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Cytotoxic Effects of Goal on Onion (*Allium cepa* L.)

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Abstract \

In the present investigation, the cytotoxic effects (mito-depressive effects) of the herbicide Goal, active ingredient: Oxyfluorfen, were examined by using Allium test assay. The roots of onion bulbs were treated with 0.125, 0.25 and 0.50ml/L of Goal for 6, 12 and 24 hours. All treatments used in the present study significantly induced the mitotic index compared to control. The inhibition of the mitotic index was dependent on the concentration and time of treatment. It was observed that the percentage of prophase, metaphase, anaphase and telophase were reduced in all treatments compared with control.

Key words: Allium test assay, cytotoxicity, herbicides, mitotic index.

Introduction \

The environmental pollution is one of the most important problems of the last few decades. One of the reasons of environmental pollution is an increasing use of pesticides and herbicides in agriculture (2, 13, 14). Herbicides are widely used in agriculture in order to minimize the loss in economic crops due to plant competition. Herbicides are compounds designed to control the development of undesirable plants that may interfere with the growing of commercial crops (6). However, herbicides, in addition to their intended effects, are sometimes found to affect non-target organisms, including humans (3, 18). Despite the beneficial effects associated with the use of herbicides and herbicides, many of these chemicals may pose potential hazards to humans and to nature. Number of

investigators had studied the side effect of pesticides and herbicides on the cell division (mito-depressive effects) through several test systems (1, 2, 6, 11, 17, 24, 25). They found that these chemicals had cytotoxic and genotoxic effects on both mitotic and meiotic divisions. In spite of that the herbicides have been used extensively to increase crop productivity. Moreover, the mutagenic and carcinogenic action of herbicides and insecticides on experimental animals is well known and several studies have shown that chronic exposure to low levels of pesticides can cause mutations (4, 8, 10, 23). The indiscriminate use of herbicides in agriculture, as well as the increase of pollution in ecosystems due to industrial development, justifies the evaluation of the toxicity of these chemicals. They can be transformed into mutagenic or carcinogenic agents by vegetables, which are the first living beings in the food chain, absorbing the nutrients of polluted environments and acting as the toxic agents' vectors to humans (3, 27). Plant bioassays are considerably more sensitive and simple in comparison with other bioassays. They have been proven to be efficient tests for the cytotoxic effects of environmental pollutants. Plant bioassays have been validated in collaborative studies by many international organizations (20, 26, 33, 35). The Allium test is one of plant bioassays that is simple and just as reliable as the method where chromosome aberrations were recorded in all types of mitotic cells (29). The Allium cepa assay is an efficient test for chemical screening and monitoring of environmental contaminants and has been widely used to study cytotoxicity and genotoxicity of many chemicals (9, 32). The herbicide Goal (active ingredient: Oxyfluorfen) is commonly used in agricultural applications in Al-Jabal Al-Akdar region, Libya. Because of the potential environmental impact connected with the introduction and heavy use of herbicides, the present study was conducted to investigate the herbicide Goal for inhibition of cell division (cytotoxicity) by using Allium test assay. Also, it carried out to add to the data on cytotoxic studies that are necessary to assess the possible health risks associated with the extensive use of herbicides.

Material and methods \

The tested compound in the present investigation was Goal herbicide with the active ingredient Oxyfluorfen which belong to diphenyl ether group. The molecular formula is $C_{15}H_{11}ClF_3NO_4$ which corresponds to a molecular weight of 361.7. For determination of EC_{50} of Goal herbicide, clean and healthy onion bulbs were set up and allowed to produce roots in distilled water for 24 h, where after the homogeneously rooted bulbs (five for each treatment) were chosen and transferred to the control (distilled water) and different Goal herbicide solutions: 0.125, 0.25, 0.50, 1.0, 2.0 and 3.0 mg/L for 96 h. The lengths of ten roots

