Pulsed electric field technology for checking aflatoxin production in cultures and corn grains

By

Eisa, (Nawal) A. , F.M. Ali** , G.M. El-Habbaa* , S.K. Abdel-Reheem and M.F. Abou-El-Ella*****

*Agric. Bot. Dept., Fac. Agric. Moshtohor, Zagazig Univ., Benha Branch, Egypt.

**Bio-Physics Dept., Fac., Science, Cairo Univ., Egypt

***Central Lab for Food and Feed, Agri. Res. Center (ARC), Giza, Egypt

ABSTRACT

Exposure of aflatoxin-producing cultures of *Aspergillus flavus* to different pulsed electric field (PEF), reduced B1 and G1 toxin production. In recently inoculated cultures, continuous as well as 12 hrs exposure, every two days, to 50 Hz PEF resulted in the highest toxin decrease. Exposure of four-day old cultures to 0.50 and 50 Hz., as a daily treatment for 0.5-24 hr, reduced production by 75.53 and 82.75%, respectively. Increasing PEF from 100 to 400 Hz caused slight decrease in production, through the remarkable increase at 800 Hz. The highest amounts of aflatoxins B1 and G1 were associated with applying PEF strengths of 500 and 400 Hz., respectively. The multiple exposure at different PEF ranging from 800 to 500 Hz decreased production by 99.0% maximum. It is worth to note that the B1 aflatoxin was undetectable at different combined PEF strengths. Multiple exposure of yellow corn grains over 21 days to a combined treatment reduced the amount of aflatoxin, in either non-inoculated or in grains inoculated with *A. flavus*, compared to the check. Negligible changes were observed in protein and carbohydrates contents of the treated grains.

Key words: *A. flavus*, aflatoxins, electric waves, stored corn grains.

INTRODUCTION

Corn (*Zea maize* L.) is one of the most important grain crops all over the world. It is used mainly for animal feeding, poultry wealth and human consumption in some growing countries. Moreover, there are a new industrial uses of corn such as the industrial corn products for clean environment (biodegradable packing peanuts, biodegradable tray and eating utensils, bunnies diaper, clam shell, and daily home uses. (U.S. Feed Grains Council, 1994). **Hesseltine (1965), Shotwell *et al.* (1980)** and **Sauer and Tuite (1986)** detected aflatoxins on some agricultural commodities i.e. rice, wheat, corn, soybean and sorghum. Whereas, **Henry *et al.* (1981)** found three mycotoxins (aflatoxin, zearalenone and T-2 toxin) in the stored grain samples during feeding of grain after storage. **Zohri *et al.* (1995)** studied the occurrence of aflatoxins and mould flora in 60 different maize snack samples in Egypt and found that all this samples were contaminated with aflatoxins B1, B2, G1 and G2 at concentration ranging from 50 to 100 µg/kg. While, **Davis *et al.* (1966)** and **Diener and Davis (1986)** and **Abramson and Clear (1996)** detected production of aflatoxins B_1 and G_1 by *Aspergillus flavus* in semi-synthetic medium and considered that *Aspergillus flavus* and *A. parasiticus* could be as aflatoxin producers only. **Eisa *et al.* (1996b)** noted that grade three of corn grains showed the highest amounts of both B_1 (986 µg/kg) and B_2 (219 µg/kg) followed by grade five 237 µg/kg of both B_1 and B_2, respectively. While, the least amount of both B_1 and B_2, were shown in grade one (32.9 and 5.48 µg/kg). **Saubois *et al.* (1998)** analyzed 37 corn samples collected from Argentina and

detected aflatoxins in 10.8% of the samples and the levels of contamination were in the range of 20.0 to 50 µg/kg. **Thompson and Henke (2000)** studied the effects of maize grain storage containers on aflatoxin production and determined that aflatoxin was produced regardless of type of storage container, time of storage and climatic condition.

