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Effects of Wastewater from Olive oil And Milk Industry on Growth and Mitosis in Allium Cepa Root Apical Meristem

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Abstract

The effects of two different kinds of wastewater samples, taken from olive oil and milk industry were investigated in the mitotic cell division in onion (Allium cepa) root tip cells during germination. Our pretreatments showed that olive oil industrial wastewater was more toxic than milk industrial wastewater. Thus we prepared different concentrations of milk industrial wastewater solutions (100%, 75%, 50%, 25%) and olive oil industrial wastewater solutions (20%, 10%, 5%, 2.5%) using modified Hoagland nutrient solution. Control groups were germinated in modified Hoagland nutrient solution. As a result all the concentrations used caused several abnormalities in mitotic cell divisions and decreased the mitotic frequency in the onion root tip cells. Chromosomal bridges, laggard chromosome, polar slip and lack of cytokinesis and some other abnormalities were observed in different phases of mitosis. Furthermore, we also observed the negative effects of wastewater samples on chromatin condensation in the interphase.

Keywords: Wastewater, toxicity, Allium cepa, root tip, mitosis

INTRODUCTION

Higher plants constitute an important material for genetic tests to monitor environmental pollutants. The species Allium cepa has been considered an efficient test organism to indicate the presence of mutagenic chemicals [1], due to its kinetic characteristics of proliferation and chromosomes suitable for this type of study (2n = 16 large chromosomes) [2]. Grant [3] and Chauhan et al. [4] describe A. cepa as an efficient test system routinely used to evaluate the genotoxic potential of chemicals in the environment, due to its sensitivity and good correlation with mammalian test systems.

Katkat et al. [5] investigated how water wastes of Gemlik fertilizer industry inc. can be made use of in agriculture. In another study, the cytological effect of the storage of the two commercial mineral waters bottled with this material in different environmental conditions on the roots of A. cepa was investigated [6]. Aybeke et al. [7] (2000) investigated Triticum aestivum (wheat) mitotic division anomalies on the root of the meristem cells and the total amount of protein by wastewater of an olive oil industry. Another study carried out in 2003 investigated the effect of the fertilization on the accumulation of toxic element in the onion (A. cepa) watered via Karakoyun river which flows through the center of Şanlıurfa and where domestic and industrial wastes are disposed of [8]. More experiments are needed to demonstrate precisely the effects of such chemicals and establish their in situ levels of toxicity. The aim of this study is to determine the influence of two different industrial (olive oil and milk industry) waste water sample in onion (Allium cepa) root tip cells during growth and mitosis.

MATERIAL AND METHODS

In Allium root growth test, the onions were grown in freshly made dH_2O for the first 24 h and afterwards exposed for 4 day to the different concentrations of wastewater samples. Experiments were carried out in triplicate. Cytological experiments were carried out using olive oil industrial wastewater (%20, %10, %5, %2.5) and milk industrial wastewater (%100, %75, %50, %25) concentrations at 48 hours, with a control for each treatment. The root tips were sampled between 08.00 and 09.00 h, as the highest mitosis frequency in the onion is recorded between 06.00 and 09.00 h (Sharma, 1983). Root-tips were placed in a solution of ethanol (99%) and glacial acetic acid (3:1) for 24 h and washed with distilled water three times. The roots were transferred to 70% alcohol and stored in refrigerator until use. The root tips were macerated in a solution of 1 N HCl at 60ºC for 5 min. Then, the roots were washed with distilled water three times. Chromosomes were stained with aceto-orcein by squashing. The squash technique was applied for the study of the mitotic index (MI) and chromosomal aberrations. Three replicates were performed for each treatment and scoring was made from the 10 roots of each replicate. A minimum of 5000 mitotic cells were counted from each of the slides. The MI was calculated for each treatment as a number of dividing cells/100 cells. In chromosome aberration test, 100 cells in anaphase or telophase were examined for aberrations per slide. Chromosome aberrations were examined in 500 cells for each treatment. The mostly seen abnormalities were presented in figures.

RESULTS AND DISCUSSION

The morphological effect of different wastewater concentrations on mitotic frequency is shown in Figure 1. and 2. All the concentrations generated negative effects on mitosis. The frequency of mitosis decreases as the concentration of wastewater samples increases (Table 1. and Table 2.). The effects of olive oil and milk industrial wastewater concentrations on the root growth of the onion root tips are given in figures 3 and 4 as mean of root lenght in cm.

Fig.1. The comparison of the effect of different concentrations of wastewater taken from olive oil industry on Allium cepa . a. Control, b. 2,5% c. 5%, d. 10%, e. 20%.

