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Ramifications Of High Frequency, Low Frequency, And Static Magnetic Field On Nucleic Acid Expressions In Wheat (*Triticum Aestivum* L.)

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Abstract: Exposure to magnetic fields can have various effects on plant biology. It has been applied in the field of agriculture and is considered as an alternative for answering global food security question. The field on magnetism affecting plants is still in its budding phase and the overall mechanism is still being discovered. We have scrutinized the effect of different types of magnetic fields like high frequency generated by cell phones' electromagnetic radiation with power density: 8.55 µW cm^{-2}; 900 MHz band width; for ½, 1 and 2 hr, low frequency and static magnetic field created by an autotransformer attached to a Helmholtz coil produced magnetic field strengths of 30, 60 and 75 Gauss for different periods of time (10, 20 and 30 minutes) on nucleic acid levels in Wheat (*Triticum aestivum* L.) seedlings which were placed inside the Faraday's cage in case of high frequency using cell phones' electromagnetic radiation and inside Helmholtz coil for low frequency and static magnetic field. The DNA and RNA isolation were done on 7th, 10th and 14th day, and estimated. The 7th day isolate which among the best, in terms of concentration, was used for depicting the nucleic acid expression difference.

Index Terms- Magnetic Field, Global Food Security, Helmholtz coil, Faraday's cage, DNA, RNA

1. INTRODUCTION

The impact of magnetic field radiation on plants, including wheat, is still not fully understood. While some studies have suggested potential negative effects, such as altered growth and reduced yield, other research has found positive or no significant impact (Tran et al., 2023, Vian et al., 2016, Roche et al., 2022, Racuciu and Creangia, 2005, Al-Kathiri et al., 2016, Rath et al., 2023a). It is essential to continue studying the effects of magnetic field radiation on crops like wheat, as they play a crucial role in global food security and the economy. Additionally, as technology advances, it is important to consider the potential consequences and take measures to mitigate any harmful effects. Farmers and policymakers should work together to ensure that crops are grown and harvested in a safe and sustainable manner, with a focus on minimizing potential risks to both human health and the environment.

While wheat is an essential crop globally, concerns have been raised about the potential impact of magnetic field radiation on plant growth, including wheat (Mekki and Badr,2013, Qureshi et al., 2017, Dhawi et al., 2009, Sarraf et al.,2020). Current research on this topic is inconclusive, and further studies are needed to ensure the safe and sustainable growth of crops. Moreover, the development of green technologies such as magnetic fields could offer promising solutions to enhance crop growth and minimize environmental toxicity. Continued research and adaptation of innovative technologies are necessary to promote safe and sustainable crop production

2. MATERIALS AND METHODS

2.1. Plant material

Wheat seeds (*Triticum aestivum* L.); variety-HD 3117, India) were collected from Division of Seed Science and Technology, ICAR-IARI, New Delhi.

2.2. Soil Sample

Soil sample was collected from regions adjoining Noamundi Iron Mines, Noamundi, Jharkhand. The soil samples were analysed (Table 1) and used in the experiment after sieving.

Table 1: Analysis result for soil sample collected from region adjoining Noamundi Iron Mines, Noamundi, Jharkhand.

Location	Soil type	Taxonomic group	pH[a]	Organic Carbon[b] (%)	Total Nitrogen[c] ($mg.kg^{-1}$)	Phosphorus[d] ($mg.Kg^{-1}$)	Potassium[e] ($mg.Kg^{-1}$)	Iron[f] ($mg.Kg^{1}$)
Noamundi Iron Mines, Noamundi, Jharkhand	Laterite	Haplustulf	6.35	0.49	345	< 3	77	< 5

[a] Measured by taking 1:1.25 soil water ratio by Elico-digital pH meter
[b] Determined by Walkey-Black method
[c] Estimated by Kjeldal method
[d] Estimated by Bray's No.1 method
[e] Estimated by Platenic-chloride gravimetric method
[f] Estimated by Citrate dithionite-bicarbonate method

2.3. Application of Low frequency and static electromagnetic field

2.3.1. Growing of Plants

Seeds were placed inside a plastic tray containing soil from Noamundi Iron Mines, Noamundi, Jharkhand and exposed to magnetic field generated by AC (Low frequency) and DC (static) output. The dimension of the plastic tray used was 20 cm x 15 cm. The seeds were placed in 5 columns and 4 rows on the soil. No extra manure or fertilizers were used (Rath et al., 2023a).

