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CYTOLOGICAL STUDIES ON THE EFFECT OF THE HERBICIDE "DUAL"* ON *ALLIUM CEPA*

By

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ABSTRACT

Dual is widely used in Egypt as an active herbicide against annual grass weeds in soybean, sunflower, sugar beet and maize. Cytological studies have been carried out to show the effect of the herbicide on cell division in the meristematic cells. Roots tips of *Allium cepa* were treated with a series of concentrations, ranging from 1000×10^{-6} ml/ml to 50×10^{-6} ml/ml for 4,12, 24, 48 and 72 hours. Dual induced a wide range of mitotic abnormalities. Their frequencies were found to depend on the concentration of the herbicide and duration of treatment. The most conspicuous effect in the metaphase was the formation of c-metaphase and non-congression.

A number of abnormalities were found at both anaphase and telophase, where a large percentage of c-anaphase, unoriented chromosomes, lagging chromosomes and chromosome and chromatin bridges were induced. Dual showed an ability to induce chromosome breakage at both metaphase and anaphase. Micronuclei and multinucleate cells were seen at interphase cells. The present results indicate the hazardous effect of the herbicide "Dual" on the environment, especially on the hereditary machinery of plants.

*Dual is a trade name for a herbicide produced by CIBA-GEIGY. It contains acetanilide as an active ingredient, and its chemical formula is 2-ethyl-6-methyl-N-(2-methoxy-1-methyl-ethyl-chloro-acetanilide).

INTRODUCTION

The application of different pesticides is widely utilized by man. The use of such pesticides could overcome a substantial loss in agricultural crop yields. However, a large number of investigations have shown the dangerous effect of these pesticides on hereditary material, for example, in inducing chromosomal aberration on both mitotic and meiotic divisions (Grover and Tyagi, 1980; Najjar and Soliman, 1982; Reddy and Rao, 1982; Soriano, 1984 and Pandita, 1986). Many of these pesticides have exhibited their mutagenic effect and could also be cancerous (Barthel, 1976; Hardell, 1979).

The aim of the present study is to investigate the cytological effects of the herbicide "Dual" on the process of mitosis in root tips of *Allium cepa*. Dual contains acetanilide as an active ingredient.

MATERIALS AND METHODS

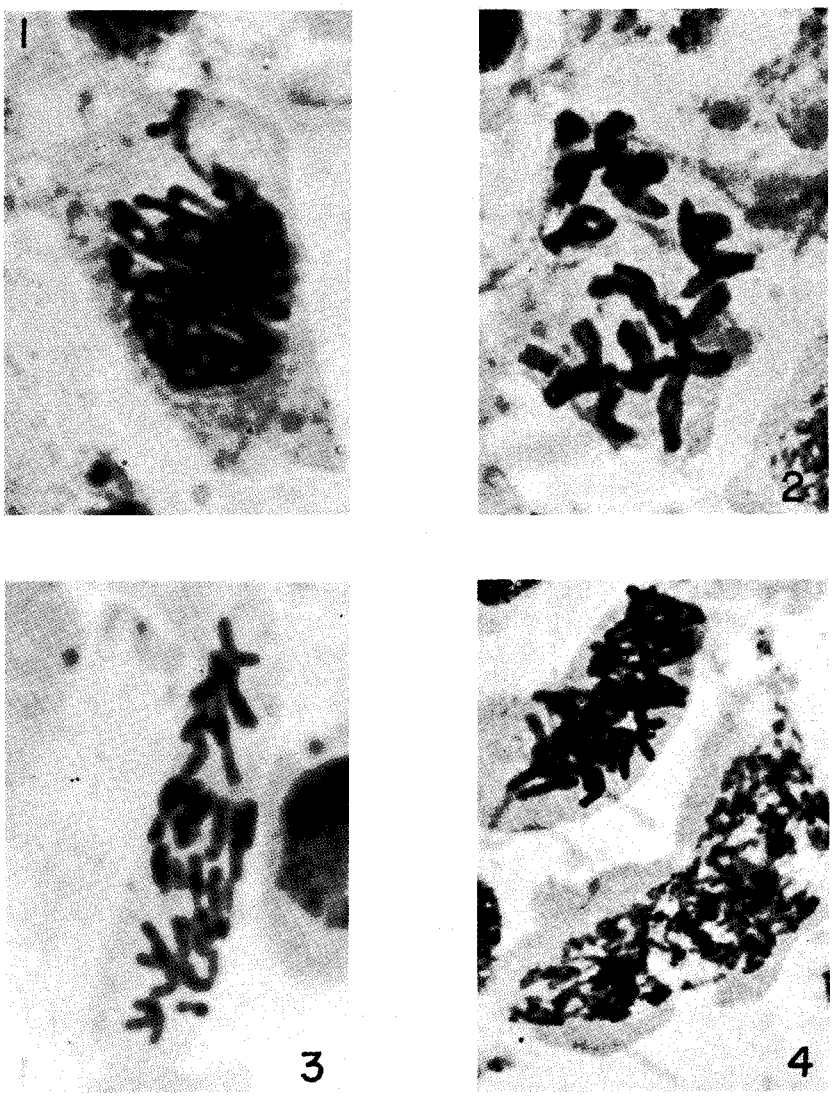
Pure strains of bulbs of *Allium cepa* (variety Giza-6) were used as plant test material. Roots, while intact, were treated with different concentrations of the Dual herbicide; ranging from 1000 to 50×10^{-6} ml/ml for 4, 12, 24, 48 and 72 hours.

