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THE CYTOGENETIC EFFECTS OF SHEFFER A, A LIQUID FERTILIZER AND GROWTH REGULATOR IN ROOT TIP CELLS OF *VICIA FABA* L.

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Abstract: The present study concerns with genotoxic effects of a liquid fertilizer and growth regulator, Sheffer A and *Vicia faba* chromosomal aberation test was used to evaluate genotoxicity. Effects of Sheffer A on mitotic division and cromosomes have been studied. 50 ml/l, 100 ml/l and 200 ml/l doses of Sheffer A were applied on *Vicia faba* roots for 3, 6 and 12 hours. It was seen that, with the application of Sheffer A in excessive doses and long periods, mitotic index was decreased significantly when compared with control. Anaphase bridges, fragments, laggards, stickiness and micronucleus formation have been observed.

Keywords: Sheffer A, Vicia faba, Mitotic index, Chromosomal abnormalities, Micronucleus.

SIVI GÜBRE VE BİTKİ BÜYÜME DÜZENLEYİCİSİ SHAFFER A'NIN *VICIA FABA* KÖK UCU HÜCRELERİNDEKİ SİTOGENETİK ETKİLERİ

Özet: Bu çalışmada bir sıvı gübre ve bitki büyüme düzenleyicisi olan Shaffer A'nın genotoksik etkisi *Vicia faba* kromozomal aberasyon testi ile değerlendirildi. Shaffer A'nın mitoz bölünme ve kromozomlar üzerine etkisi 50 ml/l, 100 ml/l ve 200 ml/l lik dozlarının 3, 6 ve 12 saat süreyle *Vicia faba* köklerine uygulandı. Yapılan incelemeler sonucunda, Shaffer A'nın aşırı dozda ve uzun süre uygulanmasının kontrole göre mitotik indeksi önemli derecede azalttığı belirlendi. Ayrıca anafaz köprüsü, parça, kalgın kromozom, yapışkanlık ve mikronükleus oluşumu gibi kromozomal anormallikler gözlendi.

Anahtar kelimeler: *Sheffer A, Vicia faba, Mitotik indeks, Kromozomal anomaliler, Mikronükleus.*

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1. Introduction

Environmental pollution is one of the most important problems of our time. One of the reasons of environmental pollution is an increasing use of pesticides in agriculture. Pesticides used in fighting with crop hazardous have some adverse effects on target organisms and other living things directly or indirectly by causing pollution in soil and water. Use of plant growth regulators and fertilizers in excess amounts to obtain higher yields of crops cause genotoxic effects on living things [1-6]. They can accumulate in the food to a toxic level and consequently affect the public health.

With the increasing concerns on genotoxicity of environmental pollutants on living things, several plant system bioassays have been used to detect their genotoxicity including *Vicia faba* test system. *Vicia faba* has six pairs of large chromosomes, easy to grow and doesn't require any sterile conditions so it is easy to observe chromosomal aberrations with this system [7].

Büyük Menderes is one of the few basins of Turkey; which has the greatest agricultural potential with its proper climatic and soil properties. In this basin, Sheffer A is widely used as a liquid fertilizer and growth regulator in cultivation of some plants such as; tomatoes, pepper, strawberry etc. in excess amounts. Such liquid fertilizer, growth regulator and other chemicals may accumulate in certain food chains and eventually reach concentrations capable of yielding toxic or genotoxic effects.

As a result, human health may be affected. Thus, a correct estimation of the environmental pollution risk needs to be done [8]. Although this liquid fertilizer is used widely, there is no research in concerning with its effect on ecosystem so we decided to study effects of this liquid fertilizer Sheffer A on *Vicia faba* root tip meristematic cells to determine its relative degree of genotoxic potencies.

2. Material and Methods

In this study, *Vicia faba* (2n=12) seeds were used as the test system. Sheffer A, which is a vegetable-fruit fertilizer and growth regulator was used as the test substance. The chemical properties of Sheffer A are given in Table 1.

