Effect of Magnetic Field Energy on Growth of *Aspergillus flavus* and Aflatoxins production

Aiman M. Ahmad, Abdul Ghani I. Yahya and Abdul Wahid Sh. Jabir
Department of Biotechnology, College of Science, Al-Nahrain University.
E-mail: Ayman881117@yahoo.com.

Abstract

The study was conducted to determine the impact of the magnetic field poles on aflatoxins produced by *Aspergillus flavus*. The subjected fungus to the northern pole, southern pole, both poles and their influences were compared with the control at which the fungus was not affected by magnetic field energy. *Aspergillus flavus* was influenced by magnetic field energy which applied through a magnet at different forces (5, 7,10,30,50 Gauss) for seven days at temperature of 27 ^{o}C. There are no different between the uses of magnate with 5 or 50 Gauss. The effect of magnetic field poles was observed on the growth of *Aspergillus flavus* on solid and liquid media. The southern pole had a positive effect on the growth of *Aspergillus flavus* by increasing the diameter of the colony or the turbidity of growth medium, while the northern pole had a negative influence on the growth of *A. flavus*, diameter or the turbidity, while the treatment of northern and southern poles together and the control treatment are equal. The most important conclusion that have been observed was the effect of magnetic field poles on the concentration of total aflatoxin produced by *A. flavus*, which was 454.73 ppb (when treated with southern pole) and 25.40 ppb (when treated with northern pole) while the control 212.46 ppb and both poles 88.33 ppb by using ELISA technique.

Keywords**:** Aflatoxin, Magnetic field, *Aspergillus flavus* , Gauss , ELISA.

Introduction

Magnetic energy is the energy of fundamental nature and it controls the spin of electrons around the nucleus of atoms and cells. Recent years have seen a growing interest in studying the impact of the magnetic field on living organisms, especially human and animal, but few studies have addressed toward the effect of magnetic field on plants and microorganisms, as well as the effect of magnetic field energy on the field of medicine, microbiology and biotechnology [1].

In addition, few studies have focused on the effect of magnetic field on growth and metabolisms of microorganisms have been published. The impact of the magnetic field energy lies in the stimulus to the events of significant changes in the characteristics of metabolic organisms, these are changes in the exchange of ions through the cell membrane in the movement of cells [2].

Aspergillus flavus is the most important species among over 185 known species within the genus *Aspergillus*. It is not the most abundant and widely distributed soil-born molds that can be found anywhere on earth but also produce aflatoxins among the carcinogenic natural products ever discover.

A. flavus produces (AFB_1, AFB_2, AFG_1, and AFG_2). These four major aflatoxins are named based on their blue (B) or green (G) fluorescence under Ultraviolet (UV) light. Aflatoxine M_1 is Hydroxylated derivative metabolized from aflatoxine B_1 by cows and screated in milk. Aflatoxin B_1 is the most frequent of these compounds present in contaminated food samples and aflatoxins B_2, G_1 and G_2 are generally not reported in the absence of aflatoxin B_1[3].

Bioelectromagnetics is the study of the interaction between electro-magnetic fields and biological entities. Common areas of investigation include animal navigation utilizing the geomagnetic field, potential effects of manufactured sources of electromagnetic fields. The term is similar to bioelectromagnetism, which deals with the ability of living cells, tissues, and organisms to produce electrical fields and the response of cells to electro-magnetic fields [4-5].

The use of magnetic fields poles as new detoxification method did not cause heating of tissues or damage to the nutritive value of the

crops, but only inhibit organisms living cells or their secondary metabolites.