for each bulb from the control and experimental sets were measured at the end of exposure time. The relative reduction of root length was calculated as the percentage of the deviation from the control (19, 36). The EC_{50} value of the Goal herbicide was determined as approximately 0.5 mg/L. Cytotoxic parameters were done by using three different concentrations of the tested herbicide: 0.5 (EC_{50}), 0.25 (1/2 EC_{50}) and 0.125 (1/4 EC_{50}) mg/L in addition to control treatment. The plant materials used for the cytotoxicity test were (*Allium cepa* L., 2n=16). Clean and healthy bulbs of onion were chosen. Before starting to the experiments, dry scales of bulbs were removed, and 0.125, 0.25 and 0.50 mg/L of herbicide Goal were used. The solutions were prepared in distilled water. The roots of onion were treated with different concentrations of Goal for 6, 12 and 24 hours. Controls were treated with distilled water for the same time periods. Five bulbs were used in the controls and in each of the treatment groups. Roots of 0.5 to 1.5 cm long were collected from control and all treatments. Only 1 mm of the root tips were executed and slit with razor blade.

For the microscopic examination, the root tips were washed with distilled water and placed in 95% ethanol: glacial acetic acid (3:1v/v) for 24 hours at room temperature for killing and fixation. The root tips were then removed from fixative solution and placed in 70% ethanol and stored in a refrigerator (4-5^{o}C) until usage. Just before making root tip squashes, tips were treated with 1N hydrochloric acid (HCl) for 10 minutes at 60^{o}C. This was followed by the preparation of crushed material with Aceto-carmine dying method (15, 16). The mitotic index was determined by scoring more than 5000 cells (more than 1000 cells per slide). Mitotic index was calculated as the percent ratio of dividing cells and total numbers of cells scored. The analysis of variance (ANOVA) was used to assess the significant differences between control and each treatment, and statistically meaningful value was considered at P0.05. If there was a significant differences, the experimental data analyzed using Duncan's multiple range test. The statistical analyses were performed by using SPSS software analysis program, version14.

Results \

Table (1) presents the results obtained after treating onion (*Allium cepa* L.) with Goal herbicide. The inhibitory effect of Goal is expressed by the reduced percentage of the cells in division (Mitotic Index) and the percentage of the different mitotic stages in direct correlation with an increased concentration and time of action (exposure time). In the control group, mitosis was normal (Fig. 1) with the value ranging between 12.10% and 12.56% of mitotic index (MI). When the different concentrations (0.125, 0.25, and 0.50 mg/L) of Goal were introduced at 6, 12, and 24

hours, the results showed that mitotic index values were lower than that of the control values at all treatments. Mitotic index (MI) significantly decreased in all concentrations of the Goal herbicide compared to control at each exposure time (Table 1). Compare the cytotoxic effects of Goal herbicide, within the applied concentrations , results of the present study showed that there were no significant differences between two exposure times (6 and 12) at concentration of 0.125mg/L in mitotic index. On other hand, MI values of 0.5 mg/L treatment was found sharply decreased. This reduction decreased as the time of treatment increased recording values of 7.10, 6.23 and 4.93 mg/L at 6, 12 and 24h respectively (Table 1). Meanwhile, MI values of 0.25 mg/L treatment were 8.10, 6.80 and 5.33 at 6, 12 and 24hours respectively.

In all phases of mitosis, the percentage of the cells in division was inverse proportion to the increased concentration and treatment period of the herbicide Goal. Compare with control, the percentage of the cells in prophase is lower ranging between 1.91% - 2.70%. Moreover, the number of the cells in metaphase is smaller (1.22% – 2.33%), as well as the cells from anaphase (1.07%- 2.00%) and telophase (0.73% – 1.53%). Results of the present investigation clearly showed that each one of the three concentration (0.125, 0.25 and 0.5 gm/L) caused a considerable reduction in the percentage of each of the mitotic stages compared with control. This reduction increased when exposure time (treatment period) increased from 6 to 24 hours for each concentration (Table 1).

Table (1): Effect of treatment with Goal herbicide on mitotic index and phase index in root tip cells of *Allium cepa* L.

