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Genotoxic Effects of the Herbicide Oxyfluorfen on the Root Meristem Cells of *Helianthus annuus* (Sunflower)

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Summary The genotoxic effects of the herbicide oxyfluorfen on the root meristem cells of *Heianthus annuus* L. (sunflower) were investigated. The roots were treated with 75, 150, and 300 ppm concentrations of oxyfluorfen for 12 and 24 h. The different genetic endpoints such as the mitotic index (MI), chromosome aberrations (CA), and micronuclei (MN) were analyzed in both control and test groups. The oxyfluorfen showed a marked mitodepressive action on mitosis. Additionally, MN were observed in interphase cells. The types of chromosome aberrations included disturbed prophase, c-mitosis, stickiness, laggards, and chromatid bridges. A pronounced toxic effect was observed at all concentrations applied. The herbicide oxyfluorfen may have genotoxic and clastogenic effects on sunflowers.

Keywords Genotoxicity, *Helianthus annuus*, Mutagenicity, Oxyfluorfen.

The use of herbicides is indispensable for weed control in the most important crops. Oxyfluorfen is a diphenyl ether-based herbicide commonly used in many agricultural practices for weed control, globally. The herbicides of the diphenyl ether group act by inhibiting protoporphyrinogen oxidase, an enzyme that acts in the synthesis of chlorophyll and cytochrome (De Vasconcelos Lima *et al.* 2019).

H. annuus L. (sunflower) is a member of the family Asteraceae and is one of the most important crops. It is considered that the sunflower is the fourth oilseed crop in the world (Nisar *et al.* 2011, Tonev *et al.* 2020). On the other hand, it is known that the sunflower fields have rich weed flora, such as *Amaranthus retroflexus* L., *Chenopodium album* L., *Polygonum aviculare* L., *Lolium multiflorum* Lam., and *Portulaca oleracea* L (Pannacci *et al.* 2007, Prashan *et al.* 2017) It known that the effective weed control is necessary to achieve high yields (Tonev *et al.* 2020). The control of the weeds in this crop is performed mainly by herbicides such as oxyfluorfen (Prashan *et al.* 2017).

The potential side effects of the herbicides, which are mutagenic and/or carcinogenic agents to the crops as non-targeted organisms, are worthy of extended studies in greater depth (Inceer *et al.* 2009). Although there are several studies on the effects of oxyfluorfen on non-targeted organisms (Hassanein 2002, Geoffroy *et al.* 2003, Dragoeva *et al.* 2012, Stagg *et al.* 2012, El-Rahman *et al.* 2019), no studies are available on genotoxic effects of oxyfluorfen on mitotic cells of *H. annuus*. This study aimed to evaluate the genotoxic effects of oxyfluorfen on the mitotic cells and somatic chromosomes of this economic plant.

Materials and methods

Three concentrations (75, 150, and 300 ppm) of 2-chloro-1-(3-ethoxy-4-nitrophenyl)-4-(trifluoromethyl) benzene (oxyfluorfen) were used for chromosome aberration assays. Test solutions were prepared using tap water. Seeds were placed directly in the test solutions, and controls were placed in only tap water. The seeds were treated with the test solutions for 12 and 24 h and were then allowed to recover for 10–15 min in tap water (Inceer *et al.* 2009). The seeds were then placed on wet filter paper in Petri dishes and left in the dark at 22±2°C (Inceer and Beyazoglu 2000). After germinating, the root tips were excised and fixed in ethanol-glacial acetic acid (3 : 1) for at least 24 h at 4°C, hydrolyzed in 1 M HCl at 60°C for 10–12 min, and then rinsed in deionized water for 2–3 min. Staining was carried out in Schiff's reagent for 1–2 h at room temperature, and squashes were made in 45% acetic acid (Inceer and Beyazoglu 2004). For each treatment, five root tip squashes were prepared, and a minimum of 500 mitotic cells were counted from each slide under a microscope (Leica DM 1000). The cells were photographed using the Leica DM 1000 microscope.

Analysis of variance (One-Way ANOVA) of the data was done with the SPSS computer program. The Dunnet *t* (2-sided) multiple range test was employed to determine the statistical significance of differences among the

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means. The statistical analysis presented in Table 1 indicates significant variation ($p=0.05$) in mitotic cells when comparing the number of normal and abnormal cells at each concentration with the control.

Results and discussion

This study presents the primary results which have been for the first time made on the genotoxicity of the herbicide oxyfluorfen on mitotic cells of *H. annuus*. The genotoxic effects of the different concentrations, *viz.* 75, 150, and 300 ppm, of oxyfluorfen on the mitotic cells, are presented in Table 1. As seen in Table 1, all of the herbicide applications significantly decreased the MI compared to their controls. Besides, the recorded MI value was particularly very low in roots treated with high concentration and prolonged exposure time. In particular, the reduction in MI at the 300 ppm concentration of oxyfluorfen was above 60% compared to the control. As already reported by Rank and Nielsen (1997) and Inceer *et al.* (2009), if the LD_{50} value is considered the highest concentration and the others are below the LD_{50} for the genotoxicity test, the MI will not decrease well below 50% of the control.