Material and Methods

Isolation and characterization of organism

Aspergillus flavus was isolated from clay soil by serial dilution method. The soil suspension was further diluted for seven serial dilutions. About 0.1 ml from the 5^{th}-10^{th} dilution was spread on potato dextrose agar (PDA) plates using a glass spreader, sterilized by dipping in 95% ethanol and flaming. (PDA) was prepared as it's instructed by the company and autoclaved at temperature of 121°C and the pressure of 1.08kg/cm^2 for 15 minutes, and after the sterilization the culture plats cooled to 45 °C and then chloramphenicol was added to prevent growth of bacteria. The plates were incubated at 27°C for 7 days. The growth of fungal colonies were observed after incubation. The individual colonies were then sub cultured. Identification was based on cell and colony morphology characteristics (morpho-logical and microscopic features).

Rose-Bengal Chloramphenicol Agar was used to confirms Alatoxine production by the isolated *Aspergillus flavus* .

Effect of magnetic poles on the growth of *Aspergillus flavus* on solid media

Aspergillus flavus was influenced by magnetic field energy which applied through a magnet with different forces (5, 7,10,30,50 Gauss).

This application was influenced by three treatments which are:

1. Influence of northern pole.
2. Influence of southern pole.
3. Influence of both northern and southern poles.

And these influences were compared with the control which is not affected by magnetic field energy. All the PDA and SDA dishes were incubated at (27 °C) for 7 days, after the end of the period the diameter of the colonies were measured in millimeter (mm) for each treatments separately and compared with the control treatment ,As shown in the Fig.(1).

Effect of magnetic poles on the growth of *Aspergillus flavus* in Liquid media, dry weight and Aflatoxin production

Inoculated flasks containing yeast extract broth were subjected by magnetic field energy of poles that applied through a magnet with different forces (5, 7, 10, 30, 50 Gauss); this application was effected by three treatments on the flasks containing yeast extract broth media which be:

1. Influence of northern pole.
2. Influence of southern pole.
3. Influence of both northern and southern poles.

Moreover, these treatments were compared with the control treatment in which there is no effect of magnetic field energy and at three replicates for each treatment and then incubated at a temperature (27 °C) for 7 days. As shown in the Fig.(2).

The effect of magnetic field poles on the growth of *Aspergillus flavus* in liquid media

Based on continuous monitoring of changes in the optical density of fungal growth after 48 hrs. the growth of *Aspergillus flavus* in liquid media was effected by three treatments. The OD_{405} of each growth was read spectrophotometrically and calculated with the following equation:

$$\text{Percentage of growth} = \frac{OD_{405}\ of\ treatment\ 1, 2\ or\ 3 \times 100}{OD_{405}\ of\ the\ control}$$

The effect of magnetic poles on the dry weight of *Aspergillus flavus*

After incubation for 7 days the cultures were filtered by Whitman filter paper No.4 each filter paper were dried until constant weight then the dry weight of mycelium was determined after drying the mycelia for 12 to 24 hrs at 70 °C.

The effect of magnetic poles on the Aflatoxin production by *Aspergillus flavus* in broth culture

The filtrate which resulted from the filtration process of yeast extract broth culture was applied to aflatoxin kit procedure to detect toxin concentration

