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50 Hz Frequency Magnetic Field Effects On *Pseudomonas Aeruginosa And Bacillus Subtilis Bacteria*

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Abstract: *The effect of electromagnetic field of different intensities on Pseudomonas aeruginosa (as gram-negative bacteria) and Bacillus subtilis (as gram-positive bacteria) was investigated to find out the effective magnetic field strength that alters the running physiological processes of every microorganism. Equal volumes of P. aeruginosa and B. subtilis suspensions were exposed for one hour at their maximum rate of active growth to the electromagnetic field (2 - 10 mT, 50 Hz). The results indicated that no remarkable differences were found in the growth of exposed P. aeruginosa. Moreover, a remarkable inhibition in the growth of exposed relative to unexposed B. subtilis cells was achieved at (4 mT) as compared with other intensities which may indicate that this magnetic field induction had a great effect on the biological activity of the cells, so more investigations were made at this magnetic field induction. Remarkable changes in the growth characteristics could be easily detected as the absorbance decreased which indicate a decrease in the cells number and consequently an inhibition case for the bacteria. Also, the antibiotic sensitivity test of B. subtilis cells indicated either inhibition or stimulation case for the bacteria depending on the drug mode of action.*

Keywords: *electromagnetic field, microorganism, Bacillus subtilis , Pseudomonas aeruginosa .*

I. Introduction

Lots of studies have been carried out concerning man-made ELF-EMFs effects on biological systems (Balcavage et al., 1996; Berg, 1999; Panagopoulos et al., 2002) to state safety limits for exposure to these fields especially with increasing daily use of electric and electronic devices even though many studies on their effects on health.

During the last decade the effects of electromagnetic fields on "smaller" biological objects started to be investigated. The objects studied were cells (Scarfi et al., 1997; Monti et al., 1991), tissues (Schimmelpfeng and Dertinger, 1993) and living organisms (Hönes, 1998). Recently, bacteria are good experimental subjects to evaluate how such prokaryote unicellular microorganisms may respond to electromagnetic fields (Markov et al., 2004; Babushkina et al., 2005). Because of the nature of microorganisms which makes them suitable for use into areas .

Such as basic molecular and cellular biology. They are simpler than plants and animals, both genetically and biochemically, they reproduce rapidly so that large numbers of organisms, with the same genetic composition, can be easily grown (Atlas, 1995).

In this field, Segatore et al., (2012) found that the exposure of E. coli and P. aeruginosa to extremely low-frequency electromagnetic fields (2mT; 50Hz) at 4, 6, and 8 h of incubation the number of cells was significantly decreased in bacteria exposed to electromagnetic field when compared with the control. Additionally, at 24 h of incubation, the percentage of cells increased (P. aeruginosa ~ 42%; E. coli ~ 5%) in treated groups with respect to control groups suggesting a progressive adaptive response. By contrast, no remarkable differences were found in the antibiotic susceptibility and on the growth rate of both bacteria comparing exposed groups with control groups.

Taqavi et al., (2012) found that the effect of low frequency (10 Hz with an intensity of 700 milli gauss) on *E. coli* showed, a significant decrease in the number of exposed cells (CFU / ml). The results of biochemical tests also showed negative effects of electromagnetic fields on the biochemical properties of *E. coli.*

Inhan-Garip et al. (2011) investigated the effect of extremely low frequency (<300 Hz) electromagnetic fields (ELF-EMF) on the growth rate of Gram-positive and Gram-negative bacteria. The results showed a decrease in the growth rate of exposed samples with respect to control.

Di Campli et al. (2010) investigated the effects of exposure to extremely low-frequency electromagnetic fields (ELF-EMF) both on biofilm formation and on mature biofilm of *Helicobacter pylori.* The ELF-EMF acted on the bacterial population during the biofilm formation displaying significant differences in cell viability. whereas, on mature biofilm, no significant differences were found when compared to the controls.

In this work we used Pseudomonas aeruginosa (gram-negative bacteria) and Bacillus subtilis (gram-positive bacteria) as experimental models for our studies. Our choice to these bacterial strains was supported by the fact that these bacteria are within easy reach, short life cycle (Nakasono and Saiki, 2000) and can grow at 37 ° C. The effect of electromagnetic field (2 - 10 mT, 50 Hz) was investigated on these strains to find out the effective magnetic field strength that alters the running physiological processes of every microorganism.