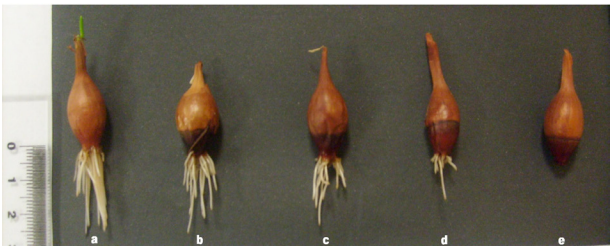

Fig.2. The comparison of the effect of different concentrations of wastewater taken from

Table.1. The effect of different concentrations of olive oil industrial wastewater concentrations on mitotic frequency and abnormal cell frequency.

Wastewater concentration % (v/v)	Mitotic Frequency (%)	Abnormal cell frequency (%)
control	5.70	0.80
2.5%	3.62	1.17
5%	2.17	2.50
10%	1.22	2.95
20%	0.54	3.58

Table.2. The effect of different concentrations of milk industrial wastewater on mitotic frequency and abnormal cell frequency.

Wastewater concentration % (v/v)	Mitotic Frequency (%)	Abnormal cell frequency (%)
control	4.84	0.75
25%	4.42	0.90
50%	3.27	1.40
75%	2.71	1.45
100%	1.59	1.80

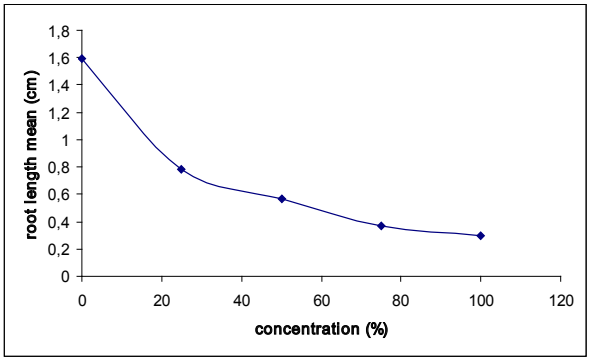

Fig.3. The effect of olive oil industrial wastewater concentrations on the root growth of the onion root tips.

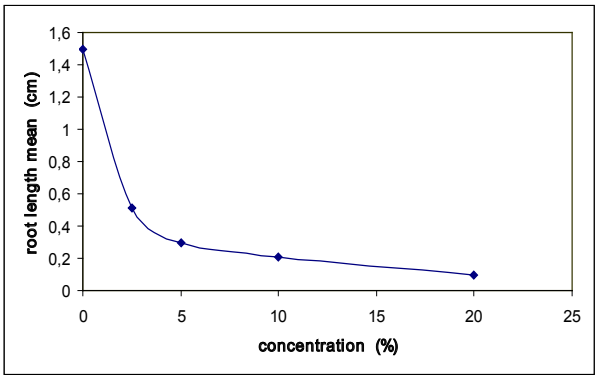

Fig.4. The effect of milk industrial wastewater concentrations on the root growth of the onion root tips.

The research revealed that all concentrations of the wastewater (pure) taken from two different industries reduced the mitotic division rates due to the increasing concentrations in the root meristem and created various chromosomal abnormalities. Among the abnormalities, the most distinctive ones and those having the highest observation frequency are polar slip at methaphase and anaphase, laggard chromosome, formation of fragments, and chromosomal bridges at anaphase (Figures 5 and 6). The mitotic index, which is 5.7% in the control samples, was found to be 1.59% in the samples germinated in 100% concentration of milk industrial wastewater and 0.54% in 20% concentration of olive oil wastewater.

The cytotoxicity level of a test compound can be determined based on the increase or decrease in the mitotic index (MI), which can be used as a parameter of cytotoxicity in studies of environmental biomonitoring [9]. The most common aberrations observed in all treatments were alterations in anaphase cells (bridges and polar slip) and changes in methaphase cells (irregular metaphase and laggard chromosome). According to Hoshina, the increase or decrease in MI can be an important indicator to monitor pollution levels in affected environments, especially those contaminated with potentially toxic and cytotoxic compounds[10]. Smaka-Kincl et al. reported that a decreased mitotic index of meristematic cells of A. cepa may be considered a reliable method to determine the presence of cytotoxic compounds in the environment, and thus be a suitable test for monitoring pollution levels [11]. In their research about the exposure of Allium cepa seeds to industrial

