Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: C. Alaca, A. Özdemir, B. Bozdağ, C. Özdemir (2020) Cytogenetic effects of C_6H_4 $(CH_3)_2$ (xylene) on meristematic cells of root tips of *Vicia faba* L. and mathematical analysis. *Caryologia* 73(1): 27-35. doi: 10.13128/caryologia-279

Received: May 31, 2019

Accepted: February 26, 2020

Published: May 8, 2020

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Cytogenetic effects of C_6H_4 $(CH_3)_2$ (xylene) on meristematic cells of root tips of *Vicia faba* L. and mathematical analysis

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Abstract. Xylene is a readily flammable and poisonous liquid with a chemical formula of C_6H_4 $(CH_3)_2$. It is used as raw material or auxiliary raw material in many industrial products such as dye, pencil, agricultural chemicals, rubber, fiber, glue and diaper. In this study, cytogenetic effects of xylene, on the meristematic cells of root tips of *V. faba* L. used as food have been investigated. For this purpose, the seeds of the plant have been treated with xylene solutions prepared in different concentrations for different time periods. Chromosomes at the root tips have been looked and the effect of xylene has been determined. The abnormalities as chromosome breaking, chromosome dispersion, bridge chromosome, chromosome adherence, ring chromosome have been observed. Abnormalities have been seen at each treatment depended on the time periods. In addition to these visible damages of xylene in the study, possible damages on chromosomes carrying genetic codes of living beings to future generations have been investigated and mathematical analyzes has been made. The results obtained have been evaluated statistically.

Keywords. Abnormalities, Chromosome, Mathematical analysis, Xylene.

1. INTRODUCTION

Xylene is a colorless, characteristic solvent odorous and liquid form raw material with a chemical formula of C_6H_4 $(CH_3)_2$ and molecular weight of 106.17 GR / MOL. It is formed by bonding two methyl groups to benzene and it is a readily flammable and poisonous liquid. Xylene may leak to surface, surface water or groundwater, where it may remain for months or more. Xylene is widely used industry and medical technology as a solvent, but concerns about its safety have been raised from time to time (Jenifer 1994). Health and safety authorities in most countries recommend a threshold limit value (TLV) of 100 ppm in the working environment. Xylene vapour is absorbed rapidly from the lungs, and xylen1. e liquid and vapour are absorbed slowly through the skin. Of the xylene absorbed, about 95%

is metabolised in the liver to MHA and 70 to 80% of metabolites are excreted in the urine within 24 hours. Differences are suspected between animal species, and between animals and humans, in the metabolism of, and sensitivity to, xylene (Langman 2009). There have been different studies about that the cytogenetic effects of some metals and chemical substances except to xylene on plant in literature. Various chemical substances which may be used in Medicine, Biology and Agricultural fields can affect negatively growth of both plants beside their positive effects (İnceer *et al*. 2003; Kıran and Şahin 2005). İnceer and Beyazoğlu (2000) have investigated cytogenetic effects of copper chloride on root cells of *Vicia hirsuta* (L.) S.F. Gray and they detected that this compund affects cell division negatively and also leads to chromosomel abnormalities. The researchers reported that compounds with mercury affect spindle threads during cell division in *V. faba* and *Allium cepa* L. (Leonard *et al*. 1983). Some researchers have made some investigations about the effects of heavy metal pollution on plants, resulted from different factors at environment and enterence of these elements into soil and plant (Çelik *et al*. 2004; Özdemir 2008; Özdemir *et al*. 2015; Şutan 2018).

In this study, we investigated the effects of xylene used as raw materials or auxiliary raw materials in many industrial products such as dye, pencil, agrochemicals, rubber, fibers, glue and diaper on chromosomes of *V. faba* used as food. Faba bean is one of the most important grain legumes in the world because of its multiple uses and its ability to grow over a wide range of climatic conditions (Kursheed *et al*.2018) The results of the research have been determined mathematically and the as statistical have been evaluated. For this purpose, xylene solutions have been prepared in different concentrations, and the seeds of the *V. faba* have been germinated with treatment with these solutions. Chromosomes at at the root tips have been looked and the effect of xylene has been determined.

The environment where xylene, a carcinogenic substance, is present at 100 ppm or more than 435 mg/m^3 in air is harmful to human health (Haglund *et al*. 1980). In addition to these visible damages of xylem in the study, possible damages on chromosomes carrying genetic codes of living beings to future generations have been investigated and mathematical analyzes have been made. The results obtained have been evaluated statistically.

