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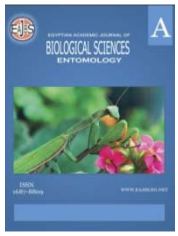

Evaluation of Electromagnetic Resonance Designed Pulses for Controlling Potato Brown Rot Caused by *Ralstonia solanacearum*

Fadel M. Ali[1], Naglaa M. Balabel[2], Kh. Gh. Elmalki,[3] A. Elmaghraby[4] and A. Amin[4]

1- Faculty of Science-Cairo university
2- Plant Pathology Res. Instit., ARC, Egypt
3- Plant Protection Res. Instit., ARC, Egypt
4- Engineering authority of Armed Forces

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ABSTRACT

One of the main problems facing potato production is brown rot. Potato brown rot is a systemic bacterial wilt disease caused by Aggressively colonize the xylem vessels causing a lethal wilting. Control of potato brown rot has proven to be a serious, very difficult and puzzling task. As a result it became one of the major obstacles in the total value of Egyptian potato exports . As potato brown rot was declared a quarantine disease of concern in the European Union.

This work aimed to asses the efficiency of very low waves to detect infestation of land and potatoes' tuber by brown potato rot as well as control that aggressive bacterium.

Rs-FAST device had been manufactured as specific detector of potato brown rot bacterium in both land and tubers. Results confirmed the efficiency of Rs-FAST device for that purpose. Results showed that, treating potato field by electromagnetic resonance pulses for one hour achieved 100% mortality of bacterium, *Ralstonia solanacearum* in soil and tubers. There was an insignificant difference between treating potato fields for one and two hours. Chemical analysis of certain potato leaves contents showed that, significant increase of total protein, total carbohydrates, nitrogen, potassium and phosphorus of treating leaves. Chemical analysis of potato tubers confirmed the results of leaves. It means that, treating potato fields by very low pulses of electromagnetic resonance improved the nutritional values of potato tubers as well as increased the crop yield by about 15% of control.

INTRODUCTION

The ability of *R. solanacearum* to survive in field soil is subject to controversy due to co-extensiveness of strains and diversity of host plants for certain races (Kelman, 1953; Buddenhagen and Kelman, 1964), latent infection (s), re-infestation of soil through irrigation water (Farag *et al.*, 1999) and last but not the least the lack of sensitive methods, at the time of the majority of studies had been carried out, for the detection of the pathogen at different strata of the soil profile. More recent studies, based in principal on molecular biology, have eliminated in part the difficulties related to the accurate sensitive detection potato lots (Elphinston e*t al.,* 1996, 2000). It could be concluded, however that ELISA, IFAS, immunofluorescence colony staining (IFC) may be considered as a good compromise between sensitivity and specificity of detection and between easy and expense of application (Robinson-Smith *et al.,* 1995;Elphinstone *et al.,* 1996).The present study was undertaken to determine the most predominant genera involved in the cross-reaction with the IFAS techniques (Balabel,2014).*R. solanacearum* is the causal organism of Potato Bacterial Wilt (PBW) or potato brown rot (PBR) a disease that, after late blight, is the second major constraint to potato production in tropical and subtropical regions worldwide (French, 1994). Regardless of the place of origin the movement of seed and/or commercial potato stocks either diseased showing symptoms or latently infected tubers is the main way for disease spreads (Graham *et al.,* 1979) and strict quarantine regulations have been applied to avoid dissemination of the disease in the member states of the European Union DirectiveNo.98/57EC.

Several detection methods, including isolation, immunofluorescence antibody staining (IFAS) analysis, enzyme-linked immunosorbent assays (ELISAs) and bioassays, though the apparent constraint for each of them, have been used for examination of potato (Elphinstone *et al.,* 1996, 2000). These methods differ from each other in the degree of sensitivities and specialization (Robinson-Smith *et al.,* 1995; Elphinstone *et al.,* 1996). Fadel *et al*. (2013) studied the effect of positive square pulsed electric field at different frequencies in the range 0.1-50 Hz, exposure periods on the growth characteristics of *P. aeruginosa*. Results indicated that exposure to PEF can inhibit bacterial growth at particular resonance frequencies 0.7 Hz and 0.5 Hz and significant increase in antibiotic susceptibility of protein and cell wall inhibitor. Also, results of DNA, dielectric relaxation and TEM indicated molecular and morphological changes. Federico *et al*. (2008) studied the effect of the application of PEFs to potato tissue on the diffusion or the fluorescent dye FM1-43 through the cell wall. Potato tissue was subjected to field strengths ranging from 30 to 500 V/cm, with 1 ms rectangular pulse, before application of FM1-43 and microscopic examination. Results showed a slower diffusion of FM1-43 in the electro pulsed tissue when compared with that in the non-pulsed tissue, suggesting that the electric field decreased the cell wall permeability. This is a FAST response that is already detected within 30 s after the delivery of the electric field.

