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Effect of gamma radiation on mitodepressive action of *Aspergillus niger* aflatoxin on *Vicia faba* root tip cells

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Abstract

In this study, the cytotoxic and genotoxic effects of aflatoxin extracted from *Aspergillus niger* grown on stored coffee bean seeds were investigated. Three concentrations were prepared (0.5%$_v$,1%$_v$,2%$_v$) and the effects were studied after the root tips of *Vicia faba* were treated for three different periods of time (6, 12 and 24 hours). Gamma radiation was used as a protective agent against afltoxin mitodepressive effect; three different dosages were used (3, 5 and 10 kGy) and compared against untreated aflatoxin and negative treatment (control). Treatment with 1%$_v$ and 2%$_v$ decreased the mitotic index and the effect was significant at 0.05, while the lowest concentration (0.5%$_v$) increased the mitotic index. In addition aflatoxin increased chromosomal aberrations, treatment with 1%$_v$ showed significant effect at 0.05. The type of chromosomes abnormalities were disturbance, sticky chromosomes during metaphase and anaphase, star metaphase, c-metaphase and tripolar in anaphase, lagging chromosomes and micronuclei. The treated aflatoxin with different dosages of gamma radiation showed less effect on mitotic index and chromosomal aberration frequency compared with untreated aflatoxin and control, gamma radiation became more effective on aflatoxin with the increase of dosage. This study encourages using gamma radiation as a protective agent against aflatoxin mitodepretion effect on cell division.

Key words: Gamma radiation, *A. niger*, mitotic chromosomes, *Vicia faba*.

Introduction

Aflatoxins belong to a class of compounds known as mycotoxins. Mycotoxins may be defined as small molecular weight compounds produced by fungi that are toxic to animals and humans. Over 300 mycotoxins are known and they are produced by several species of fungi. Most of the mycotoxins of concern are produced by three species: *Aspergillus*, *Penicillium* and *Fusarium* [1, 2].

These fungi can produce their toxic compounds on almost any food that supports their growth. Aflatoxins are secondary metabolites that are highly mutagenic and toxic for human and animal health. The main biological effects of aflatoxins are carcinogenicity, immunosuppression and teratogenicity [3,4], aflatoxin also causes dangerous histopathological changes in internal organs with mutagenic and carcinogenic effects[5, 6]. Aflatoxin produces a number of toxic effects in plant tissues, chlorophyll development in seedlings of *Lepidium sativum* and cotyledons of cotton seed is inhibited by the toxin [7, 8], it also inhibits mitosis and causes fragmentation and bridging of chromosomes in roots of *Vicia faba* [9]. Chromosomal aberrations such as clumping, fragmentation, mitotic polyploidy and euploidy were recorded in mice fed with aflatoxin-contaminated diets [10]. Several researches reported that afltoxin has effect on total lipid, protein, and carbohydrate content, inhibition of chlorophyll synthesis, and chromosomal aberration [11-13],

The harmful effects of aflatoxin led many researchers to investigate substances that provide a protective effect against aflatoxins [14-19]. Gamma radiation is one of the agents used for the protection against aflatoxin ability of toxicity. The use of gamma radiation is considered as one of the alternative solutions of traditional ways for fighting food storage harmful organisms [20, 21]. Gamma radiation is very effective and leaves no radical on treated material [22]. Several researchers investigated the effect of gamma radiation on microorganisms [23, 24] and found that 10 kGy of gamma radiation had severe effect on actinomycetes, and gamma radiation was used to control the growth of several harmful fungi [25].

In the present study, the effect of aflatoxin, extracted from *A. niger* grown on stored coffee bean seeds, on cell division (cytotoxicity) and chromosomal aberration (genotoxicity) was investigated.

Material and Methods

Aflatoxin treatments: Aflatoxin was extracted from *A. niger* mycelium grown on stored coffee bean [26]. Three samples of aflatoxin were exposed to 3, 5 and 10 kGy doses of gamma radiation at the Atomic Energy Research Institute, King Abdul Aziz City for Science and Technology in Riyadh. The treatments were compared with the effect of untreated aflatoxin on cell division and chromosomal frequency. Control (negative treatment) was distilled water only. Three concentrations were prepared for each treatment

(0.5%$_v$, 1%$_v$, 2%$_v$) for three different periods of time (6, 12, 24 hours) using *Vicia faba* seeds for monitoring cytotoxicity and genotoxicity effects.

Preparation of samples: Seeds of *Vicia faba* were procured from the local market. They were first presoaked for 24 hours in distilled water and then transported to the tested concentrations of aflatoxin for different periods of time; long secondary root tips (1-2 cm) 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 1 N HCl at 60°C for 5 min, and then washed with distilled water several times and stained with 1% acetocarmin; five temporary slides were prepared using the squash technique, five seeds were used for each treatment and two root tips on each slide were examined for the effects of the untreated and treated aflatoxin on mitotic index (MI). The same slides were analysed for the types and frequencies of chromosomal abnormalities.