Treatment period	Concentration mg/L	Mitotic Index (MI)%	Prophase %	metaphase %	Anaphase %	Telophase %
6h	control	12.40 a	3.50 a	3.20 a	2.86 a	2.83 a
	0.125	8.57 b	2.70 b	2.33 b	2.00 b	1.53 b
	0.25	8.10b	2.57 b	2.13 bc	1.93 bc	1.43 bc
	0.50	7.10 c	2.30 c	1.90 cde	1.69 de	1.23 d
12h	control	12.56 a	3.53 a	3.23 a	2.93 a	2.86 a
	0.125	7.33 c	2.30 c	1.97 cd	1.80 cd	1.27 cd
	0.25	6.80 cd	2.23 c	1.77 de	1.60 e	1.16 de
	0.50	6.23 d	2.13 cd	1.63 ef	1.50 e	0.96 fg
24h	control	12.10 a	3.43 a	2.96 a	2.87 a	2.87 a
	0.125	6.36 d	2.13 cd	1.61 ef	1.50 e	1.06 ef
	0.25	5.33 e	1.93 d	1.37 fg	1.20 f	0.83 gh
	0.50	4.93 e	1.91 d	1.22 g	1.07 f	0.73 h
Duncan's multiple range test.		0.66	0.21	0.29	0.18	0.16