In recent years, several studies have been accomplished by researchers to evaluate the effects of electric and magnetic fields on the viability of plants and biological commodities. Marinkovic *et al*. (2002) reported a 144.8% increase in potato yield as a result of electric field treatment and Navid *et al*. (2013) studied the physiological properties of maize seeds as affected by electric field intensity (2, 4, 9 and 14 KV) and exposure time (15, 45, 80 and 150 s). Results indicated that increasing the electric field intensity to 9 KV/m and increasing the exposing time caused the length of root to be decrease. The highest decrease was belonged to the80 s exposing time. Fadel, M. Ali *et al*. (2014) studied the effect of PEF on biophysical and structural properties of *R. solanacearum* at different frequencies to find out the most effective frequency that may coincide with the bioelectric signals generated during cellular technique on large scale farm field zone and the best application conditions. The results indicated maximum cell viability inhibition occurred after exposer for 60 minutes to 1.0 Hz. The diversity of soil bacterial community was not changed by the electric pulse, contrary to expectation (Yi et al., 2012) and added that, electric pulse caused an increase in lettuce growth, and improvement in nutritional soil conditions, and varied mass spectrometric patterns.

Although various control measures have been found, bacterial brown rot is still a difficult to control because of wide host range of the bacterium and long survival of the pathogen in soil (Hsu, 1991). Recommended of crop rotation with non-host plants, is not an efficient method, since *R. solanacerum* has its disseminating and survival phases in the soil and it remains viable for long time. The race and strain diversity of the pathogen has made breeding for resistant cultivars ineffective or with limited value in the control of bacterial wilt (Hanson *et al*., 1996, Wang *et al*., 1998 and Farag *et al*., 1982). Biological control methods may be used after being integrated with other practices for effective disease management at the field level (Myint & Ranamukhaarachchi, 2006).

The present study was undertaken to evaluate new technique for detecting infestation compared with the usual methods according European Union protocol No. 98/57EC. Also, to evaluate the efficacy of electromagnetic resonance waves for controlling *R. solanacearum* either in soil or in potato tubers.

The scientific principle of this new technology is based on the fact that alive systems produce electromagnetic waves (bio electricity) resulting from ionic motions during metabolic functions. The form and frequency of these electromagnetic pulses depend on the running physiological function in the specific organ. Interference between this bioelectric signals and external electric impulses can occur only when they are at resonance and the resulting wave will be the algebraic summation of the two waves. This interference will result in either enhancement or inhibition of the running physiological event, depending on the mode of interaction (Ali, F.M. 1998).

MATERIALS AND METHODS

Isolation of *R. solanacearum* from different potato fields in Egypt:

Isolates of bacterial pathogen in this study were recovered from different potato fields in Beni-Suif, Menoufia and Beheira governorates Governorates, Egypt. Sixteen samples from each location were taken from 20 cm depth, by using a sampling auger, mixed well and sieve through 2mm mesh size in the laboratory for homogenization. Tested sample was divided into (4) samples. Isolation of *R. solanacearum* from the soil was made on (SMSA) Semi Selective Media of South Africa medium (Elphinstone *et al*., 1996). Decimal dilution(s) of the suspension was made in 90 ml sterile phosphate buffer (0.05 M). Shacked for 2 hours at 15°C and were plated onto SMSA medium. The plates were incubated at 28°C for 3-6 days.

Immunofluorescent Antibody Staining (IFAS) test:

IFAS as a serological identification of *R. solanacearum* was carried out. The polyclonal antibodies were provided by Loewe Biochemical GmbH, Germany and produced in goats against *R. solanacearum* race 3, biotype II, cat. No. 07356. The anti-rabbit and anti-goat (RAG/Ig G (HCL) (FITC) antiserum is conjugated with fluorescein isothiocyanate cat. No.07200, provided by Nordic Immunological Laboratories B.V., Netherlands and being used in IFAS testing. The previous dilutions from *R. solanacearum* were used.

Real-Time PCR assay:

Real-time PCR (Taq-Man) was made according to Weller et al. (2000) by using the apparatus of Applied Biosystem7500. The same previous dilutions from *R. solanacearum* were used in this test. The reaction mixture consisted of 12.5µl of master mix, 1 µl of primer forward, 1 µl of primer reverse, 1 µl of probe and 7 µl of water and 2.5µl of nucleic acid extract. The sequence of primers and probe used is shown in Table, 1 and were provided by OPRON, USA. Positive control of DNA extracted was used and water was used as a negative control.

Table (1): Characteristics of primers and Tag-Man probe used to detect *R. solanacearum* by Real-time PCR.

Primer and Probe name	Primer and Probe Sequence (5'→3')	Length	Dye
RS-I-F	GCA TGC CTT ACA CAT GCA AGTC	22	
RS-II-R	GGC ACG TTC CGA TGT ATT ACT CA	23	
RS-P	AGC TTG CTA CCT GCC GGC GAG TG	23	FAM

Evaluate the Device (Rs-FAST) as a New Technique for Detecting *R. solanacearum* Either in Soil or in Potato Tubers:

The device (Rs-FAST) has been developed as a FAST detector of potato brown rot bacterium in soil and potato tubers (Fig., 1). Evaluation of that device had been run for two purposes; its specify and sensitivity.

