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Investigation of the Cytotoxic and Genotoxic Effects of the *Euphorbia rigida* Bieb. Extract[1]

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Abstract. The present study was conducted to evaluate and compare the cytotoxic and genotoxic effects of the aqueous extracts of *Euphorbia rigida* Bieb. which is a natural pesticide. The comparison was done using the *Allium* test to the chemical pesticides Elandor® and Goldplan®. According to *Allium* test results, it had negative impacts on mitosis and showed cytotoxic and genotoxic effects on the existing cells. The lowest level of MI (2.95 %) was observed in the 200 ppm treatment of *E. rigida* extract. Number of the aberrant cells were 88.1, 84.1 and 82.5 in the treatments with Elandor®, 50 ppm, *E. rigida* extract and Goldplan® respectively. The highest cytological anomalies were chromosome stickiness, irregular metaphase and anaphase, pole deviations and C-mitosis. According to the present results of this research, we can suggest that the extract obtained from *E. rigida* plant with water up to 50 ppm can be used as an alternative to chemicals used as biopesticide. Antibacterial, antifungal and antiviral properties of *Euphorbia* extracts are well known, so it could mean that they can be used as additives or a disinfectant for inanimate surfaces in the pharmaceutics industry. No matter what purposes the extract of this plant is used, great care should be taken while using it because it can cause damage on cells and chromosomes. Hence, through more detailed and comprehensive studies about its capacity for medical and biopesticide purposes should be investigated.

Keywords: *Allium* test, Biopesticide, Cell aberrations, Cytotoxic, Genotoxic and *Euphorbia rigida* Bieb.

INTRODUCTION

Extracts and essential oils of some naturally grown plants show antibacterial and antifungal activities and these activities are used as a biopesticide in agricultural control and nutrient preservation (Baytop 1991). In recent years, researchers have been focusing on studies to obtain compounds harmless to human health and the environment that can be used instead of chemical pesticides against plant diseases and pests, which are of great economic value. Although pesticides have a fast and strong effect in the con-

trol of pests, they cause environmental pollution and accumulate over time in all living things through the food chain, creating toxic hazard (Güler and Çobanoğlu 1997).

Most of the *Euphorbia* species are rich in phenolic compounds, aromatic esters, diterpenoids, tetracyclic and pentacyclic triterpenoids, essential oils, resins, resinoids and many bioactive compounds (Wu et al. 2009; Kumar et al. 2010; Ekeke and Ndukwu 2014; Ghareeb et al. 2018; Ghosh et al. 2019). Diterpenoids of *Euphorbia* have biological activities such as antitumor, cytotoxic, anti-viral and anti-inflammatory, but flavonoids and tannins are known for their antitumor, hepatoprotective and antioxidant activities (Wu et. al. 2009; Ghareeb et al. 2018; Ghosh et al. 2019). The plants of *Euphorbia* species are used for the treatment of hypertension, destruction of wart cures, skin diseases, gonorrhoea, migraine and intestinal parasites (Kırbağ et al. 2013; Ghareeb et al. 2018). *E. hirta* possesses antibacterial, anthelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal, and antimalarial properties (Kumar et al. 2010; Özbilgin and Çitoğlu-Saltan 2012). Compounds isolated from *E. paralias* showed moderate antiviral activity against HIV-1 replication. Seven triterpenoids isolated from *E. antiquorum* and steroids isolated from *E. chamaesyce* also have strong inhibitory activity against Epstein-Barr virus early antigen (EBV-EA) activation (Shi et al. 2008). *E. paralias, E. maschallian* and *E. myrsinites* species have different diterpene species that have been identified as antiviral compounds. Some of the other *Euphorbiaceae* species, e.g. *E. pekinensis, E. peplus*, *Phyllanthus nanus* and *P. amarus* are effective against virus infections (Gyurıs et al. 2009). The aqueous and 50% MeOH extracts of *E. hirta* shows direct antiviral effects on HIV-1, HIV-2 and SIV (mac251) reverse transcriptase (RT) activity which were determined in MT4 cells *in-vitro* (Gyurıs et al. 2009; Alam et al. 2016). The antibacterial effect of *E. hirta* (Linn.) comes from tannins, alkaloids and flavonoids contained in the ethanol extract (Ogueke et al. 2007). *E. orientalis* L. was found containing bioactive compounds that has essential antibacterial and antioxidant activities (Avcı et al. 2013). The aqueous extract of *E. hirta* also inhibits aflatoxin contamination in rice, wheat, maize, and mustard crops (Kumar et al. 2010). *E. platypyllos* L. extracts showed significant cytotoxic effect and DNA damaging effects in MCF-7 cells (Aslantürk and Aşkın-Çelik 2013). The active ingredients of 17-Acetoxyjolkinolide B and 13-hexadecanoyloxy-12-deoxyphorbol were obtained from the roots of *E. fischeriana*. Cytotoxic effects of these compounds against Ramos B cells were already determined (Wang et al. 2006; Özbilgin and Çitoğlu-Saltan 2012). Dafnan and tigliane diterpenoids isolated from latex of *E. poisonii* showed selective and potent cytotoxic effects on human kidney carcinoma (A-498) cell lines (Wang et al. 2006; Shi et al. 2008). Ingenol 3-angelate that is *Euphorbia* diterpenes, which was approved by the FDA in 2012 and the EMA in 2013 for the treatment of actinic keratosis, a precancerous skin condition. *E. retusa* extract was effective for the prevention of CCl_4-induced hepatic damage in rats (Ghareeb et al. 2018). Extracts of *E. hirta* have been found to show anticancer activity (Kumar et al. 2010). The latex and plant extracts derived from the roots of the *E. rigida* plant did not cause gene change in bacteria in two sensitive Ames test strains, such as TA 98 and TA 100, while in the Comet experiment, they showed mutagenic effects in human lymphocytes (Başaran et al. 1996). Fumigant effect of *E. aleppica* extract has been demonstrated with an average of 88% mortality against grain storage pests, *Sitophilus garanarius* and *S. oryzae* (Şahin et al. 2006). The strong molluscoidal activity of water extracts and partially purified latex of *E. pulcherima* and *E. hirta* plants is known (Shi et al. 2008). Isolated compounds from *E. paralias* had strong molluscicidal activity on *Biomphalaria alexandrina* (Ehrenberg) and antifeedant effects on third-instar larvae of *Spodoptera littoralis* (Boisd) (Abdelgalil 2002). The petroleum ether fraction of *E. hirta* is indicated as an herbicidal biopesticide with a larvicidal effect in *Anopheles stephensi* known as the malaria vectors (Huang et al. 2012). Methanolic extract of aerial parts of *E. hirta* was effective against *P. falciparum* parasites, polyphenolic extract of *E. hirta* inhibited the growth of *Entamoeba histolytica (*Kumar et al. 2010) and aqueous leaf extracts (1:10 w/v) of *E. hirta* had a maximum killing efficacy (45%) against the *Zonabris pustulata* Thunb. (Oudhia 2000).