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).

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

$$\text{Mitotic Index (MI)} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} \times 100$$

Cytotoxicity: The mitotic index of the treated cells was compared with that of the negative control group.

Genotoxicity test: Chromosomal aberration frequency per dose was examined; the percentage of cells with aberrations of each dose was compared with that of the negative control using the SPSS 16.0 for Windows statistical package. Two-way analysis of variance was used for determining the significance of difference at P = 0.05.

$$\text{Chromosomal aberration frequency (CF)} = \frac{\text{Total number of abnormal cells}}{\text{Total number of divided cells}}$$

Results and Discussion

Mitotic index (MI): MI measures the proportion of cell in the M-phase of the cell cycle and its inhibition could be considered as a cellular death or a delay in the cell proliferation kinetics [27]. Table 1 and Fig. 1 show the effects of different aflatoxin treatments on mitotic chromosomes of root tip cells of *Vicia faba*. The untreated aflatoxin affects the mitotic index after treatment with the three different concentrations for three different periods of time. Compared with control, the effect of low concentration (0.5%$_v$) increased the MI, and this effect may be resulted from shortening the duration of mitotic cycle and enhanced interphase cells enter the subsequent division stages [28, 29]. Sopova *et al.*[30] found out that low concentration of extracted tobacco leaf exerted had a stimulating effect, whereas high concentration had a mitodepressant effect.

Higher concentrations of the untreated aflatoxin (1%$_v$ and 2%$_v$) affect the mitotic index (MI) by decreasing it compared with control, and the effect was significant at 0.5%. This inhibition of MI could be explained, that aflatoxin affects the compounds of DNA synthesis and inhibits the enzyme system required for the chain reaction of DNA synthesis [11, 30, 32]. Sisman *et al.* [33] reported that aflatoxins are bound to cellular macromolecules like DNA, RNA, and protein and obstruct the synthesis of the macromolecules. McLean and Dutton [34] reported that activity of RNA polymerase connected to DNA is also blocked by aflatoxins. Also Theumer *et al.* [35] suggested that effect of aflatoxin results from increasing generation intracellular reactive oxygen species ROS which causes different damage in cell components, especially in DNA, RNA and protein. Similarly, aflatoxin caused severe damage on DNA of *Labeo rohita* fish [15]. Aflatoxin affected the production of proteins because of the damage in DNA and RNA of *Oreochromis niloticus* [18].

The effect of high concentration of untreated aflatoxin for different periods of time on mitotic index indicates that aflatoxin extracted from *A. niger* had a cytotoxic effect especially at high concentrations. Treatment with aflatoxin exposed to 3 kGy of gamma radiation decreased mitotic index after treatment with low concentration (0.5%$_v$) and was significant at $p<0.05$. Concentration of aflatoxin treated with high dosages of gamma radiation (5 and 10 kGy) increased MI compared with untreated aflatoxin, but still effect was lower than control and statistically insignificant. The effect on MI indicates that treatment with low dosage (3kGy) of gamma radiation had partial effect on *A. niger* aflatoxin. Aflatoxin treated with high dosage (5 and 10 kGy) was more effective on aflatoxin cytotoxicity, this effect was clear by the increased MI percentage compared with MI of untreated aflatoxin and control. This increase of MI may be due to the effect of gamma radiation on substance or metabolism pathway in cells of aflatoxin. Similar results were found on the protective effect of selenium[14]; the effect on the *Nigella sativa* extract [16]; the effect of curcumin [17] and the protective effect of *Rhodotorula glutinis* on toxicity of aflatoxin [19].

Chromosomal aberrations: Table 2 and Figs 2-3 show the data of chromosomal aberration recorded on root tip cells of *Vicia faba* after treatment with different concentrations of aflatoxin extracted from *A. niger*. All the tested concentrations of untreated aflatoxin induced chromosome abnormalities after exposer for different periods of time compared with control, and despite of no treatment with tested concentrations of aflatoxin, the apical meristem of *Vicia faba* treated with distilled water only showed cytological abnormalities with low frequency. This might be due to the auto mutagenic substance [36, 37]. Teas *et al.* [38] 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.

Chromosomal aberration may result by inhibition of mitotic activity and production of several aberration caused by some fungal metabolites [12]. Treatment with tested concentrations of untreated aflatoxin increased chromosome aberration frequency compared with control, and the effect was significant at $p<0.05$ after treatment with (1%$_v$). An increase in chromosomal aberrations may result from interaction of a great variety of chemical agents with DNA[39].

