UNIVERSITI PUTRA MALAYSIA

EFFECTS OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELD ON PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF SELECTED TISSUE CULTURED PLANTS

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By

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EFFECTS OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELD ON PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF SELECTED TISSUE CULTURED PLANTS

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March 2014

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The extremely low frequency electromagnetic field (ELF-EMF) occurs naturally from the Earth and artificially from human inventions. The electric power lines which lie across many plantations in Malaysia is one of the examples of the source of ELF-EMF. Earlier research demonstrated that some plants exposed to ELF-EMF respond positively in terms of growth and biochemical properties. Therefore, it was hypothesized that ELF-EMF is able to stimulate plant's growth. The objectives of this study were to develop a suitable ELF-EMF generator**,** to determine the effects of ELF-EMF on plant development**,** and to analyze the biochemical changes occurs in plants exposed to ELF-EMF. Initially, an ELF-EMF generator, the coGEM 1000 was constructed using four coils of copper wires that were connected to a transformer, a multimeter, and a rheostat. The coGEM 1000 suitable for tissue culture plants is able to produce stable and uniform 6 and 12 mT 50 Hz ELF-EMF in the four coils of the ELF-EMF generator. Four different species of tissue culture plants, namely tobacco plant, banana, orchid, and ficus were exposed to ELF-EMF. All these *in vitro* plantlets were incubated in a controlled environment prior to exposure to 6 and 12 mT of 50 Hz ELF-EMF for a period of 0.5, 1, 2, and 4 hours. The parameters observed were the number of shoots, shoot height, and number of leaves (growth characteristics) and biochemical properties, such as chlorophyll a and b content, total chlorophyll content, ratio chlorophyll a/b, carotene content, and activities of the enzymes namely catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR). For tobacco plant, the exposure to 6 mT ELF-EMF for an hour had increased the shoot height (3 cm), chlorophyll a (32.8 mg/g) and SOD activity (1.9 U/mg protein); whereas, the exposure to 6 mT ELF-EMF for 0.5 hour had increased the number of shoots (1.4) and total soluble proteins (23.3 mg/g) of the banana plant. However for orchid plants, higher ELF-EMF the exposure to (12 mT) for longer duration (4 hours) had induced a high number of shoots (3.5),

number of leaves (10.2) and CAT enzyme activity (1.0 U/mg protein). In contrast to the former, ficus plant exposed to extended periode of ELF-EMF did not induce any changes in the growth characteristics. However; the exposure of ficus plant to 12 mT ELF-EMF for 4 hours showed an increased in the activity of CAT (9.8 U/mg protein) and APX (13.2 U/mg protein). It is concluded that different plant species requires different strength and duration of exposure to ELF-EMF to promote growth and development. In addition, the exposures of the selected plant species to the ELF-EMF were able to promote the plant abilities to resist the presence of reactive oxygen species (ROS) generated in tissue culture. These have been illustrated by the increase of the various antioxidative enzymes activities (SOD, CAT and APX) presence in the selected plants species (viz. tobacco, orchid, and ficus plant, respectively). These results suggest that the application of ELF-EMF may help to promote *in vitro* plant growth through creating a plant that can resist the ROS. This findings provide a foundation for further apllication of the ELF-EMF in promoting plant growth.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

