Cytogenetic Effect of Chitosan on Mitotic Chromosomes of Root Tip Cells of *Vicia faba*

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Abstract: Chitosan is a biopolymer consisting predominantly of unbranched chain and derived from the natural product chitin. Three concentrations of chitosan during three different time periods were examined for its genotoxic and cytotoxic effects. The results show that treatments with chitosan increased the mitotic index compared with control, although treatment with high concentrations for (12&16houre) decreased the mitotic index but the effect were in significant at 005; furthermore, different treatments caused chromosomal aberrations similar to the chromosomal aberrations noticed in control which is normally observed during seed growing and before long would be insignificant. The study indicates that chitosan can be considered an excellent alternative to synthetic plants fertilizations.

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Key wards: Chitosan, Cytogenetic, *Vicia faba*, Root Tips.

Introduction

Chitosan $(C_6H_{11}NO_4)_n$ is derived from a natural product, chitine $(C_8H_{13}NO_5)_n$ (figure1-a) by deacylated chitin, cellulose-like biopolymer consisting predominantly of unbranched chain (figure 1-b), found in fungi, yeasts, marine invertebrates and arthropods, which is a principal component in the axoskeletons, and considered environmentaly friendly and easily degradable.

The Chitosan has been applied in many fields; it had been found that chitosan regulates the immune system of plants (Boonlertnirun et al, 2008); also because chitosan is a natural biopolymer it contains a lot of nitrogen molecules that enhance germination index and shoot and root dry weight (Grant et al, 2009), it promotes seedling growth, accelerates the first flowering date (Ohta et al, 2004), can increase the microbial population by large numbers, and transforms organic nutrient into inorganic nutrients that are easily absorbed by plants' roots (Somashekar and Joseph, 1996; Bolto et al, 2004), (Boonlertnirun et al, 2008) found that chitosan improved rice resistance to disease and insect infections. These studies encourage using chitosan in agriculture field with minimum side effects on plants.

In this study, the cytotoxic and genotoxic effects of chitosan were investigated using the *Vicia faba* assay.

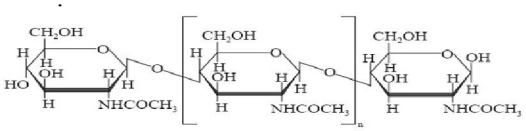


Figure (1-a): Chitine $(C_8H_{13}NO_5)_n$ Cellulose-like Biopolymer Consisting Predominantly of Un-branched Chain (The merck index, eleventh edition).

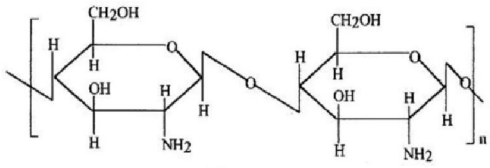


Figure (1-b): Chitosan $(C_6H_{11}NO_4)_n$ (Nair and Laurencin, 2007)

Material and Methods

1-Preparation of samples:

Seeds of *Vicia faba* were collected from the local market. They were first presoaked for 24 hours in distilled water and then transported to the tested concentrations for different periods of time, and then grown until the seedling secondary roots tip become 2- 3 cm long, then were cut and fixed in freshly prepared 3:1 (v/v) alcohol: glacial acetic acid for 24 hours. For Cytological preparations, secondary root of *Vicia faba* were hydrolyzed in 1N HCL 60c for 5 minutes, and then washed with distilled water several times and staind with 1% acetocarmin, five temporary slides were prepared using the squash technique, two root tips on each slide were examined for the effects of the chitosan on mitotic index (MI). The same slides were analysed for the types and frequencies of chromosomal abnormalities (CA).

2-Treatments:

I- Three concentrations were prepared for each treatment (0.5%$_v$, 1.5%$_v$, 2.5%$_v$) for four different periods of time (8, 12, 16, 24 hours) using *Vicia faba* for monitoring cytotoxicty and genotoxcity effects.

II- Control (Negative treatment) using distilled water only.

