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The Effects of Green Tea Leaf Extract on Cytogenetical and Physiological Parameters of *Allium cepa* L. exposed to Salinity

Dilek ÇAVUŞOĞLU¹

Abstract

The aim of this study investigated the effects of 50 mg L⁻¹ green tea leaf extract in decreasing harmful effects of 0.175 M salinity stress on the mitotic activity, chromosomal aberrations, seedling growth (fresh weight, radicle length and radicle number), micronucleus frequency which is the simplest indicator, the most effective of cytological damage and bulb germination of *A. cepa* L. In only green tea leaf extract medium, the radicle length and radicle number of bulbs were partially reduced compared to the control bulbs germinated in the distilled water medium. While their germination percentage and fresh weight statistically indicated the same values. Besides, the mitotic index and chromosomal abnormalities in the root tip meristematic cells of *Allium cepa* bulbs germinated in alone green tea leaf extract medium increased compared to germinated control bulbs in the distilled water medium, whereas the micronucleus frequency showed statistically the same value compared to the control. In other words, it can be said that salt stress significantly inhibited the seedling growth and bulb germination of *Allium cepa*. What's more, it significantly reduced the mitotic index in the root tip meristems of the bulbs and increased the number of chromosomal abnormalities and micronucleus frequency. On the other hand, inhibitory effects of salt on the mitotic activity, seedling growth, bulb germination, chromosomal abnormalities and micronucleus frequency significantly decreased with the application of green tea leaf extract. The germination percentage, radicle length, radicle number, fresh weight, mitotic index, micronucleus frequency and chromosomal aberrations of the seedlings grown in 0.175 M salinity were 23 %, 10.3 mm, 12.7, 7 g, 1.2 %, 13 % and 17 % respectively, while these values became 75 %, 13.4 mm, 17.2, 13.8 g, 6.3 %, 9 % and 9.3 % in the seedlings treated with 50 mg L⁻¹ green tea leaf extract.

Keywords: green tea leaf extract, chromosomal aberrations, mitotic index, salt stress, bulb germination

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1. INTRODUCTION

Saltiness is an expanding problem. The soil affected by salt is increasing worldwide through irrigation vegetation and clearance, both of which raise the water table bringing dissolved salts to the surface. One of the most serious peripheral factors that limit the efficiency of crop plants that have a major impact on agricultural productivity is defined as salt stress. Saltiness on plants has three potential effects: 1) conflict with the intake of essential nutrients 1) reduction of water potential, 11) direct toxicity absorbed by any Cl and Na [1, 2]. Salinity stress caused is one of the most serious environmental factors, which inhibits plant development and growth. Also it decreases crop productivity worldwide. Salt has the most harmful effect in germination cycle. In many cases, salt tolerance increases as long as plant development progresses. Plant's reactions to salt; it can vary according to the development period in which the plant is located, the concentration of salt that the stress factor, on the time it affects on the plant of salt; it may also vary depending on climate and soil properties. Primary cytogenetically effects occurring at the beginning of salt stress include retarded expansion and cell division [3]. It was found that salt had negative effects on plant growth and development by preventing the seed germination, seedling growth, enzyme activation, nucleic acid and protein synthesis and mitosis. On the other hand, salt may interfere with growth and development by disrupting endogenous hormone balance in favor of abscisic acid, increasing free radical production, and causing changes in the morphological and anatomical structures of plants. During long-term exposure to salt stress, accumulation of salt ions in plant aerial parts via the transpiration stream leads to ionic stress [3, 4]. To adaptively respond and survive under salinity, plants require changes of various cellular, physiological and metabolic mechanisms, which are controlled by the regulated expression of specific stress-related genes through cascades of complex regulatory networks [5].

Green tea is one of the most ancient consumed beverages by over 2/3 of the world population. The principal constituents of green tea leaf extract (GTLE) are tannins, essential oil and caffeine.

Green tea leaf extract contains catechin, gallacatechin, epicatechin gallate, epicatechin, epigallocatechin, epigallocatechin gallate which the most active polyphenol. GTLE has anti-inflammatory, anti-bacterial, anti-allergenic, anti-viral, anti-arthritic, anti-angiogenic, anti-mutagenic, anti-diabetic, anti-cancer properties, neuroprotective, anti-aging (defensive the skin from damage arised from ionizing radiation and others, increasing collagen volume and assisting wound healing/ insect stings), protective effect against oxidative damage-dependent diseases (i.e. Parkinson, Alzheimer, heart disease, atherosclerosis), beneficial effect in alcohol intoxication, effect of reducing the oxidation of food products and extend their shelf life, thermogenesis (enhancing weight loss), cholesterol-lowering effects, preventive effect of tooth decay [6, 7].

