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The Effects of Tartrazine in *Allium Cepa L*

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Abstract

The effect of tartrazine on *Allium cepa L.* at concentrations of 0.1 , 1, 3 and 5 ng/ml-1 were studied. Analysis focused on root growth, frequency of mitosis in a meristematic zone, and chromosomal aberrations. It was observed that tartrazine reduces root growth and the frequency of mitotic cells in meristematic zones, and increases the frequency of aberrant cells. The intensity of the effects is a function of tartrazine concentration.

Keywords: Chromosomal aberrations; Tartrazine; Proliferating activity; Root growth

Introduction

Dyes are a class of additives without nutritional value which are added to foods with the objective of providing color thus making the product more attractive and increasing its consumer acceptability. Tartrazine is one of the largest artificial dyes used in the food industry and belongs to the family of the azo dyes (-N=N-). This dye is used in baked goods, cereals, meat products, canned vegetables, soups, sauces, ice cream, desserts, candy and other sweets; it is also used for coloring drinks of orange, lemon, milk products, jellies, fillings, liqueurs, juices and yogurts [1]. On a worldwide level, the control of the use of food dyes is based on the Acceptable Daily Intake (ADI), which is based on the results of the international research and the recommendations of the Codex Committee on Food Additives and Contaminants (CCFAC) [2]. In Argentina, the maximum acceptable levels of food dyes are regulated by the Argentine Food Code (CAA) [3] for products of confectionery, culinary preparations and the bakery products with chemical leavening agent, with or without stuffing, coated or not (includes sponge cakes, cakes, puddings and other masses of pastries with chemical leavening).

In the last decade, this dye has caused controversy as to its toxic effects, and its use was prohibited in some countries such as the United States, Japan, Australia and Germany, which showed induction of chromosomal and other genotoxic effects in mammals [4-6]; in addition, it has been demonstrated in vitro the mutagenic potential in cell cultures in the human stomach [7]. However, in another study conducted in France concluded that the tartrazine does not generate an increase in the frequency of micronucleated cells in the colon of rats at concentrations of 1000 mg/kg, 200 mg/kg and 20 mg/kg [8,9]. The cytotoxicity and genotoxicity of tartrazine dye was also tested in a bioassay in *Allium cepa L.* which resulted in the significant reduction of the mitotic index and the presence of telophase, anaphase bridges, and micronuclei [10,11].

The bioassays with plants are considered to be quite sensitive and simple in the control of the cytotoxic effects of chemical compounds [12,13], and the onion (*Allium cepa*) has been indicated as an efficient system for the evaluation of the cytotoxicity [14] due to their kinetic properties of proliferation and its small number (2n=16) of chromosomes and other features, which facilitates their analysis for the detection of damage to the structure of the DNA molecule [15-17] and changes in the cell division index (mitotic index), as the increase or reduction of the proliferation of tissue cells exposed to chemical compounds [18,19], and also to demonstrate satisfactory similarity to the results obtained with other bioassays such as those conducted with animals and cell cultures [2,20-22].

In virtue of the controversy of the toxic effects of the tartrazine in different systems, the aim of this work is to determine the cytotoxic effect of tartrazine in meristematic cells of *Allium cepa* exposed to different times and concentrations, by assessing the root growth, and proliferative activity and chromosomal aberrations.

Materials and Methods

Bulbs And Adventitious Roots

Onion bulbs of the pearl variety, average weight 20 g, were used. Adventitious roots were obtained by placing the base of the bulbs in filtered water in glass tubes equipped with a constant air bubbling system (10-20 ml/min). Nursing was done in an incubator at 25º C+/- 0.5º C, in darkness. Control bulbs were incubated in filtered water. Tartrazine was administered as tartrazine solution in concentrations of 0.1, 1, 3 and 5 ng/ml-1 of this element prepared with the same kind of water. All preparations were administered when the roots were 2-3cm long. Solution were renewed every 24 h, following the applied methodology for Lerda [23].

Root Growth Measurement

Growth was determined by measuring the length of 10-12 previously identified roots per bulb, every 24 h for 96 h.

Proliferating Activity

Proliferating activity was quantified by determining the mitotic cell frequency at the root tip. Root obtained 0, 12, 24 and 48 h after the beginning of the assay were fixed in ethanol-acetic acid, 3:1 v/v at 4º C for 24h. After dyeing the roots with acetic-hydrochloric acid [24], the meristematic area was flattened. The frequency of mitotic cells was determined on 1000 flattened cells.

