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CYTOGENETIC DISTURBANCES IN GERMINATING SEEDS OF BROAD BEAN (*VICIA FABA* L. VAR. *MINOR* BECK) CAUSED BY HERBICIDE AVADEX B. W.[1]

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Summary. The effect of the carbamate herbicide Avadex B. W. (a.i. triallate) in 0.5, 1.0 and 1.5% concentrations on the genetic material of broad bean root tips cv. Nadwiślański was studied. The examined compound induced chromosomal aberrations depending on its dose. In roots of broad bean treated with Avadex B. W. a decrease in the mitotic activity was found. However, in the material fixed 48 h after the herbicide treatment a tendency towards increase of the mitotic index was observed.

Pesticides are important factors of increasing the yield of many crops but continuous studies should be conducted to investigate the mechanism of their action on living organisms.

Theoretical estimation of potential pesticide mutageniticity can be made by establishing gene mutation frequency using microbiological tests (Szarapińska-Kwaszewska et al. 1984) or by determining chromosomal aberrations in higher plant or animal organisms (including human cells) (Stroev 1970, Skorupska 1976, Pilinskaya, Kurinnyi 1980).

Mutation inducibility of pesticides is very variable and depends on the composition of their active ingredients, on their dose and on the degradation rate as well as on plant reaction (Drozd 1984).

Routine estimation of potential mutageniticity of the presently applied pesticides as well as newly synthetized pesticides permit to reject the most dangerous chemicals.

The aim of the present work was to estimate potential mutageniticity of the carbamate herbicide Avadex B. W. to broad bean (*Vicia faba* L. var. *minor* Beck). The investigations were based on the analysis of the chromosomal abberration frequency and abnormalities in the mitotic division rate of the root tip cells in germinating seeds.

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MATERIAL AND METHODS

This study was based on the laboratory experiment conducted at the Department of Plant Breeding and Seed Production of the Academy of Agriculture and Technology in Olsztyn.

Seeds of broad bean cv. Nadwiślański constituted the studied material.

Avadex B. W. was used in water solutions in concentrations of 0.5, 1.0 and 1.5%.

Seeds after 24 h soaking in water with aeration were transferred to Avadex B. W. solutions for 12 h at 20°C. Roots were fixed in Carnoy's fixative 24,36 and 48 h after the treatment and stored in 70% ethanol in a refrigerator. Some roots from each combination were treated with 0.05% colchicine for 3 hours and used to study metaphase abnormalities.

Microscopic observations were performed on sqash preparations stained according to Feuglen's method at magnification of 1000 - 4000×. The number and type of chromosomal abnormalities were studied in 200 cells of each mitotic phase in the root tips fixed 24 h after the treatment. In the roots fixed 48 h after the treatment, the frequency of micronuclei and interphase mitotic inactive nuclei was studied by analysing 1000 succeeding cells in the random part of the preparation.

The values of mitotic indices were established for each fixation time considering 1000 succeeding cells in the randomly chosen part of the preparation.

The experiment was performed in four replications and results are presented as means.

RESULTS AND DISCUSSION

EFFECT OF AVADEX B. W. ON THE CHROMOSOMAL ABERRATION FREQUENCY

In the root tips of control plants only a low frequency of the chromosome breaks and gaps during metaphase was observed. In other mitotic phases no chromosomal abnormalities were found (Tab. 1).

Treatment with Avadex B. W. at concentrations of 0.5% caused chromosomal aberrations at each phase of mitosis. About 8% of the prophase nuclei had chromosomes distinguished by irregular edges and structural changes, which indicated disturbances in the composition of the chromosomal protein complex (Phot. 1). Besides the increased number of chromosome breaks and gaps of the metaphase chromosomes in relation to the control metaphase chromatid exchanges, „minutes" and c-metaphases were also found (Phot. 2 - 7). In the remaining mitotic phases (anaphase and telophase) disturbances in the chromatid separation were observed, i.e: chromosome bridges (single or multiple) and laggards as well as chromosome fragments (Phot. 8 - 13).