While treatment with the same concentrations of aflatoxin

Table 1. Total number of examined cells , mitotic index and chromosomes aberration frequency after treatment with different concentrations of *A. niger* aflatoxin for different periods of time on root tip cells of *Vicia faba.*

treatment	Examined concentration %	Ttime of duration / hour	No. of counted cells	No. of divided cells	No. of abnormal cells	Mitotic index	Abnormal frequency
Negative control	0	6	2745	271	26	10	0.1
	0	12	2038	215	24	11	0.11
	0	24	2104	201	17	10	0.1
aflatoxin	0.5	6	2287	321	57	14	0.2
	0.5	12	2086	197	29	9	0.2
	0.5	24	2203	172	55	8	0.3
	1	6	2129	138	40	7	0.3
	1	12	2125	84	13	4	0.2
	1	24	2119	74	20	4	0.3
	2	6	2372	171	56	7.2	0.3
	2	12	2207	149	44	6.8	0.3
	2	24	2319	96	15	4.1	0.1
treatment with Gamma radiation							
aflatoxin treated with 3 kGy	0.5	6	2042	166	24	8.1	0.1
	0.5	12	2078	154	41	7.4	0.3
	0.5	24	2175	188	35	6	0.2
	1	6	2098	220	23	11	0.1
	1	12	2068	135	24	6.5	0.2
	1	24	2042	129	32	6.3	0.2
	2	6	2140	201	25	9.4	0.12
	2	12	2041	140	39	7	0.3
	2	24	2072	185	27	9	0.2
aflatoxin treated with 5 kGy	0.5	6	2014	236	37	12	0.2
	0.5	12	2289	103	22	4.5	0.2
	0.5	24	2081	146	26	7	0.2
	1	6	2171	147	29	7	0.2
	1	12	2055	83	15	4	0.2
	1	24	2096	167	33	8	0.2
	2	6	2170	166	34	8	0.2
	2	12	2087	151	23	7.24	0.2
	2	24	2076	148	35	7.13	0.2
aflatoxin treated with 10 kGy	0.5	6	2095	237	26	11.31	0.11
	0.5	12	2160	210	19	9.7	0.1
	0.5	24	2244	179	24	8	0.1
	1	6	2076	143	6	6.9	0.04
	1	12	2191	126	11	6	0.1
	1	24	2091	192	37	9.2	0.2
	2	6	2085	132	24	6.3	0.2
	2	12	2119	242	30	11.4	0.1
	2	24	2093	141	14	7	0.1

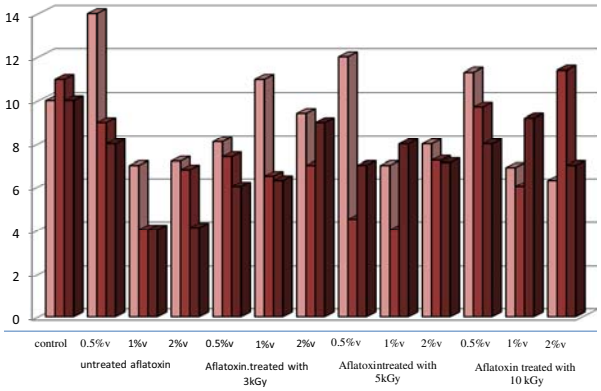

Figure 1. Effect of aflatoxin treatments on mitotic index of root tip cells of *Vicia faba*.

exposed to gamma radiation caused chromosomal aberration but in low percentage compared with untreated aflatoxin and close to chromosomal aberration percentage of control, and the effect was significant at $p< 0.05$ after treatment with ($0.5\%_v$) for 6 hours, also different concentrations treated with 5 kGy of gamma radiation were significant at $p<0.05$. These different effects of treated aflatoxin indicate that gamma radiation affects the ability of aflatoxin to induce chromosomal aberration, and this may be due to the effect of gamma radiation on metabolic process and the secondary substance that effect the chromosomes, also the data show that the harmful effect decrease with the increase of gamma radiation dosage. Gamma radiations affect the growth of fungus by inhibiting the metabolism of the fungi cells [40, 41].

The chromosomal aberration percentage depends on the concentration and time of exposure, and several types of chromosomal aberration raised after treatments: sticky chromosomes during metaphase, anaphase and telophase. The metaphase with sticky chromosomes lose their normal appearance and appear to have a sticky "surface" which cause chromosome agglomeration [42], possibly due to the effects on chromatin and chromosome organization. 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[43]. Disturbance of chromosomes during meta- and anaphase, star metaphase, C-metaphase and tripolar in anaphase stage, arise because of unfunctional spindle, and this indicates that aflatoxin may have poisoning effect on spindle formation, also the disturbance could be genes responsible for assembly and function of microtubules [44].

Lagging chromosome areas as a result of chromosome stickiness and subsequent failure of anaphase separation [45] also could be happened because of chromosome breakage and reunion [46, 47]. Micronuclei (MN) was scored according to following criteria [48] (1) the diameter of MN should be one third of the main nucleus, (2) should be on the same plane focus, (3) should have a chromatin structure similar to that of the main nuclei, (4) should be smooth oval or round shape and (5) clearly separated from the main nucleus. MN often results from acentric fragments or lagging chromosome that fail to incorporate into daughter nuclei during telophase of mitotic cells, and this can cause cellular death due to the deletion of primary genes [49-51], also it may be due to the genotoxity of aflatoxin [52].

