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### STATIC ELECTROMAGNETIC FIELD (EMF) OF LOW FREQUENCY ENHANCES SEED GERMINATION AND PLANT GROWTH AT EARLY STAGES OF DEVELOPMENT

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#### KEYWORDS

EMF

Bio – stimulation

Germination

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Seedling

Low frequency

#### ABSTRACT

Magnetic fields have influenced the life and growth of organisms on this earth. Extremely low frequency electromagnetic radiation is the designation for radiation with frequencies from 1-300 Hz and its influence on animal and human systems are found to be hazardous. On the contrary, low electromagnetic fields (EMF) have been found to increase the seed germination rates and accelerate the plant growth, protein biosynthesis and root development. The present study aims at determining the impacts of low electromagnetic field (EMF) exposures on seed germination rates and the plant growth phases. For the study, seeds of *Pisum sativum*, *Zea mays*, *Solanum Lycopersicum*, *Cyamopsis tetragonoloba*, *Cajanus cajan* were germinated at low electromagnetic fields ( $380 \pm 20 \mu\text{T}$ ). The control groups were kept away from the influence of low EMF while the test groups were germinated under the EMF influence. The observations of present study indicated a significant effect of EMF on germination rates and an enhanced growth period in the test groups. The pot studies were carried out to monitor the seedling growth. The growth rates for the test group seedlings were faster in comparison to the control group. The influence of EMF acting as a biostimulant displays a positive response on seed germination speeding up the germination rates leading to a faster and enhanced plant growth thus reducing the time period for growth leading to a faster crop yield.

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## 1 Introduction

Magnetic fields may have influenced the life and growth of organism on this earth. Extremely low frequency electromagnetic radiation is the designation for radiation with frequencies from 1-300 Hz and its influence on animal and human systems are found to be hazardous. On the contrary, Earth's geomagnetism is an inevitable attribute to all the organisms' present on the earth. There are possible aspects of geomagnetic fields inducing the growth of species on this plant. So the magnetic fields may have played a role in acting as a bio-stimulant for growth of plants. Plants are considered as better models for investigations in regard to the EMFs as they are ideally suited because of their immobility and their pattern of development which can be constantly monitored in an electromagnetic field (Vian et al., 2007). Still the knowledge about the effects of extremely low frequency magnetic field (ELF MF) on living organisms is limited. The first studies reported on the effects of Magnetic fields (MF) on plants were conducted by Krylov & Tarakonova in 1960. They proposed an auxin like effect of MF on germinating seeds, by calling this effect as magnetotropism (Krylov & Tarakonova, 1960). A similar study by Boe & Salunkhe also explains about an Auxin like effect on the ripening of tomato fruits (Boe & Salunkhe, 1963).

Weak or low MF ranges from 100 nT to 0.5 mT, whereas super weak or zero also known as magnetic vacuum is referred to MFs below 100 nT. Electromagnetic Fields effects on biological systems have caught attention since its first reporting in 1960 and still pursued. Studies using the EMF for plant growth and stimulating their growth responses were proved (Orchard, 1977; Pittman, 1963; Galland & Pazur, 2005; Vashisth & Nagarajan, 2010; Alikamanoglu & Sen, 2011). Changes in the duration of the cell division phases RNA, protein synthesis under the EMF conditions were altered (Atak et al., 2014; Berteau et al., 2015). This particular study focuses on the use of static EMF with a lower frequency to study the seed germination rates under the influence of EMF growth rates. The plant growth is believed to be enhanced during its growth period and also studies have reported enhanced metabolism (Roux et al., 2008). Germination indices give us the detailing about the ELF MFs' influence on a seed's early growth right from stage 0 (beginning) which helps in monitoring the entire growth period.