In the material from this combination fixed 48 h after Avadex B. W. treatment, micronuclei and smaller than normal interphase nuclei with compact and intensi-

Table 1. The effect of the herbicide Avadex B. W. on the chromosomal aberration frequency in *Vicia faba* L. var. *minor* Beck roots (in the material fixed 24 h after the treatment)

Stage of mitosis	Aberration type	Concentration of herbicide Avadex B. W.								
		Control			0.5%			1.0%		
		number of		damaged cells (%)	number of		damaged cells (%)	number of		damaged cells (%)
		analyzed cells	aberrations		analyzed cells	aberrations		analyzed cells	aberrations	
Prophase	Changes of chromatin structure	200	–	–	200	16	8	110	20	18
Metaphase	Single gaps		3			21			43	
	Breaks		1			26			35	
	Chromatid exchange	200	–	2	200	2	23	200	6	38
	Minutes		–			3			2	
	C-metaphases		–			8			13	
	Chromosome bridges		–							
Anaphase	a) Single					18			21	
	b) Multi	200	–	–	160	10	21	85	12	43
	Chromosome fragments		–			6			4	
	Chromosome bridges		–			11			8	
Telophase	Chromosome fragments	200	–	–	110	4	14	70	7	21

vely stained chromatin were seen (Tab. 2). Such nuclei were not observed to have mitotic division and were recognized mitotically inactive (Phot. 14).

The occurrence of various disturbances in the chromosomal structure and mitosis of plant cells under effect of herbicides with some chemical groups (including carbamates) were also found by other investigators (Stroev 1970, Skorupska 1976, Klein et al. 1985, Samborska-Cianía 1985).

Table 2. The effect of the herbicide Avadex B. W. on the frequency of micronuclei and mitotically inactive nuclei in *Vicia faba* L. var. *minor* Beck. (in the material fixed 48 h after the treatment)

Concentration of Avadex B.W.	Frequency of	
	micronuclei	mitotically inactive nuclei
Control	2	0
0.5%	60	46
1.0%	152	252
1.5%	181	312

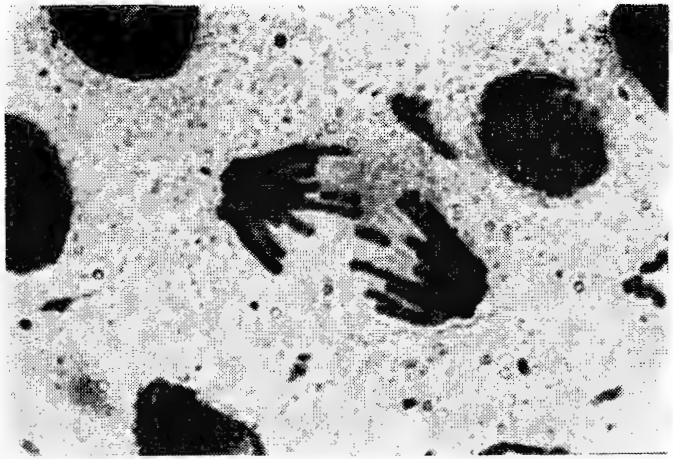
Moreover, Wuu and Grant (1966) as well as Tomkins and Grant (1972) proved that herbicides could considerably disturb the course of meiosis in plant cells and Pilinskaya (1970, 1981) found that pesticides are capable of causing chromosomal aberrations in human cells.

Some of the papers deal with correlations between the chemical dose and effect. This correlation is clearly seen also in this paper.

