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Evaluation of Genotoxic Effect of Phloxine by Allium Test

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ABSTRACT

Phloxine is used as a food dye. In this study, genotoxicity of phloxine at the root tip of *Allium cepa* L. was investigated. *A. cepa* L. meristematic root tip cells were treated with ten different doses of phloxine. In this way, the EC50 value was determined. Then, phloxine was applied to root tips at EC50/2, EC50 and EC50×2 doses. Treatment time was determined as 24, 48 and 72 hours. As a result, it was revealed that phloxine caused chromosomal aberrations in cells in mitotic cycle at the root tip of *A. cepa*. There are equatorial plate shifting in metaphase, laggard chromosome, disturbed spindle, chromosome stickiness, C-mitosis, polar shifting among the observed chromosomal aberrations. It was stated that the % chromosomal aberration index (CAI) increased depending on concentration increase. It has been demonstrated that the highest % chromosomal aberration index occurred at the EC50×2 dose for 72 hours. According to the research, it was revealed that phloxine has a genotoxic effect on the root cells of *A. cepa*. For this reason, it can be emphasized that care should be taken in its use in foods.

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Allium Testi ile Floksinin Genotoksik Etkisinin Değerlendirilmesi

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ÖZ

Floksin, gıda boyası olarak kullanılmaktadır. Bu çalışmada, kırmızı renkli olan floksinin *Allium cepa* L. kök ucundaki genotoksik etkisi araştırılmıştır. *A. cepa* L. meristematik kök ucu hücreleri, on farklı dozda floksin ile muamele edilmiştir. Bu şekilde EC50 değeri belirlenmiştir. Daha sonra kök uçlarına EC50/2, EC50 ve EC50×2 dozlarında floksin uygulanmıştır. Floksin ile muamele süresi 24, 48 ve 72 saat olarak belirlenmiştir. Araştırmanın sonucunda, *A. cepa* kök ucu mitotik döngüsündeki hücrelerde floksinin kromozomal aberasyonlara neden olduğu ortaya konmuştur. Mitotik hücrelerde gözlenen kromozomal aberasyonlar arasında metafazda ekvatoral plak kayması, gecikmeli kromozom, bozulmuş iğ iplikleri, kromozom yapışkanlığı, C-mitoz, kutup kayması bulunmaktadır. Kök uçlarına uygulanan floksin dozu artışına bağlı olarak, % kromozomal aberasyon indeksinin arttığı belirlenmiştir. En yüksek % kromozomal aberasyon indeksinin ise, EC50×2 dozunun 72 saat süresince uygulanması ile meydana geldiği ortaya konmuştur. Araştırmanın sonucuna göre, floksinin *A. cepa* kök ucunda genotoksik etkisi olduğu saptanmıştır.

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Introduction

Food additives are substances used by food manufacturers. They are added into food in small quantities through production to enhance the flavor quality of the foods and extend their storage time (Magnuson et al., 2013). Food additives are not regularly eaten as food or/and utilized as food fixings. However, they are substances used as additives in foods to gain certain effects in the foods (Sharma, 2015). Food additives can be used for various aims such as preservatives, colorants, ingredient developers, antioxidants, non-nutritive sweeteners, etc. Therefore, they are classified according to their functions (Güngörmüs and Kılıç, 2012). As the world's population continues to increase, consumers are worried about acquiring new food sources and preserving these sources for longer periods without compromising their characteristics (Kumar and Panneerselvam, 2007). Usually using of food additives has shown the best effect of extending the shelf life of food sources. However, it has been reported that food additives used to protect foods are genotoxic (Luca et al., 1987). However, they have also antioxidant effects when applied at different concentrations (Ito and Hirose, 1989; Kahl, 1984).

Phloxine is a color additive used in foods (Rasooly, 2005). Sasaki et al. (2002) proposed that as a red food dye, phloxine is one of the most effective gene toxins in the gastrointestinal organs. Phloxine has been reported to cause concentration related DNA damage in colon, bladder, glandular stomach. It leads to DNA damage at low doses (10 or 100 mg/kg) in the gastrointestinal organs. Sako et al. (1980) expressed that phloxine was explicitly harmful to cell development and a concentration-response relationship was determined between phloxin concentration and hepatocyte development. Tanaka and Kitahara (1975) demonstrated that phloxine caused chromosome deviations in human leucocytes.

