THE EFFECT OF ELECTROMAGNETIC FIELD ON PROTEIN MOLECULAR STRUCTURE OF *E. COLI* AND ITS PATHOGENESIS

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Abstract. Effects of electromagnetic field 50 Hz frequency and strength 2 mT on each of growth characteristics and pathogenicity of *E. coli* cells have been studied. Also, the changes in the molecular structure of water soluble protein (WSP) extracted from *E. coli* bacteria exposed to demonstrated magnetic field were investigated through measuring each of their dielectric relaxation in the frequency range 1 kHz → 4 MHz at 4±0.05 ºC and molecular weight distribution using SDS polyacrylamide gel electrophoresis. In addition the absorption spectra of the WSP were measured at wavelength range 300–350 nm. Equal volumes of *E. coli* suspension were exposed to magnetic field for different periods. Most effective periods, namely of 6 and 16 h, were chosen for all our experimental studies (direct and late effect – 2 h). The results indicated that there are pronounced changes in the growth characteristic curve, where suppressive effect was observed on the cell growth. Number of cells at stationary phase markedly decreased after exposure period of 6 h, but there was a slight increase in the cells number at stationary phase after an exposure period of 16 h. Further, more remarkable changes happened in the molecular structure of extracted protein molecules after exposure periods, 6 and 16 h such as average molecular radii, shape, relaxation time and dielectric increment. Also, there were sharp decreases in the number of protein bands and in their protein amount after an exposure period of 6 h. However, after an exposure period of 16 h, number of protein bands and protein amount increased relative to the unexposed cells. Mortality rate recorded after injection with *E. coli* suspension exposed to 6 h was 40%, but after an exposure period of 16 h it was 80%.

Key words: electromagnetic field, *E. coli*, growth rate, dielectric relaxation, molecular weight distribution, pathogenicity.

INTRODUCTION

Some scientists allege that exposure to magnetic fields generated by power delivery systems is responsible for some diseases.

Therefore, it is both appropriate and important to evaluate the possible effects of man-made electromagnetic field on living organisms. In this field Fadel *et al*. [7]

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reported that the main damaging role of 50 Hz magnetic field may be on the cellular membrane that strongly affects, not only the cellular physiological functions, but also the cell-to-cell communications.

Rodriguez *et al*. [26] found that growth of *E. coli* could be altered (stimulated or inhibited) under magnetic field, induced effect. *E. coli* cultures exposed to 0.1 T during 6.5 h exhibited changes in its viability as compared to unexposed cells, which was 100 times higher than the control.

Delcosta *et al*. [6] concluded that the extremely low frequency electromagnetic field (1 mT, 50 Hz, SMF, PMF) influences the synthesis of HSPs in *E. coli* in a way that critically depends on the signal characteristics.

Fojt *et al*. [9] found that *E. coli*, bacteria adecarboxy and *Staphylococcus aureus* viability affected with the magnetic field (10 mT, 50 Hz). They also found that the decrease of the colony forming units (CFU) starts immediately after the magnetic field was switched on. Mei *et al*. [20] studied the inactivation of microorganisms by a pulsed magnetic field. It was reported that the application of electromagnetic pulses evidently causes a lethal effect on *E. coli* cells suspended in buffer solution.

Yonemoto *et al*. [34] studied the non-thermal sterilization by using the self-designed generator of magnetic field. The results showed that the magnetic flux density which had the greater effect on *E. coli* was 1 T. The greater destruction rate of *E. coli* was 78% under 8 hours of magnetic field (1 T) treatment.

This work is concerned with the study of the biological effect of magnetic fields, as a component of the non-ionizing radiation on the unicellular system. Pathogenic microorganisms, especially *Escherichia coli* which is chosen to be our experimental model for many reasons, it is widely distributed in the environment such as soil, water and air. *E. coli* is a member of the normal intestinal flora of humans. It causes several diseases such as urinary tract infection; wound infection, traveler's diarrhea. It reaches blood stream and causes sepsis and meningitis [25]. *E. coli* are rapidly growing, Gram-negative, rod-shaped cells measuring approximately 0.5×2 μm length [23]

In the light of the pathogenic effects of *E. coli* bacteria, we aim to study the effect of different exposure periods to a magnetic field (50 Hz, 2 mT) and choose the period which affects the pathogenesis of *E. coli* cells. Such a process would supply an easy and elegant method to get rid of those bacteria, especially for sterilizing medical instruments.

Moreover, we intend to take two exposure periods for investigating the effect of magnetic field, 20 G, 50 Hz (direct and late effect – 2 h), on the growth rate, the molecular structure of water soluble protein (WSP) extracted from the *E. coli* cells, and on its pathogenicity.

MATERIAL AND METHOD

BACTERIAL STRAIN

Escherichia coli ATCC ≠ 25992 was cultivated over night on nutrient broth at 37 °C, each ml of bacterial suspension containing 13×10^3 CFU/ml.

SURVIVAL CURVE

To study the bacterial growth, a standard survival curve was plotted between the absorbance of volume A (unexposed cells) at 600 nm and the concentration of cells (number of cells/ml). For cell counting the plate count technique was applied [31] and appropriate dilutions of the bacterial cells were used to inoculate nutrient agar plates. Inoculated plates were then incubated at 35 °C for 24 h 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.

MAGNETIC FIELD EXPOSURE FACILITY

E. coli suspension containing 13×10^3 CFU/ml was exposed to a homogeneous magnetic field generated by a solenoid consisting of 320 turns from electrically insulated 2 mm copper wire thickness wound in a homogeneous way around a copper cylinder 2 mm thick, 40 cm diameter and 25 cm length. The cylinder wall was well grounded to eliminate electric field components effects. The magnetic field generator was temperature controlled during the exposure period by using a water pump as shown in Fig 1. The temperature during the exposure periods was 37 °C. The tubes of the exposed bacteria were put in the middle of the solenoid by using supports inside it to get a homogeneous and higher magnetic fields strength. The ends of solenoid were connected to variac fed from the mains (220 V, 50 Hz). The magnetic field was measured by means of hand held Gauss/Tesla meter and the magnetic flux density was 20 Gauss. The unexposed bacteria cells were put in similar conditions, but without magnetic field.

GROWTH CHARACTERISTICS OF EXPOSED AND UNEXPOSED CELLS

Ten equal volumes from the suspension were incubated for 18 h and then exposed to different exposure periods. The first volume was exposed to two hours, the second volume was exposed to four hours, the third volume was exposed to six hours and so on. For each exposure volume, there was a corresponding control volume. The absorbance of each volume was measured and the two volumes exposed to the two periods, 6 h and 16 h, were chosen for additional investigations.

Four volumes were used in this study A, B, C and D for investigating the effect of magnetic field (20 G, 50 Hz) on the growth rate, the molecular structure of water soluble protein (WSP) extracted from the *E. coli* cells and on its pathogenesis. Volumes A and C are control of volumes B and D, exposed to 6 and 16 h respectively. Half of each volume for the direct effect were named A_1, B_1, C_1, D_1, and the other half were named A_2, B_2, C_2, D_2, for the late effect (2 h).