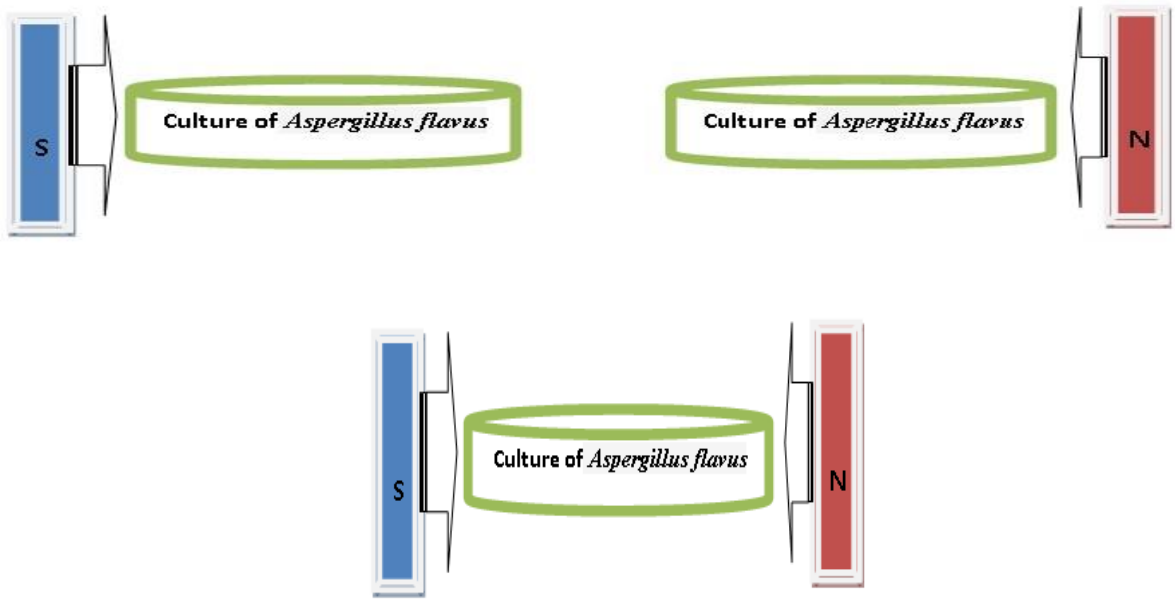

Fig.(1) Show the position of magnetic bar at different treatments (N and S for northern and southern pole) when affected on the growth of Aspergillus flavus on solid media.

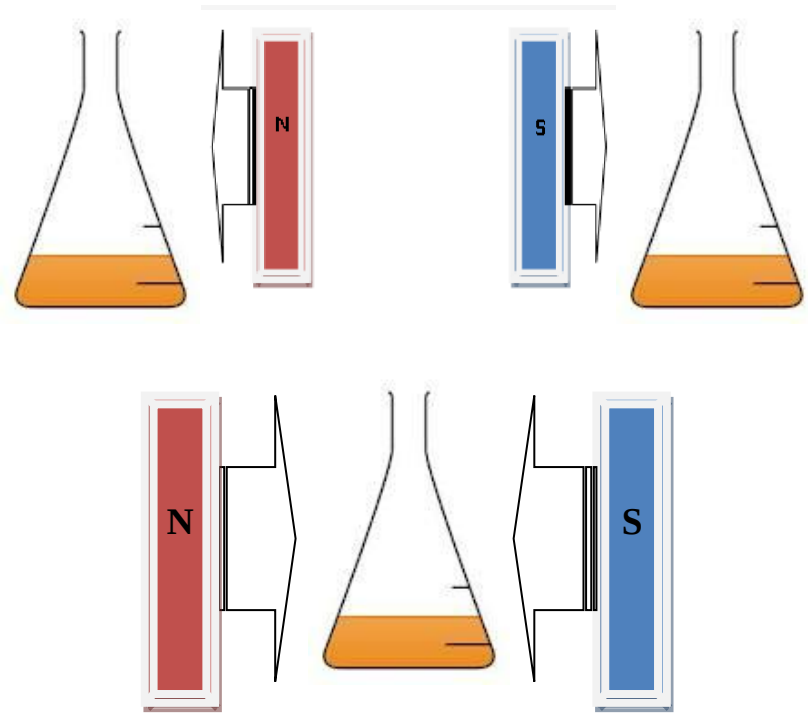

Fig.(2) Show the position of magnetic bar at different treatments (N and S for Northern and Southern pole) when affected on the growth of Aspergillus flavus on liquid medium.

.

Results and Discussion

Identification of Fungal isolates

Aspergillus flavus isolate was identified macroscopically on the solid media PDA and SDA as yellow to dark yellowish-green colonies, consisting of a dense felt of conidiophores. While microscopically was confirmed as it found in [6].