3-Scoring of slides and data analysis

The slides were viewed under light microscope (Phenix P H 50 DB047VU) using the 40X objective lens immersion. The most representative ones for each structural aberration were photographed using (Phenix micro Image analyzer Software 2008 En V2, 2).

4-Mitotic index:

On one slide for each treatment, a total of 2000 cells, were scored. The mitotic index (MI) is expressed as the number of dividing cells per total cells scored.

5-Cytotoxicity:

The mitotic index of the treated cells at each dose of chitosan were compared with that of the negative control group

6-Genotoxicity test:

Chromosomal aberration per dose were examined; the percentage of cells with aberrations of each dose were compared with that of the negative control using the (SPSS 16.0 for Windows statistical package). Two-way Analysis of Variance was the statistical method used for determining the significance of difference at P = 0.05.

Result and Discussion:

1-Mitotic index:

Table (1) and figure (2 & 3) show the effect of different concentrations of chitosan for different time periods on mitotic index and chromosomes aberration frequency.

Mitotic index (MI) measures the proportion of cells in the M-phase of cell cycle, inhibition could be considered as cellular death or delay in the cell proliferation kinetics (Rojas et al, 1993). The treatment with (0.5 $\%_v$) of chitosan for (12,16,24 hours) caused increase in (MI), while treatment for 8 hours decreased the (MI) compared with control and it was in significant at 0.05. The increase in mitotic index may be due to the effect of chitosan on biochemical activities as (Mondal et al, 2012) reported that foliar application of chitosan enhanced the biochemical activities and led to plant growth and development. (Apiradee et al, 2007) reported that chitosan can be used as a plant growth enhancer for immature plants or in tissue culture. The plants grow result from cells division, also chitosan increased the growth rates of roots and shoots of *Raphanus sativa* L. (Tsugita et al, 1993), (Boonlertnirun et al, 2012) found that chitosan stimulates plant growth and enhances yield of *Oryza sativa*. (Wanichpongpan et al, 2001) reported that chitosan significantly enhanced growth factors in terms of the average values of flower-stem length, the number of growing leaves, including leaf width and length as well as the number of flowers per bush. Also (Apiradee et al, 2007) conclude that chitosan may induce a signal to synthesize plant hormones such as gibberellins, and may induced secondary metabolites that enhances growth. (Guan et al, 2009) reported that chitosan contained a lot of nitrogen molecules and can enhance germination index and shoot and root weight. The nitrogen molecules is important for amino acids building compounds that inter in proteins synthesis. Similar result of the effect of chitosan on growth reported by (Abdel-Mawgoud et al, 2010; El-Sawy et al, 2010).

High concentrations of chitosan (1.5% $\%_v$ & 2.5% $\%_v$) decrease the (MI) compared with control, this decrease of (MI) were in significant at 0.05. The effect of chitosan on plant growth is similar to the effect of gibberellic acid a plant hormone that enhance cell division.

Table (1): Total Number of Examined Cells, Mitotic Index and Chromosomes Aberration Frequency after Treatment with Different Concentrations of Chitosan for Different Time Periods on Root Tip Cells of *Vicia faba*

treatment	Type of treatment & duration(h)	test concentration	Total cells observed	total number of divided cell	total number of abnormal cell	Mitotic index %	ch . Fr
control	8	d. water	2393	333	10	13.9	0.03
	12	d. water	2642	289	9	10.9	0.03
	16	d. water	2174	235	14	10.8	0.06
	24	d. water	2121	101	9	12	0.09
chitosan	8	0.5	2076	204	7	9.8	0.034
	12	0.5	2057	328	46	15.9	0.14
	16	0.5	2015	330	55	16.4	0.17
	24	0.5	2254	316	31	14	0.1
	8	1.5	2135	240	26	11.2	0.1
	12	1.5	2014	237	25	11.7	0.1
	16	1.5	2811	307	12	10.9	0.03
	24	1.5	2125	223	24	10.5	0.1
	8	2.5	2031	235	12	11.6	0.05
	12	2.5	2156	188	19	8.7	0.1
	16	2.5	2083	171	20	8.2	0.11
	24	2.5	2152	253	25	11.8	0.09