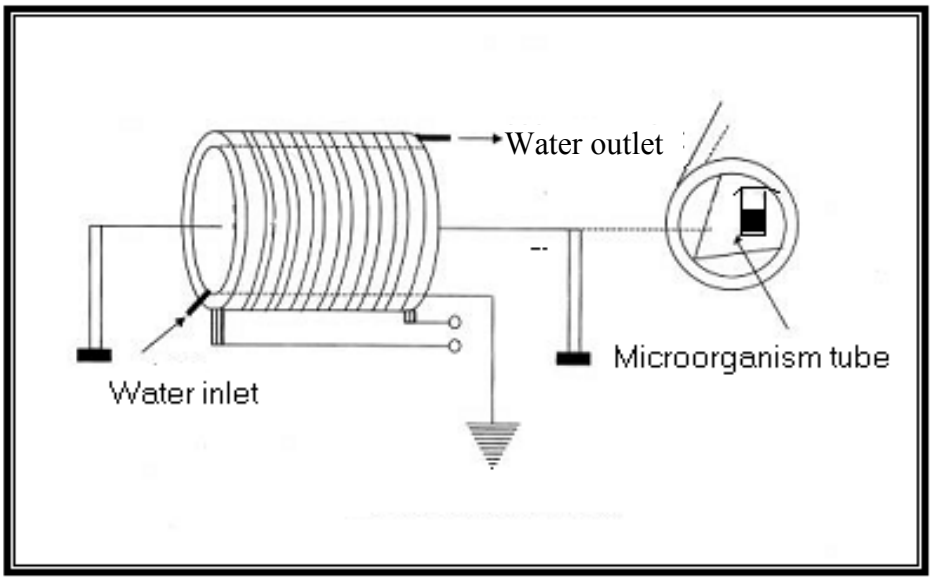


Fig. 1. Magnetic field exposure facility.

GROWTH RATE

The growth rates of all volumes (direct and late effect) were studied through measuring the absorbance of the viable cells after 2, 4, and 6 until 22 h. The absorbance of the volumes was measured and then plotted as a function of time. Spectronic 20+ Series Spectrophotometer (USA) was used for this purpose.

THE DIELECTRIC MEASUREMENTS OF (WSP)

About 50 ml from each volume were centrifuged for 15 min at 5000 rpm. Cells were collected in cold phosphate buffer of pH 7.4. Cells were disrupted by an ultrasonic homogenizer. Water soluble protein samples were extracted from the cells by using ammonium sulphate according to the method of Schleif and Wensink [28].

The concentrated protein solution was then diluted with bi-distilled water at a ratio 1:20. Loffer *et al*. [18] calculated the dielectric properties of protein and its solvent (comparison). They found that the coupling between the dielectric relaxation of the peptide and that of the water-component is particularly important for correctly describing the dielectric constant of the peptide. Also Bai *et al*. [2] and Brunton *et al*. [4] used the same study to define the changes in the molecular structure of another protein molecule.

Dielectric measurements for the protein extracted from all the volumes were carried out at the fixed temperature of 4ºC, with an incubator type 2771, Katter Mann, Germany, and cell types (PW 950/60) manufactured by Philips. The cell has two parallel square platinum black electrodes of 0.8 cm side each, and area 0.64 cm^2, A, with an inter-electrode distance, *d*, of 1 cm. The dielectric relaxation was measured in the frequency range 1 kHz → 4 MHz using RLC Bridge 3235 Hioki – Japan. The measured values of capacitance, *C*, and resistance, *R*, were used to calculate the real part, ε', the dielectric constant or relative permittivity of the material, representing the energy stored per cycle, and the imaginary part, ε″, the dielectric loss factor, representing the energy dissipated per cycle of the complex permittivity (Polk and Postow [24]).

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \tag{1}$$

Using the following equation:

$$\varepsilon' = \frac{Cd}{\varepsilon_0 A} \tag{2}$$

where ε_0 is the permittivity of free space,

$$\varepsilon'' = \varepsilon' \tan \delta \tag{3}$$

$$\text{tag } \delta = \frac{1}{2\pi fRC} = \frac{\varepsilon''}{\varepsilon'} \tag{4}$$

The a.c. conductivity was calculated from equation

$$S = \frac{\sigma}{\varepsilon_0} = \omega\varepsilon'' = 2\pi f \varepsilon'' \tag{5}$$

and

$$\sigma = 2\pi f \varepsilon'' \varepsilon_0 \tag{6}$$

where ω and σ are the angular frequency and the actual conductivity, respectively. The molecular radius (*R*) of the protein molecules was estimated through the use of equation (6) (Polk and Postow [24]).

$$R^3 = \frac{kT\tau}{4\pi\eta} \tag{6}$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the protein solution, and τ the relaxation time, namely, the time at which the dielectric molecule has the ability to relax under the effect of the applied field and can be calculated from the relation

$$\tau = \frac{1}{2\pi f_c} \tag{7}$$

where f_c is the critical frequency corresponding to mid point of the dispersion curve.

The difference between the values of ε'_s and ε'_∞ at low and high frequencies is called the dielectric dispersion $\Delta\varepsilon' = (\varepsilon'_s - \varepsilon'_\infty)$. This quantity is a measure for the shape and volume of the non polar solution consisting of protein and bound water Hasted [13]. The dielectric spectrum of a biological macromolecule such as protein at high frequency (hundreds of Kilohertz) are known as β dispersion which came from the polarization of protein and organic macromolecules (Gabriel *et al*. [10]).

In case of bimolecular, such as proteins, the dielectric relaxation shows broader dispersion curves and lower maxima than those predicated by Debye model, and the ε'' versus $\acute{\varepsilon}$ curves fall inside the semicircle, so Cole & Cole [5] introduced a new parameter, α, and modified Debye equation. It was shown by Cole and Cole that the angle $C\ \varepsilon'_\infty\ \varepsilon'_0 = \theta = \alpha\pi/2$. This in turn enables one to estimate the Cole parameter α, experimentally, so $\alpha = 2\theta/\pi$.

QUALITATIVE ANALYSIS OF WSP BY (SDS) POLYACRYLAMIDE GEL ELECTROPHORESIS

Gel electrophoresis technique is a useful method to separate, clarify and identify different types of proteins. Richard *et al*. [27], and Rodriguez-Justo *et al*. [26] classification of the water soluble protein extracted from each volume of *E. coli* cells (direct and late effect) was carried out by discontinuous electrophoresis Laemmli [17]. The molecular weights of the protein bands were estimated by SDS polyacrylamide gel electrophoresis according to the methods of Weber [33]**.** Six standard protein markers of known molecular weights were used as standard protein. Similar amounts of WSP were placed in each lane of the gel. The gel was stained with compassion blue R 250.

The data were identified and analyzed by using gel pro analyzer version 3Media Cybernetic imaging experts software.

PATHOGENESIS TEST

For detecting virulence of bacterial volumes (direct effect only), pathogen city test was carried out, using 40 albino mice (each mouse weighing 20–30 g). The animals were classified into four groups each of 10 mice. The first and third groups were used as control. The second group was injected (I/P) with 1 ml of *E. coli* suspension 6 h exposure periods (0.1 ml for each mouse). The forth group was injected with 1 ml of *E. coli* exposed for 16 h.

STATISTICAL EVALUATION

The statistical analyses of the data were used according to Harnet [15] by calculating arithmetic means and standard deviations for dielectric measurements. The average readings of 5 runs were used.