Seeds of *Vicia faba* were taken from Adnan Menderes University, Faculty of Agriculture. They were soaked in tap water for 24 hours at 25^0C and allowed to germinate in moist perlite in the dark for 4 days at 25^0C. At this time, seedlings were grown 3-5 cm long primary roots. The seeds coats were removed and shoot and primary root tips (5 mm) were cut off. The seedlings were incubated at 25^0C to allow the development of secondary roots. After 4 days, secondary roots had grown to 1-2 cm., which is a suitable length to be used in the experiments. The seeds were placed in petri dishes and secondary roots were treated with Sheffer A at the concentrations of 50, 100 and 200 ml/l for 3, 6 and 12 hours. Solutions were diluted with distilled water and tap water was used as control.

Table 1: Chemical content of Sheffer A

Specific gravity :kg/l (25ºC)	1.20
Storage temperature (min. temp.)	16
Ammonium nitrogen (N-NH_4) %	2.8
Nitrate nitrogen (N-NO_3) %	4.2
Total nitrogen (N-TOT) %	7
Phosphorous (P_2O_5) %	3
Iron (Fe) ppm	7
Manganese (Mn) ppm	300
Potassium (K_2O) ppm	150
Zinc (Zn) ppm	75

Roots of control and root of treated seeds were fixed in ethanol: acetic acid (3:1), stained with aceto-orcein and squashed. The

mitotic indexes, chromosomal aberrations and micronuclei were evaluated.

Analysis of variance of data was performed with SPSS. To determine statistical significance of data, one-way ANNOVA test was used. Table 2 indicates significant variation ($P<0.05$) in mitotic indexes (MI) and aberration comparing the number normal and aberrant cells at cells at each concentration and time with control. Table 2 shows the effect of Sheffer A on micronucleus formation in *Vicia faba* at different concentrations and time. 100 ml/l for 6 hours application is suggested dose for farmers but in this region farmers use excess amounts in order to obtain higher yields of crops. Therefore we decided to analyse effects of half and twice doses of Sheffer A on mitotic division and chromosomes and we applied suggested time (6 hours), half and twice time to observe effects of application time on division and chromosomes.

3. Results and discussion

Mitotic index can be used as a parameter in evaluating cell division frequency. Effects of Sheffer A on *Vicia faba* root tip cells in different doses and times are given in Table 2 and Figure 1. As it is seen in Table 2, 3 hours application of Sheffer A in 50, 100, 200 ml/l doses and 6 hours application of Sheffer A in 50, 100 ml/l doses caused some changes in mitotic index of *Vicia faba* but when compared with control these effects are not important statistically. 6 hours application of Sheffer A in 200 ml/l dose and 12 hours application of Sheffer A in all doses caused an important decrease of mitotic index and these effects important statistically ($P<0,05$).

Use of Sheffer A in excessive doses and long periods caused a significant decrease in mitotic indexes. Similar results were obtained by application of different herbicides, insecticides, plant growt regulators and chemical substances [2, 9-13]. Mitotic inhibition caused by different chemicals can be related with an increase in G_2 period [14]. According to our findings, excessive and long period application of Sheffer A has such an effect.

In addition of Sheffer A's effects on mitotic index, it causes some chromosomal aberrations as it is seen in Table 2 and Figure 2. Most observed chromosomal aberrations are; fragments, chromosomal stickness, anaphase bridges and formation of micronuclei. Similar results were obtained by using other tests [3, 15-17]. Chromosomal aberrations formed by application of Sheffer A are similar to aberrations formed by other pesticides, plant growt regulators and chemical mutagens. Anaphase bridge formation is a result of dicentric chromosomes and chromosomal stickeness. Stickeness occurs as a result of subchromatic bridges [18]. Effects of chemicals on spindle apparatus cause some abnormalities such as; stickeness, multipolar anaphase, chromosome bridges and unequal chromosome distributions [14,19]. Such chromosomal irregularities can affect the vigour, fertility, yield or competitive ability of the exposed plants [20].

Application of Sheffer A in all doses and times caused formation of micronucleus and important statistically (Table 2). Micronucleus formation is the result of acentric fragments or loggers being excluded from nucleus during mitosis [17]. They are small cytoplasmic substances, which form as a result of chromosome breaks and aneuploidi during cell division [21]. Micronucleus formation is considered to be one of the most economical, quickest and most effective way in determining genotoxicity of different chemicals [2, 17, 22, 23]. In conclution, as it has been above, Sheffer A has harmful effects on the root tip cells of *Vicia faba*. In addition to these findings, increase of soil and water pollution can lead to certain irreversible

cytogenetic effects in plants and even in higher organisms.

