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Cytological Effects of Herbicide Butachlor 50 EC on Somatic cells of *Triticum aestivum* L.

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ABSTRACT

The widespread use of the herbicides for weed control and crop productivity in modern agriculture exerts a threat on economically important crops by way of cytological damage to the cells of crop plant or side effects. In the present study the cytotoxic effects of herbicide butachlor were investigated in the somatic cells of wheat. The wheat grains were treated with different concentrations of herbicide (0.15-1.0ppm) at room temperature. The percentage mitotic index decreased significantly as the concentration of the herbicide increased when compared to control. The chromosomal abnormalities were found to be increased as the concentration of the herbicide increased when compared to control. The observed chromosomal irregularities were sticky chromatin, chromosomal bridge, nuclear lesion, scattered chromosome, fragmented metaphase, fragmented anaphase, multipolar chromosome and micronuclei. According to our findings we can say that butachlor can produce negative side effects on mitotic division in somatic cells of wheat.

1. INTRODUCTION

In agricultural practice many herbicides are directly applied to soil to control weeds and other competitive plants that grow with the main crop. This is a major problem in developing countries with agro-based economics, including India. The use of herbicide in cereal crop have become very popular and common for the reason that crop protection, satisfactory residual action, wide range of weed control, flexibility in application timing and the most important cost effective. The use of herbicides in modern agriculture exerts a threat on crop plant by a way of cytological damage to the cells of the crop plant [1]. Wheat is one of the most abundant sources of energy and its increased production is essential for food security [2]. Wheat is characterized by large genome size (approximately 1700MB). Herbicides are selective, cost effective, easy to apply and flexibility in application time as well, if applied in proper dose they become eco-friendly. In India 96% of herbicides are moderately toxic, while more than 70% insecticides are extremely toxic [3]. Herbicides are metabolic inhibitors and their mode of action can be classified in to different groups; Photosynthetic inhibitor, Cell growth disrupters (mitotic inhibitors), Growth regulators, Lipid biosynthesis inhibitor, Carotenoid biosynthesis inhibitor and Branch chain amino acid inhibitor [4]. The herbicide butachlor, (N-[butoxymethyl]-2-chloro-2,6'-diethylacetanilide), the chloroacetanilide herbicide, affects seed germination, lipid metabolism, pigment and

Gibberlic acid synthesis, cell division, cell permeability, mineral uptake and disturb the absorption and incorporation of amino acid in to protein [5], spindle inhibitor [6], very long chain fatty acid (VLCFA) synthesis [7], lipid biosynthesis, RNA synthesis [8]. In present study, an attempt has been made to assess the cytotoxic effect of butachlor on wheat variety HD-2189.

2. MATERIALS AND METHODS

Wheat (*Triticum aestivum* L.) variety HD-2189 was obtained from University of agricultural sciences, Dharwad. The herbicide butachlor 50 EC was obtained from Mateshwari pesticides Ltd. Meerut (UP). The present study was carried out with different concentrations of herbicides (0.15, 0.25, 0.5, 0.75 and 1.0ppm). Seeds were germinated in petridishes with different concentrations of herbicides prepared in Hoagland's nutrient solution and the control group was treated only with Hoagland's nutrient solution.

The root tips (size-1-1.5cm) of both treated and control germinated seedling were collected and rinsed with distilled water, fixed in carnoy's solution II (alcohol: chloroform: acetic acid in 6:3:1 ratio) for 24 hours. The fixed root tips were preserved in 70% ethanol in a refrigerator for further studies. Mitotic index and frequency of abnormalities was calculated following method [9]. Three replicates were made for each concentration. The slides were observed under microscope and photographed.

2.1 Statistical analysis

The data were subjected to analysis using SPSS package ver.16 with Tukey's HSD significant test at 5% level.