Several investigators indicated that it may be possible to use pulsed electric field (PEF) in future applications in order to produce safe products **(Wouters *et al.*, 1999)**. As for, using PEF as a new effective technique on pathogenic fungi is considered a new trend for controlling myco-organisms. Lacking of literatures in this field, pushed us to use the available literatures on bacteria and other micro-organisms in order to support our study. **Dunn (1996)** discussed two new technologies for use in the food industry, the first method uses intense pulse of light. This pulsed light (pure Bright) process uses short duration flashes of broad-spectrum (white) light to kill all exposed microorganisms, including vegetative bacteria, microbial and fungal spores, viruses, and protozoan oocysts. The intensity of each flash of light is about 20000 times the intensity of sunlight at the earth's surface. The second method discussed uses multiple short duration, high intensity electric field pulses to kill vegetative microorganisms in pumpable products. This pulsed electric field (or cool pure) process can be applied at modest temperatures at which no appreciable thermal damage occurs and the original taste, color, texture, and functionality of products can be retained. **Grahi and Mark (1996)** indicated to the lethal effects of pulsed electric fields (PEF) on suspensions of various bacteria, yeast and spores in buffer solutions and liquid foodstuffs. Living-cell counts of vegetative cell types were reduced by PEF treatment by up to more than four orders of magnitude (>99.99%). On the other hand, endo- and ascospores were not inactivated or killed to any great extent. The killing of vegetative cell types depends on the electrical field strength of the pulses on the treatment time (the product of the pulse number and decay time constant of the pulses). In addition, they studied also the inactivation of microorganisms, the effect of PEF on food components such as whey proteins, enzymes and vitamins, and on the taste of foodstuffs. The degree of destruction of these food components by PEF was very low or negligible. Hence, PEF treatment is excellent process for inactivation of microorganisms in acid and in thermosensive media, but not for complete disintegration of microbial cells. **Kekez *et al.* (1996)** reported that the proposed model assumes that the criteria leading to the lethal breakdown of microorganisms suspended in a continuous medium depend on two parameters: (a) the applied electric field must exceed the critical field of membrane to create holes and (b) the Joule energy (deposited in the membrane) must exceed the minimum value beyond which the cell can not recover. The first parameter initiates (reversible) breakdown and second one, the completion of the (irreversible) electrical breakdown leading to death of the cell. **Calderon *et al.* (1999)** reported that pulsed electric field (PEF) is a nonthermal preservation method used to inactivate microorganisms mainly in liquid foods. PEF is known to inactivate microorganisms by causing dielectric breakdown of the cell membrane, thus altering the functionality of the membrane as semi-permeable barrier. The extent of damage of the cell membrane, whether visible in the form of pore or as loss of membrane functionality leads to the inactivation of the microorganisms. **Evrendilek *et al.* (1999)** studied the effect of high voltage pulsed electric field (PEF) treatment on *Escherichia coli* in apple juice. PEF is a promising technology for the inactivation of *E. coli* in apple juice. **Jeantet *et al.* (1999)** reported that high intensity electric field have been successfully applied to the destruction of *Salmonella enteritidis* in diaultrafiltered egg white. **Jeyamkondan *et al.* (1999)** found

that use of pulsed electric fields (PEFs) for inactivation of microorganisms is one of the more promising non-thermal processing methods. Inactivation of microorganisms exposed to high-voltage PEFs is related to the electromechanical instability of the cell membrane. Electric field strength and treatment are the two most important factors involved in PEF.**Wouters *et al.* (1999)** studied the effect of pulsed electric field (PEF) treatment and processing factors on the inactivation kinetics of *Listeria innocua* by using a pilot plant (PEF) unit with a flow rate of 200 L./hr. The electric field strength, pulse length, number of pulses, and inlet temperature were the most significant process factors influencing the inactivation kinetics. **Ramsted *et al.* (2000)** recorded the effect of 50 Hz sinusoidal electric currents and magnetic field on the Gram positive skin bacterium *Propionibacterium acnes*. Magnetic field generated by Helmholtz Coils for up to 30 min. changed in (Ca). Current densities greater than 800 A/m (2) were required for a reduction in pH. However, a pH gradient across the cell membrane (inside alkaline) was maintained even when exposure resulted in less than 0.2% survival thus, dissipation of the pH gradient across the cell membrane and changes in [Ca (2+)] were not a consequence of cell inactivation by 50 Hz electric currents.

This study aimed to indicate that it may be possible to use pulsed electric field (PEF) in future applications as a promising technology for destroying aflatoxins and other mycotoxins produced in stored grains in order to produce safe products and to minimize using fungicides in this field.

MATERIALS AND METHODS

I- Use of electric waves 10 MHz Amplitude modulated *in vitro*:

Mode of action

Electric charge produced electric fields around it. Also, electric currents produce magnetic fields. The direction of the magnetic field around a wire carrying an electric current is perpendicular to the direction of the current. Since the magnetic field produced by a current depends on the direction and the value of the current, the passage of an alternating current in a wire will produce an alternating magnetic field. On the other hand, an alternating current in a wire will produce alternating electric field. Both magnetic and electric fields generated by the current are perpendicular on each other and on the direction of the current. On the other hand, we can say a changing electric field will produce a magnetic field and changing magnetic field will produce an electric field we can imagine a self perpetuating disturbance of electric and magnetic fields will be produced. Action of magnetic field causes either an intensified growth of living organisms or a slow down of their growth or even death. The actual response of living organisms depends on the magnetic field strength and on the characteristic of the living organisms since time immemorial. There are two possible modes for ELF magnetic fields to interact with biological systems: induced electric fields that are produced in accord to Faraday's Law of induction and the direct effects of the magnetic field on the magnetic particles and crystals that are present in the organisms. There are a number of possible interaction mechanisms including resonance effects have been proposed. The induced current loops in biological systems resulting from exposures to time varying magnetic fields can involve electrochemical processes in the tissue that influence cellular functions. Moreover, these ELF (electric field) and current circulating into the extracellualr medium can alter ion binding to membrane macromolecules, influence iontransport across the membrane and modify legend receptor interactions at the cell membrane surface (*e.g.*

the binding of hormones, mitogens, *etc*.). These changes in membrane properties will lead to changes in the ion transport mechanism and hence affect cellular growth state. There are several resonance models that involve the action of external ELF (magnetic field) with the ionic motion and transport through biological membranes. Several studies have been conducted to detect changes in DNA synthesis and cell proliferation in cell exposed to magnetic fields. It was found elevation in DNA synthesis in the cultured cells. This increase was independent of the current density produced by magnetic field in the cell culture medium **(Ali, 1998)**.