2. MATERIAL AND METHODS

In our study, we determined the concentration of xylene by taking into consideration the application period and the level of harm to human health in the literature. The amounts given in the literature belong to the direct xylene effect of the human. We tried to determine this effect by applying on seed of *V. faba*, which commonly used by people as food. Thus, we used 10 ml / L, 10 ml / L, 12h and 24h values for the application. The seeds of *V. faba* have been treated with these concentrations of xylene during 12 and 24 hours. Then, the seeds have been has beenhed by distiled water and germinated in petri dish at 20-25 °C. The root tips obtained, have been put in 70 % ethyl alcohol after the fixation of they. Stock root tips have been stained by Feulgen method (Darlington and La Cour 1976) and have been got ready for microscopic examination. Homoligous areas have been chosen on these preparations for cytogenetic examination; the cells in these areas have been counted and the number of mitotic cells have been also detected. Chromosomel abnormalities have been tried to detected in the cells counted. Preparates has been photographed with motorized Leica DM 3000 microscope. Chromosome abnormalities detected in the study have been coded as A, B, C, D, E, F, G, H and I (Table 3). The concentrations and times of treatment have been coded as 1-4

(10 ml/L -12h): 1, (10 ml/L -24h): 2, (100 ml/L -12h): 3, (100 ml/L -24h): 4

We used following formulas for calculating the mitotic index and the percentage of total abnormalities. In this the study the cell numbers in per unit area (24x24mm) have been considered.

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \text{ x } 100$$

$$\text{\% of total abnormalities} = \frac{\text{Number of cells with abnormal chromosome}}{\text{Total number of cells}} \text{ x } 100$$

Statistical analyses have been performed using MINITAB software package.

3. RESULTS

At the end of the study, it has been observed that different concentrations of xylene treatment on the seeds have been increased mitotic cell division at the different periods of time, compared with the control group (Figure 1). This situation has been reached on the top point at 12 hour of 100ml/L treatment. At the 12 hour

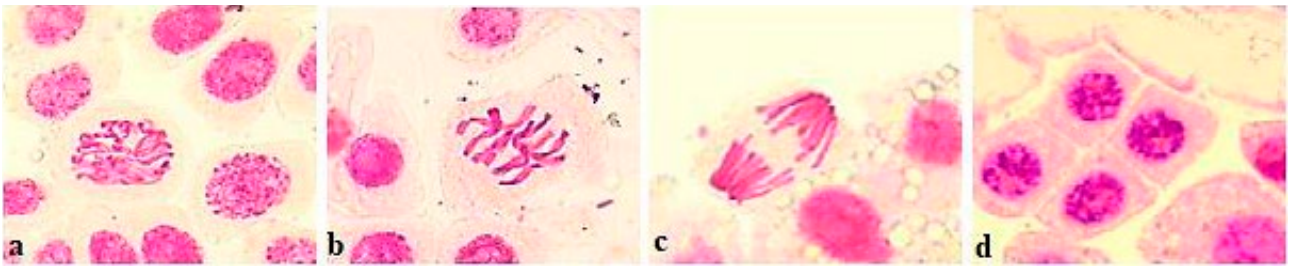

Figure 1. Photomicrographs of *V. faba* root tip meristem cells. Normal mitotic phases: (A) prophase, (B) metaphase, (C) anaphase, and (D) telophase.

Table 1. The mitotic index and total chromosome abnormalities in the root tip cells of *V. faba*.

	Control	10 ml/L		100 ml/L	
		12h	24h	12h	24h
Mitotic index (%) ± SD	12.02 ± 7.84	25.23 ± 11.12	20.02 ± 10.12	32.02 ± 17.14	27.03 ± 19.64
Total abnormalities (%)	0.00	03.13	10.21	07.05	18.03
The number of different chromosome abnormalities	0.00	2	9	6	4

S.D.- Standart Deviation, Time (h): hour.

Table 2. Number (%) of cells in each mitotic stage of *V. faba* roots treated with xylene.

Stages (%)	Control	10 ml/L		100 ml/L	
		12h	24h	12h	24h
Prophase	10.00	22.12	18.00	27.06	20.02
Metaphase	1.30	2.03	1.20	1.60	1.70
Anaphase	0.82	0.72	0.87	0.13	1.20
Telophase	0.42	0.02	0.60	1.12	2.02

Time (h): hour.

for 100ml/L of treatment, mitotic cell division has been decreased. Mitotic division increased again at the 12 and 24 hour of 100ml/L of treatment (Table 1,2). In the cells of the root tips of treated with xylene investigated seeds various chromosomel abnormalities as sticky chromosome, ring chromosome, chromosome breaking, bridge chromosome, vagrant chromosome, polar deviation, binucleated cell and scattered anaphase at different stages of mitotic division have been detected (Figure 2-8). Total abnormalities (%) has been observed high level all treatment for 24 hours of time according to 12 hours of time (Table 1-3). The number of different chromosome abnormalities have been observed the highest level at 10ml/L and 24 hour treatment time. Whereas the at least number of different chromosome abnormalities have been observed at 10ml/L and 12 hour treatment time. The chromosomel abnormalities with the highest percentage have been seen in sticky chromosome and ring chromosome. Bridge chromosomes have been seen as single, binary, triple and multiple bridge shaped in the all treatment except to control and 12h- 100ml/L treatment. Chromosome shrinking, ring chromosome and chromosome breaking have been seen all treatment times except to 10ml/L and 12 hour and control. Binucleated cell and Scattered anaphase have been seen in the all treatment except to control and 12h- 10ml/L treatment (Table 1-3; Figure 3,7)