## 2 Materials & Methods

### 2.1 Seed collection & EMF Exposure

Commercially available seeds of *Solanum lycopersicum*, *Cyamopsis tetragonoloba*, *Zea mays*, *Vigna radiata*, *Cicer arietinum*, *Cajanus cajan* were purchased from APMC market Ahmedabad, Gujarat, India. Control group (seeds) was kept for germination at normal room conditions away from any high EMFs

or electric gadgets. Test sets were kept at low electromagnetic fields of 3m T inside a locally built solenoid. Solenoid generated a variable homogeneous magnetic field with frequency of 3mT. A locally built solenoid (560 turns) was used for the static EMF. Fields were strong inside the center of the solenoid. Magnetic field did not alter the temperature of the seeds under stimulation. Three replicates of 50 seeds in each were spread on petri plates containing moist cotton beds. Emergence of radicle and plumule marked the event of seed germination for which seed germination indices were recorded. Germinated seedlings were transferred for pot studies and monitored for 12 weeks.

### 2.2 Germination indices

Growth parameters like shoot length, root length, fresh weight, and dry weight of leaves were studied. Germination rates were also studied for all the seeds kept under the influence of low ELF MF.

1. Mean Germination Time (MGT) was calculated by the method given by Bench et al. (1991), the detail is as follows

$$MGT = \frac{\sum f \cdot x}{\sum f}$$

$f$  = Seeds germinated on day  $x$

2. Germination Index (GI) was calculated by the Jones & Sanders (1987) method, as mentioned below

$$GI = (10 \cdot n_1) + (9 \cdot n_2) + \dots + (1 \cdot n_{10})$$

$n_1, n_2, \dots, n_{10}$  = No. of germinated seeds on the first, second, and the subsequent days until the 10<sup>th</sup> day, 10, 9, ... and 1 are weights given to the number of germinated seeds on the first, second, and subsequent days, respectively.

Therefore, the GI emphasizes on both the percentage of germination and its speed. A higher GI value denotes a higher percentage and the rate of germination.

3. Coefficient Velocity of Germination (CVG) Theoretically, the highest CVG possible is 100. In present study it was calculated by Esehie (1994) method

$$CVG = \frac{N_1 + N_2 + \dots + N_x}{100} \times \frac{1}{N_1 T_1 + \dots + N_x T_x}$$

$N$  = No. of seeds germinated each day;  $T$  = No. of days from seedling corresponding to  $N$

4. Seed Vigour Index was calculated by the following formula

$$\text{SVI} = \text{Sapling Length} \times \text{GP}\%$$

GP= Germination Percentage

## 2.3 Biochemical indices

### 2.3.1 Total Protein

Total protein was estimated by Lowry method (Lowry et al., 1951), for this 0.5gm fresh leaf tissue collected from interveinal area was homogenized in 1.0 ml 50mMTris-HCl buffer, pH8.0. The homogenate was centrifuged at 15000g for 10 minutes and proteins were precipitated from the supernatant with 5% trichloroacetic acid (TCA).The proteins were pelleted by centrifugation and hydrolyzed in 1M NaOH at 37°C overnight. An aliquot of the lysate was used to determine the protein content 660(nm).

### 2.3.2 Superoxide dismutase

Superoxide dismutase was estimated by Kakkar et al., method (Kakkar et al., 1984).The leaves (0.5 gm) were ground with 3.0ml of potassium phosphate buffer, centrifuged at 2000g for 10min and the supernatant was used for assay. The intensity of the chromogenin the butanol layer was measured at 560nm in a spectrophotometer. One unit of enzyme activity is defined by the amount of enzyme that gives 50% inhibition of Nitrobluetetrazolium dye reduction in 1 minute.

### 2.3.3 Lipid peroxidation

LPO levels in the leaf tissue were determined in terms of malondialdehyde (MDA, a product of LPO) by Heath &Packer method (Heath &Packer, 1968). For this 0.26 gm fresh leaf sample was homogenized in 5.0 ml 0.1% TCA. This homogenate was centrifuged at 10,000g for 5 minutes. The reagent mix containing 4.0 ml 20% TCA and 0.5 % TBA were added to 1.0 ml aliquot of supernatant. This mixture was then heated at 95°C for 30 minutes and then quickly cooled in an ice bath.

After centrifuging at 10,000g for 10 min the absorbance of the supernatant was read at 542 nm and the value for the non-specific absorption at 600nm was subtracted. The concentration of MDA was calculated using its extinction coefficient of 155mM<sup>-1</sup>cm<sup>-1</sup>.