Fig. 1: Rs-FAST detector of *R. solanacearum*

Evaluate (Rs-FAST) Device Specify:

To evaluate (Rs-FAST) specification, different strains of bacteria have been tested such as, *R.solanacearum, Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas brassicacearum subsp. Brassicacearum, Halotolerans strain [Brevibacterium], Bacillus subtillis, Bacillus polymyxa, Pectobacterium carotovorum* and *Serratia marcescens* were prepared and exposed to the (Rs-FAST) device. That exposers were separately and together.

Evaluate (Rs-FAST) Device Sensitivity:

A bacterial suspension of *R. solanacearum* had been prepared in the concentration of (O.D =1.6 (650 nm) 1x $l0^9$ cfu /mL). Serial dilutions were prepared from 10^{-1} till 10^{-45} . Also two methods were used to be compared with (Rs-FAST) device. These methods were the Immunofluorescent Antibody Staining (IFAS) test according to Janse, 1988 and the test of Real-Time PCR according to Weller et al., 2000.

Evaluate (Rs-FAST) Device for detecting infested soil and potato tubers in field and storage :

Fields chosen by the specialists of Potato Brown Rot Project out of the pest free areas were tested for evaluating the (Rs-FAST) device as a detector. Also, a storage of seeds in Menoufia Governorate was chosen to the same purpose.

Application of Electromagnetic Resonance Pulses for Controlling Potato Brown Rot Caused by *Ralstonia solanacearum* in fields :

Six experiments were conducted at four districts belong to 3 Governorates; Beni-Suif, Meoufia and Beheira. The first experiment was at Alwastta District, Beni-Suif (area of 5 Fiddans), the second and third ones were at Tala District, Menoufia (areas of 4 & 5 fiddans), the fourth was at Altawfykia Destrict, Beheira (area of 5 feddans), the fifth one was at Kom Hamadah District, Beheira (area of 5 feddans) and the last one was at Nobaria District, Beheira (area of 6 feddans). That last district was newly reclaimed land.

Table (2): Time of electromagnetic Resonance Pulses Application Under Potato Fields Conditions at Experimental Districts

Exper. Districtx	Area (Feddan)	Planting Date	First Exposure		Second Exposure		Crop Harvest	
			Date	Plant age	Date	Plant age	Date	Plant age
Beni-Suef	5	2016/10/02	2016/10/22	20	2016/12/10	69	2017/01/15	105
Tala1	5	2016/10/03	2016/10/27	24	2016/12/14	72	2017/01/31	120
Tala2	4	2016/10/13	2016/10/27	14	2016/12/14	62	2017/02/25	135
Etay-Albaroud	6	2016/10/11	2016/11/05	25	2016/12/17	67	2017/02/04	116
Koum-Hamada	7	2016/10/22	2016/11/09	18	2016/12/31	70	2017/02/22	123
Nobaria	3	2016/10/15	2016/11/26	42	2016/12/21	67	2017/02/15	123

Statistical design was Complete Randomized Blocks. Each area was Divided into 3 blocks and each block had 3 treatments. Treatments were; exposed area of 6 replicates cultivated by potato for one hr., and after interval of 10 days 3 replicates exposed again for one hr. as the second treatment of 2 Exposures and one treatment left without exposure as a control.

Samples of soil were taken before treatment the soil and examined at the Brown Potato Rot Project by the specialists. On the harvest day another soil samples and 75 potato tubers were collected randomly form each replicate (225 tubers for treatment) according the proses of Brown Potato Rot Project to be examined according to European Union Protocols. Another 15 potato tubers of each replicate (45 tubers of treatment) and leaves samples were collected randomly and transported to Analytical Unit at Plant Protection Research Institute to assume the contents of total protein, total carbohydrates, Potassium, Calcium and Phosphorus rather than Chlorophyll in leaves. The weight of potato tubers collected from fixed area of each replicate chosen randomly was recorded.

RESULTS

Evaluate (Rs-FAST) Device as a Direct Potato Brown Rot Detector

1. **Evaluate (Rs-FAST) Device Specify:**

As shown in Table (1), (R-FAST) device recognized only bacteria causing the brown potato rot, *R. solanacearum* amongst the other 8 species of bacteria. It means that this new device (R-FAST) specific only for detecting brown potato rot bacteria, *R. solanacearum.*

Table (3): Ability of the new device (R-FAST) for responding with certain bacteria species.

Bacteria Species	Respond
Ralstonia solanacearum	+
Pseudomonas putida	-
Pseudomonas fluorescens	-
Pseudomonas aeruginosa	-
Pseudomonas brassicacearum subsp. Brassicacearum	-
Halotolerans strain [Brevibacterium]	-
Bacillus subtillis	-
Bacillus polymyxa	-
Pectobacterium carotovorum	-
Serratia marcescens	-

2. **Evaluate (R-FAST) Device Sensitivity:**

Data in Table (2) and Figs (2 &3) reveal that, while the two methods developed according to the protocols of European Union Directive No. 89/57EC and routinely used in Potato Brown Rot Project; IFAS and Real Time PCR tests detected bacterium of brown potato rot at concentrations from 10^9 till 10^4 only. While (R-FAST) device detected that bacteria at dilution from 10^9 till 10^{-39} in high response and weak response at dilution of 10^{-40} and very weak response at 10^{-41}. In other words, the sensitivity of (R-FAST) device for detecting *R. solanacearum* was about 10 folds of the two another tested methods (IFAS & Real Time PCR). So, (R-FAST) device could be easy and accurate method for detecting brown potato rot bacteria. This device advantage is that, it could detect infestation either in soil or potato tubers directly without needing any analysis or transportation samples to the lab. It could be used in field, storages, packing stations and customs.