II. Materials and Methods

Samples and tested organisms

Subcultures of *P. aeruginosa* and *B. subtilis* originally from cultures maintained at the microbiology laboratory were used during this investigation. All bacterial strains were cultivated over night on nutrient broth at 37 ^{o}C. Inoculums of these strains were used to inoculate nutrient agar plates and incubated at 37 ^{o}C till used.

Preparation of the bacterial cultures for exposure:

- Bacterial cells were inoculated to sterilized nutrient broth in screw capped test tubes filled to 2/3 of their total volume and incubated overnight at 37 ^{o}C.
- The bacterial cells were cultivated in 500 ml screw capped flasks containing 150 ml of sterilized nutrient broth.
- The cultures were incubated at 37 ^{o}C and allowed to grow.
- Each culture obtained was divided under sterile conditions into two groups, one exposed to ELF-EMF and the other remained as control.
- The part of the culture to be exposed is sub-divided into three aliquots. These aliquots will be exposed simultaneously to the same field characteristics and used as replicates in each experiment.

Magnetic field exposure facility

A homogenous magnetic field generated by a solenoid consisting of 320 turns from electrically insulated 2 mm cupper wire wound in a homogenous way around a cupper cylinder 2 mm thick, 5 cm diameter and 10 cm length. The temperature during the exposure period was 37 ^{o} C; it was controlled using air conditioner. The Tubes of the exposed bacteria were putted in the middle of the coil by using supports to get a homogenous and higher magnetic field strength. The ends of the solenoid were connected to variac fed from the mains (220 V, 50 Hz), as shown in figure (1a).

The magnetic field intensity was measured by means of hand held Gauss/Tesla meter, as the result proved its homogeneity among all the volume and was within ± 5% at the ends. The magnetic flux density was varied, as the field strength was adjusted by changing the current through the coil. The magnetic field exposure system was manufactured locally, in the faculty of Science Mansoura University.

Equal volumes of the bacterial strains were exposed for an hour at their mid-to-late exponential phase (maximum rate of active growth) to the magnetic field at different intensities in the range of (2 - 10 mT) to specify the most effective magnetic field intensity on the organisms. Then, the organisms were exposed for an hour at (2, 4, 6 …24) hr at this magnetic field intensity, after exposure; part of the sample was used to evaluate the direct effect and the rest was incubated at 37 ^{o}C for an hour to study the recovery of the magnetic field. The unexposed bacterial cells were put in similar conditions, but without magnetic field.

Fig. (1a): The electromagnetic field exposure system.

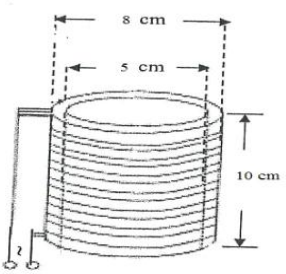

Fig. (1b): A diagram of the solenoid characteristics.

Measurement of the bacterial growth

Count-Absorbance calibration curve (survival curve)

To study the bacterial growth, standard survival curves were plotted between the absorbance of the samples (unexposed cells) at 600 nm and the concentration of cells (number of cells / ml). Spectro SC LaboMed, Inc. was used for absorbance measurements. For cell counting the plate count technique was used (Stainer et al., 1986).

III. Method:

- Appropriate dilutions of the bacterial cells were used to inoculate nutrient agar plates.
- Inoculated plates then incubated at 37 ^{o} C for 24 hr.
- By counting the number of colonies developed after incubation and multiplying it with the dilution factor, the number of cells in the initial population is determined.

Growth characteristics of exposed and unexposed cells

Equal volumes from every bacterial strain were incubated for 24 hr. These strains exposed at different periods, the first volume exposed after two hours of incubation, the second volume exposed after four hours, the third volume exposed after six hours and so on until 24 hr. For each exposure volume there was a corresponding control volume.The absorbance of each volume was measured.

Growth rate

The growth rates of the selected volumes (direct and late effect) were studied through measuring the absorbance at wavelength 600 nm of the viable cells after (2, 4 and 6…… 24 hr) and then plotted as a function of time.