2.3.2. Design of Helmholtz coil

Single axis-Helmholtz coil was made based on simulation in MATLAB (Rath et al., 2023a, Gyawali, 2008). The three-dimensional plot obtained from MATLAB shows a region of uniform magnetic field around the centre of the coil. (Figure 2). The material used for the frame was plywood and was made according to the specification avoiding any type of metals and nails (Table 2). Only glue was used. 10 Standard wire Gauge (SWG) copper wire was used for winding a total of 100 turns in each coil and both the coils were connected in series. A flat base was made using the same plywood and was put at the centre of both the coils for exposure to uniform magnetic field (Figure 1). The magnetic field inside the Helmholtz coil was calculated theoretically and the same was measured practically using a gauss meter. This was done for accuracy in obtaining magnitude and uniformity data.

Table 2: Helmholtz coil specification

Type	Monoaxial
Coil radius	47.5 Cm
Number of turns per coil	100
Coil height	130 Cm
Copper wire (Gauge)	10 SWG Copper
Material	Plywood

(a) **(b)**

Figure 1 (a and b): Helmholtz coil with base for magnetic field exposure

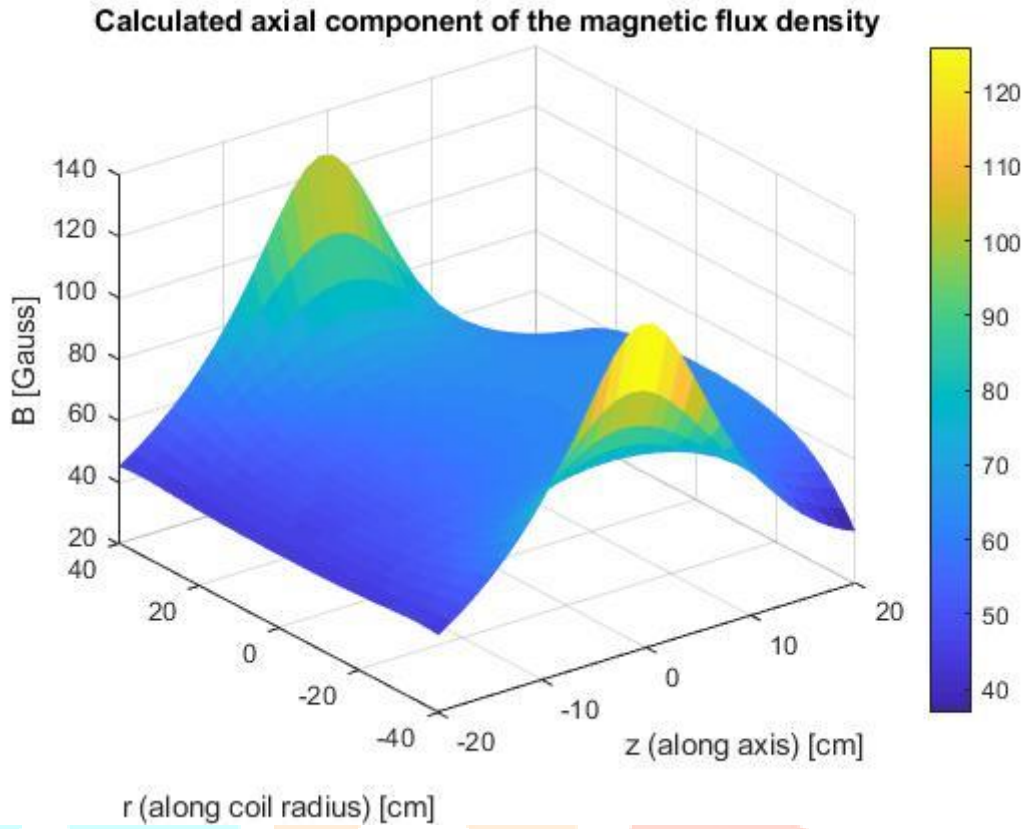

Figure 2:
Three-dimensional plot obtained from MATLAB showing region of homogeneity around the centre of the Helmholtz coil

2.3.3. Design of Autotransformer

The autotransformer was of variate kind and designed in order to avail multiple output voltage from a single AC and DC input of 220 Volt, 50 Hertz. This caused multiple output current to pass through the Helmholtz coil and resulted in different flux densities of magnetic field (30 G, 60 G and 75G). DC output was generated using a bridge rectifier consisting of four diodes connected in a series and attached to the autotransformer (Rath et al., 2023a).

2.3.4. Experimental methods

As a treatment, three very different magnetic flux densities (30 G, 60 G and 75G) generated by both AC and DC outputs for three different time intervals (10, 20 and 30 minutes) were applied to seeds till they germinate and turn to wheat grass in the experiment for fourteen days. Test conditions were carried out and compared with control (Plants grown under Earth's magnetic field (GMF)) (Rath et al., 2023a).

