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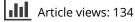
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Cytogenetic Effects of Urginea maritima L. Aqueous Extracts on the Choromosomes by Using Allium Test Method

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Abstract — The genotoxic and cytotoxic effects of extracts of *Urginea maritima* L., belonging to the family Liliaceae, were investigated with Allium test which is a plant test method. The bulbs of *Allium cepa* were treated with 2%, 4%, 6% of *U. maritima* aqueous extracts and a 20 ml/l solution of Vydate which is a chemical pesticide. Meristem cells of *A. cepa* root tip were prepared according to Feulgen squash procedure after stained with feulgene to identify the chromosomal aberrations (breaks, fragments, sticky). All observed data were compared statistically. As a result, the chromosomal aberrations were increased by the increase of dose and application time in all extracts of *U. maritima* and Vydate solution and the mitotic index (MI) was significantly reduced. The extracts of *U. maritima* were less genotoxic and cytotoxic than Vydate. It is stated that the usage of *U. maritima* extracts will be less harmful than chemical pesticides in plant protection.

Key Words: Allium test, Biopesticide, Cytogenetics, Urginea maritime.

INTRODUCTION

It is necessary to battle plant diseases, pesticides and competing weeds in order to have a quality and an abundant yield in agricultural endeavors. Pesticides are commonly used in the modern agriculture practices. However, as a result of the extended and prolonged use of pesticides, have emerged some problems in agriculture (DELEN 2003).

Pesticides may create a tolerance in harmful organisms as a result of intense use. They also have the potential to harm non-target organisms, thus they can cause perturbations in the balance of nature. The downstream effects of ecological disruption are manifold ranging from shifting of natural pollination mechanisms to environmental pollution whereby pesticides leave their compounds and breakdown products on plants or in the soil, air and underground bodies of water, so that they pose threats for the health of people (MADANLAR *et al.* 2002; DELEN *et al.* 2005).

Because of the aforementioned risks and damages of synthetic pesticides, in recent years there has been a great increase in the number of the studies carried out to examine the effects of biopesticides in the agricultural context. Plant extracts, especially, the compounds of terpenoids, alkaloids and phenolics have been examined recently with respect to their effects on the growth and development of harmful insects (ERTÜRK et al. 2004). While many of these natural plant substances share some of the potent characteristics of commercial synthetic pesticides, most natural plant products do not have the problem of creating harmful residues or breakdown materials that would damage plants or harm human beings and animals. In fact, they hardly have any harmful effects on the plant-animal relationships in nature. However, much of the biological activity of natural products is still not well-documented. Some of the questions that remain regarding some compounds of the alkaloids, phenolics and terpenoids include: how long it takes for these natural products to disintegrate or disappear from nature (i.e. underground water, soil)? What is their role in the food web as a whole? Are there significant reactions with other living organisms in water and land? With which chemicals do they form synergistic or antagonistic interactions? What are the treatments or toxicity or antidote levels for animals including humans? The purpose of this study is to investigate the genotoxic and cytotoxic effects of Urginea maritima bulb extracts which have properties which allow for potential use as a biopesticide by using the Allium test method.

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MATERIALS AND METHODS

Collection of the Plants and their Extraction - The material used in this study is the extract of *Urginea maritima* L. Baker bulbs which has medicinal and insecticidal activity properties from the family of Liliaceae (PASCUAL and FERNANDEZ, 1999).

Urginea maritima bulbs were collected from Ortaca-Muğla (Turkey) in February-March, 2004. After collection, the bulbs were broken into smaller parts then dried in a cool, dry environment with no direct sunlight. Extracts were obtained from dried *Urginea maritima* bulbs in a Soxalet mechanism through a hot water (100 °C) extraction method. Distilled water (100 ml) was slowly stirred with 2 g *U. maritima* bulb for 4 h (CIVELEK and WEINTRAUB, 2004). The extracts obtained were stored at + 4 °C' in a fridge.