E. fischeriana has been used as an anthelmintic and insecticide in China (Lee et al. 1991). The aqueous extract of *E. hirta* reduced the egg counts of intestinal parasites in Nigerian dogs' feces as a potential anthelmintic and antiparasiticide agent (Huang et al. 2012). The ethanol extracts from the leaves and flowers of *E. cyparissias* L., had the highest larvacidal activity against the codling moth (*Cydia pomonella* L.) with strong acaricidal activity on the treated population of two-spotted spider mite (*Tetranychus urticae* Koch.) on the third day post treatment (Velcheva 2001). Extracts of *E. hirta* and the root exudate exhibit nematicidal activity against juveniles of *Meloidogyne incognita* (Kumar et al. 2010). *E. myrsinites* extracts against root-knot nematodes (Nematoda: Meloidogyne spp.) in greenhouse tomato cultivation were found to be significantly more effective than synthetic pesticide Cyromazine ($C_6H_{10}N_6$) (Civelek

and Weintraub 2004). Most plant extracts have been used as topical antiseptics, or have been reported to have antimicrobial properties (Avcı et al. 2013).

Most of the *Euphorbia* species and other plant extracts, which are used for some medicinal and agricultural purposes, show potentially toxic, mutagenic, carcinogenic and teratogenic effects. For this reason, it is necessary to test the potential harmful effects of plant extracts to be used in both medical (antibacterial, antifungal, antiviral, antitumor, antioxidant, antiseptic, anthelmintic etc.,) and agricultural purposes (biopesticide, insecticidal, acaricidal, antiparasitic, larvicidal, molluscoidal, antimalarial, antifeedant etc.,). In the current study, the cytotoxic and genotoxic effects of *E. rigida* (Bieb.) being used for medical and biopesticide purposes were investigated. The objective of this study was to evaluate and compare the cytotoxic and genotoxic effect of the extract of *E. rigida*, a natural pesticide, with the chemical pesticides, Elandor® and Goldplan®.

MATERIALS AND METHODS

Collection of the plants and their extraction

The aerial parts of *Euphorbia rigida* (Bieb.) in the flowering period were collected from Sıtkı Koçman University campus (GPS:37°09'40,1"N 28°22'34"E) in Muğla at Turkey. The taxanomic identification of plant materials was confirmed and deposited in the herbarium by voucher specimen Dr. Olcay Ceylan at the Department of Biology at Muğla Sıtkı Koçman University (Herbarium number: O0388). The samples were air-dried at room temperature and protected from direct sunlight. Seventyfive (75 g) grams of dried and powdered aerial parts of *E. rigida* were extracted with 2.5 L boiling water for 60 min. Decoction (aqueous phase) was filtered with a 2.5 µm filter paper (Whatman No. 42) to remove suspended particles and the extract was kept at least 3 days at -20°C and later lyophilized to obtained crude (6.72 g) extract which was stored at -20°C.