KESAN MEDAN ELEKTROMAGNET FREKUENSI SANGAT RENDAH KE ATAS CIRI FIZIKAL DAN BIOKIMIA PADA KULTUR TISU TUMBUHAN TERPILIH

By
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Medan elektomagnet frekuensi yang sangat rendah (ELF-EMF) berlaku secara semula jadi dari bumi dan buatan dari ciptaan manusia. Talian kuasa elektrik, yang terletak di banyak ladang-ladang di Malaysia adalah salah satu contoh sumber ELF-EMF. Kajian yang terdahulu menunjukkan bahawa sesetengah tumbuhan terdedah kepada ELF-EMF bertindak balas secara positif dari segi pertumbuhan dan sifat biokimia. Oleh itu, ia telah membuat hipotesis bahawa ELF-EMF mampu untuk merangsang pertumbuhan pokok. Objektif kajian ini ialah untuk membangunkan sesuai penjana ELF-EMF, untuk menentukan kesan ELF-EMF kepada pertumbuhan tumbuhan, dan untuk menganalisis perubahan biokimia berlaku dalam tumbuhan yang terdedah kepada ELF-EMF. Pada mulanya, penjana ELF-EMF, coGEM 1000 telah dibina menggunakan empat gegelung wayar tembaga yang dihubungkan dengan transformer, multimeter, dan reostat. coGEM 1000 yang sesuai untuk tisu kultur tumbuhan mampu menghasilkan 6 dan 12 mT 50Hz ELF-EMF dalam empat gegelung penjana ELF-EMF yang stabil dan seragam. Empat spesies yang berbeza kultur tisu tumbuhan, iaitu tumbuhan tembakau, pisang, orkid, dan ficus tlah didedah kepada ELF-EMF. Semua anak pokok *in vitro* telah dieram dalam persekitaran yang terkawal sebelum pendedahan kepada 6 dan 12 mT 50 Hz ELF-EMF untuk tempoh 0.5, 1, 2, dan 4 jam. Parameter diperhatikan adalah bilangan pucuk, ketinggian pucuk, dan bilangan daun (ciri-ciri pertumbuhan) dan sifat-sifat biokimia, seperti kandungan klorofil a dan b, jumlah kandungan klorofil, nisbah klorofil a/b, kandungan karotena, dan aktiviti enzim catalase (CAT), askorbat peroksidase (APX), superoksida dismutase (SOD), dan glutation reductase (GR). Untuk tanaman tembakau, pendedahan kepada 6 mT ELF-EMF selama satu jam telah meningkatkan ketinggian tunas (3 cm), klorofil a (32.8 mg/g) dan aktiviti SOD (1.9 U/mg protein); manakala, pendedahan kepada 6 mT ELF-EMF selama 0.5 jam telah meningkatkan

bilangan tunas (1.4) dan jumlah protein larut (23.3 mg/g) tanaman pisang. Walau bagaimanapun, bagi tanaman orkid, pendedahan ELF-EMF yang lebih tinggi kepada (12 mT) untuk tempoh yang lebih lama (4 jam) telah mendorong peningkatan bilangan pucuk (3.5), bilangan daun (10.2) dan CAT aktiviti enzim (1.0 U/mg protein). Berbeza dengan tanaman sebelumnya, tanaman mas cotek terdedah kepada periode panjang ELF-EMF tidak mencetuskan apa-apa perubahan dalam ciri-ciri pertumbuhan. Walau bagaimanapun; pendedahan tumbuhan mas cotek kepada 12 mT ELF-EMF selama 4 jam menunjukkan peningkatan dalam aktiviti CAT (9.8 U/mg protein) dan APX (13.2 U/mg protein). Ia membuat kesimpulan bahawa spesies tumbuhan yang berbeza memerlukan kekuatan ELF-EMF dan jangka masa pendedahan yang berbeza untuk menggalakkan pertumbuhan. Di samping itu, pendedahan spesies tumbuhan terpilih kepada ELF-EMF dapat menggalakkan kebolehan tumbuhan untuk menentang kehadiran spesies oksigen reaktif (ROS) yang dihasilkan dalam kultur tisu. Ini telah digambarkan oleh peningkatan pelbagai aktiviti enzim antioksidan (SOD, CAT dan APX) dalam spesis tumbuhan terpilih (iatu tembakau, orkid, dan mas cotek). Keputusan ini menunjukkan bahawa penggunaan ELF-EMF boleh membantu untuk menggalakkan dalam pertumbuhan tumbuhan *in vitro* dengan mewujudkan tumbuhan yang dapat menahan ROS. Penemuan ini menyediakan asas bagi penggunaan ELF-EMF selanjutnya dalam menggalakkan pertumbuhan tumbuhan.