Bridges during anaphase and telophase were noticed and this raised when the chromosomes fail to separate. Stickiness usually leads to the formation of anaphase and telophase bridges and this end up inhibiting metaphase and cytokinesis, respectively,

Table 2a. Type of chromosomal aberrations on root tip cells of *Vicia faba* after treatment with different concentrations of *A. niger* aflatoxin for different periods of time.

			Metaphase						Anaphase							Telophase								
pesticide	Concentration%	duration of time (h)	sticky	disturb	c-metaphase	fragment	s- metaphase	lagging	sticky	disturb	lagging	bridge	polar	fragment	S-anaphase	sticky	lagging	chromatine bridge	bridge	polar	fragment	mic-nucli	binucleat	AF%
control	Distell water	6	0.06	0.02						0.004		0.01										0.004		10
		12	0.02	0.06						0.009		0.009					0.009							11
		24	0.02	0.06								0.004												8
positive control	0.5	6	0.04	0.07								0.05				0.009								18
		12	0.005	0.08					0.005	0.02		0.04			0.005									15
		24	0.06	0.13	0.004				0.009	0.009		0.05							0.004			0.009		27
	1	6	0.03	0.2	0.04					0.01		0.04		0.01					0.007					29
		12		0.11	0.01						0.01						0.01					0.01		16
		24	0.07	0.11	0.04							0.03				0.03								27
	2	6	0.04	0.14	0.05					0.02	0.01	0.04				0.006				0.02	0.006			33
		12	0.03	0.2	0.007					0.01							0.01		0.01			0.007		30
		24	0.01	0.06					0.01			0.07												16
aflatoxin treated with 3kGy	0.5	6	0.05	0.07						0.01								0.006	0.006					15
		12	0.11	0.1	0.25					0.006		0.006				0.02			0.006					26
		24	0.08	0.1					0.005															19
	1	6	0.02	0.05	0.005					0.02		0.01												11
		12	0.007	0.08						0.02		0.07												18
		24	0.05	0.13	0.02					0.02		0.008					0.02							25
	2	6	0.02	0.04						0.01		0.04										0.005		12
		12	0.11	0.11	0.007			0.007		0.03						0.01								28
		24	0.07	0.04								0.02				0.02								15

Table 2b. Type of chromosomal aberrations on root tip cells of *Vicia faba* after treatment with different concentrations of *A. niger* aflatoxin for different periods of time.

			Metaphase						Anaphase						Telophase								
pesticide	Concentration%	duration of time (h)	sticky	disturb	c-metaphase	fragment	s- metaphase	lagging	sticky	disturb	lagging	bridge	polar	fragment	S-anaphase	sticky	lagging	chromatine bridge	bridge	polar	fragment	mic-nucli	binucleat	AF%
aflatoxin treated with 5kGy	0.5	6	0.05	0.06						0.004		0.04			0.004		0.008							16
		12	0.03	0.08								0.06		0.01								0.03		21
		24	0.01	0.11						0.007		0.05												18
	1	6	0.05	0.1	0.007					0.03		0.01												19
		12		0.12						0.04		0.005										0.005		18
		24	0.04	0.11		0.006		0.006				0.03							0.006			0.006		20
	2	6	0.01	0.12	0.01					0.006	0.01	0.02		0.006			0.006				0.006			21
		12	0.007	0.12						0.007		0.01					0.007							15
		24	0.02	0.08	0.03		0.02			0.05		0.02						0.007	0.007					24
aflatoxin treated with 10kGy	0.5	6	0.02	0.04						0.03			0.01								0.004			11
		12		0.08								0.01												9
		24	0.006	0.08								0.03											0.01	13
	1	6	0.03	0.01																				4.20
		12	0.02	0.05																		0.02		9
		24	0.05	0.09	0.005					0.005		0.05					0.005							19
	2	6	0.05	0.07	0.007					0.03	0.007								0.007			0.007	0.007	19
		12	0.04	0.08	0.004					0.004		0.004												12
		24	0.007	0.05								0.04												1

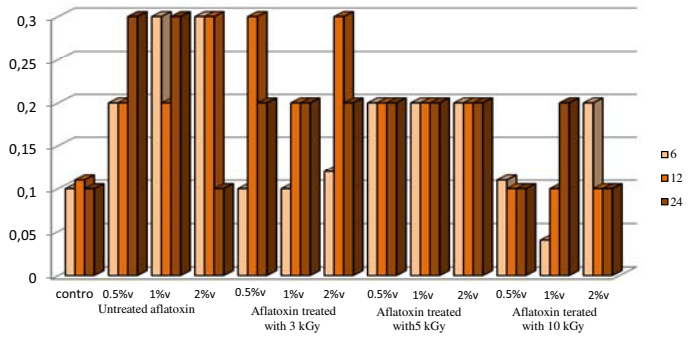

Figure 2. Effect of treatments of *A. niger* aflatoxin on chromosomal abnormalities frequency in root tip cells of *Vicia faba*.

and thus hampering cell division [53, 54], also it could be due to broken chromosome ends exhibiting a tendency to fuse and form dicentric chromosome [55]. Similar results were observed in chromosomes of *Vicia faba* root tip cells [9, 11, 56].