Allium test method

The United Nations Environment Program (UNEP) and the US Environmental Protection Agency (USEPA) have standardized the use of plants as bioindicators in the determination of toxicity (Sivas and Gökbayrak 2011; Girasun et al. 2019). UNEP and the International Chemical Safety Program (IPCS) certified the *Allium cepa* (onion) root tip test in 1991 as a highly effective biotester for imaging mutagenic effects (Oney-Birol and Gündüz 2019). The onion genotoxicity test provides for easy screening of chemicals or toxic agents with genotoxic, cytotoxic, physiologic, clastogenic and aneugenic effects, especially to plants. The *A. cepa* assay is an efficient test for chemical screening and *in situ* monitoring for genotoxicity of environmental contaminants (Sivas and Gökbayrak 2011; Pandey et al. 2014; Girasun et al. 2019; Adhikari 2019). The test has been used widely to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems of *A. cepa*. The most important advantage of this test is that it is a low budget method, which besides being fast and easy to handle, it also yields reliable results (Fiskesjö 1985, Rank 2003; Çelik and Aslantürk 2006; Eren et al. 2017; Karaismailoğlu 2016; Adhikari 2019; Liman et al. 2020). *Allium* test results show a good correlation with the other eukaryotic and prokaryotic test results (Bonciu et al. 2018; Pirdal and Liman 2019; Oney-Birol and Gündüz 2019; Adhikari 2019).

The effects of the extract on cells and chromosomes were investigated through *Allium* test (Fiskesjö 1981). For the *Allium* test, *A. cepa* were purchased from the market. 0, 50, 100, 200 and 400 ppm (v/v) solutions of *E. rigida* extract were prepared with distilled water and also 200 ppm Elandor® (Imidacloprid) and 400 ppm Goldplan® (Acetamiprid) solution (v/v) (recommended doses) was prepared. Elandor® and Goldplan® are commercially available pesticides, which are contact and systemic insecticide for the control of *Hemiptera*, especially aphids, *Thysanoptera* and *Lepidoptera* (Öncüer 2004).

All onions were grown in distilled water for first 48 hours. Then *A. cepa* bulbs were placed in beakers including 0 (control= distilled water), 50, 100, 200 and 400 ppm (v/v) solutions of *E. rigida*, 200 ppm Elandor® and 400 ppm Goldplan® (v/v) solution (for the treatment 24 h). At the end of 24 h (after total 72 h), the root number and length of *A. cepa* were determined. Also the least ten root tips from each treatment were fixed in Carnoy solution (absolute alcohol: chloroform: glacial acetic acid, 6:3:1). Thereafter, roots tips were applied to cold hydrolysis and the meristem tissue cells were painted with Feulgen, then squashed and examined under the light microscope (Nikon UFX-2A) (Elçi 1994). From these squashed root tips, ten random areas were selected for the observation at 10X40 microscopic magnification (approximately 2000 cells were counted in an area), minimum 20.000 mitotic cells were counted from each of the slides. On each slide, abnormalities of chromosome stickiness, binucleate cells, micronuclei, C-mitosis, lagging chromosome, fragmentation in the metaphase, bridges and pole deviations in anaphase, irregular metaphase or anaphase etc. were detected. Moreover, the

mitotic index (MI) was calculated for each treatment as a number of dividing cells/100 cells (Metin and Bürün 2008).

Statistical analysis

Statistical analyses were performed using the SPSS 14.0 Software Package Program, data were evaluated with One-Way ANOVA and LSD (Least Significant Difference) tests.

RESULTS AND DISCUSSION

The effects of the extract of *E. rigida*, a natural pesticide were compared with Elandor® and Goldplan®, chemical pesticides. Aqueous extracts of *E. rigida* plants (50, 100, 200 and 400 ppm), 200 ppm Elandor® and 400 ppm Goldplan® were observed to cause the occurrence of aberrant cells and considerably decrease cell division depending on the increase in extract concentration.

The number and length of root according to treatments

The roots of all onions left for rooting in distilled water for 48 hours were healthy and well developed, and the differences were observed in the average root length and the total number of roots due to the onion's own characteristics. In the 24 hours following the first 48 hours, the development of the roots of onions exposed to different doses of *E. rigida* extracts and chemical pesticides slowed down, the number of roots did not change and the root lengths were in different statistical groups ($p<0.05$) (Table 1). Changes in root length of the treatments were compared to the control at the inhibition percent. Growth in onion root apical meristems of the treatment samples was 45% less than that of the control. Accordingly, the extracts and chemicals applied most likely contain toxic substances with a sublethal effect ($p<0.05$). Inhibition of root growth could be not only related to apical meristematic activity but also cell elongation during differentiation or enzyme activation that promote the elongation and loosening of the cell wall in the differentiation process (Eren et al. 2017; Pirdal and Liman 2019).

