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Possible Control of Sugarbeet Pathogen *Sclerotium rolfsii* Sacc. By Elf Amplitude Modulated Waves

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Abstract: The effect of extremely low frequency (ELF) amplitude modulated (AM) square waves in the frequency range 0.5 to 20 Hz on the *S. rolfsii* activity was studied. The effect of time of exposure to ELF AM waves on the linear growth and biomass gain of the fungus *in vivo* and *in vitro* was also included. The results indicated significant reduction in the linear growth and biomass gain of *S. rolfsii* after being exposed to square AM waves for 24 h *in vivo* or *in vitro*. The number of sclerotia, percentage of germination and germ tube length was dramatically affected. Sensitization of the fungus for 24 h counteracted the potency of the fungus exposed to frequencies above 0.5 Hz. 5.0 and 15 Hz were even stimulatory to seedling emergence of sugarbeet to values around the control non-infected plants.

Key words: *Sclerotium rolfsii*, sugarbeet, electromagnetic waves

Introduction

There are different models describing the interaction mechanism of electromagnetic waves with biological system (Liboff *et al.*, 1987; Lednev, 1991; Blanchard and Blackman, 1994). However, none of these models succeeded to explain with enough confidence, all biological changes following exposures to these electromagnetic waves. Recently Fadel (1998) suggested the biomagnetic resonance model to explain the mechanism of interaction of electric and magnetic field with the biological system. The model indicated that interaction of electromagnetic wave with biological phenomena could occur only when the frequency of the wave is at resonance frequency with the electromagnetic wave generated from specific physiological phenomena in the biological system. A successful application for this model to stop Ehrlich tumor growth in mice after being exposed to 4.5 Hz and 5 Hz square amplitude modulated waves is recently published (Fadel and Khater, 2002; Ghannam *et al.*, 2002). In a more recent work carried out in the same laboratory, it was possible to stop *Pseudomonas* microorganism cell division and generation of toxin after exposure of the microorganism *in vivo* or *in vitro* to 0.5 Hz AMW (El-Hag, 2002).

Pulsed electric field (PEF) processed orange juice, apple juice and tomato juice demonstrated superior quality than those thermally pasteurized. Microbial inactivation studies with three scales validate the ability of PEF process to scale up. With improved quality PEF process cost is very close to that of thermal processing.

The aim of this work is to find out whether or not the

different field frequencies (Hz) and/or exposure time will affect the biology of one of the pathogenic fungi hoping to apply it as a safe mean for control of *Sclerotium rolfsii* pathogen in sugar beet as well as others organisms in the future.

Materials and Methods

Sclerotium rolfsii Sacc., causing root rot of different sugarbeet cultivars grown in Egypt, was isolated from the saline soils and rhizosphere of beet cultivars and maintained on Czapek-Dox medium (El-Abyad *et al.*, 1988). To study the effects of extremely low frequency (ELF) electromagnetic (EM) waves on the activity of biological cellular function; it was necessary to carry these waves on a high frequency wave carrier. The biological system has very high impedance (X_c) for ELF EM waves because

$$X_c = \frac{1}{2\pi FC}$$

where F is the frequency of the wave and C is the capacitance of the electrodes applying the field. Therefore, in this work a high frequency sine wave amplitude modulated by square ELF EM wave which is known by amplitude modulated waves (AMW). Two-day-old fungus samples were exposed to square AMW which has a modulation depth of 1 Vpp and frequencies with the range 0.5 to 20 Hz. The modulated wave carrier was a sine wave of frequency 10 MHz. These waves were generated from two generators types (TTi- TGA 1230 synthesized arbitrary wave from generator and Sony Tektronics AFG

310 arbitrary function generator, Japan) at the Biophysics Department, Faculty of Science, Cairo University; for 1 and 24 h applying 0.5, 1.0, 5.0, 10.0, 15.0 or 20.0 Hz.

Growth criteria of the exposed fungus were then compared with those of non-exposed controls. *In vitro* linear growth was determined on sterile Czapek-Dox plates inoculated with a disc of agar bearing mycelium of exposed to the above-mentioned Hz and controls. The plates were incubated at 27°C and three plates were set up for each treatment. Colony diameters were measured daily.

Method of El-Abyad and Saleh (1971) was used to study the *in vivo* growth of the exposed fungus to different AMW (Hz). Growth was measured daily between 2 and 7 days and compared with control.