YES medium (yeast extract sucrose) was used to produce aflatoxins B_1 and G_1 by *A. flavus*, as described by **Park and Bullerman** (**1981**). Each 25 ml YES broth medium was transferred to 100 ml Erlenmeyer flasks, autoclaved at 121°C for 15 min, inoculated with 0.5 ml spore suspension of *A. flavus* (10^6 spore/ml). The inoculated flasks with *A. flavus* were exposed as follows:

1- For 1 hr after 4 days from inoculation with *A. flavus*:

The inoculated flasks were incubated for 4 days before exposing to wave generator (GA-1230, 30 MHz synthesised ARBitarary, Japan), at Faculty of Science, Biophysics Dept., Cairo Univ, Giza, Egypt under suppervision of **Prof. Dr. Fadel M. Ali,** for 1 hr at different exposure doses as follows, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40 and 50 Hz. Then, the flasks were incubated for 10 days.

Aflatoxins were extracted from the broth with chloroform, according to **Davis *et al.* (1966).** Aflatoxin B_1 and aflatoxin G_1 levels were determined by comparing the unknown samples to quantitative standards on thin-layer-chromatography (TLC). The aflatoxins B_1 and G_1 production was compared with the control results and the reduction rates due to the use of electric waves were then calculated as follows.

Reduction%=

$$\frac{\text{Concentration of aflatoxin in control - concentration of aflatoxin in exposed flask}}{\text{Concentration of aflatoxin in control}} \times 100$$

2- Use of 50 Hz wave after 1 hr from inoculation with *A. flavus* and for different period exposed.

YES flasks (25 ml/each) are inoculated with 0.5 ml of a spore suspension of ***A. flavus,*** incubated for 1-hr and then exposed to electric waves at rate 50 Hz for different exposure time (i. e. 0.5, 1.0, 3.0, 4.0, 12 and 24 hrs) every 2 days during 14 days incubation. Aflatoxins were extracted as mentioned before. The reduction in aflatoxins B_1 and G_1 production as compared with control flasks were calculated as previously determined.

3- Different electric waves (Hz) for 2 hrs.

The same previous technique was done till inoculation with *A. flavus* spores then, incubated for 1-hr and exposed to electric waves at rates 100, 200, 300, 400 and 500 Hz for 2 hrs only.

4- Different electric waves at different exposure periods for 21 days

The same previous technique was done till inoculation with *A. flavus* spores then, incubated for 1-hr and exposed daily to electric waves at rates 600, 700 and 800, Hz for 2 hrs, 700+ 800 Hz for 4 hrs (2 then 2 hrs respectively), 600+700+ 800 Hz for 6 hrs (2, 2 then 2 hrs respectively) and 500+600+700+800 Hz for 8 hrs (2, 2, 2 then 2 hrs respectively) for 21 days continuos incubation period.

5- Electric wave 700 Hz:

In this experiment, the flasks were exposed daily for 3 days to electric wave at 700 Hz directly before inoculation with *A. flavus* spores for 4 and 6 hrs exposure time.

Then the flasks were inoculated with *A. flavus* and incubated for 21 days at 25°C. Aflatoxins were extracted as mentioned before.

II- Use of electric waves 10 MHz Amplitude modulated *in vivo*

Imported corn grain samples (grade 2) were scratched by shaking with sand for 1 min (**Eisa *et al.* 1996a**), disinfested by immersing in 5% sodium hypochlorite for 2 min, washed thoroughly with sterilized water and dried in hot-air oven at 44°C for 42 hrs **Osman (1982)**. The moisture content of grains was adjusted to 25% as described by **Eisa *et al.*, (1996a),** divided into two groups, the first inoculated with ***A. flavus*** (aflatoxin producer isolate **Abou-El-Ella, 2002**) at rate 3000-3500 spore/g grains (**Eisa *et al.* 1996a**), comparing with control grains (un-inoculated), and then exposed to electric waves (800+700+ 600+500 Hz) for 2 hrs of each wave every day for 21 days continously. The second group at same moisture content was leaved without inoculation and exposed to the same electric waves for 2 hrs of each wave every day for 21 days continously. The control treatment of this group is un-exposed grains. Aflatoxins (B_1, B_2, G_1 and G_2) were determined according to **A.O.A.C. (1990),** 100-grams of imported corn grain samples were homogenized in 200 ml methanol: water solution, (8 : 2) in blender at high speed for 3 min. The samples were filtered by using filter paper No. 1 then, cleaned using 50 ml of clean up solution (150 g zinc sulphate + 50 g phosphotungestic acid then dissolved in 1000 ml distilled water) and filtered again by using filter paper No. 4. About 75-ml of collected filtrate were put in separating funnel containing 15-ml benzene, then shaked for 5 min. The upper layer was collected in a glass beaker and evaporated till dryness under steam of nitrogen.