Detection the effect of magnetic field energy on the growth of *Aspergillus flavus* on solid media

The results showed that southern pole, which carries the positive charges had a positive effect on the growth of the fungus under study by increasing the diameter of the colony developing on solid media and the number of spores formed, while for the northern pole, which holds negative charges had a negative influence on the growth of *Aspergillus flavus*. The colony diameter developed after the southern pole was 73-80 millimeter, however colony developed at northern pole had reach 35-40 millimeter, while the treatment of northern and southern poles together and the control treatment showed diameter 50-60 millimeter (N.B). These results were the average for three replicates for each treatment) as shown in Table (1). There are many scientific reports on the influence of magnetic or electromagnetic fields on living organisms. Many physiological responses to electromagnetic field pulses have been studied, but not much of this work has addressed growth and morphogenesis [7].

Table (1)
Effect of magnetic field energy on the growth of Aspergillus flavus on solid media.

Effect of magnetic poles	*Repeats (cm)*			*Mean (cm)*	*Stander error*	*LSD (≤ 0.05)*
	A	*B*	*C*			
Control	5.2	5	5.3	5.2	0.88	0.77
Northern	3	3	3.2	3.1	0.66*	0.00
Southern	6.8	7.3	7	7	0.12*	0.00
Both	5.4	5.3	5.6	5.4	0.88	0.77

the mean difference is significant at the 0.05 level.

The results suggest that the effect of the magnetic field may have an important environmental factor affecting of the growth as well as the morphology of the examined fungus [8] .

It has been demonstrated experimentally [9] that the application of a low-frequency, weak magnetic field, both static and time-varying, induces considerable changes in the metabolism characteristic of tested organisms. These changes are manifested primarily in altered ion flow through cell membranes and in the motion of cells. Such results indicate the role of resonance. Swedish researchers found that a magnetic field of extremely low frequency affected the fluctuation of cellular calcium content [10].

Detection the effect of magnetic field energy on the growth of *Aspergillus flavus* in liquid media

The Optical Density at 405 $_{nm}$ (OD_{405}) of each Influence after 48 h for *A. flavus* was measured spectrophotometrically. The percentage of growth at each magnetic pole treatment. The growth is increased by the influence of southern pole because the turbidity and so the absorbance of the spectro-photometer while its decreased through the effect of northern pole on the other hand the control (which had no effects) and both poles are the same in optical density.

Table (2)
The effect of magnetic field energy on the growth of Aspergillus flavus in liquid medium (percentage of growth).

Effect of magnetic poles	*Repeats%*			*Mean %*	*Stander error*	*LSD (≤ 0.05)*
	A	*B*	*C*			
Control	0.73	0.69	0.75	0.72	0.1520	0.5
Northern	0.49	0.46	0.52	0.4.9	0.0173*	0.02
Southern	1.1	1.2	1.6	1.3	0.0177*	0. 04
Both	0.86	0.77	0.83	0.8.1	0.0352*	0.03

the mean difference is significant at the 0.05 level.

The main theories that try to discuss the biological effects of Magnetic fields are based on the possible effects on the permeability of the ionic channels in the membrane; this can affect ion transport into the cells and result in biological changes in the organism [10-11].

The membrane phospholipids are large molecules. One end consists of hydrophobic (water hating) hydrocarbon chains. The other end has a negatively charged phosphate group and is hydrophilic (water loving). In a watery medium, they arrange themselves spontaneously to form double-layered membranes with a central core made from their water hating ends. Their water loving phosphate ends face outwards towards the water [12].

The affinity that the central hydrophobic parts have for one another helps hold the membrane together but the negatively charged phosphate groups on the outside repel each other and try to tear it apart. Normally, the membrane is stabilized by positive ions that fit in between the negative phosphate groups, so that they do not repel each other. However, not all positive ions stabilize the membrane equally well. Calcium ions are particularly good because of their double positive charge, but monovalent potassium, with just one charge, is only ordinary [10-13].