DNA and RNA were isolated from sample leaves harvested for 60 G and 30 minutes on 7^{th}, 10^{th} and 14^{th} Day After Treatment (DAT). The nucleic acids were estimated, and quality checked. The readings were tabulated and analysed. The experiments were carried out in triplicate to ensure consistent results and to establish the reliability of the findings.

2.4. Application of High frequency electromagnetic field

2.4.1. Design of Faraday's Chamber

The experimental setup called for a shielded chamber designed to act as a Faraday cage (Rath et al., 2023b, Sharma et al., 2009) which followed the pattern of a Mode Stirred Reverberation Chamber (MSRC). A thermocol chamber was designed with plywood frame covering its walls completely with Faraday fabric to obtain an environment free from outside EMF interferences. The Faraday fabric (thickness: 0.09 mm and surface resistance: < 0.05) was purchased from J J CARE, USA, a military grade shielding fabric made of polyester fibre, metallic copper, and metallic nickel (Figure 3). Two GSM cell phones operating at the 900 MHz frequency band, with modulated voice of 217 and low-frequency signals of 8.34 Hz were used in the present study (Hyland, 2000). These cell phones were placed inside the chamber (47.5 cm x 27 cm x 17.5 cm) to expose seeds to a homogeneous electromagnetic field. The exposure had a field strength of 5.7 V/m and an average power density (PD) of 8.549 µW/cm² measured using a RF meter (GQ EMF-390, USA). Cell phones were placed in talk mode, with earphones attached. This adaptation simulated a real-world scenario in which cell phones are actively transmitting and receiving RF signals during the talk mode. The aim of the study was to observe the effects of RF exposure on the seed samples. For a continuous operation during the exposure period, the phone batteries were charged with a help of a 12 V DC, 220 V AC adapter. The adapter was placed 2 meters away from the cell phones to minimize any interference or impact on the exposure conditions inside the chamber.

(a) **(b)**

Figure 3: (a and b): Shielded chamber for RF electromagnetic radiation exposure

2.4.2. Growing of Plants

A total of hundred pre-soaked seeds with distilled water for 8 hours was placed in a petri dish having a diameter of 4.5 cm. petri dish was positioned between the cell phones at approximately 8 cm distance away from both the phones. These seeds were exposed to RF radiation generated from the cell phones for durations: ½ hour, 1 hour and 2 hours. A control group constituting another set of 100 seeds were placed inside a separate chamber without cell phones, hence no RF exposure for this group. The control group was the baseline and accounted for any changes or effects that may occur due to factors other than the RF exposure. Prior care was taken to eliminate all other electromagnetic field radiation (EMF radiation) sources both inside and outside the exposure laboratory during the treatment time. This ensured that the observed effects on the seeds can only be attributed to the RF radiation from the cell phones. Also, both the exposure chamber (with cell phones) and the control chamber (without cell phones) had a constant room temperature of 25°C (Rath et al., 2023b)

Exposed and control seeds were allowed to germinate in plastic trays containing the soil from regions adjoining Noamundi Iron Mines, Noamundi, Jharkhand, for a period of fourteen days with constant temperature, humidity, and light.

DNA and RNA were isolated from sample leaves harvested on 7th, 10th and 14th Day After Treatment (DAT). The nucleic acids were estimated, and quality checked. The readings were tabulated and analysed. The experiments were carried out in triplicate to ensure consistent results and to establish the reliability of the findings.

2.5. Isolation of Nucleic Acid

2.5.1. Isolation of DNA

100 mg of fresh plant leaves were used to isolate DNA using FastDNA® SPIN kit (MP Biomedicals, India) (Figure 4). The standard procedure, which was provided by the KIT, was followed. The DNA obtained was further checked for its quality. Absorbance helps determine the concentration of the DNA preparation and detect any possible contaminants. DNA absorbs the most at 260 nm, while proteins absorb the most at 280 nm. Organic compounds and chaotropic salts, on the other hand, absorb maximally at 230 nm. The A260/A280 ratio can serve as an indicator of DNA purity, and the ideal range for this number is between 1.8 and 2.0.