The *Allium cepa* bioassay, which was applied in this study, has excellent correlation with mammalian systems. These bioassay is a fast, inexpensive, sensitive method and easily handled toxicity test, which has advantages over other short-term tests that require previous preparations of tested samples. The *Allium cepa* test facilitates testing different toxicity endpoints viz. mitotic index, root growth inhibition [8], the chromosomal aberrations, nuclear abnormalities and occurrence of micronuclei [9]. For this reason, *Allium cepa* was used as a test material in this study. There is no study on the influences of green tea leaf extract on the seedlings growth, bulb germination, chromosomal aberrations, mitotic activity and micronucleus frequency under saline and normal conditions. Therefore, this work was designed to investigate the efficiency of green tea leaf extract on the chromosomal aberrations, seedling growth, micronucleus frequency, bulb germination and mitotic activity in *Allium cepa* L. subject to salt stress.

2. MATERIALS and METHODS

2.1. Green Tea Leaf Extract, The Bulb and Salt Concentrations

Uniform and healthy small bulb (*Allium cepa* L., 2n=16) that used for the assay were obtained from Erdoğan Ekinci Ltd. Şti., Antalya, Turkey. Green tea leaf extract (60 capsules of 380 mg) was purchased from Sepe Natural. By a preliminary investigation carried out, firstly it was determined as 0.175 M salt concentration (tried out concentrations of 0.10, 0.125, 0.15, 0.175, 0.20, 0.225, 0.25, 0.275, 0.30 M) which largely preventing the germination of *Allium cepa* L. Then it was designated as 50 mg L⁻¹ green tea leaf extract concentration (tried out concentrations of 1, 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg L⁻¹ dose of green tea leaf extract) alleviating the adverse effects of this salt concentration (0.175 M) on the bulb germination and seedling growth. Thus, 50 mg/L green tea leaf extract and 0.175 M NaCl (salt) concentration used for this study.

2.2. Germination of the Bulbs

The germination assay of the bulbs was carried out with *Allium cepa* bulbs, that are physiologically homogeneous. Bulbs were germinated at incubated at 20°C in the darkness and they were surface-disinfected in 2.5% sodium hypochloride solution for ten minutes. Then, they washed in ultra-distilled water for 24 hours. For germination, 20 bulbs from each treatment group were placed in 1700-mL plastic boxes. For 7 consecutive days, the study included four groups of boxes with bulbs:

- Control (group I), to which bulbs were treated with distilled water
- Group II, to which bulbs were treated with alone 0.175 M NaCl
- Group III, to which bulbs were treated with a 50 mg L⁻¹ dose of GTLE
- Group IV, to which bulbs were treated with both GTLE (50 mg L⁻¹) and NaCl (0.175 M)

These bulbs into plastic boxes were germinated at incubated. When the roots reached about a length

of ten cm (approximately 7 days after the beginning of the assay), their radicle numbers and germination percentages were recorded. The radicle lengths were measured in mm, additionally the fresh weights in g/seedling were determined.

2.3. Cytological Analysis

For cytological and physiological studies, root tips were excised after a few days. Cytological preparations pretreated with saturated para-dichlorobenzene for 4 hrs, carried out by fixation of roots in a mixture of 3: ethanol / 1: glacial acetic acid at room temperature for 24 hrs. Then stored at 70 % ethanol in 4°C until used for analyses. Hydrolysis were done by 5 N HCl for 45 min, root tips were stained using the Feulgen, squashed and then smashed in a drop acetic acid of 45% [10]. Microscopic slides made permanent by mounting in balsame. Mitotic index was expressed in percentage by counting cells of different mitotic phases in total number of cells. Mitotic phases were also expressed as percentage of total number of cells. Chromosomal aberrations were recorded. The mitotic index was calculated by means of this formula, 2,000 cells (three slides = 6,000 total cells).

Mitotic index (%) = $\frac{\text{Number of cells in mitosis} \times 100}{\text{total number of cells}}$

Observations of counted cells in each application groups were performed using an Olympus CX41 microscope and photographed at X500 magnification.

