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Bahtiyar Vahabzade'nin Ana Dili Uğrunda Verdiği Mücadele
Erdal Karaman

Azerbaycan 1988-1995: Sancı, Kargaşa ve İktidar
Ömer Faruk Ünal

Problems of Young People Related to Anthropometric Measures of Ready-Made Clothes
Canan Yertutan

Using of Income in the Family Life Cycle: A Turkish Sample
Seval Güven

Azərbaycan-Türkiyə Xarici İqtisadi Əlaqələri və Azərbaycan Sənayesində Türkiyə Kapitalının Rolu
Ayhan Erdal

Köktürk Ədəbi Dilində Samit Səslərin İmlası
Elşən Şükürlü

Proximate And Fatty Acid Composition of Apricot (Prunus armeniaca L.) Kernels
Musa Özcan

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AN INVESTIGATION ON CYTOLOGICAL EFFECTS OF TONIFRUIT ON VICIA *FABA* L.

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ABSTRACT

The effects of Tonifruit on the mitotic division, chromosomes and nuclear DNA amount have been investigated in *Vicia faba L.* 300 ppm, 600 ppm and 900 ppm of Tonifruit doses were applied to *V. faba* roots for 6 and 24 hour periods.

It was observed that the substance caused a decrease in mitotic index when compared to the control group. It was also observed that this substance caused abnormalities on chromosomes such as C-metaphases, anaphase bridges, breakages, stickiness and fragments. In conclusion, different doses and period of Tonifruit treatment of the roots of *V. faba* increased the nuclear DNA amount when, compared to control the control group.

Key Words: Tonifruit, mitotic division, Vicia faba L.

TONİFRUİT'İN *VICIA FABA* L. ÜZERİNDEKİ ETKİLERİNE DAİR BİR ARAŞTIRMA

ÖZET

Tonifruit'in *Vicia faba* L.'da mitotik bölünme, kromozomlar ve çekirdek DNA miktarı üzerine etkileri araştırılmıştır. Tonifruit'in 300 ppm, 600 ppm ve 900 ppm'lik dozları 6 ve 24 saat sure ile *V. faba* köklerine saat uygulanmıştır.

Yapılan incelemelerde, uygulanan maddenin mitotik indeksi kontrole göre azalttığı saptanmıştır. Aynı zamanda bu maddenin kromozomlar üzerinde C-metafaz, anafaz köprüsü, kırılma, yapışkanlık ve fragment gibi anormalliklere de neden olduğu gözlenmiştir. Ayrıca, Tonifruit'in farklı süre ve dozlarla *V. faba* köklerine uygulanması sonucu da çekirdek DNA miktarı kontrole göre artmıştır.

Anahtar Kelimeler: Tonifruit, mitotik bölünme, Vicia faba L.

INTRODUCTION

In our country (Turkey), different pesticides and plant growth regulators are being used extensively in modem agriculture, the importance of which is undoubtedly economic due to its low cost and easy of application.

Though the use of these chemicals has become a necessity, their frequent and indiscriminate use has proved to have many undesirable consequences in culture plants. A number of workers have carried out studies to show the cytological effects different agrochemicals on different plants species (1-4)

Tonifruit (% 1.18 Alphanaphtylacetamide, % 0.43 Alphanaphtyiacetic acid), which is a plant growth regulator, is being widely and frequently used in speeding the development of fruits such as aubergine, tomato, watermelon, muskmelon, strawberry, squash etc. (5).

The aim of present work is to determine the effect of Tonifruit on root-mitosis of *V. faba* root-treatments.

MATERIAL AND METHODS

The seeds of *V. faba* were soaked in distilled water for 24 hours and then germinated on a filter moistened with distilled water in petri dishes at 25° C. Germinated seeds of *V. faba* were treated with 300 ppm, 600 ppm and 900 ppm doses of Tonifruit for 6 and 24 hours (the effective dose of Tonifruit used in agriculture is 600 ppm). Following all treatments, root-tips were excised and fixed in freshly made 3/1 absolute ethyl alcohol/glacial acetic acid (v/v) for 24 hours at 4°C. Root-tips were washed in distilled water for 30 minutes. Thereafter, they were hydrolysed in 1N HCI for 5 minutes and stained in aceto-orcein solution for one hour. The stained root-tips were squashed in 45% acetic acid (6) and observed under the microscope. In addition, the control root-tips were simultaneously treated *with distilled water and the same stain* procedure was also applied.