### 2.3.4 Chlorophyll

Chlorophyll was extracted in DMSO and the absorbance was read at 663 nm and 645 nm by Hiscox & Israelstam (1979) method. Absorption coefficients were used to calculate the amount of chlorophyll (Arnon, 1949).

$$\text{Chlorophyll}_a = 12.7(\text{A663}) - 2.69(\text{A645})$$

$$\text{Chlorophyll}_b = 22.9 (\text{A645}) - 4.68 (\text{A663})$$

$$\text{Total chlorophyll mg/l} = 20.02 (\text{A645}) + 8.02 (\text{A633})$$

## 2.4 Statistical analysis

Experiments were performed in sets of triplicates. The data were statistically analyzed using one-way analysis of variance (ANOVA). The level of significance was accepted with  $P < 0.05$ . Quantitative data were expressed as mean  $\pm$  SD for all the indices i.e. Germination as well as biochemical.

## 3 Results

### 3.1 Germination indices

A lower value of MGT depicts a faster germination rate which was observed for test sets. Coefficient velocity of germination was higher in test sets which denote the rapid growth in the test sets than controls. Germination Percentage denotes the growth of seeds per day which was found to be lesser in controls group of seeds. Seed Vigor Index were higher in test sets compared to the control sets. Fresh and dry weights were higher for EMF treated seedlings grown under the influence of EMF compared to the controls (not kept in EMF fields) for *Z. mays*, *C. tetragonoloba* & *S. lycopersicum* whereas only for *C. cajan* the control sets were having higher fresh-dry weights (Table 1).

### 3.2 Biochemical assays

The protein content was expressed in  $\mu\text{gm/ml}$  and the protein content estimated by Folin Lowry's method showed increased protein values for *C. cajan* & *V. radiata* in the test sets while the controls expressed lesser protein content (Figure 1). While the total MDA concentration in *S. lycopersicum*, *V. radiata* and *C. cajan* for test groups were greater than controls (Figure 2). In case of SOD Units, it was reported higher for all control sets compared to the test sets (Figure 3). Similarly, total chlorophyll content was higher in test groups than the control groups except for *V. radiata* and *C. cajan* (Figure 4).

## 4 Discussion & Conclusion

Studies have pointed out towards EMFs playing a growth promoting role in plant growth right from its germination stage and our results. The calculated germination indices for this study were on a positive scale for the EMF treated seeds e.g. a faster germination rate for EMF treated seeds compared to control seeds not under the influence of any static EMFs which suggests a promotional effect of EMF influence on its growth (Fischer et al., 2004; Cakmak et al., 2010; Shine et al., 2011; Payez et al., 2013; Mahajan & Pandey, 2014). The dry and fresh weights were also higher than the control seedlings (Florez et al., 2007; Florez et al., 2012). Pre-treating the seeds before sowing also shows improved