Also, according to the statistical results derived, there is a considerable positive relation between the increase concentration of treatment and the mitotic index (%). On the other hand, there are positive relation between the time of treatment and the chromosome abnormality (%) (Table1-3).

The statistical analysis of the results are shown in Tables 1-8. According to Table 4 and 8 based on the Pearson's correlation and analysis of variance method

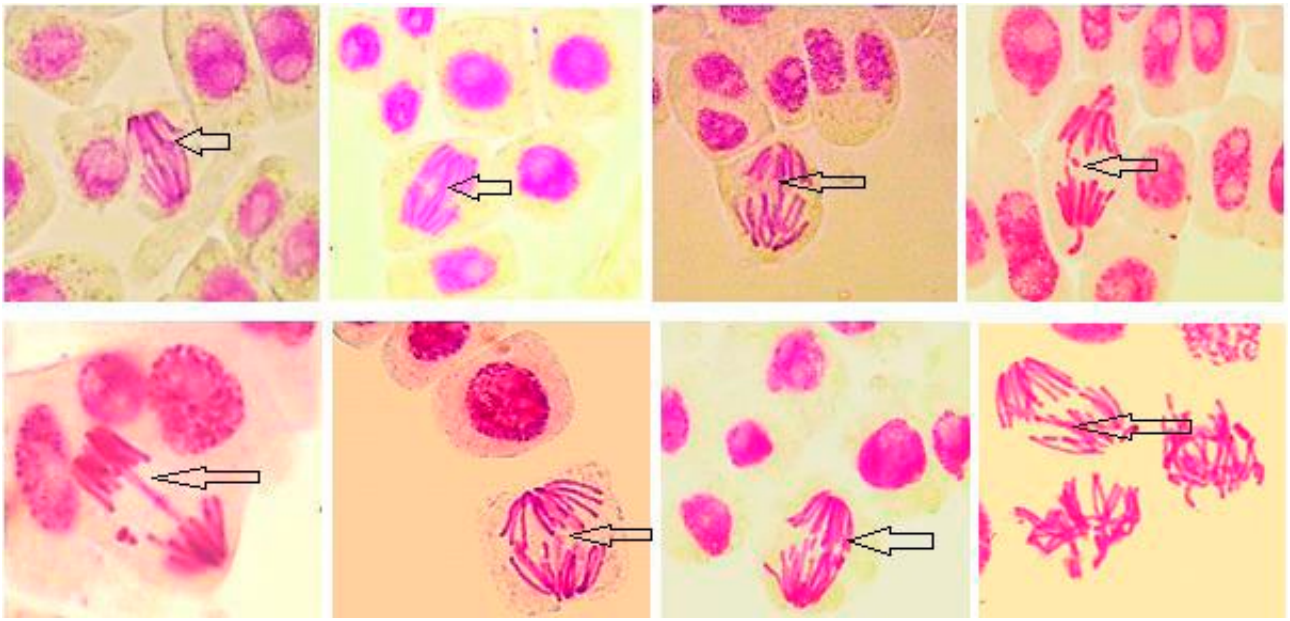

Figure 2. The xylene induced abnormalities: Anaphase bridges

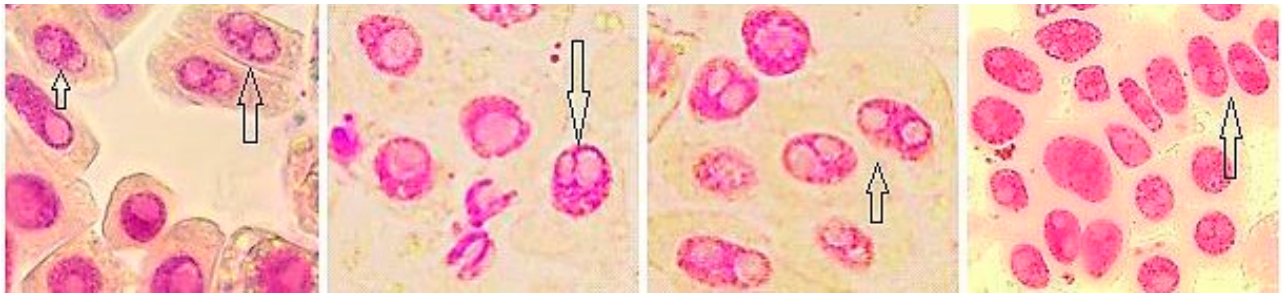

Figure 3. The xylene induced abnormalities: Binucleated cells

Table 3. The xylene induced chromosome abnormalities in the root tip cells of *V. faba.*