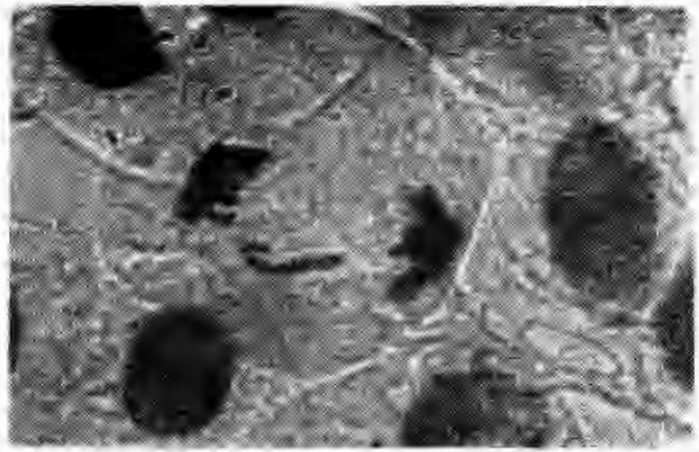
A 1% concentration of Avadex B. W. caused an increase in the abnormality frequency at each phase of mitosis (Tab. 1). The number of simple types of these abnormalities, i.e. breaks and gaps of metaphase chromosomes, increased to the highest extent, but the frequency of the remaining types of abnormalities was considerably higher than that in the roots treated with a lower herbicide dose (0.5%).

A detailed analysis of the number and type of abnormalities in the root tip cells treated with Avadex B. W. at the highest concentration, i.e. 1.5% appeared to be impossible because of too small number of division figures in these roots. On the other hand, the number of micronuclei as well as mitotic inactive nuclei in relation to other combinations showed a further increase of the chromosomal abnormality frequency (Tab. 2).

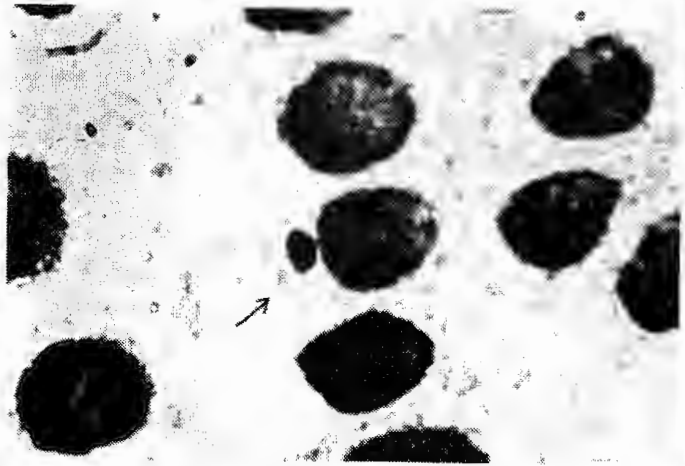
In spite of relatively numerous papers dealing with the effect of herbicides on plant, animal or human genetic material, the mechanism of their action remained unknown. However many authors agree that chromosomal aberrations are induced by disturbances in the basic metabolic processes in the cellular level and, first of all, by disorders in the enzyme synthesis (Alison, Paton 1965, Moreland, Boots 1971), by disturbances in auxin and gibberellin level (Narshingham, Kumar 1971) or in nucleic acids and protein synthesis (Bartels, Wolf 1965, Black, Myers, 1966, Moreland et al. 1969).



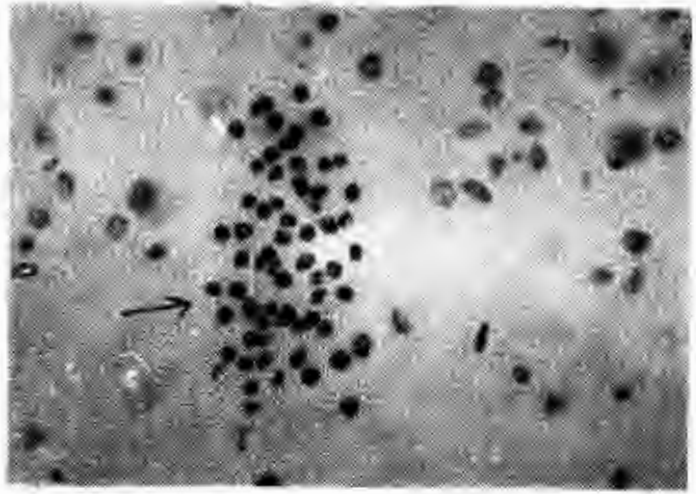
Phot. 12. Chromosome fragments in anaphase (1000×)