Allium Test - The effects of extracts on cells and chromosomes were examined through a very cheap, simple and sensitive assay called the Allium test (FISKESJÖ, 1981). In order for Allium *cepa* to produce roots whereby their growth could be monitored, it was placed into beakers including 2, 4, 6% doses of Urginea maritima aqueous extract (v/v) and 2% (v/v) vydate solution. Tap water was used as a control, for every application of the four replicate experiment mechanisms. This rooting experiment was conducted at 20 \pm 2°C room temperature. Vydate is a commercially available pesticide whose active ingredient is Oxamly ((N,N-dimethyl-2-methylcarbamoyloxyimino-2-(methylthio) acetamide) and which has contact and systematic insecticide, acaricide and nematocide properties. In our experiments, a standard dose of Vydate, (2%), was used. At the end of the 48, 72, and 144 h of exposure, at least ten root tips from each treatment were examined in Carnoy solution (absolute alcohol: chloroform: glacial acetic acid, 6:3:1) for four h and then exposed to cold hydrolysis in 5 N HC1 for 10 min. At the end of the hydrolysis process, after removing HCl from root tips with 70% ethyl alcohol and distiled water, they were kept at least 24 h + 4°C in darkness and painted with feulgene. Until they were examined under the light microscope, they were stored in the same conditions. Root tips were prepared according to squashing technique (ELCI, 1994) and from these squashed prepared root tips, ten random places were selected for observation. The number of total cells, divided cells and mitotic phases, and the fragments in divided cells, chromosome stickiness, pole deviations, nucleus aberrantly, numerical chromosome changes. similar chromosome anomalies were determined

for each examined area. Photographs were taken, and ratios were calculated. Moreover, for each dose and treatment period, the ratio of divided cells to total cells, mitotic index (MI), was calculated.

Statistical Analysis - The statistical analyses were performed by using SPSS 14.0 software analysis program and every time-period was evaluated within itself, the significance level was accepted to be *P< 0.05 and One-Way ANOVA and LSD tests were used in the analyses.

RESULTS

This study examined the cytogenetic effects of all the extracts *U. maritima*, which is 2, 4, 6%, and 2% vydate on the growth of *Allium* roots.

Effects on the Number and Length of Roots According to Their Treatment Dose and Period - According to the treatment period, while the control, the Allium root tips were the healthiest, the root tips of Allium rooted in the U. maritima aqueous extracts were less healthy than those of control. Vvdate solution, which is a chemical pesticide was found to be the unhealthiest. It was observed that the emerging root tips of Allium bulbs in U. maritima extracts were shorter and thicker when compared to those in the control group depending on treatment period. Moreover, Allium root tips were observed to soften when the treatment period was increased. The decrease, seen in the root elongation dependent on the dose is thought to result from the decrease in the number of the total divided cells in meristem tissue due to toxic effect of the extract (Table 1).

Effects on the Mitotic Index (MI) according to their Treatment Dose and Period - At the end of the 48 h treatment period, the highest level of MI (4.9%) was observed in vydate solution, and this was followed by control (1.7%). At the end of the 72 h treatment period, the highest level of MI (3.3%) was observed in the control and this was followed by 6% *U. maritima* extract (2.6%), 2% *U. maritima* extract (2%) and in vydate treatment (1.45%). At the end of the 144 h treatment period, the highest level of MI (17.01%) was observed in 2% *U. maritima* and this is followed by 4% *U. maritima* (16.4%) and in the control (10.8%) (Table 1).

All the extracts caused to the appearance of aberrant cells in root meristem cells in line with the increase seen in the dose and period when compared to the control, yet it was also observed that these treatments caused statistically significant decreases in MI (Table 1).