Chromosome abnormalities (%)		10 ml/L		100 ml/L	
		12h (1)	24h (2)	12h (3)	24h (4)
Sticky chromosome	**A**	1.30	2.30	1.50	3.20
Ring chromosome	**B**	1.30	3.20	1.20	2.10
Chromosome breaking	**C**	0.53	2.80	0.60	1.50
Bridge chromosome	**D**	0.00	1.21	1.10	4.30
Vagrant chromosome	E	0.00	0.00	1.40	3.20
Polar deviation	F	0.00	0.00	0.30	2.20
Binucleated cell	G	0.00	0.20	1.30	0.70
Scattered anaphase	**H**	0.00	0.10	0.10	0.80

Treatment time (h): hour. Abbreviations: A-H: Codes of chromosome abnormalities.
Abbreviations: 1-4 : Codes of Treatment (10 ml/L -12h): 1, (10 ml/L -24h): 2, (100 ml/L -12h): 3, (100 ml/L -24h): 4.

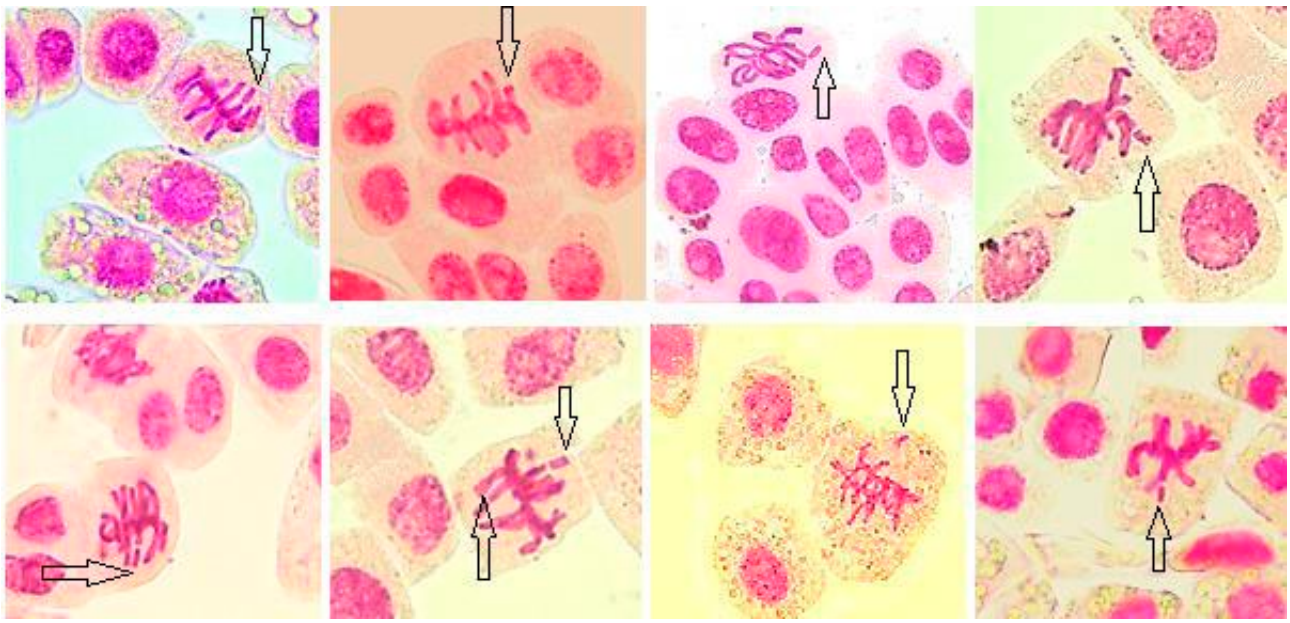

Figure 4. The xylene induced abnormalities: Chromosome breaking.