2.4. Statistical Analysis

Data collected from physiological and cytogenetical parameters using the SPSS program according to Duncan's multiple range test in triplicate, all statistical analyses were performed [11].

3. RESULTS

3.1. Effects of Green Tea Leaf Extract on the Seedling Growth and Bulb Germination

Table 1 results clearly demonstrate that while germination percentage and fresh weight of group III showed statistically the same values as control (group I), their radicle number and radicle length partly decreased according to control bulbs germinated in distilled water.

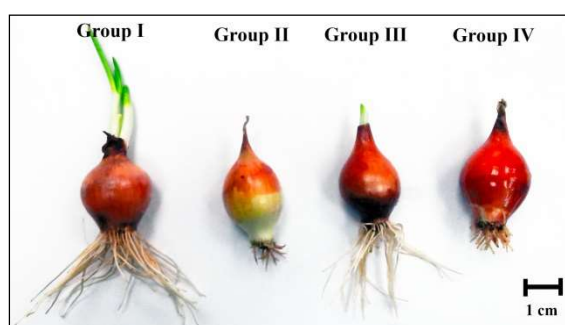


Figure 1. Root tip cells of *Allium cepa* showing germination situations at the end of 7 day. Group I (control): distilled water, Group II: 0.175 M NaCl alone, Group III: 50 mg L⁻¹ GTLE and Group IV: 50 mg L⁻¹ GTLE+0.175 M NaCl, Scale bar = 1 cm

NaCl showed an inhibitory activity on all growth parameters examined. For instance, control bulbs germinated in distilled water after 7 days showed 100% germination, whereas this value was 23 % in group II bulbs germinated at 0.175 M salinity. That is to say, NaCl prevented 77 % *Allium cepa* bulb germination. The restricting effect of NaCl stress on the bulb germination markedly mitigated by green tea leaf extract (GTLE) application. Group IV bulbs treated with GTLE at said salt level showed 75 % germination (Fig. 1). In addition, GTLE continued its success on the seedling growth parameters like fresh weight, radicle number and radicle length. Radicle length, radicle number and fresh weight of group II bulbs grown in 0.175 M salted were 10.3 mm, 12.7 and 7.0 g, respectively while these values became 13.4 mm, 17.2 and 13.8 g in group IV (Tab. 1).

Table 1. Effect of green tea leaf extract on some growth parameters of *Allium cepa*

Groups	Growth parameters			
	Germination percentage (%)	Radicle length (mm)	Radicle number	Fresh weight (g/seedling)
Group I	*100 ± 0.0 ^c	63.5 ± 0.5 ^d	63.2 ± 0.6 ^d	14.2 ± 0.8 ^b
Group II	23 ± 2.8 ^a	10.3 ± 0.3 ^a	12.7 ± 0.5 ^a	7.0 ± 0.5 ^a
Group III	100 ± 0.0 ^c	53.9 ± 0.2 ^c	39.4 ± 0.6 ^c	15.4 ± 1.2 ^b
Group IV	75 ± 5.0 ^b	13.4 ± 0.6 ^b	17.2 ± 0.5 ^b	13.8 ± 0.6 ^b

* The difference between the values in each column and the same letters isn't significant at the 0.05 level (±SD). Group I (control): distilled water, Group II: 0.175 M NaCl alone, Group III: 50 mg L⁻¹ GTLE and Group IV: 50 mg L⁻¹ GTLE + 0.175 M NaCl

3.2. Effects of Green Tea Leaf Extract on The Mitotic Activity, Chromosomal Aberrations and Micronucleus Frequency

Exposure to 0.175 M salt revealed significant inhibition of the mitotic index and induction of the chromosomal aberrations and micronucleus frequency. That is to say, the mitotic index in root-tip meristematic cells of *Allium cepa* germinated in containing 0.175 M salt media showed a 89% reduction compared to group I bulbs and the mitotic aberrations and micronucleus frequency flashily increased. Micronucleus frequency of group III bulbs germinated in only GTLE medium was remained the same compared to group I (control) samples. This application increased the mitotic index and chromosomal aberrations (Tab. 2). Simultaneous GTLE+NaCl treatment (group IV) may be successful in improving reverse effects of salt on all cytogenetical parameters. Statistically, all values mentioned here are highly significant at level of significance $P \leq 0.05$. Table 2 summarizes all cytogenetic parameters obtained from the control and other treated bulbs.