RESULTS AND DISCUSSION OF BIOLOGICAL RESPONSE

The results obtained in this work concern the induced changes in the structure and the characteristic behavior of *E. coli* results from the exposure to the demonstrated magnetic field. These results may be of a great importance for evaluating the benefits as well as the hazards of the exposure to low frequency low level magnetic field.

Also the importance of this work lies in the fact that *E. coli* as a microorganism is a unit cell behaving as a complete alive biological system

SURVIVAL CURVE

The sample absorbance measured at 600 nm versus the number of microorganisms in cfu/ml is shown in Figure 2. The results show the linear dependence of the absorbance on the number of microorganisms in count/ml (C).

By using this relation we can calculate the number of microorganisms / ml (C) from the measured value of its absorbance (A). The liner dependence can be easily expressed by the relation $C = 9\times10^{11}$ A.

GROWTH CHARACTERISTICS CURVE

Fig. 3 shows the changes in the absorbance of bacterial suspension as a function of the time of exposure to the magnetic field.

It is clear from the figure that the exposure periods of 2, 4, 6, 8, 10, 12 and 14 h decreased the absorbance and, in accordance with equation (1), indicate a decrease in the cells number and consequently an inhibition case for the bacteria. However,

at the exposure periods of 16 and 18 h the absorbance increased relative to their control indicating an increase in the cells number and a stimulation case. These results are in a good agreement with Mohamed *et al*. [21], where the number of cells of *S-typhi* microorganism exposed to 20 G magnetic fields for 2 hours increased relative to the unexposed one.

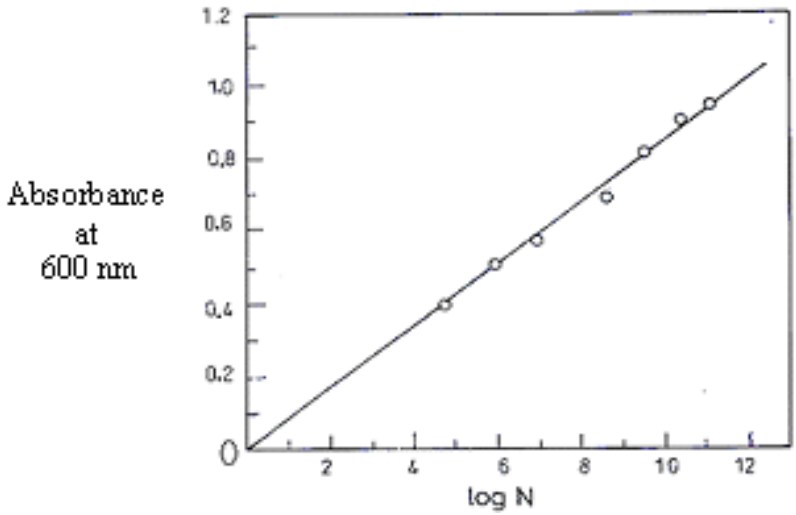


Fig. 2. Calibration curve between the log number of bacteria cells/ml and absorbance at 600 nm.

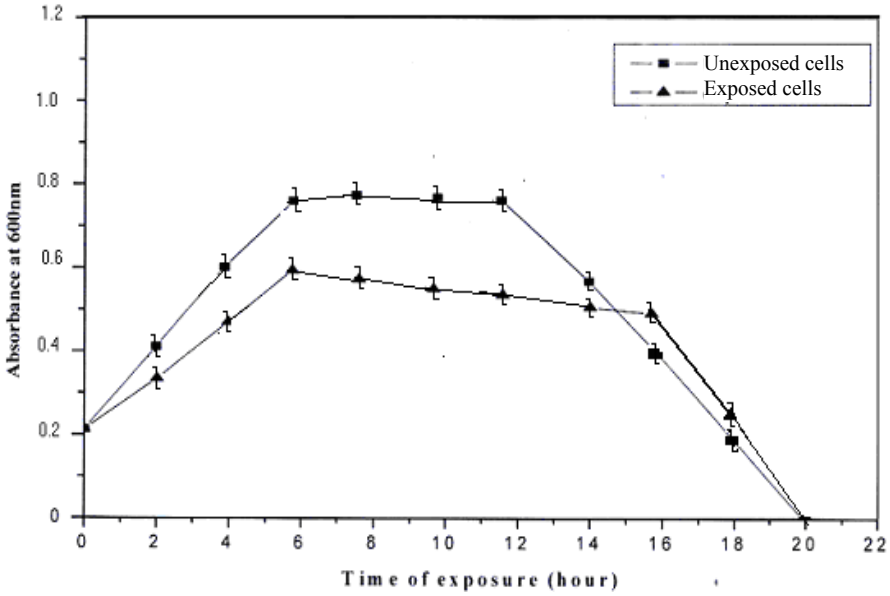


Fig. 3. Biological response of *E. coli* to electromagnetic field.

For this reason we used the exposure period 6 h (volume B_1) as an inhibition case where the number of cells was 10^8 and became 10^7 cells/ml also the exposure period 16 hours (volume D_1) as stimulation case where the number of cells was 3.5×10^2 and became 3.5×10^4 cells/ml.

Moreover, we intend to take the two exposure periods for investigating the effect of the magnetic field (20 G, 50 Hz) on the growth rate, the molecular structure of water soluble protein (WSP) beig extracted from *E. coli* cells.

THE GROWTH RATE

Fig. 4 shows the growth rate of volumes A_1, B_1 and B_2 It is clear from the figure that there is a decrease in the growth rate of *E. coli* cells exposed to 6 h relative to the unexposed ones.

Fig. 5 explains the growth rate of volumes A_2, D_1 and D_2. It is clear from the figure that there was a slight increase in the growth rate of the exposed *E. coli* cells relative to those unexposed.

It is clear from the figures and the calculated data in Tables 1 and 2 that there are considerable changes in the growth rate of *E. coli* cells for the two exposure periods of 6 and 16 h. For an exposure period of 6 h (volume B_1), the maximum growth occurred at 16 h, while for the unexposed cells at 18 h. Also, the maximum number of microorganism decreased to 2×10^7 cells/ml as compared with the unexposed cells 8×10^9 cells/ml.

For the late effect (Table 2), the maximum growth occurred at 18 h similarly to the unexposed, but the number of cells was still lower.

However, for an exposure period of 16 h (volume D_1), the maximum growth occurred at 14 h, with increasing the maximum number of microorganisms to 2×10^{10} cells/ml, as shown in Table 1, but for the late effect (Table 2), the maximum growth occurred at 16 h, and the number of cells was still higher than in the unexposed.

Moreover, from these results one sees how the period of active growth (log phase) decreased for the two volumes B_1 and D_1, which became 12 and 10 h (direct effect) respectively 12 h for the two volumes B_2 and D_2 (late effect) while it was for the unexposed cell 14 h and also the lag phase was short for all the volumes. In spite of these facts, the exposure period of 16 h increased the cell division rate in a good agreement with Nascimento *et al*. [22], who concluded that the electromagnetic field (8 h, 5 G, 60 Hz) had a positive effect in the consumption of glucose and growth of *E. coli*. They attributed the increase in the growth to the shortening of lag phase and excitement of log phase.