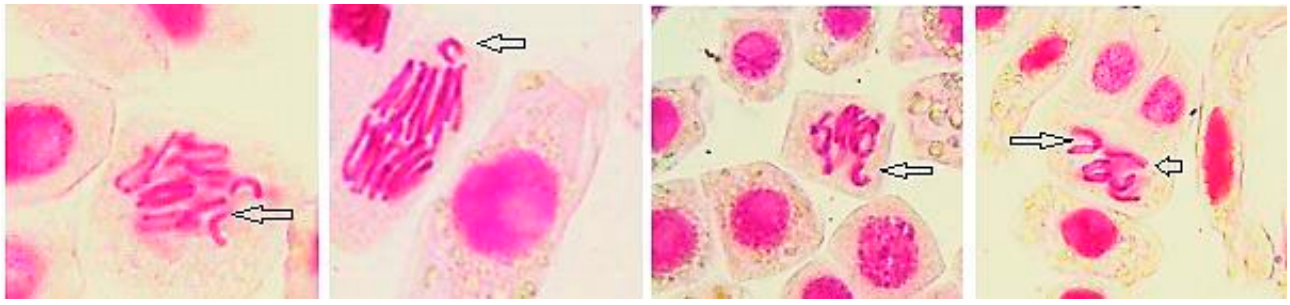

Figure 5. The xylene induced abnormalities: Vagrant chromosome.

Table 4. Pearson's correlation based on chromosome abnormalities.

	A	B	C	D	E	F	G
B	0,966 0,034*						
C	0,938 0,062	0,894 0,106					
D	0,012* 0,988	0,195 0,805	0,264 0,736				
E	0,342 0,658	0,539 0,461	0,117 0,883	0,916 0,084			
F	0,266 0,734	0,419 0,581	0,031* 0,969	0,964 0,036	0,950 0,050*		
G	0,220 0,780	0,441 0,559	0,313 0,687	0,326 0,674	0,579 0,421	0,301 0,699	
H	0,142 0,858	0,298 0,702	0,161 0,839	0,985 0,015	0,921 0,079	0,991 0,009**	0,249 0,751

* Significant at the level of P< 0.05. ** Significant at the level of P< 0.01. Abbreviations: A-I : Codes of chromosome abnormalities.

Table 5. Pearson's correlation based on chromosome abnormalities.

	1	2	3
2	0,851 0,007**		
3	0,412 0,310	0,272 0,514	
4	0,028* 0,762	0,135 0,750	0,470 0,239

* Significant at the level of P< 0.05. ** Significant at the level of P< 0.01. Abbreviations: 1-4 : Codes of Treatment.

(Correlation), there are important correlations among (A-B, D; C-F; D-F, D-H; E-F; F-H) the investigated chromosome abnormalities at levels of 0.01 and 0.05 (Table 4, 8). According to Table 5, based on the Pearson's cor-

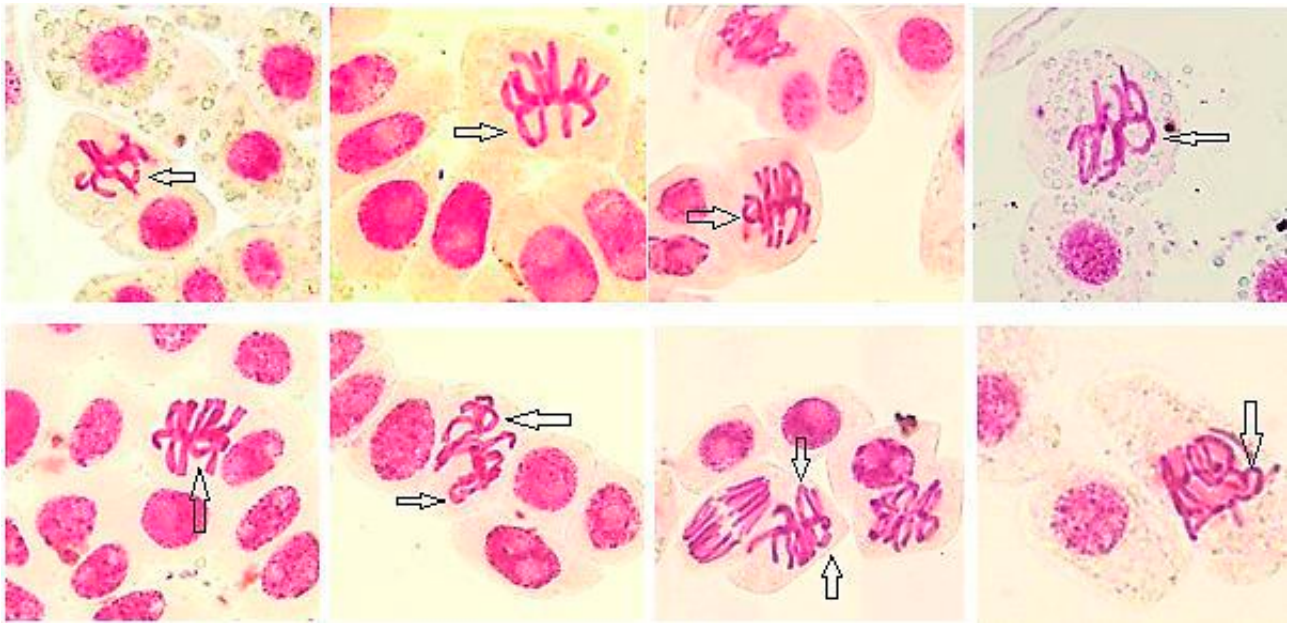

Figure 6. The xylene induced abnormalities: Ring chromosome.