Method:

- By using a sterilized platinum loop, bacterial cells (either control or exposed) from selected volumes were inoculated into sterilized 500 ml screw-capped flasks containing 150 ml sterilized nutrient broth.
- The cultures are incubated at 37 ^{o} C, but the incubation was interrupted each two hours for about 1 min, as a sample is taken for absorbance measurements (each reading was taken three times and the average was taken).
- The absorbance of the cultures then plotted as a function of the incubation time.

Antibiotic sensitivity test

Selected isolates of pathogenic bacteria were subjected sensitivity testing using 5 different antimicrobial agents, which represents susceptibility breakpoint for all antimicrobial agents used in the antibacterial sensitivity testing.

Discs as well as zone reading chart were supplied by BBL™. The sensitivity tests were carried twice for each microorganism, before and after exposure to magnetic fields. Antimicrobial sensitivity tests were carried out and performed by the procedure outlined by the National Committee for (Clinical Laboratory standards, 1984).

The sensitivity test was done using Iso-Sensitest agar (Oxide), this medium is used primarily for the antimicrobial disc-agar diffusion procedure, the so-called disc method, which is one of the most useful and widely used tests for antimicrobial susceptibility of microorganisms.

In this work the antibiotic sensitivity test was made as a comparative study between unexposed bacterial cells and the exposed ones, and nutrient agar was used as culture medium. The antibiotics used in this study were chosen to be with different modes of action, and they were: Amikacin, 30 µm, Rifampin, 5 µm, Norfloxacin, 10 µm, Ceftriaxone, 30 µm and Rifampin, 5 µm.

The method used to measure the sensitivity of the bacterial cells toward different antibiotics was disc method by Bauru-Kirby technique (Baker et al., 1980). The diameters of the inhibition or stimulation zone were measured after 24 hr.

Method:

- Using 150 × 15 mm plates, about 60 ml of sterile molten nutrient agar was poured into each plate. To give a uniform agar layer of depth 4 mm.
- The plates were allowed to dry at 37 ^{o} C for 30 min.
- Using sterile cotton-wool swab, the micro-organisms were equally distributed over the agar surface.
- As soon as possible and not later than 15 min after the inoculation of plates, the antibiotic disks were applied in order that diffusion and growth proceed simultaneously. Disks were arranged at least 15 mm from the edge of the plate and 20 mm apart of each other.
- The plates were incubated at 37 ^{o} C for 24 hr.
- The diameter of each zone of inhibition was then measured to the nearest mm using ruler stick.

Statistical evaluation

The statistical analysis of the biological data was used according to (Harnet, 1994) by calculating arithmetic means and standard deviations for dielectric measurements. The average readings of 5 runs were used.

IV. Results

***P. aeruginosa* studies**

Survival curve:

Fig. (2) illustrates the variation of the sample absorbance measured at 600 nm as a function of the number of microorganisms in cfu / ml (N). The plot shows a linear dependence of the absorbance on the number of microorganisms in count / ml. By using this relation we can calculate the number of microorganisms / ml from the measured value of its absorbance (A).

The linear dependence can be easily represented by the relation;

$$N = 5 \times 10^{12} A \qquad (1)$$

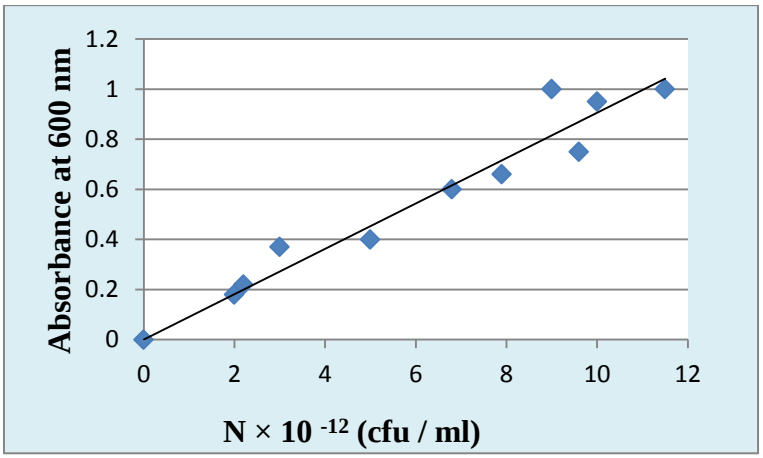

Fig. (2): Calibration curve between the number of *P. aeruginosa* cells / ml and absorbance at 600 nm.