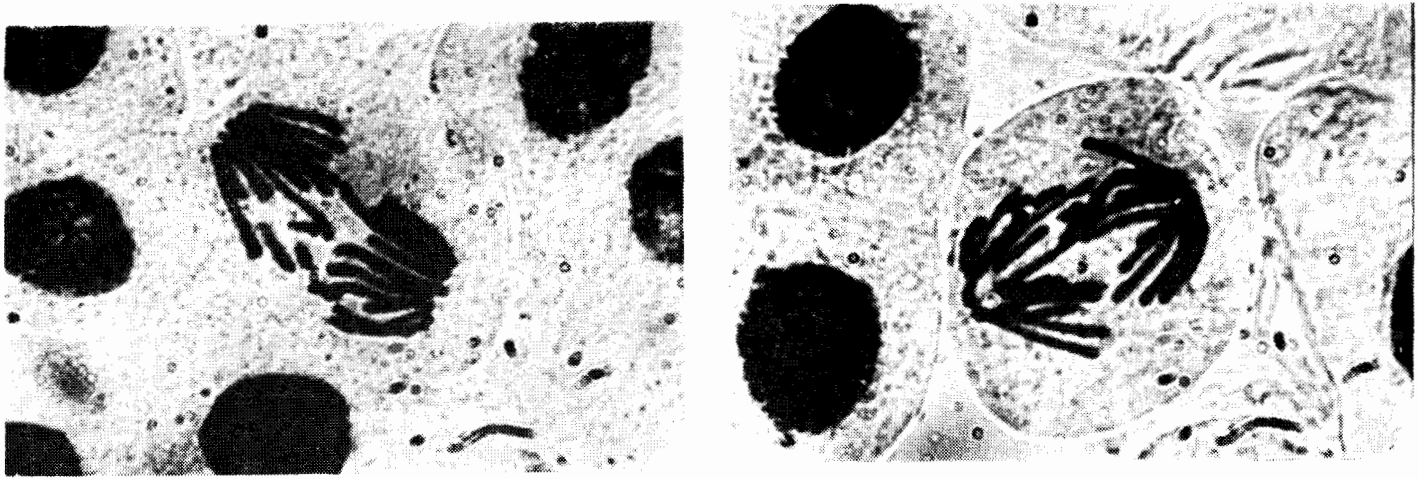
Phot. 13. Chromosome fragments in telophase (1000×)



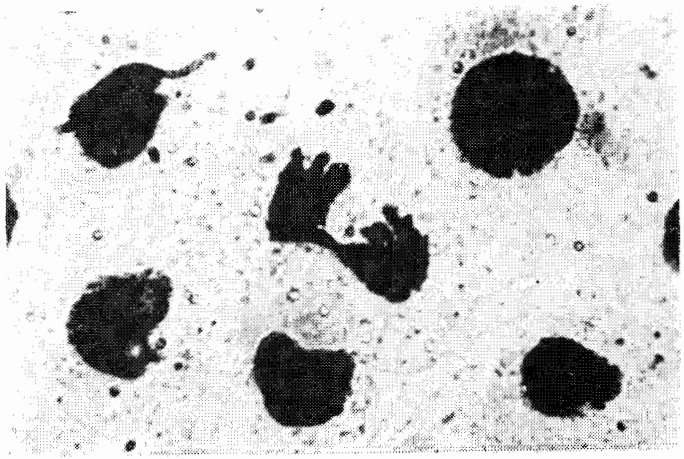
Phot. 14. Micronuclei (1000×)