The lowest root length (highest inhibition) in 24-hour treatment of extract and chemicals was observed in 400 ppm *E. rigida* treatment ($p<0.05$) (Table 1). The root elongation % inhibition of Elandor® and Goldplan® treatments remained below the 50 ppm dose of the extract ($p<0.05$). Root elongation inhibition increased with concentration increase of plant extract.

This means that the plant extract prevents mitosis by showing toxic effects on the meristematic cells of *A. cepa*. Heavy metal induced toxicity and mutagenicity on various plant species have been already reported. Primary toxic effect of *Pb* in higher plants had been the inhibition of root growth possibly due to the inhibition of cell division in the root tip region. The reduction in root lengthening is strongly correlated to the mitotic index of the root tips of *Lathyrus sativus*. The reductions in the number of mitotic cells in root tips of seeds exposed to *Pb* could be due to its mechanism of action on cell cycle progression (Adhikari 2019).

Effect on the mitotic index (MI) of treatments

Mitotic index (MI) is a cytogenetic parameter that helps to measure the proliferation (M phase) of mitotic cells (Oney-Birol and Gündüz 2019). After applications, the highest level of MI (15.70 %) was observed in the Elandor® treatment, and this was followed by the Goldplan®, 50 ppm, *E. rigida* extract and control. The lowest level of MI (2.95 %) was observed in the 200 ppm treatment of *E. rigida* extract ($p<0.05$) (Table 2). *A. cepa* meristem cells are not affected by the level of toxicity of these chemical pesticide solutions. In contrast, some chemicals involved in Elandor® and Goldplan® or the lowest plant extracts (*E. rigida* 50 ppm) may promote the cells into mitosis. However, by increasing the treatment doses of plant extracts, the toxic impact prevents cell division, and, as a result MI decreases ($p<0.05$) (Table 2). The decline in MI value shows interference in the cell cycle (Oloyede et al. 2009). The reduction in number of the dividing cells in the roots shows the cytotoxic effects of the substances that are found in the plant aqueous extracts. MI, number of aberrant cells and its percent were high in the control and *A. cepa* meristematic tissue cells which were exposed to *E. rigida* 50 ppm, Elandor and Goldplan solutions. This indicates that at the end of 24-hour exposure, treatment doses have enough influence on stopping the mitosis except the *E. rigida* 200 ppm ($p<0.05$). On the other hand, in *E. rigida* 100 ppm and *E. rigida* 400 ppm treatments, the defence systems preventing mitosis become active and this results in the decrease of MI. The high MI exposed to Elandor, Goldplan and 50 ppm *E. rigida* extract indicates that the damage on living cells from these extracts can be recoverable and tolerable. Significant reduction in MI may be due to disturbed cell cycle such as blockage of G1 phase and suppressing DNA synthesis or inhibition of DNA synthesis at the S phase or blocking of G2 phase preventing the cell from entering in mitosis or mitotic phase changes (Pandey et al. 2014; Karaismailoğlu 2017a;

Table 1. Mean root length (mm) and number of *Allium cepa* before and after the treatment with the different doses of *E. rigida* extract and the chemical pesticides.

First 48 h (Before the treatment)		After the treatments (After 72 hours)				
At the end of 48 h Root length (mean) (mm)± SD	Treatments and Doses (ppm)	At the end of 72 h Root length (mean) (mm)± SD	Increase in root length (mm ±SD)	Increase in percent of root length (%)		Root number ± SD
				% Growing	% İnhibition	
19.00 ± 5.25 d	Control (0)	22.00 ± 5.31 e	3.00	100	0	71 f
14.60 ± 4.70 c	*E. rigida* (50)	16.10 ± 4.64 c	1.50	50	50	30 a
19.34 ± 6.08 d	*E. rigida* (100)	20.23 ± 6.12 de	0.89	29.6	70.4	47 e
9.48 ± 2.51 a	*E. rigida* (200)	10.33 ± 2.43 a	0.85	28.3	71.7	33 c
12.40 ± 4.33 b	*E. rigida* (400)	13.00 ± 4.32 b	0.60	20	80	37 d
10.73 ± 2.58 ab	Goldplan (400)	12.76 ± 3.14 b	2.03	67.6	32.4	30 a
17.62 ± 4.96 d	Elandor (200)	19.34 ± 5.02 d	1.72	57.3	42.7	32 b

Variability around the mean was represented as ± SD (Standart Deviation). Data having the same letter in a column were not significantly differed by LSD's multipli comparison test (P<0.05).

Table 2. The number of normal, total normal and total aberrant dividing cells and percentage of total aberrant dividing cells in mitotic phases and mitotic index (MI %) for the chemical pesticides and the different treatment doses of *E. rigida* extract.