Growth curve characteristics for unexposed *P. aeruginosa* sample

Fig. (3) illustrates the changes in the absorbance of the bacterial suspension as a function of incubation time for 24 hr. It is clear from the figure that the lag phase ended after two hours followed by exponential growth period ended after 14 hr. and followed by the stationary phase.

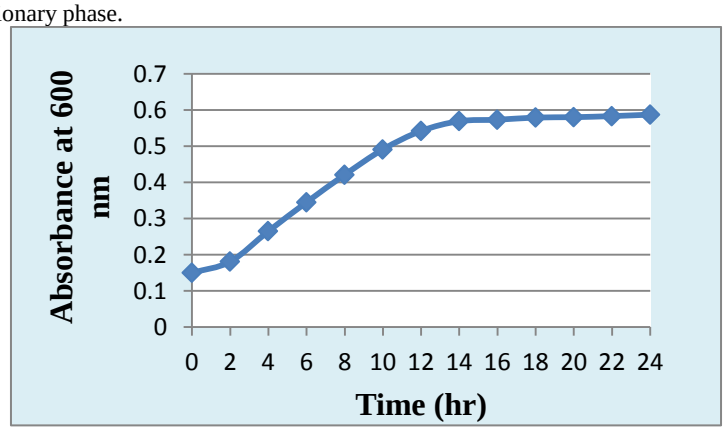

Fig. (3): Growth curve characteristics for unexposed *P. aeruginosa*.

Effects of ELF electromagnetic field on *P. aeruginosa* cells

Fig. (4a) shows a histogram for the absorbance at 600 nm of the bacterial samples after being exposed at their maximum rate of active growth, after (10 hrs) of incubation, to (50 Hz) electromagnetic field of different intensities in the range of (2 - 10 mT) for one hour relative to their control.

Fig. (4b) shows a histogram for the absorbance at 600 nm of the bacterial samples one hour after being exposed at their maximum rate of active growth after (10 hrs) of incubation to (50 Hz) electromagnetic field of different intensities in the range of (2 - 10 mT) for one hour relative to their control.

The results indicated that there were convergent results for both exposed and recovery study as compared with unexposed. This may indicate that none of the used magnetic field inductions (2 - 10 mT) has an obvious effect on the growth of *P. aeruginosa.* Therefore, the experiment had been stopped at this stage of findings.

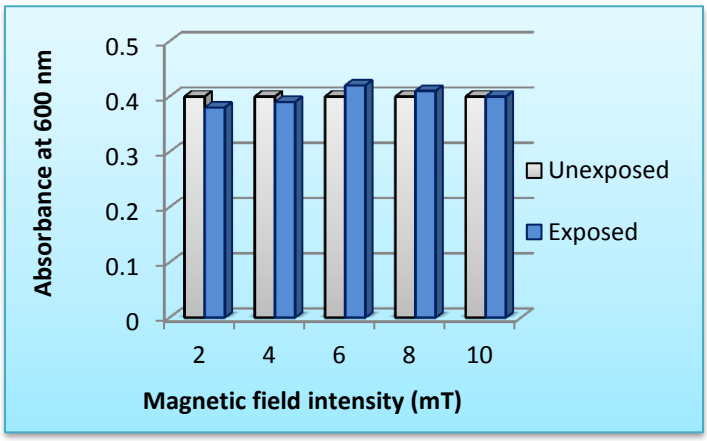

Fig. (4a): Effect of exposure to electromagnetic field of different intensities on the growth of *P. aeruginosa.*

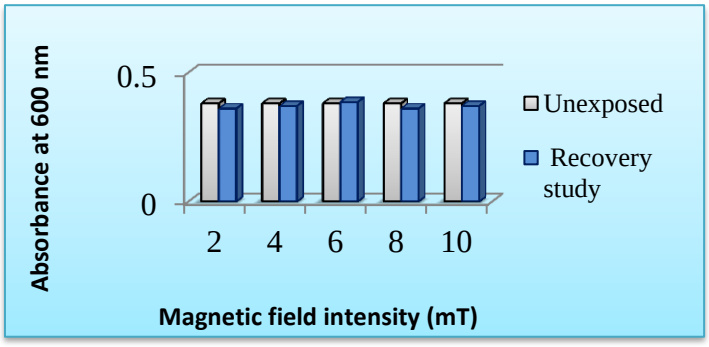

Fig. (4b): Effect of exposure to electromagnetic field of different intensities on the growth of *P. aeruginosa* one hour after exposure.