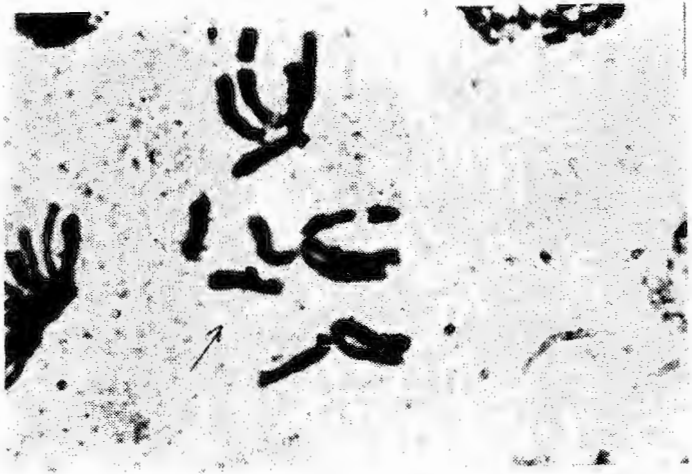
Phot. 15. Mitotic inactive nuclei (100×)



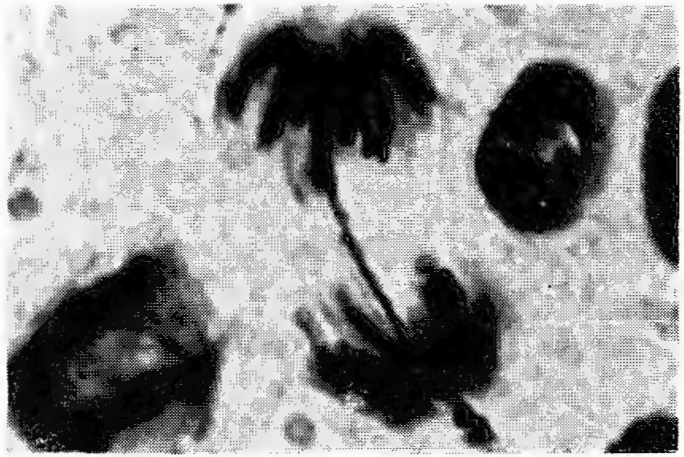
Phots 9 - 10. A multibridges in anaphase (1000×)



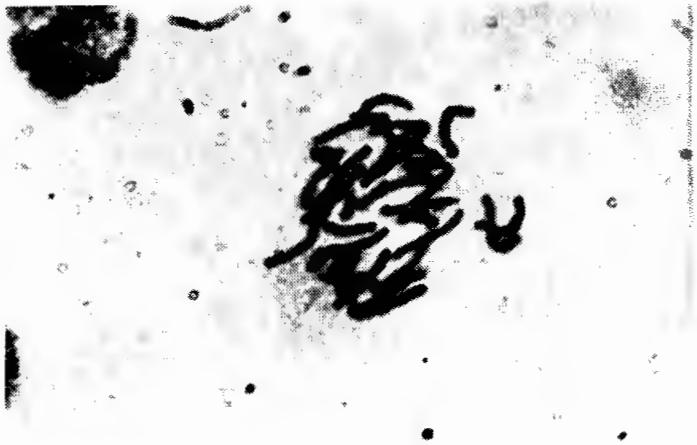
Phot. 11. A single bridge in telophase (1000×)



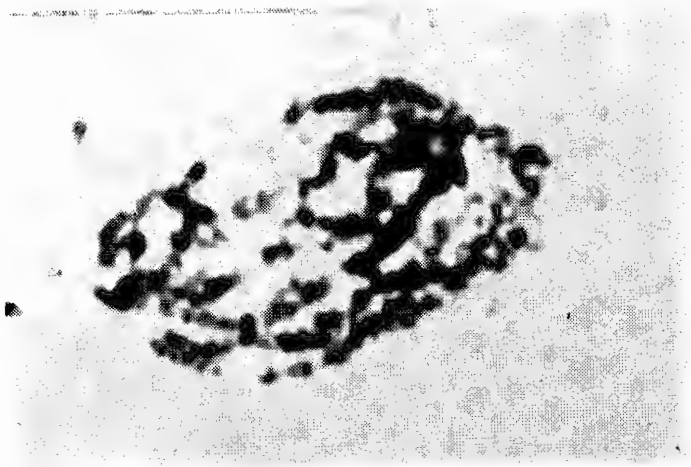
Phot. 5. Aberration "minutes" i metaphase chromosome (1000×)