Therefore, when northern pole of magnetic fields is applied it will swap membrane‑bound calcium for potassium, it weakens the membrane (These membranes are only a hundred thousandth of a millimeter thick) and it becomes more prone to accidental tearing and the formation of transient pores [13]. Fortunately, these pores are usually self‑healing and the dam-age to the membrane is not permanent. How-ever, during electro-magnetic exposure there will be more tears, slower repair and consequently more overall leakage. In contrast the effect of southern pole of magnetic field is increase the backup of calcium ions in the cell membrane and enhances the cell metabolism. According to the results the influence of magnetic field on the growth in liquid media is very similar to the growth on solid media [13-14-15].

Detection the effect of magmatic field energy on the dry weight of *Aspergillus flavus* for seven days.

According to the recorded results, as for the biomass has reached a dry weight of biomass for the treatment of the Southern (4.1 g) and (0.23 g) of the Northern, (0.81g and 0.8 g) for each treatment of the poles together and the control treatment, respectively. As shown in Table (3).

These results came in the same rate of those in the effect of magnetic field energy on the growth on solid media, so when we try to explain the decrease and increase in biomass, it will regarded to the effect of MF on the calcium signal transduction [9-15].

Table (3)
The effect of magnetic field energy on the dry weight of Aspergillus flavus for seven days.

Effect of magnetic poles	*Repeats (gm)*			*Mean (gm)*	*Stander error*	*LSD (≤ 0.05)*
	A	*B*	*C*			
Control	0.82	0.77	0.85	0.81	0.024*	0.01
Northern	0.18	0.21	0.26	0.21	0.023*	0.01
Southern	4.7	3.8	4	4.1	0.272	0.947
Both	0.86	0.8	0.75	0.8	0.031*	0.02

the mean difference is significant at the 0.05 level.

Detection the effect of Magmatic poles energy on the total aflatoxine concentration produced by *Aspergillus flavus*

Aflatoxins analyses of 12 samples were analyzed by using ELISA technique. Analyses were repeated two times. The influence of magnetic field energy on the aflatoxine production was observed by the concentration of total aflatoxins in part per billion (ppb) which produced by *Aspergillus flavus* in yeast extracts broth for 7days which approximately 168 hours.

In ELISA analyses, there was evidence about the total aflatoxins. Despite there was no encounter of any of the aflatoxin B_1, ELISA analysis results of total aflatoxin were given in Tables (4). According to *Product Specifi-cations* Lower limit of detection: 0.5 ppb- Range of quantization: 1 ppb–8 ppb -Controls provided: 0, 1, 2, 4 and 8 ppb - Antibody cross-reactivity: Total aflatoxins (B_1, B_2, G_1, G_2).

The data shows that concentration of total Aflatoxine production by *A. flavus* react to the magnetic field, Cultures exposed to a magnetic field exhibited changes in aflatoxine production compared to unexposed cells, which was 454.73 ppb (when treated with southern pole) and 25.40 ppb (when treated with Northern pole) while the control 212.46 ppb and both poles 88.33 ppb.

The main theories that explain the biological effects of MFs are based on the possible effects on the permeability of the ionic channels in the membrane [16]. This can affect ion transport into the cells and result in biological changes in the organism. The concentration of calcium ions in the cytosol (the main part of the cell) is normally kept about a thousand times lower than that outside by metabolically driven ion pumps in its membranes. Many metabolic processes are then regulated by letting small amounts of calcium into the cytosol when needed.

Other possible effects are the activity of correlative enzymes entering in the biosynthesis of aflatoxine by the formation of free radicals due to magnetic field exposure. Free radicals are atoms or groups of atoms containing at least one unpaired electron in their orbitals. Once formed these highly reactive radicals can start a chain of reaction, like dominoes. Their main danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs [17]. Most common free radicals are reactive oxygen (ROS) and Reactive nitrogen (RNS) species. The spin of the odd electron of a free radical, when placed in a magnetic field, may have two, and only two, orientations, one with and the other against the field [18].

As a result, these radicals might effect on the biosynthesis of Aflatoxine by one of the two stages from malonyl CoA, first with the formation of hexanoyl CoA, followed by formation of a decaketide anthraquinone.