Effects on the growth characteristics curve of *P. aeruginosa* bacteria

Fig. (5) illustrates the changes in the absorbance at 600 nm of the bacterial suspensions as a function of the incubation after being exposed to the electromagnetic field (4 mT - 50 Hz) for one hour relative to their control.

It is clear from the figure that the growth of the microorganism had not been differ broudly from exposed samples, which indicated that the irradiation of the samples with electromagnetic fields caused very week effect on the growth of *P. aeruginosa* bacteria.

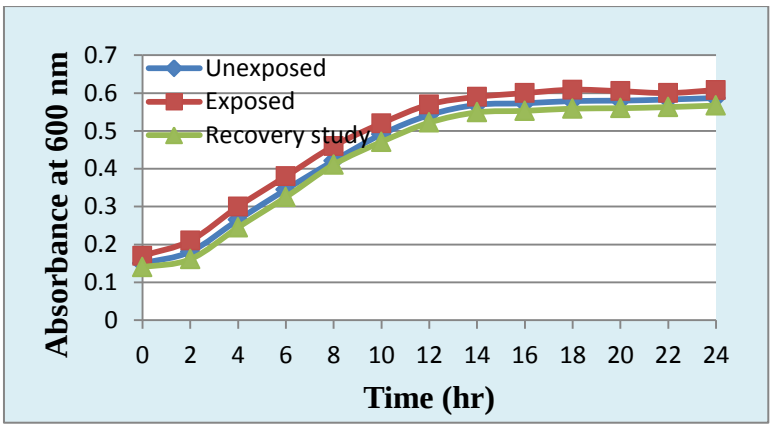

Fig. (5): Growth curve characteristics for unexposed *P. aeruginosa* cell.

Effects on the antimicrobial sensitivity

Table (1) illustrates the antibiotic sensitivity of *P. aeruginosa* cells after exposure to electromagnetic field as compared with unexposed at the periods of (14 hr). The antibiotics used have different modes of action on the microorganism.

It is clear from the table that there was a little decrease in the sensitivity of exposed cells to the antibiotics Amikacin, Ceftriaxone, Norfloxacin, Rifampin and Ciprofloxacin as revealed in the increase of the zone diameter of the microorganism. These results indicate that the viability of cells exposed at (14 hr) decreased as compared with the unexposed cells as an inhibition case.

All these results indicate that there are small effects of the used electromagnetic field to drug mode of action on bacterial cell.

Table (1)

The antibiotic sensitivity of *P. aeruginosa* cells after exposure to electromagnetic field as compared with unexposed at the period of (14 hr).

Antibiotic	Disc content (µg)	Inhibition zone diameter in (cm)		
		Mode of action	Unexposed	Exposed (14 hr)
Amikacin (AN)	30	Inhibition of protein synthesis	1.5	1.5
Ceftriaxone (CRO)	30	Inhibition of cell wall synthesis	2	1.9
Norfloxacin (NOR)	10	Inhibition of DNA respiration	2.9	2.8
Rifampin (RA)	5	Inhibition of RNA transcription	1.1	0.9
Ciprofloxacin (CIP)	5	Inhibition of DNA synthesis	1.5	1.4

***B. subtilis* studies**

Survival curve

Fig. (6) illustrates the variation of the sample absorbance measured at 600 nm as a function of the number of microorganisms in cfu / ml (N). The plot shows a linear dependence of the absorbance on the number of microorganisms in count / ml. By using this relation we can calculate the number of microorganisms / ml from the measured value of its absorbance (A).