Table 2. Effect of green tea leaf extract on some cytogenetic parameters of *Allium cepa*

Groups	Mitotic index (%)	Micronucleus frequency (%)	Chromosome aberrations (%)
Group I	*11.6 ± 1.0 ^c	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
Group II	1.2 ± 0.2 ^a	13.0 ± 1.0 ^c	17.0 ± 0.4 ^d
Group III	16.2 ± 0.6 ^d	0.0 ± 0.0 ^a	4.6 ± 1.0 ^b
Group IV	6.3 ± 0.1 ^b	9.0 ± 1.0 ^b	9.3 ± 0.3 ^c

* The difference between the values in each column and the same letters isn't significant at the 0.05 level (±SD). Group I (control): distilled water, Group II: 0.175 M NaCl alone, Group III: 50 mg L⁻¹ GTLE and Group IV: 50 mg L⁻¹ GTLE+0.175 M NaCl

The micronucleus formations observed during the microscopic examination of root tip meristematic cells of *Allium cepa* are shown in Figure 2 and abnormal mitosis phases are shown in Figure 3. The chromosomal abnormalities were observed nuclear disintegration, accumulation of micronuclei in cell, chromosomal rings, ball metaphase/telophase, stickiness in metaphase, metaphase/anaphase with chromosome losses, telophase/anaphase with chromosome bridges, vagrant chromosome in anaphase/telophase with broken chromosome bridges, disorientation at anaphase, diagonal at telophase. The greatest abnormalities percentage in root-tip cells treated with salt or GTLE were nuclear peak and micronucleus in this study.

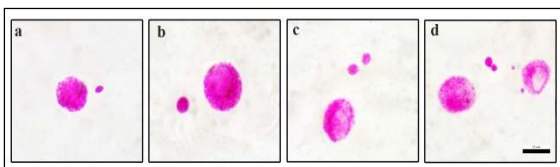


Figure 2. The micronucleus formations observed in *Allium cepa* L. meristematic cells exposed to Group II and Group IV; a, b: micronucleus c, d: accumulation of micronuclei in cell, Scale bar=10 µm

4. DISCUSSION

4.1. Physiological and Cytogenetical Effects of Green Tea Leaf Extract under Non-Stress Conditions

Unless there are generally stress conditions, there is no need for exogenously add any plant growth regulator during germination. Exogenously addition of a plant growth regulator in non-stress conditions can cause negative or positive effect on the seedling growth and bulb germination [12]. But, there is no study about the influences of green tea leaf extract on the seedling growth and bulb germination under non-stress conditions. Therefore, for the first time, the effects of GTLE application on the seedling growth, bulb germination, chromosomal aberrations, mitotic activity and micronucleus frequency under stress-free conditions requested to be tested in the laboratory study. Results of this study showed that the radicle number and radicle length of the bulbs germinated in only GTLE treatment were partially reduced but their fresh weight and germination percentages of the mentioned bulbs statistically showed the same values compared to those of the control bulbs germinated in distilled water (Table 1).

Moreover, some growth regulators may cause particularly cell disortions, chromosomal aberrations and mitotic irregularities especially if stress conditions aren't present [13, 14]. Although a number of researchers state that some plant extracts that have been widely used in recent years have significant mutagenic effects [15], there is no extent study relating to the impacts of GTLE on the chromosomal abnormalities, micronucleus frequency and mitotic activity subject to non-stress conditions. For this reason, this study was examined the first time whether GTLE affected these parameters at normal conditions. This data obtained in the present study indicated that the mitotic index in root tip meristem cells of *Allium cepa* (Group III) bulbs exposed to GTLE application in non-stress conditions increased 39% according to ones of the group I bulbs germinated in distilled water medium. That is, 50 mg L⁻¹ GTLE treatment showed a triggering

influence on the mitotic activity by accelerating cell division.