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I certify that a Thesis Examination Committee has met on March 2014 to conduct the examination of Riry Prihatini on her thesis entitled Effects of Extremely Low Frequency Electromagnetic Field (LF-EMF) on Physical and Biochemical Characteristics of Selected Tissue Cultured Plants in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A)106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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TABLE OF CONTENTS

		Page
ABSTRACT		ii
ABSTRAK		iv
ACKNOWLEDGMENTS		vi
APPROVAL		vii
DECLARATION		ix
LIST OF FIGURES		xiv
LIST OF TABLES		xvii
LIST OF ABBREVATIONS		xviii
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	3
	2.1. Electro Field Versus Electromagnetic Field (EMF)	3
	2.2. Extremely Low Frequency Electromagnetic Field (ELF-EMF)	4
	2.3. Engineering Aspect of The ELF-EMF Generator	5
	2.4. Factors effecting Plant Growth	6
	2.5. Other Factors Affecting Plant Growth	9
	2.5.1. Ultraviolet	9
	2.5.2. Gravity	10
	2.5.3. ELF-EMF	10
	2.6. Plant *In Vitro system*	11
	2.7. Some Parameters That Can Be Used To Determine Development and Biochemical Properties in *In Vitro* Plants	14
	2.7.1 Plant developmental parameters	14
	2.7.2. Plant Biochemical Properties Parameters	15
	A. Protein soluble protein	15
	B. Chlorophyll content	17
	C. Antioxidant enzymes activities	18
	D.	
3	**MATERIAL AND METHOD**	21
	3.1. Materials	21
	3.1.1. Equipments Used to Construct ELF-EMF Generator	21
	3.1.2. Tissue Culture Media and Chemicals	22
	3.1.3. Plants Materials	22
	A. Tobacco Plantlets	22
	B. Banana Plantlets	22
	C. Slipper Orchid Plantlets	22
	D. Ficus Plantlets	22

3.2. Methodology 22
3.2.1. Development of ELF-EMF Generator 22
3.2.2. Exposure to ELF-EMF to Plant Materials 25
3.2.3. Growth Parameters 25
3.2.3. Biochemical Tests 26
A. Total chlorophyll content 26
B. Total soluble protein content 27
i. Total protein standard curve 27
ii. Total soluble protein assays 27
C. Crude leave extract 27
i. Catalase (CAT) enzyme activity assay 28
ii. Ascorbate peroxidase (APX) enzyme activity 28
iii. Superoxide dismutase (SOD) enzyme assay 28
iv. Glutathione reductase (GR) enzyme activity 29
3.3. Experimental design and statistical analysis 29

4 RESULT AND DISCUSSION 30
4.1. Development of ELF-EMF Generator 30
4.2. Effects Of ELF-EMF (Extremely Low Frequency Electromagnetic Field) on the Developmental Characteristics and Biochemical Properties of Selected Tissue Cultured Plants 35
4.2.1. Effect of the ELF-EMF (Extremely Low Frequency Electromagnetic Field) on the Tissue Cultured Tobacco Plants 39
A. Effect of ELF-EMF on the Developmental Characteristics of the Tissue Cultured Tobacco Plants 39
B. Effect of ELF-EMF on The Biochemical Parameters of Tissue Cultured Tobacco Plants 42
4.2.2. Effect of The ELF-EMF (Extremely Low Frequency Electromagnetic Field) on The Tissue Cultured Banana Plants 45
A. Effect of ELF-EMF on The Developmental Characteristics of The Tissue Cultured Banana Plants 45
B. Effect of ELF-EMF on The Biochemical Parameters of Tissue Cultured Banana Plants 45
4.2.3. Effect of The ELF-EMF (Extremely Low Frequency Electromagnetic Field) on The Tissue Cultured Slipper 51