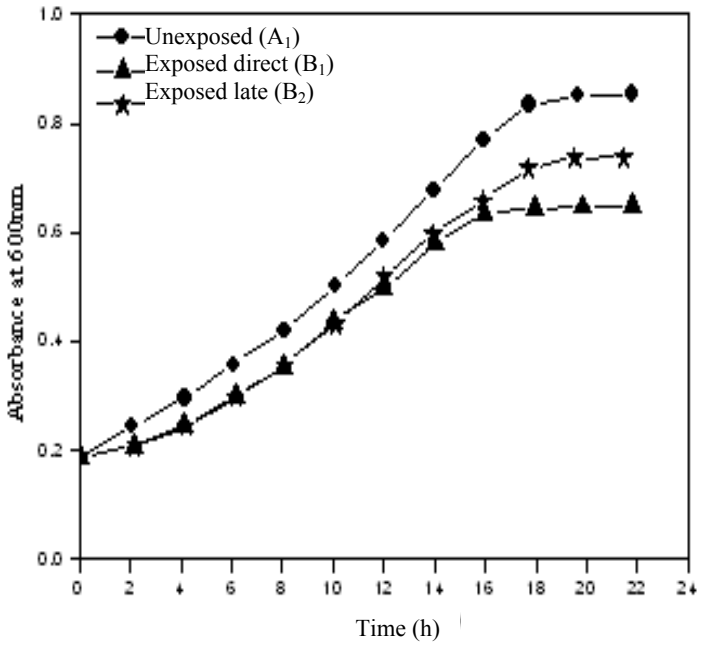


Fig. 4. Growth rate of *E. coli* before (unexposed) and after (exposure) period 6 h.

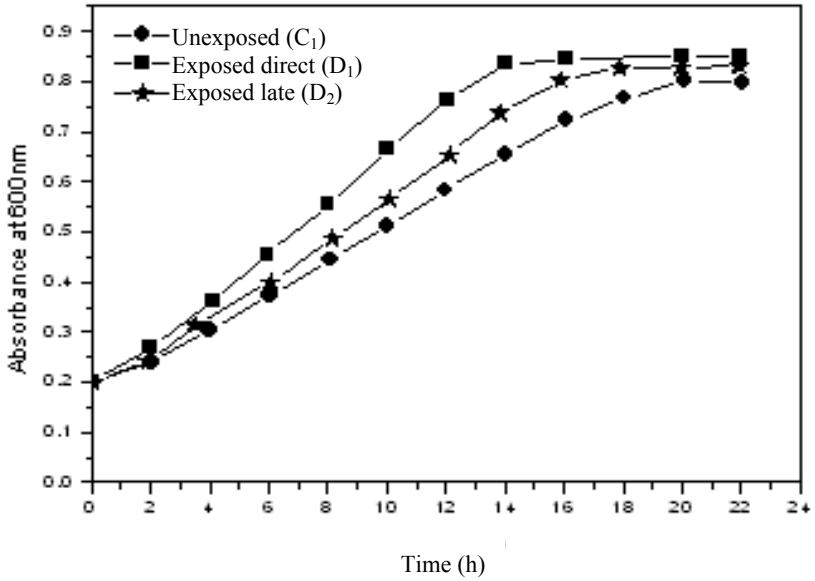


Fig. 5. Growth rate of *E. coli* before (unexposed) and after a period of 6 h (exposed).

Table 1

Growth characterization of *E. coli* before and after exposing to the magnetic field for 6 h

Volumes	Log phase (h)	Stationary phase (h)	No. of cells/ml at stationary phase
A_1	14	18	10^{10}
B_1	12	16	8×10^{7}
B_2	14	18	10^{9}

Table 2

Growth characterization of *E. coli* before and after exposing to the magnetic field for 16 h

Volumes	Log phase (h)	Stationary phase (h)	No. of cells/ml at stationary phase
C1	16	20	8×10^{9}
D1	10	14	5×10^{10}
D_2	14	18	2×10^{10}

The inhibitory effect of EMF after an exposure period of 6 h on the growth of bacteria may be due to the interaction between electric charges induced by EMF and that of the cytoplasm membrane resulting in a partial abolishment of the electric potential of the cytoplasm membrane with a subsequent decrease in the macromolecular biosynthesis. Also EMF may cause damage of bacterial DNA and inhibition of its replication [12, 16, 29].

Since the present data proved the cellular membrane of the microorganism had been affected by the external magnetic field, then one expects a disturbance in their metabolic activity and consequently a change in their cell division in a good agreement with Mohamed *et al*. [21] who reported that exposing *S-tyhi* to 20 G magnetic field increased their cell division and cell number.

RESULTS AND DISCUSSION OF THE DIELECTRIC METHOD

Figure 6a illustrates the variation of the permittivity ε′ and the dielectric loss ε″ plotted on the left Y axis and the conductivity (σ) on the right Y axis as a function of the applied frequency for volumes A_1, B_1 and Fig.7a for volumes C1 and D1 (direct effect). Figure 6b illustrates the Cole-Cole plots (ε′ *versus* ε″) for volumes A_1, B_1 and Figure 7b for volumes C_1 and D_1.

Table 3

Values of dielectric increment (Δε), conductivity (σ), relaxation time (τ), viscosity (η), average molecular radii (*R*), and Cole-Cole parameter (α) for the WSP as a function of the time of exposure (direct effect)

Volumes	Conductivity $\sigma\times 10^8$ (S/m)	Dielectric increment $\Delta\varepsilon\times 10^6$	τ(μs)	η(Poise)	*R*(nm)	α
A_1	10.2±0.3	5.6±0.2	5.55	0.133	2.24	0
B_1	9.8±0.2	5.7±0.1	3.88	0.124	2.17	0.05
C_1	9.6±0.2	5.7±0.1	5.88	0.134	2.27	0
D_1	9.4±0.1	5.6±0.1	3.54	0.134	2.05	0.09

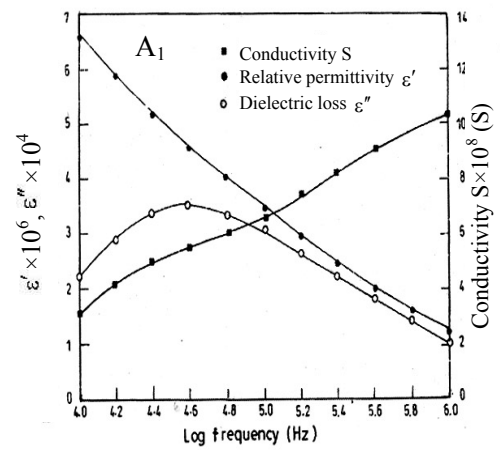
Table 4

Values of dielectric increment (Δε), conductivity (σ), relaxation time (τ), viscosity (η) average molecular radii (*R*), and Cole-Cole parameter (α) for the WSP as a function of the time of exposure (late effect)

Volumes	Conductivity $\sigma\times 10^8$ (S/m)	Dielectric increment $\Delta\varepsilon\times 10^6$	τ(μs)	η(Poise)	*R*(nm)	α
A_2	9.5±0.2	5.5±0.1	5.31	0.125	2.4	0
B_2	9±0.1	5.1±0.1	4.55	0.118	2.33	0.07
C_2	9.5±0.2	5.7±0.1	5.31	0.125	2.5	0
D_2	8.5±0.3	4.9±0.1	3.98	0.127	2.17	0.14

Figure 8a illustrates the variation of the permittivity ε′ and dielectric loss ε″ plotted on the left Y axis and the conductivity (σ) on the right Y axis as a function of the applied frequency for A_2, B_2, while Figure 9a is for volumes C_2 and D_2, (late effect). Figure 8b illustrates the Cole-Cole plots (ε′ *versus* ε″) for volumes A_2 & B_2. Figure 9b illustrates the Cole-Cole plots (ε′ *versus* ε″) for volumes C_2 & D_2. It is clear from the figures that the permittivity ε′ passed through a dielectric dispersion and the decrease in the value of ε′ was accompanied by an increase in the value of conductivity (σ) which we considered as indicating confidence in the measurements Fadel *et al*. [7].