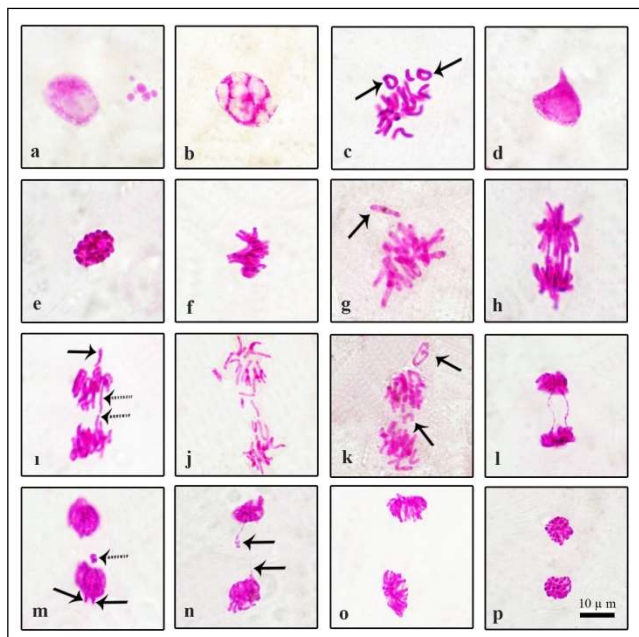


Figure 3. Different types of chromosomal aberrations observed in *Allium cepa* L. meristematic cells exposed to Group II, Group III and Group IV; a: accumulation of micronuclei in cell b: nuclear disintegration c: chromosomal rings= arrows d: nuclear peak e: ball metaphase f: stickiness in metaphase g: metaphase with loss chromosome= arrow h: anaphase with chromosome bridges i: vagrant chromosome (arrow) in anaphase with broken chromosome bridges= patterned j: disorientation at anaphase k: anaphase with chromosome losses= arrows l: telophase with chromosome bridges m: vagrant chromosomes (arrows) in telophase with micronucleus= patterned n: telophase with broken chromosome bridges= arrows o: diagonal at telophase p: ball telophase (Scale bar = 10 µm)

4.2. Physiological and Cytogenetical Effects of Green Tea Leaf Extract under Saline Conditions

It is well-known that saltinity stress has diverse influences on plant physiological processes such as increased ion toxicity and respiration rate, mineral distribution, change in plant growth, membrane permeability [16] and decreased of photosynthesis efficiency [17].

The results from table 1 clearly demonstrated that as expected, the seedling growth and bulb

germination of *Allium cepa* inhibited under salinity medium. Salinity stress can be preventive in many ways. Seed germination can be prevented by causing to change in water situation of the seed, thus prevent water intake [18]. Results of the present study displaying the diminish in water content and the fresh weight of the seedlings in salted conditions can be explained by the inability of roots to receive enough water due to high osmotic pressure in medium. Restrictive treatment of salt on the fresh weight, radicle number and radicle length might result from reducing cell division, protein synthesis and nucleic acid [19].

On the contrary, by GTLE application, inhibitory effect of salt stress on parameters such as the bulb germination, seedling growth (fresh weight, radicle number and radicle length) was significantly eliminated (Tab. 1). Unfortunately, to date, there isn't extant literature data relating to effects of GTLE on the seedling growth and bulb germination exposed to saline conditions. This GTLE alleviates salt stress on the bulb germination and seedling growth could be noticed by decreasing the osmotic influence of salt. For example, at 0.175 M NaCl treatment, GTLE application raised markedly growth parameters of seedlings compared to the control indicates this probability (Tab. 1).

Effects of the determined concentrations of a test chemical on the chromosome aberration and mitotic index are used respectively as parameters of cytotoxicity and genotoxicity [20]. The inhibitory and cytotoxic effects of salt stress on the mitotic activity have long been known. A high concentration of salt causes total inhibition of the chromosomal abnormalities and mitotic activity in root-tip cells according to some researchers [21]. With this study, it should be noted that salinity negatively affects the mitotic activity, chromosome behaviors and micronucleus frequency in *Allium cepa* root meristem cells. Data of this study showed that salt showed a greater number of the chromosomal abnormalities and micronucleus frequency compared to controls and reduced the mitotic index by 89 % and this reduced was achieved by reducing the number of cells entering mitotic division. For example, the micronucleus frequency and chromosomal aberration in the root tip meristematic cells of the

bulbs germinated exposed to distilled water were 0.0 %, 0.0 % while it were 13.0 %, 17.0 % at 0.175 M saltness (Tab. 2). Besides, simultaneous GTLE+NaCl application could be succeeded in alleviating of the detrimental influence of salinity on the chromosome aberration, micronucleus frequency and mitotic activity. Limit of mitotic inhibition by this treatment reached to 6.3 %. So, the chromosomal aberration decreased by 45%, the frequency of micronucleus decreased by 30% with the application of simultaneous GTLE+NaCl. This result shows GTLE repair role against salt injuries during *Allium cepa's* mitosis.