Treatments and Doses (ppm)	Prophase ± SD	Metaphase ± SD	Anaphase ± SD	Telophase ± SD	The number of total normally dividing cells ± SD	Total Aberrant Cell number ± SD	% Aberrant Cells ± SD	Total dividing cell number ± SD	% MI (Mean ±SD)
Control (0)	81 ± 11.97 c	61 ± 10.08 c	28.2 ± 6.95 c	42.9 ± 11.60 c	213.1	60.7	22.16	273.8	13.69 d
E. rigida (50)	82 ± 18.13 c	57.8 ± 18.89 c	30.3 ± 9.26 cd	31.7 ± 7.70 b	201.8	84.1	29.41	285.9	14.29 d
E. rigida (100)	59 ± 11.97 b	41.3 ± 10.39 b	17.9 ± 7.74 b	23.8 ± 9.02 b	142	48.5	25.45	190.5	9.52 c
E. rigida (200)	18.2 ± 6.12 a	9.6 ± 3.83 a	6.3 ± 2.54 a	7.3 ± 2.35 a	41.4	17.6	29.83	59	2.95 a
E. rigida (400)	56.1 ± 8.99 b	39.9 ± 10.99 b	18 ± 4.26 b	24.4 ± 6.61 b	138.4	46.5	25.14	184.9	9.24 b
Goldplan (400)	90.3 ± 14.29 c	55.2 ± 20.38 c	36.7 ± 11.47 d	46.3 ± 12.32 c	228.5	82.5	26.52	311	15.55 e
Elandor (200)	88.5 ± 8.51 c	57.3 ± 18.01 c	37 ± 8.89 d	43.2 ± 11.69 c	226	88.1	28.04	314.1	15.70 e

Variability around the mean was represented as ± SD (Standart Deviation). Data having the same letter in a column were not significantly differed by LSD's multipli comparison test (P<0.05).

Liman et al. 2020). The causes of the decrease in the MI can be physiological response of cells that have entered the mitotic cycle and are not protected against extract components; partial inhibition of energy, protein, RNA and DNA synthesis in treatment groups and inhibition or postponement of the mitotic spindle formation in treated groups due to the high percentage of prophase in some concentrations (Karaismailoğlu 2016).

Formation aberrant cells according to treatments

Increase in the frequency of C-mitosis cells, multipolar anaphases, sticky and diffuse chromosomes together with the decrease in the mitotic index are defined as cytotoxic, and other nuclear abnormalities as genotoxic (Kanev et al. 2017). Chromosome abnormalities occur as a result of damage at the DNA level and are considered to be highly reliable analyzes for the evaluation of genotoxicity.

The abnormal cells in mitosis were observed at different levels in all treatment doses of extract and in the pesticides treatments. The highest number of aberrant cells was observed in Elandor® treatment with 88.1 aberrant cells, followed by the 50 ppm *E. rigida* extract with 84.1 and Goldplan® with 82.5 aberrant cells (Table 3). Total abnormal cell formation was the highest in applications where MI was high. Total abnormal cell counts were also observed to be the highest in these treatments as there was no toxic effect at the lowest dose of extract (*E. rigida* 50 ppm) and chemical pesticides. When the

Table 3. Type and percentage of mitotic abnormalities observed in the treatments with pesticides and different doses of *E. rigida* extract.

Doses (ppm)	Stickiness ± SD.	Fragment ± SD	Irregular Metaphase ± SD	C-Mitos ± SD	Laggard Chromosome ± SD	Irregular Anaphase ± SD	Pole Deviation ± SD	Bridge ± SD	Binucleus ± SD	Micronucleus ± SD	Other Anomalies ± SD	Total Aberrant Cell Number ± SD
Control (0)	23.4 ± 6.94 bcd	0.2 ± 0.63 a	12.5 ± 5.08 bc	1.4 ± 1.34 a	0.8 ± 0.91 ab	12.3 ± 5.27 b	6 ± 0.81 d	0.6 ± 0.84 ab	1.3 ± 1.05 ab	0.4 ± 0.69 a	0 ± 0 a	60.7
E. rigida 50	31.2 ± 13.72 d	0.7 ± 0.82 a	19 ± 11.46 cd	10.6 ± 11.85 b	2.3 ± 2.35 c	12.7 ± 4.85 b	2.9 ± 1.96 b	0.3 ± 0.48 ab	3 ± 1.76 bc	1.3 ± 0.82 b	0.1 ± 0.31 a	84.1
E. rigida 100	18.4 ± 7.80 bc	0.4 ± 0.69 a	9.5 ± 4.06 ab	3.6 ± 4.37 a	2 ± 2.10 bc	6.8 ± 4.61 ab	2.7 ± 2.66 b	0.2 ± 0.42 a	2.7 ± 2 abc	2.2 ± 1.54 c	0 ± 0 a	48.5
E. rigida 200	5.4 ± 2.22 a	0.4 ± 0.51 a	4.6 ± 1.77 a	0.6 ± 0.69 a	0.8 ±0.76 ab	3.2 ± 1.03 a	0.8 ± 0.78 a	0.3 ± 0.48 ab	1.2 ± 0.78 a	0.3 ± 0.48 a	0 ± 0 a	17.6
E. rigida 400	16.2 ± 8.13 b	0.3 ± 0.48 a	9.5 ± 4.30 ab	1 ± 0.69 a	0.4 ± 0.51 a	10.4 ± 6.71 b	3.6 ± 2.11 bc	0.5 ± 0.52 ab	3.5 ± 2.54 c	1 ± 0.66 ab	0.1 ± 0.31 a	46.5
Goldplan 400	25.1 ± 9.76 cd	0.6 ± 0.84 a	22.3 ± 9.42 d	3.1 ± 2.55 a	1.2 ± 0.91 abc	21.5 ± 8.80 c	5 ± 1.82 cd	1.2 ± 1.93 b	1.9 ± 1.1 abc	0.4 ± 0.51 a	0.2 ± 0.42 a	82.5
Elandor 200	27.5 ± 7.36 d	1.6 ± 2.01 b	23.3 ± 9.40 d	1.8 ± 0.91 a	1 ± 0.66 abc	23 ± 7.93 c	5.2 ± 2.82 cd	1 ± 0.66 ab	3.2 ± 2.2 c	0.5 ± 0.70 a	0 ± 0 a	88.1