OrchidPlants
A. Effect of ELF-EMF on The Developmental Characteristics of The Tissue Cultured Slipper Orchid Plants 51
B. Effect of ELF-EMF on The Biochemical Parameters of Tissue Cultured Slipper Orchid Plants 54
4.2.4. Effect of The ELF-EMF (Extremely Low Frequency Electromagnetic Field) on The Tissue Cultured Ficus Plants 57
A. Effect of ELF-EMF on The Developmental Characteristics of The Tissue Cultured Ficus Plants 57
B. Effect of ELF-EMF on The Biochemical Parameters of Tissue Cultured Ficus Plants 60

5 SUMMARY, CONCLUSIONS, AND RECOMMENDATION FOR FURTHER STUDIES 63

REFERENCES 65

APPENDIX

A Composition of Murashige and Skoog (1962) Media 84

B1 Bradford Standard Curve 85

B2 SOD Standard Curve 85

C1a MANOVA Test Result for Effect of ELF-EMF on Tobacco, Banana, Slipper Orchid, and Ficus Physical Characteristics 86

C1b MANOVA Test of Between-Subjects Effects for Effect of ELF-EMF on Tobacco, Banana, Slipper Orchid, and Ficus Physical Characteristics 86

C2a MANOVA Test Result for Effect of ELF-EMF on Tobacco, Banana, Slipper Orchid, and Ficus Biochemical Characteristics 86

C2b MANOVA Test of Between-Subjects Effects of ELF-EMF on Tobacco, Banana, Slipper Orchid, and Ficus Biochemical Characteristics 87

D The Calculation of The Significantly Affected Parameters To The Different Strength and Duration of ELF-EMF Exposures Compared To The Control Group 88

BIODATA OF STUDENT 89

LIST OF PUBLICATIONS 90

LIST OF FIGURES

Figure		Page
2.1	The pathway of catalase (CAT), ascorbate peroxidase (APX), glutatione reductase (GR), and superoxide dismutase (SOD) enzymes to combat reactive oxygen species on plant	20
3.1	Electrical circuit diagram of the ELF-EMF generator	25
4.1	The calibration curve of magnetic field generated from one coil connected in series	31
4.2	The calibration of magnetic field generated from two coils connected in series	31
4.3	The calibration of magnetic field generated from three coils connected in series	33
4.4	The calibration curve of magnetic field generated from two coils connected in parallel	33
4.5	The calibration curve of magnetic field generated from four coils connected in series and parallel	34
4.6	The CoGEM 1000, an ELF-EMF generator consists of 4 coils connected in series and parallel with AC supply, Amp meter, and rheostat	34
4.7	The graph showing electromagnetic field stability over 5 hours duration at 60 Gauss (6 mT) strength	36
4.8	The graph showing electromagnetic field stability over 5 hours duration at 120 Gauss (12 mT) strength	37
4.9	Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the developmental characteristics of the tissue cultured tobacco plantlets 4 weeks after cultures	40
4.10	The morphology of the tissue cultured tobacco plantlets after being exposed to different strength and duration exposure of ELF-EMF (extremely low frequency electromagnetic field) 4 weeks after cultures	41
4.11	Effect of different strength and duration exposure to ELF-	43

EMF (extremely low frequency electromagnetic field) on the pigment content of the tissue cultured tobacco plantlets 4 weeks after cultures

4.12 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the biochemical properties of the tissue cultured tobacco plantlets 4 weeks after cultures 44

4.13 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the developmental characteristics of the tissue cultured banana plantlets 8 weeks after cultures 46

4.14 The morphology of tissue cultured banana plantlets after being exposed to different strength and duration exposure of ELF-EMF (extremely low frequency electromagnetic field) 8 weeks after cultures 47

4.15 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the pigment content of the tissue cultured banana plantlets 8 weeks after cultures. 48

4.16 Effect of different strength of duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the biochemical properties of the tissue cultured banana plantlets 8 weeks after cultures. 50