Treatment	Treatment	Avarage	Mean	Mean	Mean	Mean	%
Doses	Period	root lengts	Prophase	Metaphase	Anapahese	Telophase	MI
	(hour)	$(mm) \pm SD$	± SD	± SD	± SD	± SD	\pm SD
С	48	25 ± 0.63 e	14 ± 3.37 a	8 ± 2.07 b	7.6 ± 2.19 d	2.2 ± 0.47 bc	1.7 ± 2.44 ab
V	48	6.5 ± 0.34 a	41 ± 4.62 b	18 ± 2.45 c	$4.6 \pm 0.60 \text{ ab}$	$3.2\pm0.29~\mathrm{c}$	4.9 ± 0.34 b
2%	48	14 ± 0.36 d	$7 \pm 1.08 \text{ a}$	1.2 ± 0.20 a	1.2 ± 0.13 a	1.1 ± 0.17 ab	0.7±0.05 a
4%	48	11.5 ± 0.37 b	7 ± 0.70 a	1.5 ± 0.43 a	1.2 ± 0.36 a	0.6 ± 0.19 a	0.7 ± 0.13 a
6%	48	12 ± 0.69 c	6.1 ± 1.04 a	4 ± 1.43 ab	3 ± 0.86 a	1.8± 0.61 b	1 ± 0.24 ab
		df: 4.49	df: 4.49	df: 4.49	df: 4.49	df: 4.49	df: 4.49
		F:193.958	F: 29.452	F: 18.079	F: 5.428	F: 6.802	F: 2.507
С	72	45 ± 0.64 d	38 ± 8.22 b	24 ± 6.30 a	2.2 ± 0.71 ab	0.7 ± 0.15 a	3.3 ± 0.75 a
V	72	7.5 ± 1.96 a	$10.7 \pm 1.01 \text{ a}$	8 ± 3.70 a	2.3 ± 1.71 ab	1.1 ± 0.42 a	1.45 ±7.07 a
2%	72	17 ± 0.42 c	19 ± 8.41 ab	14 ± 6.35 a	0.8 ± 0.30 a	0.6 ± 0.15 a	2 ± 0.73 a
4%	72	14 ± 0.70 b	10 ± 2.90 a	7 ± 2.80 a	1.4 ± 0.32 a	0.5 ± 0.18 a	$1.3 \pm 0.44 a$
6%	72	15 ± 0.93 b	27 ± 4.85 ab	13 ± 2.39 a	4.2 ± 0.53 b	3.8 ± 0.53 b	2.6 ± 0.40 a
		df: 4.44	df: 4.44	df: 4.44	df: 4.44	df: 4.44	df: 4.44
		F: 504.694	F: 2.659	F: 1.303	F: 2.494	F: 18.993	F: 0.907
С	144	70 ± 4.46 c	125±11.15 c	46 ± 3.89 bc	14 ± 2.62 bc	19.1 ± 2.23 c	10.8 ± 1.08
V	144	7.5 ± 0.50 a	13.3±1.90 a	4.5 ± 0.80 a	1.8 ± 0.59 a	1.3 ± 0.26 a	1.5 ± 0.27 a
2%	144	21 ± 1.19 b	198 ± 23.76 d	64 ± 8.08 cd	19 ± .20 c	25.6 ± 4.89 c	17.01 ± 2.40
4%	144	16 ± 0.97 b	209 ± 15.27 d	74 ± 10.38 d	12 ±1.82 b	$8.8\pm1.87~\mathrm{b}$	16.4 ± 1.48
6%	144	16.5 ± 1.64 b	60.3 ± 12.46 b	33.3 ± 6.27 b	5.11 ± 0.94 a	4.9 ± 1.20 ab	5.02 ± 1.12
		df: 4.39	df: 4.39	df: 4.39	df: 4.39	df: 4.39	df: 4.39
		F: 108.619	F: 40.767	F : 15.586	F: 11.433	F: 19.466	F: 27.017

Table 1 — Average root length, mitosis phases (prophase, metaphase, anaphase, telophase) and MI % according to treatment dose and period.

*Significant at 0.05 level. C= Control. V= Vydate.

Aberrant Cells Observed according to Treatment Dose and Period of the Extracts - In all of the doses and periods of treatment in the study, various abnormal cells were observed. Anomalies that were observed in the study are as follows: stickiness (3.85%, Figure 1.a), fragmentation (3%, Figure 1.b), irregular metaphase (1.55%), bridge occurrence in anaphase (1.2%, Figure 1.c), anaphase pole deviations (1.15%, Figure 1.d) and irregular anaphase (0.65%, Figure 1.e) (Table 2).

