



## Increase of Anthocyanin, Vitamin C, and Flavonoid Content in Red Lettuce (*Lactuca Sativa L.*) using Alternating Magnetic Field Exposure

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### Abstract

Red lettuce (*Lactuca sativa L.*) is widely consumed for its health benefits. With the a growing global population and limited land resources, there is a need to increase the nutritional content and bioactive compounds of vegetables or fruits. This research aims to increase the growth, productivity, and the content of anthocyanin, vitamin C and flavonoids in red lettuce leaves through exposure to magnetic fields during seeding. The research utilized red lettuce seeds as the research sample. Exposure was carried out using an alternating magnetic field (MF) with an effective magnetic flux density (MFD) of 0.4 mT, a frequency range of 25-125 Hz, and a daily exposure time of 15 minutes. The treatment of each sample group was repeated for five days. The results showed that exposure to a MF with a frequency of 75 Hz resulted in optimum growth and weight, an increase of 65.15% and 60.18%, respectively. The optimum anthocyanin, vitamin C, and flavonoid content were obtained at an exposure to a frequency of 100 Hz, an increase of 22.52%, 40.70%, and 32.50%, respectively. Exposure to seeds uses an alternating MF; frequency is an important parameter for producing optimum enhancement.



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### Introduction


Vegetables generally contain many antioxidants, including vitamin C, carotenoids, anthocyanin, and flavonoids,<sup>1</sup> which can reduce the number of free radicals and the potential risk of developing carcinogenic diseases. Consuming antioxidants from natural foods such as vegetables is a good

way to prevent cardiovascular diseases, especially arteriosclerosis.<sup>2</sup> Lettuce is widely consumed fresh in salad and other dishes. In its fresh state, lettuce contains vitamin C of around 3.9 mg/100g<sup>3</sup> and anthocyanins of around 351.3 µg/g.<sup>4</sup> Currently, the global population is increasing, while the availability of agricultural land is decreasing. To meet nutritional

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needs, it is necessary to improve the agricultural properties of vegetables or fruits to increase the nutritional content and bioactive compounds that are useful for human health. Effective cultivation management strategies can help plants become more resistant to microbes, enhance their tolerance to environmental stress, increase production yields,<sup>5</sup> and elevate the active biomolecule content.<sup>6</sup>

Various methods have been used to increase the productivity and nutritional content of vegetables or fruits, some of which are organic or inorganic fertilization, biofortification, nanotechnology, and others. The application of a nitrogen fertilizer at a dose of 60 kg Nha<sup>-1</sup> resulted in increased levels of calcium and magnesium in cherry leaves, while at a dose of 120 kg Nha<sup>-1</sup>, the levels decreased.<sup>7</sup> However, the inappropriate use of mineral fertilizers in many productive soils has decreased their ability to function.<sup>8</sup> When using litter compost with livestock manure at a rate of 25 Mg ha<sup>-1</sup> combined with nitrogen fertilizer, there was an increase in the fresh weight and the number of leaves per mustard plant. Conversely, there was no significant difference without the addition of nitrogen fertilizer.<sup>9</sup> However, it is worth noting that the use of organic fertilizers generally requires large amounts to achieve the desired effect;<sup>10,11</sup> moreover, it involves significant labor investment, and the quality still needs improvement.<sup>12</sup> Biofortification aims to increase the concentration of nutrients in food,<sup>13</sup> which is beneficial for human health,<sup>14</sup> but it often comes with high costs, and the availability of biofortified foods is generally limited.<sup>15</sup> The unutilization of nanotechnology in agriculture is highly expensive, making it difficult for farmers to access.<sup>16</sup> Meanwhile, the use of microbial fertilizers (MFs) is a cost-effective and environmentally friendly method.