The linear dependence can be easily represented by the relation;

$$N = 14 \times 10^{8} A \qquad (2)$$

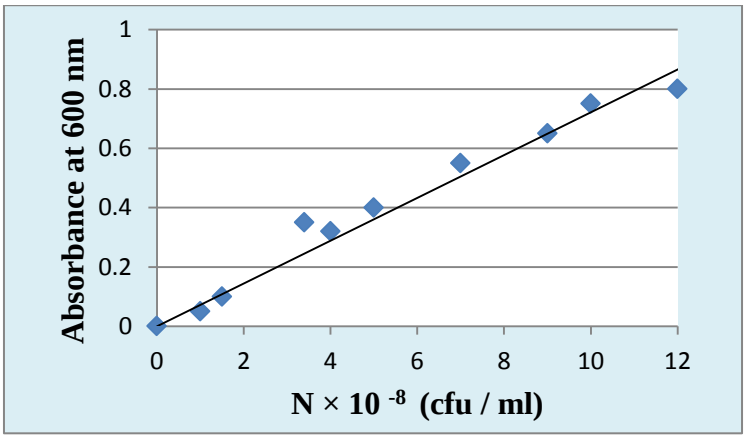

Fig. (6): Calibration curve between the number of *B. subtilis* cells / ml and absorbance at 600 nm.

Growth curve characteristics for unexposed *B. subtilis* sample

Fig. (7) illustrates the changes in the absorbance of the bacterial suspension as a function of incubation time for 24 hr. It is clear from the figure that the lag phase ended after two hours followed by exponential growth period ended after 14 hr and followed by the stationary phase.

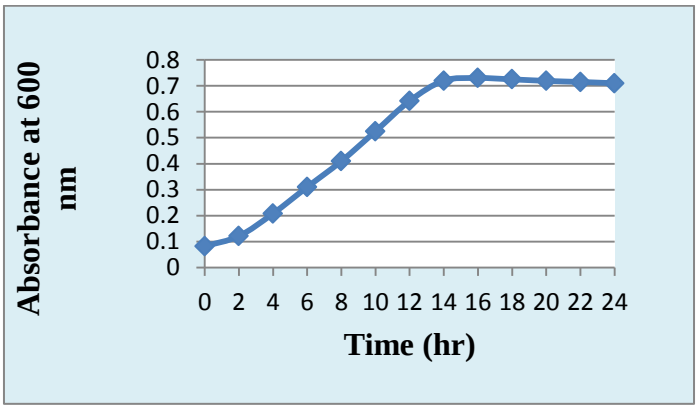

Fig. (7): Growth curve characteristics for unexposed *B. subtilis.*

Effects of ELF electromagnetic field on *B. subtilis* cells

Fig. (8a) shows a histogram for the absorbance at 600 nm of the bacterial samples after being exposed at their maximum rate of active growth, after (10 hrs) of incubation, to (50 Hz) electromagnetic field of different intensities in the range of (2 - 10 mT) for one hour relative to their control.

Fig. (8b) shows a histogram for the absorbance at 600 nm of the bacterial samples one hour after being exposed at their maximum rate of active growth after (10 hrs) of incubation to (50 Hz) electromagnetic field of different intensities in the range of (2- 10 mT) for one hour relative to their control.

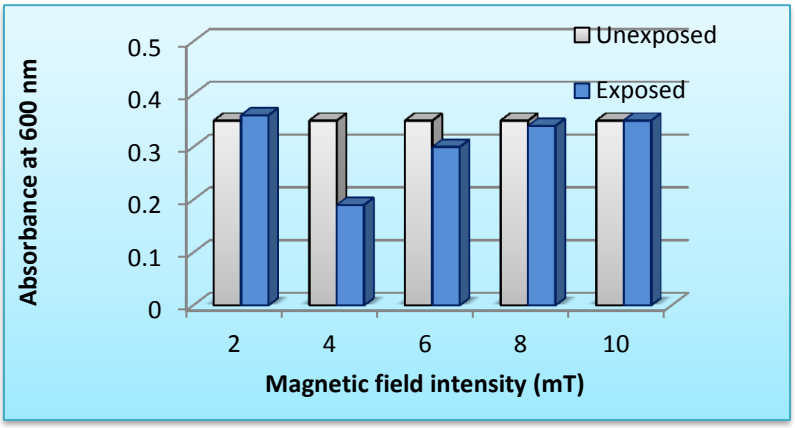

Fig. (8a): Effect of exposure to electromagnetic field of different intensities on the growth of *B. subtilis*.