In particular, as a result of growth in vydate solution, splits in interphase nucleus (Figure 1.f) can be referred to nucleus deformation and nucleus vacuolization (Figure 1.g). The other observed anomalies were not statistically significant. These included: micronuclei occurrences (Figure 1.h), changes in the number of chromosomes (aneuploidy, polyploidy etc.) (Figure 1.1,i), C-mitosis (Figure 1.k), bi-nucleate cells (Figure 1.l) and increases in the number of nucleolus in the nucleus. According to the types of treatments, at the end of 48-h treatment period, the highest rate of aberrant cells was obtained in the Vydate treatment (31.5) and this was followed in order by the 6%, 4%, 2% *U. maritima* treatments. At the end of the 72 h treatment period, the highest rate of aberrant cells was observed in 4% *U. maritima* extracts and vydate (7 cells). At the end of 144 h treatment period, the highest rate of aberrant cells was observed 2% (35 cells) and 4% (24 cells) *U. maritima* extracts (Table 2).

DISCUSSION

There were a number of interesting results for examining the effects of *U. maritima* bulb extract on plant growth indicators. In the 48 h treatment period, the highest rate of aberrant cell formation was observed in vydate solution where MI is the highest. At the end of 72 h treatment period, the highest rate of aberrant cell formation was observed in 4% *U. maritima* extracts. At the end of the 144 h treatment period, the highest rates of aberrant cell