Variability around the mean was represented as ± SD (Standart Deviation). Data having the same letter in a column were not significantly differed by LSD's multipli comparison test (P<0.05).

dividing cells were suddenly exposed to treatments after 48 hours, these cells, which were not affected by the lowest dose of extract and chemical pesticides at a toxic level, completed their division with abnormalities. On the other hand, in the cells exposed to high doses (100, 200 and 400 ppm) of plant extract, toxic effects and mitodepressive effects were observed, MI decreased and fewer abnormal cells were recorded.

The highest anomalies were chromosome stickiness (Figure 1a), irregular metaphase (Figure 1b), irregular anaphase (Figure 1c), pole deviations in the anaphase (Figure 1d), C-mitosis and aneuploidy (hipoploidy) (Figure 1e) (Table 3). Other anomalies observed in this study were fragmentation in the metaphase (Figure 1f), lagging chromosome (Figure 1g), bridges in anaphase (Figure 1h), binucleate cells (Figure 1i), micronuclei (Figure 1j). In addition to these anomalies, granulation in the prophase nucleus (Figure 1k), irregular prophase (Figure 1m), split in the interphase nucleus (Figure 1n), increases in the number of nucleolus in the nucleus (Figure 1o), nucleus erosion and granulation in the interphase nucleus (Figure 1p), nucleus vacuolization in the prophase (Figure 1q), multipolar anaphase with polyploidy (Figure 1r), polyploidy with C-Mitosis and fragments (s) were observed ($p<0.05$). Micronucleus (MN) occurs as a result of clastogenic and aneugenic effects (Andrade-Vieira et al. 2012; Adhikari 2019; Rosculete et al. 2020). Micronucleus analysis has an important role in assessment of the genotoxic and cytotoxic impacts of chemicals or pesticides (Karaismailoğlu 2015; 2017b). Disturbed ana-telophase and chromosome laggards may result from deformation of the spindle structure or degraded microtubules and remaining acentric chromosome fragments (Türkoğlu 2007; Andrade-Vieira et al. 2012; Pirdal and Liman 2019; Rosculete et al. 2020). Laggard chromosomes are considered indicators of spindle poisoning (Rank 2003). The induction of spindle disturbances in the cell of *A. cepa* by extracts may lead to aneuploidy and lagging chromosome(s) or micronucleus formation at the next stage of cell division. The lagging chromosome(s) may be lost or form nuclear membrane around itself thereby forming micronucleus (Grant 1978). The lagging chromosome(s) usually arises from irregular separation of chromosomes at anaphase thereby making some chromosomes to reach the poles before the other (Grant 1978; Pandey et al. 2014; Adhikari 2019; Rosculete et al. 2020). C-mitosis, binucleate cells, and increases in the number of nucleolus in the nucleus were also observed.