Chromosomal Aberrations

When roots were 3-5cm long, they were exposed to the tartrazine concentration (0.1, 1, 3 and 5ng/ml-1) for 48 h. Later on, they were exposed to the action of colchicine at 0.1 % for 3h. Roots were then cut and fixed in ethanol-acetic acid (3:1 v/v) at 4° C for 24h. Afterwards, they were dyed with acetic orcein. Approximately 5000 cells were scored for frequency and type of chromosomal aberrations. Ethyl Metil Sulfoxide (EMS) at 0.2% was used for positive control. The frequency (percentage) of aberrant cells was determined on the basis of the total of counted cells, and the number of dividing cells. To determine significant differences between treated and control roots, the Irwin-Fischer (Z) assay for exact probability were used.

Results

The effect of different tartrazine concentrations on the longitudinal growth of the root was analyzed (Figure 1). It was observed that concentrations of 5ng/ml-1 stop the growing process after 24 h, however, this being due to root death. At concentrations of 0.1, 1 and 3 ng/ml-1 the root growth rate is reduced compared to control roots. These finding confirm that tartrazine cause an inhibition of root growth whose intensity depends on the concentration in a given milieu.

In the studying of the cell proliferation, it was observed that the frequency of mitotic cells was progressively reduced with increasing tartrazine concentrations (Figure 2). Minimun values are reached after 24h. This inhibition was transient, as recovery of the proliferation activity was observed after 48h of tartrazine incubation. These findings suggest that tartrazine blocks the cell division cycle at a stage before mitosis. Onion bulbs exposed to 0.1, 1 and 3 ng/ml-1 tartrazine had a frequency of mitotic cells similar to that of the control bulbs.

Tartrazine induces clastogenic effects in the meristematic roots of *Allium cepa.* Data regarding the frequency of aberrant cells are presented in Table 1. An increase in the frequency of aberrant cells was observed with the highest tartrazine concentration (5 ng/ml-1). The Irwin-Fischer (Z) assay for exact probability was carried out with the total of aberrant cells since at the concentration of 5 ng/ml-1 only bridges and binucleate cells occurred, thus differing from the positive control. The result was $Z=7.10$ (> 1.96) significant at $P < 0.05$.

Discussion

These findings suggest that tartrazine inhibits the longitudinal growth of the root as a function of its concentration. Tartrazine blocks the cell division cycle at a stage prior to mitosis. It was also found that onion bulbs possess tartrazine elimination or neutralization mechanisms. Cytologic aberrations observed in the *Allium* root-tip assay, at a concentration de 5 ng/ml-1, showed that Tartrazine was able to induce genotoxicity at the chromosome level. The most of the common abnormalities were bridges. These are probably produced by the disruption and binding of the chromosomes or chromatids [25] or as a result of the rigidity of the chromosome or that could be attributed to translocation or unequal inversion of chromosome segments [26]. Silvia [10] conducted an investigation of the cytotoxicity of tartrazine in *A. cepa* and obtained a reduction of the mitotic index of 10.5 at its control 7.6 (0.4mL-24 hours); in addition, observed the presence of telophase anaphase bridges, and micronuclei. This would indicate that the tartrazine has an antiproliferative activity in meristematic cells of *A. cepa.* Ulloa Carbajal et al. [11], also found alterations in the mitotic index. It should be noted, at the same time, that these concentrations are above the ADI of tartrazine (0-7.5 mg/kg body weight-day).

This dye is controversial in relation to its toxic activity and attracts the interest of toxicologists and allergists. It is considered to be responsible for various reactions that cause hives or even asthma. It is estimated that one person in 10 thousand have adverse reactions to these dyes [27]. According to Sayed et al. [28], they suspect that there is a significant correlation between the mutagenic effect of food dyes of the azo group and the trigger of various human pathologies.

The results obtained in the present investigation reinforce the importance of *Allium cepa,* as in this study, the results are similar to those obtained with other bioassays. It is also important to note, as has been mentioned by Lerda [23,29], that even if the plant metabolism is different, the results of *Allium cepa* are excellent cytotoxic analysis parameters, and that the observation of the chromosomal alterations in the cell cycle of this species has been used as an indicator to warn people about the consumption of certain foods [30-32].

