	0.86	2.91	77.27
Endoxan	3mg/ml	230	28	9.00	1.09	10.85
	6mg/ml	239	29	8.72	1.06	10.82
	9mg/ml	249	30	8.62	1.04	10.75

B. Chromosomal aberrations induced by Endoxan and E.M.S.

It has been indicated earlier that both Endoxan and E.M.S. caused several types of chromosomal aberrations . These aberrations were recorded in Figure 1.

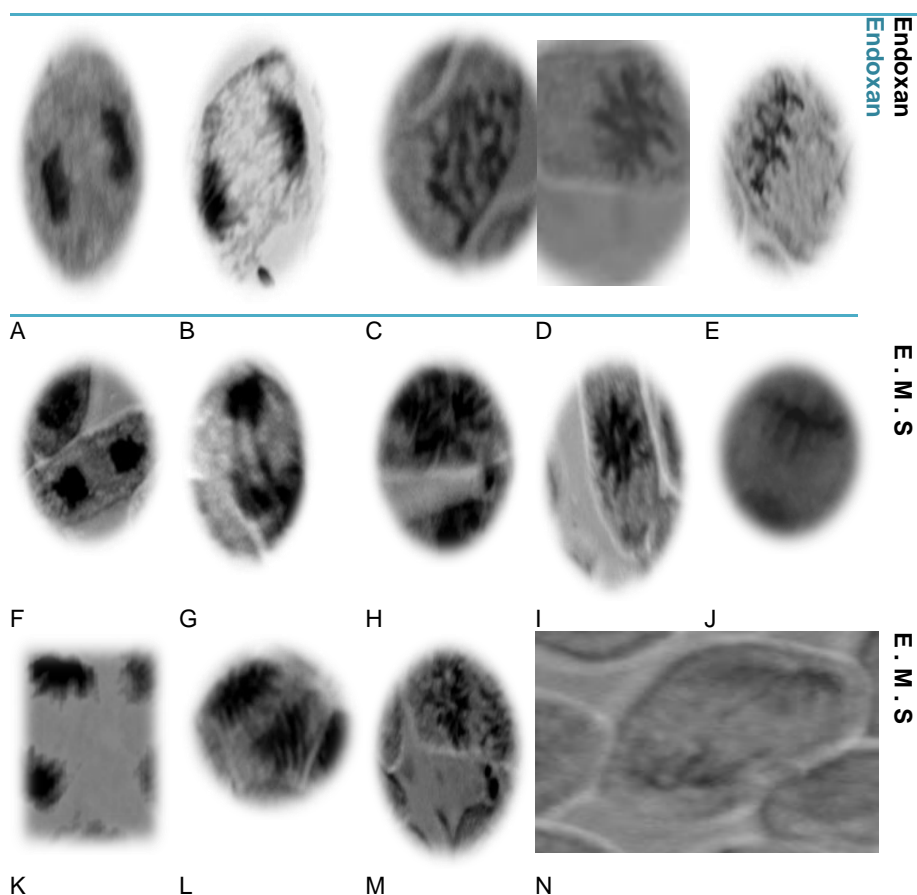


Figure 1: Different chromosomal aberration that resulted in mitotic division of onion root tips due to the effect of Endoxan and EMS.

The figure shows the different chromosomal aberration as follows :

A,F - Telophase sticky chr B,G - Telophase bridge chr C,H- Metaphase disrupted chr

D,I- Metaphase star chr E,J - Metaphase sticky chr k- Anaphase sticky chr

L- Anaphase bridge chromosomes M- Anaphase disrupted chr N-

Anaphase lagging chr Both Endoxan and EMS caused the similar trend in chromosomal aberrations with different ratio and different appearance .

Endoxan and EMS showed disrupted type of chromosomal aberrations which appeared during metaphase stage. It appeared that disrupted metaphase varied from Endoxan to EMS. In addition , EMS caused disrupted chromosomes in anaphase which did not occur with Endoxan . This agreed with Grundmann (1979) , who found that the normal course of mitosis may be disrupted by various pathological processes . In addition , Gisselsson *et al* . (2002) found that chromatin bridges may be implied as a diagnostic marker for cancer.

Both Endoxan and EMS caused abnormal mitosis which appeared as sticky chromosomes. Endoxan caused sticky chromosomes in during metaphase and telophase . Similarly, EMS showed sticky chromosomes during metaphase , anaphase and telophase. These results indicated that EMS had strongest effect on chromosomal behaviour during mitosis and exerted more chromosomal damage . Indeed , sticky chromosomes would caused the death of those cells. Similar results were obtained by authors among them: Gaulden, mary (1989) who found that chromosomal aberrations caused by the physical stretching and breaking of chromatids at the sticky sites .