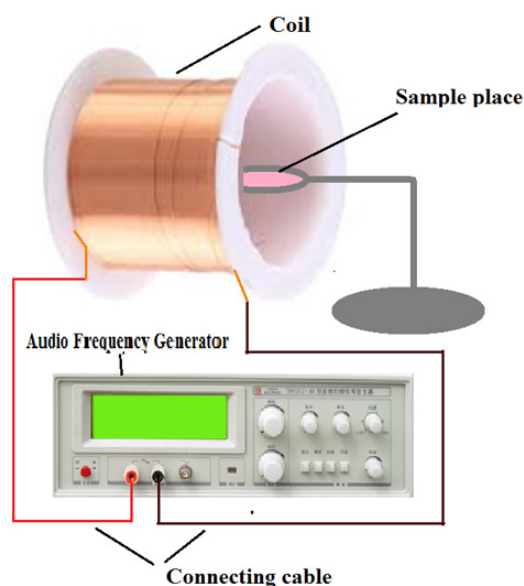
Several studies have shown that treatment with MFs can increase plant tolerance to biotic (living)<sup>17</sup> and abiotic (dead)<sup>18</sup> stresses, resulting in observable responses from their activation.<sup>19</sup> Research using MFs in plants has been carried out using various MFs, including low-frequency, static, and pulsed MFs.<sup>20</sup> For example, Shabrangi & Majd<sup>21</sup> used a static MF with an intensity of 0.1 mT, while Iqbal *et al.*<sup>22</sup> applied MFs with low frequencies, specifically MFs of 60 mT and 180 mT. However, no research has yet reported the frequency of MFs that can optimize plant growth, productivity,

and antioxidant content. It has been reported that the cell membranes of biological systems contain both positive and negative ions, thereby generating electric fields in and around the cell membrane.<sup>23</sup> It was further revealed that external MFs could interact resonantly with the internal electric fields of biological systems,<sup>24</sup> suggesting the potential for significant impacts on red lettuce plants' growth, productivity, and antioxidant content. Given these findings, this research aims to identify a MF frequency that can optimize plant growth, productivity, and the content of anthocyanin, vitamin C, and flavonoids.

## Materials and Methods

### Sample preparation

In this study, samples of red lettuce seeds produced by PT Bisi International Tbk- Indonesia were utilized. Seeds of nearly uniform size were selected for the experiment. These seeds were divided into six groups, each containing ten seeds. The seeds were initially soaked in water for 10 minutes and then placed on cotton that had been moistened.



**Fig.1: The arrangement of the seed exposure device using an alternating magnetic field**

### Research Tool Design

In this study, an alternating MF was employed as a research tool. The MF was generated using a solenoid coil, which was connected to an alternating current source in the form of a sinusoid of the

frequency audio generator. The solenoid was made of copper wire measuring 1.0 mm and consists of 216 turns. The solenoid had a radius of 2 cm. In its implementation, the solenoid is connected to a frequency audio generator, and the seeds are placed in the middle of the solenoid (Figure 1). Once assembled, the audio frequency generator was activated by selecting a sinusoidal voltage source. The current and frequency were adjusted in such a way as to produce an effective MFD of 0.4 mT. The MFD was measured using a Kanetec TM801 Tesla Meter.

### Exposure to Magnetic Fields

The seeds, which had been moistened and placed in cotton, were exposed to an alternating MF with an effective flux density of 0.4 mT and a different frequency according to the group. The MF frequency used was 0 -125 Hz with an exposure time of 15 minutes each, repeated daily for up to five days. Exposure to MFs was conducted at the Biophysics Laboratory of Maulana Malik Ibrahim State Islamic University, Malang.

### Seeding and Planting

Seeding was conducted in polybags measuring 6 x 6 cm filled with a mixture of husk charcoal and sand in a ratio of 1: 1, with a depth of 4 cm. The seeds sown in polybags were watered twice daily, every morning and evening. Seedlings were initially grown in nursery polybags, and at 15 days of age, were transferred into larger 25 cm x 25 cm polybags. Plants in these polybags were watered every morning. The planting took place in the Green House of the Maulana Malik Ibrahim State Islamic University, Malang under environmental conditions featuring a temperature range of 21-30°C and air humidity levels of 70-85%