Allium cepa test is used to evaluate the genotoxicity of food additives. *Allium cepa* L. genome has been recognized as an important genetic material for testing and monitoring environmental pollution and the impact of various chemical substances (Grant, 1978); Accepted as a biological indicator (Rank et al., 2003; Leme and Marin-Morales, 2009); a) Plant roots grow in direct contact with related foreign substances; b) Used to predict possible DNA damage in eukaryotes. As pointed out by Camparoto et al. (2002) and Rank and Nielsen (1994), the cell division and DNA damage in *A. cepa* and mammalian cells are highly related (82%). Peron et al. (2008), Fachinetto and Tedesco (2009) and Geras'kin et al. (2011) stated that even though plant metabolism differs from animal metabolism, the results obtained using the test system count as good toxicity analysis parameters at the cells and it has been utilized for a long time to remind people to be careful in the use of natural medicines, certain synthetics and some foods.

Studies on phloxine have shown that phloxine has a phototoxic effect. It can damage cell membrane and DNA. These results show that phloxine is allowed to be evaluated on the usage dose in the cosmetic and food industry (Valenzeno and Pooler, 1982; Inbaraj et al., 2005; Qi et al., 2012).

In this research, it was intended to evaluate genotoxic effect of phloxine food dye on *A. cepa* root cells by Allium cepa test. Phloxine was applied with different dose and times in *A. cepa* root tip cells. To determine the genotoxicity, % chromosomal aberration index (CAI) was calculated.

Material and Method

Material

Allium cepa L.and phloxine was used as a plant material and a food preservative, respectively. Phloxine (CAS registry no:18472-87-2, purity: ≥95) was purchased from Cesa Chemical Industry Trade Limited Company. *A. cepa* bulbs was purchased from a nearby market.

Method

Treatment with Phloxine on Allium cepa L. Roots

To determine the effective concentration reducing the root prolongation by 50%, the EC50 value was determined by treating the root tips for 72 h with ten different doses of phloxine (0.15, 0.30, 0.45, 0.60, 0.75, 0.90, 0.105, 0.120, 0.135, 0.150 g/l) dissolved in water. EC50 value was determined by root length estimation (Fiskesjö, 1993). Ten onion bulbs were used for each phloxine concentration and the control group (distilled water). The roots of each bulb were suspended on phloxine EC50/2 (0.15 g/l), EC50 (0.30 g/l), 2XEC50 (0.60 g/l) doses inside test tubes for 24, 48, 72 h at room temperature.

Preparation of mitotic slides

Root tips were cut and placed in fixative comprising of ethanol and glacial acetic acid (3:1). The root tips were hydrolyzed according to the method provided by Souguir et al. (2008). Samples were prepared by fixing a mash preparation with 2% acetocarmin (w/v). At least 5000 cells were counted for all treatment groups. The cells in the mitotic division stage were monitored with microscope at 1000X objective.

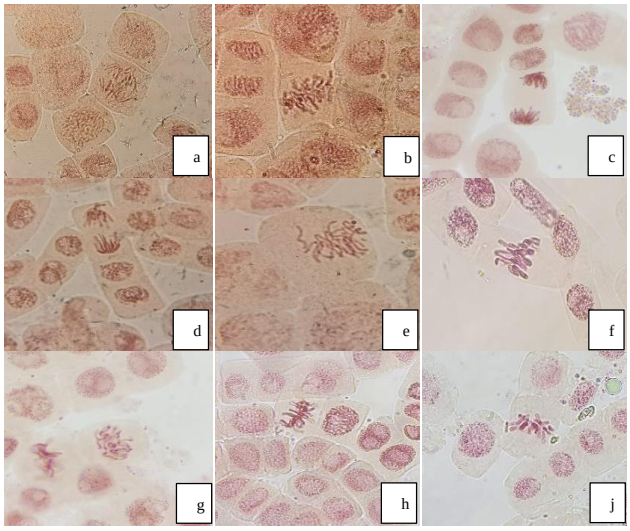

Figure 1. Chromosomal aberrations in *A. cepa* root tip meristematic cells a) Laggard chromosome in anaphase, b) Equatorial plate shifting in metaphase, c) Laggard chromosome and polar shifting in anaphase, d) Laggard chromosome in anaphase, e) Disturbed spindle, f) Chromosome stickiness, e) Disturbed spindle, h) Equatorial plate shifting in metaphase, j) C-mitosis

