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Changes in *Ralstonia solanacearum* Cells and its Pathogenicity Due to Exposure to Extremely Low Frequency Electromagnetic Field

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Significant ultrastructure changes in *Ralstonia solanacearum* cells were observed using transmission electron microscope (TEM) after exposure to extremely low frequency (ELF) electromagnetic (EM) (1 Hz QAMW for 1 h) as well as DNA mutations and genetic alterations using RAPD PCR technique were detected. TEM was used to examine the changes in *R. solanacearum* cells exposed to 1 Hz QAMW (quantitative amplitude modulated waves) for 1 h in which elongation, deformation, heterogeneous appearance of cytoplasm disruption, disintegration of cell wall and retraction of cytoplasmic membrane were observed. Three of five RAPD PCR primers screened showed the appearance of new bands at 600, 450 and 550bp in the RAPD patterns for primers, P2, P3 and P4, respectively, after the exposure of *R. solanacearum* to 1 Hz QAMW. Potato tubers were kept immersed, for 30 minutes at room temperature, in bacterial suspension that exposed to QAMW at resonance frequency of growth inhibition then dried for 10 minutes and planted under greenhouse conditions. Symptoms of wilt disease did not appear on plants treated with exposed bacteria compared with those treated with non-exposed. Although, the tubers of plants treated by exposed bacteria were small compared with healthy one, the disease did not appear in these tubers. The results indicated that, extremely low frequency of electromagnetic field modifies the cell structure and the DNA sequences which could inhibit the growth of *R. solanacearum* and affect the pathogenicity of the microbe.

Keywords: DNA, electromagnetic field, electron microscope, growth inhibition and *Ralstonia solanacearum*.

Root and tuber crops are the most important food commodities produced in many subtropical and tropical countries. World production figures show that root and tuber cultivation is increasing and the rich sources of carbohydrates in the tropical and subtropical region, where clonal reproduction and poor soils give those advantages for subsistence agricultures and are occupied the level second to cereals in total world supply (Scurrah *et al.,* 2005). In Egypt, potato has an important position among all vegetable crops, where about 20% of total area devoted for vegetable production is cultivated with potato. In addition, total cultivation of potatoes reached 197.250 feddan (4200 m²) which produced 2,039,350 tons of tubers with an average yield of 10.34 tons/Feddan (Abd-Elgawad *et al.,* 2008).

This crop is economically important to Egypt and any disturbance in its production affects severely its local and more importantly export impact. During their seasonal plantations (Summer, Nili and Fall), potato plants are subjected to numerous pathogens and insect pests which cause considerable loss in Egyptian quantitative and qualitative potato yield. Such pathogenic and insect problems include the fungal pathogens (and their diseases): *Alternaria solani* (early blight) and *Phytophthora infestans* (late blight); bacterial pathogens: *Ralstonia* (*Pseudomonas*) *solanacearum* (brown rot or bacterial wilt), *Erwinia carotovora* subsp. *atroseptica* (black leg and rot Erwinia), *Clavibacter michiganense* subsp. *sepedonicum* (ring rot) and *Streptomyces scabies* (common scab); nematode diseases especially those caused by *Meloidogyne incognita*, *M. javanica* and *M. arenaria* and virus diseases.

Thus, a key barrier to the improvement of potato in Egypt is the reduction in yield and tuber quality caused mainly by potato pests and pathogens (Abd-Elhak *et al.*, 2000 and Abd-Elhak, 2005). *R. solanacearum* is a strictly aerobic, non-spore forming, Gram-negative organism, with a wide and diverse host range affecting several hundred plant species from 44 families, including the Solanaceae, Compositae and Leguminosae. Host plants of economic importance include potato, tomato, tobacco, pepper, eggplant, groundnut and banana.