### Determination of Anthocyanin Content

To determine the anthocyanin content of lettuce leaves, the pH-differentiation method was employed.<sup>25,26</sup> The measurements were taken on the third leaf from the top when the plants were 38 days old. The leaves were thoroughly mashed until they released liquid, and then as much as 10 grams of this liquid were collected in a Duran brand Erlenmeyer. To this liquid, 25 mL of 1% HCl (Brand of Merck- Supelco) solution was added. The solution was macerated for 1 minute and then shaken for

5 minutes. Subsequently, it was filtered and then placed in a test tube, covered with aluminum foil and left in the refrigerator for 24 hours. The samples that had precipitated or cooled were returned to room temperature for 2 hours. The samples were divided into two parts, each measuring 0.2 mL. The first part was added with a 4.8 buffer solution at pH 1.0, while the second part was added with a 4.8 buffer solution at pH 4.5. These mixtures were homogenized using a vortex (Brand of D-LAB; MX-S Type). Absorption measurements were carried out at wavelengths of 400 nm and 700 nm using a Phywe P3070101 UV-Vis spectrometer. The absorbance determination of the dissolved sample (A) was carried out using equation (1).

$$A = (A_{\lambda_{400 \text{ nm}}} - \lambda_{700 \text{ nm}}) \text{ pH } 1.0 - (A_{\lambda_{400 \text{ nm}}} - \lambda_{700 \text{ nm}}) \text{ pH } 4.5 \quad \dots(1)$$

Meanwhile, the determination of the anthocyanin content was carried out using equation (2)

$$\text{Anthocyanin content} : (A \times \text{BM} \times \text{FP}) / (\epsilon \times l) \times 1000 \quad \dots(2)$$

where: A = absorbance, l = cuvette thickness (1 cm),  $\epsilon$  = molar absorptivity (26900 L/(mol.cm)), FP = dilution factor, BM = molecular weight (449.2 g/mol).

### Determination of Vitamin C Content

Vitamin C content was measured using a Phywe P3070101 UV-Vis spectrometer.<sup>27</sup> This analysis was conducted on leaf samples taken the third position from the top, collected when the plants were 38 days old. To establish as standard reference, ascorbic acid was prepared as a standard solution by dissolving 50 mg of ascorbic acid in 100 mL of distilled water to form a solution with a concentration of 500  $\mu\text{g}/\text{mL}$ . The maximum wavelength was determined using a solution with a concentration of 7  $\mu\text{g}/\text{mL}$ , and measurements were taken at a wavelength of 200-400 nm; <sup>28</sup> the results were obtained at a wavelength of 265 nm. For the calibration of vitamin C measurements, a calibration curve was made using various concentrations, namely 3,5,7,9, and 11  $\mu\text{g}/\text{mL}$ . This calibration curve was generated from the measurement results, yielding a regression equation,

$$Y = 0.056X + 0.079 \quad \dots(3)$$

where  $x$  is the concentration of vitamin C, while  $y$  is the absorbance.

To prepare the extract 10 g of finely ground sample were mixed with 50 mL of 5% metaphosphoric acid acetic acid (Brand of Merck–Supelco) solution. Subsequently, the mixture was transferred to a conical flask, to which 50 mL of phosphoric acid solution was added. Then, the resulting solution was then filtered using Whatman filter paper. The filtrate obtained from this process was collected to determine the vitamin C content. The vitamin C concentration was calculated using equation (3).

#### Determination of Flavonoid Content

The determination of total flavonoid content was performed using a Phywe P3070101 UV-Vis spectrometer.<sup>29</sup> For this analysis, aluminum chloride was used as a reagent, while quercetin was used as the comparator compound used.<sup>30</sup> Sample preparation was conducted by dissolving 0.5 mL of extract solution with a concentration of 200  $\mu\text{g}/\text{mL}$  in 1.5 mL of ethanol. Then, 0.1 mL of 10%  $\text{AlCl}_3$  and 0.1 mL of 1M potassium acetate were added to the solution. The mixture was subsequently diluted to a total volume of 10 mL using distilled water and allowed to stand for 30 minutes at room temperature. The absorbance of the mixed solution was measured using a UV-Vis spectrometer at a wavelength of 428 nm.