The strong dielectric dispersion in the B region (0.01–10 MHz) for the samples is mainly due to protein and DNA, and counter ion molecular relaxation Polk and Postow [24]. From the figures, it is also clear that the conductivity (σ) of all groups is frequency dependent, and shows the conductivity dispersion which is due to the interfacial polarization Bordi *et al*. [3]. In addition, the values of the conductivity and permittivity for 16 h were higher than for 6 h.



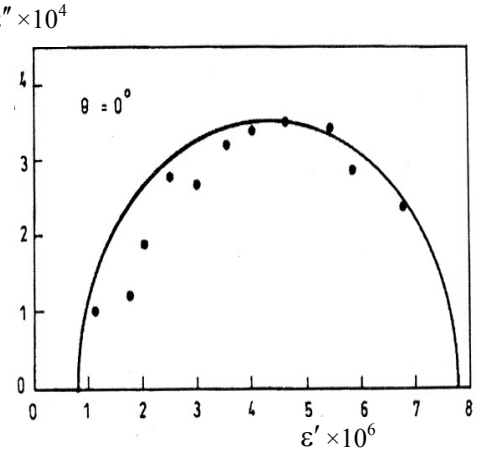
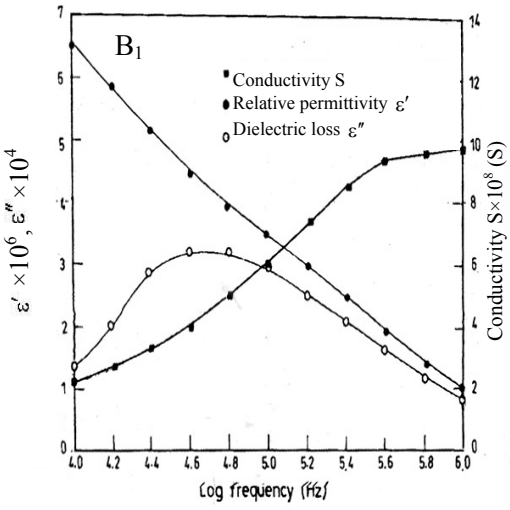


Fig. 6a. The variation of the relative permittivity ε′, dielectric loss ε″, and conductivity σ as a function of the applied frequency for samples A_1 and B_1 (direct effect).



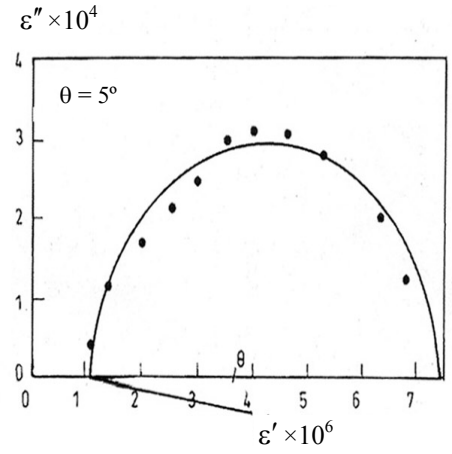
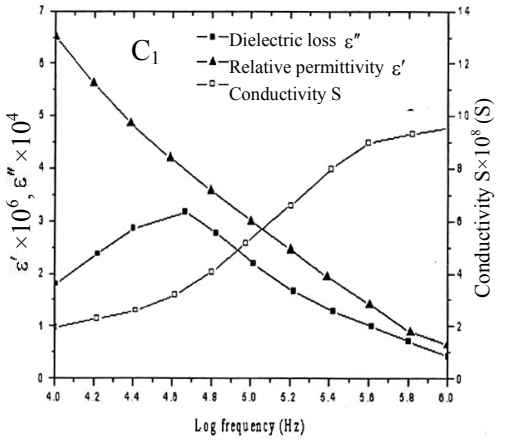
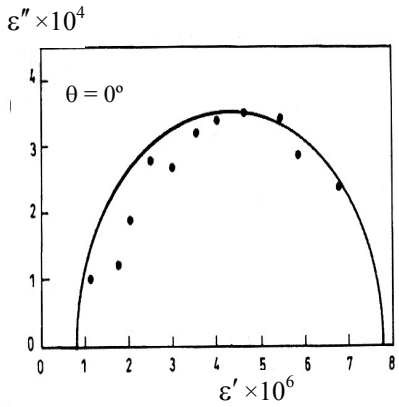
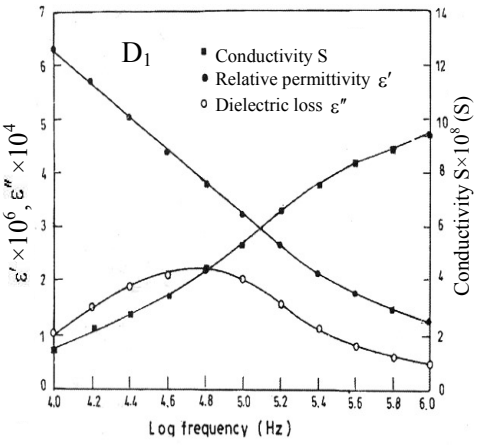


Fig. 6b. The Cole-Cole plot ε′ *versus* ε″ for volumes A_1, B_1 (direct effect).







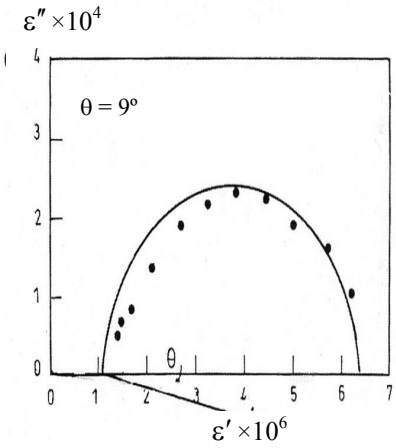
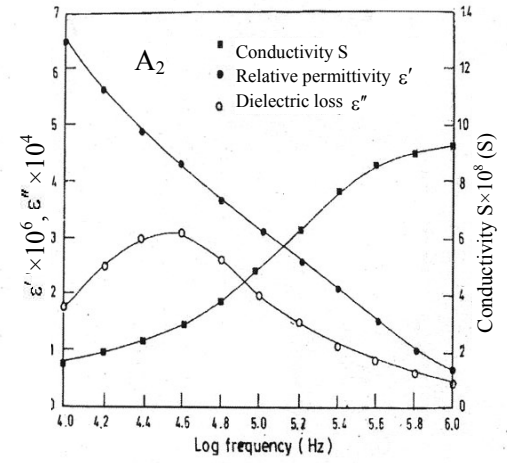
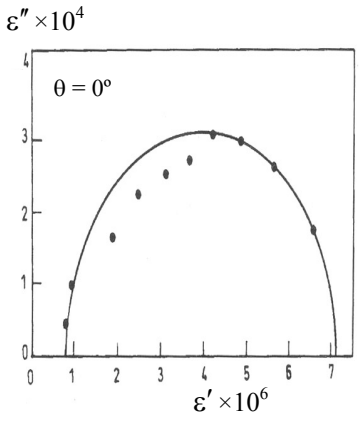