In examination under the microscope, from each slide three field of view were randomly selected and total number of dividing cells, total number of abnormal cells as well as the number of cells with C-metaphase, anaphase bridges, breakages, stickiness and fragments were scored. The chromosome abnormalities were photographed with a Nikon AFX-DX.

For Feulgen cytophotometric estimation of 4C nuclear DNA contents the fixed root-tips were hyrolysed in 1 N HCI for 12 minutes at 60 °C and stained in Feulgen solution for one hour. The stained root-tips were washed in three changes of $S0_2$ water for 10 minutes each and dried briefly on absorbent paper. Darkly stained root-tips were squashed in a drop of 45 % glacial acetic acid. Cytophotometric measurement of 4C metaphase nuclei were made, using a Reichert-Zetopan microspectrophotometer, at a wavelength of 550 nm. On average, 35 4C metaphase nuclei were measured in each of three replicates in every doses of Tonifruit. Measurement were converted to absolute amounts using *Allium cepa L.* as a standard (4C = 67 pg) (7).

The mitotic index (M.I.), chromosome aberration and 4C nuclear DNA contents of *V. faba* were tested by analysis of variance (ANOVA) and comparisons between means were performed with Tukey test (8).

RESULTS

The effect of different treatments with Tonifruit on mitotic index, chromosomes and 4C nuclear DNA content in root meristems of *V. faba* are shown Table 1, 2 and Figure 1, 2. As presented in Table 1, the mitotic index decreased with increasing concentrations and exposure time when compared to means of control values. It was observed that the difference between each other doses was not significant for treatment of 6 and 24 hours periods. In addition, it was observed that chromosome aberrations on root-tips of *V. faba* at treatment with of Tonifruit for 6 and 24 hours. The most common type of abnormality was C-metaphase. In addition, anaphase bridges, breakage, stickiness and fragments occurred on chromosomes (Table 2, Figure 3 a, b, c).

Abnormal cell numbers were generally increased due to concentration of doses and this increase was significant when compared to the control groups. In addition, statistical analysis showed that the difference between doses of 300 ppm, 600 ppm and 900 ppm of Tonifruit was significant for 6 hours. On the other hand, difference between 300 ppm and 900 ppm doses was significant for 24 hours. The most abnormal (dividing) cells were observed at 900 ppm dose for 24 hours.

4C nuclear DNA contents in picograms at 300 ppm, 600 ppm and 900 ppm for 6 and 24 hours are presented in Table 1. 4C nuclear DNA content of *V. faba are* found to be 57.58±0.36 picograms. In this table, we observed that mean 4C nudear DNA contents of *V. faba* were increased with increasing concentration and periods, when compared to control groups. In addition, 4C nudear DNA contents were approximately two times increase in treatment with three different doses of Tonifrurt for 24 hours when compared to 6 hours.

Table 1. Total dividing cells, mitotic index (M.I.) and 4C nuclear DNA content after treatments of *V. faba* roots with Tonifruit

Doses	Number of cells scored	Total dividing cells	M.I. Mean±SE	4C Nuclear DNA Content (pg) Mean±SE *
Control	3043	344	1 1 .38±0.40 a	57.58±0.36 a
300 ppm (6 h)	3078	129	4.42±0.50 b	64.56±0.01 b
600 ppm (6 h)	3104	96	3.32±0.70 bd	59.52±0.30 ae
900 ppm (6 h)	3113	87	2.81±0.09 cd	62.63±0.70 be
300 ppm (24h)	3085	62	2.00±0.20 c	102.57±1.08 c
600 ppm (24h)	3152	79	2.47±0.10 cd	102.88±1.04 c
900 ppm (24h)	3044	79	2.67±0.20 cd	109.81±2.72 d

S.E.: Standart Error; pg : Picogram.

*: Means with the same letters do not significantly differ at 0.05 level.