Chromosome aberrations (CAs) are change in chromosomal material or exchange in the structure of the chromosome resulting from breakage. CAs induction could affect the fertility, vigour, competitive or yield ability of the exposed plants [22]. The presence of nuclear disintegration (Fig. 3b) offer cytological evidences for the inhibitory action on DNA biosynthesis [23]. Diagonal orientation (Fig. 3o) is caused by a slight tilt in the spindle apparatus [24]. Vagrant chromosomes (Fig. 3i, m) which might arise as a consequence of disturbances in the mitotic spindle were categorized as indicatives of aneugenic action. The micronucleus (Fig. 3a, m) is composed either of all of the chromosomes that do not migrate during anaphase as a result of spindle dysfunction or of small chromatin fragments which arise as a consequence of chromosomal break. The loss chromosomes (Fig. 3g, k) are typically associated with mitotic spindle malfunction [25]. Chromosomal ring (Fig. 3c) is the result of chromosome losses in the telomere domain [26]. The ball metaphase (Fig. 3e) may be due to the localized activity of spindle apparatus in the centre, so that the centromeres remain in the equator and arms spread in different directions in the form of a ball. The bridges (Fig. 3h, l) are probably caused by joining and interruption to chromatids or chromosomes. Stickiness in metaphase (Fig. 3f) is an indicates that chemical substance has a high toxicity and might cause the cell deaths by inducing unrecoverable damages [27]. Disorientation at anaphase (Fig. 3j) may be due to spindle apparatus disturbance which allows that the chromosomes to spread irregularly over the cell [28]. Shortly, green tea leaf extract may

be function as a stimulator triggering the protein synthesis required for the normal cell division and accelerate the mitotic cycle.

There is no literatures data on the influences of GTLE application under salted conditions on cytogenetical and physiological parameters examined in the study. Therefore, for the first time, this study results have been reported particularly in saline conditions. As a result, this study shows that GTLE can significantly increase the activations such as the seedling growth and bulb germination in either alone or saline conditions. But the mechanisms by which salt inhibits growth are controversial and complex, also they might vary according to cultivar and species. An universal mechanism hasn't been established yet. While the causes of salty have been determined, it is still very poor to understand the mechanisms by which salty prevents plant growth. Therefore further work should be done to learn more about the effect of GTLE on cell division, cell cycle and germination molecular metabolism. This literature study can serve to present new conceptual tools for designing salt tolerance hypotheses in plants.

5. REFERENCES

- [1] T.J. Flowers and A.R. Yeo, "Ion relations of plant under drought and salinity", Australian Journal of Plant Physiology, vol. 13, pp. 75–91, 1986.
- [2] T.J. Flowers and A.R. Yeo, "Breeding for salinity resistance in crop plants: where next?", Australian Journal of Plant Physiology, vol. 22, pp. 875–884, 1995.
- [3] P. Carillo, M.G. Annunziata, G. Pontecorvo, A. Fuggi, and P. Woodrow, "Salinity stress and salt tolerance", in: Abiotic stress in plants - mechanisms and adaptations, pp. 21–38, 2011.
- [4] A.K. Parida and A.B. Das, "Salt tolerance and salinity effects on plants: a