After treatment, the roots were fixed in Carnoy (3 parts absolute ethyl alcohol and 1 part acetic acid glacial) for 24 hours. Cytological investigations were carried out from permanent slides prepared by the Feulgen squash technique (Darlington & La Cour, 1976).

RESULTS

I. Mitotic abnormalities induced by Dual

Dual was found to induce a wide range of mitotic abnormalities in meristematic cells of *Allium cepa* root tips. Their frequency was found to depend on the concentration of the chemical and the duration of treatment. At prophase stage, the chromosomes appeared irregular (Fig. 1). A high percentage of cells showed scattered chromosomes in the cytoplasm giving the c-metaphase (Fig. 2). C-anaphase resulted as a consequence of the c-metaphase as seen in Fig. 3. On reconstitution this led to the production of polyploid cells (Fig. 4). Lagging chromosomes (Fig. 5) as well as chromatid and chromosome bridges (Fig. 6) were also well noticed during anaphase and telophase. Moreover, Dual showed its ability to cause chromosome or chromatid breaks at different stages of mitosis (Figs. 7 & 8). Micronuclei, multinucleate and polyploid cells were of common occurence at interphase, due to the effect of Dual (Figs. 9 & 10).



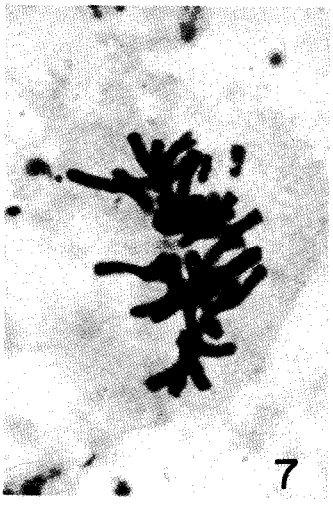
Figs 1 - 4, Mitotic abnormalities induced by "Dual" in root tips of *Allium cepa.*

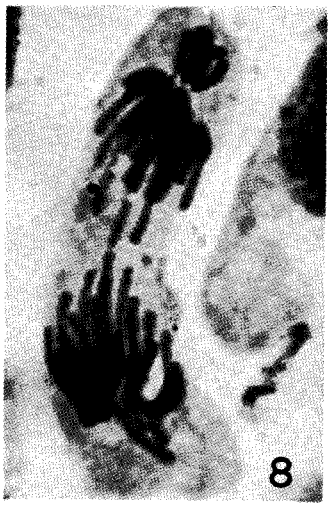
1 - Irregular appearance of prophase chromosomes after treatment with 10×10^{-6} ml/ml for 72 hours.