Figure 4: FastDNA® SPIN kit (MP Biomedicals, India)

DNA estimation was done using UV-Vis spectroscopy (Dash et al.,2020). DNA quality and quantity was checked using UV-Vis spectroscopy. The DNA was estimated using Beer-Lambert's law (Swinehart, 1962) where

$$A = \epsilon CL$$

A = Absorbance

ϵ = Extinction coefficient, for DNA it is 0.020 $(mg/ml)^{-1}$ / cm^{-1}

C = concentration of DNA

L = Path length of spectrophotometer cuvette

2.5.2. Isolation of RNA

100 mg of fresh plant leaves were taken and modified Trizol method was used to isolate RNA (Rio et al., 2010). For every 100 mg of plant leaves 1 ml of Trizol (TRIzol-T Reagent, SRL, India) used for homogenisation and incubation for 2 to 3 min. Later, the tube was centrifuged for 15 minutes at 20000g at 4°C. The clear upper aqueous layer containing RNA, was transferred to a new 1.5 mL MCT, and 0.5 mL of isopropanol per millilitre of initial Trizol was added. The MCT was inverted 5 times and incubated for 10 minutes at room temperature. The sample was further centrifuged for 15 minutes at 20000g at 4°C. The supernatant was discarded, and the pellet resuspended in 1000 µL of 80% ethanol. The sample was again centrifuged for 5 minutes at 15000g at 4°C. The extra ethanol was removed in orderly fashion first with a 200 µL pipette and later with a 2 µL pipette. The remaining pellet should have a glass like texture. Total RNA isolated was eluted by 20 µL of nuclease-free water (Genetix Biotech Asia Pvt LTD.) (Amirouche et al., 2021).

RNA quality and quantity was checked using UV-vis spectroscopy. The A260/A280 ratio is also used to assess RNA purity. An A260/A280 ratio of 1.8 to 2.1 indicates highly purified RNA. The RNA was estimated using Beer-Lambert's law (Swinehart, 1962, Okamoto and Okabe, 2000), where

$$A = \epsilon CL$$

A = Absorbance

ϵ = Extinction coefficient, for RNA it is 0.025 $(mg/ml)^{-1} / cm^{-1}$

C = concentration of RNA

L = Path length of spectrophotometer cuvette

2.6. DNA and RNA profiling on agar gel

Leaves treated with 60G for 30 minutes were selected for the both DNA and RNA profiling. Such a selection was based on previous result during germination, post germination studies and current studies of both DNA and RNA quantification and quality. (Rath et al., 2023a, Rath et al., 2023b)

2.6.1. Preparation of Gel

Agarose gel electrophoresis is a widely used technique for separating DNA and RNA fragments by size. To prepare an agarose gel, weighing of 0.5 grams of agarose was done into an Erlenmeyer flask. The concentration of the gel will depend on the sizes of the DNA and RNA fragments to be separated. 0.5% agarose gel was prepared. Addition and swirling of running buffer to the flask was carried out. Gel running buffers used was TAE (40 mM Tris-acetate, 1 mM EDTA). The agarose/buffer mixture was melted by heating in a microwave, swirling at 30 s intervals until all the component dissolved. Addition of ethidium bromide (EtBr) to a concentration of 0.5 µg/ml was done when agarose gel was just about to be solidified (Lee et al., 2012).

The agarose gel was cooled by incubation it in a 65 °C water bath. Failure to do so will warp the gel tray. The liquified gel was placed on the tray inside the casting apparatus. An appropriate comb was placed into the gel mould to create the wells. The molten agarose was poured into the gel mould. The agarose gel was set at the room temperature to solidify. After solidification, the comb was removed and placed in the gel box. Alternatively, the gel can also be wrapped in plastic wrap and stored at 4 °C until use (Lee et al., 2012).

2.6.2. Setting up of Gel Apparatus and Separation of DNA and RNA

The loading dye was added to about 20 µl of the DNA and RNA samples prepared for the separation. Gel loading dye was prepared with a 6X concentration (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol). These help to track how far the DNA and RNA samples has travelled, and also allows the sample to sink into the gel. The power pack is programmed to desired voltage (1-5V/cm between electrodes). Enough running buffer was added to cover the surface of the gel. It is highly recommended to use the same running buffer as the one used to prepare the gel. The leads of the gel box to the power supply were attached. A check was carried out by turning on the power supply and verifying that both gel box and power supply are working. The lid was removed and the DNA and RNA samples into the gel were slowly and carefully loaded. The lid to the gel box was replaced. Care was taken for the positioning of gel where cathode (black leads) was closer to the wells than the anode (red leads). Double checks were done before the electrodes are plugged into the correct slots in the power supply. The power was turned on and the gel was run until the dye had migrated to an appropriate distance (Lee et al., 2012).

2.6.3. Visualization of separated DNA and RNA fragments

When electrophoresis has completed, the power supply was turned off and the lid of the gel box, removed. The gel was removed from the gel box. The excess buffer was drained off from the surface of the gel. The gel tray was placed on paper towels to absorb any extra running buffer. The gel from the gel tray was exposed to uv light in Geldock systems. DNA and RNA bands showed up as orange fluorescent bands. Picture was taken of the gel run which was later analysed. The gel and running buffer were properly disposed of as per regulations (Lee et al., 2012).