Samples and standard aflatoxins (B_1, B_2, G_1 and G_2), (Sigma, USA) were spotted on thin layer chromatography (TLC) plates at different concentrations: 2, 5, 7 and 10 µl, the spotted samples on TLC plates were eluted in eluting jar (contained, diethyl ether-methanol-water 96:3:1, respectively) for running. The running of samples were stopped when elution solvent reached the end line then TLC plates dried and examined under ultraviolet detector (UV) wavelength 365 nm.

Mycotoxin µg/kg samples = (S x Y x V) / (X x W)

Where: S = µl mycotoxin std. equal to unknown

Y = concentration of std. mycotoxins (aflatoxins) µg/ml

V= µl of final dilution of sample

X = µl sample extraction spotted giving flourescent intensity equal to S (mycotoxins such as aflatoxins)

W = weight of sample (100 g).

The reduction percentages were calculated as mentioned before.

III- Chemical determinations in exposed grains to electric waves 10 MHz Amplitude modulated:

a- Determination of total protein:

Protein was determined by Kjeldahl method according to **A.O.A.C (1990)**. One gram from each sample of grains was digested using a mixture of concentrated sulphuric acid and hydrogen peroxide (40%) Five grams mixture composed of potassium sulphate and selenium (in proportion of 999 grams + 1 gram, respectively) was used as a catalyst. Digestion was carried out for 45 min. Cooling the samples and added 75 ml sodium hydroxide (NaOH) solution 10 N, steam distillation and titrated by sulphuric acid (1%). The resulted volume of sulphuric acid required titration was multiplied by a factor (1.99) which gives the percentage of crude protein **AOAC (1990)**.

b- Determination of total carbohydrates:

Carbohydrate was determined using the phenol-sulphuric acid method described by **Dubois *et al.* (1956)**. Simple sugars, oligosaccharides, polysaccharides, and their derivatives, including the methyl ethers with free or potentially free reducing group, give an orange-yellow colour when treated with phenol and concentrated sulphuric acid. The reaction is, sensitive and colour is stable.

A known weight of sample (20 mg) was mixed with 10 ml of 1.0 N-sulphuric acid in a tube, sealed and placed in an oven at 100°C for 16 hours.

One ml hydrolyzed solution which taken from 100 ml hydrolyzed sample in distilled water, 1 ml of 5% phenol in water and 5 ml of concentrated sulphuric acid were pipetted into test tube. The tubes were allowed to stand for 10 min, then shaked. The resulted colour is stable for several hours. The highly absorbency value of the characteristic yellow-orange colour was measured colorimetrically at a wavelength of 490 nm. The amount of carbohydrate was determined by using a standard curve for glucose, which prepared. Fifty mg of anhydrous glucose was dissolved in distilled water, completed to 100 ml. In measured flask. A series of dilutions were carried out. The colorimetric method of the phenol sulphuric acid was followed. The absorbance was plotted against the corresponding concentration of the sugar to obtain a standard curve. The percentage of carbohydrate in a particular sample was calculated according to the following formula:

$$\% \text{ of Carbohydrates} = \frac{\text{Read out of S} \times \text{concentration of st}}{\text{wt. of S} \times \text{Read out of the st}} \times 100$$

where:

S = the sample st = the standard wt = weight (grams)

RESULTS

I- Effect of electric waves 10 MHz Amplitude modulated *in vitro* on the aflatoxins production in YES media inoculated with *A. flavus:*

1- One hr and after 4 days from inoculation with *A. flavus*:

The obtained data in **Table** (1) indicate that there was a remarkable reduction percentages in aflatoxin (B_1+G_1) production as a result for exposing *A. flavus* (aflatoxin producer isolate) to different electric waves. Where, the reduction percentage in aflatoxins (B_1&G_1) was gradually increased after exposing the fungus to doses started from 0.5-50Hz. The highest reduction percentage in aflatoxins (B_1&G_1) was recorded with 50Hz while the least reduction percentage was recorded with 0.5 and 1.0 Hz.

Table (1): Effect of electric waves 10 MHz Amplitude modulated *in vitro* on the aflatoxins production in YES media inoculated with *A. flavus*

*Exposure Dose (Hz)	Aflatoxin production μg/ml			Reduction %
	B_1	G_1	Total B_1 + G_1	
0.5	16.04	15.16	31.20	1.01
1.0	16.00	15.20	31.20	1.01
2.0	16.40	14.40	30.80	2.28
4.0	15.60	14.60	30.20	4.18
6.0	14.80	15.20	30.00	4.80
8.0	14.80	15.60	30.40	3.55
10.0	14.40	15.20	29.60	6.09

12.0	14.00	14.80	28.80	8.63
14.0	13.60	15.04	28.64	9.14
16.0	14.60	13.96	28.56	9.39
18.0	13.60	14.80	28.40	9.90
20.0	13.20	14.84	28.04	11.00
30.0	12.40	14.80	27.20	13.70
40.0	12.00	13.20	25.20	20.00
50.0	10.00	12.00	22.00	30.20
Control	15.97	15.55	31.52	

* Inoculated flasks were incubated for 4 days before exposing to electric waves for 1 hr.