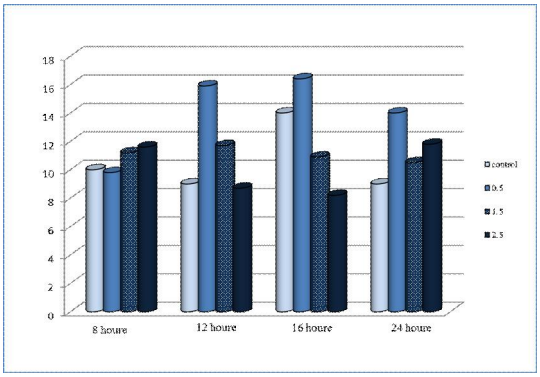


Figure (2): Effect of chitosan on mitotic index of *Vicia faba* root tip cells

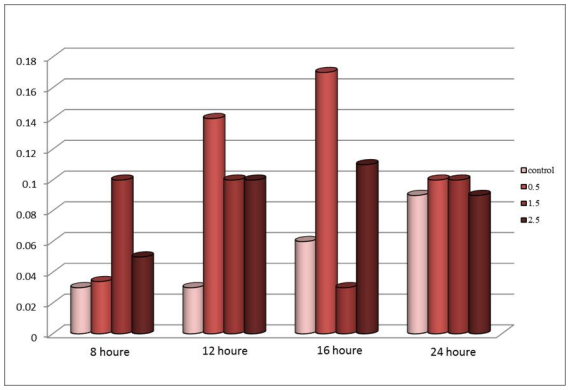


Figure (3): Effect of chitosan on chromosomes aberration frequency of *Vicia faba* root tip cells

2-Chromosomal aberration:

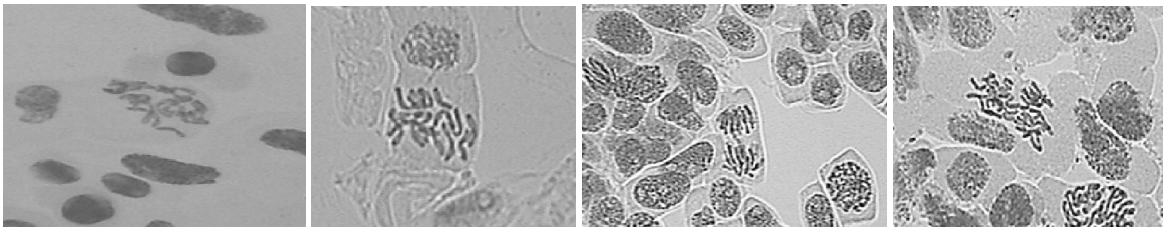
Table (2) and figure (4) show the result of different treatment of chitosan on cells of root tip of *vicia faba.* (Tülay, 2012) reported that an increase in chromosomal aberrations may be resulted from interaction of a great variety of chemical agents with DNA.

Different treatments of chitosan cause increase in chromosomal aberration frequency with the increase of concentration and duration of treatment, except for high concentration (2.5%v) decrease the chromosomal aberration frequency compared with control, this decrease may be due to the decrease of cell division, and the effect were in significant at 0.05.

Figure 4 show the chromosomal aberrations that was noticed, the type of chromosomal aberrations were similar to the chromosomal aberrations recorded in the control sample. Despite of no treatment, the apical meristem showed cytological abnormalities with low frequency; this might be due to the auto mutagenic substance (Dubinin and Scerbako, 1962; Kaushik, 1996). (Teas et al, 1965) suggested that as seedling roots increase in length, the aberrations are less likely to continue mitosis and when root become 2 to 3 cm in length; the aberration caused in control condition become insignificant, and this indicate that the effect of chitosan are similar to the effect of natural substance that effect seeds growing and which the genotoxic effect of these substance became in significant.