2.7. Statistical Tools

A minimum of three replicates were conducted for each experimental group, and the results were calculated as mean ± standard error. Two-way ANOVA with P>0.01 significance was carried out using SPSS Version 27. Correlation analysis with 95 percent confidence interval was carried out using R-statistical tool version 4.3.0 and R-Studio version 2023.03.1-446.

3. RESULTS AND DISCUSSION

3.1. Estimation of DNA

All the quality of DNA had a quality range from 1.8 to 2.0. Among the wheat grass leaf sample collected on 7th, 10th and 14th day, plants treated with low frequency magnetic field had the highest DNA content (Table 3 and Figure 5). This may be due to the positive effect of magnetic treatment. Wheat grass treated with high frequency magnetic field showed reduced DNA content. It implies that the high frequency magnetic field affected negatively in plant growth. The results show that exposure to low frequency and static electromagnetic field increased DNA concentration, while exposure to high frequency decreased it. Additionally, the effects were more pronounced in plants exposed for longer durations. But the significant difference of treated and its control can be observed on the 7th day samples. This factor played major role for being selected for DNA gel run analysis to showcase a typical DNA profiling illustration.

Table 3: Different sample days with respective treatment and concentration of DNA isolated from per milligram of fresh leaves along with its standard error mean

Sample	Concentration (ng of DNA/ mg of fresh plant sample)	260/280
7th day Control	112.14±0.91	1.82±0.03
7th day Low Frequency	214.03±0.52	1.86±0.04
7th day Static	195.38±1.02	1.92±0.03
7th day High Frequency	97.40±0.78	1.82±0.04
10th day Control	177.41±0.67	1.89±0.05
10th day Low Frequency	221.69±0.38	1.88±0.06
10th day Static	209.31±0.65	1.82±0.04
10th day High Frequency	109.72±0.18	1.93±0.05
14th day Control	200.89±0.14	1.89±0.03
14th day Low Frequency	231.93±0.64	1.80±0.03
14th day Static	221.43±0.25	1.80±0.02
14th day High Frequency	133.17±0.47	1.83±0.03

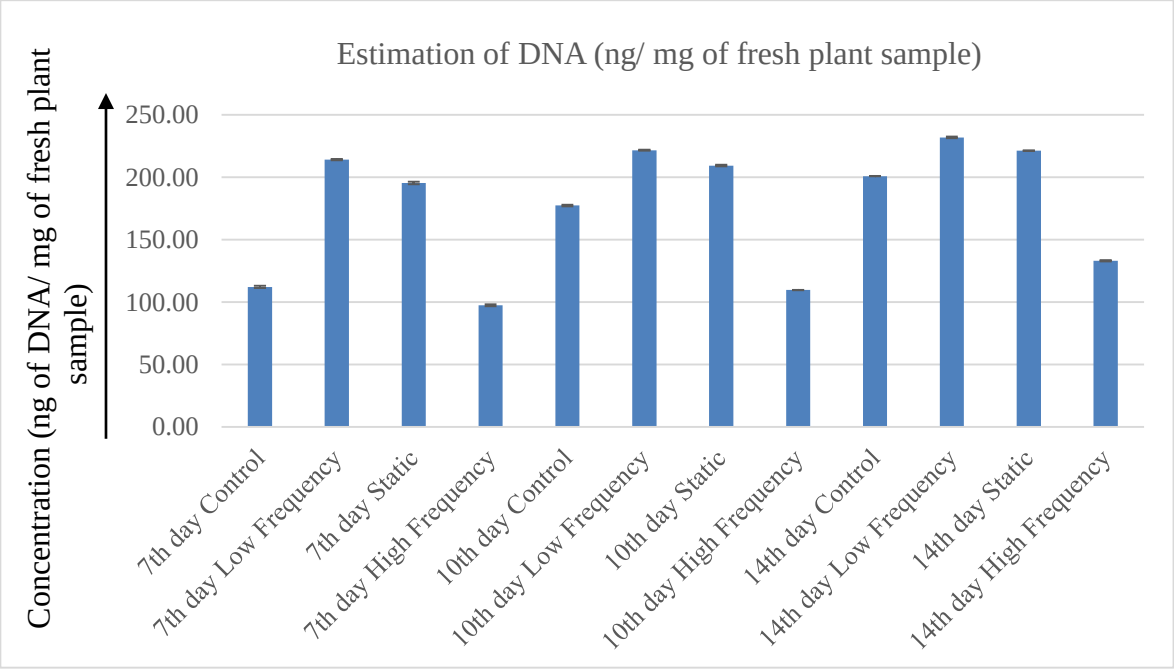

Figure 5: Illustration of the measure of DNA concentration per mg of fresh plant leaves obtained in different days with different treatments