MI is an important indicator of cell division, and a significant reduction in MI is related to mitodepressive effect of the substances tested (Akinboro and Bakare 2007, Sharma and Vig 2012, Smirnova and Korovkina 2021), which was observed in the study. These changes in the mitotic phase show that the herbicide oxyfluorfen affects the relative duration per phase, as compared with the controls. Similar results were obtained after treating *H. annuus* root tip cells with the herbicide linuron and the insecticide cypermethrin (Inceer *et al.* 2004, 2009).

The results of CA in meristematic cells of sunflower based on oxyfluorfen applications are shown in Table 2 and Fig. 1. The herbicide treatments yielded five types of the common CA: disturbed prophase, c-mitosis, stickiness, laggards, and chromatid bridges. The present results indicate that as the concentration of the herbicide increased, the percentage of CA gradually increased due to spindle failure. According to Fiskesjö and Levan (1993), some CA can form an irreversible and genotoxic influence. Similarly, various CA occurred in different phases of mitotic cell division in *H. annuus* meristematic stem cells treated with other herbicides, such as linuron and quizalofop-*p*-ethyl (Inceer *et al.* 2004, Karaismailoglu *et al.* 2013). Additionally, the inhibition of spindle formation has been shown to lead to severe chromosome aberrations in *H. annuus* based on the herbicide applications (Inceer *et al.* 2004, 2009, Karaismailoglu *et al.* 2013).

It is known that MN provides important information to evaluate the action mechanisms of an agent about its effects on the genetic material (clastogenic and/or aneugenic effects). Besides, the MN is an important parameter to evaluate environmental contamination. Therefore, MN has been considered by many authors as the most effective and simplest genetic endpoint to analyze the mutagenic effect promoted by chemicals (Leme and

Table 1. Effect of oxyfluorfen on mitotic cells in root tips of *H. annuus*.

Time (h)	Concentration (ppm)	Number of cells examined	MI±SD	Total prophase (%)	Abnormal prophase (%)	Total metaphase (%)	Abnormal metaphase (%)	Total ana-telophase (%)	Abnormal ana-telophase (%)
12	control	3,400	27.47±1.08	67.65±0.50	0.7±0.22	20.78±0.15	0.7±0.11	11.57±0.10	0.7±0.35
	75	3,749	15.78±0.64*	67.57±0.53	1.8±0.27	14.84±0.18	2.7±0.18*	11.70±0.23	0.9±0.18
	150	3,354	14.19±0.48*	46.76±0.42*	13.66±0.48*	8.56±0.16*	18.06±0.34*	10.88±0.11	1.8±0.24
	300	3,117	10.88±0.53*	31.37±0.18*	2.46±0.14*	6.27±0.55*	30.63±0.13*	4.43±0.16*	5.08±0.65*
24	control	3,575	25.02±2.21	69.55±0.26	0.61±0.24	26.68±0.46	0.28±0.43	3.15±0.34	0.28±0.46
	75	3,499	17.52±1.03*	65.94±0.62	5.8±0.33	20.83±0.54	4.52±0.17*	2.71±0.38	2.17±0.31
	150	3,184	12.88±0.3*	40.03±0.13*	22.47±0.15*	20.10±0.65	8.61±0.27*	2.63±0.41	4.23±0.28*
	300	3,284	6.26±1.03*	37.16±0.34*	27.53±0.62*	9.97±0.14*	19.03±0.32*	1.11±0.62*	5.74±0.51*

* Significant from the control $p=0.05$

Table 2. Chromosome aberrations in mitotic cells of root rips of *H. annuus* exposed to oxyfluorfen.

Time (h)	Concentration (ppm)	Disturbed prophase (%)	Chromatid bridge (%)	C-mitosis (%)	Stickiness (%)	Laggards (%)	Total abnormality (%)
12	control	0.36±0.28	—	—	0.36±0.64	0.36±0.54	0.14
	75	33.33±0.14	10.33±0.25	3.00±0.35	19.30±0.36	4.00±0.28	0.32
	150	38.61±0.48	21.75±0.25	2.84±0.22	30.66±0.55	5.10±0.24	4.14
	300	47.53±0.26	27.77±0.38	7.55±0.44	36.27±0.60	9.33±0.65	6.05
24	control	0.58±0.48	—	—	0.40±0.38	0.40±0.25	0.19
	75	38.37±0.4	16.00±0.6	3.70±0.46	11.52±0.43	2.70±0.18	1.97
	150	54.17±0.38	18.72±0.48	6.05±0.55	14.98±0.25	3.30±0.38	4.83
	300	61.03±0.36	36.74±0.17	11.45±0.42	24.75±0.35	6.45±0.42	6.56