The dry weight yield was determined by inoculating 80 ml of Czapek-Dox medium flasks with 6 mm agar disc bearing mycelium of the 2 days old fungus directly after the exposure to different AMW field of different frequencies Hz. Control flasks with unexposed fungus were also included. All flasks were incubated at 27°C for 12 days and the mycelial felts were dried to constant weight at 80°C and the dry weight estimated.

Percentage germination of sclerotia and length of the germ tubes were carried out after 10 days growth; sclerotia were maintained on agar water medium for 3 days at 27°C applying the method of Abeygunawardena and Wood (1957). The production of sclerotia by *S. rolfsii* was

studied employing the agar medium described by Rodriguez-Kabana (1969).

In vivo experiment: This experiment was conducted in order to study the effect of the pathogenic fungus (*S. rolfsii*) on the emergence of seedlings under different field frequencies (Hz). The soil used in this study was obtained from the Agricultural Research Center, Giza, of pH 7.6 and salinity of 0.340 millimohse, measured by electric-conductivity meter (Model LF 90, Germany). Pots containing 1 kg clayey soil inoculated with *S. rolfsii* maintained on barely grains directly after exposure of the fungus to different field frequencies (Hz). The pots were left for 3 days before the seeds were sown.

Ten seeds of either Poly N, Farida or Raspoly sugarbeet cultivars were sown in each pot. Pots were left for 4 days after which the number of emerged seedlings was recorded for 15 days.

Statistical analysis: The obtained data were carried out according to Snedecor and Cochran (1980), using LSD to compare the significance of the results.

Results and Discussion

It should be recalled that 24 h exposure of 3 mm discs of *Sclerotium rolfsii* to AMW field means 24 h (1 day) growth under continuous treatments which means that

Table 1: Effect of various levels and exposure periods to amplitude modulated waves (AMW) frequencies (Hz) on the *in vitro* linear growth of 3-mm diameter disc of *Sclerotium rolfsii* (in mm) during 5 days incubation period at 27°C

Frequency (Hz)	Exposure time (h)	Incubation period (days)				
		1	2	3	4	5
Control (0)	1	14.0	33.0	56.3	75.6	90
	24	19.0	29.0	48.3	72.0	90
0.5	1	14.3	27.0	50.0	71.0	90
	24	18.3	30.6	52.0	79.3	90
5.0	1	14.6	32.0	56.6	74.6	90
	24	18.0	26.3	44.0	58.0	88
10.0	1	14.6	32.3	55.3	77.0	90
	24	18.3	28.3	50.3	70.6	90
15.0	1	14.3	36.3	59.0	80.3	90
	24	17.0	31.3	53.0	80.0	90
20.0	1	143.0	33.0	55.0	74.3	90
	24	19.6	31.0	51.0	77.6	90
5%		1.5	2.2	2.3	1.8	0.0
LSD (exposure period 1 hr.)						
1%		2.9	3.3	3.6	2.8	0.0
5%		2.0	1.4	2.0	6.4	0.0
LSD (exposure period 24 hrs.)						
1%		3.1	2.8	3.1	10.9	0.0

Table 2: Effect of different levels and exposure periods to amplitude modulated waves (AMW) frequencies (Hz) on the *in vivo* linear growth (in soil tubes) of *S. rolfsii* (in mm) after 7 days incubation period at 27°C

Exposure period (h)	Frequency (Hz)							LSD	
	0	0.5	1.0	5.0	10.0	15.0	20.0	5%	1%
1	47.6	48.6	46.6	51.3	48.0	34.0	32.6	2.0	3.0
24	47.6	28.6	20.0	21.6	25.8	22.6	20.7	10.6	16.0

Table 3: Effect of different levels and exposure periods to AMW frequencies (Hz) on the dry biomass gain (mg) by *S. rolfsii* after 12 days incubation period at 27°C

Exposure period (h)	Frequency (Hz)							LSD	
	0	0.5	1.0	5.0	10.0	15.0	20.0	5%	1%
1	151	137.7	125.7	105.3	147.6	148	118.6	3.8	5.0
24	151	110.0	108.0	063.0	115.3	149.3	091.6	3.9	5.7