Table 2: Effects of Sheffer A on mitotic index , number of aberrant cells and micronuclei of *Vicia faba*

Application time	Application dosage	Total number of cells scored	Total dividing cells	Mitotic index (MI)	Tot.div. abnormal cells	MCN/1000 cells
Control	Control	6403	653	10.2±3.41	4	0.33
3 hour	50 ml/l	4813	556	11.6±3.20	13*	8.2*
	100 ml/l	5190	518	10.0±4.29	24*	16.7*
	200 ml/l	5017	529	10.5±3.25	56*	29.6*
6 hour	50 ml/l	5042	590	11.7±2.37	32*	13.8*
	100 ml/l	4630	553	11.9±3.22	42*	30.2*
	200 ml/l	4760	16•	0.34±3.18*	10*	34.4*
12 hour	50 ml/l	4760	128•	2.7±4.57*	56*	15.0*
	100 ml/l	4710	16•	0.34±3.20*	8*	40.0*
	200 ml/l	5048	8•	0.16±3.01*	6*	36.0*

* important statistically (P<0,05)
• toxic, inhibited mitosis

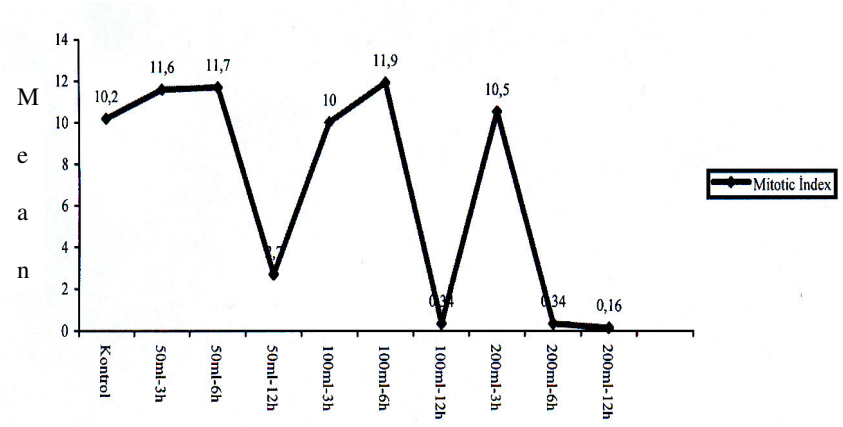

Figure 1: Mitotic index of root-tip cells of *V. faba* treated by Sheffer A with different concentrations and time.

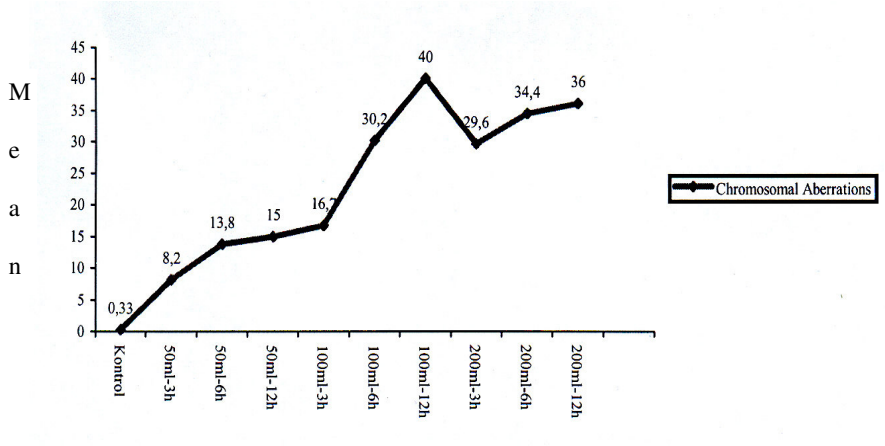

Figure 2: Aberrations of root-tip cells of *V. faba* treated by Sheffer A with different concentrations and time.

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