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3. RESULTS AND DISCUSSION

Mitotic index is an important microscopic parameter that can be used as biomonitor to assess the effect of stress [10]. The effect of different treatments with butachlor on the mitotic index in the root tip cells of wheat is given the table 1. There was a significant change in the mitotic index of the dividing cells. As could be seen in the table the mitotic index decreased significantly with increase in herbicide concentration, when compared to control. Maximum value of the mitotic index was observed in the control (6.48), while the minimum value of the mitotic index was observed at 1ppm concentration (2.08). The mitotic index decreased from 5.72 to 2.08 at dosage 0.15 to 1ppm concentration of butachlor respectively. However the mitotic cells observed in treated root tips, was relatively lower than the control. It was supported by the decreased mitotic index in treated plants. Similar results have been reported by [10] who observed that dithiopyr caused a cessation of root elongation results in swelling of root tips in wheat, mitotic index decreased as the concentration of the herbicide increased and mitotic cells were arrested in late pro metaphase. A sharp reduction in the number of dividing cells of wheat as the concentration of the herbicide dicamba increased [11]. The effect of 2,4-D and isoproturon on wheat root tips of 3 varieties (HUW-234, HUW-648 and HUW-533), showed a decrease in mitotic index as the concentration of the herbicide increased when compared to control[12]. The interphase chromosome volume increased as the concentration of ethylene glycol increased in somatic cells of wheat [13]. There was an exponential relationship between the percentage of aberration and concentration of the pesticides [14]. On the other hand herbicide illoxan significantly increased the abnormal cell frequency at all concentrations. The mitotic index decreased in all the treatments when compared to control. The decrease in mitotic index is slightly dose dependent, it does not affect the percentage of mitotic stages [15]. On the contrary the mitotic index were high as well as low in most of the treatments compared to that of control but did not show any clear relationship based on increasing or decreasing doses of herbicides as reported by [16]. In the present study the mitotic divisions were found to be inhibited at higher concentration. The percentage of prophase and metaphase were increased as the herbicide concentration increased. Our results are in line with those of earlier studies such as effect of tribunil on *Allium cepa* [17] showed that an increase in prophase in root meristamatic cells. The different kinds of chromosomal abnormalities induced by butachlor in the present study increase with increased in herbicide concentration.

In root tip cells of wheat, butachlor arrested the mitosis at metaphase and anaphase (Table 2). In control sets prophase was 50.72%, while it was 55.63% in 1ppm treatment. However there was no much significant difference in prophase between control and 0.15ppm concentration of butachlor. Among the treatment the percentage of anaphase and telophase were decreased as the concentration of the herbicide increased, when compared to control. On the other hand the percentage of prophase and

metaphase were increased as the concentration of the herbicide increased, when compared to control. Maximum mean value of prophase and metaphase were 55.63% and 34.54% at 1ppm concentration of butachlor respectively, when compared to control. The different types of chromosomal abnormalities induced by different concentration of butachlor in wheat are presented in the table 3. The percentage of chromosomal abnormalities increased as the concentration of the herbicide increased, when compared to control.

The most common types of abnormalities observed were sticky chromosomes [Fig. A], Chromosomal bridges [Fig. B], Nuclear lesions [Fig. C], Multipolar chromosomes [Fig. D], Fragmented metaphase [Fig. E], Fragmented anaphase [Fig. F], Fragmented chromosomes [Fig. G], Scattered chromosomes [Fig. H] & Micronuclei [Fig.I]. Highest frequency of sticky chromosomes were observed at 1ppm concentration (1.09%), while it was not seen in control. Butachlor is effective in the formation of the bridge especially in higher concentration, while control and 0.15 did not show any chromosomal bridge. Nuclear lesion were observed in all the concentration expect in control, on the other hand the percentage of the Nuclear lesion were increased as the herbicide concentration increased. The maximum percentage of Nuclear lesion observed at 1ppm concentration (0.9 %). Occurrence of multipolar chromosome were maximum (0.15%) at dosage 0.75 ppm concentration, while it was minimum (0.06%) at dosage 0.15ppm concentration of butachlor. Fragmented metaphase and fragmented anaphase were maximum (0.23 and 0.31%) at dosage 1ppm concentration of butachlor respectively. Fragmented chromosomes were observed in all the concentration expect in control and 0.15 ppm concentration of butachlor, the maximum percentage of fragmented chromosome were observed at 1ppm concentration of butachlor (0.19%). The scattered chromosomes were observed at 0.5, 0.75 and 1ppm concentration of butachlor. Micronuclei (0.57%) were seen in only 1ppm concentration of butachlor treatment.

Chromosomal stickiness arises from improper folding of the chromosome fiber in to a single chromatids and chromosomes. As a result there is a intermingling of the fibers, the chromatids become attached to each other by means of subchromatid bridges [18]. Chromosome stickiness were reported following of treatment with number of pesticides including illoxan on *Allium cepa* [15], imazethapyr on wheat [19], 2, 4-D on wheat [20] nd 2,4-D and isoproturon on wheat[21]. The distinct views have been given by the different workers. It had been suggested that stickiness was the physiological effects of the herbicide [22] and also might be the complicate tangling of interchromosomal chromatin thread. This brings to the sub chromatid association; apart from the above stickiness might be the result of action of herbicides on the protein of the chromosome [23]. Chromosomal bridges have been reported following treatment with number of chemicals including 2,4-D and isoproturon on wheat [21], Fieldr and Ronstar on wheat [16], imazethapyr on wheat [19], ethylene glycol on wheat[28], putrescine on wheat[24], maleic hydrazide on *Trigonella* [25], illoxan on *Allium cepa* [15].