Table 1. Chromosomal aberration index (CAI) and chromosome aberrations

Application period (h)	Concentration (g/l)	Chromosome aberrations (%)		
		C-Mitosis	E.Plte Shftng	Plte Shftng
24	Control	18	12.23	18.12
	EC50/2	22.15	23.19	25.14
	EC50	26	34.16	28.45
	EC50X2	25.18	28.64	34.15
48	Control	0	21.18	11.26
	EC50/2	41.12	22.41	17.42
	EC50	35.19	18.25	24.74
	EC50X2	51.16	8.15	39.17
72	Control	28.42	5.11	28.34
	EC50/2	32.25	16.13	37.24
	EC50	39.54	21.28	42.15
	EC50X2	45.29	23.18	48.09

Application period (h)	Chromosome aberrations (%)			CAI % (Mean±Std. Error)
	Laggard	Chromosome stickiness	D.spindle	
24	19.12	12.45	0	1.105 ± 0.253^{Db}
	25.06	23.56	6.14	12.154 ± 0.421^{Cb}
	18.24	27.06	8.34	19.257 ± 0.620^{Bcb}
	32.18	25	21.04	25.648 ± 0.647^{Acb}
48	17.21	0	4.27	1.526 ± 0.269^{Da}
	27.15	34.18	0	15.230 ± 0.108^{Cb}
	34.27	0	0	27.147 ± 0.345^{BAb}
	41.08	42.2	18.13	31.109 ± 0.287^{Ab}
72	10.17	0	0	1.357 ± 0.430^{Da}
	9.04	39.14	19.27	24.310 ± 0.435^{Ca}
	18.12	27.32	30	41.027 ± 0.08^{BAa}
	29.01	42	0	57.141 ± 0.291^{Aa}

Note 1: During the same period, the difference between the doses stated in various capital letters is significant. Note 2: At the same dose, the difference in treatment periods given in various small letters is significant. Abbrevations: E.Plte Shftng: Equatorial plate shifting, Plte Shftng: Polar shifting, Laggard: Laggard chromosome, D. Spindle: Disturbed spindle, CAI: Chromosomal aberration index, Std error: Standard error

Examination of Genotoxicity

Five thousand cells were counted for each treatment group. Actively dividing cells were defined by counting the phases of mitosis in which they were found and the mitotic aberrations were monitored in these phases by a compound light microscope. Chromosomal aberration index (CAI) percentage was calculated by the formula of CAI % = Number of cells with chromosomal aberrations/Total number of cells.

Statistical Analysis

The TUKEY multiple comparison test was used. Repeated measurement of two-factor ANOVA was utilized to examine the effects of concentration and duration on the genotoxic effect ($P \leq 0.05$).

Results and Discussion

Chromosomal aberrations observed as a result of the application of phloxine food dye at the root tip of *A. cepa* at certain exposure times and doses are shown. C-Mitosis, equatorial plate and polar shifting, laggard chromosome, disturbed spindle are the observed chromosomal aberrations. Observed chromosomal aberrations are indicated in Table 1 with CAI %. According the Table 1, administration of phloxine for 72 h significantly increased the chromosomal aberration index compared to the administration doses at 24 and 48 h. Chromosomal aberrations observed in meristematic cells of *A. cepa* root tips because of the application of phloxine are indicated in Figure 1. The observed chromosomal aberrations are laggard chromosome in anaphase, equatorial plate shifting in metaphase, polar shifting in anaphase, disturbed spindle, chromosome stickiness and C-mitosis.

Das et al. (2021) stated that saccharin induced genotoxicity in *A. cepa* root tips. Öztürk et al. (2020) specified that fragments and adherent chromosomes were the most common chromosomal abnormalities observed in *A. cepa* meristematic cells after potassium bromate administration. Pandey and Kumar (2021) detected genotoxicity induced by butylated hydroxytoluene and butylated hydroxyanisole at *A. cepa* root tips. It has been noted that the frequency of chromosome abnormalities raised after application with these food preservatives. Yoosuf et al. (2020) determined that sunset yellow caused an increase in chromosomal abnormalities depending on dose and time in the root tips of *A. cepa*. It was reported that chromosomal aberrations were formed due to sunset yellow treatment. Ertushi and Noori (2021) stated that the percentage of chromosomal abnormalities increased with increasing metabisulfite concentrations. Farheen et al. (2021) observed sticky metaphase, sticky anaphase, abnormal prophase, C-mitosis and laggard anaphase at each administered dose of allura red. It was reported that forwarded anaphase, dislocated metaphase and bridge anaphase were detected in *A. cepa* root tips applied with sunset yellow, tartrazine, and fast green. Frâncica et al. (2021) reported that synthetic chocolate food flavoring applied at concentrations between 100.00 and 0.50 µL/L caused micronuclei, nuclear buds and chromosomal breaks