2. metaphase after treatment with 40×10^{-6} ml/ml for 24 hours.

3. C-anaphase after treatment with 80×10^{-6} ml/ml for 12 hours.

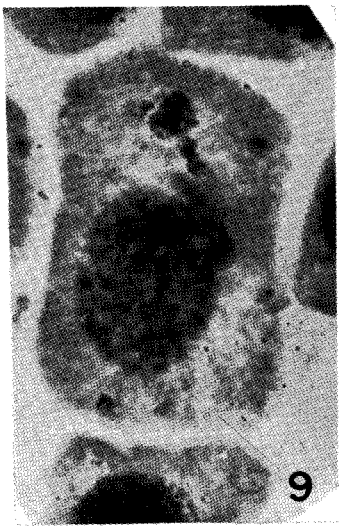
4. Polyploid cells after treatment with 30×10^{-6} ml/ml for 72 hours.

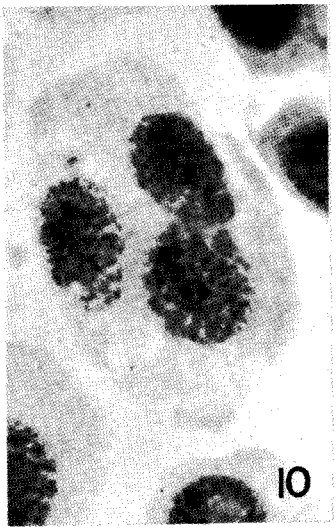




Figs 5 - 10, Mitotic abnormalities induced by "Dual" in root tips of *Allium cepa*.

5. Lagging chromosomes at anaphase stage after treatment with 30×10^{-6} ml/ml for 24 hours.
6. Chromosome bridges at anaphase stage after treatment with 10×10^{-6} ml/ml for 72 hours.
7. Chromosome breaks at metaphase stage after treatment with 5×10^{-6} ml/ml for 24 hours.
8. Chromosome breaks at anaphase stage after treatment with 30×10^{-6} ml/ml for 72 hours.





9. Micronuclei at interphase stage after treatment with 80×10^{-6} ml/ml for 48 hours.

10. Multinucleate cell at interphase stage after treatment with 80×10^{-6} ml/ml for 72 hours.

The total abnormal metaphase and anaphase appeared to increase with the increase in concentration and the duration of treatment (Figs. 11, 12, 13, 14 & 15).

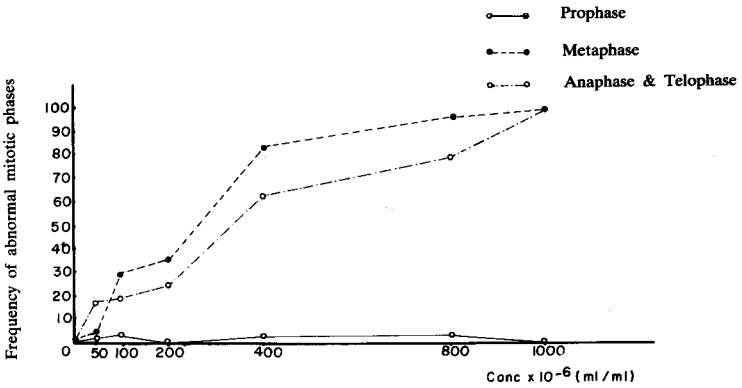


Fig. 11: Frequency of abnormal mitotic phases after treating *Allium cepa* root tips with different concentrations for 4 hours.