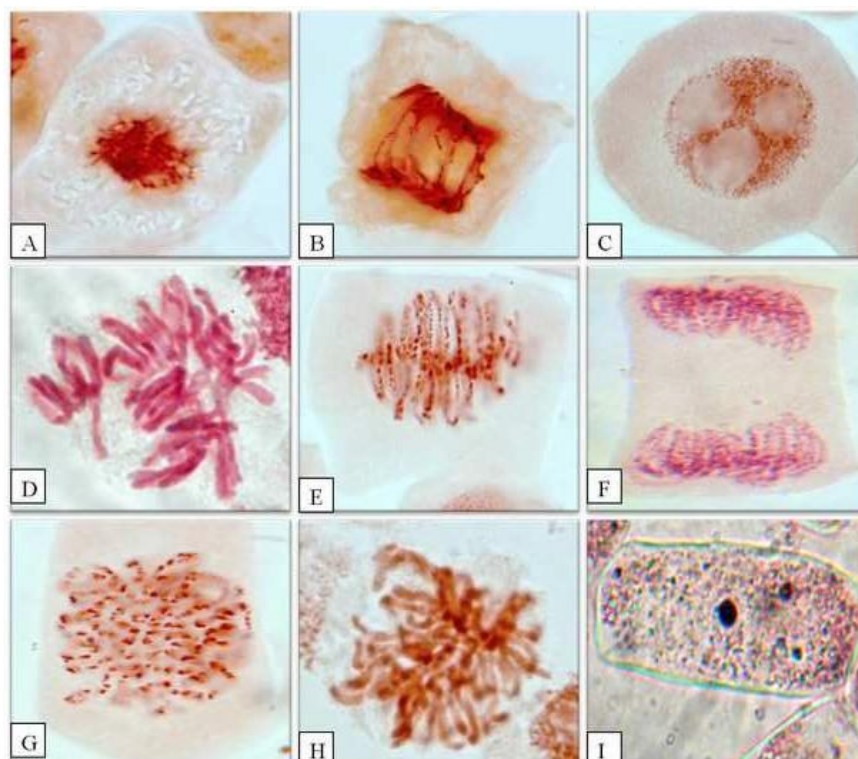


Fig. 1: Different types of abnormalities observed in the mitosis following the treatment of butachlor: A-Sticky chromosome (0.5 PPM), B.- Chromosomal bridges (1 PPM), C- Nuclear lesion (0.75 PPM), D- Multipolar chromosome (0.75 PPM), E- Fragmented metaphase (0.15 PPM), F- Fragmented anaphase (1 PPM), G- Fragmented chromosome (1 PPM), H- Scattered chromosome (0.75 PPM) and I- Micronuclei (1 PPM).

Table 1: The effect of different concentrations of butachlor on mitotic index of root tip cells in wheat.

Butachlor Concentration (ppm)	Total number of cells	Number of dividing cells	Mitotic index
Control	5780	364	6.48±0.32 ^a
0.15	5564	303	5.72±0.06 ^b
0.25	5074	223	4.25±0.20 ^c
0.5	4786	174	4.18±0.01 ^d
0.75	4568	120	2.67±0.06 ^e
1.0	2098	43	2.08±0.05 ^f

Mean ± SD followed by the same superscript are not statistically significant between the concentration, when subjected to SPSS package ver.16.0 according to Tukey's mean range test at 5% level significance.

Table 2: The effect of different concentrations of butachlor on different stages in root tip cells of wheat

Butachlor Conc. (ppm)	Prophase(%)	Metaphase(%)	Anaphase (%)	Telophase (%)
Control	50.72±0.35 ^d	20.46±0.12 ^f	16.21±0.15 ^a	12.41±0.17 ^a
0.15	50.47±0.43 ^d	19.07±0.04 ^e	15.60±0.05 ^b	12.27±0.04 ^a
0.25	51.52±0.59 ^c	22.71±0.54 ^d	14.02±0.28 ^c	11.46±0.15 ^b
0.5	54.76±0.44 ^b	25.34±0.15 ^c	13.19±0.06 ^d	8.20±0.11 ^c
0.75	54.92±0.10 ^b	29.57±0.26 ^b	11.13±0.16 ^c	7.226±0.86 ^d
1	55.63±0.17 ^a	34.54±0.45 ^a	6.543±0.24 ^f	5.62±0.20 ^e

Mean ± SD followed by the same superscript are not statistically significant between the concentration, when subjected to SPSS package ver.16.0 according to Tukey's mean range test at 5% level significance

Table 3: Somatic chromosomal abnormalities (%) in root tip cells of wheat induced by different concentrations of butachlor