Table 4: Effect of different levels and exposure periods to AMW frequencies (Hz) on the number of sclerotia/cm² of mycelial felt of *S. rolfsii* after 10 days incubation period at 27°C

Exposure period (h)	Frequency (Hz)							LSD	
	0	0.5	1.0	5.0	10.0	15.0	20.0	5%	1%
1	24	24	21	25	19	27	26	1.8	2.7
24	24	10	11	10	13	18	20	5.7	9.0

Table 5: Effect of different levels and exposure periods to AMW frequencies (Hz) on the percentage germination of sclerotia of *S. rolfsii* after 3 days on tap water agar at 27°C

Exposure period (h)	Frequency (Hz)							LSD	
	0	0.5	1.0	5.0	10.0	15.0	20.0	5%	1%
1	100	100	100.0	100	100.0	100	95	0.0	0.0
24	100	100	36.6	60	66.6	45	80	6.3	9.6

Table 6: Effect of different levels and exposure periods to AMW frequencies (Hz) on the germ tube length of sclerotia of *S. rolfsii* after 3 days on tap water agar at 27°C

Exposure period (h)	Frequency (Hz)							LSD	
	0	0.5	1.0	5.0	10.0	15.0	20.0	5%	1%
1	18	10.3	20.0	20.0	21.3	26.6	13.6	0.8	1.3
24	18	11.7	11.3	15.7	3.3	3.0	2.0	1.4	2.0

Table 7: Percentage emergence of 3 sugarbeet cultivars exposed to infection by *S. rolfsii* treated with different levels and exposure period to AMW frequencies (Hz), 15 days post sowing

Exposure period (h)	Cultivars	Frequency (Hz)							LSD	
		0	0.5	1.0	5.0	10.0	15.0	20.0	5%	1%
1	Poly N	25.0	66.6	20.8	33.3	50.0	58.3	25.0	3.9	5.7
	Farida	54.1	16.6	58.3	58.3	54.1	0.0	0.0	3.2	4.9
	Raspoly	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	Poly N	25.0	45.8	29.1	25.0	66.6	75.0	54.1	19.8	30.9
	Farida	54.1	54.1	62.5	95.8	70.8	95.8	70.8	4.0	6.2
	Raspoly	0.0	0.0	0.0	8.3	21.9	4.2	5.3	2.8	4.4
Plain soil:	Poly N = 79.1	Farida = 66.6			Raspoly = 100.0					

these treatments are older than those exposed for 1 hour by one day, thus they are not comparable unless by the rate of growth which was limited by the diameter of the petri dishes (9 cm).

Within one hour exposure (Table 1), the *in vitro* linear growth was hardly affected by the AMW at 15 Hz where a significant increase in length dominated during the 3rd and 4th post treatment. 24 hours exposure was also stimulatory to linear growth at 0.5 and 1.0 Hz, inhibitory at 5.0 Hz and mostly without effect at the larger frequencies. The action of magnetic field causes either an intensified growth of living organisms or a slow down of their growth or even death (Sale and Hamilton, 1967; Zhang *et al.*, 1995; Jia *et al.*, 1999). On the other hand, data shows that the frequency and/or time of exposure played a significant role in reducing the *in vivo* linear growth of *S. rolfssii* (Table 2). Exposures to frequencies of the AMW (up to 10.0 Hz) for one hour insignificantly affected *in vivo* linear growth whereas larger frequencies were suppressive. On the other hand, longer exposure time (24 hours) favoured a sever drop *in vivo* growth of the fungus even at 0.5 Hz application; a response that was not affected by increasing the dose above 1 Hz. Wouters *et al.* (1999) and Eisa *et al.* (2002) reported that the growth of *Aspergillus flavus* and aflatoxin production (%) were reduced by exposure of the fungus to different frequencies of electric waves. These findings may be due to either biophysiological and chemophysiological changes, genetic variations in the fungus or reduction of the viability of cells. Table 3 further shows that 1 or 24 h exposure to electromagnetic waves lowered the biomass gain by 12-day-old *S. rolfssii*; a response that was lessened or alleviated by 10-15 Hz; above which a drop was noticed again (Table 3). Under all conditions, the effect was more prominent after 24 h exposure. Pulsed electric field was effective in inactivation of *Zygosaccharomyces bailii* and its ascospores showing a 4-log reduction of the yeast (Romine *et al.*, 1999). The results (Table 4) also reveal that the different field frequencies hardly affected the number of sclerotia/cm² of 10-days-old mycelial felts of *S. rolfssii* after 1 hour exposure; with 10 Hz being the exception which significantly reduced the number of sclerotia. Increasing the exposure time dramatically lowered the number of sclerotia/cm²; a response that was slightly alleviated by increasing the frequency to 15 or 20 Hz. Reina *et al.* (1998), Jeantet *et al.* (1999) and Ramsted *et al.* (2000) indicated that PEF induced greater reduction of viable cells of microorganisms and the use of high voltage PEF is a promising technology for inactivation of pathogenic