- review”, *Ecotoxicology and Environmental Safety*, vol. 60, pp. 324–349, 2005.
- [5] U. Deinlein, A.B. Stephan, T. Horie, W. Luo, G. Xu, and J.I. Schroeder, “Plant salt-tolerance mechanisms”, *Trends Plant Science*, vol. 19, pp. 371–379, 2014.
- [6] D.G. Nagle, D. Ferreira, and Y.D. Zhou, “Molecules of interest epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives”, *Phytochemistry*, vol. 67, pp. 1849–1855, 2006.
- [7] S.Y. Asadi, P. Parsaei, M. Karimi, S. Ezzati, A. Zamiri, F. Mohammadzadeh, and M. Rafieian-Kopaei, “Effect of green tea (*Camellia sinensis*) extract on healing process of surgical wounds in rat”, *International Journal of Surgery*, vol. 11, no. 4, pp. 332–7, 2013.
- [8] G. Fiskesjo, “The *Allium* test as a standard in environmental monitoring”, *Hereditas*, vol. 102, no. 1, pp. 99–112, 1985.
- [9] W.F. Grant, “Chromosome aberration assays in *Allium*: a report of the united states environmental protection agency’s genotox program”, *Mutation Research/Reviews in Genetic Toxicology*, vol. 99, pp. 273–291, 1982.
- [10] P.C. Sharma and P.K. Gupta, “Karyotypes in some pulse crops”, *Nucleus*, vol. 25, pp. 181–185, 1982.
- [11] D.B. Duncan, “Multiple range and multiple F tests”, *Biometrics*, vol. 11, pp. 1-42, 1955.
- [12] D. Çavuşoğlu, “Effects of L-lysine on cytogenetical and physiological parameters in *Allium cepa* L. under salt stress”, *Bangladesh Journal of Botany*, vol. 48, no. 3, pp. 625-632, 2019.
- [13] D. Çavuşoğlu, “Physiological and cytogenetical effects of L-tyrosine in *Allium cepa* L. exposed to NaCl stress”, *Fresenius Environmental Bulletin*, vol. 28, no. 12A, pp. 9753-9759, 2019.
- [14] K. Çavuşoğlu, S. Cadıl, and D. Çavuşoğlu, “Role of potassium nitrate (KNO₃) in alleviation of detrimental effects of salt stress on some physiological and cytogenetical parameters in *Allium cepa* L.”, *Cytologia*, vol. 82, no. 3, pp. 279-286, 2017.
- [15] MC. Karaismailoğlu, “Investigation of the cytotoxic and genotoxic effects of *Artemisia annua* methanol extract with the *Allium* test”, *Ekoloji*, vol. 23, pp. 64-74, 2014.
- [16] N.K. Gupta, S.K. Meena, S. Gupta, and S.K. Khandelwal, “Gas exchange, membrane permeability, and ion uptake in two species of Indian jujube differing in salt tolerance”, *Photosynthetica*, vol. 40, pp. 535-539, 2002.
- [17] O.H. Sayed, “Chlorophyll fluorescence as a tool in cereal crop research”, *Photosynthetica*, vol. 41, pp. 321-330, 2003.
- [18] T.J. Flowers, and T.D. Colmer, “Plant salt tolerance: adaptations in halophytes,” *Annals of Botany*, vol. 115, no. 3, pp. 327-331, 2015.
- [19] K.F. Mccue and A.D. Hanson, “Drought and salt tolerance: towards understanding and application”, *Trends Biotechnology*, vol. 8, pp. 358-362, 1990.
- [20] H. Nefic, J. Musanovic, A. Metovic, and K. Kurteshi, “Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by *alprazolam*”, *Medical Archives*, vol. 67, pp. 388–392, 2013.
- [21] S. Radic, M. Prolic, M. Pavlica, and B. Pevalek-Kozlina, “Cytogenetic effects of osmotic stress on the root meristem cells of *Centaurea ragusina* L.”, *Environmental and Experimental Botany*, vol. 54, pp. 213-218, 2005.
- [22] M. Kara, M.A. Tanda, and A. Ateş, “Cytogenetic effects of the insecticide cypermethin on the root meristems of *Allium*

cepa L.” Turkish Journal of Biology, vol. 18, pp. 323–331, 1994.

- [23] F.I. Akaneme and I.V. Iyioko, “Mutagenic potentials of the sterilizing fluid-purital on root tip mitosis of *Allium cepa*”, Bio-Research, vol. 6, pp. 293–297, 2008.
- [24] P.K. Renjana, S. Anjana, and J.E. Thoppil, “Evaluation of genotoxic effects of baking powder and monosodium glutamate (MSG) using *Allium cepa* assay”, International Journal of Pharmacy and Pharmaceutical Sciences, vol. 5, 132-139, 2013.
- [25] D.M. Leme and M.A. Marin-Morales, “*Allium cepa* test in environmental monitoring: a review on its application”, Mutation Research, vol. 682, pp. 71–81, 2009.
- [26] N. Khanna and S. Sharma, “*Allium cepa* root chromosomal aberration assay”, Indian Journal of Pharmaceutical and Biological Research, vol. 1, pp. 105-119, 2013.
- [27] S. Türkoğlu, “Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L.” Mutation Research, vol. 626, no. 1-2, pp. 4-14, 2007.
- [28] S.M. Amer and E.M. Alı, “Cytological effects of pesticides. V. effects of some herbicides on *Vicia faba*”, Cytologia, vol. 39, pp. 633-643, 1974.