in root tips. Bhattacharjee (2020) determined that tartrazine (E102) caused a raise in the number of mitotic chromosomal aberrations at the root tips of *Vicia faba*. Kaya (2020) and Kaya (2021) stated that chromosomal aberrations were caused by triacetin (glycerol triacetate) and ferrous gluconate, respectively. The observed chromosomal aberrationswere C-mitosis, polyploidy, polar shifting equatorial plate shifting, laggard chromosome. Therefore, it was determined that triacetin and ferrous gluconate were genotoxic. Pasileva et al. (2021) revealed that food preservative sodium benzoate, food sweetener aspartame and food colorant carmoizine have genotoxic effects. It was stated that these food additives have an inhibitory effect on cell division. It was determined that the frequency of chromosomal aberrations increased with increasing food additive concentration. It was determined that all food additives stimulated the formation of C-mitosis, laggard and vagrant chromosomes, fragments, and micronucleus. Bhattacharjee (2020) stated that tartrazine caused mitotic chromosomal aberrations at the root tip of *V. faba*. Consistent with the results of these studies, it was determined that phloxine food dye was genotoxic to *A. cepa* root tip cells at certain doses and application times in this research. In addition, consistent with other studies (Kaya, 2020; Ertushi and Noori, 2021; Kaya 2021; Pasileva et al., 2021), it was determined that the percent chromosomal aberration increased because of increasing dose and treatment time of phloxine. Therefore, it can be stated that the genotoxic effect increased depending on the treatment time and concentration increase.

Tanaka and Kitahara (1975) demonstrated that phloxine caused chromosomal aberrations in metaphase cells such as chromatid break, isochromatid break, fragment, pericentric inversion, dicentric, chromatid exchanges in human leucocytes. Chromosomal aberrations in all mitotic phases (prophase, metaphase, anaphase and telophase) were examined in this research. These chromosomal aberrations were C-mitosis, equatorial plate shifting, polar shifting, laggard chromosome, disturbed spindle in *A. cepa* root tips. While Sako et al. (1980) determined cytotoxic effect of phloxine, in this research it was stated genotoxic effect of phloxine.

Inhibition of the mitotic index at chemically treated root tips is caused by inhibition of DNA synthesis or/and blocking of the G2 phase in the mitosis (Sudhakar et al., 2001), as well as arrest of mitotic phases that prevent cells from entering the mitosis cycle (Kabarity and Mallalah, 1980). Likewise, the portion subordinate change in prophase (Liman et al., 2011). The genotoxicity of any synthetic lies in any undesirable substitute in chromosome shape regularly connected with breakage or change of chromosome known as chromosomal variation (Debnath et al., 2020). Besides, underlying changes in the foundations of DNA cause mismatches, arrangement of essential districts, unstable DNA design and single strand breaks (Kumar et al., 2018). These anomalies cause cell cycle interruptions and different sorts of chromosomal variations (Öztürk et al., 2020). It may occur because of sticky chromosomes, protein attachment (Patil and Bhat 1992), depolymerization of DNA, nucleoprotein disintegration, breakage and change in the development of essential collapsing fiber units of chromatids and stripping of the protein covering the DNA (Mercykutty and Stephen 1980). Fiskesjö (1985) detailed that chromosomal stickiness can have an irreversible and profoundly poisonous impact on the cell and this may ultimately prompt cell death. The rise of C-mitosis suggests that these food additives behave like colchicine, which represses the arrangement of mitotic spindle strands. In addition, formation of laggard chromosomes is because of chromosome development or acentric pieces (Farheen et al., 2021).

Conclusion

It was assessed that phloxine has genotoxic potential in *A. cepa* meristematic root cells. It has been resolved that phloxine may have a genotoxic impact when applied more than specific dosages and exposure times. It was expressed that the genotoxic impact expanded relying upon the expanding exposure time and portion. Therefore, it can be said that phloxine causes genotoxic effects when used as food additive.

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