In addition, several ornamental plants and weeds can act as host reservoirs of infection. *R. solanacearum* is very complex and highly variable. Strains of *R. solanacearum* are grouped into five races according to the host or hosts primarily affected and five biovars according to the use of selected biochemical properties (Kabeil, 2005). Of the five races, 1 and 3 cause symptoms on potato, with major yield losses from rotting tubers (brown rot) and wilting with subsequent death of the plant (bacterial wilt). Race 3 is adapted to pathogenesis at lower ambient temperatures and is believed to have originated in the temperate highlands of Peru and Bolivia. It is closely associated with the potato and is responsible for the present brown rot outbreaks in Europe and North Africa (Russell, 2008). Although *R. solanacearum* (race 3) is a soil borne pathogen that persists in wet soils, deep soil layers (75 cm) and reservoir plants (Van Elsas *et al.*, 2001), its distribution in potato fields can be spotty and is commonly found in tomato caused by *R. solanacearum* (race 1, biovar areas that have poor drainage (Stevenson *et al.*, 2001). Infection of the potato plant commonly occurs via the soil, where bacteria enter the root system of the plant at root emergence points, at wound sites, *e.g.* caused by nematode activity or soil particle abrasion, or via infected mother tubers. The pathogen has a quarantine status in the European Union (EU) to restrict its spread, as infections can be very destructive and cause considerable yield losses. In Egypt, potato plants are considered one of the most important hosts of *R. solanacearum*. In addition to considerable qualitative and quantitative yield losses caused by the brown rot disease, the existence of *R. solanacearum* in soil hinders the cultivation of potato, in such a soil, for the production of seed tubers or exportation. As *R. solanacearum* is a quarantine organism there can be large costs due to disease testing and administration of seed production to control the disease. In March and April of 1996, France, Finland, Spain and Denmark banned imports of potatoes from Egypt on the basis of

continued interceptions of potato containing *R. solanacearum*. By Decision 96/301/EC of May 3, 1996 the European Commission (EC) imposed a series of "additional restrictions" on imports of potatoes from Egypt.

Previously, Abdelbacki *et al.* (2011) found out the resonance frequency of the electromagnetic waves that inhibit the activity of *R. solanacearum* and its ability to make division [1 Hz QAMW (quantitative amplitude modulated waves) for 1 h]. Consequently, this study was designed to investigate the changes that may occur in the molecular level and changes in bacterial cells as a result of exposure to ELF-EMFs using RAPD PCR technique and transmission electron microscope (TEM), respectively.

Materials and Methods

R. solanacearum test strains:

R. solanacearum isolate was obtained from the Plant Pathol. Dept., Fac. of Agric., Cairo Univ., Egypt. Physiological and biochemical tests were carried out according to the methods described by Hayward (1964), meanwhile bacteriological characteristics have been done according to Bergey's manual of systematic bacteriology (Palleroni, 1984 and Abdelbacki *et al.*, 2011).

Measurements of bacterial growth:

As described in Abdelbacki *et al.* (2011).

ELF EM field application:

As described in Abdelbacki *et al.* (2011).

Transmission Electron Microscope (TEM) examination:

The morphological changes of control group and group exposed to 1 Hz QAMW for 1 h Hz QAMW have been determined using TEM. In order to prepare the bacterial sample to be examined by TEM, the sample should undergo some processing (Demicheli *et al.*, 2007). The processing of bacterial cells (control and exposed) began after 1h of exposure, where the bacterial cells were collected and washed three times in PBS. The pellet was suspended in 2% glutaraldehyde in phosphate buffer, pH 7.2, and fixed overnight in the refrigerator. After fixation, the pellet rinsed three times in phosphate buffer, pH 7.2. The resulting pellet containing the cells was fixed in 1% osmium-tetroxide in phosphate, pH 7.2, for 3 h at 4°C and dehydrated with increasing concentrations of ethanol. After the 100% ethanol washes, cells were washed with 100% acetone and infiltrated with resin. Semi thin sections were prepared on glass slides through cutting at 1 um using the ultramicrotome. Sections were stained with Toludine blue for 5min. examined by light microscope model M-200M. Ultra-thin sections were cut using ultramicrotome Leica model EM-UC6 at thickness 90nm, mounted on copper grids (400 mish). Sections were stained with double stain (Uranyl acetate 2% 10 min followed by Lead citrate for 5min and examined by transmission electron microscope JEOL (JEM-1400) at the candidate magnification. Images were captured by CCD camera model AMT, optronics camera with 1632 x 1632 pixel format as side mount configuration. This camera uses a 1394 fire wire boarded for acquision. This work was done in TEM Lab. FARP., Fac. of Agric. Res. Park, Cairo Univ.