A series of highly organized oxidation-reduction reactions then allows formation of aflatoxin several specific enzyme activities associated with precursor conve-rsions in the aflatoxin pathway have been partially, whereas others such as methyltransferases.

Also free radicals could effect on the enzymes which are involved in aflatoxin

biosynthesis such as a reductase and a cyclase because they attack sites of increased electron density like the nitrogen atom present in proteins and carbon-carbon double bonds present in polyunsaturated fatty acids and phospholipids [19].

Table (4)
The influence of magnetic field energy on the Aflatoxine concentration produced by A. flavus after 168 hrs.

Effect of magnetic poles	*Repeats (ppb)*			*Mean (ppb)*	*Stander error*	*LSD (≤ 0.05)*
	A	*B*	*C*			
Control	189.1	214.1	234.2	212.46	13.04*	0.04
Northern	20.4	11.7	44.1	25.400	9.68*	0.00
Southern	485.5	472.5	406.2	454.73	24.55*	0.00
Both	66.6	44.8	153.6	88.33	33.23*	0.04

****the mean difference is significant at the 0.05 level.***

Conclusions

The Southern pole of magnetic field which have the positive charge while induced the calcium signal transduction and such signaling will induce and accelerate the growth of *A. flavus*, In contracts when influenced by northern pole (which had the negative charge) it will inhibits the calcium signal transduction and so causing inhibition to the growth. There is undeniable experimental proof that magnetic field can remove bound calcium ions from cell membranes. There is also no doubt that bound calcium ions are essential for the stability of these membranes. Consequently, their loss will increase temporary pore formation under the mechanical stresses from pressure differences within the cell and abrasion by its moving contents. This very simple conclusion can account for virtually all of the known biological effects of electromagnetic fields, including changes in metabolism and genetic damage.

The quality of the effect is the same but the quantity of the effect is dependent on the chemical structure of metabolites.

Recommendations

Studying the effect of magnetic fields poles on the production of Aflatoxin B1 produced by *Aspergillus flavus*. Studying the effect of magnetic fields poles on the DNA of *Aspergillus flavus* genes.

References

[1] Wilson, B.W., and Stevens, R.G., Extremely low frequency electromagnetic fields: the question of cancer, Battelle Press, Columbus, Ohio, 1990.

[2] Dobson, S.T.; Pierre, T.J.; Weiser, H.G. and fuller, M.R., changes in paroxysmal brainwave patterns of epileptics by weak field magnetic stimulation, Bioelctromagnetics J., 21 : 423 – 430, 2000.

[3] Goldbatt, L.A., Aflatoxin, Academic Press, New York. Pp1-40, 1969.

[4] Garcia R.F. and Arza Pascual L.D., influence of stationary magnetic field on water relations in lettuce seeds, Bioelctromagnetics J.,22: 589 – 595, 2001.

[5] Gao, M.; Zhang, J. and Feng, H., Extremely low frequency magnetic field effects on metabolite of Aspergillus Niger, Bioelctromagnetics J., 32 : 73 – 78, 2011.

[6] Atlas, R.M.; Parks, L.C. and Brown, A.E., Laboratory manual of experimental microbiology. 1st ed. Mosby-Year Book Inc. U.S.A, 1995.

[7] Andrew Goldsworthy. The Biological Effects of Weak Electromagnetic Fields. Mosby-Year Book. Inc. U.S.A., 2007.

[8] Crombie, C.N.; Gow, A.R. and Gooday, G.W., Influence of applied electrical field on yeast and hyphal growth of candida albicans. Microbio. Gene J., 136:311 – 317, 1990.

nitrosamines on the induction of mycotoxins synthesis by nontoxigenic moulds isolated from feed samples, Bioelctromagnetics J., 2 : 118-121, 2008.