Table 2. Total abnormal dividing cells and the different types of the abnormalities occurring in the mitosis of *V.* faba roots after treatments with Tonifruit

Doses	Number of cells scored	Total dividing cells	Total dividing abnormal cells	Abnormal dividing cells (%) Mean±S.E. *	C-M	A.B	B	S	F
Control	3043	344	0	0.00 a	0	0	0	0	0
300 ppm (6 h)	3078	129	31	1.16±0.08 b	30	1	0	0	0
600 ppm (6 h)	3104	96	60	1.92±0.30 c	56	0-	2	1	1
900 ppm (6 h)	3113	87	77	2.59±0.20 d	77	0	0	0	0
300 ppm (24 h)	3085	62	53	1.86±0.10 c	50	0	0	0	3
600 ppm (24 h)	3152	79	71	2.23±0.20 cd	56	0	0	0	15
900 ppm (24 h)	3044	79	84	2.72±0.20 d	72	1	0	0	11

C-M: Metafaz; **A.B:** Anaphase Bridges; B: Breakage; S: Stickiness; F: Fragment; S.E : Standart Error *: Means with the same letters do not significantly differ at 0.05 level.

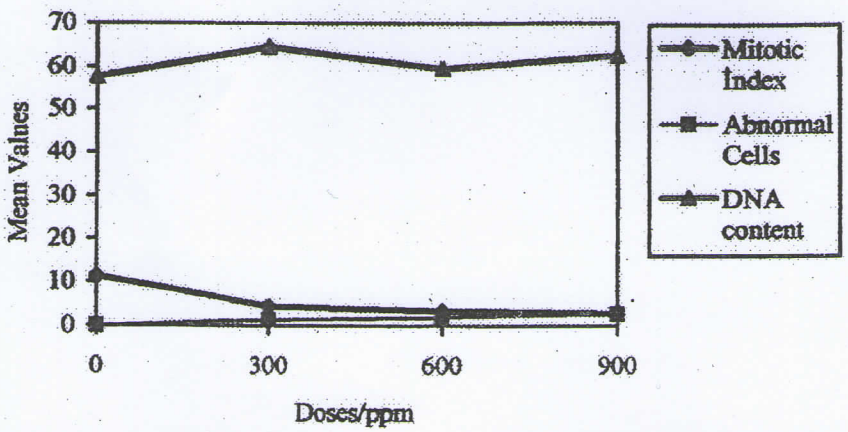

Figure 1. Relationship between dividing cells, abnormal cells, 4C nuclear DNA contents and different concentrations of Tonifruit on *V. faba* root-tips for 6 hours.

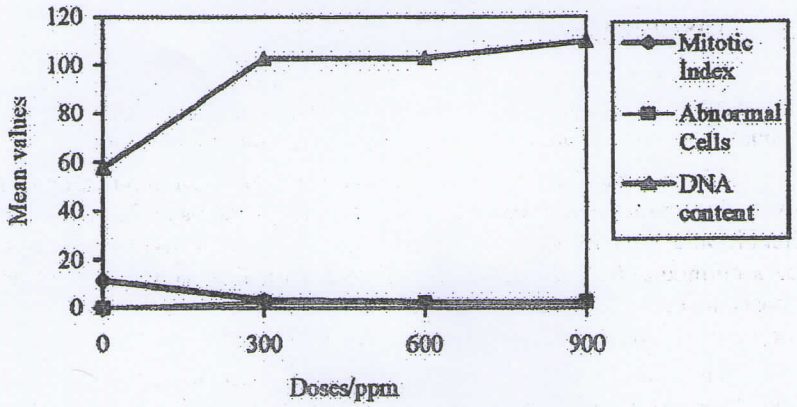

Figure 2. Relationship between dividing cells, abnormal cells, 4C nuclear DNA concentrations of Tonifruit on *V. faba* root-tips for 24 hours.