4.17 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the developmental characteristics of the tissue cultured slipper orchid plantlets 16 weeks after culture 52

4.18 The morphology of the tissue cultured slipper orchid plantlets after being exposed to different strength and duration exposure of ELF-EMF (extremely low frequency electromagnetic field) 16 weeks after cultures 53

4.19 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the pigment content of the tissue cultured slipper orchid plantlets 16 weeks after cultures. 55

4.20 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the biochemical properties of the tissue cultured slipper orchid plantlets 16 weeks after cultures 56

4.21 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the developmental characteristics of the tissue cultured ficus plantlets 8 weeks after cultures 58

4.22 The morphology of the tissue cultured ficus plantlets after being exposed to different strength and duration exposure of ELF-EMF (extremely low frequency electromagnetic field) 8 weeks after cultures 59

4.23 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the pigment content of the tissue cultured ficus plantlets 8 weeks after cultures 61

4.24 Effect of different strength and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the biochemical properties of the tissue cultured ficus plantlets 8 weeks after cultures. 62

LIST OF TABLES

Table		Page
1.1	The tabulated data on research of effect of ELF-EMF on plants	13
4.1	Tabulated data for Multivariate analysis of variance (MANOVA) of the effect of different strength and duration exposure of the ELF-EMF (Extremely Low Frequency Electromagnetic Field) on the developmental parameters of the tissue cultured tobacco, banana, slipper orchid, and ficus plantlets	38
4.2	Tabulated data for Multivariate analysis of variance (MANOVA) of the effect of different strength dan duration exposure of the ELF-EMF (Extremely Low Frequency Electromagnetic Field) on the biochemical parameters of the tissue cultured tobacco, banana, slipper orchid, and ficus plantlets	38

LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

µM	Micromolar
µT	Micro Tesla
ºC	Celsius
2,4-D	2,4-dicholophenoxyacetic acid
Å	Amstrong
A/m	Ampere per meter
AC	Alternating current
APX	Ascorbate peroxidase
BA or BAP	6-benzyladenine
CAT	Catalase
CITES	Convention on International Trade in Endangered Species
Cm	Centimeter
CO	Carbon monoxide
CO_2	Carbon dioxide
CoGEM1000	Coil Generator Electromagnetic Field 1000
Cu	Cuprum (copper)
DMRT	Duncan Multiple Range Test
DTNB	5-5'-dithio-bis-(2-nitro-5-thiobenzoic acid)
EDTA	Ethylenediamine tetraacetic acid
ELF	Extremely Low Frequency
ELF-EMF	Extremely Low Frequency Electromagnetic Field
EMF	Electromagnetic Field
G	Gram
gl^{-1}	Gram per liter
GR	Glutathione reductase
GSH	Glutathione
GSSG	Oxidized glutathione
H_2O	Water
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
Hz	Hertz
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
IU	International unit
Kcal	Kilo calories
kV/m	Kilo volt per meter
M	Meter
M	Molar

MANOVA	Multivariate Analysis of Variance
mg/ml	Milligram per milliliter
mGauss	Milli Gaus
mgl^{-1}	Milligram per liter
mM	Millimolar
mRNA	Messenger Ribonucleaic acid
MS	Murashige-Skoog
mT	MilliTesla
NAA	α-naphthylacetic acid or 1-naphthalene acetic acid
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
NBT	2-nitro-5-thiobenzoic acid
NH	Nitrogen hydroxide
nm	Nanometer
O_2	Oxygen
ODC	Ornithine decarboxylase
pA	Pico ampere
PAL	phenylalanine ammonialyase
PCD	Programmed Cell Death
PEMF	Pulsed Electromagnetic Field
PGR	Plant Growth Regulator
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SPSS	Statistical Package for Social Science Studies
TDZ	Thidiazuron
U/ml	Unit per milliliter
UNITEN	Universiti Tenaga Nasional
V/m	Volt per meter
v/v	Volume per volume

CHAPTER 1

INTRODUCTION

Earth, as a habitat of many living organisms, is controlled by natural physical forces in order to support life continuously. One of the Earth's fundamental interactions is electromagnetism, which is an interaction between electrically charged particles. On a resting state, these particles interact through electrostatic force, but when the particles are relatively on a moving state to each other, they interact with both electric and magnetic forces. The electric and magnetic forces are different with each other, but they are associated with one another to form an electromagnetic field (EMF).