Table (4): Sensitivity of (R-FAST) device to brown potato rot bacteria compared with other methods developed by UA

Bacteria Dilution	Response of Tested Methods		
	IFAS	Real time PCR	R-FAST
10^{9}	+	+	+
10^{8}	+	+	+
10^{7}	+	+	+
10^{6}	+	+	+
10^{5}	+	+	+
10^{4}	+	+	+
10^{3}	-	-	+
10^{2}	-	-	+
10^{1}	-	-	+
10^{-1}	-	-	+
10^{-2}	-	-	+
10^{-3}	-	-	+
10^{-4}	-	-	+
10^{-5}	-	-	+
10^{-6}	-	-	+
10^{-7}	-	-	+
10^{-8}	-	-	+
10^{-9}	-	-	+
--------	-	-	+
10^{-40}	-	-	Weak +
10^{-41}	-	-	V. weak+
10^{-42}	-	-	-
10^{-43}	-	-	-

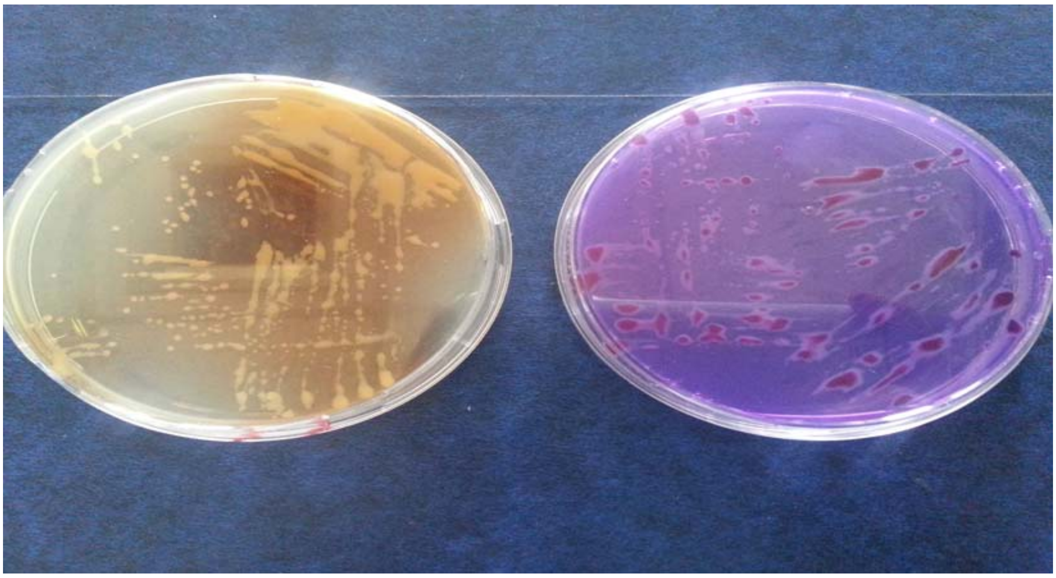

Fig. (2) : Typical colonies of *R. solanacearum* on King's B and SMSA media

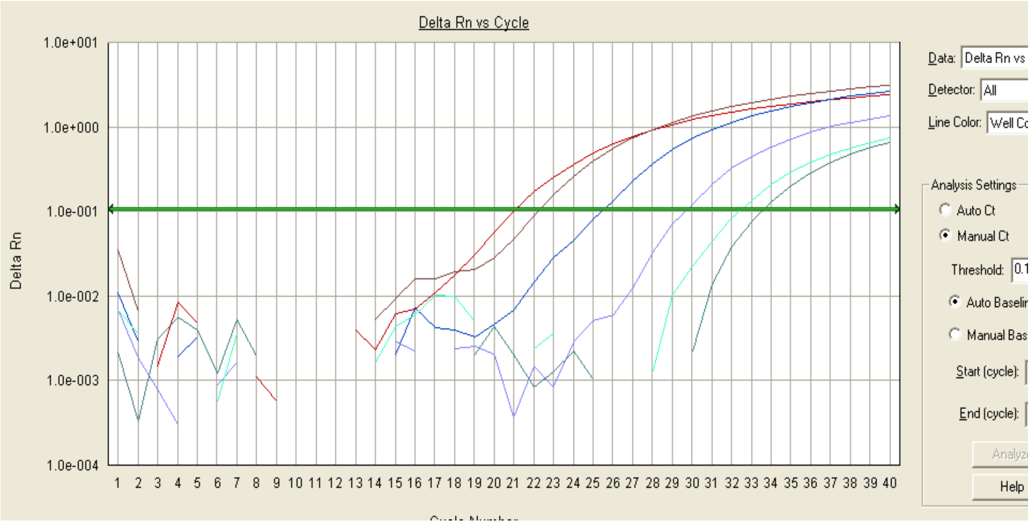

Fig. (3): Amplification of DNA extract of *R. solanacearum* from different concentration by Taq Man polymerase chain reaction (PCR).
Negative and positive controls were included. The curves exceed the threshold considered positive.