3.1.1 Two-way ANOVA between samples and treated for DNA isolates

Table-4 shows the results of a two-way analysis of variance (ANOVA) conducted on plant samples exposed to different frequencies for different durations. The table provides information on the sum of squares (SS), mean sum of squares (MSS), and F-ratio for between samples, between treatment, within error, and the total. The two-way ANOVA results indicate that there is a significant difference between the treatment groups, as the F-ratio is greater than the critical value. The effects of

frequency exposure on DNA concentration are more pronounced in plants exposed for longer durations, as evidenced by the higher MSS for between samples in the longer duration treatment groups.

Table 4: Showing two-way ANOVA between samples and between treatment with confidence interval of 99.9%.

	df	SS	MSS	F-ratio
Between Samples	2	10755.276	5377.638	29.560
Between Treatment	3	65739.208	21913.069	120.452
Within Error	30	5457710	181.924	
Total	35	81852.194		

Two-way ANOVA between the samples and treatment did not proof to be significant as the calculated significance was found below <0.001 which explains that both the days of sample collection and the intensity of treatment were affecting the DNA replication in experimental plants.

3.1.2. DNA Gel Run

A single DNA isolate from leaves treated with 60 Guass of low-frequency magnetic field for thirty minutes was selected due to its significant levels during DNA estimation per mg of leaves from all other isolates. This profiling establishes that DNA of treated was more per µl of sample load, thus supporting the claim of increase in DNA amount due to treatment. (Figure 6)

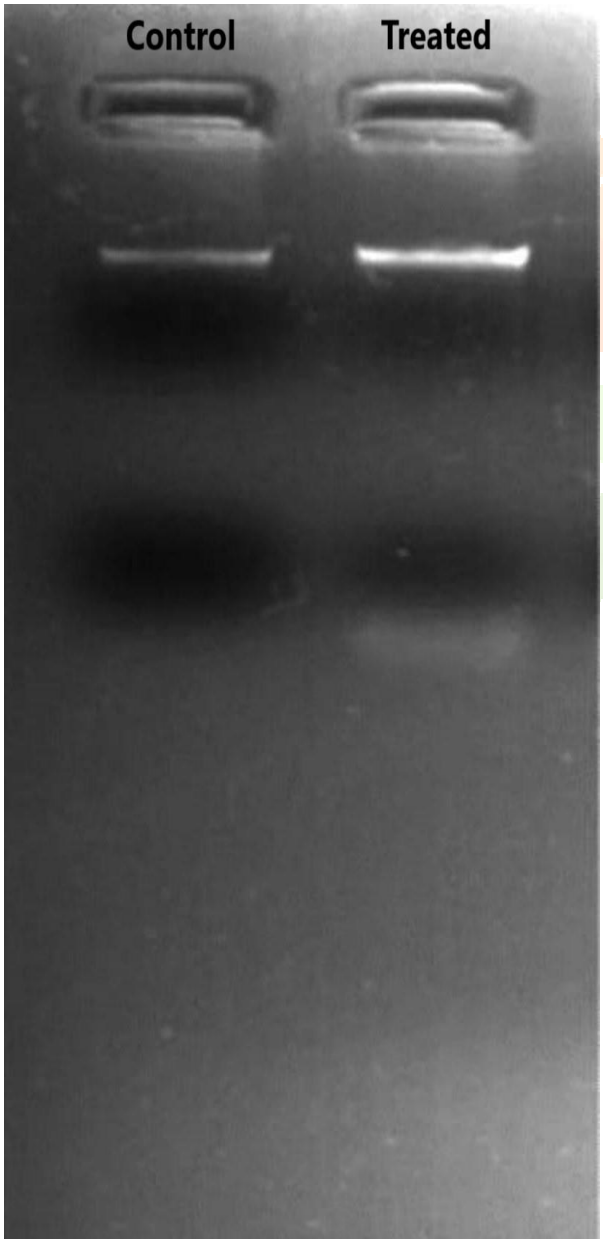

Figure 6: DNA profiling of control and treated sample (60 Gauss, Low frequency electromagnetic radiation for 30 minutes) in 0.5 % Agarose gel run

3.2. Estimation of RNA

Table 5: Different sample days with respective treatment and concentration of RNA isolated from per milligram of fresh leaves along with its standard error mean