الخلاصة

أجريت هذه الدراسة لتحديد تأثير أقطاب المجال المغناطيسي على الأفلاتوكسين التي ينتجها *Aspergillus flavus*. تعرض الفطر إلى القطب الشمالي، القطب الجنوبي، كلا القطبين وقورنت تأثيراتهم مع معاملة السيطرة التي لا يتأثر الفطر بطاقة المجال المغناطيسي. تأثر *Aspergillus flavus* بطاقة المجال المغناطيسي التي تطبق من خلال مغانط مختلف القوى (٥،٧ ،١٠،٣٠، ٥٠غاوس) لمدة سبعة أيام في درجة حرارة ٢٧ درجة مئوية. ولوحظ تأثير المجال المغناطيسي أقطاب على نمو *Aspergillus flavus* على الوسائط الصلبة وفي السائلة. كان القطب الجنوبي له تأثير إيجابي على نمو *Aspergillus flavus* عن طريق زيادة القطر أو التعكر، في حين القطب الشمالي كان له تأثير سلبي على نمو *A. flavus* في العكارة أو القطر، في حين معاملة القطبين الشمالي والجنوبي معا ومعاملة السيطرة كانت متساوية . اهم استنتاج التي لوحظت كان تأثير اقطاب المجال المغناطيسي على التركيز الكلي للافلاتوكسين التي تنتجه *A. flavus*، التي كانت ٤٥٤،٧٣ جزء في البليون (عند تعامله مع القطب الجنوبي) و ٢٥،٤٠جزء في البليون (عند تعامله مع القطب الشمالي) في حين أن السيطرة ٢١٢,٤٦ جزء في البليون وكلا القطبين ٨٨،٣٣ جزء في البليون باستخدام تقنيةELISA .

[9] Blank M and Goodman R, Stimulation of stress response by low frequency electromagnetic fields: possibility of direct interaction with DNA, Bioelctromagnetics J., 28: 168 - 172, 2000.

[10] Matthews E.K., British Medical Bulletin, Calcium and membrane permeability, Microbio. Gene J., 42: 391 - 397, 1986.

[11] Galvanoskis, J. and Sandblom, J., Theortical studies of the effects of low – frequency field on the magnitude of oscillation, Bioenergy J., 46: 161 – 174, 1998.

[12] Ivanova, V.; Hristov, J.; Dobreva, E. and Al Hassan, Z., Appl. performance of a magnetically stabilized bed reactor with immobilized yeast cells, Biochem. & Biotechnol. J., 59 : 187 – 198, 2011.

[13] Ivancsits, S., Diem E., Pilger A., Rüdiger H. W. and Jahn O. , Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts, Mutation Research J., 519: 1–13, 2002.

[14] Jin, M., H. ; Lin, L.I.; Maurer ,M.S. and Goodman R.B., Biological and technical variables in myc expression in HL60 cells exposed to 60 Hz electromagnetic fields, Bioelectrochemistry and Bioenergetics J., 44, 111–120, 1997.

[15] May, A.E.; Snoussi, S.R. ; Miloud, N.B. ; Maatouk, L.B. and Aissa, R.B., Foodborne Effect of static magnetic field on cell growth, viability, and differential gene expression in salmonella, pathog. Dis. J., 6: 547 – 552, 2009.

[16] Mehedintu, M. and Berg, H., Proliferation response of yeast Saccharomyces cerevisiae on electromagnetic field parameters, Bioelectrochem. Bioenerg. J., 43 : 67 - 70,1997.

[17] Ramon, C.; Martin, J.T and Powell, M.R., Low–level, magnetic field induced growth modification of Bacillus subtillis, Bioelectromagnetics J., 8 : 275–282, 1987.

[18] Ramchand, C.N.; Priyadarshini,P. and Metha, R.V., Application magnetic field in medicine and biotechnology, Indian Journal of pure and Applied physics, 39: 683 – 686, 2001.

[19] Aziz, N. H. and Smyk, B., Nahrung, Influence of UV radiation and