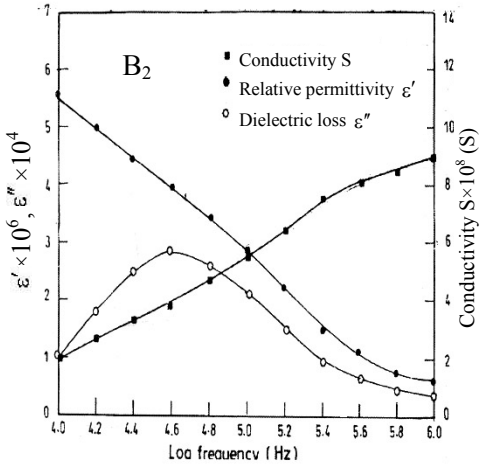
Fig. 7a. The variation of the relative permittivity ε', dielectric loss ε'' and conductivity σ as a function of the applied frequency for samples C_1 and D_1 (direct effect).

Fig. 7b. The Cole-Cole plot ε' *versus* ε'' for volumes C_1, D_1 (direct effect).

From the data obtained from the figures and by using equations (1), (2), (3), (4) and (5), the values of relaxation time τ, the dielectric increment $\Delta\varepsilon'$, the average molecular radii *R*, the Cole-Cole parameters (α) and of the conductivity (σ) for direct effect were calculated and given in Tables 3 and 4.







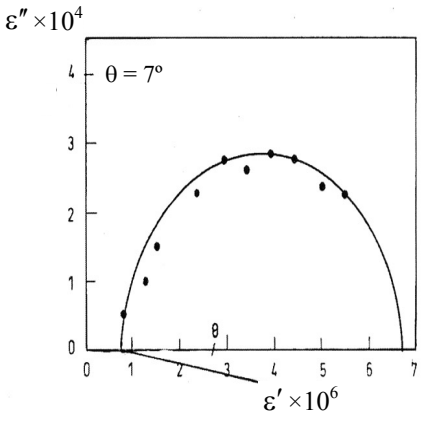


Fig. 8a. The variation of the relative permittivity ε', dielectric loss ε'', and conductivity σ as a function of the applied frequency (late effect).

Fig. 8b. The Cole-Cole plot ε' *versus* ε'' for volumes A_2, B_2 (late effect).

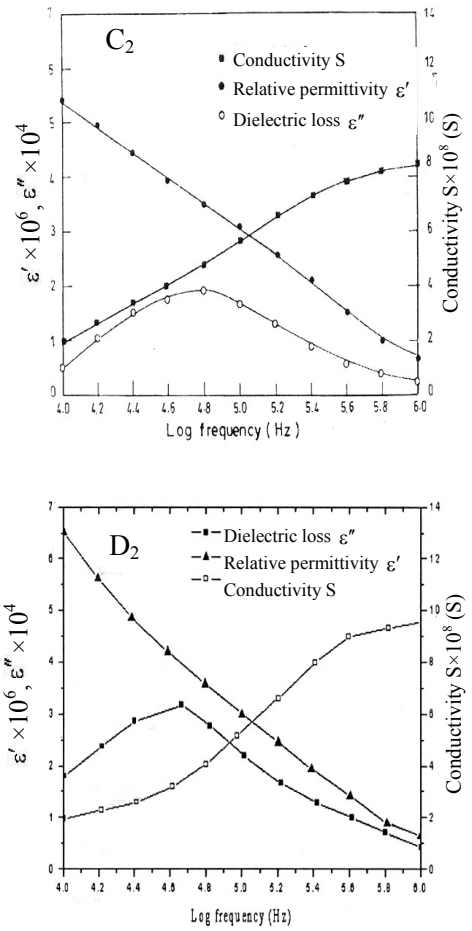


Fig. 9a. The variation of the relative permittivity ε′, dielectric loss ε″, and conductivity σ as a function of the applied frequency (late effect).

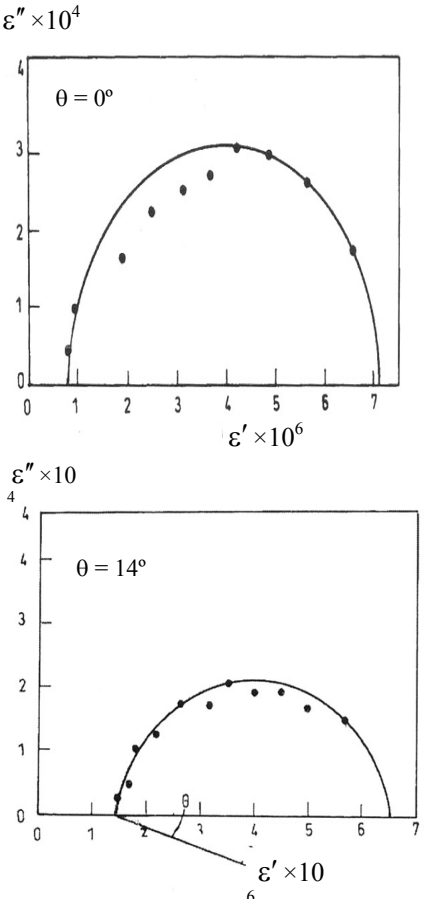


Fig. 9b. The Cole-Cole plot ε′ *versus* ε″ for volumes C_2, D_2 (late effect).

It is clear from Table 3 that the relaxation time τ decreased from 5.55 μs for unexposed to 3.88 μs and 3.54 μs for exposed microorganisms to 6 h and 16 h respectively. This decrease in τ is an indicator for the decrease of the molecular weight of the protein molecule. Moreover, the value of the Cole-Cole parameter α was 0 for protein molecules extracted from unexposed cells, which indicates that the water soluble protein molecules have a spherical form. Exposure of the

microorganism to magnetic field of 6 h and 16 h caused the increase of the value of α to be 0.05 and 0.09, respectively, which indicates the shape of the molecule is changed and that there is a change in the charge distribution on the protein molecules, that the protein molecules are changed from spherical to form semi-circle respectively.

Since the changes in the dielectric increment Δέ are function of the changes in the dipole moment of the protein macromolecules, which means changes in the center of mass of the charge distribution of molecules. Hence, one may conclude that there are some biochemical changes run within the protein molecules resulting from the irradiation process which led to the destruction of protein macromolecules and caused changes in both shape and volume [13].

For the late effect, the results in Table 4 indicate the progressive increase of τ and α in addition to the decrease of Δε and conductivity.