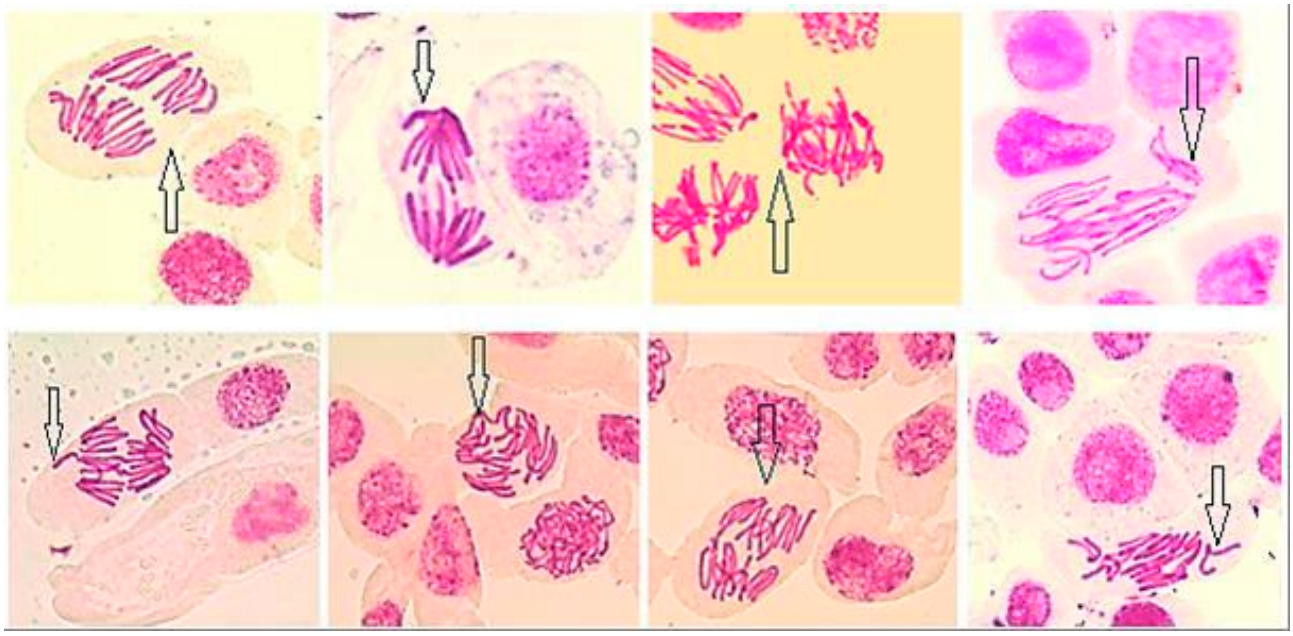

Figure 7. The xylene induced abnormalities: Polar deviation and Scattered anaphase.

relation method there are important correlations among (1-2; 1-4) the treatment time and treatment concentrations at levels of 0.01 and 0.05 According to Table 9, based on the analysis of variance method, there are important correlations among only 1-2 the treatment time and treatment concentrations at levels of 0.01.

4. DISCUSSION

In this study, we studied the cytogenetic effects of xylene on which human beings are exposed in different way on chromosome of plant. For this aim we used meristematic cells of root tips belonging to the *V. faba*