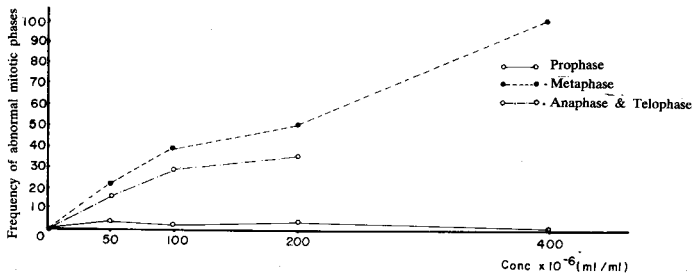


Fig. 12: Frequency of abnormal mitotic phases after treating *Allium cepa* root tips with different concentrations for 12 hours.

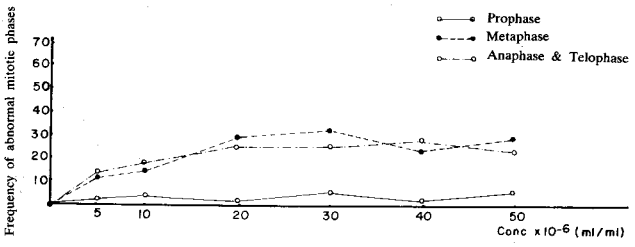


Fig. 13: Frequency of abnormal mitotic phases after treating *Allium cepa* root tips with different concentrations for 24 hours.

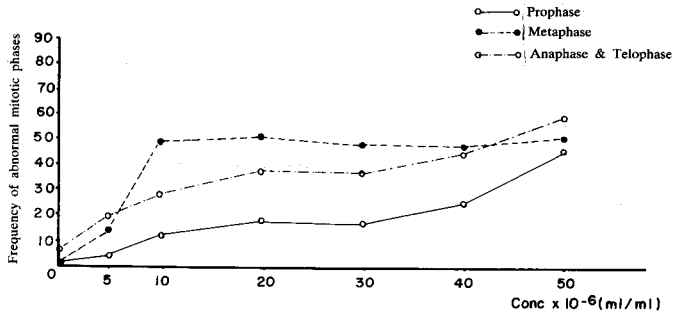


Fig. 14: Frequency of abnormal mitotic phases after treating *Allium cepa* root tips with different concentrations for 48 hours.

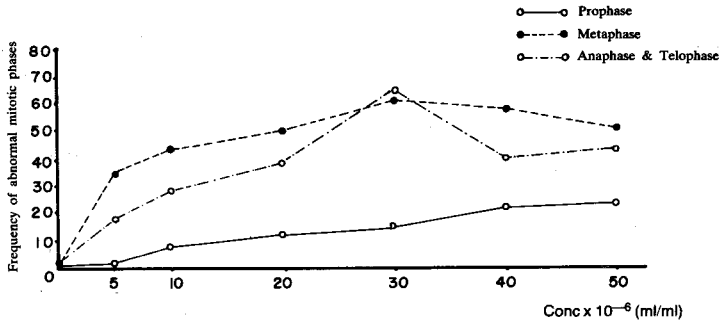


Fig. 15: Frequency of abnormal mitotic phases after treating *Allium cepa* root tips with different concentrations for 72 hours.

II. Effect on the frequencies of mitotic stages

The frequency of the different mitotic stages varied according to the concentration applied (Tables 1 & 2). The frequency of prophase fluctuated from that of the control.

Metaphase, on the other hand, increased in roots treated for a longer duration than that of the control. Generally, an inverse correlation appears to exist between the frequency of prophase and that of metaphase. It reached its lower value in roots treated with high concentrations.

III. Effect on mitotic index

With all the treatments used in the present study, Dual reduced the mitotic index in *Allium cepa* roots. However, there was a slight increase in roots treated with a low concentration after 48 and 72 hours (Figs. 16, 17, 18, 19 & 20).