DNA study:

DNA was extracted from 50 mg of fresh *R. solanacearum* suspension (either control or exposed) according to the method developed by Dellaporta *et al.* (1983). The quantity of extracted DNA was measured by means of agarose gel electrophoresis and confirmed by spectrophotometer (Hoisington *et al.,* 1994). The extracted DNA subjected to amplification reaction via the polymerase chain reaction (PCR) manufactured by Thermocycler T1, Biometra, Germany. The PCR mixture consists of PCR beads tablet (manufactured by Amessham Pharmacia Biotech). The Cod, nucleotide sequence and G+C percentage of tested primers used in the random amplified polymorphic DNA (RAPD) reactions are shown in Table (1). The amplified DNA of all groups was electrophoresed using electrophoresis unit (wide mini-sub-cell GT Bio-RAD) on 2% agarose containing 0.5 µg/ml of ethedium bromide, at a constant 75 volt and 60 mA, and visualized with UV trans-illuminator. Then DNA gel was scanned for band, using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6 C, Fullerton, CA 92631). The different molecular weights of bands were determined against a DNA standard (100bp DNA ladder, Stratagene, Canada) with molecular weights 80, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1030bp. The similarity level was determined by un-weighted pair group method based on arithmetic mean (UPGMA).

Table 1. Code, nucleotide sequence and G+C (%) of arbitrary primers used in the RAPD reactions

Primer	Sequence	G+C %
P1	5′- GGTGCGGGAA- 3′	70
P2	5′- GTTTCGCTCC- 3′	60
P3	5′- GTAGACCCGT- 3′	60
P4	5′- AAGAGCCCGT- 3′	60
P5	5′- AACGCGCAAC- 3′	60

In vivo study:

In order to infect potato with *R. solanacearum*, 480 ml of bacterial suspension has a concentration of 10^{12} CFU/ml was prepared and divided equally into two groups, one group kept as control and the other one exposed to QAMW at resonance frequency of growth inhibition for a period of 1 h. At the end of the exposure period, the bacterial suspension of each group undergo to centrifugation at 14,000 rpm and 4°C for 10 minutes under sterilized conditions, the pellets were then harvested, washed with sterile deionised water twice more and finally re-suspended in sterile deionised water. Healthy potato tubers were brought and divided into three groups; the first one immersed in the bacterial suspension that exposed to QAMW at resonance frequency of growth inhibition, the second group immersed in the non-exposed bacterial suspension and the last group was the control group where the potato immersed in deionised water. All groups kept immersed for 30 minutes at room temperature. After that the potato tubers of all groups were lifted, dried for 10 minutes and planted under greenhouse conditions. Symptoms were then

followed-up after planting in order to note the appearance of any symptoms on both; leaves and tubers of plants treated with bacterial suspension exposed to QAMW at resonance frequency of growth inhibition and those of plants treated with non-exposed bacterial suspension to compare them with normal plants (treated by deionised water).

Results

Bacterial images by TEM:

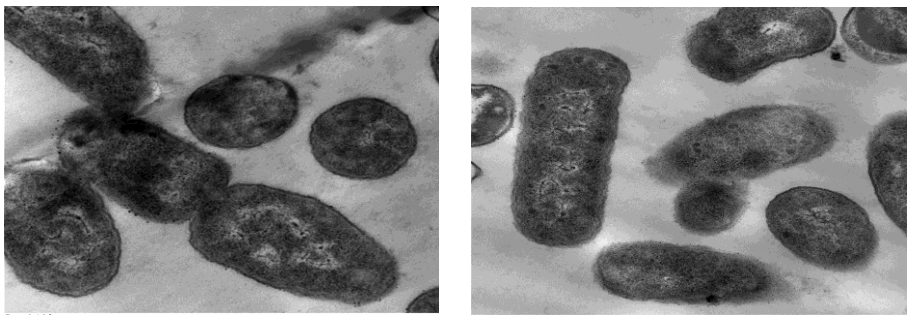
The bacterial cells of *R. solanacearum* were examined using TEM. The examination of bacterial cells including both control group (non-exposed) and group exposed to 1 Hz QAMW for 1 h. The normal *R. solanacearum* cells as viewed by TEM are shown in Fig. (1a). It is clear from the figure the appearance of flagella, cell wall, cell membrane, ribosomes which give the cytoplasm of bacteria a granular appearance, and bacterial DNA. Significant ultrastructural changes were observed on the morphological shape of bacterial cells after exposure to 1 Hz QAMW for 1 h. such as; elongation and deformation of the bacterial shape in addition to fragmentation of DNA (Fig. 1b), a less dense electron space and a heterogeneous appearance of cytoplasm which is an indicative to the dissolution of the cell wall, disruption and disintegration of cell wall, retraction of cytoplasmic membrane (may have been one of the reasons leading to disintegration of cell wall) and presence of *R. solanacearum* ghost cell representative of retracted cytoplasmic material are seen in Fig. (1b), an extrusion of cytoplasmic contents from cell wall and almost completely dissolution of the cytoplasm are also observed in Fig. (2). Furthermore, disintegration of cell wall and cytoplasmic membrane, retraction of cytoplasmic membrane and almost completely dissolution of the cytoplasm of exposed bacterial cells during the binary fission process are seen in Fig. (3a) comparing with the binary fission of non-exposed bacterial cell (Fig. 3b).