2- Different exposing periods:

Results in **Table (2)** revealed that increasing exposure periods from ½ - 24 hrs increased gradually the reduction percentage in total aflatoxin (B_1+G_1) production compared with control. The highest reduction percentages were 73.8, 71.4, 70.7, 69.7, and 68.68%, after exposing the inoculated media for 24, 12, 4, 3 and 2 hrs for continuos 14 days respectively. Meanwhile, exposing inoculated media for ½ hr gave the least reduction percentage in aflatoxins production at the same conditions.

3- Effect of different electric waves (Hz):

Results in **Table**s (3) showed that exposing the inoculating medium with *A. flavus* to different electric waves started from 100-500 Hz decreased the total aflatoxin amounts (B_1&G_1) than un-exposed ones (control). Moreover, the highest reduction percentages in aflatoxins production by using different exposure doses was 37.2% at both 100 and 200Hz whereas the least reduction percentage were 5.9 and 19.9% at 400 and 500Hz respectively. It was noticed that the last doses increased to somewhat the amount of G_1

Table (2): Effect of 50 Hz electric wave (for different periods after 1 hr post inoculation YES medium with *A. flavus*) on the aflatoxin production (B_1 and G_1).

***Exposure period (hrs)**	**Aflatoxins production µµg /ml**			**Reduction %**
	B_1	G_1	Total $B_1 + G_1$	
½	7.45	5.71	13.21	56.68
1	6.25	5.00	11.25	63.10
2	4.05	5.50	9.55	68.68
3	3.00	6.25	9.25	69.70
4	2.88	6.04	8.92	70.70
12	4.80	3.92	8.72	71.40
24	3.92	4.40	8.80	73.80
Control	15.5	15.00	30.50	

*Exposure to electric waves was done by exposing the inoculating medium seven times every two days for 14 days continuos. (for different periods after 1 hr post inoculation YES medium with *A. flavus*)

Table (3): Effect of different electric wave doses (Hz) on aflatoxin production (B_1 & G_1)

Exposure dose (Hz)	**Exposure time (hr)**	**Aflatoxin production µg/ml**			**Reduction %**
		B_1	**G_1**	**Total $B_1 + G_1$**	

100	2	40.0	40	80.0	37.25
200	2	24.0	56	80.0	37.25
300	2	24.0	64	88.0	30.98
400	2	40.0	80	120.0	5.90
500	2	42.0	60	102.0	19.90
Control		52.5	75	127.5	

*Exposure to different electric waves was done by exposing the inoculating medium with *A. flavus* one time for 2 hrs only.

4- Effect of different electric waves (Hz) under daily exposure condition.

The obtained data in **Table** (4) revealed that the reduction percentages in aflatoxins production was greatly high as a result for exposing the inoculating medium with *A flavus* to daily exposure with electric waves at doses ranging from 600-800 Hz (alone or in combination) for 21days. The highest reduction percentages were 99.0, 98.0 and 95.1% at the combined electric wave doses i.e., (800+700+600+500Hz), (800+700+600Hz) and (800+700Hz) respectively. Meanwhile, aflatoxin B_1 could not be detected at the same aforementioned combined electric wave doses. On the other hand, exposing the inoculated medium with *A. flavus* for 21 days continuous to individual doses of electric waves (600, 700 and 800Hz), resulted in also high reduction percentages in aflatoxins production. Where, the reductions were 60.4, 68.3 and 72.5% respectively, but lesser than the combined doses of electric waves at the same incubation period. It is clear also that increasing exposure time from 2-8 hrs increased gradually the reduction percentages in total aflatoxin production

Table (4): Effect of electric wave (different wave Hz) on aflatoxin production (B_1 and G_1) (YES) inoculated with *A. flavus* exposed after 1 hr post inoculation (different period) and incubated for 21 days at 25°C.

Exposure dose (Hz)	Exposure time (hrs)	Aflatoxin production µg/ml			Reduction %
		B_1	G_1	Total $B_1 + G_1$	
600	2	30.0	20.4	50.4	60.4
700	2	20.0	20.4	40.4	68.4
800	2	10.0	25.0	35.0	72.5
800+700	2+2	00.0	6.24	6.24	95.1
800+700+600	2+2+2	00.0	2.50	2.50	98.0
800+700+600+500	2+2+2+2	00.0	1.26	1.26	99.0
Control		52.0	75.0	127.5	

5- Effect of electric wave (700 Hz) on aflatoxins production (B_1 and G_1) in YES medium before inoculation with *A. flavus*:

Data in **Table** (5) showed that exposing YES medium to electric wave dose (700Hz) 6 hrs for 3 successive days before inoculation with A. flavus (aflatoxin

producer isolate), led to whole inhibition of aflatoxin B_1 and reduced the produced amount of aflatoxin G_1 to lower level comparing with control (un-exposed). Meanwhile, the reduction percentage in total aflatoxins (B_1 + G_1) was 94.1% as a result for the previous treatment. Therefore, exposing the same medium for 700Hz. 4 hrs for 3 successive days resulted in 84.1% reduction percentage in aflatoxin (B_1&G_1) production and reduced also the produced total amount of aflatoxins (B_1&G_1) to low level comparing with the control treatment. Generally, exposing medium to 700Hz, 6 hrs was better than 4 hrs for 3 successive days.