A chromatid bridge would occur as a result of the weakness of the spindle fiber . Bridge as an aberration occurs due to treatment by both EMS and Endoxan.

The results agreed with Abo El Khier and Abo El Khier (1992) , who . Investigated the effects of harmol and harmine alkaloids extracted from the medicinal plant peganum harmala L. on the root tips of *Allium cepa* L. and found that alkaloids used caused an increase of mitotic index in *Allium cepa* roots and induced high percentage of abnormalities such as : abnormal prophase , sticky chromosome , scattered chromosome , lagging chromosome and disturbed anaphase .

During abnormal chromosomal behavior of mitosis , spindle fiber can not to attract one chromosome , this chromosome remains near the middle of the cells . This phenomenn called lagging chromosome and resulted genome aneuploidy $2n-1$. This kind of aberration did not occur by Endoxan treatment as appeared in this study . This agreed with Aydemir *et al* . (2008) , 4, 6-Dinitro-o-cresol (DNOC) on the root tips of *Allium cepa* L. , who found that Large number of c-mitosis in root tips of *Allium cepa* L. as a result of treatment by 4 , 6 – nitro – o – cresol(DNOC) which strongly inhibitor spindle fibers whereas, other types of chromosomal aberrations types such as, breaks, bridges etc were shown .

Among the chromosomal aberrations caused by Endoxan or EMS. the formation of star type of chromosomes was shown . Both Endoxan and EMS caused this type of aberration .

In conclusion , the treatments by endoxan and EMS caused different types of chromosomal aberrations with variable percentages than the normal cells in control experiment the same time there were differences of the percentage ratio of each . This indicated that both chemical agents are dangerous . Although , EMS was more dangerous than Endoxan because of cytotoxicity delaying mitosis and inducing mass chromosomal aberrations .

REFERENCES

- Abo El Khier , zakia A . and Gihan M . Abo El Khier (1992) . Cytological Effects of Certain Active Constituents of Peganum harmol and Harmine Alkaloids on Mitosis of *Allium cepa* , L . J.King Saud Univ ., 4, Science (1) : 37- 45.
- Aydemir . N; S . elikler ; S. Summak and D . Yilmaz (2008) . Evaluation of Clastogenicity of 4, 6-Dinitro-*o*-cresol (DNOC) in *Allium* Root Tip Test. J. Biol . Environ. Sci ., 2(5) : 59-63.
- Dube, K.G ; A. S. Bajaj and A.M. Gawand (2011) . Effect of gamma rays and ethyl methane sulphonate in alone and combination treatments on mitotic chromosomal aberrations in *Cyamopsis tetragonoloba* (L.) Var. sharada . Asiatic Journal of Biotechnology Resources; 2 (5) : 577-581.
- Gaulden , mary E. (1989) . Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. *Mutagenesis* 2 (5): 357-365.
- Gisselsson , D . ; T. Jonson ; C . Martins ; N. Mandahl ; J . Weigant ; J. Y. Jin ; F. Mertens and C . Jin (2002) . Centrosomal abnormalities, multipolar mitoses, and chromosomal instability in head and neck tumours with dysfunctional telomeres. *British Journal of Cancer* , 87(2): 202–207.
- Green , lydia C .; P. Kalitsis ; M . Cipetic ; J. H . Kim ; O. Marshall and D. F. Hudson (2012). Contrasting roles of condensin I and condensin II in mitotic chromosome formation . *Cell Sci* .,125 : 1591-1604. doi: 10.1242/jcs.097790.
- Grundmann E .(1979) In *Handbuch der allgemeinen Pathologi c* . vol. 2, part 1. Edited by H. Altman. Berlin-Heidelberg-New York. Pages: 282–479.
- Hahn , S . and D.S.Kim (1979) . The production of micronuclei from chromosome aberration by chemical carcinogens in mice . Yonsei medical journal , 20(2):105-112.
- Hassan , L . and S.D. Ahmad (2000) . Chromosomal Aberrations Induced by Chemical Mutagens in *Allium* . Pakistan Journal of Biological Sciences 3 : 1186 – 1189 .
- Inceer, H .; O. Beyazoglu and H. A. Ergul (2000) . Cytogenetic Effects of Wastes of Copper Mine on Root Tip Cells of *Allium cepa* L. Pakistan