Evaluate (Rs-FAST) Device for detecting infested soil and potato tubers in field and storage:

The observations show that, all of the pointed areas by Rs-FAST device were infested by *R. solanacearum.* The lab tests in the Potato Brown Rot Project confirmed that result. Also, that device pointed to many stored tuber seeds and routine tests of the Project and examination of some of that seeds by hand confirmed that all these seeds were infested by potato brown rot disease. So, this (Rs-FAST) device was adopted by Ministry of Agriculture as a means for detecting potato brown rot disease either in soil or in tubers.

Evaluation of Electromagnetic Resonance Pulses for Controlling Potato Brown Rot Caused by *Ralstonia solanacearum* in fields :

This technology of the electromagnetic resonance waves has been developed by Prof. Dr. Fadel Mohamed Aly for several years. This was the first time to evaluate the efficiency of that technology under field conditions so, three parameters were considered to evaluate that method for controlling the potato brown rot under field condition. 1) the effect of this technology on the population of bacteria in the field, 2) the effect of that method on the nutrient contents of the potato leaves and tubers and 3) the effect of this technology on the crop yield.

Effect of Electromagnetic Resonance Pulses on The Population of Bacteria, *R. solanacearum* in fields:

This experiment was carried for 6 times in 3 Governorates; Beni-Suef, Menoufia and Beheira. The total field areas was 30 feddans all of them were chosen by the Potato Brown Rot Project as infested areas. Infestation confirmed by routine methods applied in the Project and the (Rs-FAST) device. The two treatments of apply electromagnetic resonance desidned pulses either for one exposure or for two exposures achieved 100% disappear of the potato brown rot disease while the control treatment was infested. It means that all tests carried out in the Potato Brown Project could not record any colony of bacterium either in soil or potato tuber samples. The (Rs-FAST) device gave that result directly after each treatment. It could be concluded that applying the electromagnetic resonance waves on fields of potato infected by potato brown rot achieved disappearing this disease after one hour. So, this new technology of electromagnetic resonance waves developed by Prof. Dr. Fadel M. A. was adopted by Egyptian Ministry of Agriculture as an efficient method for controlling potato brown rot in the field when applied for one hour.

Effect Electromagnetic Resonance Pulses on Potato Leaves and Tubers Nutrient Contents:

1. Effect Electromagnetic Resonance Pulses on Potato Leaves and tubers Nutrient of Total Carbohydrates Contents:

As shown in Table (5) treating potato fields with electromagnetic resonance waves for one hour achieved a highest total carbohydrates contents either in potato leaves or tubers followed by treating soil for 2 hours and control in order. Total carbohydrates contents in potato leaves after one hour of treatment were 40.1, 23.1, 40.8, 33.6, 36.96 and 32.8 µg/g at the experimental districts of Beni-Suef, Tala1, Tala2, Etay-Albaroud, Koum-Hamada and Nobaria respectively. Potato tubers contents of total carbohydrates after expose the potato field for one hour were 341.7, 494, 377 353.7 465.7 and 604 µg/g for the fore-mentioned districts in order. Statistical analysis revealed that, there was significant differences between the first treatment (one hour) and the other two treatment of (2 hours) and control in all experimental districts except Beni-Suef and (Kom Hamada) as there was insignificant differences between first and second treatments. That insignificance in Beni-Suef due to that after the first treatment experimental area had been irrigated with agricultural drainage water so, it could be said that this area treated only for one hour. The obtained results of potato tubers contents of total carbohydrates came in the same trend of that mentioned before in potato tubers. Almost, there was significant differences between the first treatment (one exposure) and control (Table 5).

Table (5): Total Carbohydrates in potato leaves and tubers After treatment of electromagnetic resonance waves In field applications.

Analysis Sources	Treatments	Total Carbohydrates (µg/g) Contents in Experimental Districts					
		Ben.	Tala1	Tala2	Etay	Koum	N
Potato Leaves	1 Exposure	40.1[a]	23.1[a]	40.8[a]	33.6[a]	36.96[a]	32.8[a]
	2 Exposures	39.1[ab]	19.5[b]	34.6[b]	29.6[b]	36.6[ab]	31.6[ab]
	control	36.7[bc]	19.2[bc]	15.5[c]	16.8[c]	24.1[c]	22.3[c]
	"F"	3.3	8.8	100.8	61.7	74.3	39.2
	L.S.D.	3.25	2,56	4.54	3.87	3.87	3.18
Potato Tubers	1 Exposure	341.7[a]	494.0[a]	380[a]	397.3[a]	487.0[a]	604.0[a]
	2 Exposures	333.3[ab]	494.0[ab]	377[ab]	353.7[bc]	465.7[b]	569.7[b]
	control	315.3[c]	409.3[c]	347[c]	349.7[c]	427.0[c]	557.3[c]
	"F"	11.3	73.3	24.4	9.3	49.5	25.2
	L.S.D.	13.8	19.8	12.97	18.3	14.97	16.7