The EMF is naturally produced by the Earth, but it can also be released by human inventions, in the form of electrical and electronic devices, and through transmission and distribution of power lines. The electric distribution usually operates at frequencies of 50 and 60 Hz. The EMF produced from 30-300 Hz electric current is generally referred to as Extremely Low Frequency-EMF (ELF-EMF).

The ELF-EMF's potential effects on organisms have become a subject to many extensive researches. Although the data from these researches have shown different results, the World Health Organization (WHO) recommends that environmental ELF-EMFs are harmless to human health. However, high density of ELF-EMF (more than 12 mT) may potentially induce cancer, leukemia, depression, cardiovascular disorder, and other physiological dysfunctions as shown from studies on laboratory animals (Ravazzani 2008).

The outcomes of the studies on the impact of ELF-EMF on plants have shown that ELF-EMF may have positive, neutral, or negative effects on plant growth and development depending on the plant's genotype (Table 1.1). The positive effect of ELF-EMF on plants implies that this environmental factor may increase seed germination percentage, plant growth, and resistance to virus. For some plants species, ELF-EMF may not affect plant growth (neutral). On the contrary, other studies have also reported that ELF-EMF may have negative impact on plants, such as inducing abiotic stress, which may reduce the growth rate and seed germination of the plants.

Most studies on the impacts of ELF-EMF on various crop plants have been conducted using native plants which are grown under natural condition. However, it is difficult to measure the true impacts of ELF-EMF on these plants since other abiotic factors may be presence and contributed to the measurement. In order to eliminate these other factors and to measure the true impacts of ELF-EMF on the plants, *in vitro* culture system was selected for this research.

To proceed with the investigation on the effects of ELF-EMF on *in vitro* plants, it is important that a stable ELF-EMF generator is available. The commercial ELF-EMF generators mostly present as a Helmholtz coil. This expensive device is often used to measure magnets, rather than to create the electromagnetic field. Using the same principle, we have developed an ELF-EMF generator that can be used to investigate the impact of ELF-EMF on *in vitro* plants. This generator was built using copper wire coils that were connected to electrical devices. Various aspects were considered during the development of the ELF-EMF generator, including accuracy, uniformity, stability, and safety issues.

In order to examine the effects of ELF-EMF on *in vitro* plants, growth and biochemical parameters that represent the developmental characteristics of plants need to be analyzed. The growth parameters include number of shoots, number of leaves, and shoot height; whereas biochemical parameters includes the assessment of chlorophyll content and several antioxidant enzymes activities (catalase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase).

Many plantation and agriculture areas in Malaysia lies beneath high power transmission lines. Generally, the crops planted beneath the transmission power lines grow rapidly, greener, and bigger compared to those planted in other places. Thus, to confirm this assumption and to determine the actual impacts of ELF-EMF on plants, this study was initiated on a range of tissue culture plants. It is hypothesized that the ELF-EMF treatments (i.e. interaction between ELF-EMF strength and duration of exposure) promote plant growth. Since the effect of ELF-EMF varied with different plant genotype, this study was conducted on four different species of plants.

Thus, the objectives of this study were:

1. To develop a suitable ELF-EMF generator to study the effects of ELF-EMF on selected plants under controlled environment.
2. To determine the effects of different strength and time exposure of ELF-EMF on the development of four different plant species.
3. To analyze the biochemical changes that occurin four different plant species exposed to ELF-EMF.

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