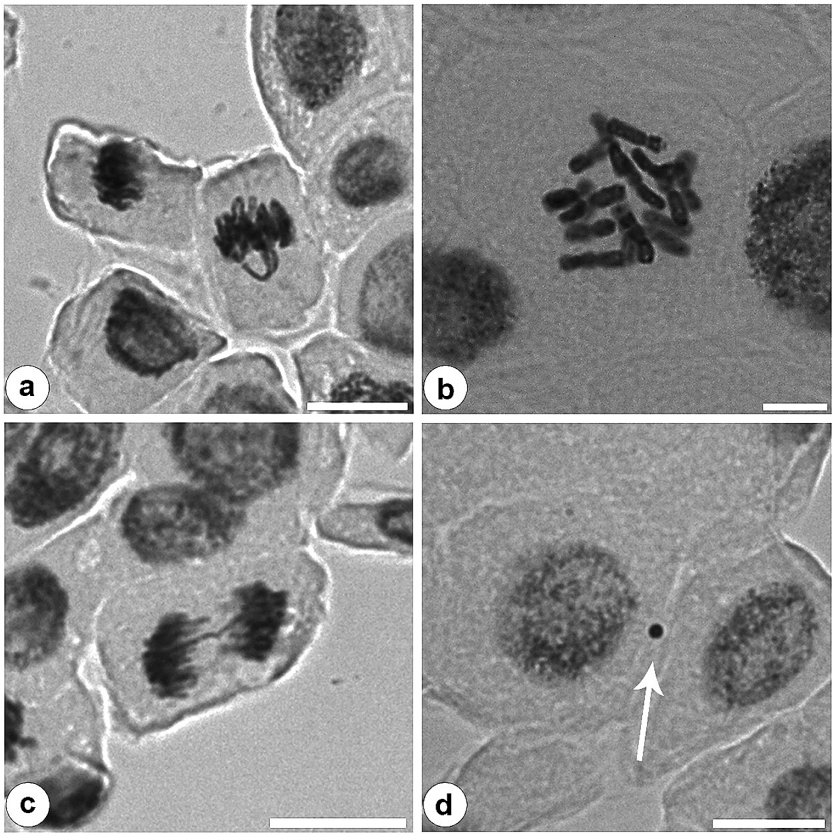


Fig. 1. Mitotic abnormalities induced by oxyfluorfen in root tips of sunflower. a; Stickiness. b; C-mitosis. c; Chromatid bridge. d; Micronucleus indicated by an arrow. Scale bars=10 μm.

Table 3. Effect of oxyfluorfen on MN formation in mitotic cells of *H. annuus*.

Time (h)	Concentration (ppm)	Number of cells examined	MN (%)
12	control	2,758	—
	75	2,709	0.04±0.42
	150	2,680	0.10±0.15
	300	2,408	0.18±0.55
24	control	2,801	—
	75	2,957	0.07±0.45
	150	2,854	0.13±0.20
	300	2,790	0.21±0.16

Marin-Morales 2009). Our results obtained from the MN analysis are presented in Table 3 and Fig. 1. The present results show that as the concentration of the herbicide increased, the percentage of the MN in interphase cells gradually increased. In addition, the MN observed in the cells is small. As already reported by (Leme and Marin-Morales 2009), small MN may indicate a clastogenic action resulting from chromosome break. However, other cytogenetic techniques, such as chromosomal banding (C-banding, Ag-NOR staining, and base-specific fluorochrome banding) and *in situ* hybridization, should be applied to make the analysis more reliable and accurate.

Higher plants present characteristics that make them perfect genetic models to assess environmental pollutants (Leme and Marin-Morales 2009). In particular, the higher plants such as *Allium cepa* L., *Vica faba* L., *Tradescantia* L., *H. annuus*, *Crepis capillaris* (L.) Wallr., and *Hordeum vulgare* L. are commonly used to evaluate environmental contamination caused by chemicals, such as pesticides and metals (Zakia *et al.* 1990, Grant 1994, Inceer *et al.* 2004, 2009, Gadeva and Dimitrov 2008, Leme and Marin-Morales 2009, Cavusoglu *et al.* 2014). On the other hand, it is known that the chemicals used in the genotoxicity tests inhibit mitosis in plants. Similarly, our findings reveal that oxyfluorfen has a marked mitodepressive action on mitosis (Table 1). Such a significant decrease in the MI indicates that oxyfluorfen interferes with the normal sequence of the cell cycle to reduce the number of cells starting to divide at interphase. The reduction in the mitotic activity could be due to the inhibition of DNA synthesis, which is one of the major prerequisites for a cell to divide (Yuzbasıoglu *et al.* 2003, Inceer *et al.* 2004, Ergin *et al.* 2020).

Rank and Nielsen (1997) reported that if a chemical can cause damage to the chromosomes in a reliable plant assay, then the chemical should be considered as having the potential to damage the chromosomes of other organisms in the environment. In the present study, clastogenic types of the herbicide oxyfluorfen should be regarded as an agent that shows mutational activity in *H. annuus*.

The present results indicate that oxyfluorfen, like other pesticides in the environment, can be absorbed by higher plants and may adversely affect their genomes, thus causing damage to plants. The wide application of herbicides for the control of weeds in agricultural practices is a potential threat to the genetic constitution of economically important plants like sunflowers. Therefore, it is necessary to test the genotoxic effects of herbicides on plants and other systems before considering their applications for agricultural purposes.

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