Table (5): Effect of electric wave (700Hz) on aflatoxin production (B_1&G_1) in YES medium before inoculation with *A. flavus*.

*Exposure time (days)	Aflatoxin production µg /ml			Reduction %
	B_1	G_1	Total B_1 + G_1	
4 hrs/3 days	4.28	16.0	20.28	84.1
6 hrs/3 days	Zero	7.5	7.50	94.1
Control**	52.50	75.0	127.5	

*YES medium was exposed to 700Hz for 3 successive days, then inoculated with *A. flavus* and incubated for 21 days at 25ºC.
** Control = un-exposed medium

II- Effect of exposing inoculated and non-inoculated corn grains with *A. flavus* to electric wave 10 MHz Amplitude modulated on aflatoxin production:

Results in **Table (6)** showed that the exposing the inoculating corn grains with *A. flavus* (producer isolate for aflatoxins) to different electric waves (800 + 700 + 600+500 Hz) for 8 hrs continuously for 21 days gave % reduction in aflatoxins (B_1, B_2, G_1 and G_2) production reached 12.36% compared with control (without exposure). While, exposing natural infected corn grains to electric wave (800 + 700 + 600 + 500 Hz) decreased aflatoxin production (B_1, B_2, G_1 and G_2) to about 21.16% reduction comparing with control.

Table (6): Effect of exposing inoculated and non-inoculated corn grains with *A. flavus* at 25% moisture to electric wave 10 MHz Amplitude modulated on aflatoxins production

Exposure dose (Hz)	Aflatoxin production mg/100 g grains				Total	Reduction (%)
	B_1	B_2	G_1	G_2		
Inoculated with *A. flavus*						
800 + 700 + 600 and 500	92.46	109.58	171.20	136.98	510.22	12.36
Control	102.74	130.14	188.86	160.96	582.20	
Without inoculation						
800 + 700 + 600 and 500	27.4	60.19	126.90	125.69	340.18	21.16
Control	27.4	82.19	171.23	150.68	431.50	

III- Effect of electric waves 10 MHz amplitude modulated on total protein and carbohydrate percentage in yellow corn grains (inoculated with *A. flavus*).

Data in **Table (7)** showed that exposing yellow corn grains that inoculated with *A. flavus* to electric waves 800 + 700 + 600 + 500 Hz for 2 hrs of each wave every day for 21 days continuously, did not do any changes in protein and carbohydrate contents of treated corn grains comparing with control (un-exposure). While, results showed that exposing natural infected yellow corn grains (25%

moisture content) to electric waves 800 + 700 + 600 + 500 Hz for 2 hrs of each wave every day for 21 days continuously, did not change the protein and total carbohydrate contents of treated corn grains comparing with control.

Table (7) Effect of electric waves 10 MHz amplitude modulated on total protein and carbohydrate percentages in inoculated and non-inoculated yellow corn grains with *A. flavus*.

Exposure dose (Hz)	Crude protein (%)	Carbohydrate (%)
Inoculated with *A. flavus*		
800 + 700 + 600 + 500	8.4	72.9
Control (1)	8.9	73.6
Without inoculation		
800 + 700 + 600 + 500	8.3	73.0
Control (1)	8.6	73.0
*Control (2)	8.3	70.2

* = Analyzed corn grains at zero time just before starting the experiment.

DISCUSSION

Corn (*Zea mays* L.) is one of the most important grain crops all over the world. A wide variety of microorganisms are present on and in grain kernels. The kinds and abundance of these microorganisms depend on factors such as the climate under which the grains are produced, the conditions of storage, and the portion of the grains of which the products are composed. The most common species of storage fungi are mostly species of *Aspergillus* and *Penicillium* **(Abo El-Ella, 2002)**.

In contrast, exposure the inoculated semi-synthetic medium (YES) with *A. flavus* to electric waves (for 1 hr and after 4 days from inoculation) resulted in a remarkable reduction percentages in aflatoxin (B_1+G_1). The reduction percentage in aflatoxins (B_1&G_1) was gradually increased after exposing the fungus to doses started from 0.5-50Hz. The superior reduction percentage in aflatoxins (B_1&G_1) was recorded with 50Hz while the least reduction percentage was recorded with 0.5 and 1.0 Hz. On the other hand, exposing for different periods after 1 hr post inoculation with *A. flavus* using 50Hz, results revealed that increasing exposure periods from ½-4 hrs increased gradually the reduction percentage in total aflatoxin (B_1+G_1) production compared with control. Meanwhile, exposing inoculated media for ½ hr gave the least reduction percentage in aflatoxins production at the same conditions. While, exposing the inoculating medium with *A. flavus* to different electric waves started from 100-500 Hz decreased aflatoxins production amounts especially the total aflatoxin amounts (B_1&G_1) in comparing with the un-exposed ones (control). Moreover, the highest reduction percentages in aflatoxins production by using different exposure doses was at both 100 and 200Hz whereas, the least reduction percentage was at 400 and 500Hz especially G_1. Un-published data of **Ali (1998)** could be used for interpreting our findings in this respect, where he mentioned that matching between frequency of electric field currents and frequency of ionic metabolism of cell activity of microorganisms cause an enhancement (increase) in its activity which reflected in form of increasing the toxin production. In the same time, it will happen antagonism between the frequency of electric field currents and frequency of ionic metabolism in active cells of microorganisms, which reflect by causing inhibition in activity for microorganisms as the production of toxins, decreased.