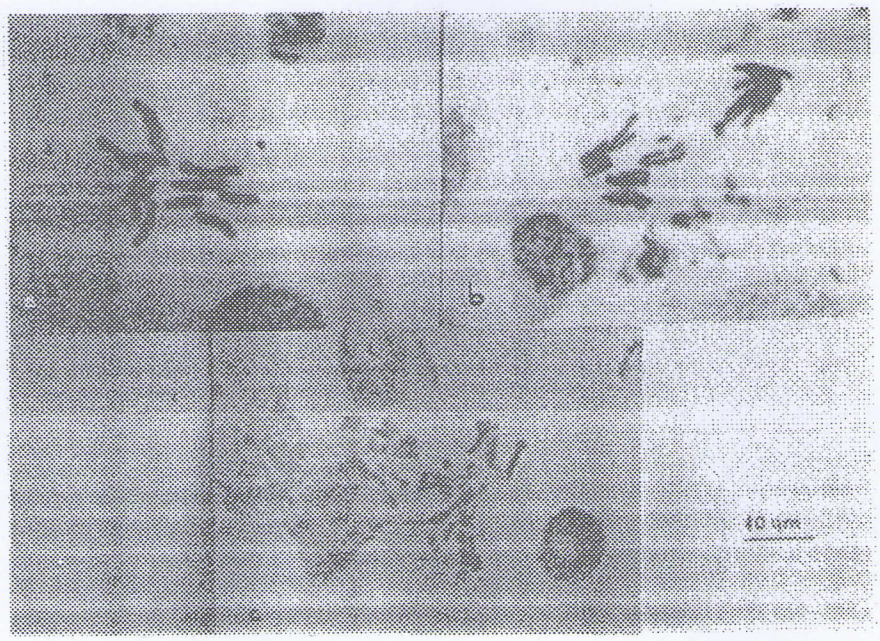

Figure 3. Chromosomal abnormalities induced by Tonifruit on *V. faba.* a) C-Metaphase, b) Breakage, c) Fragment

DISCUSSION

In root-meristem cells of *V. faba* treatment with Tonifruit at three different doses for 6 and 24 hours resulted in several chromosomal abnormalities, reduction in mitotic index and increase in 4C nuclear DMA content.

Mitotic inhibition by chemicals has been attributed to blocking of mitotic cycle during interphase which may result from a prolonged G_2 period (or to the inhibition o* DNA synthesis) (10-13). The reduction of mitotic activity seem to be a common effect of most chemical tested for their action on mitosis. As a consequence, we conclude that inhibition of mitotic activity was correleted with total cytogenetic damage to the chromosome in *V. faba.*

Tne total percentage of abnormalities increased with increasing durations ano doses o^4 Tonrfruit treatment (Table 2). The chromosomal abnormalities produced by Tonifruit are C-metaphase, anaphase bridges, breakages, fragments and stickiness. The colchicine type C-metaphase of configurations was the major mitotic abnormality produced in the root of *V. faba* treated with Tonifruit. The inhibition of spindle apparatus by Tonifruit may be due to the effect of this chemical on the proteins constituting the spindle apparatus. It has been reported the chromosomal abnormality by various studies (12-15). The formation of anaphase bridge may be due to chromosome breakage and reunion. On the other hand, bridges produced by Tonifruit may be attributed to the stickiness of chromosomes, which makes their separation, and free movements complete and thus they remain connected by bridge.

Chromosomal breakage, stickiness and fragments also occurred in root-tips of *V. faba* in a low percentage. The heterochromatic regions all chromosomes before all else break (16). It has reported the certain regions on some chromosomes of *V. faba* firstly may react with chemical substances (17). These regions are the breaking points on chromosomes of *V. faba.* Therefore, we think that Tonifruit is effective on heterochromatic regions. The occurrence of fragments at mitosis may be attributed to the failure of broken chromosomes to recombine (18). Stickiness has been attributed to the improper folding of chromosome fibers, which makes chromatids connected by means of subchromatid bridges (19). However, the sticky nature of chromosomes may be due to a delay in chromosomes could not reach the poles and remained scattered in the cytoplasm and appeared condensed and sticky.

We observed that 4C nuclear DNA contents of *V. faba* increased after treatments with Tonifruit (Table 1, Figure 1, 2). The increase in 4C nuclear DNA content of *V. faba* with Tonifruit can have attributed to an effect like colchicine, which has inhibitory effect on spindle. It has shown that the plant growth regulatory substances have effect on nucleic acids and proteins (20). Hormones are among the substances known to induce nuclear DNA variation in plants. In particula-gibberellic acid can stimulate extramitotic DNA synthesis during the elangation of the epicotyl of Lens *culinaris* (21); analogously in the hypocotyl of *Cucumis sativus* a relationship has been hypothesized between

growth substances and the increase of DNA (22); in vegetative stem segments of *Nicotina tabacum a* direct correlation between indole-3-acetic acid and DNA synthesis has been observed (23). Finally, the influence of external elements such as growth factors, chemical substances, culture medium composition, or different stimuli of environmental character, can have on the quantitative variation of nuclear DNA sequences.

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