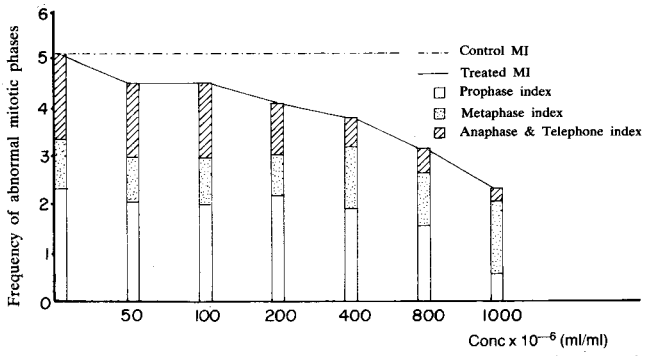


Fig. 16: Changes in the mitotic stages indices (M.S.I.) and mitotic index (M.I.) treating *Allium cepa* root tips with different concentrations of dual for 4 hours.

Table 1

Mitotic Index (M.I.) and percentage of mitotic phases after treating *Allium cepa* root tips with different concentrations of "Dual" for 4 hours, 12 hours and 24 hours.

Treatment period	Conc $x10^{-6}$ (ml/ml)	Total cells examined	No of inter-phase	Total mitosis	Prophase %	Metaphase %	Ana & Telophase %	M.I %
4 hrs	1000	10252	10010	242	23.14	69.42	7.44	2.36
	800	12326	11942	384	49.48	34.90	15.68	3.12
	400	11276	10846	430	50.70	35.35	13.95	3.81
	200	10782	10338	444	53.60	20.72	25.68	4.12
	100	9646	9205	441	43.08	21.32	35.60	4.57
	50	9308	8886	422	51.18	19.43	29.38	4.53
	0.00	11998	11380	618	45.95	20.71	33.34	5.15
12 hrs	400	8608	8596	12	0.00	100.00	0.00	0.14
	200	11072	10780	292	32.53	31.85	35.62	2.64
	100	9612	9327	285	36.84	23.86	39.30	2.97
	50	8826	8388	438	51.60	20.55	27.85	4.96
	0.00	9829	9261	568	48.59	19.72	31.69	5.78
24 hrs	50	7841	7546	295	49.10	21.07	29.83	3.76
	40	9276	8961	315	56.20	20.63	23.17	3.40
	30	8989	9671	298	52.01	24.50	23.49	3.32
	20	8623	8224	399	47.12	23.06	29.82	4.63
	10	7119	6813	306	44.12	24.18	31.70	4.30
	5	9378	8988	390	45.38	20.00	34.62	4.16
	0.00	7212	6840	372	50.54	21.51	27.95	5.16

Table 2

Mitotic Index (M.I.) and percentage of mitotic phases after treating *Allium cepa* root tips with different concentrations of "Dual" for 48 hours and 72 hours.

Treatment period	Conc $x10^{-6}$ (ml/ml)	Total cells examined	No of inter-phase	Total mitosis	Prophase %	Metaphase %	Ana & Telophase %	M.I %
	50	6420	6122	298	47.99	28.19	23.82	4.64
	40	7763	7417	346	45.66	24.86	29.48	4.46
	30	8825	8443	382	43.98	23.82	32.20	4.33
48 hrs	20	7836	7401	435	48.28	20.23	31.49	5.55
	10	9047	8602	445	46.74	23.60	29.66	4.92
	5	7190	6657	533	51.22	18.20	30.58	7.41
	0.00	9124	8640	484	49.59	19.63	30.78	5.30
	50	8848	8480	368	43.48	28.80	27.72	4.16
	40	9972	9662	310	51.61	26.77	21.61	3.11
	30	8242	7861	381	43.04	25.20	31.76	4.62
72 hrs	20	7786	7281	505	48.51	25.15	26.34	6.49
	10	9174	8607	567	48.85	21.87	29.28	6.18
	5	7890	7299	591	48.22	19.63	32.15	7.49
	0.00	8475	8007	468	50.21	19.23	30.56	5.52