#### Statistical Analysis

Statistical analysis involved the use of analysis of variance (ANOVA) to examine the significance of the effect of changes in MF frequency on growth, plant weight, and the content of anthocyanin, vitamin C, and flavonoids. Subsequently, if a significant effect was found, The Duncan Multiple Range test was applied to determine the significance of differences between treatments. All statistical analysis was conducted using SPSS Version 20.

### Results

#### Plant Height Growth

Plant height growth was measured from the time of transfer to polybags until the plants reached 38 days old. Growth was determined by calculating the difference between the height of plants aged 38 days and the height of control plants aged seven days. Notably, plants experienced varying height growth in each treatment in response to different MF frequencies (Figure 2). Optimum growth was observed

in plants exposed to a MF with a frequency of 75 Hz. In the absence of exposure to a MF (control), the growth measured  $10.56 \pm 0.92$  cm. However, when exposed to a MF with a frequency of 75 Hz, the growth increased to  $17.44 \pm 1.31$  cm or an increase of 65.15%. Exposure to a frequency of 50 Hz resulted in lower plant growth compared to exposure to a frequency of 25 Hz, with plant growth measuring  $13.08 \pm 1.17$  cm for 50 Hz and  $12.7 \pm 0.35$  cm for 25 Hz. Likewise, exposure to a frequency of 100 Hz resulted in lower growth than exposure to a frequency of 125 Hz, namely  $14.76 \pm 0.60$  cm and  $16.26 \pm 1.50$  cm, respectively.

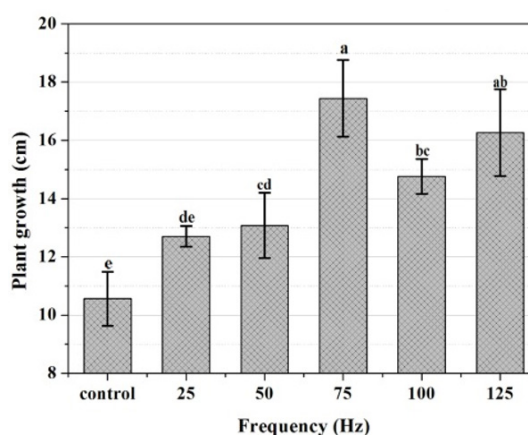


Fig. 2: Growth of red lettuce plants at the age of 38 days exposed to a MF with a frequency of 25-125 Hz

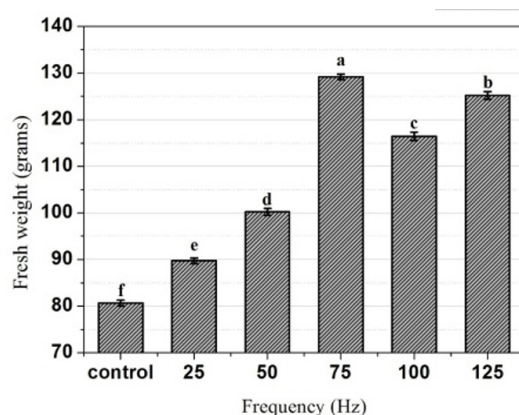


Fig. 3: Fresh weight of lettuce exposed to a MF with a frequency of 25-125 Hz

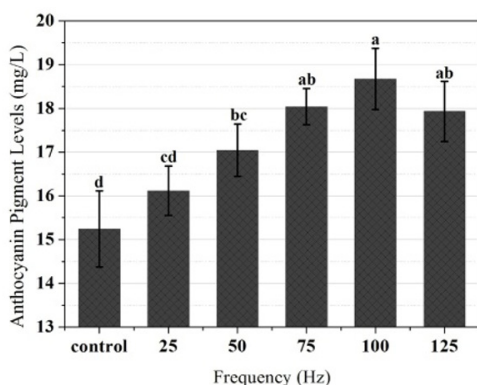
#### Plant Weight

Lettuce plant weight, excluding the roots, was measured immediately after harvest from the field