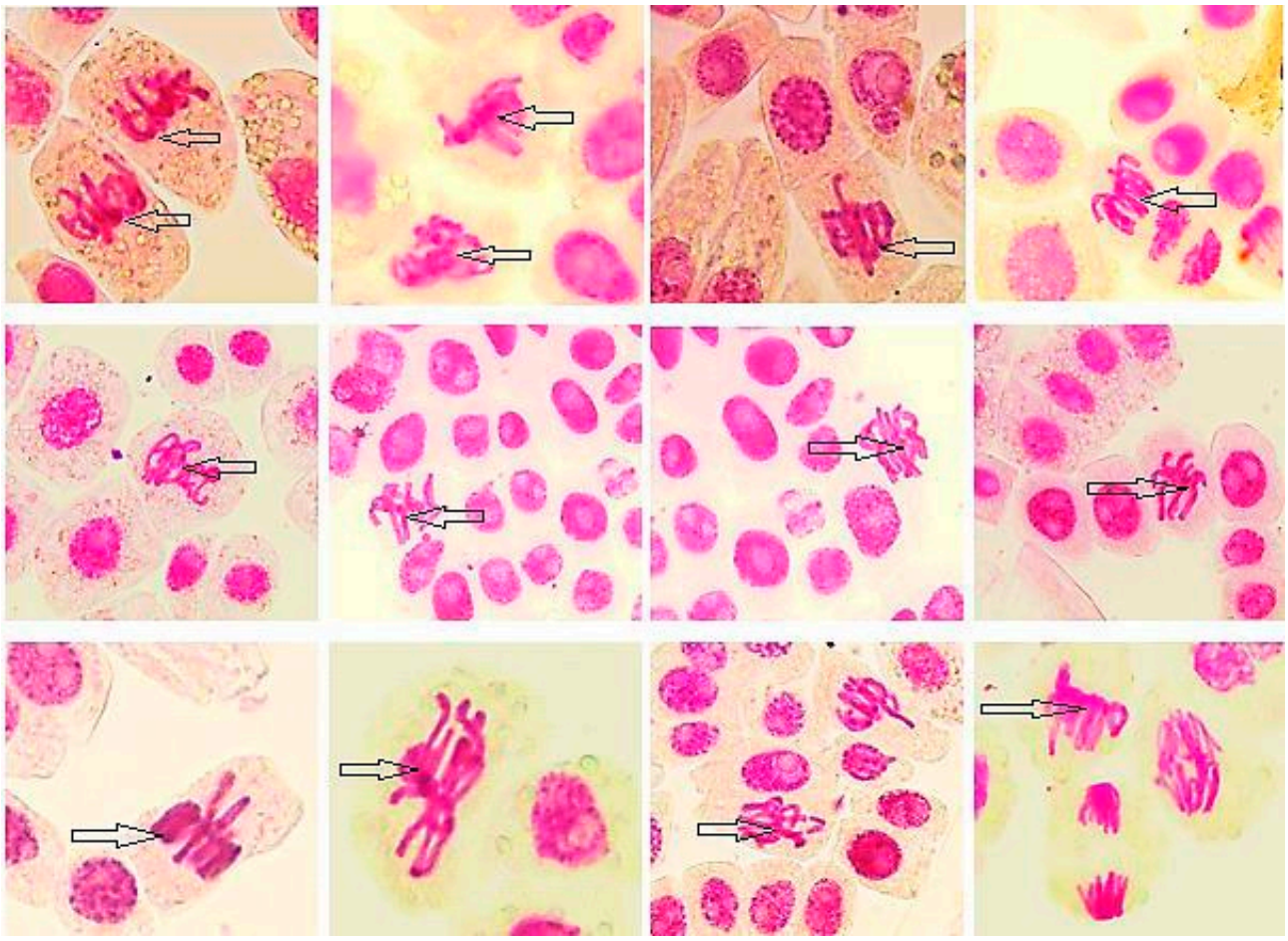

Figure 8. The xylene induced abnormalities: Sticky chromosome.

Table 6. Regression Analysis: **A** versus **B**.

The regression equation is C5 = 0,899 + 0,435 C6				
Predictor	Coef	SE Coef	T	P
Constant	0,8995	0,1588	5,66	**0,030**
C6	0,43508	0,08255	5,27	0,034
S = 0,140751; R-Sq = 93,3%; R-Sq(adj) = 89,9%				

Analysis of Variance					
Source	DF	SS	MS	F	P
Regression	1	0,55038	0,55038	27,78	0,034
Residual Error	2	0,03962	0,01981		
Total	3	0,59000			

Abbreviations: A-B : Codes of chromosome abnormalities

Table 7. Regression Analysis: **1** versus **2**.

The regression equation is C1 = - 0,065 + 0,370 C2				
Predictor	Coef	SE Coef	T	P
Constant	-0,0654	0,1645	-0,40	0,705
C2	0,36970	0,09332	3,96	0,007
S = 0,334601; R-Sq = 72,3%; R-Sq(adj) = 67,7%				

Analysis of Variance					
Source	DF	SS	MS	F	P
Regression	1	1,7570	1,7570	15,69	**0,007**
Residual Error	6	0,6717	0,1120		
Total	7	2,4288			

Abbreviations: 1-2: Codes of Treatment.

which widely used as food by humans. The researchers have pointed out that *Allim cepa* has as an advantage due to its large chromosomes, easily observed with a light microscope (Bonciua 2019). For the same reason,

Table 8. Correlation between 8 investigated chromosome abnormalities (Analysis of Variance).

	MS	F-value	Probability	Significance
A-B	0.5503	27.78	0.034	*
A-C	0.5187	14.56	0.062	NS
A-D	45.134	482.2	0.050	*
A-F	0.0418	01.15	0.734	NS
B-F	0.5120	0.430	0.581	NS
B-H	0.2580	0.190	0.702	NS
C-D	0.2370	0.150	0.736	NS
D-F	9.5300	26.47	0.036	*
D-H	9.9522	66.84	0.015	**
E-F	6.2427	18.71	0.050	*
F-H	3.3103	115.7	0.009	**
G-H	0.0624	0.130	0.751	NS

MS: Mean Square; *P<.05; **P<.01; A-H: Codes abnormalities; NS: Not Significant.