Table 1 Seed germination indices for test group (EMF) and control group

Test and Control Plant Varieties	Mean germination time $\pm$ SD	Germination Index	Coefficient Velocity of Germination	Germination Percentage %	Seed Vigour Index
<i>Cajanus cajan</i> (Control)	0.053 $\pm$ 0.77	11.52 $\pm$ 4.9	0.31 $\pm$ 0.02	51	6124 $\pm$ 5.74
<i>Cajanus cajan</i> (test)	0.090 $\pm$ 1.12	24.27 $\pm$ 10.8	0.35 $\pm$ 0.14	86	8036.6 $\pm$ 4.16
<i>Solanum lycopersicum</i> (control)	0.59 $\pm$ 0.62	16.44 $\pm$ 12.9	0.18 $\pm$ 0.03	59	5340.00 $\pm$ 11.13
<i>Solanum lycopersicum</i> (test)	1.22 $\pm$ 1.61	23.43 $\pm$ 16.7	0.28 $\pm$ 0.09	83	5870.33 $\pm$ 5.50
<i>Cyamopsis tetragonoloba</i> (Control)	0.79 $\pm$ 0.83	10.25 $\pm$ 4.5	0.018 $\pm$ 0.027	53	2349 $\pm$ 5.56
<i>Cyamopsis tetragonoloba</i> (test)	2.56 $\pm$ 3.71	16.16 $\pm$ 8.6	0.29 $\pm$ 0.09	66	3178 $\pm$ 7.21
<i>Zea mays</i> (control)	1.62 $\pm$ 2.17	4.29 $\pm$ 1.29	0.23 $\pm$ 0.11	72	6440.33 $\pm$ 4.16
<i>Zea mays</i> (test)	2.73 $\pm$ 3.78	27.35 $\pm$ 1.59	0.37 $\pm$ 0.25	79	7789 $\pm$ 7.81
<i>Vignaradiate</i> (control)	0.19 $\pm$ 0.06	13.55 $\pm$ 15.99	0.35 $\pm$ 0.20	78	8174.66 $\pm$ 5.13
<i>Vignaradiate</i> (test)	0.30 $\pm$ 0.07	18.58 $\pm$ 1.78	0.29 $\pm$ 0.17	89	8278 $\pm$ 7.32
<i>Cicer arietinum</i> (control)	0.31 $\pm$ 0.15	11.49 $\pm$ 4.76	0.35 $\pm$ 0.20	52	7403 $\pm$ 3.60
<i>Cicer arietinum</i> (Test)	0.38 $\pm$ 0.21	6.36 $\pm$ 2.52	0.48 $\pm$ 0.32	68	8440.66 $\pm$ 7.76

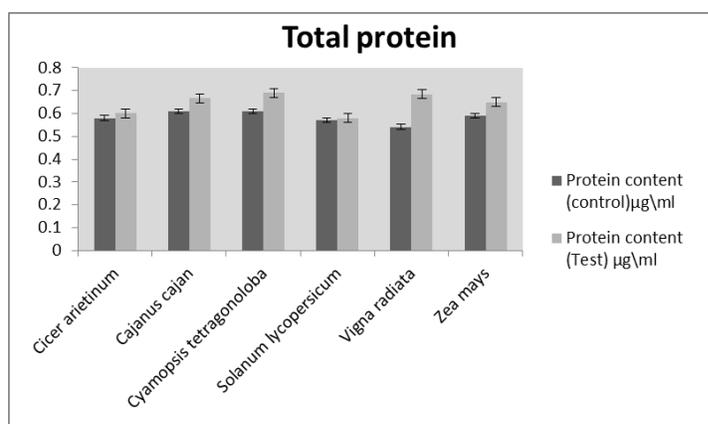


Figure 1 Total protein content for test &amp; control groups

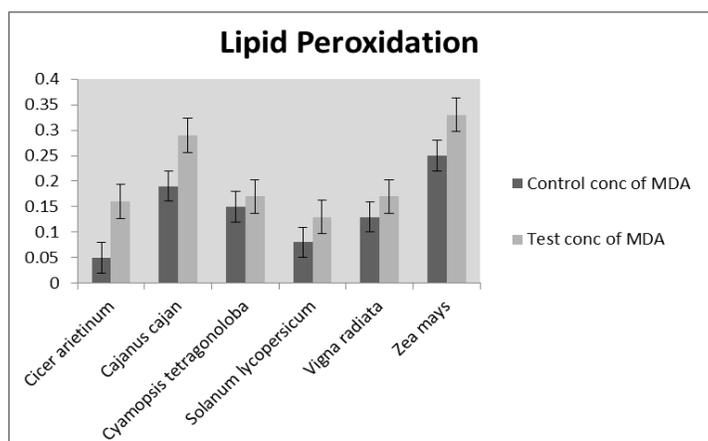


Figure 2 Concentration of MDA - product of LPO for control and test groups

results on seed germination and growth factors. Pre-treating the corn seeds with pulsed EMFs for varying time periods have shown an improved germination percentage, seed vigour index, chlorophyll content, leaf area, fresh & dry weights & the grain yield at full maturity (Bilalis et al., 2012). However, contrasting results have also been reported which show reduced mean germination time for rice seeds exposed to MF strengths for different time periods or chronic exposure (Florez et al., 2004). The continuous exposure gave better results than intervals of exposure (Carbonell et al., 2011). The physiological parameters in which the early growth stages of the seedling such as the shoot length, root length, fresh weight, dry weight, and leaf area showed uniformity in growth pattern in all the test groups. The results were consistent in all the triplicates. Total protein levels, MDA concentration, Superoxide dismutase (SOD) activity and chlorophyll content in test groups were more significant and considerably increasing in the test groups in comparison to the controls (Radhakrishnan & Kumari, 2013). This method and its variations i.e. magnetized water for irrigation, magnetizing the seeds before sowing are now being used for agriculture in some countries and varying as well as static magnetic fields and their variations are also