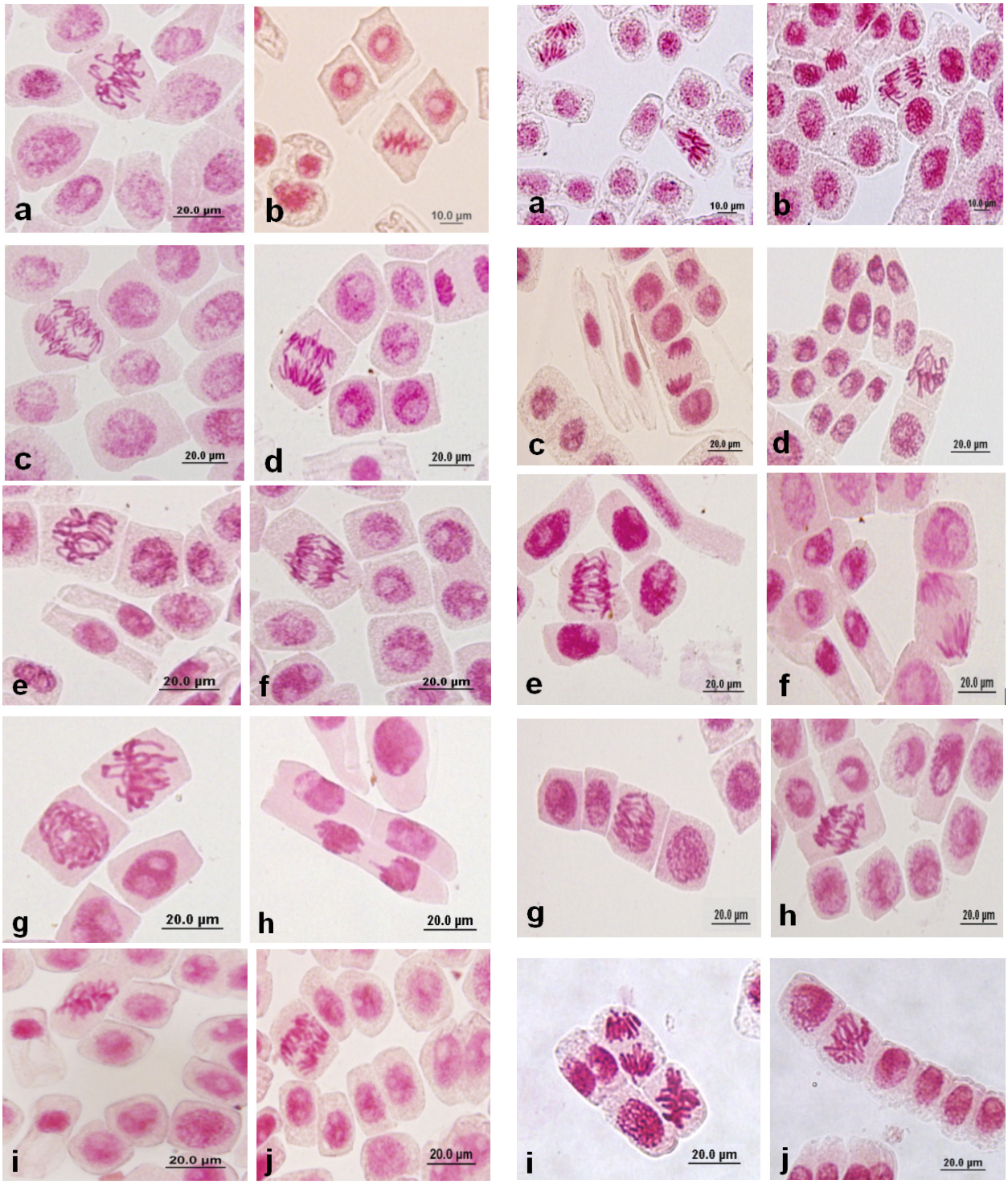

Fig.5. Samples of the abnormalities observed in the mitosis following treatment with different concentrations of olive oil wastewater; (a) irregular metaphase (2,5% conc.), (b) polar slip (2,5% conc.), (c) chromosomal bridge in anaphase (2,5% conc.), (d) chromosomal bridge in anaphase (5% conc.) (e) irregular metaphase (5% conc.), (f) polar slip and chromosomal bridge in anaphase (5% conc.), (g) polar slip and irregular metaphase (5% conc.), (h) laggard chromosome in telophase (5% conc.), (i) polar slip in metaphase(10% conc.), (j) chromosomal bridge in anaphase (10% conc.)

Fig.6. Samples of the abnormalities observed in the mitosis following treatment with different concentrations of milk industrial wastewater; (a) polar slip in anaphase (25% conc.), (b)) laggard chromosome (25% conc.), (c), polar slip (50% conc.) (d) irregular distribution of chromosomes in metaphase (50 % conc.), (e) chromosomal bridge (75% conc.), (f), polar slip in anaphase (75% conc.) (g) chromosomal bridge (75% conc.), (h) polar slip and chromosomal bridge (75% conc.), (i) polar slip in metaphase and anaphase (100% conc.), (j) irregular distribution of chromosomes in metaphase (100% conc.)

effluents contaminated with azo dyes, Carita and Morales found chromosome and nuclear aberrations, such as abnormal anaphases (multipolar, with bridges, delayed, etc.), fragments and loss of chromosomes, c-metaphases, micronuclei, and multinucleated cells [12].

In our experiments the negative side effects of two different wastewater samples on onion root tip cells during mitosis were investigated. Treatment of the onion tip cells with waste water caused several negative effects in mitotic cell division. According to us, the reason of the change in the plane of the cell division (polar slip) is the effect of waste water samples on the polymerization of microtubules. As a result of our study it is found that olive oil industrial wastewater was more genotoxic than milk industrial wastewater on root meristematic cells of A.cepa.

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