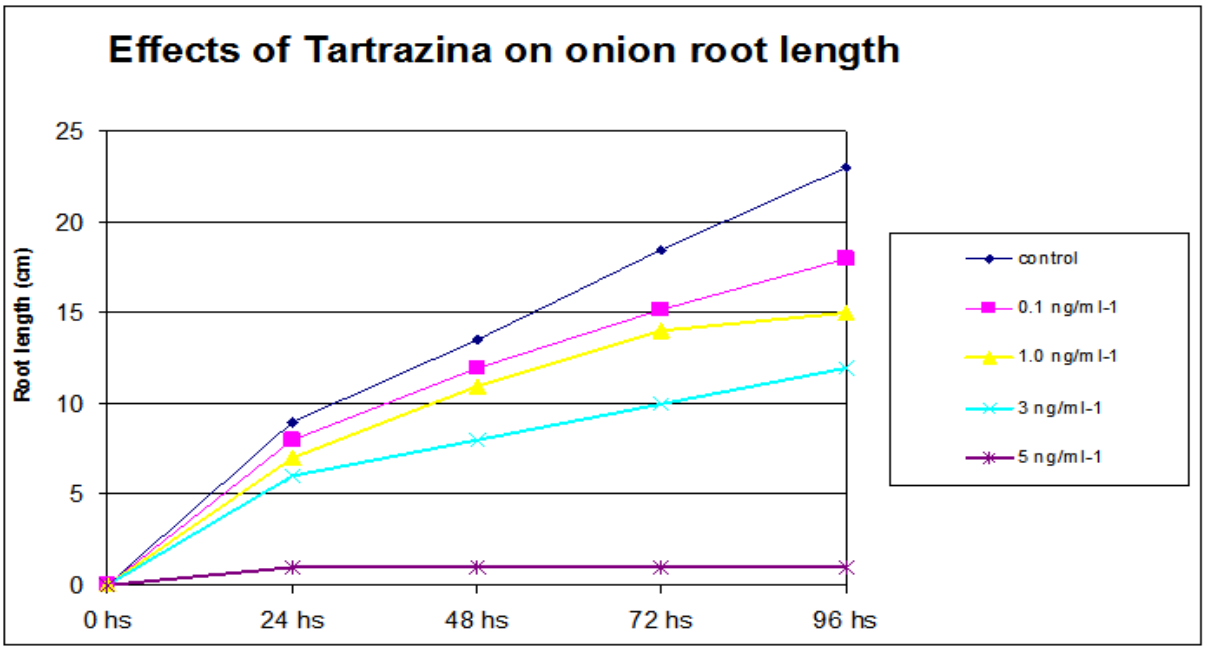

Figure1: Effect of Tartrazine on Root Growth Control; 0.1 ng/ml^{-1}; 1.0 ng/ml^{-1}; 3 ng/ml^{-1}; 5 ng/ml^{-1}