Table 9. Correlation between treatment time and concentrations (Analysis of Variance).

	MS	F-value	Probability	Significance
1-2	1,7570	15,69	**0,007**	**
1-3	0,4128	1.230	0,310	NS
1-4	0,0398	0.100	0,762	NS
2-3	0,9520	0.480	0,514	NS
2-4	0.2340	0.110	0,750	NS
3-4	0.4380	0.710	0,239	NS

MS: Mean Square; **P<.01; Abbreviations: 1-4: Codes of Treatment; NS: Not Significant.

we have used *V. faba* which has this feature in this study. We think that the results of the study are quite important. Because chromosomes are the passwords that keep the viability. We have been determined that xylene has been caused to some chromosamal abnormality on root tip cells of the plant as bridge chromosome, chromosome breaking, sticky chromosome, ring chromosome, vagrant chromosome, scattered anaphase. Similarly, the researchers investigated the cytotoxic effect of benzene and thinner, the toxic chemical used in the painting of steel furniture such as xylene. The effect were evaluated using root tip cells of *Allium cepa*. They observed Chromosomal abnormalities induced were early separation, exclusion, laggard, sticky bridge and persistent bridge (Barbhuiya *et al*. 2018). Some other the researchers reported that copper chloride has caused to some chromosamal abnormality on root tip cells of *Vicia hirsuta* (L.) Gray. According to the same researchers the most observed abnormalityes have been chromosome adherence and bridge chromosome (İnceer and Beyazoğlu 2000). In other study, it has been determined that increase of the lead (PbCl2) concentrations cell division has been decreased, several mitotic anomalies such as c mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges on root tip cells of *Lens culinaris* Medik (Kıran and Şahin 2005). In another study, It has been determined that the frequency of mitotic cell division have been affected by uranium depending on the different treating time and uranium led to chromosomel abnormalities in the *V. faba* cells (Özdemir *et al*. 2008). Similarly, in our study, the mitotic division of the seed treated with xylene gradually increased in comparision to the control group. On the other hand, the chromosome abnormalities vary in parallel with the concentration while the chromosome abnormalities changes irregularly with the time periods of the treatment in our study. As a result of the study, xylene has been shown to induce cleavage in plant meristematic cells and cause abnormal cell division and chromosomel abnormalities. Statistical analysis have been performed using Analysis of Variance, Regression and Pearson Correlation tests. The differences have been evaluated with the same tests. The reason for the application of this statistical method is to see if there is a difference between the groups on the variables studied. These statistical methods have been used to test the differences between two or more groups for our investigated. The results have been taken into account in the significance evaluations at P <0.05 and P <0.01 levels. Thus, we have tried to prove and evaluate the results obtained from laboratory studies numerically. Also, according to the statistical results derived, different concentrations of xylene treatment on the seeds have been increased mitotic cell division at the different periods of time, compared with the control group. However, mitotic division of these treated seeds decreased with increasing application time. This result shows that the application time in mitotic division is important. On the other hand there is a considerable positive relation between the treatment time (hour) and the chromosome abnormality (%). As shown in the tables According to analysis of variance, regression analysis and pearson correlation tests it has been found that there have been statistically important differences at levels of 0.01P between Scattered anaphase and Polar deviation (Table 4,6,8). We can say that these chromosomal abnormalities are interrelated and trigger each other.

We did not find any detalied study on the effect of xylene on plant chromosomes except for a few studies on the effect of xylene on animal cells in our literature review (Mohtashamipur *et al*. 1985; Dean 1985; Nise 1991). In literatüre some researchers have done studies

on the health damages of xylene through various routes of exposure. The researchers indicated that xylene, an aromatic hydrocarbon, is widely used in industry and medical laboratory as a solvent and it is a flammable liquid that requires utmost care during its usage. Also researchers pointed that prolonged exposure to xylene leads to a significant amount of solvent accumulation in the adipose and muscle tissue (Rajan and Malathi 2014). Thus, we think that we are trying to close this deficiency in the literature with this study.

The researchers observed the damage of chromosome in bone marrow cells of rats after dosing with xylene (Lebowitz *et al*. (1979). Similarly Donner *et al*. (1980) exposed rats to technical-grade xylene by inhalation at 300 ppm, 6 h/day, 5 days/week for up to 18 weeks. The chromosome damage has been detected in animals examined after 9, 14 or 18 weeks exposure. The occurrence of these disorders in the chromosomes shows that this negativity will be transmitted from generation to generation. People who have been reluctantly exposed to xylene could have similar effects. Therefore, the use of this chemical in our lives should make us think.

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