Ben. = Beni-Suef, Etay = Etay-Albaroud, Koum= Koum-Hamada & N=Nobaria

Effect Electromagnetic Resonance pulses on Potato Leaves and tubers Nutrient of Total Protein Contents:

When potato fields exposed to electromagnetic resonance pulses for one hour total protein contents in potato leaves and tubers exceeded than that exposed for 2 hours and control (Table 6). Total protein contents of leaves after one hour of exposure were 28.8, 34.5. 29.9, 24.6, 32.7 and 31.9 µg/g while they were 37.7, 35.97, 42.3, 38.7, 39.7 and 32.5 µg/g as potato tubers protein at Beni-Suef, Tala1, Tala2, Etay-Albaroud, Koum-Hamada and Nobaria respectively. Statistical analysis cleared that, there were significant differences between the treatment of one hours and the other two treatments of 2 hours exposure and control. While there was insignificant difference between the treatment of 2 hours and control in both leaves and tubers analyzed for protein contents.

Table (6): Total Protein contents in potato leaves and tubers After treatment of electromagnetic resonance waves In field applications.

Analysis Sources	Treatments	Total Protein (μg/g) Contents in Experimental Districts					
		Ben.	Tala1	Tala2	Etay	Koum	N
Potato Leaves	1 Exposure	28.8^{a}	34.5^{a}	29.9^{a}	24.6^{a}	32.7^{a}	31.9^{a}
	2 Exposures	19.4^{b}	30.8^{b}	27.5^{b}	22.7^{ab}	31.9^{ab}	29.4^{b}
	control	18.2^{bc}	30.1^{bc}	26.3^{bc}	16.9^{c}	30.8^{bc}	28.2^{bc}
	"F"	3.3	8.8	100.8	61.7	74.3	39.2
	L.S.D.	3.25	2,56	4.54	3.87	3.87	3.18
Potato Tubers	1 Exposure	37.7^{a}	35.97^{a}	42.3^{a}	38.7^{a}	39.7^{a}	32.5^{a}
	2 Exposures	33.9^{b}	35.3^{ab}	41.97^{ab}	37.7^{ab}	37.9^{ab}	31.3^{ab}
	control	30.4^{c}	34.6^{bc}	40.4^{bc}	36.8^{bc}	33.6^{c}	30.8^{bc}
	"F"	11.3	73.3	24.4	9.3	49.5	25.2
	L.S.D.	13.8	19.8	12.97	18.3	14.97	16.7

Ben. = Beni-Suef, Etay = Etay-Albaroud, Koum= Koum-Hamada & N=Nobaria

Effect Electromagnetic Resonance pulses on Potato Leaves and Tubers Nutrient of Nitrogen Contents:

Table(7) show that, nitrogen content in leaves after one hr. of exposure were 5.4, 5.2, 3.7, 4.8, 5.8 and 3.4 μg/g in leaves collected from Beni-Suef, Tala1, Tala2 (Menoufia) Etay-Albaroud, Koum-Hamada and Nobaria (Beheira) in order. They were 5.3,6.4,5.8,6.04, 6.8 and 6.01 μg/g in tubers collected from the mentioned districts respectively. Statistically, there was significant differences between one treatment of 1 exposure, 2 exposures. and control at Nobaria district, beheira only may be because of their light sandy soil.

Table (7): Nitrogen contents in potato leaves and tubers After treatment of electromagnetic resonance waves In field applications.

Analysis Sources	Treatments	Nitrogen Contents(μg/g) at Experimental Districts					
		Ben.	Tala1	Tala2	Etay	Koum	N
Potato Leaves	1 Exposure	5.4^{a}	5.2^{a}	3.7^{a}	4.8^{a}	5.8^{a}	3.4^{a}
	2 Exposures	4.8^{b}	5.2^{ab}	3.69^{b}	4.6^{ab}	5.3^{b}	3.26^{b}
	control	4.6^{bc}	5.0^{bc}	2.6^{bc}	4.4^{bc}	4.9^{bc}	3.09^{bc}
	"F"	29.3	2.2	108.97	5.4	18.9	5.5
	L.S.D.	0.25	0.22	0.19	0.35	0.34	0.248
Potato Tubers	1 Exposure	5.3^{a}	6.4^{a}	5.8^{a}	6.04^{a}	6.8^{a}	6.01^{a}
	2 Exposures	5.3^{ab}	6.3^{ab}	5.8^{ab}	5.9^{ab}	6.7^{ab}	5.5^{b}
	control	5.2^{abc}	5.6^{bc}	5.7^{abc}	5.8^{abc}	6.3^{bc}	4.9^{c}
	"F"	0.07	12.3	0.93	0.89	8.8	36.3
	L.S.D.	0.37	0.3	0.32	0.28	0.31	0.32

Ben. = Beni-Suef, Etay = Etay-Albaroud, Koum= Koum-Hamada & N=Nobaria

Effect Electromagnetic Resonance pulses on Potato Leaves and Tubers Nutrient of Potassium Contents:

The potassium contents of the potato leaves collected form Beni-Suef, Tala1, Tala2, Etay-Albaroud, Koum-Hamada and Nobaria after soil exposure to electromagnetic pulses for one hr. were 51.03, 80.3, 63.7, 51.7, 36.7 and 45.97 μg/mol. These contents of potato tubers were 6.6, 4.7, 4.2, 2.5, 3.5 and 2.1 μg/mol. for the same districts respectively (Table 8). Analysis of Variance showed that there was significant differences between treatment of one hr. exposure and control either in leaves or in tubers. It means that treating potato fields with electromagnetic pulses increased potato leaves and tubers potassium contents. In other words that application of that technology improved potato tubers nutrient characters.