Abnormalities	Concentration (ppm)					
	Control	0.15	0.25	0.5	0.75	1.0
Total abnormalities	0.00±0.00 ^e	0.82±0.03 ^e	1.04±0.05 ^d	1.48±0.07 ^c	1.57±0.10 ^b	4.48±0.19 ^a
Sticky chromosome	0.00±0.00 ^e	0.32±0.02 ^c	0.29±0.03 ^c	0.23±0.06 ^c	0.17±0.01 ^b	1.09±0.10 ^a
Chromosomal bridge	0.00±0.00 ^e	0.00±0.00 ^e	0.17±0.06 ^{bd}	0.32±0.05 ^{cd}	0.12±0.04 ^b	0.43±0.04 ^a
Nuclear lesion	0.00±0.00 ^e	0.00±0.00 ^e	0.35±0.01 ^d	0.48±0.03 ^c	0.57±0.04 ^b	0.90±0.05 ^a
Multipolar chromosome	0.00±0.00 ^c	0.06±0.02 ^b	0.00±0.00 ^c	0.00±0.00 ^c	0.15±0.02 ^a	0.00±0.00 ^c
Fragmented metaphase	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	0.12±0.03 ^b	0.08±0.04 ^c	0.23±0.01 ^a
Fragmented anaphase	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	0.08±0.02 ^b	0.06±0.02 ^c	0.31±0.20 ^a
Fragmented chromosome	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	0.18±0.05 ^b	0.13±0.02 ^c	0.19±0.05 ^a
Scattered chromosome	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^c	0.04±0.02 ^b	0.14±0.03 ^a
Micronuclei	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.0±0.00 ^b	0.00±0.00 ^b	0.57±0.01 ^a

Mean ± SD followed by the same superscript are not statistically significant between the concentration, when subjected to SPSS package ver.16.0 according to Tukey's mean range test at 5% level significance

Presence of chromosomal bridge may be due to stickness or formation of dicentric chromosome caused by breakage and reunion [25]. Sticky bridges might be also the result of incomplete replication of the chromosome by defective and less active replication enzymes [26]. Chromosomal bridges mainly arises due to the non disjunction of sticky chromosome or breakage and reunion during separation at anaphase [27]. Interchromatid connections have been reported by [28] in *Tradescantia* and *Vicia faba* treated with mercurial fungicide. Chromatin fibers which join two sister chromatids at metaphase and presumably hold the chromatids together until anaphase have been termed interchromatid connections [29]. Chromosomal fragmentation arises as a result of multiple breaks of the chromosome in which there is a loss of chromosomal integrity. Fragmentation can range from partial to total disintegration of chromosome.

Fragmentation occurs in prophase, metaphase and anaphase. Chromosome fragment in plant cell have been reported only rarely after treatment with pesticides [18]. Pentachlorophenol induces fragmentation of both mitotic and meiotic chromosome of *Vicia faba* [30]. Other pesticides which have been reported to induce fragmentation include ferbam in *Allium cepa* [31] and Simazine in *Vicia faba* [32]. Multipolar chromosome arises as a consequence of incomplete suppression of spindle function [21]. The occurrence of multipolar chromosome was reported by [20] in wheat which respond to 2,4-D and in *Allium cepa* and *Allium sativum* which respond to isoproturon and Carbofuron respectively [33]. Scattered chromosomes have been reported by [34] in *Allium cepa* which respond to copper mine. They have also reported that such chromosomal regulation affect the vigour, fertility and yield of the exposed plants. Nuclear lesion have been reported by [35] in *Allium cepa* treated with sodium benzoate. Binucleate cell formation probability have been reported by [12] in wheat that respond to 2, 4-D and isoproturon. The herbicide spoiled the phragmoplast microtubule that would not assign the limited amount of cell plate establishment precisely and owing to that binucleate cell might be formed, cell plate may be absent in longer exposure of herbicides consequently [36]. Pesticides are mitodepressive at higher concentration and mitopromoter in lower concentration and induce variety of chromosomal abnormalities in higher concentrations in *Vicia faba* L [14]. The herbicide butachlor act as potent spindle inhibitor. The herbicides bind to tubulin, a major microtubule protein. The herbicide –tubulin complex inhibits the polymerization of microtubule, leading to loss of microtubule structure and function. As a result spindle apparatus is absent, thus preventing the alignment and separation of the chromosome during mitosis. In addition to this cell plate cannot be formed [7].

4. CONCLUSION

Herbicides drastically influence all aspects of primary and secondary metabolism in crop plants when given to control undesired weeds. The present investigation clearly showed that there was a significant reduction in the mitotic index of the

dividing cells and the chromosomal abnormalities were found to be increased as the concentration of the herbicide increased when compare to control.

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