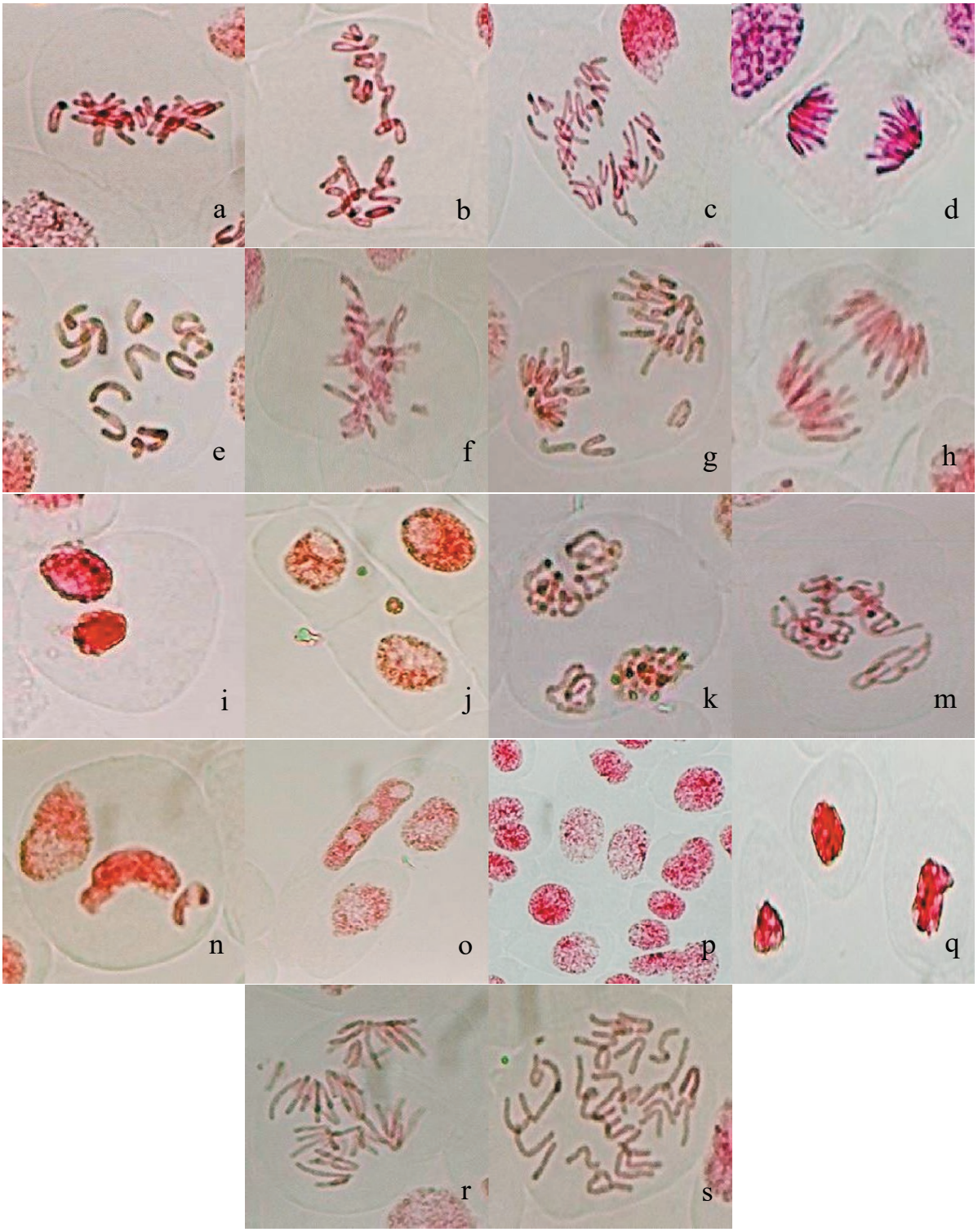

Figure 1. (a) Stickiness in Metaphase (100 ppm *E. rigida*) (b) Irreguler Metaphase (100 ppm) (c) Irreguler Anaphase, (100 ppm) (d) Pole deviation (Goldplan) (e) C-Mitosis and Aneploidy (100 ppm) (f) Fragments (400 ppm) (g) Laggard chromosome (50 ppm) (h) Bridge in Anaphase, (Goldplan) (i) Binucleus (200 ppm) (j) Micronucleus (50 ppm) (k) Granulation of nucleus (50 ppm) (m) Irreguler Prophase (100 ppm) (n) Nucleus deformation (Goldplan) (o) Increase in the number of Nucleolus in the Nucleus (Goldplan) (p) Nucleus erosion in interphase (200 ppm) (q) Vacualation of nucleus in interphase (200 ppm) (r) Multipolar Anaphase (Elandor) (s) Polyploidy with C- Mitosis and fragments (50 ppm) (10X40).