Different types of chromosomal aberration that raised after treatment of untreated aflatoxin, insure that aflatoxin had genotoxic and cytotoxic effect, and its toxicity could be through induction of free radicals [57], and the toxicity is related to its metabolic fate in a complicated network of mutually competing pathways in the target tissue [58].

Treated aflatoxin with high dosage of gamma radiation degree the genotoxicity of aflatoxin comparing with untreated aflatoxin

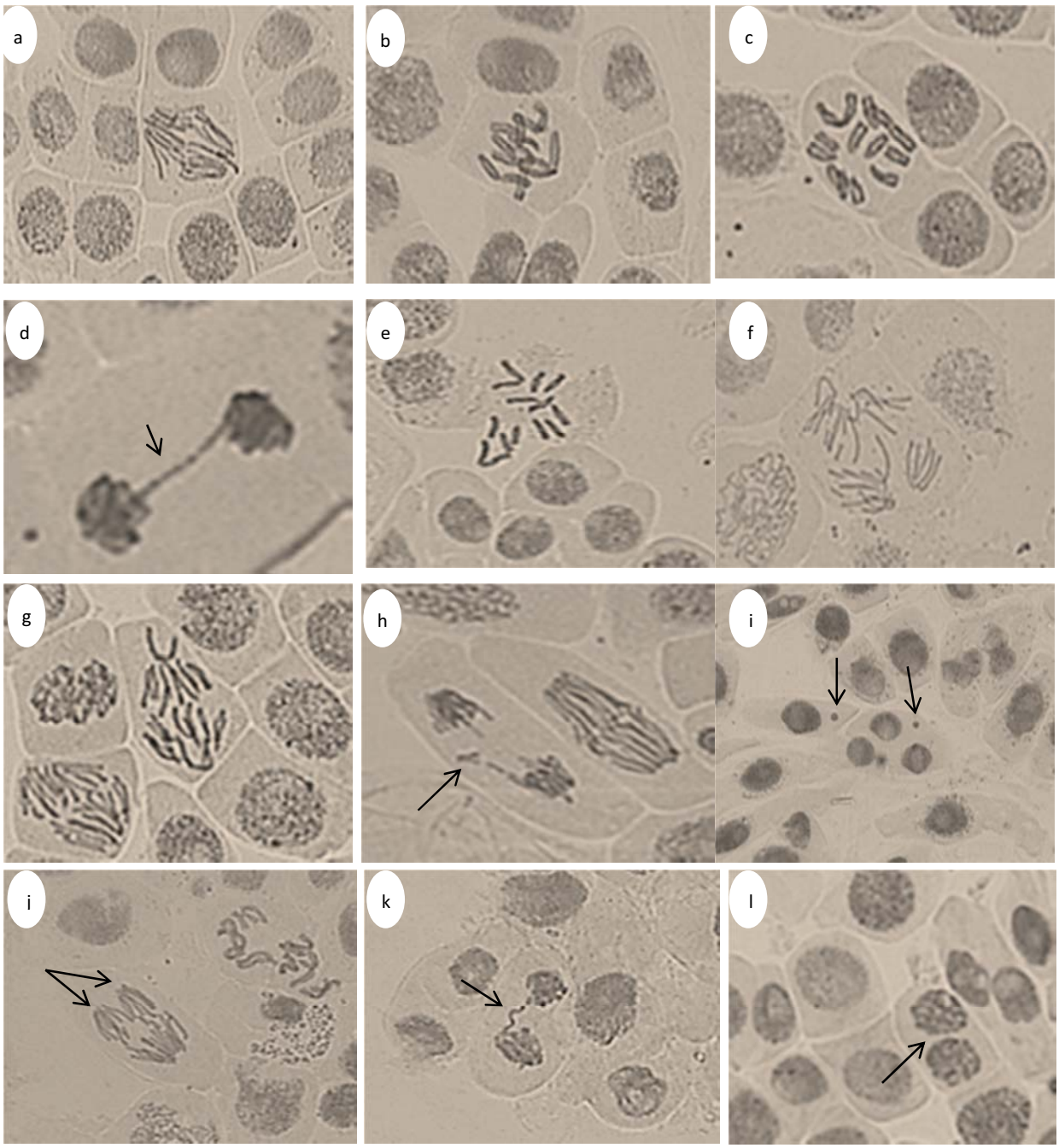

Figure 3. Type of chromosomal aberrations (a- k) after treatment with different concentrations of *A. niger* aflatoxin on root tip cells of *Vicia faba* : a- Anaphase bridge, b &c- C- metaphase, d - Telophase bridge, e- disturbed metaphase, f- Disturbed & tri polar anaphase, g- Lagging chromosome during anaphase, h- Fragment in telophase, i- Micronuclei, j- Tripolar at anaphase, K- Lagging chromosome during telophase, l- Bi-nucleated cell.