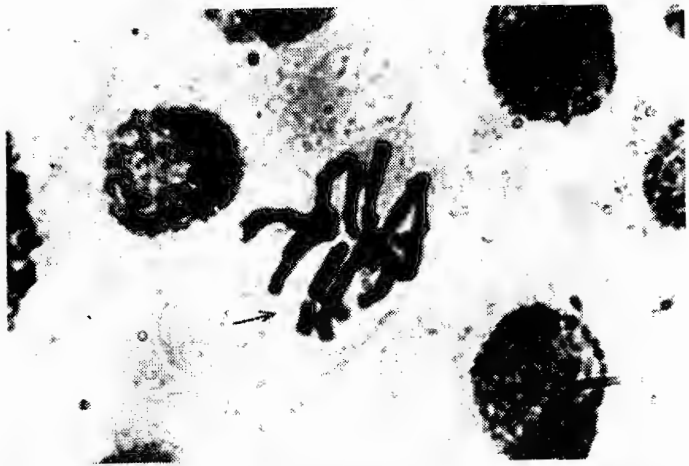
Phot. 8. Anaphase single bridge (1000×)



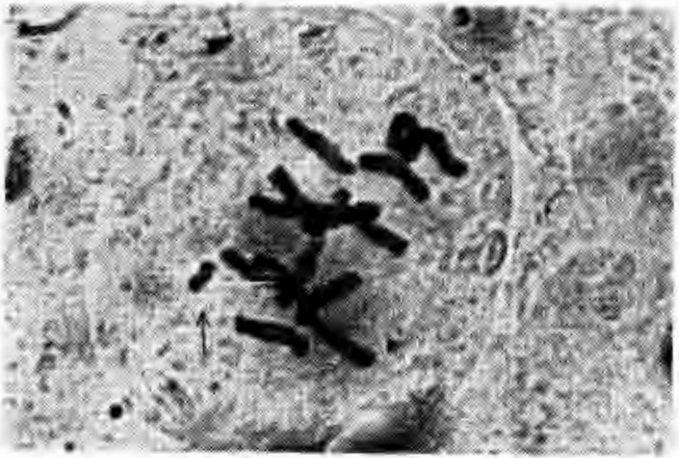
Phots 6 - 7. C-metaphase (1000×)



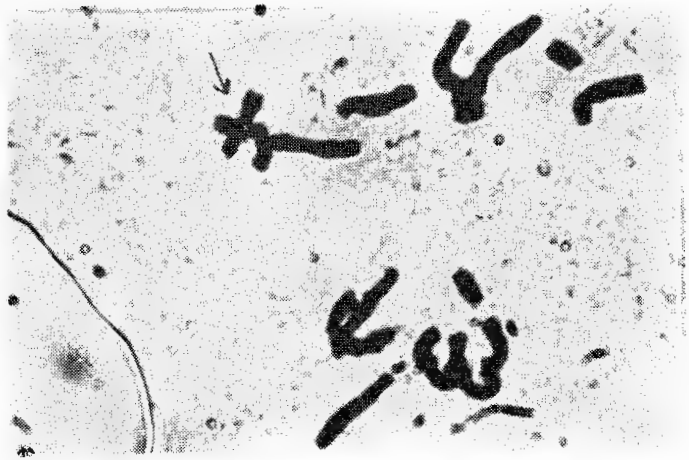
Phot. 1. Changes of chromatin structure in the prophase chromosomes (1000×)



Phot. 2. Single gap in metaphase chromosome (100×)



Phot. 3. Breaks in metaphase chromosome (1000×)



Phot. 4. Chromatid exchange in metaphase (1000×)

Effect of Avadex B. W. on mitotic division rate

The values of mitotic indices in the root tips of the control plants remained on a constant level (for various fixation times) and ranged from 9.6 to 10.1%.