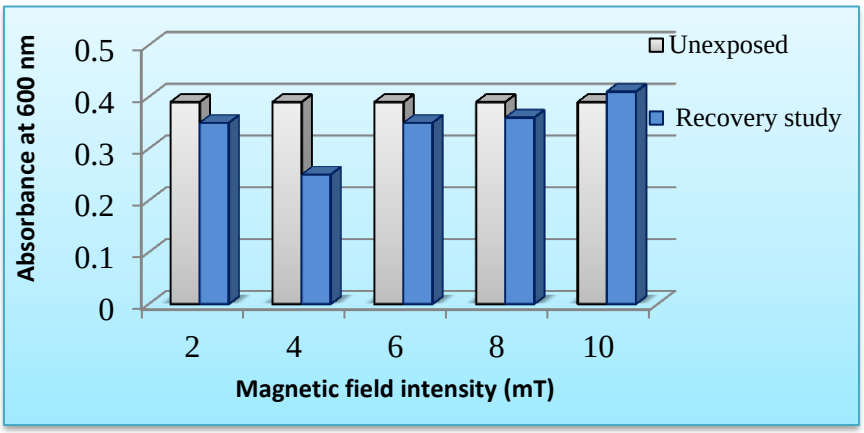

Fig. (8b): Effect of exposure to electromagnetic field of different intensities on the growth of *B. subtilis* one hour after exposure.

The results indicated that the maximum inhibition in growth was for samples exposed at (4 mT) for both exposed and recovery study as compared with unexposed. Therefore the further studies were concerned only with this magnetic field induction effects.

Effects on the growth characteristics curve of the *B. subtilis bacteria*

Fig. (9) illustrates the changes in the absorbance at 600 nm of the bacterial suspensions as a function of the incubation after being exposed to the electromagnetic field (4 mT - 50 Hz) for one hour relative to their control.

It is clear from the figure and table (2) that the lag phase for the growth of the microorganism had been shifted to higher values for exposed samples, which indicated that the irradiation of the samples with electromagnetic fields for one hour caused lately active growth for the microorganism.

Also, the absorbance of the exposed sample decreased and in accordance with equation (2), there is a decrease in the cells number and consequently an inhibition case for the bacteria.

Moreover, we used the period of 14 hr for investigating the effect of the electromagnetic field (4 mT - 50 Hz) on the antibiotic sensitivity of *B. subtilis* cells.

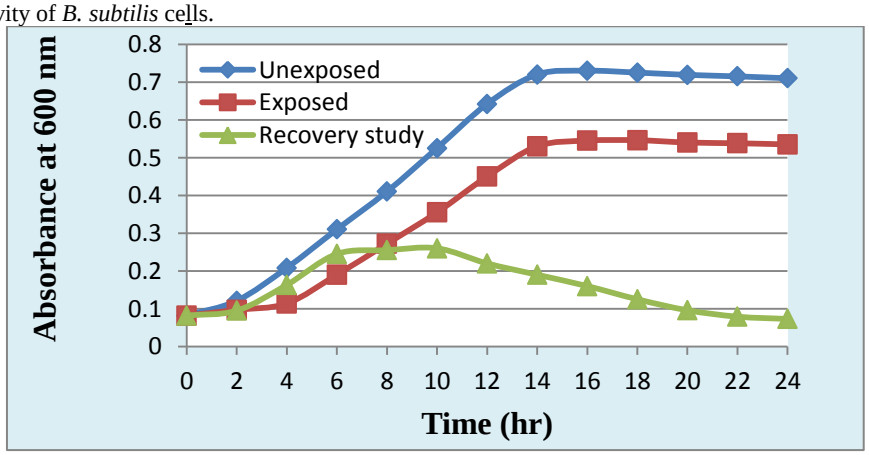

Fig. (9): Growth characteristics curve for *B. subtilis* after exposure to electromagnetic field as compared with unexposed.

Table (2)
Growth characteristics of *B. subtilis* cells before and after exposure to the electromagnetic field.

Samples	Lag phase (hrs)	Exponential phase (hrs) (Active Growth)	Stationary phase (hrs) (Maximum Growth)	No. of cells / ml at Stationary phase
Unexposed	1	10	14	10.08×10^{8}
Exposed	4	10	14	7.42×10^{8}
Recovery study	2	4	6	3.43×10^{8}

Effects on the antimicrobial sensitivity

Table (3) illustrates the antibiotic sensitivity of *B. subtilis* cells after exposure to electromagnetic field as compared with unexposed at the periods of (14 hr). The antibiotics used have different modes of action on the microorganism.

Table (3)
The antibiotic sensitivity of *B. subtilis* cells after exposure to electromagnetic field as compared with unexposed at the period of (14 hr).