$ \begin{array}{cccccccccccccccccccccccccccccccccccc$									
$\begin{array}{c c c c c c c c c c c c c c c c c c c $					-		-	0	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Doses		± 5D	± 5D	-	-	-	-	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	С	48	1 ± 0.21 a	$0.7 \pm 0.18 \text{ a}$	$0.1 \pm 0.08 \text{ a}$	0.2 ± 0.16 a	0.6 ± 0.22 a	$0.09 \pm 0.83 \text{ a}$	2.6 ± 0.51 a
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	V	48	10.4 ± 1.45 b	4 ± 0.57 b	$6.2\pm1.03~\mathrm{b}$	2 ± 0.21 c	$6.3\pm4.32~\mathrm{b}$	$2.6\pm0.52~b$	31.5 ± 3.53 b
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	2%	48	$1.2 \pm 0.13 \text{ a}$	$1 \pm 0.10 \text{ a}$	$0 \pm 0.00 a$	1 ± 0.23 ab	$0 \pm 0.00 a$	$0.1 \pm 0.10 \text{ a}$	3.1 ± 0.34 a
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	4%	48	1.3 ± 0.39 a	$1~\pm 0.27~\mathrm{a}$	$0.4 \pm 0.18a$	0.5 ± 0.19 a	$0.1~\pm 0.08~a$	$0.3 \pm 0.18 \text{ a}$	3.5 ± 1.03 a
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	6%	48	3 ± 1.07 a	0.4 ± 0.16 a	$0 \pm 0.00 a$	$1.5 \pm 0.47 \text{ bc}$	$0.4~\pm 0.16~a$	0 ± 0.00 a	5.2 ± 1.67 a
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			df: 4.49	df: 4.49	df: 4.49	df: 4.49	df: 4.49	df: 4.49	df: 4.49
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			F: 25.185	F: 19.154	F: 36.646	F: 8.014	F: 2.158	F: 19.894	F: 35.258
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	С	72	0.1 ± 0.09 a	0.5 ± 0.28 a	0.1 ± 0.00 a	$0 \pm 0.00 a$	$0.3 \pm 0.14 a$	$0 \pm 0.00 a$	1 ± 0.30 a
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	V	72	15 ± 0.37 b	1.1 ± 0.28 a	1.5 ± 0.29 b	0.8 ± 0.32 b	0.5 ± 0.17 a	0.9 ± 0.20 b	7 ± 1.23 ab
	2%	72	0.2 ± 0.12 a	4.3 ± 1.12 ab	0.4 ± 0.20 a	0.01 ± 0.09 a	$0.3 \pm 0.14 \text{ a}$	0.1 ± 0.09 a	5.4 ± 1.45 ab
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	4%	72	0.9 ± 0.22 ab	6 ± 3.31 b	$0 \pm 0.00 a$	0.3 ± 0.16 ab	$0 \pm 0.00 a$	$0 \pm 0.00 a$	7.2 ± 3.52 b
F: 7.794F: 3.025F: 16.644F: 3.274F: 1.015F: 15.609F: 2.034C144 4.4 ± 1.02 a 4 ± 0.84 ab 0 ± 0.00 a 1.7 ± 0.26 ab 1.8 ± 0.41 abc 0 ± 0.00 a 11.6 ± 1.52 abV144 3 ± 1.17 a 1 ± 0.17 ab 3 ± 2.00 b 0.7 ± 0.21 a 0.6 ± 0.16 ab 2 ± 0.33 b 9.1 ± 2.42 a2%144 11 ± 2.64 b 17 ± 5.11 c 0 ± 0.00 a 2.9 ± 1.05 b 3.7 ± 1.65 c 0.4 ± 0.42 a 35 ± 9.86 c4%144 11.3 ± 2.33 b 7 ± 1.86 b 0 ± 0.00 a 2.5 ± 0.83 b 3 ± 0.86 bc 0.4 ± 0.37 a 24 ± 4.79 bc6%144 0.3 ± 0.22 a 0 ± 0.0 a 0 ± 0.00 a 0.4 ± 0.16 a 0.12 ± 0.11 a 0 ± 0.00 a 0.7 ± 0.37 adf: 4.39df: 4.39df: 4.39df: 4.39df: 4.39df: 4.39df: 4.39	6%	72	$2.6 \pm 0.71 \text{ c}$	0.4 ± 0.22 a	$0 \pm 0.00 a$	$0.8 \pm 0.32 \text{ b}$	$0.4 \pm 0.22 a$	$0 \pm 0.00 a$	4.2 ± 0.82 ab
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			df: 4.44	df: 4.44	df: 4.44	df: 4.44	df: 4.44	df: 4.44	df: 4.44
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			F: 7.794	F: 3.025	F: 16.644	F: 3.274	F: 1.015	F: 15.609	F: 2.034
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	С	144	4.4 ± 1.02 a	4 ± 0.84 ab	$0 \pm 0.00 a$	1.7 ± 0.26 ab	1.8 ± 0.41 abc	0±0.00 a	11.6 ± 1.52 ab
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		144	3± 1.17 a	1± 0.17 ab	3 ± 2.00 b	0.7± 0.21 a	0.6± 0.16 ab	2 ± 0.33 b	9.1±2.42 a
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			11 ± 2.64 b		$0 \pm 0.00 a$	2.9 ± 1.05 b	3.7 ± 1.65 c	0.4 ± 0.42 a	35 ± 9.86 c
df: 4.39						0.4 ± 0.16 a	0.12 ± 0.11 a	$0 \pm 0.00 a$	
			df: 4.39	df: 4.39	df: 4.39	df: 4.39	df: 4.39	df: 4.39	df: 4.39
			F: 9.560		F: 18.835	F: 4.010	F: 3.997	F: 10.305	F: 8.847

Table 2 — The mean number of aberrant cells during mitosis with respect to the treatment dose and period.

*Significant at 0.05 level. C= Control. = Vydate.

formation were observed in 2%, 4% U. *maritima* extracts and then the control and vydate solutions.

The effects of vydate and *U. maritima* extracts in parallel to the increase in dose at the end 48 h treatment period could be clearly seen. The aberrant cell formation at the end of 48 h treatment period was observed in vydate solution and *U. maritima* extracts while the MI was the highest. Because the *Allium cepa* meristematic tissue cells, undergoing mitosis, were affected by vydate and *U. maritima* extracts but the cells that have tendency to division were not complately affected yet. Cells toxically affected by various chemicals, remain in G₁, S or G₂ phases of the cell cycle, and this happens with the involvement of the defense systems that can hinder the cell from mitosis (AK-PINAR *et al.* 2001).