when the plants were 38 days old. The measurement results showed that the frequency of exposure to the MF affected the weight of the lettuce plants (Figure 3). The largest weight was obtained by plants exposed to a MF with a frequency of 75 Hz, measuring  $129.19 \pm 0.61$  grams, representing a substantial increase of 60.18% compared to no exposure to MF (control), which had a weight of  $80.65 \pm 0.67$  cm. In contrast, exposure to a frequency of 100 Hz resulted in a lower weight than exposure to a frequency of 75 and 125 Hz, measuring  $116.42 \pm 0.91$  grams. Plants exposed to a frequency of 125 Hz had a weight of  $125.2 \pm 0.79$  grams. The results of the statistical analysis showed that changes in the MF frequency significantly ( $p \leq 0.05$ ) affected the weight of the lettuce plants produced.

#### Anthocyanin Content

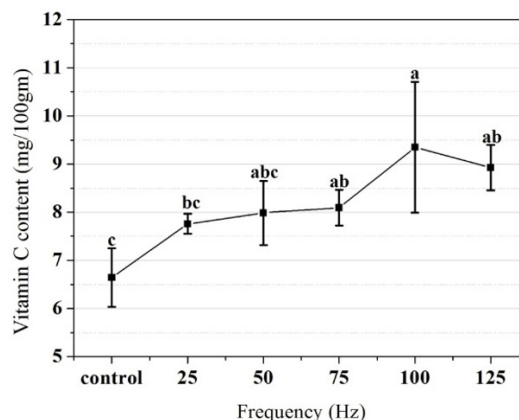
Red lettuce leaves exhibit a variety of colors, including red, green, and yellow. Measurements were taken by mixing all the colors found on the third leaf from the top. The measurement results show that the frequency of exposure to the MF affects the anthocyanin content. The highest levels of anthocyanins were observed in plants exposed to a MF frequency of 100 Hz, measuring  $18.67 \pm 0.69$  mg/L, an increase of 22.52% compared to the control group, which had no exposure to a MF, measuring  $15.24 \pm 0.87$  mg/L (Figure 4). Conversely, exposure to a higher MF frequency, 125 Hz, resulted in anthocyanin content lower than 100 Hz, measuring  $17.93 \pm 0.69$  mg/L. The results of statistical analysis showed that the 50-125 Hz MF frequencies had a significant effect ( $p \leq 0.05$ ) on the anthocyanin content of the red lettuce leaves.



**Fig. 4: The anthocyanin content of red lettuce leaves exposed to a MF frequency of 25-125 Hz**

#### Vitamin C Content

The use of an alternating MF during the seeding red lettuce plants affects the vitamin C content in the leaves as they grow. Exposure to an alternating MF with an effective MFD of 0.4 mT and a frequency of 25-125 Hz led to increased vitamin C content compared to plants without exposure. Different frequencies of MF used for exposure resulted in varying vitamin C contents in red lettuce leaves (Figure 5). The highest vitamin C content was observed in plants exposed to a MF frequency of 100 Hz. Exposure using an MF frequency of 100 Hz increased the vitamin C content by 40.70% compared to no exposure. The vitamin C content of lettuce leaves without MF exposure measured  $6.64 \pm 0.61$  mg/100gm, while exposure to a MF frequency of 100 Hz measured  $9.35 \pm 1.36$  mg/100gm. In contrast, exposure to a frequency of 125 Hz yielded a lower vitamin C content than that of 100 Hz, measuring  $8.93 \pm 0.47$  mg/100gm. The results of statistical analysis found that exposure to MF frequencies of 75-125 Hz had a significant effect ( $p \leq 0.05$ ) on the vitamin C content of red lettuce leaves.