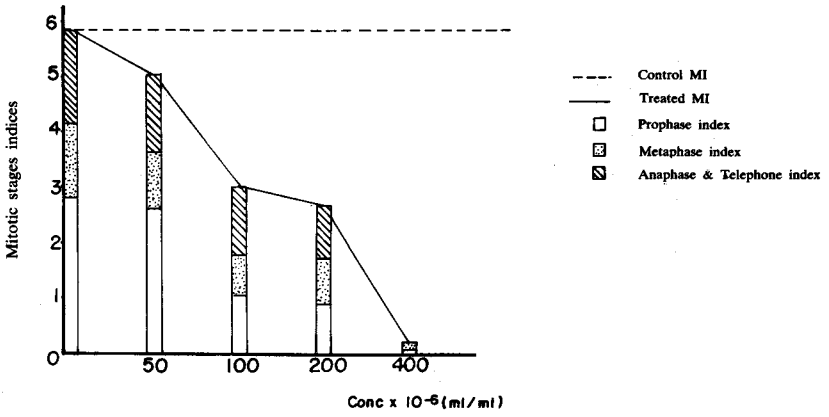


Fig. 17: Changes in the mitotic stages indices (M.S.I.) and mitotic index (M.I.) treating *Allium cepa* root tips with different concentrations of dual for 12 hours.

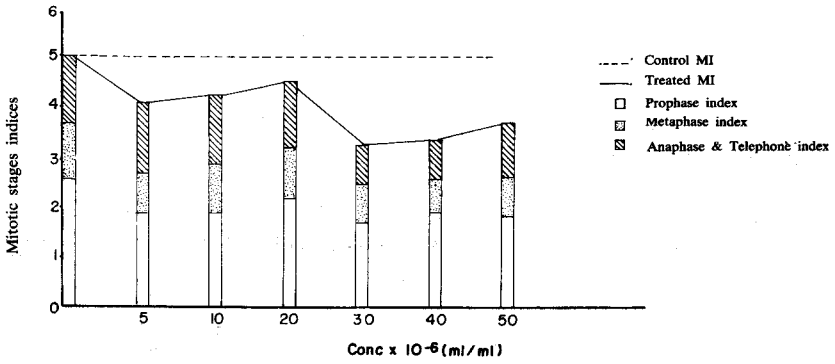


Fig. 18: Changes in the mitotic stages indices (M.S.I.) and mitotic index (M.I.) treating *Allium cepa* root tips with different concentrations of dual for 24 hours.

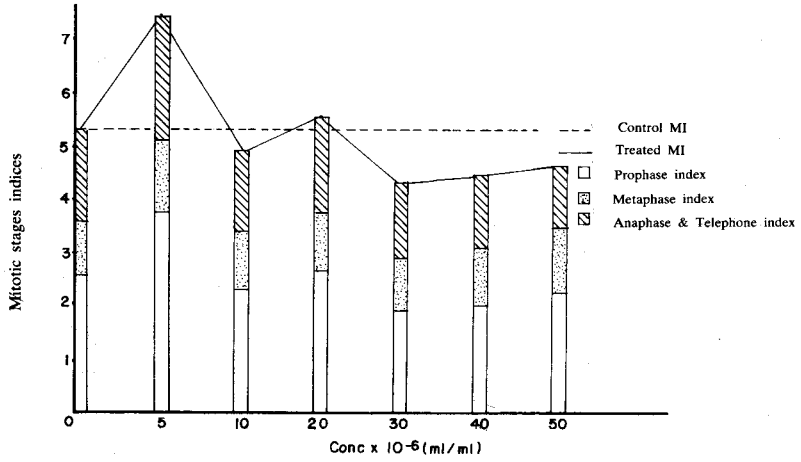


Fig. 19: Changes in the mitotic stages indices (M.S.I.) and mitotic index (M.I.) treating *Allium cepa* root tips with different concentrations of dual for 48 hours.