Tülay (2012) explained that the possible ways in which inhibitors of genotoxic agent can act include the inhibition of interaction between genes and biochemically reactive genotoxic agent and the inhibition of metabolic activation of indirectly acting toxicant. The chromosomal aberration that raised after treatment were chromosome stickiness that means loss of normal appearance, and they are seen with sticky surface causing chromosomes agglomeration (Babich et al., 1997); also stickiness might be due to the deplolymerization and partial dissolution of nucleoproteins, breakage and exchange of the basic folded units of chromatids and the stripling of the protein covering of DNA in chromosomes (Onyenwe, 1983). Disturbance during metaphase, anaphase and telophase appears because of the effect of the treatment on the spindle lead to failure of spindle mechanism (Yadav, 1986).

Chromosomes bridges during anaphase and telophase raised when the chromosomes fail to separate because of chromosomes stickiness (Yadav, 1986). Other chromosomal aberrations were observed in low percentage, c-metaphase appeared because of the inactivation of the spindle followed by a random scattering of the chromosomes over the cell (Auti et al, 2010), anaphase tri-polar resulted from the failure of the spindle apparatus to organize and function in a normal way (Ozlem *et al*, 2008); in addition, lagging chromosome which raise from centromere adhesion causing abnormality of chromosomes movement towards the equatorial (Bader *et al*,1992) was noticed.



A B C D

Figure (4): Type of Chromosomal Aberration:– (A & B) Disturbed metaphase. (C) Bridge at anaphase. (D) Disturbed anaphase.

Table (2): Type of Chromosomal Aberrations after Treatment with Different Concentrations of Chitosan for Different Time Periods on Root Tip Cells of *Vicia faba*

	pesticide	control				chitosan											
		Distilled water				.5mlL/100ml				1.5ml/100ml				2.5ml/ml			
	concentration &duration(h)	8	12	16	24	8	12	16	24	8	12	16	24	8	12	16	24
metaphase	stiki	0.02	0.014	0.009		0.0049	0.054	0.06	0.031	0.029	0.0084	0.009	0.013	0.009	0.031	0.017	0.016
	distirb	0.006	0.014	0.039	,08	0.014	0.04	0.03	0.04	0.063	0.046	0.02	0.076	0.04	0.021	0.04	0.02
	lagging	0.003		0.004													
	fragment																
	colch									0.0042	0.0084						
	stare metaphase																
anaphase	stiki																
	distirb			0.004		0.0049				0.0083	0.0042	0.003	0.0045			0.018	0.004
	lagging							0.009	0.003						0.01		
	fragment																
	colch																
	bridg					0.0049	0.042	0.03	0.012	0.0042	0.013	0.006	0.012	0.004	0.026	0.023	0.02
	tri-polar										0.013					0.018	
	sta																
telophase	stiki																
	distirb							0.03									0.016
	lagging				0.001	0.0049			0.009						0.01		
	fragment																
	colch																
	bridg			c.004			0.003		0.003								0.023
	polar																
	sta																
nucli			0.003														
%		0.03	0.03	0.06	0.08	0.034	0.14	0.17	0.1	0.108	0.11	0.03	0.12	0.05	0.1	0.11	0.09

Conclusion:

The three concentrations of chitosan (0.5%$_v$, 1.5%$_v$, 2.5%$_v$) to evaluate the cytotoxic and genotoxic effect for four different time periods (8, 12, 16, 24 hours) showed that low concentration (0.5%$_v$) increased the mitotic index with the increase of treatment time, while higher concentrations (1.5%$_v$) decreased the mitotic index after treatment for 16 hour and (2.5%$_v$) after treatment for (12&16 hour) and were in significant at 0.05. The result indicate that low concentration of chitosan stimulate plant growth by enhancing cell division similar to gibberellins (plant hormone that enhance cell division), also chitosan causes chromosomal abnormalities similar to the chromosomal aberration that recorded in control. The result of this study indicates that chitosan can be used in agriculture fields with less harm on plants, and safe alternative of chemical fertilizations

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