Seed treatment with Avadex B. W. caused a considerable decrease in the mitotic rate (Tab. 3), which was directly proportional to the chemical dose. The antimitotic action of the herbicide was mainly based on the reduction of the number of cells which initiated division, because a regular decrease of the prophase number was observed (as herbicide concentration had increased). The number of prophases in the root tips treated with the highest concentration of Avadex B. W. was equal or even lower than the number of metaphases.

Table 3. The effect of the time of fixation of different concentration of the herbicide Avadex B. W. on the mitotic index in *Vicia fab* L. var. *minor* Beck.

Concentration of Avadex B.W.	Mitotic index (%)			P/M		
	time of fixation					
	24h	36h	48h	24h	36 h	48h
Control	9.6	10.1	9.8	2.6	2.7	2.5
0.5%	5.2	5.8	6.0	1.2	1.4	1.8
1.0%	3.6	3.8	4.5	0.8	1.0	1.3
1.5%	1.3	1.2	2.1	0.9	0.7	1.0

A decrease in the mitotic rate of plant meristem cells under the effect of pesticides was also reported by other authors. Stroev (1970) found that atrazine caused the mitotic index decrease in barley root tips. Klein et al. (1985) observed a similar phenomenon after treating the onion root meristem with some pesticides.

A comparison of the mitotic index values for various fixation times (for each Avadex B. W. concentration) indicated that it is possible to overcome a negative influence of the herbicide. In the roots tips fixed at 48 h after the treatment, a small increase in the mitotic index values as well as in the prophase number in relation to earlier times of fixation (24 and 36 h) was observed. This tendency was seen even when seeds were treated with the highest (1.5%) concentration of Avadex B. W.

CONCLUSIONS

1. Avadex B. W. used in 0.5, 1.0 and 1.5% concentrations was capable to induce chromosomal aberrations.
2. The number and type of abnormalities depended on the chemical dose.
3. In the root tips exposed to action of Avadex B. W. the number of dividing cells decreased and some cells lost their mitotic activity.
4. 48 h after the treatment the tendency to overcome an antimitotic action of herbicide was observed.

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ZMIANY CYTOGENETYCZNE W KIEŁKUJĄCYCH NASIONACH BOBIKU (*VICIA FABA* L. VAR. *MINOR* BECK) WYWOŁANE DZIAŁANIEM HERBICYDU AVADEX B. W.

Streszczenie

Badano wpływ herbicydu karbaminianowego Avadexu B. W. w stężeniu 0.5, 1.0, i 1.5% na przebieg mitozy w stożkach wzrostu bobiku odmiany Nadwiślański. Celem badań było ustalenie, czy badany preparat jest zdolny do wywoływania zmian w aparacie genetycznym roślin uprawnych. Obserwacje prowadzono na preparatach rozmazowych.

Stwierdzono, że Avadex B. W. indukuje abberacje chromosomowe we wszystkich fazach cyklu mitotycznego oraz obniża tempo podziałów komórkowych. Działanie herbicydu było w znacznym stopniu zależne od jego stężenia. Zwiększanie dawki powodowało wzrost częstości abberacji chromosomowych oraz spadek wartości indexu mitotycznego.

ЦИТОЛОГИЧЕСКИЕ НАРУШЕНИЯ В ПРОРАСТАЮЩИХ СЕМЕНАХ КОНСКИХ БОБОВ (*VICIA FABA* L. VAR. *MINOR* BECK), ВЫЗВАННЫЕ ГЕРБИСИДОМ AVADEX B. W.

Резюме

В работе исследовалось влияние карбаминового гербицида Avadex B. W. в концентрации 0.5, 1.0 и 1.5% на процесс митозы в конусах роста корней прорастающих семян конских бобов сорта Nadwiślański. Наблюдения проводились на препаратах-мазках.

Было обнаружено, что исследуемый препарат индуцирует хромосомные аберрации во всех фазах митотического цикла, а также снижает темп клеточных делений. Частота и род аберраций, а также значение митотического показателя зависили в большой степени от дозы гербицида.