- Journal of Biological Sciences , 3(3) : 376- 377.
- Ivanova , E .; T. A. Staikova and I . Velcheva (2005) . Cytogenetic testing of heavy metal and cyanide contaminated river waters in a mining region of Southwest Bulgaria. Journal of Cell and Molecular Biology , 4: 99-106 .
- Iwegbue , C.M.A .; F.O .Arimoro and G.E . Nwajei , (2007) . The Genotoxicity Screening of Simulated Leachate from Semi- urban Waste Dumps in the Niger Delta , Nigeria Analysed by Allium Test . Research Journal of Environmental Sciences, 1(6) : 310-316.
- Kayraldiz , A .; H. B . Ila and T. Cakmak (2001) . The Cytogenetic Effects of Sodium Metabisulfite, a Food Preservative in Root Tip Cells of *Allium cepa* L. Turk J . Biol ., 25 : 361-370.
- Khan , Z .; H. Gupta ; M . Ansari and S. Chaudhary (2009) . Methyl methanesulphonate induced chromosomal variations in a medicinal plant *Cichorium intybus* L. during microsporogenesis. *Biology and Medicine*, 1 (2): 66-69.
- Maraj-ud-din and Niamat Ali (2011) . Genotoxic effect of ethyl methane sulphonate (EMS) in *Rattus norvegicus* . Volume 6, Issue 2, January – February ; Article-013.
- Sehgal , R .S. R. and V. L. Kumar (2006) . Evaluation of cytotoxic potential of latex of *Calotropis procera* and Podophyllotoxin in *Allium cepa* root model. Biocell , 30(1): 9-13.
- Sultan , A . O. and T. A . Celik (2006) . Protective Effect of Lycopene on Ethyl Methane Sulfonate induced Chromosome Aberrations in *Allium cepa*. Caryologia , 59 (3) : 220-225.
- Sultan , A . O. and T. A . Celik (2009). Genotoxic and Antimutagenic Effects of *Capparis spinosa* L. on the *Allium cepa* L. Root Tip Meristem Cells, Caryologia , 62 (2) : 114-123.
- Swann P. F (1990). Why do O6-alkylguanine and O4-alkylthymine miscode? The relationship between the structure of DNA containing O6-alkylguanine and O4-alkylthymine and the mutagenic properties of these bases. Mutat Res, 233(1-2):81-94.
- Svenja K. B .; Kerstin . R. ; Kirsten . B . and Wagner T . (2006) . Chloroacetaldehyde: mode of antitumor action of the ifosfamide metabolite Cancer Chemotherapy and Pharmacology March 2006, 57 (3) : 349-356.
- (see From Wikipedia, the free encyclopedia website).
 - The free chemical database : (Chem Spider ID: 5887)

تأثير الإندوكسان والإيثايل ميثان سلفونيت على السلوك الكروموسومي ومعدل
الإنقسام الميتوزي في الخلايا الجسمية للبصل
على ماهر العدل ، كوثر سعد قش ، خليفه عبد المقصود زايد و ميرفت إبراهيم كمال
قسم الوراثة- كلية الزراعة – جامعه المنصورة .

يهدف هذا البحث إلى دراسة السلوك الميتوزي الشاذ الناتج عن المعاملة
بالإيثايل ميثان سلفونيت كمادة مطفرة مقارنة بذات التأثير الناتج عن المعاملة بالإندوكسان
كمادة مثبطة للسرطان وذلك على مستنبتات البصل التجارى كمادة تجريبية لإختبار أثر
المركبين على معدل الإنقسام الميتوزي للخلايا ، حيث تم إنبات جذور البصل تحت تأثير
تركيزات مختلفة من الإيثايل ميثان سلفونيت والإندوكسان ومقارنتها بالإنبات فى الماء
العادى ككنترول . أوضحت النتائج وجود فروق معنويه بين المعاملات والكنترول ، وقد
تأثر معدل الإنقسام الميتوزي حيث كان أعلى معدل للخلايا المنقسمه فى حالة الإنبات بالماء
العادى حوالى ١٥% بينما كان فى حالة المعامله بالإندوكسان ١٠% وفى حالة المعامله
بالإيثايل ميثان سلفونيت ٤% مما يدل على أن الإيثايل ميثان سلفونيت أقوى تثبيطاً لنشاط
الإنقسام الميتوزي ، وعلى ذلك فإن الإندوكسان والإيثايل ميثان سلفونيت لهما تأثير مثبط
لنشاط الإنقسام الميتوزي مقارنة بالكنترول ، هذا بالإضافة إلى مقدرتهم على إستحداث أنواع
متعدده من الشذوذات الكروموسوميه مثل : البعثرة الكروموسومية ، الجسور الكروموسومية
، التلكأ الكروموسومى ، اللزوجه الكروموسوميه والكروموسومات النجمية بنسب متفاوتة ،
الأمر الذى يعكس مدى خطورة هذه المركبات على الخلايا الجسمية المنقسمة ميتوزياً .

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
مركز البحوث الزراعية

أ.د / اشرف حسين عبد الهادى
أ.د / محمد محمد عبد الرحمن