DNA analysis:

Electrophoretic RAPD patterns of *R. solanacearum* DNA extracted from control and treated group by the inhibiting resonance frequency 1.0 Hz QAMW for 1 h showed that only three out of five screened primers indicated the appearance of new bands at 600, 450 and 550bp for primers, P2, P3 and P4 respectively after the exposure of *R. solanacearum* to 1.0 Hz QAMW, (Fig. 4a, b and c). There is no difference in the RAPD patterns for both control and treated groups appeared for primers P1 and P5.

In vivo results:

The *in vivo* study has been carried out on 100 potato plant and tubers originated from tubers treated by deionised water, tubers treated by non-exposed bacterial suspension and tubers treated by bacterial suspension exposed to 1 Hz QAMW for 1 h. The results indicated that the symptoms of potato brown rot disease appeared on infected plants after 20-30 days from planting. Fig. 5 (A, B, C, & D) show the symptoms of potato brown rot disease in plant treated by non-exposed bacteria. The symptoms include; sudden wilting, droopy appearance of branches and the branches gradually turn bronze and die, unusual browning of vascular bundles in the stem.



a b

Fig. 1. TEM image of normal *R. solanacearum* cells (a). TEM image of exposed *R. solanacearum* cells; showing elongation and deformation of bacterial cells (b) (Magnification 30000x).

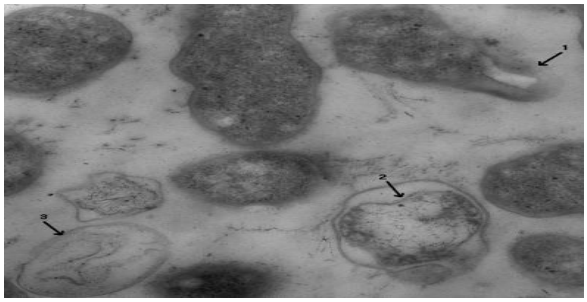
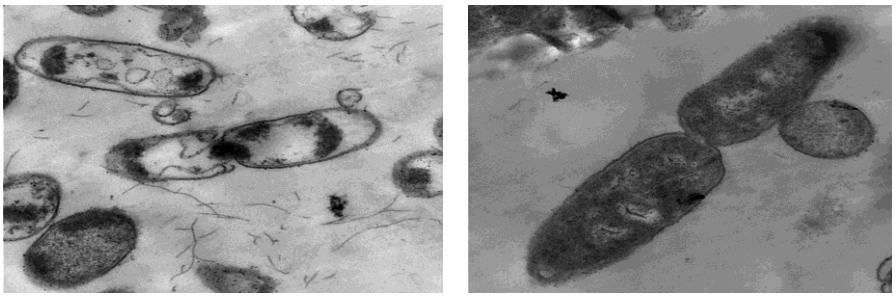
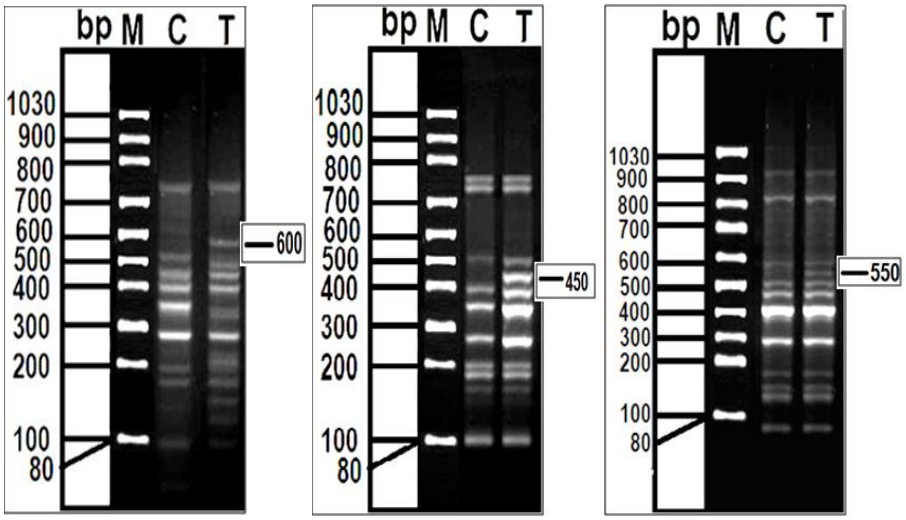