Table (8): Potassium contents in potato leaves and tubers After treatment of electromagnetic resonance waves In field applications.

Analysis Sources	Treatments	Potassium Contents (µg/mol) at Experimental Districts					
		Ben.	Tala1	Tala2	Etay	Koum	N
Potato Leaves	1 Exposure	51.03^{a}	80.3^{a}	63.7^{a}	51.7^{a}	36.7^{a}	45.97^{a}
	2 Exposures	50.2^{ab}	34.4^{b}	52.7^{b}	35.3^{b}	32.3^{b}	43.3^{ab}
	control	48.7^{bc}	26.3^{c}	38.0^{c}	34.3^{bc}	31.6^{bc}	34.3^{c}
	"F"	0.92	184.7	19.7	20.9	4.96	10.99
	L.S.D.	4.3	7.4	10.03	7.3	4.3	6.4
Potato Tubers	1 Exposure	6.6^{a}	4.7^{a}	4.2^{a}	2.5^{a}	3.5^{a}	2.1^{a}
	2 Exposures	6.3^{ab}	4.3^{b}	3.5^{b}	2.2^{b}	2.6^{b}	2.1^{ab}
	control	3.4^{c}	3.1^{c}	3.5^{bc}	2.02^{bc}	2.4^{bc}	2.1^{abc}
	"F"	295.8	109.2	25.1	13.4	47.3	0.32
	L.S.D.	0.4	0.3	0.3	0.24	2.03	0.2

Ben. = Beni-Suef, Etay = Etay-Albaroud, Koum= Koum-Hamada & N=Nobaria

Effect Electromagnetic Resonance pulses on Potato Leaves and Tubers Nutrient of Phosphorus Contents:

The phosphorus contents of the potato leaves collected form Beni-Suef, Tala1, Tala2, Etay-Albaroud, Koum-Hamada and Nobaria after soil exposure to electromagnetic pulses for one hr. were 40.1, 23.1, 40.8, 33.6, 36.96 and 32.8 µg/g. These contents of potato tubers were 341.7, 494.0, 380, 397.3, 487.0 and 604.0 µg/g for the same districts respectively Table (9). Analysis of Variance showed that there was significant differences between treatment of one hr. exposure and control either in leaves or in tubers. It means that treating potato fields with electromagnetic pulses increased potato leaves and tubers phosphorus contents. In other words that application of that technology improved potato tubers nutrient characters.

Table (9): Phosphorus contents in potato leaves and tubers After treatment of electromagnetic resonance waves In field applications.

Analysis Sources	Treatments	Phosphorus Contents (µg/g) at Experimental Districts					
		Ben.	Tala1	Tala2	Etay	Koum	N
Potato Leaves	1 Exposure	40.1^{a}	23.1^{a}	40.8^{a}	33.6^{a}	36.96^{a}	32.8^{a}
	2 Exposures	39.1^{ab}	19.5^{b}	34.6^{b}	29.6^{b}	36.6^{ab}	31.6^{ab}
	control	36.7^{bc}	19.2^{bc}	15.5^{c}	16.8^{c}	24.1^{c}	22.3^{c}
	"F"	3.3	8.8	100.8	61.7	74.3	39.2
	L.S.D.	3.25	2,56	4.54	3.87	3.87	3.18
Potato Tubers	1 Exposure	341.7^{a}	494.0^{a}	380^{a}	397.3^{a}	487.0^{a}	604.0^{a}
	2 Exposures	333.3^{ab}	494.0^{ab}	377^{ab}	353.7^{bc}	465.7^{b}	569.7^{b}
	control	315.3^{c}	409.3^{c}	347^{c}	349.7^{c}	427.0^{c}	557.3^{c}
	"F"	11.3	73.3	24.4	9.3	49.5	25.2
	L.S.D.	13.8	19.8	12.97	18.3	14.97	16.7

Beni = Beni-Suef, M = Menoufia, B= Beheira & N=Nobaria

Effect of Electromagnetic Resonance pulses on Potato Leaves Chlorophyll Contents:

As shown in Table (10), potato leaves contents of chlorophyll were 1.76, 2.46, 1.0, 0.97, 2.08 and 1.01 µg/g after one exposure to electromagnetic resonance pulses at the experimental districts of Beni_Suef, Tala1, Tala2, Etay-albaroud, Koum-Hamada and Nobaria respectively. These contents were 1.07, 2.05. 0.92, 0.57, 1.78 and 0.89 µg/g at the experimental districts in order. Statistical analysis revealed that, there was insignificant difference between the first treatment of one exposure to electromagnetic resonance designed pulses for one hr. (one exposure) and the other two treatments of twice exposure and control.