organisms. Yeast and mold cells of orange juice were less resistant to PEF process than bacteria (Jia *et al.*, 1999).

Table 5 further shows that all test frequencies of AMW did not affect the percentage of germination of sclerotia at one hour exposure. Increasing the exposure time suppressed the germination percentage that was most apparent at 1 Hz and least apparent at 20 Hz. Inspection of germination percent, the germ tube length (Table 6) was very sensitive to the AMW when applied for 24 hours. The sharp drop in tube length remarkably increased with increased dose application. One hour application effective only at 0.5 and 20 Hz whereas the other frequencies were ineffective if not stimulating to germ tube length. Hamilton and Sale (1967) used PEF and Dunn (1996) used intense pulse of light (pure bright) to kill all exposed microorganisms including vegetative bacteria, microbial and fungal spores, viruses and protozoan oocysts. Grahi and Mark (1996) studied the effect of PEF on bacteria, yeast spores in buffer solution and foodstuffs. They reported that the counts of vegetative cells were reduced. Killing of vegetative cell types depended on the electric field strength of the pulses and time of exposure.

Results showed that, under normal condition, Raspoly beet cultivar showed 100% germination of seeds whereas Farida cultivar was least germinated (66.6%). Soil inoculation with *S. rolfssii* reduced seedling emergence of either Poly N or Farida cultivar, more prominently for the former whereas germination of Raspoly cultivar was completely arrested (Table 7).

One-hour exposure of the fungus to amplitude modulated waves noticeably alleviated the fungal virulence (increasing the seedling emergence), most prominently at 0.5, 10 and 15 Hz frequencies but not reaching the control. The exposed fungus to AMW was more virulent than the control in lowering the percentage of germination causing complete arrest of seed germination of cultivar Farida at 15 or 20 Hz.

Sensitization of the fungus by AMW for 1 or 24 h did not affect its potency against the Raspoly cultivar except for a slight attenuation, at the large frequency applied reaching maximum at 10 Hz. The potency of the fungus was attenuated by 24 h application increasing with applied frequency up to 15 Hz augmented or arrested infection then dropped.

Sensitization for 24 h exposure counteracted the potency of the fungus exposed to frequencies above 0.5 Hz. 5.0 and 15 Hz were even stimulatory to seedling emergence to values around the control non-infected plants. The dielectric breakdown of the cell membrane occurs when the microbial cells are exposed to the high voltage PEF

(Zimmermann, 1986; Tsong, 1991).

Chow and Tung (1999) found that the exposure to magnetic field causes mutations to microorganisms and demonstrated that magnetic field can actually enhance the efficiency of DNA repair. Calderon *et al.* (1999) and Evrendilek *et al.* (1999) agreed that pulsed electric field (PEF) is a promising technology for inactivation the pathogenic microorganisms.

From these results one can assume that the application of an appropriate frequency of AMW for an appropriate time can be used for controlling the growth and virulence of *S. rolfii* to sugarbeet cultivars grown in Egypt.

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References

Abeygunawardena, D.V.W. and R.K.S. Wood, 1957. Factors affecting the germination of sclerotia and mycelial growth of *Sclerotium rolfii* Sacc. Trans. Brit. Mycol. Soc., 40: 211-223.

Blanchard, J.P. and C.F. Blackman, 1994. Clarification and application of an ion parametric resonance model of magnetic field interactions with biological system. J. Bioelectromagnetic, 15: 217-238.

Calderon, M., G.V. Barbosa and B.G. Swanson, 1999. Inactivation of *Listeria innocua* in skim milk by pulsed electric field and nisin. Int. J. Food Microbiol., 51: 31-38.