and control, and this effect increased with the increase of gamma radiation dosage. Most of the chromosomal abnormalities recorded were disturbance in metaphase and anaphase, sticky chromosomes and bridges in anaphase and telophase.

Conclusions

Aflatoxins were produced naturally from *A. niger* affected stored coffee bean seeds. The high concentrations of untreated aflatoxin for different periods of time caused mitotic decrease compared with control; while aflatoxin treated with different dosage of gamma radiation causing disturbance in aflatoxin metabolism led to decrease of the cytotoxicity of tested concentrations. Also untreated aflatoxin caused increase in chromosomal abnormalities frequency compared with control while treated aflatoxin with gamma radiation decreased the genotoxic and the chromosomal abnormalities frequency. The most type of chromosome abnormalities recorded after treatment with untreated aflatoxin were disturbance in metaphase and anaphase, bridges in anaphase and telophase, micronucleus, lagging chromosome, stickiness and binucleated cells. The chromosomes most abnormalities scored after treatment with treaded aflatoxin were disturbance and sticky chromosomes. The results show that aflatoxin treated with different dosage of gamma radiation affect its cytotoxicity and genotoxicity on mitotic chromosomes of *Vicia faba*.

The results of this study encourage using gamma radiation to sterilize seeds before storage and recommended for more physiological and morphological studies on growing seeds treated with gamma radiation, to insure safety.

References

[1]Wogan, G. N. 1999. Aflatoxin as a human carcinogen. Hepatology **30**(2): 573-575.

[2]CAST 2003. Mycotoxins: Risks in plant, animal, and human systems. Council of Agricultural Science and Technology, CAST, Ames, IA. Task Force Report No. 139. 199 p.

[3]Betina V. 1989. Biological effects of mycotoxins. In Betina, V. (ed.). Mycotoxins: Chemical, Biological and Environmental Aspects. Elsevier, Amsterdam, pp. 42-58.

[4]Abdel-Wahhab, M. A., Nada, S. A., Farag, I. M., Abbas, N. F. and Amra, H. A. 1998. Potential protective effect of HSCAs and bentonite against dietary aflatoxicosis in rat: with special reference to chromosomal aberration. Natural Toxins **6**:211- 218.

[5]Jantrarotai, W. 1991. Acute and subacute toxicity of AFB1 and cyelopiazonic acid to channet catfish. Dissertation Abstracts International Part B: Science and Engineering **51**:132.

[6]Thorgaard, H. G., Arbogast, N. D., Hendricks, J. D., Pereira, C. B. and Baily, G. S.1999. Tumor suppression in triploid trout. Aquatic Toxicol. **46**:121-126.

[7]Black, H. S. and Altschul, A. M.1965. Gibberellic acid-incuced lipase and α-amylase formation and their inhibition by aflatoxin. Biochem. Biophys. Res. Commun. **19**:661-664.

[8]Schoental, R. and White, A. F. 1965. Aflatoxins and 'albinism' in plants. Nature **205**:57-58.

[9]Lilly, L. J. 1965. Induction of chromosome aberrations by aflatoxin. Nature **207**:433- 434.

[10]Bilgrami, K. S. and Sinha, S. P. 1988.Mutagenic Effects of Mycotoxins. Final Technical Report of U.G.C. Research Project, Bhagalpur University, Bhagalpur, India.

[11]Abdou, R. F., Megalla, S. E., Moharram, A. M., Abdal, K. M., Sherif, T. H. I., El-Syed-Mahmood, A. L.and Lottfy, A. E. 1989. Cytological effects of fungal metabolites produced by fungi isolated from Egyptian poultry feedstuffs. J. Basic. Microbiol. **29**:131-139.

[12]El-Zawahri M., Morad, M. and Khishin, A. F. 1990. Mutagenic effect of aflatoxin G_1 in comparison with B_1. J. Environ Pathol. Toxicol. Oncol. **10**:45-51.

[13]El-Naghy, M. A., Fadl-Allah, E.M. and Samhan, M. 1999. Effect of aflatoxin G_1 on germination growth and metabolic activities of some crop plants. Cytobios **97**:87-93.

[14]Agar, G. and Alpsoy, L. 2005. Antagonistic effect of selenium against aflatoxin G_1 toxicity induced chromosomal aberrations metabolic activities of two crop plants. Bot. Bull. Acad. Sin. **46**:301-305.