Fig. 2. TEM image of exposed *R. solanacearum* cells. Arrow 1 indicates a disruption and disintegration of cell wall, arrow 2 indicates retraction of cytoplasmic membrane and arrow 3 indicates *R. solanacearum* ghost cell (Magnification 30000x).



a b

Fig. 3. TEM images of binary fission of *R. solanacearum* cell. (a) Exposed cells show lyses during the binary fission and extrusion of cytoplasmic contents from cell wall and almost complete dissolution of the cytoplasm and , and DNA fragmentation. (b) control cells. (Magnifications, 50000x for image a, and 40000x for image b).



a **b** **c**

Fig. 4. Electrophoretic RAPD patterns of *R. solanacearum* DNA extracted from control and treated groups by 1.0 Hz QAMW for 1 h. a) RAPD pattern using P2 showing appearance of a new band at 600bp for treated sample. b) RAPD pattern using P3 pattern showing appearance of a new band at 450bp for treated sample c) RAPD pattern using P4 showing appearance of a new band at 550bp for treated sample.

*** M= DNA Ladder (DNA Marker); C= control sample DNA; T= treated sample DNA.**

It was found that these symptoms did not appear on plants treated with exposed bacteria comparing with those treated with non-exposed bacteria and were appeared completely like healthy plants treated with water as shown in Fig. (6A, 6B, & 6C). At the end of the season tubers of the plants were collected and examined for brown rot appearance. It was found that the tubers of plants treated with water were completely healthy (Fig. 7a). Although the tubers of plants treated by exposed bacteria were small compared with healthy ones, the brown rot ring symptom did not appear on 95 tubers from 100 tubers were screened (Fig. 7b). Almost all plants treated by non-exposed bacteria were hit by the brown rot disease and the brown rot ring symptom was clearly appeared (Fig. 7c).

Fig. 5. Symptoms in infected plants treated with non-exposed bacteria after 30-45 days from planting, A) wilting of the plant, B) droopy appearance and wilt, C) branches gradually turned bronze, D) unusual browning of vascular bundles in the stem.

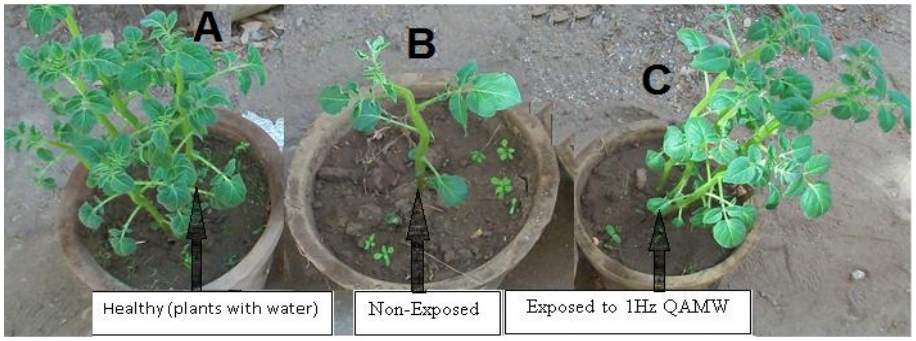


Fig. 6. Symptoms on the plants after 30-45 days from planting, A) plants treated with water, B) plants treated with non-exposed bacteria and C) plants treated with exposed bacteria to 1 Hz QAMW for 1hr.

a **b** **c**

Fig. 7. Potato tubers, a) plant treated by water, b) plant treated by exposed bacteria, and c) plant treated by non-exposed bacteria.