The obtained data revealed that the reduction percentages in aflatoxins production were greatly high as a result for exposing the inoculating medium with *A*

flavus to daily exposure with electric waves at doses ranged between 600-800 Hz (alone or in combination) for 21days. The highest reduction percentages were at the combined electric wave doses i.e., (800+700+600+500Hz), (800+700+600Hz) and (800+700Hz) respectively. Meanwhile, aflatoxin B_1 could not be detected at the same aforementioned combined electric wave doses. On the other hand, exposing the inoculated medium with *A. flavus* for 21 days continuous to individual doses of electric waves (600, 700 and 800Hz), resulted in also high reduction percentages in aflatoxins production but lesser than the combined doses of electric waves at the same incubation period. It is clear also that increasing exposure time from 2-8 hrs increased gradually the reduction percentages in total aflatoxins production. Data showed also that exposing YES medium to electric wave dose, 700Hz/6 hrs/3 days continuos before inoculation with *A. flavus* (aflatoxin producer isolate), led to whole inhibition of aflatoxin B_1 and reduced the produced amount of aflatoxin G_1 to lower level comparing with control (un-exposed). Therefore, exposing same medium for 700 Hz/4 hrs/3 successive days realized also a good reduction percentage in aflatoxin (B_1&G_1) production and reduced also the produced total amount of aflatoxins (B_1&G_1) to low level comparing with control treatment. Generally, exposing medium to 700Hz, 6 hrs was better than 4 hrs for 3 successive days. **Wouters *et al.* (1999)** indicated that it might be possible to use pulsed electric field (PEF) in future applications in order to produce safe products. As for, using electric waves at different doses affected growth and aflatoxins production of *A. flavus.* This finding may be due to the bio-physiological and chemo-physiological changes, genetic variations in the fungus under stress or that these electric waves reduce the viability of cells. Results showed also that exposing the inoculating corn grains with *A. flavus* (producer isolate for aflatoxins) to different electric waves (800 + 700 + 600+500 Hz) for 8 hrs continuously for 21 days gave % reduction in aflatoxins (B_1, B_2, G_1 and G_2) production reached 12.36% compared with control (without exposure). Meanwhile, exposing natural infected corn grains to the same treatment decreased aflatoxin production (B_1, B_2, G_1 and G_2) to about 21.16% reduction comparing with control. On the other hand, exposing yellow corn grains that inoculated with *A. flavus* to electric waves 800 + 700 + 600 + 500 Hz for 2 hrs of each wave every day for 21 days continuously, did not do any changes in protein and carbohydrate contents of treated corn grains comparing with control (un-exposure). While, exposing natural infected yellow corn grains (25% moisture content) did not change also, the protein and total carbohydrate contents of treated corn grains comparing with control. In this respect, **Grahi and Mark (1996)** reported that living-cell counts of vegetative cell types of bacteria, yeast and spores in beffere solutions and liquid foodstuffs were reduced by PEF treatment. As well as, the killing of vegetative cell types depends on the electrical field strength of the pulses and time. On the other hand, **Reina *et al.* (1998)** indicated that PEF resulted in greater reduction of viable cells and the use of a high-voltage PEF is a promising technology for inactivation of food-borne pathogens. Also, the studies of **Calderon *et al.* (1999), Evrendilek *et al.* (1999), Jeantet *et al.* (1999)** and **Ramsted *et al.* (2000)** verified our results and agreed together that the high voltage of PEF is a promising technology for inactivation the pathogenic microorganisms. Also, Prof. Dr. Ali, F. explained that in light of we have here two possibilities: The first is an effect of electric and magnetic fields on the media components YES itself, so there are a great reduction ranged between 84.1 and 94.1%, this reduction may be due to the effect of electric and magnetic field on Ca and Na iones exchange of yeast metabolites. The second is electric and magnetic

fields affect the component of medium and this may be make the medium non-un-suitable to fungus so it makes him un-active thus, no toxin production.

Finally, we can conclude that the dose of exposure and the time of exposure are the main effective factors.

In general, all the discussed data give us uncompleted imagination view to what is happened?. This make us to think deeply to draw a future plan for further work concentrated in two directions: (1) On the effect of electric field on the fungus. (2) On the effect of electric field on the media.

REFERENCES

Abou-El-Ella, M.F. (2002): Studies on maize grains deterioration under Egyptian conditions. Ph.D Thesis, Fac. Agric., Moshtohor, Benha Branch, Zagazig Univ. Egypt, 139 pp.