[15]Madhusudhana, N., Kavithalakshmi, K., Radha, S. S. and Shanmugasundaram, K. R. B. 2006. Aflatoxin B_1- induced DNA damage in *Labeo rohita*: Protective effect of an antioxidant supplement, Amrita Bindu. Basic Clin. Pharmacol. Toxicol. **98**:473-479.

[16]Anwar, M., Najwa, F. S., Husni, F. and Wafa, M. B. 2007. Effect of *Nigella sativa* extract and oil on aflatoxin production by *Aspergillus flavus*. Turk. J. Biol. **31**:155-159.

[17]Neeta. M. and Ramtej, J. V. 2007. Ameliorative effect of curcumin on aflatoxin-induced toxicity in DNA, RNA and protein in liver and kidney of mice. Acta Poloniae Pharmaceutica - Drug Research **64**(6):497-502.

[18]Manal, I. B. 2008. Aflatoxin B_1 induced changes in protein electrophoretic pattern and DNA in *Oreochromis niloticus* with special emphasis on the orotective effect of rosmary and parsley extracts. American-Eurasian J. Agric & Environ. Sci. **4**(3):381-390.

[19]Inas, S. G., Hassnane, M., Ahmed, E. S., Haggag, W. M., Nada, S. A. and Farag, I. M. 2010. Cytogenetic and biochemical studies on the protective role of *Rhodotorula glutinis* and its autoploidy against the toxic effect of aflatoxin B_1 in mice. Nature and Science **8**(5):28-38.

[20]Uijl, C. H. 1992. Beating the bugs. International Food Ingredients **3**:9-11.

[21]Pszczola, D. E. 1997. 20 Ways to market the concept of food irradiation. Food Technol. **51**:46-48.

[22]Tilton, E. W. and Brower, J. H. 1987. Ionizing radiation for insect control in grain and grain products. Cereal Food World 32:330- 335.

[23]Aziz, N. H. and Youssef, A. Y. 1991. Occurrence of aflatoxin and aflatoxigenic producing in fresh and processed meat in Egypt. Food Additives and Contaminants **3**:321-331.

[24]Aziz N. H., El-Fouly, M. Z., Abu-Shady, M. R. and Moussa, L. A. A. 1997. Effect of gamma radiation on the survival of fungal and actinomycetal florae contaminating medicinal plants. Appl. Radiat. Isot. 48:71-76.

[25]Sedlackova, J.1992. Ionizing radiation in the food industry. Food Industry Potravinarstvi, Prague (Czechoslovakia). Stredisko Potravinarskych Informaci **166**:82-96.

[26]Vicam 2000. The leading mycotoxin testing solutions. Available source: Http://Vicam. Com/home.

[27]Rojas, E., Herrera, L. A., Sordo, M., Gonsebatt, M. E., Montero, R., Rodriguez, R. and Ostrosky-Wegman, P. 1993. Mitotic index and cell proliferation kinetics for the identification of antineoplastic activity. Anticancer Drugs **4**:637- 640.

[28]Haroun, S. A. and Al-Shehri, A. M. 2001. Cytogenetic effects of *Calotropis procera* extract on *Vicia faba*. Cytologia **66**:373-378.

[29]Haroun, S. A. 2010. Mutagenic effects of *Kochia indica* extract on *Vicia faba* L. Journal of American Science **6**(7):292-297.

[30]Sopova, M., Sekovski, Z. and Jovanovska, M. 1983. Cytological effects of tobacco leaf extract on root-tip cells of *Allium sativum*. Acta Biol. Med. Exp. **8**:49-56.

[31]Smith, L. H. and Sullivan, M.1960. Feedback inhibition by fluorinated pyrimidines. Biochem. Biophys. Acta **39**:554-560.

[32]Taylor , J. H., Hant, W. F. and Tung, J. 1962 Effect of fluorodeoxyuridine on DNA replication, chromosome breakage and reunion. Proc. Natl Acad. Sci. USA **48**:190-198.

[33]Sisman, T., Handan, U. and Hakan, A. 2005. The clastogenic effect of aflatoxin B_1 (AFB1) on polytene chromosomes of *Drosophila melanogaster*. Dros. Inf. Serv. **88**:1–6.

[34]McLean, M. and Dutton, M. F. 1995. Cellular interaction and metabolism of aflatoxin. Pharm. Ther. **65**:163-192.

[35]Theumer, M., Cànepa, M., López, A., Marya, A., Dambolenac, J. and Rubinstein, H. 2012. Subchronic mycotoxicoses in Wistar rats: Assessment of the *in vivo* and *in vitro* genotoxicity induced by fumonisins and aflatoxin B_1, and oxidative stress biomarkers statue. Toxicology **268**:104-110.