Discussion

Results of Abdelbacki *et al.* (2011) demonstrated that *R. solanacearum* lost capacity for normal division and unable to form colonies after exposure of to 1.0 Hz QAMW for 1 h. and showed growth inhibition by 66.43%. Fojt *et al.* (2004) observed a decrease in the number of viable bacteria following exposure to low-frequency electromagnetic fields at 50 Hz. Strašák *et al.* (2005) observed a decrease of optical densities for different bacterial strains exposed to a 50 Hz electromagnetic field at room temperature. David *et al.* (2006) reported that application of the ELF magnetic field allowed the deviating of the metabolic pathway of *Lactococcus lactis* subsp. *lactis* in order to intensify or to inhibit the nisin production. Cellini *et al.* (2008) demonstrated that the exposure of *E. coli* to a 50 Hz electromagnetic field acts as a stressing factor leading to phenotypical and transcriptional changes. The underlying biochemical effects of electromagnetic fields have not been clarified until now (Cellini *et al.*, 2008). It may be presumed from the present results that the effects of ELF-EMF on bacterial growth (*R. solanacearum*) are due to the interference of these fields (according to the frequency) with the bioelectric signals generated from physiological functions of bacterial cell. The results of these interference reactions depend on the mode of interference which may lead to inhibition (destructive mode) or enhancement (constructive mode) to the running physiological process. All these may lead to changes in cells chap, DNA and proteins structures.

The TEM examinations for both control bacteria and that exposed to 1 Hz QAMW for 1 h Hz QAMW showed significant morphological alterations which have been induced after the exposure of *R. solanacearum* cells to 1 Hz QAMW for 1 h. These alterations include; elongation and deformation of the bacterial shape and fragmentation of DNA. The bacterial cell elongation and deformation these occurred, for the treated cells indicate changes in the packing properties of the phospholipids bilayer macromolecules forming the bacteria cellular membrane. This analysis is supported by previous findings of Zhang *et al.* (2001), where he reported that if the charge on one side of the membrane is changed by a direct current electric field (DC-EF) the membrane tension also changes, resulting in a modified curvature of the membrane. Such flexoelectric effects have already been demonstrated on voltage-clamped cells. Additionally, osteoblasts and osteoblast-like cells undergo processes of retraction and elongation that ultimately realign their long axis perpendicular to the EF (Curtze *et al.*, 2004). Moreover, the other morphological changes caused by exposure to 1 Hz QAMW for 1 h. are similar to those reported by Ayse *et al.* (2011) and also similar to those induced by cationic peptides, where the less dense electron space and a heterogeneous appearance of cytoplasm (which indicative of the dissolution of the cell wall) resemble morphological changes produced by cationic peptides on *S. epidermidis* and *Staphylococcus aureus* (Friedrich *et al.*, 2000). Also, Rodriguez *et al.* (2004) have previously observed abnormal septation in *S. aureus* when a cationic preservative was applied which agrees with the present observation. The disruption and disintegration of cell wall, retraction of cytoplasmic membrane (may have been one of the reasons leading to disintegration of cell wall) and presence of *R. solanacearum* ghost cell

representative of retracted cytoplasmic material and the extrusion of cytoplasmic contents from cell wall and almost completely dissolution of the cytoplasm, all of these morphological changes are in agreement with those reported by Ayse *et al.* (2011) and also similar to those induced by cationic peptides.

DNA analysis where five primers were used to study the genetic sequences between the control and exposed bacteria, three primers showed different electrophoretic RAPD patterns. New bands were observed at 600, 450 and 550, for primers P2, P3 and P4 respectively. The DNA fragmentation observed after the exposure of *R. solanacearum* cells to 1.0 Hz QAMW for 1 h is due to the possibility of ELF-EMF to cause DNA damage. These results are in agreement with several experimental studies which reported both single and double strand breaks in DNA and other chromosome damage after exposure to ELF fields (Lai and Singh, 1997; Ivancsits *et al.,* 2005; Diem *et al.,* 2005 and Winker *et al.,* 2005). The changes in DNA initiated by ELF fields cannot be explained by thermal effects. Ultimately, these changes are due to the interaction of electric and magnetic fields with charges and magnetic dipoles of the DNA. Blank and Goodman (2004) and Blank (2005) reported that, relatively little energy is needed for effects on electron transfer where the electrons have very high charge to mass ratio and are most likely to be affected even by weak electric and magnetic fields. Hence the low energies in the ELF range perturb DNA by effecting on the electrons which probably found in the Hydrogen bonds (H-bonds) that hold the two chains of DNA together. The appearance of new bands in the DNA RAPD pattern of exposed bacteria prove that the DNA sequences have been changed under the effect of ELF EMF in a way that the primers used find a new binding sequences which not present in the control bacteria. The result indicated that the genetic sequences of bacterial DNA were modified due to the exposure to 1.0 Hz QAMW and subsequently protein and enzyme synthesis expressed from theses modified sites which may affect the bacterial growth (Van der Wolf *et al.,* 2004; Salanoubat *et al.,* 2001 and Lee *et al.,* 2001). Maercker (2005) and Lupke *et al.* (2006) detected similar changes in gene expression in cells after exposure to either radio-frequency electromagnetic; ELF or static fields. Since both hydrophobic and electrostatic interactions are involved in maintaining the organization of the membrane (Ingram and Buttke, 1984), it is reasonable to speculate that ELF-EMF has altered the electrostatic balance of the membrane components in a manner similar to that caused by cationic molecules, producing loss of integrity and/or disorganization and thus triggering growth arrest or death of bacterial cell.