Abramson, D. and R.M. Clear (1996): A convenient method for assessing mycotoxin production in cultures of *Aspergilli* and *Penicillia.* J. of Food Protection, **59**: 642-644.

Ali, F. M. (1998): Principles of Biophysics. 2nd Edit., Special Print for Biophysics Dept., Fac. Sci., Cairo University.

A.O.A.C (1990): Official methods of Official analysis, 15th ed, Kenneth Helrich edit, published by the Association of Official Analytical Chemists, Inc., Virginia, USA.

Calderon, M.; G.V. Barbosa-Canovas and B.G. Swanson (1999): Inactivation of *Listeria innocua* in skin milk by pulsed electric field and nisin. Int. J. Food Mictobiol., **51**: 31-38.

Davis, N.D.; U.L. Diener and D.W. Eldridge, (1966): Production of aflatoxins B_1 and G_1 by *Aspergillus flavus* in a semi-synthetic medium. Applied Microbiology, **14**: 379-381.

Diener, U.L. and N.D. Davis, (1986): Biology of *Aspergillus flavus* and *A. parasiticus.* Aflatoxin in maize. A proceeding of workshop, Mexico, April, 7-11, page 33-44.

Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Repers, B.A. and Smith, F. (1956): Colorimetric method for determination of sugar related substances. Analytical Chemistry, **28**:350-356.

Dunn, J. (1996): Pulsed light and pulsed electric field for food eggs. Antimicrob Agents Chemother., **40**: 2012.

Eisa (Nawal), A.; S.K. Abdel-Reheem; A.E. Badr and M.F. Abol-Ela, (1996a): Pathological studies on deterioration of yellow corn during storage and its control. I-Associated fungi, percentage of infection and its control. Al-Azhar, J. Agric. Res., **24**: 65-81.

Evrendilek, G.A.; Q.H. Zhang and E.R. Richter (1999): Inactivation of *Escherichia coli* in apple juice by pulsed electric fields. J. Clin. Microbiol., **37**: 2858-2862.

Grahi, I.T. and I.H. Mark (1996): Killing of microorganisms by pulsed electric fields. Biochemistry, **35**: 3328-3359.

Henry, M.S.; F.R. Paul and O. Wilford, (1981): Some mycotoxin levels in farm-stored corn. J. Agric. Food Chem., **29**: 207.

Hesseltine, C.W. (1965): A millennium of fungi food, and fermentation. Mycologia, **57**: 149-197.

Jeantet, R.; F. Baron; F. Nan; M. Roignant and G. Brule (1999): High intensity pulsed electric field applied to egg white: Effect on *Salmonella enteritidis* inactivation and protein denaturation. FEBS, Lett, **462**: 447-452.

Jeyamkondan, S.; D.S. Jayas and Holley (1999): Pulsed electric field processing of foods. Infect control Hosp. Epidemiol., **20**: 598-603.

Kekez, M.M.; P. Savic and B.F. Johnson (1996): Contribution to the biophysics of the lethal effects of electric field on microorganisms. J. Bacteriol., **178**: 1113-1119.

Osman, A.R. (1982): Studies on fungi associated on the sorghum grains during storage. Ph. D. Thesis, Fac. of Agric., Cairo Univ.

Park, K.Y. and R. Bullerman, (1981): Increased aflatoxin production by *Aspergillus parasiticus* under conditions of cycling temperature. J. of Food Science, **46**: 1147-1151.

Ramsted, S.; C.M. Futsaether and A. Johansson (2000): Effects of 50 Hz electric currents and magnetic fields on the prokaryote *Propionibacterium acnes*. Int. J. Syst. Evol. Microbiol., **50**: 417-427.

Reina, L.D.; Z.T. Jin; Q.H. Zhang and A.E. Yousef (1998): Inactivation of *Listeria monocytogenes* in milk by pulsed electric field. Res. Microbiol., **149**: 361-372.

Saubios, A.; T. Tanaka; M.C. Nepote; M.L. Wagner and Y. Ueno (1998): Aflatoxins, Type B trichothecenes and toxigenic fungi in Indian corn from a region of Argentina. Microbiologia, Aliments Nutrition, **16**: 61-69.

Sauer, B.D. and J. Tuite, (1986): Condition that affect growth of *Aspergillus flavus* and production of aflatoxin in stored maize. Aflatoxin in maize. A proceedings of Workshop, El-Batan, DF. Mexico, April, 7-11: pp.41-50.

Shotwell, O.L.; M.L. Gouldern; C.W. Hesseltine; J.W. Dickens and W.F. Kwolek, (1980): Cereal Chem., **57**: 206-208.

Thompson, C. and S.E. Henke (2000): Effect of climate and type of storage container on aflatoxin production in corn and associated risks to wildlife species. J. of Wildlife Diseases, **36**: 172179.

Wouters, P.C.; N. Dutrux; J.P. Smelt and H.L. Lelieveld (1999): Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. J. Food Prot., **62**: 1381-1386.

Zohri, A.A.; M.A. Abdel-Sater and M.A. Ismail (1995): Incidence of aflatoxins and mould flora in corn snacks. J. of Food Sci. and Technology, Mysore, **32**: 289-294.