

SHORT ARTICLE

EVALUATION OF CYTOTOXIC EFFECT OF PHLOXINE ON *Allium Cepa L.*

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ABSTRACT

Background: The inclusion of food additives in the food supply is extremely important. The addition of food additives can occur in the manufacturing, processing, packaging, transportation, and even storage of foods. **Objective:** In this study, the cytotoxicity of phloxin at the root tips of *Allium cepa* was investigated. The EC50 value was determined. Then, phloxin was applied to the root tips at EC50/2, EC50, and EC50X2. The treatment period was determined as 24, 48 and 72 h. **Results:** We revealed that phloxine decreased the mitotic index (MI) in the root tip mitotic cycle cells of *A. cepa*. Also the percentage of mitotic index decreased with increasing dose and application period. The lowest percentage mitotic index was found at the EC50X2 dose and after treatment during 72 hour. We have revealed that phloxin has a cytotoxic effect on the root tip of *A. cepa*. However, it can be said that care should be taken when using phloxine in food.

Keywords: *Allium cepa*, cytotoxicity, phloxine, mitotix index.

1. INTRODUCTION

Food additives are used by food manufacturers and are added to foods for a variety of reasons, including to add taste and texture, to increase shelf life and to improve the appearance of foods [1]. Any chemical or substance solution is not the main component of the food and is added to food according to scientific regulations that is considered a food preservative. The inclusion of food additives in the food supply is extremely important. The addition of food additives can occur in food manufacturing, processing, packaging, transportation, and even food storage. Thus, this additives are used to preserve the flavor and color of the food. Food additives can be used to keep food in good conditions and prevent it from being damaged by microbes. Shelf life of foods can be extended with food additives [2]. Food additives can preserve nutrients for a certain period of time, especially those with protective properties against bacteria that often have negative effects on human health. This has been proven in different test systems. For example, it has been determined that food additives with increasing concentrations and different ranges in plant cells change the mitotic index values. Thus, the entire rate of mitotic stages is altered. In addition, it was found that the percentage of chromosomal aberrations increased [3].

The Allium test is used to investigate the cytotoxicity of food additives. *Allium cepa L.* chromosomes are known as an important genetic material for testing the effects of various chemicals [4]. In the Allium cepa test, plant roots are in direct contact with the exposed exogenous substance. In addition, this test is used to predict possible DNA damage in eukaryotes [5, 6]. Cell division and DNA damage in Allium and mammalian cells were found to be closely related [7, 8]. Phloxine used in food, cosmetics and medicine [9]. Sasaki et al (2002) stated that phloxine – a red food coloring – is one of the most potent genotoxins in the gastrointestinal organs. We aimed to evaluate the cytotoxic effect of phloxine on root tip cells of *A. cepa*.

2. MATERIAL AND METHOD**2.1 Material**

Allium cepa L. and phloxine was used in this research.

2.2 Method**2.2.1 Treatment with Phloxine on *Allium cepa L.* roots**

Phloxine was dissolved in water. The EC50 value was determined by treating the *A. cepa* root tips with different concentration of phloxine.

Bulbs were treated for each concentration and the control (distilled water). The roots of each bulbs were placed on phloxine EC50/2 (0,15 g/l), EC50 (0,30 g/l), 2XEC50 (0,60 g/l) inside glass tubes for 24, 48, 72h.

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2.2.2 Preparation of Mitotic Prepare

Root tips were placed in a farmer fixative and then were hydrolyzed (Souguir et al., 2008). Root samples were colored with acetocarmine. Five thousands cells were counted for all treatment groups. The cells in the mitotic stage were monitored with microscope in immersion oil.

2.2.3 Examination of Cytotoxicity

Cytotoxicity was assessed by the mitotic index (MI). MI was numbered by formula with $MI = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$. Five thousand cells were numbered for each application groups in microscope.

2.2.4 Statistical analysis

The ANOVA and TUKEY multiple comparison test was used.

3. RESULTS AND DISCUSSION

As a result of the application of phloxine at EC50/2, EC50 and EC50X2 during 24, 48 and 72 hours, it was revealed that the mitotic index in the root tips decreased depending on the increasing in dose and treatment period. The observed mitotic phases (prophase, metaphase, anaphase and telophase) are indicated (Table 1). At least mitotix index was detected EC50X2 dose and 72h application period.

Table 1: Mitotic phases and mitotix index (%).

Treatment period (h)	Dose (g/l)	Mitotic Phases (%)				Mitotic index % (Mean±Std. Error)
		Prophase	Metaphase	Anaphase	Telophase	
24	Control	72.34	33.1	23.2	15.22	$35.06 \pm 0.810\text{Aa}$
	EC50/2	58.41	27.54	25.06	16.12	$27.01 \pm 0.560\text{Ba}$
	EC50	48.75	21.85	19.13	13.08	$19.56 \pm 0.208\text{Ca}$
	EC50X2	53.42	24.08	17.12	9.15	$13.81 \pm 0.361\text{Da}$
48	Control	66.14	46.03	27.41	18.05	$38.51 \pm 0.240\text{Aa}$
	EC50/2	59.62	39.15	35.12	14.09	$25.86 \pm 0.080\text{Bab}$
	EC50	61.25	29.34	37.24	10.34	$17.64 \pm 0.107\text{Cab}$
	EC50X2	43.15	19.21	21.13	8.25	$8.60 \pm 0.119\text{Db}$
72	Control	64.58	26.45	19.1	20.37	$36.20 \pm 0.164\text{Aa}$
	EC50/2	42.61	31.02	20.17	15.71	$19.08 \pm 0.084\text{Bc}$
	EC50	54.35	21.3	14.12	9.01	$10.13 \pm 0.475\text{Cc}$
	EC50X2	41.23	15.08	10.03	7.15	$7.61 \pm 0.261\text{Db}$

Note: During the same period, the difference between the concentration stated in various capital letters is important. At the same concentration, the difference in periods given in various small letters is important

Cytotoxicity at the root tip of *A. cepa* was shown to be caused by butylated hydroxytoluene and butylated hydroxyanisole, synthetic food flavor of chocolate, ferrous gluconate, respectively. [12, 13, 14]. It was found that the mitotic index decreased with increasing concentrations of applied sodium metabisulfite. [15]. The Sodium benzoate, aspartame and carmoizine have been found to have cytotoxic potential. It has been stated that these food additives have an inhibitory effect on cell division [16]. Sunshine yellow causes a dose- and time-dependent decrease in the mitotic index in the root tips of *A. cepa* [17]. The Tartrazine (E102) caused a decreasing in the mitotic index in the root tips of *Vicia faba* [18]. In addition, Triacetin (glycerol triacetate) has a cytotoxic effect in *A. cepa* [19] and tartrazine caused a reduction in MI at the root tip of *Vicia faba* [18].

4. CONCLUSION

In the present study, we have found that phloxine has cytotoxic potential in *A. cepa* root tips. In addition we revealed also that phloxin decreased the mitotic index value depending on the increase in the application dose and period. This research can be evaluated as a pre-study for animal and human researches.

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