The *in vivo* results showed that the symptoms of potato brown rot did not appear on plants treated with exposed bacteria comparing with those treated with non-exposed bacteria where the symptoms of potato brown rot disease were clear. Also, it was found that the tubers of plants treated with water were completely healthy, the brown rot ring symptom did not appear in more than 90% of the tubers of plants treated by exposed bacteria and almost all plants treated by non-exposed bacteria were hit by the brown rot disease. Based on these finding, it could be suggested that ELF EMF effect on the physiological behaviour and pathogenic genes of *R. solanacearum* which in turn affect the pathogenicity of the bacteria. This presumption could be confirmed by our results where the appearance of new bands

in DNA pattern after exposure to 1 Hz QAMW is an indicator for the genetic alteration that followed by change in gene expression and protein synthesis which effect on the physiological functions including cell division and pathogenicity of bacterial cell. All of these changes causing weaken of the exposed bacterial cells which in turn give a chance to the defence mechanism of plant to overcome the pathogen and prevent the disease.

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تغيرات فى خلايا بكتريا رالستونيا سولاناسيرام وكذلك فى قدرتها المرضية نتيجة التعرض للترددات المتناهية الصغر من المجال الكهرومغناطيسي

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لوحظت تغييرات معنوية لخلايا رالستونيا سولاناسيرام بإستخدام الميكروسكوب الإلكتروني نتيجة التعرض للترددات المنخفضة جداً من المجال الكهرومغناطيسي (1 Hz QAMW for 1 h) وكذلك تم رصد طفرات في الحمض النووي وتغييرات جينية باستخدام تكنيك RAPD PCR. تم استخدام الميكروسكوب الإلكتروني لمعرفة التغييرات التي يمكن أن تحدث لخلايا بكتريا رالستونيا سولاناسيرام لو عرضت للتردد 1 Hz QAMW لمدة ٦٠ دقيقة و لوحظ استطالة وظهور غير طبيعي للسيتوبلازم وكذلك تغير الجدار الخلوي والغشاء السيوبلازمي بالإضافة لتقطيع الحمض النووي لأجزاء صغيرة. بالنسبة لتكنيك RAPD PCR من بين ٥ بوادئ استخدمت أظهرت ثلاثة منها اختلافات حقيقية بين البكتريا غير المعرضة والأخري المعرضة وهي P2, P3 and P4 حيث ظهرت باندات جديدة عند وزن جزئيئ ٦٠٠ و ٤٥٠ و ٥٥٠ على التوالي. تم عدوي درنات بطاطس بالبكتريا المعرضة للمجال الكهرومغناطيسي ثم زراعتها لمعرفة تاثير ذلك علي مرضية الميكروب مقارنة بغير المعرضة. التجارب الحقلية أثبتت أن البكتريا المعرضة للمجال الكهرومغناطيسي كانت أقل خطورة علي نباتات البطاطس مقارنة بالأخري غير المعرضة اضافة لعدم ظهور العفن البني في درنات البطاطس الناتجة من نباتات تم عدواها بالبكتريا المعرضة مقارنة بتلك الناتجة من نباتات تم عدواها ببكتريا غير معرضة. النتائج أكدت أن الترددات المنخفضة جداً من المجال الكهرومغناطيسي تغير من شكل و تركيب الخلية البكتيرية و كذلك شكل و ترتيب قواعد النتروجين داخل نيوكليوتيدات الحمض النووي لخلايا بكتريا رالستونيا سولاناسيرام مما قد يؤدي لتثبيط نموها و كذلك يؤثر بشكل ملحوظ علي قدرتها المرضية.