The changes in the molecular structure of the water soluble protein extracted from the microorganism following its exposure to magnetic fields may be one marker of the genetic changes in the DNA of *E. coli* cells. The data of the dielectric relaxation, as given in Tables 3 and 4, may spotlight the changes that occurred in the molecular structure of the protein.

RESULTS AND DISCUSSION OF ELECTROPHORESIS

The disc electrophoresis pattern and the molecular weight distribution of the WSP extracted from *E. coli* cells are shown in Figures 10a and 10b, for volumes A_1, B_1 and D_1 (direct effect), and in Figures 11a and 11b, for volumes A_2, B_2 and D_2 (late effect).

The scanning profiles of the electrophoresis separation indicate that WSP extracted from unexposed *E. coli* separated into 23 fractions having molecular weight in the range 160→ 20 kDalton but after an exposure period of 6 h the number of fractions became 6 only while for an exposure period of 16 h, the number of fractions became 26.

Since there are changes in the molecular weights of the fractions where there are fluctuations in the fractions towards higher molecular weights for exposure periods of 16 h and 6 h relative to unexposed cells. The increase or decrease in fractions intensity in the present study after exposure to the magnetic field can be interpreted on the basis of the gene mutation at regulatory systems that control the concerned structural genes [1]

This result is also in a good agreement with Hassan [14] where the changes in the protein electrophoresis profiles have been attributed to the occurrence of gene mutation and may change due to activation of stress protein production.

Figures 12 and 13 illustrate the absorption spectra for the water soluble protein extracted from volumes A_1, B_1 and C_1, D_1 respectively.

The results indicate the characteristics of the isolated protein by absorption band at wavelength 306 nm for volume A_1 with intensity 1.56, but for volume B_1, by a broad band with intensity 1.4 at wavelength 308 nm. For volume D1 the results indicate the broadening of the characteristic band to expand till 350 nm in addition to the increase in the intensity to become 1.6 at 314 nm.

Figures 14 and 15 illustrate the absorption spectra for the water soluble protein collected 2 hours post exposure of the *E. coli* volumes A_2, B_2 and C_2, D_2, respectively. The results indicate that the intensity of the band of volume B_2 was 1.45 at wavelength 308 nm, but for volume D_2, it was 1.64 at wavelength 313 nm.

Further, the results of the pathogenesis test, shown in Table 5, revealed the fact that there was a lowering effect in the virulence of exposed bacteria than in unexposed ones. The mortality rate recorded after the injection with *E. coli* exposed for 6 h was 40% whereas it was 80% for the cells exposed for 16 h.

RESULTS AND DISCUSSION OF MORTALITY RATE

One may find out from the above mentioned results that the molecular structure of the water soluble proteins from the microorganism, after being exposed to the magnetic fields, has changed. This change in the structural properties of the proteins resulting from the exposure to magnetic field may be due to the resonance interference of the applied magnetic field with the metabolism of these proteins. Moreover, the magnetic field at such a frequency (50 Hz) may also be in resonance with one of the metabolic activities that might occur through the *E. coli* membrane.

Table 5

Mortality rate of exposed and unexposed *E. coli*

Condition	Group number	Number of animals	Dose of injection/animal	Mortality rate	
				No.	%
Unexposed	1	10	0.1 ml saline	5	50
Exposed 6 h	2	10	0.1 ml	4	40
Unexposed	3	10	0.1 ml	6	60
Exposed 16 h	4	10	0.1 ml	8	80

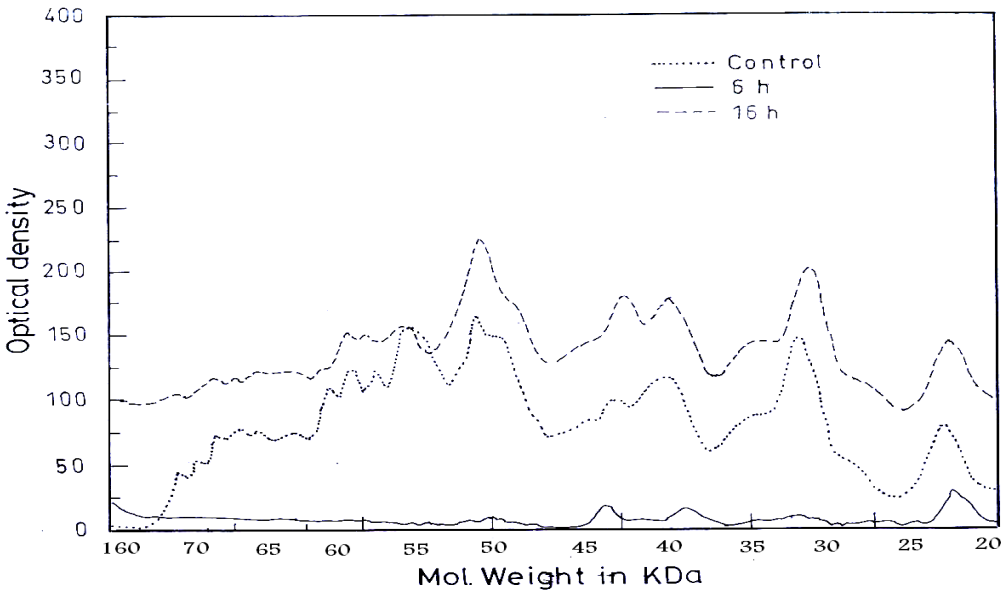


Fig. 10a. The molecular weight distribution of WSP extracted from *E. coli* (direct effect).

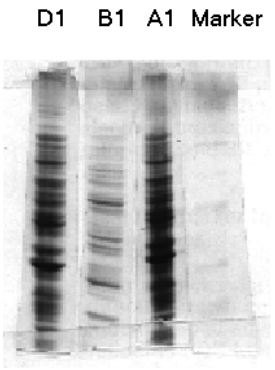


Fig. 10b. The electrophoresis pattern of WSP extracted from *E. coli* (direct effect).

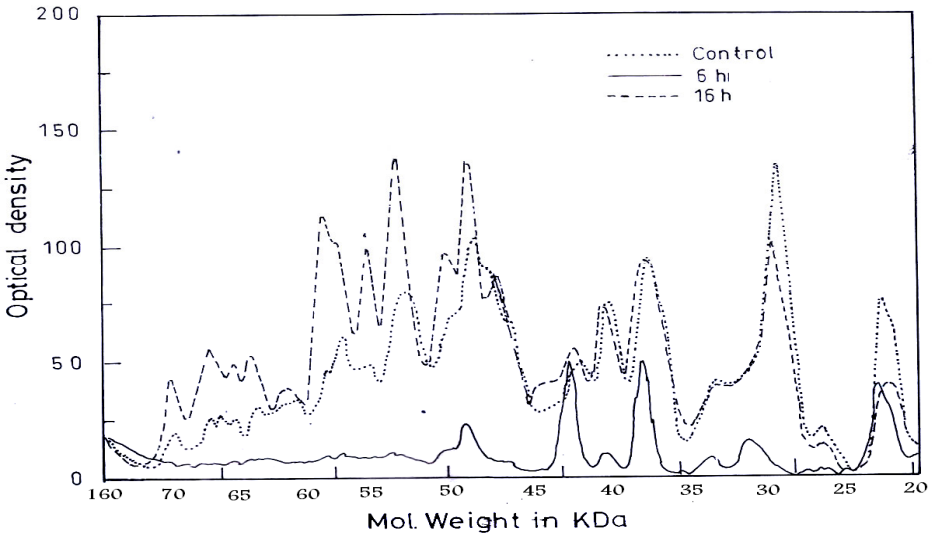


Fig. 11a. The molecular weight distribution of WSP extracted from *E. coli* (late effect).