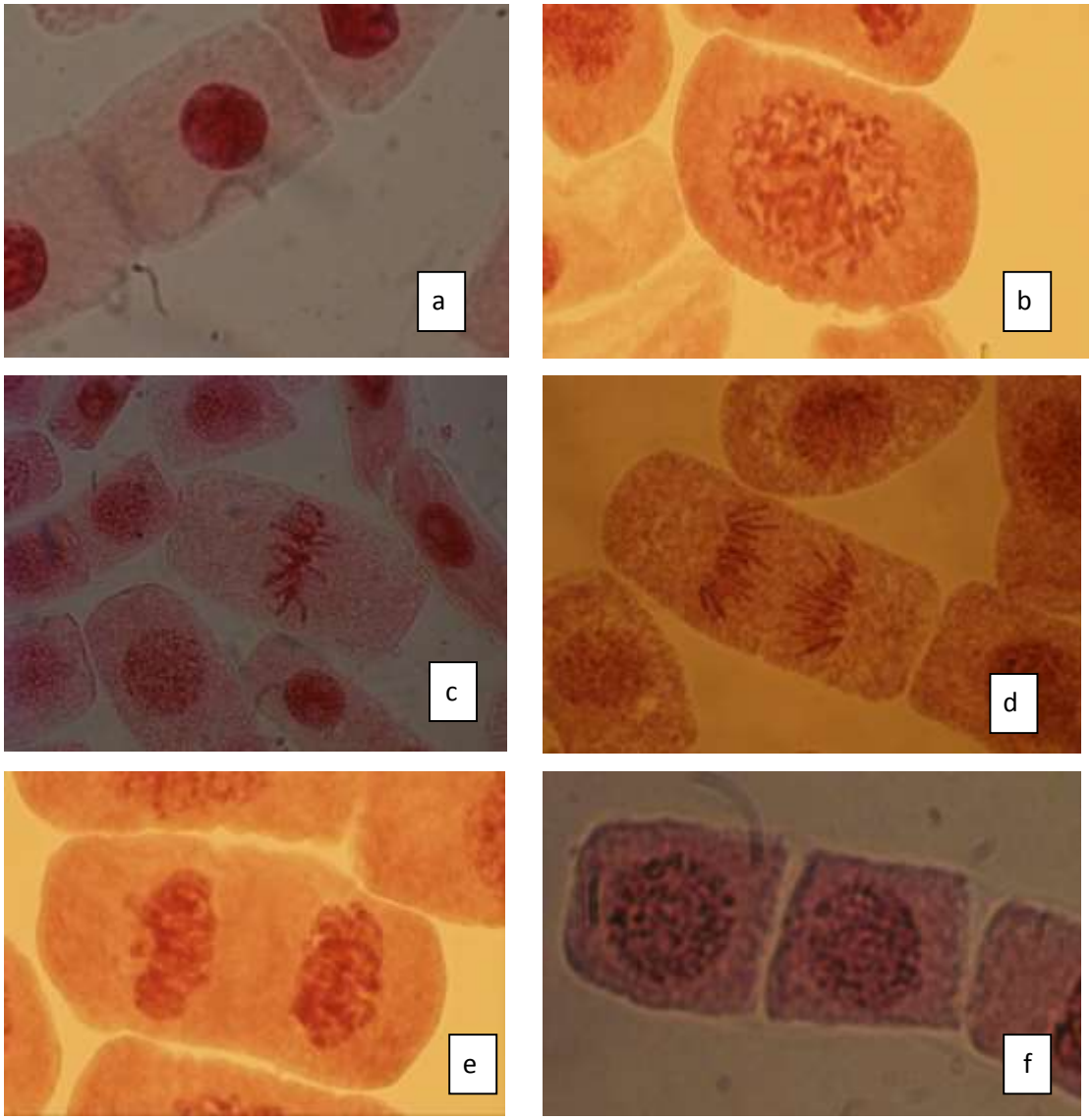

Fig. (1): Photographs of normal mitosis in meristematic cells of onion (*Allium cepa* L.).

a) Interphase b) Prophase c) Metaphase

d) Anaphase e)Telophase f) Two daughter cells

Generally, the herbicide Goal increased the percentage of the cell in pre-mitotic stage (interphase) stage and decreased the percentage of the prophase, metaphase, anaphase and telophase stages when compared with the control group in all concentrations and treatment periods (Table 1).

Discussion \

The present study investigated cytotoxicity of the herbicide Goal in onion root tip cells. The commercial form of the herbicide was examined because this is the form that is utilized in agriculture and introduced into the environment. Onion (*Allium cepa* L.) was used as the test system because plant bioassays are considerably more sensitive and simple in

comparison with others bioassays. They have been validated in international collaborative studies under the United Nations Environment Program (UNEP), World Health Organization (WHO), and US Environmental Protection Agency (US-EPA). Plant bioassays have been proven to be efficient tests for cytotoxicity monitoring of environmental pollutants(20, 26, 35). The obtained results of the present study showed gradual decrease in the mitotic index (Mito-depressive effects) either by increasing the concentrations of Goal or the time of treatment (exposure time). This decrease of mito-depressive effects was significant in all treatments when compared to the control sets. From the obtained results, it may be concluded that the increases in the concentration and time significantly increase mitotic inhibition and ensures the harmful effect of the herbicide Goal on the mitotic division . A similar results has been observed from the treatment of the herbicides Phomac (2), Illoxan (37), Atrazine (7), and Arsenal (21).

There are four possible mechanisms for chemically decreased mitotic index in cells. The first one is that a decrease in MI might be due to blocking of G1 suppressing DNA synthesis (30). The second possible mechanism is a blocking of G2 preventing the cell from entering mitosis (34). The third one is that a decrease in MI could be achieved by the inhibition of DNA synthesis at the S-phase (31). The last one is that some chemicals inhibits DNA and RNA synthesis by reducing oxidative phosphorylation in plants, resulting in lower levels of ATP (12). The obtained results showed that all treatments caused a considerable decrease in the percentage of all mitotic stages: prophase, metaphase, anaphase and telophase. The results are in agreement with many other previous studies(3, 5, 11, 36). In contrast, the percentage of stage index at pre-mitosis (interphase) increased which indicate the accumulation of cells at this stage (interphase) which consequently affect the following stage indexes (prophase, metaphase, anaphase and telophase). The percentage of stages showed positive relationship with the applied concentrations and the time of treatment. It increases as the concentration increases and the time of treatment is prolonged as previously reported by many investigators (2, 22, 27, 28).

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الملخص العربى /

التاثيرات السمية الخلوية لمبيد Goal على البصل *Allium cepa* L.

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في هذا البحث تمت دراسة تأثيرات السمية الخلوية (mito-depressive effects) لمبيد Goal، -المكون الفعال Oxyfluorfen وذلك باستخدام اختبار Allium test assay. جذور الأبصال عوملت بالتركيزات 0.125 و 0.25 و 0.50 mg/L من المبيد و بفترات تعرض 6 و 12و 24 ساعة . جميع المعاملات المستخدمة في البحث الحالي خفضت من دليل الانقسام الميتوزي مقارنة بمجموعة المراقبة. الانخفاض في معدل الانقسام كان معتمد علي التركيز و زيادة فترة التعرض. أيضا كان من الواضح أن نسب الطور التمهيدي و الطور الاستوائي و الطور الانفصالي و الطور النهائي جميعها انخفضت مقارنة بمجموعة المراقبة.

الكلمات الدالة: السمية الخلوية، مبيدات الحشائش، معدل الانقسام.