being experimented with (Ali et al., 2014; Hozayn et al., 2016; Zúñiga et al., 2016).

In the present study, ELF MF exposure to the seeds right from the beginning to the seedling stage resulted in enhanced growth. The major increases occurred when seeds were continuously exposed to the EMFs. The results we obtained for the germination indices as well as the chlorophyll content show an enhanced growth rate occurring in plants. A deeper understanding into the pathways or gene expressions affected by it could open new ways to fully develop this technique for use on an economically possible way.

New developments in this field are focused on the genetic transformations or epigenetic regulations playing their role in the growth promoting effects of EMFs on plant growth and its development (Rammal et al., 2014; Szafranski et al., 2017). EMF stimulation could be developed into an efficient method for an agricultural system devoid of the exploitation from the chemical fertilizers and a step towards a sustainable agriculture with better yields. Moreover, to upscale the application of these methods into agricultural systems would need a much wider exploration of EMFs and their effects on plants and other biological systems.

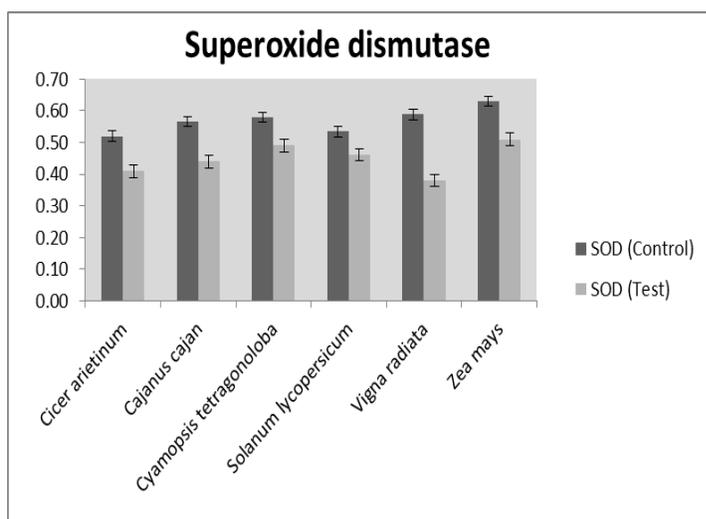


Figure 3 SOD units for control and test groups

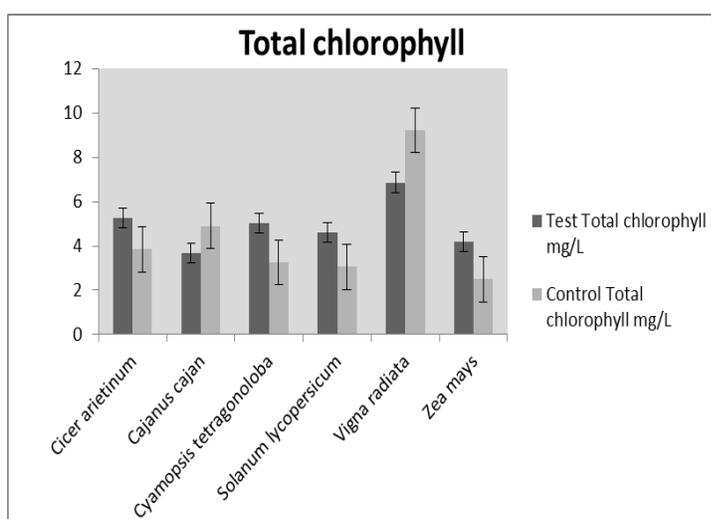


Figure 4 Total chlorophyll mg/L or test and control groups

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### Conflict of Interest

Nil

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