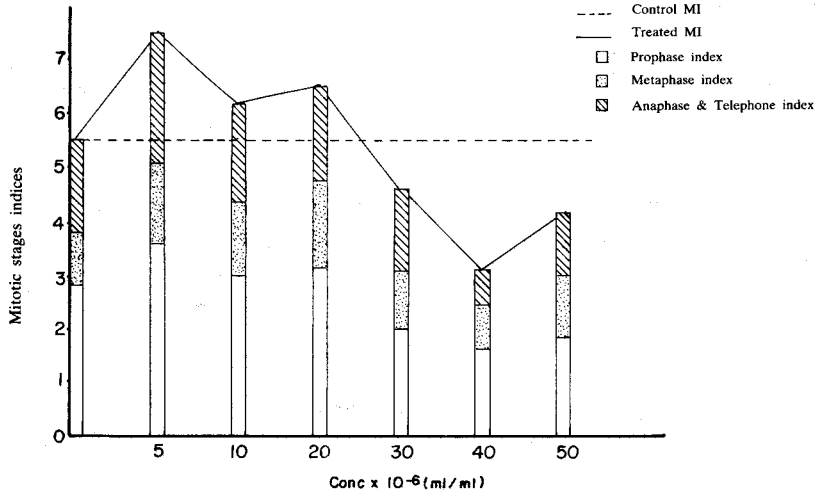


Fig. 20: Changes in the mitotic stages indices (M.S.I.) and mitotic index (M.I.) treating *Allium cepa* root tips with different concentrations of dual for 72 hours.

DISCUSSION

The results herein reported showed that Dual has the ability to affect the mitotic division of the meristematic cells of *Allium cepa* root tips. Its effect appeared to range from the production of a number of mitotic abnormalities to complete inhibition of cell division.

A drop in mitotic activity was clearly observed when the roots were treated with high concentrations. Such a drop in the mitotic index indicates that Dual interferes with the normal sequence of cell division, thus preventing or reducing the number of cells from entering the prophase stage from interphase. Such reduction in the mitotic activity could be due to inhibition of DNA synthesis (Schneiderman *et al.*, 1971). In support of this conclusion Beu *et al.* (1976) showed that exposing the root tips of *Vicia faba* to high concentrations of herbicide (paraquate), led to inhibition of D.N.A. synthesis.

The herbicide in the present study caused a change in the frequencies of the different stages. Their frequencies appeared to be a function of the duration of treatment and the concentration of the chemical applied. Thus, the frequency of prophase increased after treating the root tips of *Allium cepa* for 4 hours with low concentrations. With higher concentrations and long periods of treatment, the frequency of prophase dropped and an increase in the metaphase frequency was noticed. These changes in the stages of mitosis indicate that "Dual" affects the relative duration of each stage, as compared with the control. Similar results were obtained after treating the cells with pesticides (Amer and Farah, 1975), and papaverine hydrochloride (El-Bayoumi *et al.*, 1977).

Roots treated for a long period with high concentrations of the herbicide, a high level of chromosomal stickiness took place. Such stickiness occured in almost all the mitotic stages. Darlington and McLeish (1951) and La Cour and Tutishauser (1954) attributed such stickiness to the process of depolymerization of DNA, thus causing the chromosome surface to become sticky. Similar results were obtained after treatment with morphine sulphate and cannabis (Kabarity *et al.*, 1976 and 1980).

Another abnormality noticed in the present study is the appearance of anaphase and telophase bridges in root tips of *Allium cepa* involving one or more chromosomes. In such a case, the bridge may be due to the general stickiness of the chromosomes at metaphase stage. This is quite apparent in roots treated with high concentrations, where "Dual" caused chromosome stickiness. In this respect, these results are similar to those obtained by koerting-kieffer and Mickey (1969). In certain cases, these bridges may also be produced as a result of chromosome

breakage. In *Allium cepa* root tips, few chromosome breaks appeared after treatment with "Dual". Chromosome bridges, in such cases, appeared as a result of the occurence of a break at any stage before anaphase, followed by sister chromatid and non-sister rejoining (El-Bayoumi et al, 1984).

The appearance of chromosome fragments and micronuclei at different stages in the present study indicates that "Dual" has the ability to cause chromosome breakage in root tips of *Allium cepa*. Chromosome breakage has been induced by other chemicals, such as organic halogenides (Fiskesjo, 1969), caffeine derivatives (kihlman and kronberg, 1972), Valium (El-Bayoumi *et al.*, 1980), and trichloroacetic acid (Amer and Ali, 1980).