Table (10): Chlorophyll contents in potato leaves after treatment of electromagnetic resonance pulses In field applications.

Analysis Sources	Treatments	Chlorophyll Contents (µg/g) at Experimental Districts					
		Ben.	Tala1	Tala2	Etay	Koum	N
Leaves	1 Exposure	1.76[a]	2.46[a]	1.0[a]	0.97[a]	2.08[a]	1.01[a]
	2 Exposures	1.07[ab]	2.05[b]	0.92[b]	0.57[b]	1.78[ab]	0.89[ab]
	control	0.85[bc]	0.89[bc]	0.83[c]	0.54[c]	1.75[c]	0.87[c]
"F"		38.2	90.8	5.28	8.7	5.49	4.97
L.S.D.		0.27	0.297	0.13	0.16	0.27	0.11

Ben. = Beni-Suef, Etay = Etay-Albaroud, Koum= Koum-Hamada & N=Nobaria

Effect of Electromagnetic Resonance Designed Pulses on Potato Yield:

As illustrated in Fig. (4) It could be said that, potato crop yield resulted in all experimental districts after exposed potato fields to electromagnetic resonance designed pulses for one hr. was the highest. It followed by potato fields exposure twice each of one hr. and treatment of control. Percentages of increase yield over control after one exposure (1 hr.) were 13%, 7%, 12%, 13%, 27.5% at Beni-Suef, Tala1, Tala2, Etay-Albaroud, Koum-Hamada and Nobaria. After exposure soil twice The increase of potato yield over control were 2.3%, 0.34%,7%,7%, 18% and 2% at the experimental fields in order.

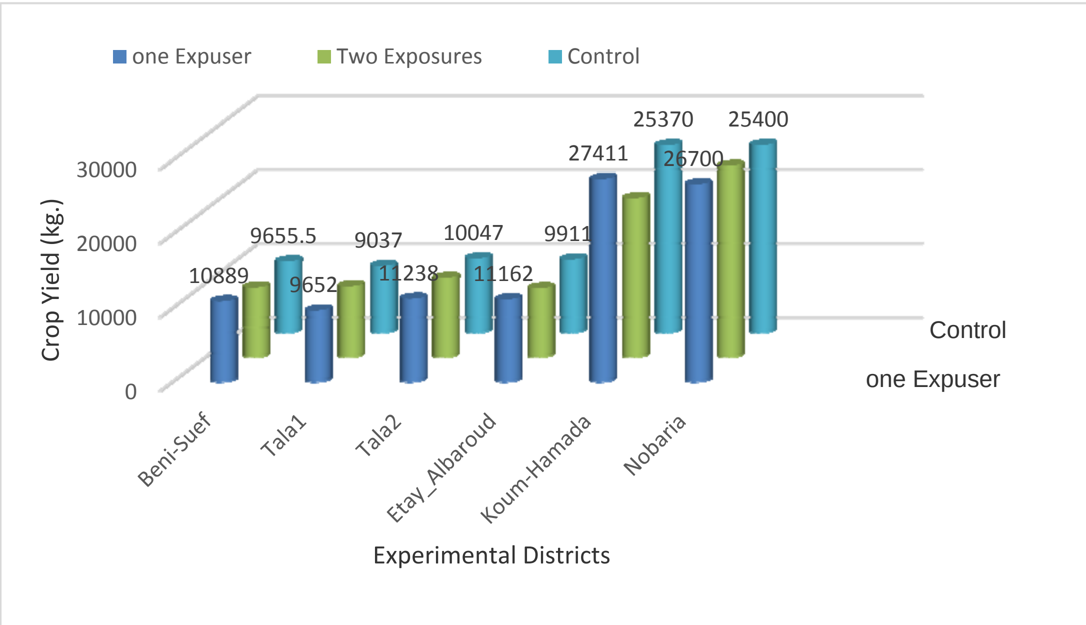

Fig. (4) : Potato Crop Yield Resulted After potato Fields Exposure to Electromagnetic Resonance pulses at Experimental Districts

DISCUSSION AND CONCLUSION

1.Electro/magnetic resonance pulses is a new technology could be applied in fields to control potato brown rot and other diseases.

2.This technology is safe for human, animals and plants. Also, it is specific for each plant disease such as potato brown rot, onion and garlic white rot and nematodes.

3.Electro/magnetic resonance pulses caused disappearing of R. *solanacearum* causing the potato brown rot disease after one hr. of soil exposure. Rather than, it achieved improvement of potato tubers nutrient contents of total carbohydrate, total protein, nitrogen, potassium and phosphorus.

4.Also, treating potato fields by this technology caused increase of crop yield by about 5-15% over control fields. This variation may be due to the type of land, irrigation water, agricultural practices, date of planting, date of application as shown in Table (2) and potato varieties.

So, this new technology of electromagnetic resonance waves developed by Prof. Dr. Fadel M. A. was adopted by Egyptian Ministry of Agriculture as an efficient method for controlling potato brown rot in the field when applied for one hour. Also, (Rs FAS) device was adopted as a direct detector of potato brown rot either in soil or in potato tuber.

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