At the end of 72 h treatment period, the highest rate of abarrent cell formation was observed in 4% *U. maritima* extracts and vydate, where MI was the lowest and result this proves that the cells undergoing mitosis are toxically (cytotoxic and genotoxic) affected by these doses. At the end of this period, by increasing levels of toxicicity, various chromosome-related anomalies increase toxically affected, in the cells exposed to 6% *U. maritima* extract and vydate solution. Depending on the toxic effect by the activation of the defense mechanisms at (or of) cell, the cell cycle stops, and thus the MI decreases.

The reason of the observation of aberrant cell formation and the high ratio of MI at the end of 144 h treatment period in 2% and 4% *U. maritima* extracts could be explained by the stimulate effect of *U. maritima* extracts on the cells that have tendency for mitosis like phenolic compounds (AYBEKE *et al.* 2000). At the end of 72 h treatment period cell defense mechanisms can only be triggered for 6% *U. maritima* extract and vydate solution (AKPINAR *et al.* 2001).

As a result, in the treatments where MI was the highest, the rate of aberrant cell formation was found to be the highest too. In the 48 h treatment period, with the increase in the dose, the number of the aberrant cells also increases, at the end of 72 and 144 h treatment periods, the cells treated with 6% *U. maritime* and vydate remained in one of G_1 , S or G_2 phases of cycle (AKPINAR *et al.* 2001).

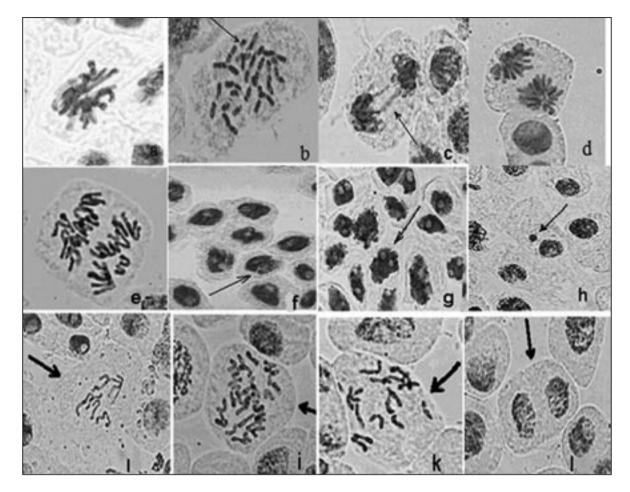


Fig 1 — a) stickiness in metaphase, b) fragment and breakage in metaphase, c) bridge in anaphase, d) pole deviation in anaphase, e) irregular anaphase, f) split in nucleus in interphase g) Vacuolization of nucleus, h) micronuclei, 1) decrease in the number of chromosome (aneuploidi), i) Polyploidy, k) C-mitosis l) bi-nucleic cell.

Such effects including changes among mitosis phases (changes seen in the ratios of prophasemetaphase and anaphase-telophase) were also observed in studies conducted with tridemorph fungicide (CORTES et al. 1982), thirom fungicide (VREA LOPER et al. 1990), marshal fungicide (TOPAKTAS and RENCÜZOĞULLARI 1996). The decrease of mitotic index results from DNA synthesis being pressured or metabolic activities' completely stopping to hinder the cell from mitosis (SUDHAKOR et al. 2001, cited in YÜZBAŞIOĞLU 2003). According to HIDALGO et al., cited in YÜZBAŞIOĞLU (2003), the reason of inhibition of cell cycle is the damage of chromosome areas containing special proteins by the pesticides. The reason of this inhibition is the lack of DNA polymerase. Besides DNA polymerase enzyme, the lack of enzymes and proteins required for spindle apparatus to work properly can be the direct reason for the inhibition of the cell cycle. Mitotic toxicity causes irregular distribution or existence of spindle apparatus, so that C-mitosis is observed (GRISOLIA 2004). It is proposed that C-mitosis is brought about by the impact of chemical poisons on spindle fibers and turbogenic events (SHAHIN and EL-AMAADI 1991; cited in YÜZBAŞIOĞLU 2003).