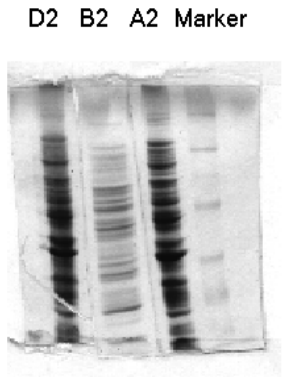


Fig. 11b. The electrophoresis pattern of WSP extracted from *E. coli* (late effect).

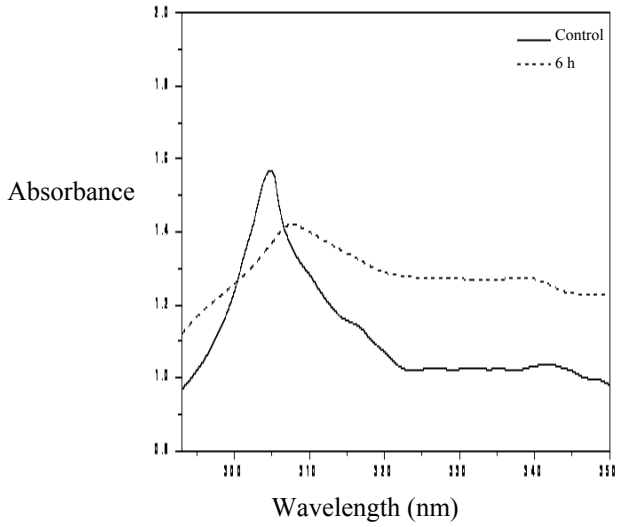


Fig. 12. The U.V absorbance spectrum for water soluble protein extracted from *E. coli* (6 h), direct effect.

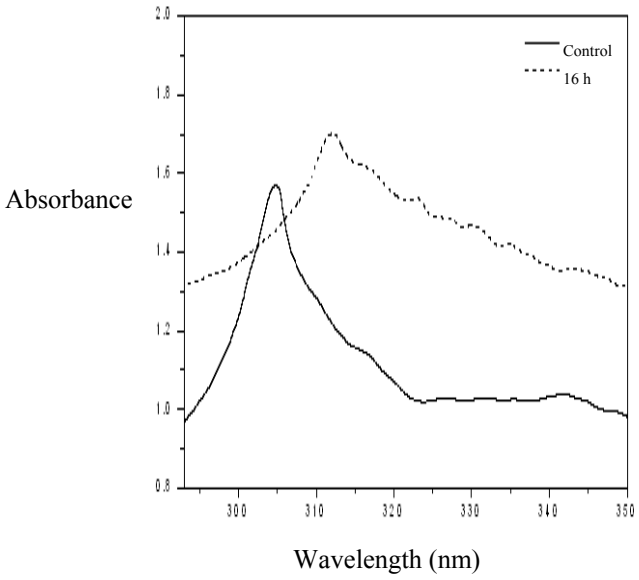


Fig. 13. The U.V absorbance spectrum for water soluble protein extracted from *E. coli* (16 h), direct effect.

The results also proved that the exposure time to magnetic field is one parameter of the induced changes in the molecule structure and also in the cell division. This finding may be concluded from the studies of the late effects of the magnetic field which indicated that the induced change in the growth characteristics of *E. coli* and structural properties of the extracted proteins begin to disappear.

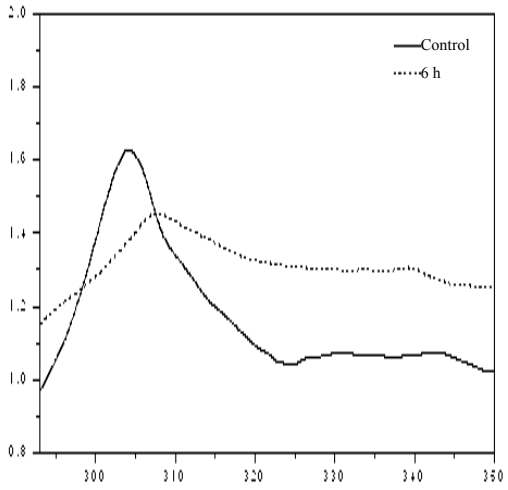


Fig. 14. The U.V absorbance spectrum for water soluble protein extracted from *E. coli* (6 h), late effect.

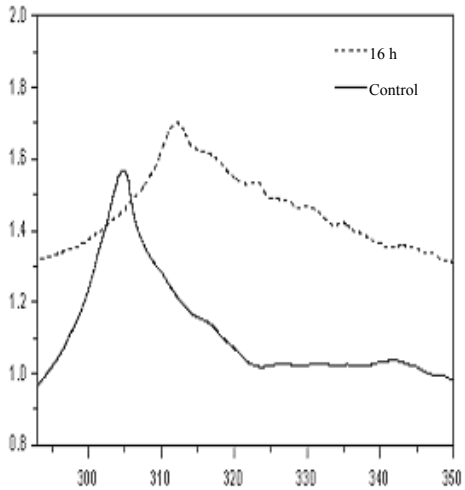


Fig. 15. The U.V absorbance spectrum for water soluble protein extracted from *E. coli* (16 h), late effect.

To get a better insight into the interaction mechanism of the magnetic field with the biological systems the understanding of the bioelectrical signals resulting from the biological system during metabolic activity is required. Mohamed *et al*. [21] reported that the bioelectrical signals from the microorganism were normally carried out through bending of their cellular membranes which generate an electric impulse through phenomena known as flux electricity. The amplitude and the frequency of these impulses depend on the magnitude and frequency of bending. These impulses travel through the medium separating the microorganisms and are received by the signal receptors at the surface and that impeded the cell membrane. Therefore, the flexibility of the membrane is the most important parameter for generation of these signals. There is also mentioned that the bio magnetic field from the biological system associated to the bioelectrical signals from the membrane of the cells through its metabolic function is very weak in nano Gauss range (20×10^{-8} G). When the biological systems exposed to an external magnetic field whose strength is very large relative to the bio magnetic field of the cells, a disturbance in their metabolic function will be expected which leads to death of the cells or increases their cell division, Fadel *et al*. [7] and Shin-Ichiro *et al*. [29].

From the present data it is easily deduced that the cellular membrane of the microorganism had been affected by the external magnetic field, in a good agreement with Fadel *et al*. [7]. The disturbance of cell division and, hence, a change in the number of cells per ml, and the measured change in the membrane sensitivity to antibiotic demonstrate also the change in the internal structure of the cells.

CONCLUSION

From this work it is concluded that the electromagnetic field (20 G) affected considerably the virulence of *E. coli* cells. 6 h exposure time was found to cause an inhibition case, whereas 16 h exposure time enhanced the virulence.

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