[36]Dubinin, N.P. and Scerbakov, V. K. 1962. Control of the natural mutation process by means of cysteamine and streptomycin. Proc. Acad. Sci. USSR. **145**:427-429.

[37]Kaushik, S.C. 1996. Cytological effects of *Lantana camara* L. leaves extract on *Vicia faba* root tip cells. Advanced Plant Science **9**:159-164.

[38]Teas, H. J., Sax, H.J. and San, K. 1965. Cycasin: Radiomimetic. Science **149**:541-542.

[39]Celik, T. A. 2012. Potential genotoxic and cytotoxic effects of plant extracts. In Bhattacharya, A. (ed.). A Compendium of Essays on Altrenative Therapy. InTech, Croatia, pp. 233-250.

[40]Geweely, N. S. I. and Anwar, L. S. 2006. Sensitivity to gamma irradiation of post-harvest pathogens of pear, fungicidal effect of gamma radiations. Int. J. Agric. Biol. **8**:710-716.

[41]Jones, H.E., West, H.M., Chamberlain, P.M., Parekh, N.R., Beresford, N.A. and Crout, N.M. 2004. Effect of gamma irradiation on *Holcus lanatus* (Yorkshire fog grass) and associated soil microorganisms. J. Env. Rad. **74**:57-71.

[42]Babich, H., Segall, M. A. and Fox, K. D. 1997. The *Allium* test- A simple, eukaryote genotoxicity assay. Am. Biol. Teacher **59**:580-583.

[43]Onyenwe, C. N. 1983. Cytological effects of seed extracts of *Abrus procatorius* on the mitosis of *Allium cepa* and the effect of root extracts of *Boerhaavia diffusa* on mitosis of *Crassocephalum biafrae*. University of Port Harcourt, Port Harcourt.

[44]Onfelt, A. 1986. Mechanistic aspects on chemical induction of spindle disturbances and abnormal chromosome numbers. Mut.Res. **168**:249-300.

[45]Abraham, S. and Koshy, M. P. 1979. Mutagenic potential of green chilies. Cytologia **44**: 221-225.

[46]El-Khodary, S., Habib, A. and Haleim, A. 1990. Effect of the herbicide tribunal on root mitosis of *Allium cepa*. Cytologia **55**:209-215.

[47]Abou-Rawash, A. A., Abdel-Megged, M. S. and Azza, K. 2008. Effects of enzyme modulation on aflatoxin cytotoxicity and carcinogenicity in rats: Cytogenetics and histopathology. Egypt. J. Comp. Pathol. & Clin. Pathol. **21**(2):175-200.

[48]Tolber, P. E., Shy, C. M. and Allen, J. W. 1991. Micronucleus and other nuclear anomalies in buccal smears: A field test in snuff users. Am. J. Epidemiol. **134**:840-850.

[49]Albertini, R. J., Anderson, D. and Douglas, G.R. 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogen in humans. Mutation Research **463**(2):111-172.

[50]Krishna, G. and Hayashi, M. 2000. *In vivo* rodent micronucleus assay: Protocol, conduct and data interpretation. Mutation Research **455**(1-2):155-166.

[51]Tülay, A. C. and Ozlem, S. A. 2010. Evaluation of cytotoxicity of *Inula viscosa* extracts with *Allium cepa* test. Journal of Biomedicine and Biotechnology **2010**:1-7.

[52]Najah Al-Terehi, M. 2012. Effect of aflatoxin B_1 on DNA structural characteristic. Research in Pharmacy **2**(2):35-38.

[53]Ndubuisi, M. C. and Bosa, E. O. 2010. Cytological effects of the root extracts of *Boerhaavia diffusa* on root tips of *Crinum jagus*. Eur. Asian Journal of BioSciences **4**:105-111.

[54]Yadave, S. K. 1986. Antimitotic and cytological activities of tropical forest tree: *Tamarindus indica*. Journal of Tropical Foresty **2**(1):53-58.

[55]Werner, J. E., Kota, R.S., Gill, B. S. and Endo, T. R. 1992. Distribution of telomeric repeats and their role on the healing of broken chromosome ends in wheat. Genome **35**:844-848.

[56]Reis, I. 1971. Chromosome Aberration in den Wurzelspitzen von *Allium cepa* durch Aflatoxin B_1. Experientia **27**:971- 972.

[57]Towner, R. A., Oian, S. Y., Kadiiska, M. B. and Mason, R. P. 2003. In *vivo* identification of aflatoxin-induced free radicals in rat bile. Free Rad. Biol. Med. **35**:1330-1340.

[58]Smela, M. E., Hamm, M. L., Henderson, P. T., Harris, C. M., Harris, T. M. and Essigmann, J. M. 2002. The aflatoxin B (1) formamidopyrimidin adduct plays a major role in causing the types of mutations observed in human hepatocellular carcinoma. Proc. Natl Acad. Sci. USA. **99**:6655-6660.