Even a weak C-mitotic effect can prevent spindle fibers to reach a chromosome, and as a result of this, appearance of retarded chromosomes. It was reported that benomyl fungicide destroys cell division and causes C-mitosis by polymerizing the microtubules that carry the cell elements to poles and it also inhibits the microtubules and thus it prevents the cytokinesis (DANE and DALGIC 2003). Chromosome fragmentations, breakages and inhibition of spindle fibers result from various chemicals' preventing some proteins essential for spindle apparatus (KAYMAK 2005).

Chromosome stickiness may result from chromatin fibers' sticking to each other or breaking due to erroneous or inadequate condensation of these fibers, as a consequence of this, movement of mitotic spindle fibers together with inner-chromosome stickiness when the chromosome is drawn to the pole causes secondary anomalies (bridge and fragment occurrence). Some clastogenic occasions can not directly affect DNA, but they indirectly affect by inducing stickiness in chromosomes. It is claimed that stickiness in chromosomes is induced by chemicals regarded to be clastogenic agents (KLASTERSKA 1976, cited in KAYMAK 2005). Stickiness in chromosomes is an indication of the high toxicity of the chemical substance and usually this may kill the cells with the irreversible damages (FISKESJÖ 1985). When all the results and data obtained from the treatments in all doses and times are compared, it is seen that the highest level of stickiness in chromosomes occurs especially in vydate solution. Except for the144 h treatment of U. maritima, in particular 2% and 4% doses were found to be less cytotoxic than vydate as a chemical pesticide; however, it has a cytotoxic effect though less than control (Table 1). At the end of 144 h treatment period, in the cells exposed to 2% and 4% U. maritima extracts, high level of chromosome stickiness was observed, this is believed to be because of the fact that the defense mechanisms are not activated in these cells and accordingly the cells that are spontaneously liable to divide can complete their mitosis divisions due to some chromosome damages. This is also the cause of the high level of MI. According to AH-MAD and YASMIN (1992), micronuclei are formed as a result of lagging chromosomes or acentric breakages (YUZBAŞIOĞLU 2003). Mitosis anomalies such as bridge, breakage and micronuclei result from clastogenic effects on nucleus chromosomes (GRANT 1978 cited in YÜZBAŞIOĞLU 2003). GRISO-LIA and at al. (2004) state that the retarded chromosomes and aneuploid occurrence are the primary proofs of genotoxic influence (BAVICH *et al.* 1977). Abnormal chromosome intensities result from the inhibition of enzymes and histone proteins (DANE and DALGIC 2003).

Vacuolization observed in vydate treatment is thought to result from the fact that chemical pesticide is a more destructive and comprehensive mutagen and is of a high-density solution. Increase observed in the number of nucleoluses in a nucleus and changing sizes depending on this, can not be directly related to the extract used for treatment. *U. maritima* treatments of all the doses and times were found to be influential on mitosis. Apart from some exceptional cases (as it is in 48 hour treatment period), in our study, parallel to the increases seen in the dose and time (as it is in 72-hour treatment period). MI decreases and aberrant cell occurrence increases. In the study carried out by CIVELEK and WENTRAUB (2004), U. maritima extract that can be used as a biopesticide against root tumor nematods was found to exhibit its effects best at 4% dose in green house conditions. At the end of the present study, while the highest rate of aberrant cell formation in 48 h treatment period was found to occur in vydate, a chemical pesticide, in U. maritima extract treatment, parallel to the increase in the dose, the formation of aberrant cells increases. At the end of 72 h treatment period, it was found that while vydate affects the cells exposed to itself at a toxic level, U. maritima extract, with increasing time, increases its cytotoxic influence and promotes the formation of normal cells as much as vydate, chemical pesticide. At the end of 144 h treatment period, U. maritima extract continues to affect the cells cytotoxically and accordingly the formation of aberrant cells by the cells inclined to divide is increased. Meanwhile, vydate, a chemical pesticide with a high toxicity, affects the cells at toxic level, so nearly stops their division, thus the formation of aberrant cells hardly occurs as they do not survive.

In our study, when vydate, which is routirely used against root tumor nematods (commercially suggested dose of 2%), is compared to the doses of *U. maritima* extracts, it is seen that *U. maritima* extracts are less mutagenic than vydate.