Chow, K.C. and W.L. Tung, 1999. Magnetic field exposure enhances DNA repair through the induction of Dnak/J. synthesis. J. Mol. Microbiol. Biotechnol., 1: 79-86.

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

Eisa, N.A., F.M. Ali, G.M. Habbaa, S.K. Abdel-Reheem and M.F. Abou-Ella, 2002. Pulsed electric field (PEF) as a promising technology for destroying aflatoxins production potentiality of *Aspergillus flavus* in stored corn grains. Proc. 2nd Conf. Foodborne Contamination and Egyptians Health, El-Mansoura, Egypt, 67-82.

El-Abyad, M.S., H. Hindorf and M.A. Rizk, 1988. Impact of salinity stress on soil-borne fungi of sugarbeet. I. Pathogenicity implications. Plant and Soil, 110: 27-32.

El-Abyad, M.S. and Y.E. Saleh, 1971. Studies with *Fusarium oxysporum* f.sp. *vasinfectum*, the cause of cotton wilt in Egypt: germination, sporulation and growth. Trans. Brit. Mycol. Soc., 57: 427-437.

El-Hag, M., 2002. Effect of ELF electric field on the biophysical properties and biophysical activity of some microorganisms. Ph.D. Thesis, Faculty of Science, Cairo University, Egypt.

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

Fadel, M.A. and I. Khater, 2002. A new method for treatment of solid Ehrlich tumors by extremely low frequency electromagnetic radiation (*in vivo* study). Proceedings of the 7th International Meeting on Progress in Radio-Oncology ICRO/ÖGRO 7, Salzburg, Austria, May 10-19, pp: 663-670.

Fadel, M.A., 1998. A new metabolic biomagnetic resonance model to describe the interaction of ELF-EM field with biological system, International School on Theoretical Biophysics, Moscow, May (1998).

Ghannam, M.M., R.H. El-Gebaly, M.M. Gaber and M.A. Fadel, 2002. Inhibition of Ehrlich tumors growth in mice by ELF electric impulses (*in vivo* studies). J. Electro and Magneto Biology, (in press).

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

Hamilton, W.A. and A.J.H. Sale, 1967. Effects of high electric fields on microorganisms. II: Mechanism of action of the lethal effect. Biochem. Biophys. Acta, 148: 789-800.

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

Jia, M., Q.H. Zhang and M.B. David, 1999. Pulsed electric field processing effects on flavor compounds and microorganisms of orange juice. Food Chemistry, 1: 8 (FOCH 2198).

Lednev, V., 1991. Possible mechanism for the influence of weak magnetic field on biological system. J. Bioelectromagnetic, 12: 71-75.

Liboff, A., R. Rozek, M. Sherman, B. Macleod and S.D. Smith, 1987. Ca45 cyclotron resonance in human lymphocytes. J. Bioelectricity, 6: 13-22.

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

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

Rodriguez-Kabana, R., 1969. Enzymatic interactions of *Sclerotium rolfii* and *Trichoderma viride* in mixed soil culture. Phytopathology, 59: 910-921.

- Romine, A., S. Li and H. Zhang, 1999. Inactivation of *Zygosaccharomyces bailii* by pulsed electric field technology. The International Meeting, Food Science and Technology (IFT), Ohio State Univ. Columbus, Ohio. July (1999).
- Sale, A.J.H. and W.A. Hamilton, 1967. Effects of high electric fields on microorganisms. I: Killing of bacteria and yeasts. *Biochem. Biophys. Acta*, 148: 781-788.
- Snedecor, G.W. and W.G. Cochran, 1980. *Statistical Methods*. 7th Ed. Iowa State Univ. Press, USA, 255-269.
- Tsong, T.V., 1991. Electroporation of cell membranes. *J. Biophys.*, 60: 297-306.
- Wouters, P.C., N. Dutruix, J.P. Smelt and H.L. Lelieveld, 1999. Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. *J. Food Prot.*, 62: 1381-1386.
- Zhang, Q.H., B.L. Qin, G.V. Barbosa-Canovas and B.G. Swanson, 1995. Inactivation of *E. coli* for food pasteurization by high intensity short duration pulsed electric fields. *J. Food Process Preserv.*, 19: 103-118.
- Zimmermann, U., 1986. Electric breakdown, electroporation and electrofusion. *Rev. Physiol. Biochem. Pharmacol.*, 105: 175-256.