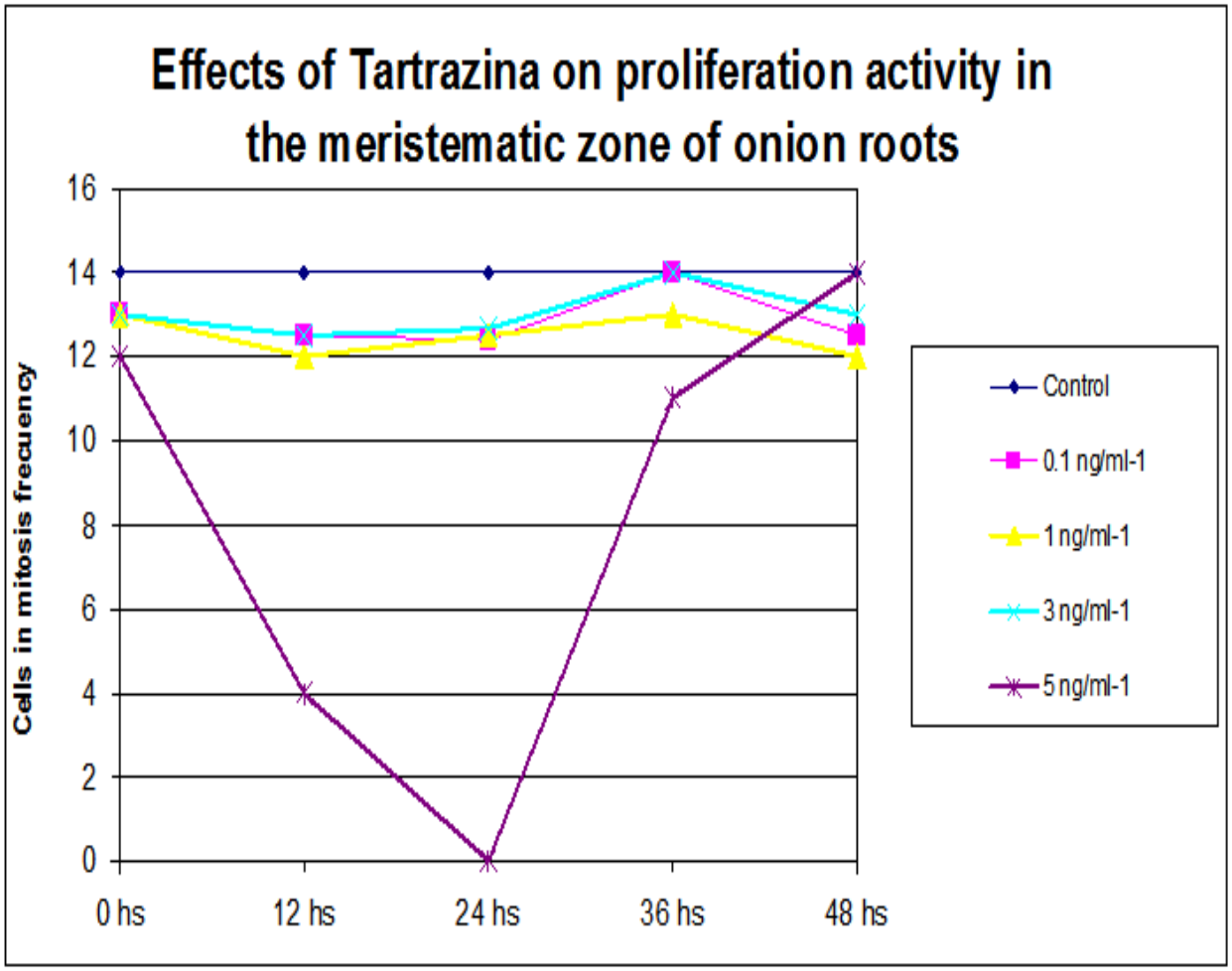

Figure 2: Effect of tartrazine on proliferative activity in the meristematic zone of the roots. Control; 0.1 ng/ml^{-1}; 1ng/ml^{-1}; 3 ng/ml^{-1}; 5 ng/ml^{-1}

Table 1: Frequency and cytological aberrations induced by tartrazine in the *Allium cepa* assay system

Treatment	Number cells	Cells in div	Abnormalities					Frequency of aberrant cells (%) based on	
			Breaks	bridges	stickiness	Binucleate Cells	total aberrant cells	total cells scored	Number of dividing cells
Tartrazine									
0.1 ng/ml^{-1}	5000	310	-	-	-	-	-	-	-
1.0 ng/ml^{-1}	5000	325	-	-	-	-	-	-	-
3.0 ng/ml^{-1}	5000	380	-	-	-	-	-	-	-
5 ng/ml^{-1}	5000	305	-	4	-	4	8 *	0.16	2.62
Negative Control	5000	355	-	-	-	-	-	-	-
Positive Control[a]	5000	360	7	8	15	3	33	0.66	9.2

a Ethyl metil sulfoxide (0.2 %)
*Z= 7.10, significant at P< 0.05

It is well known that the food industries are increasingly used food additives, which is an ingredient added intentionally to foods to modify its physical, chemical, biological or sensory without a nutritional purpose. Given that food dyes are classified as additives and, therefore, are only directly related to the improvements in the appearance of the food, which is why this and other research on the effect of the dyes on the health, you should warn the authorities to exercise greater control over the food industry, in the use of dyes.

Conclusions

The results obtained in this study indicate the cytotoxic effects and genetic damage of tartrazine in the meristematic cells of the root of *Allium cepa.* Given that this dye of common use, is widely used in the world and still raises a number of doubts about its toxicity, further studies are needed to determine their mutagenic, carcinogenic and genotoxic. This and other research, should serve to warn about the inappropriate use of these chemicals cause adverse effects to the health of the consumers and particularly in children.

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