**Fig. 5: The content of vitamin C in red lettuce leaves exposed to a MF frequency of 25 -125 Hz**

#### Flavonoid Content

Flavonoids are antioxidants and are found in many plants, including red lettuce. The content of flavonoids in red lettuce can be changed by exposing it to an alternating MF during seeding. The flavonoid content in red lettuce exposed to alternating MF changes depending on the frequency of MF used (Figure 6). The highest flavonoid content was found in plants exposed to a frequency of

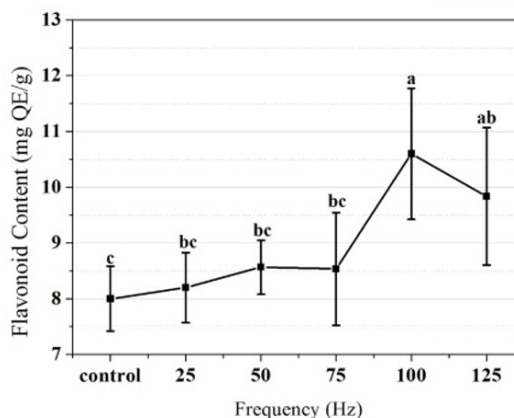


100 Hz, measuring  $10.60 \pm 1.175$  mgQE/g, while those without exposure had a content of  $7.99 \pm 0.58$  mgQE/g, representing an increase of 32.50%. Exposure to a MF with a frequency of 125 Hz resulted in a lower flavonoid content compared to 100 Hz, measuring  $9.83 \pm 1.23$  mgQE/g, while at frequencies of 25, 50, and 75 Hz, the flavonoid content measured  $8.19 \pm 0.63$  mgQE/g,  $8.54 \pm 0.49$  mgQE/g, and  $8.53 \pm 1.01$  mgQE/g, respectively. The results of the statistical analysis showed a significant effect when exposed to frequencies of 100 Hz and 125 Hz compared to no exposure to a magnetic field.

### Discussion

Exposure to an alternating MF with an effective MFD of 0.4 mT and a frequency of 25-125 Hz during the growth of red lettuce seeds affected the growth, productivity, and the content of anthocyanin, vitamin C, and flavonoid. Meanwhile, the changes in anthocyanin, vitamin C, and flavonoid content were directly proportional to the frequencies of 25-100 Hz, while the 100-125 Hz frequency showed an inverse relationship (Table 1). This suggests that

changes in the MF frequency do not linearly affect the productivity and content of anthocyanins, vitamin C, and flavonoids. Similar findings have also been reported by Usanov *et al.*<sup>31</sup> and Wójcik-Piotrowicz *et al.*<sup>32</sup>



**Fig. 6: The content of flavonoid in red lettuce leaves exposed to a MF frequency of 25-125 Hz**

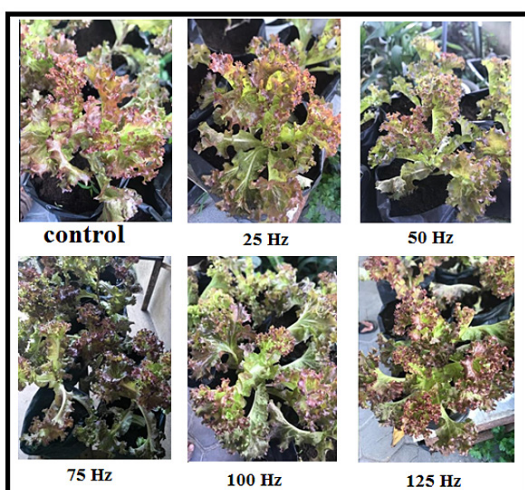
**Table 1: Percentage increase in plant growth, fresh weight, and content of anthocyanins, vitamin C, and flavonoids on exposure to a magnetic field frequency of 25-125 Hz**

Magnetic field frequency (Hz)	Increase (%)				
	plant growth	fresh weight	anthocyanin content	vitamin C content	flavonoid content
25	20.27	11.26	5.75	16.77	2.50
50	23.86	24.33	11.82	20.18	7.09
75	65.15	60.18	18.37	21.84	6.67
100	39.77	44.35	22.52	40.70	32.50
125	53.98	55.23	17.65	34.36	22.92