It is thought that the formation of binucleate cells may result from incomplete cell division or wall fusion as a result of halting protein synthesis (Sivas and Gökbayrak 2011). C-mitosis constitutes with stopping spindle form (Badr 1983). Mitotic toxicity affects spindle mechanism therewith C-mitosis is observed (Yüzbaşıoğlu 2003; Grisolia et al. 2004; Türkoğlu 2007; Sivas and Gökbayrak 2011; Andrade-Vieira et al. 2012; Pandey et al. 2014). The cytotoxic effects of urea, fungicides (Afugan, Carbendazim)(Yüzbaşıoğlu 2003; Bonciu et al. 2018), synthetic plant growth regulators (2,4-Dichlorophenoxyacetic acid)(Özkul et al. 2016), pesticides, herbicides, insecticides (Pentachlorophenol, Maleic Hydrazide, Dichlorvos and Glyphos)(Grant 1978; Kaymak 2005; Fındıklı and Türkoğlu 2010), heavy metal (Chromium=K2Cr2O7, Pb(NO3)2)(Güler et al. 2018; Girasun et al. 2019) and some plant extracts containing bioactive compounds (triterpenoids, tannins etc.)(Başaran et al. 1996; Shi et al. 2008) were observed from the occurrence of fragments on DNA doubled spindles (Çavuşoğlu 2019). Fragmentation might have arisen due to stickiness of chromosomes and consequent failure of separation of chromatids to poles. In addition to this, DNA double strand breaks induced by reactive oxygen species can lead to chromosome fragments (Adhikari 2019; Liman et al. 2020). It is claimed that the stickiness, bridges and fragments which are scored as indicators of clastogenicity in chromosomes are induced by chemicals regarded as clastogenic agents ($p<0.05$) (Rank 2003; Kaymak 2005; Sivas and Gökbayrak 2011; Andrade-Vieira et al. 2012; Adhikari 2019). These alterations can form an irreversible and genotoxic influence (Fiskesjö and Levan 1993). Stickiness aberration forms as a result of chromatid irregularity (Badr 1983). Stickiness in the chromosomes is an indication that chemical substance has a high toxicity, and may cause the death of cells by inducing unrecoverable damages (Fiskesjö 1985; Türkoğlu 2007; Andrade-Vieira et al. 2012; Pandey et al. 2014). Chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to unequal translocation or inversion of chromosome segments (Gömürgen 2005; Türkoğlu 2007; Sivas and Gökbayrak 2011). In this study, the highest stickiness in the chromosomes was seen in *E. rigida* 50 ppm, Elandor and Goldplan ($p<0.05$). Nucleus deformation increases depending on the increase in extract concentration, which indicates that the cells are affected cytotoxically and genotoxically, DNA synthesis is pressured. Vacuolisations were observed in *E. rigida* 200 ppm extract treatment, then this indicated that the chemical pesticides are more destructive and comprehensive mutagens, and those high concentrations of *E. rigida* 200 ppm extract give the same results. The anomalies occurred in all doses of *E. rigida* extract, but the highest anomalies was usually observed in *E. rigida* 50 and 100 ppm. Elandor and Goldplan are routinely used against control of *Hemiptera,* especially aphids, *Thysanoptera* and *Lepidoptera* (Öncüer 2004). When these pesticides were compared to extract of *E. rigida*, it was seen that even the high doses of *E. rigida* extract are more mutagenic ($p<0.05$). In this study, 200 and 400 ppm *E. rigida* extracts were found to be more cytotoxic and genotoxic than 200 ppm Goldplan or 400 ppm Elandor ($p<0.05$).

It is obvious from the results of the present study and based on the literature that the production of ATP is suppressed in the cells of *A. cepa* meristem tissue exposed to increased doses of *E. rigida* extract and chemical pesticides for 24-hour. Also, the metabolic activities in the cell slow down or stop. At the same time, depending on the clastogenic and aneugenic effects of plant extract and chemical pesticides, cells that tend to enter mitosis are eliminated. Thus, mitosis was suppressed in tissues and cells exposed to high doses and less abnormal cell formation were observed.

E. paralias, E. antiquorum and *E. chamaesyce* showed moderate antiviral activity (Shi et al. 2008). *E. paralias, E. maschallian* and *E. myrsinites* species have antiviral compounds. *E. pekinensis, E. peplus*, *Phyllanthus nanus* and *P. amarus* are effective against virus infections and *E.hirta* shows direct antiviral effects on HIV-1, HIV-2 and SIV (mac251) reverse transcriptase (RT) activity (Gyurıs et al. 2009; Alam et al. 2016). *E. rigida* plant did not cause gene change in bacteria such as TA 98 and TA 100, they showed mutagenic effects in human lymphocytes (Başaran et al. 1996). *E. rigida* extract may also have strong effects on viruses' capsid, reverse transcriptase (RT) and DNA/RNA structures. Although *E. rigida* extract is a natural and organic product, it is clear that it is more toxic, cytotoxic and genotoxic.

Therefore, the possibility of using *E. rigida* extract as an antiseptic for sterilization or disinfection of large and inanimate surfaces can be explored (Avcı et al. 2013).

According to the data obtained from cytotoxic and genotoxic tests in this study, the aqueous extract up to 50 ppm of *E. rigida* is seen to be promising for using as biopesticide purposes as an alternative to the chemicals we use in our experiments. However, studies on the evaluation of systematic toxicity and safety of *Euphorbia* species are very few. In the studies that have been conducted, only the organs that are targeted and side effects are emphasized (Huang et al. 2012). Although *E. rigida* extract is a natural and organic product, it is clear that it can be dangerous because it is more toxic, cytotoxic and

genotoxic than Goldplan and Elandor used as a chemical pesticide in agricultural control ($p<0.05$). In order to reach more information and certain conclusions on this subject, however, further research should be performed with different test systems.

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