Antibiotic	Disc content (µg)	Inhibition zone diameter in (cm)		
		Mode of action	Unexposed	Exposed (14 hr)
Amikacin (AN)	30	Inhibition of protein synthesis	2	2.7
Ceftriaxone (CRO)	30	Inhibition of cell wall synthesis	1.6	1.9
Norfloxacin (NOR)	10	Inhibition of DNA respiration	2.2	1.8
Rifampin (RA)	5	Inhibition of RNA transcription	1.4	1.6
Ciprofloxacin (CIP)	5	Inhibition of DNA synthesis	1.5	1.1

It is clear from the table that there was a decrease in the sensitivity of exposed cells to the antibiotics Amikacin, Ceftriaxone and Rifampin as revealed in the increase of the zone diameter of the microorganism. These results indicate that the viability of cells exposed at (14 hr) decreased as compared with the unexposed cells as an inhibition case. And, also it illustrates an increase in the sensitivity of exposed cells to Norfloxacin and Ciprofloxacin as revealed in the decrease of zone diameter of the microorganism of that volume. These results indicated that the exposed cells at (14 hr) became more resistant to these antibiotics, as a stimulation case.

All these results indicate that there are effects of the used electromagnetic field to drug mode of action on bacterial cell through inhibition of protein synthesis, cell wall synthesis, RNA transcription and DNA respiration and synthesis.

V. Discussion

From the data concerning the effects of ELF-EMF on *P. aeruginosa* convergent results for exposed samples relative to unexposed as shown in figures (4.1 a &b). This may indicate that none of the used magnetic field inductions (2 - 10 mT) has an obvious effect on the growth of *P. aeruginosa.* Therefore, the experiments had been stopped at this stage of findings.

These results could be explained according to the fact that *P. aeruginosa* known to enter into a dormant state referred to as the "**V**iable **B**ut **N**on **C**ulturable state" **(VBNC)** as well as to form biofilm, constituted of sessile aggregated cells embedded in a matrix in which cells organize themselves into microbial communities establishing a sort of "free multicellularity" ***(Donlan and Costerton, 2002; Oliver, 2005)***, to adapt themselves against stress induced by the exposure to ELF-EMF.

It is worthy to mention here that the solenoid available in our laboratory could generate magnetic flux density of maximum (10 mT) that intensities exceed this value would be accompanied with a large amount of heat that could affect on our results. Therefore, it was not possible to study the effect of higher intensities on the microorganisms.

From the data concerning the effects of ELF-EMF on *B. subtilis* a remarkable inhibition in the growth of exposed relative to unexposed cells was achieved at electromagnetic field of (50 Hz – 4 mT) as compared with other intensities, as shown in figures (4.2a & b), which may indicate that this magnetic field induction had a great effect on the biological activity of the cells.

In addition, remarkable changes in the growth characteristics could be easily detected as the absorbance decreased which indicate a decrease in the cells number and consequently an inhibition case for the bacteria.

Also, the antibiotic sensitivity test of *B. subtilis* cells indicated that the used electromagnetic field could cause either a decrease or an increase in the sensitivity of exposed cells resulted in either inhibition or stimulation case for the bacteria depending on the drug mode of action on the bacterial cell.

All the results of the recovery study indicated that the studied microorganisms could switch on new pathways to adapt themselves against stress induced by exposure to EMFs aimed at their preservation ***(Bjedov et al., 2003)*** as the transposition which represents an important source of genetic variability can be induced ***(Lamrani et al., 1999; Del Re et al., 2004)***. In

this manner, bacteria try to find their adaptation through intra-strains variability as the benefits of heterogeneity among a cell population enhances the persistence of bacteria ***(Avery, 2005)***.

VI. Conclusion

It could be concluded from the present work that:-

- The effects of exposure to extremely low frequency electromagnetic fields depend on the magnetic field intensity, frequency and the period of exposure.
- The interference of the applied electromagnetic field with the bioelectrical signals generated during the physiological processes may affect the biological functions causing either enhancement or inhibition of the running processes.
- Each organism responds to the exposure to ELF-EMF in a specific manner depending on the adaptation mechanism of each organism.
- The exposure to ELF-EMFs may have beneficial effects as well as harmful effects, that the safety limits for exposure to these fields are field strength and frequency dependent.

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