REFERENCES

- AKPINAR N., TÜRKOĞLUŞ., KOCA S., 2001 An Investigation on Cytological Effects of Tonifruit on Vicia faba L. Journal of Qafqaz University, 8: 191-198.
- AYBEKE M., OLGUN G., SIDAL U., KOLANKAYA D., 2000 — The Effect of Olive Oil Mill Effluent on the Mitotic Cell Division and Total Protein Amount of the Root Tips of Triticum aestivum L. Turk J. Biol., 24: 127-140.
- CIVELEK H.S., WEINTRAUB P.G., 2004 Effects of Two Plant Extracts on Larval Leafminer Liriomyza trifolii (Diptera: Agromyzidae) in Tomatoes. J. Econ. Entomol., 97(5): 1581-1586.
- DANE F., DALGIÇ Ö., 2003 The Effects of Fungicide Benomyl (BENLATE) on Growth and Mitosis in Onion (Allium cepa L.) Root Apical Meristem. Acta Biologica Hungarica, 56(1–2): 119-128.
- DELEN N., 2003 Türkiye de Tarım İlacı Kullanımından Kaynaklanan Sorunlar ve Çözüm Önerileri. Tarımsal Savaş Uygulamalarında Sorunlar ve Çözümler Çalıştayı. E.Ü.Z.F. Tarım Makinaları Bölümü Çalıştayları Dizisi, 7: 75-81.

- DELEN N., DURMUŞOĞLU E., GÜNCAN A., GÜNGÖR A., TURGUT C., BURÇAK A., 2005 — Türkiye'de Pestisit Kullanımı, Kalıntı ve Organizmalarda Duyarlılık Azalışı Sorunları. Türkiye Ziraat Mühendisliği 6. Teknik Kongresi: 629-648.
- ELÇI Ş., 1994 Sitogenetik Araştırma Yöntemleri ve Gözlemler. 100. Yıl Ünv. Yayınları, Van, Yayın No:18, Fen Edeb. Fak. Yayın, 16: 52-83.
- ERTÜRK Ö., KARA Ö., SEZER E., ŞAN G., 2004 Toxicity Effect of Some Plant Extracts on Development of Larvae of Plutella xylostella L. (Lepidoptera;Plutellidae) Ekoloji, 13(50): 18-22.
- FISKESJÖ G., 1981 The Allium Test on Copper in Drinking Water. Vatten, 17(3): 232-240.
- FISKESJÖ, G., 1985 The Allium test as a Standard in Enviromental Monitoring. Hereditas, 102: 99-112.
- GRISOLIA C.K., BILICH M.R., FORMIGLI M.L., 2004 A Comparative Toxicologic and Genotoxic Study of the Herbicide Arsenal, its Active Ingredient Imazapyr and the Surfactant Nonylphenol Etoxylate. Ecotoxicology and Environmental Safety, 59: 123-126.
- KAYMAK F., 2005 Cytogenetic Effects of Maleic Hydrazide on Helianthus annuus L. Pakistan Journal of Biological Sciences, 8(1): 104-108.

- MADANLAR N., YOLDAŞ Z. ve DURMUŞOĞLU E., 2002 İzmir'de Sebze Seralarında Zararlılara Karşı Doğal Pestisitlerle Savaş Olanakları. Türk Entomol Derg., 26(3): 181-195.
- ÖZMEN A., SÜMER Ş., 2004 Cytogenetic Effects of Kernel Extracts from Melia azedarach L. Caryologia, 57(3): 290-293.
- PASCUAL-VILLALOBOS M.J., FERNANDEZ M., 1999 Insecticidal Activity of Ethanolic Extracts of Urginea maritima (L.) Baker Bulbs. Industrial Crops and Products, 10: 115–120.
- YÜZBAŞIOĞLU D., ÜNAL F., SANCAK C., KASAP R., 2003 — Cytological Effects of the Herbicide Racer ' Flurochloridone" on Allium cepa. Caryologia, 56(1): 97-105.
- YÜZBAŞIOĞLU D., 2003 Cytogenetic Effects of Fungucide Afugan on the meristematic Cells of Allium cepa L.. The Japan Mendel Society, Cytologia, 68(3): 237-243.

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