The most common type of abnormality observed with all the concentrations and periods of treatment was c-metaphase. This type of abnormality was produced as a result of inhibition of spindle fibre formation. In this case, it appeared to cause an arrest, mainly at metaphase stage. Such an arrest may be one of the causes for M.I. inhibition. In this respect, "Dual" appeared to have a similar action to that of colchicine (Levan, 1954).

The roots treated in the present study showed a large number of cells appearing in the c-anaphase, which is usually the next step after c-metaphase. These results are in agreement with those obtained for chemicals similar in action to colchicine, e.g. mercury compounds (Ramel, 1969), pesticides (Amer and Farah, 1975), trifluraline (Delcourt and Deysson, 1976), and Valium (El-Bayoumi *et al.*, 1980). On restitution, the latter type of abnormality appeared to lead to the formation of a tetraploid cell. Thus, a number of polyploid cells that were observed after the herbicide treatment could be due to such restitution.

Generally, these changes in mitotic division, which were observed after treatment with the herbicide "Dual", are not uncommon to other pesticides. Pesticides of Isopropyl-N-phenyl-carbamate and Duphar caused some mitotic abnormalities in *V. faba* and *G.* barbadense (Amer and Farah, 1975). Other cytological effects of pesticides like phosdrin (as an insecticide) and bladex (as herbicide) were studied (Ahmed and Grant, 1972). The pesticide "Sevin" (N-methyl-I-naphthyl carbamate) produced a number of chromosomal aberrations (Amer et al, 1971). Trifluralin, which is one of the herbicides, produced mitotic abnormalities and caused changes in the frequencies of mitotic division (Delcourt and Deysson, 1976). Similar results were obtained with the herbicide dinitroamine (El-Bayoumi and Hassan, 1979), monochloroacetic and trichloroacetic acids on *V. faba* (Amer and Ali, 1980), Reglone, Grammoxone and Balan on roots of *V. faba* (Dimitrova-Ruseva, 1979).

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دراسات سيتولوجية عن تأثير مبيد الحشائش «دوال» على نبـــات البصـــل

عبد العزيز السعيـد البيومي - انطوانيت عبـده حبيب
و محمـد سليمـان

يستخدم مبيد الحشائش دوال بكثرة في مصر لمقاومة الحشائش الحولية في نباتات الفول ، عباد الشمس ، قصب السكر والذرة . ولقد اجريت هذه الدراسات السيتولوجية لتوضح تأثير هذا المبيد على مسار الانقسامات الخلوية في الخلايا المرستيمية . ولقد عوملت جذور نبات البصل بسلسلة من التركيزات التي تتراوح بين ١٠٠٠ × ١٠ $^{-٦}$ مل/مل و ٥٠ × ١٠ $^{-٦}$ مل/مل لمدة ٤ ، ١٢ ، ٢٤ ، ٤٨ و ٧٢ ساعة .

وقد أوضحت الدراسة قدرة المبيد على احداث العديد من الشذوذ في الانقسام غير المباشر . وتختلف نسبة هذه الشذوذ ونوعيتها تبعاً لتركيز المبيد ومدة المعاملة . وأكثر التأثيرات وضوحا هو احداث الطور الاستوائي الكولشيسيني بالاضافة إلى عدد من الشـذوذ التي ظهرت في المـرحلة الانفصـالية ومن بينهـا الطور الانفصـالي الكولشيسيني ، الجسور الكروماتينية والكروموسومية ، الكروموسومات المتخلفة . وقد أدت المعاملة بالمبيد إلى احداث كسور في الكروموسومات في المرحلتين الاستوائية والانفصالية . ونتيجة لهذه الشذوذ فقد ظهرت خلايا ذات أنوية صغيرة أو خلايا متعددة الانوية في المرحلة البينية .

وقد أوضحت هذه الدراسات التأثير الضار لهذا المبيد في البيئة وخاصة على النظام الوراثي للنبات .