Exposure to seeds using a MF with a changing MFD can lead to the induction of currents and electric fields.<sup>33</sup> These induced electric fields and currents have the potential to change the behavior of biological systems, given that living things depend on electrical signaling and charge flow.<sup>23</sup> Furthermore, magnetic fields have been shown to directly affect ion channels in cells.<sup>34</sup> It was also revealed that exposure to magnetic fields with changing flux densities could result in changes in the dielectric and acoustic properties of the aqueous medium, which correlated with changes in several characteristics

of chemical and biological systems.<sup>31</sup> MFs have the capacity to polarize the water atoms, leading to changes in the physicochemical properties of water.<sup>35</sup> Such changes in the physicochemical properties of water, both inside and outside the cells, will affect the activities of the cellular metabolism of organisms. The extent of the influence of the MF depends on the frequency used. MFs with resonant frequencies have the potential to affect the photosynthesis process; however, the magnitude of this varies among different plant species.<sup>36</sup> The probability of Brownian motion occurring in plant

seeds is influenced by the Lorentz force generated by the MF, and the magnitude of this effect depends on the relationship between the Larmor frequency and dynamic friction.<sup>37</sup> Furthermore, magnetic fields can interact resonantly with endogenous alternating electric fields in biological systems.<sup>24</sup> As a result, changes in the MF frequency have a non-linear effect on the height and weight of red lettuce and also affect plant physiology. Notably, plant physiology shows that the color of plant leaves becomes greener when exposed to a MF with a frequency of 25-75 Hz, while the opposite occurs at a frequency of 75-125 Hz (Figure 7).



**Fig. 7: Physiology of red lettuce plants at the age of 35 days exposed to an MF with a frequency of 25-125 Hz and control**

Exposure to a MF on seeds will produce pairs of spin-correlated free radicals that are chemically and biologically influential.<sup>38</sup> One form of free radicals is Reactive Oxygen Species (ROS), which affect several physiological processes in living things.<sup>39</sup> The formation of ROS can induce the oxidative degradation of cytotoxic lipids because they initiate a chain reaction in the cell membrane.<sup>40</sup> This condition increases the production of proline to protect the osmolyte compound.<sup>41</sup> Thus, the cellular structure is strengthened by lowering the osmotic potential, retaining proteins from denaturation, and reducing ROS levels.<sup>42</sup> Another potential mechanism of

action involved an increase in the activity of certain enzymes, which is a biological response to MF exposure. Antioxidant storage mechanisms can be used to scavenge excess ROS.<sup>43</sup> This system that suppresses ROS with antioxidants consists of enzymatic and non-enzymatic components that function across various partitions of the subcellular.<sup>44</sup> ROS scavenging can be assisted by complementary agents consisting of non-enzymatic antioxidants, which can be obtained by exposure to a magnetic field. Therefore, exposure to magnetic fields can affect the content of anthocyanin, vitamin C, and flavonoids in red lettuce leaves.

Exposure to an alternating magnetic field with an extra low frequency and a magnetic flux density of 0.4 mT principally affects human cell growth.<sup>45,46</sup> It may cause health problems if used over a long time. Using a solenoid coil makes the magnetic flux density of 0.4 mT localized on the inner solenoid axis (Figure 1), while the outer part is small and tends to zero. Therefore, exposure using the magnetic field generated by the solenoid is safe for the user and the environment. Using a magnetic field with a magnetic flux density of 0.4 mT requires a current flow of  $\leq 0.15$  A for 15 minutes per exposure. Therefore, low electrical energy is required so that economically, it requires low electricity costs.

### Conclusion

Exposure to an alternating MF on seeds affects water content and electrical signaling, and it can generate free radicals. As a result, it affects the growth, productivity and content of red lettuce leaves. Optimum growth and productivity were obtained with exposure to a MF frequency of 75 Hz, while anthocyanin, vitamin C, and flavonoid content were maximized at 100 Hz. It is worth noting that the effect of MF frequency on growth, productivity, and the content of anthocyanin, vitamin C, and flavonoids is not linear; instead, it is influenced by resonant frequencies, which determined the extent of the observed effects.

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### Conflicts Of Interest

Authors declare no conflicts of interest.

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