Sample	Concentration (ng of RNA/ mg of fresh plant sample)	260/280
7th day Control	78.12±0.49	1.89±0.04
7th day Low Frequency	118.68±0.29	1.90±0.03
7th day Static	109.95±0.14	1.97±0.06
7th day High Frequency	53.05±0.81	1.89±0.03
10th day Control	88.054±1.09	1.94±0.03
10th day Low Frequency	128.85±0.21	1.98±0.07
10th day Static	124.49±0.57	2.04±0.07
10th day High Frequency	76.36±0.35	2.05±0.05
14th day Control	100.73±0.28	1.85±0.04
14th day Low Frequency	139.11±0.51	1.90±0.02
14th day Static	134.78±0.10	1.91±0.03
14th day High Frequency	88.18±0.40	2.00±0.04

RNA had a quality range from 1.8 to 2.1. The concentration of RNA in different samples of fresh plant leaf at different time intervals 7th, 10th and 14th day was estimated. The samples were exposed to different frequencies of magnetic field. The control samples were not exposed to any magnetic field. The concentration of RNA was measured in ng/mg of fresh plant sample. The data (Table 5 & Figure 7) suggests that exposure to low frequency magnetic field resulted in the highest concentration of RNA, while exposure to high frequency magnetic field resulted in the lowest concentration of RNA. The results also show that the concentration of RNA increased with time, with the highest concentration observed on the 14th day. But low frequency treated sample of 7th day showed significant difference of RNA content when compared with its control. This difference of RNA content was considered when RNA profiling through gel run analysis was done.

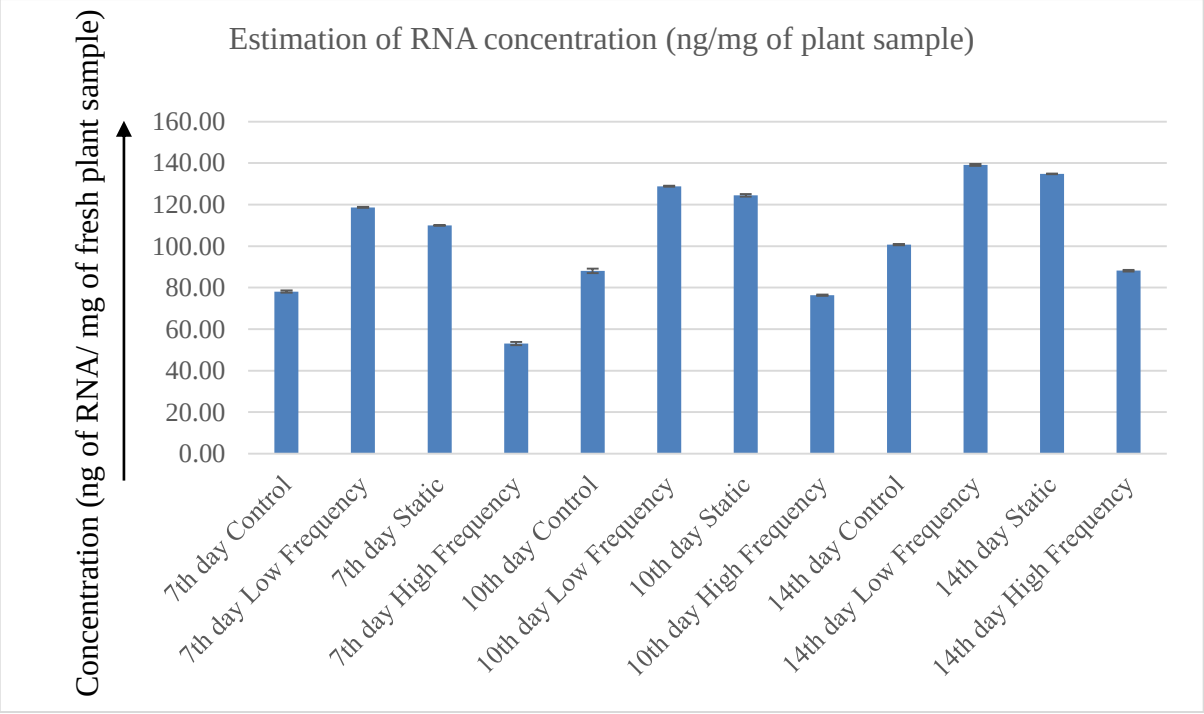

Figure 7: Illustrating the measure of RNA concentration per mg of fresh plant leaves obtained in different days with different treatments

3.2.1. Two-way ANOVA between samples and treated for RNA isolate

Table-6 shows the results of an analysis of variance (ANOVA) for RNA concentration in fresh plant tissue samples exposed to different frequencies of magnetic field at different time intervals. The two-way ANOVA compares the variation between samples, between treatments, and within the error. The F-ratio is the ratio of the variation between the samples or treatments to the variation within the error. A higher F-ratio indicates a greater difference between the means of the samples or treatments. In this case, the F-ratio for the between treatment is much higher than the F-ratio for the between samples, indicating that the treatment has a significant effect on RNA concentration.

Table 6: Showing two-way ANOVA between samples and between treatment with confidence interval of 99.9%.

	df	SS	MSS	F-ratio
Between Samples	2	4000.016	2000.008	216.797
Between Treatment	3	19778.717	6592.906	714.658
Within Error	30	276.758	9.225	
Total	35	24055.491		

Two-way ANOVA between the samples and treatment didn't proof to be significant as the calculated significance came below <0.001 which explains that both the days of sample collection and the intensity of treatment were affecting the RNA generation in experimental plants.

3.2.2.RNA Gel run

A single RNA isolate from leaves treated with 60 Guass of low-frequency magnetic field for thirty minutes was selected due to its significant levels during RNA estimation per mg of leaves from all other isolates. This profiling establishes that RNA of treated was more per µl of sample load, thus supporting the claim of RNA amount increase due treatment. (Figure 8)

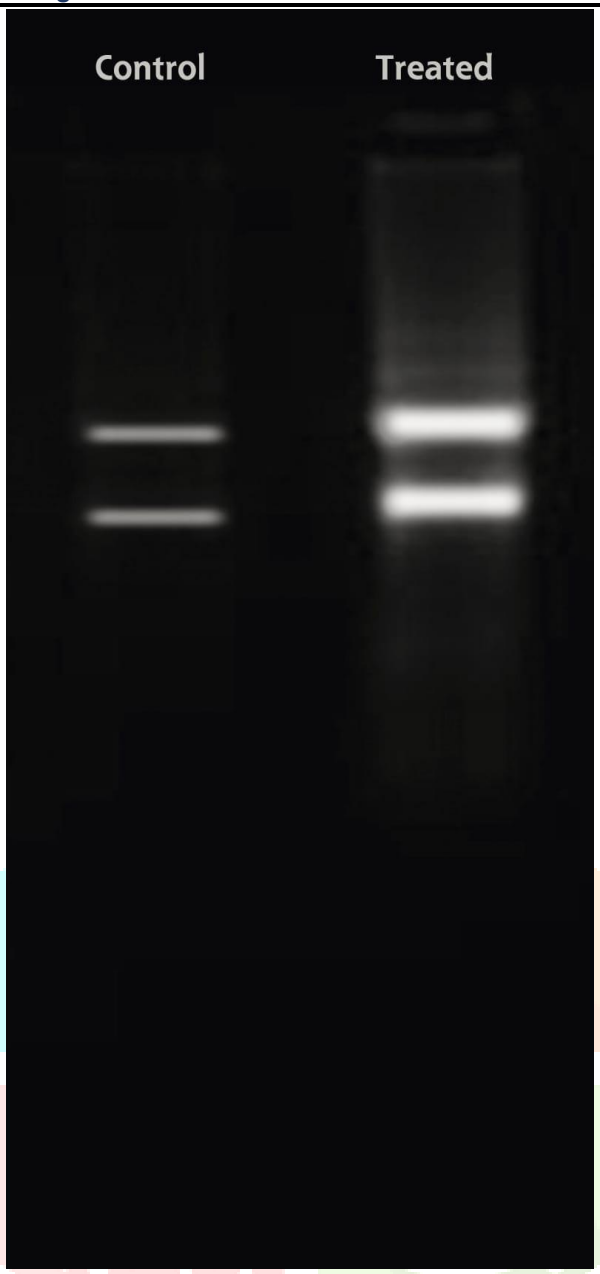

Figure 8: RNA profiling of control and treated sample (60 Gauss, Low frequency magnetic radiation for 30 minutes) in 0.5 % Agarose gel run

4. CONCLUSION

Among all the treatments given, the DNA and RNA production were found to be higher on 7th day due to low frequency electromagnetic field. Our findings suggest increase in DNA and RNA production due to Static and low frequency magnetic field. High frequency magnetic field adversely affected the level of both DNA and RNA. It can be inferred that with increase in both DNA and RNA levels, protein levels will increase and thus the overall metabolism. This treatment will mature crops better and faster which will benefit the population in tackling with global hunger and avoid environment polluting, non-degradable chemical fertilizers. We are working to study further the exact nature of different metabolic pathways and various compounds generated along the way which may help develop pharmaceutically important compounds (Rath et al., 2023c).

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