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# Biomass Production And Morpho-Phsysiological Effects On Sunflower Plants (*Helianthus Annuss* L.) Under Induced Magnetic Fiels

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One of the major concerns at a global level relies on the influence that global warming has had and will continue having and therefore climate change on different economic sectors, mainly in developing countries, which are more vulnerable and with less resilience in their population. Nowadays, it is evident the global trend of searching different sources of raw material with potential to be used for biomass production. Considering this issue, this project analyzed the effect produced by the magnetic fields induced on plants *Helianthus annuss* L, as an enhancer of biomass production, as well as the morpho-physiological variations presented. Exposure of seeds to different intensities of magnetic fields (intensity of 14 µT and 422 µt) was: 1, 3 and 5 hours a day for 15 days, permanent exposure for 15 days, using as a witness seeds without exposing. Planting was carried out in 24 $cm^3$ sockets with coconut fiber previously disinfected and moisturized as substrate. The planting trays were placed in semi-controlled growth zone to a light intensity of 3,000 lux with 12h light cycles at a temperature of 23 ° C and an average relative humidity of 43%. The variables studied are percent germination, growth dynamics, number of roots, production of fresh mass and dry biomass, chlorophyll content a, b and total as well as carotenoids. Physiologically the relative growth rate, net assimilation rate, leaf area index, crop growth rate, absolute growth rate, duration of leaf area and specific leaf area were determined. A simple analysis of variance (ANOVA) between the average of the samples per treatment was performed with a significance level of 95% (α = 0.05) in order to establish if there are significant differences for the variables in evaluation. In the case of not existing meaningful differences among the samples, a multiple range test was performed using the statistical package Statgraphics Centurion. The best results were obtained in seeds treated with permanent magnetic fields with intensity of 14 µT seeds, which demonstrated a significant increase in the value of most indicators studied, being more evident in germination percentage, stem length, number of roots, accumulation of fresh and dry mass as well as chlorophyll. An increased level of chlorophylls gives an indirect measure of the high potential that these plants have to produce biomass.

## 1. Introduction

Today, the advent of new alternative energy consumption is constantly increasing, the that opened new possibility of solution to the traditional origin. Thus, the used the biomass represent a source possible more complete and less polluting. In this case, is that for a producer small, medium or large, having quality seeds has always been a determining factor in the expectation of good yields in biomass per unit area. (Paniagua-Pardo et al., 2015) explained that the reduction of the germination power of the seeds is associate to different factors productive and environment, among which is a poor post-harvest management.

Nonetheless, it is urgent to search for methods or technologies to improve the quality of the seed, which becomes a challenge of vital importance considering that this type of biological material is prone to loss of vigor and viability during the prolonged storage. Faced with this situation, the farmer is with seeds with a reduced germinative power, which translates into the use of a larger volume of these per unit area or a decrease in the number of plants in the same. Therefore, arises the search for tools to stimulate seed embryos with low viability by forging germination, biomass production, inducing even, some resistance to exogenous factors that limit their development (manifest factors in climate change). (Suarez et al., 2016 and Ortiz et al., 2015) propose one treatment with magnetic field as technological alternative for stimulating the post-germination of seeds. It this case, is important to note that the main advantages of applying the magnetic field in seeds are the fact that they have a greater ease of manipulation with minimal risks to possible damages compared to leaf, root or stem tissues. But also, other of the fundamental reason why treatment with magnetic fields has spread worldwide is the impact it has had on the recovery of seeds with low quality in certain plant species (Suarez et. Al, 2016), the exposure of sunflower seeds (Helianthus annuus L.) to induced magnetic fields seeks to increase their germination power, providing a lower time and a higher percentage of the same, to the already known, increase the biomass and yield of plants.

## 2. Materials and methods

### 2.1 Characteristics of vegetable material.

For the experiment, were used lots of seeds of *Helianthus annuus* L. supplied by Recolsemillas S.A.S. Initially the seeds were seeded in germination trays of 200 holes and 25 cm3 each, with coconut fiber previously disinfected and moistened as a substrate, in order to provide the seeds with optimum conditions for their germination and to facilitate the later transplant. Each tray was labeled with the respective treatment. providing one treatment per tray was placed for a total of 9 experimental units, and as a reference a control plant. Afterward, the 11 days a germination, the transplant was performed; 30 random seedlings were selected for each treatment; each seedling in stage ll is an experimental unit, which was located in flowerpot with a capacity of approximately 3 kg of soil, 3 plants were located per flowerpot, 10 per treatment, recording daily process of number leaves, height and foliar area of the plant.

### 2.2 Application of magnetic fields in sunflower seedlings (*Helianthus annuus* L.)

Before, to planting of the seeds, the assembly was carried out for the application of magnetic fields in these, using coils of 300 and 1200 turns respectively for each intensity (14μT and 422μT) and exposure time (60min, 180min, 300min Min and 15 days). The circuit consists of 8 coils and 4 power sources, the final assembly of the circuit for the application of magnetic fields according to each treatment and located at the center of the tray, with the force lines of the magnetic field coming in of the solenoid, revised with a compass. During the seed germination and exposure to magnetic fields, the trays were kept in a specialized laboratory at Uniagraria where they were they remained at a temperature of 23ºC and relative humidity of 43%, including the control tray.

### 2.3 Evaluation of the germination process of the seeds of *Helianthus annuus* L

The interpretation of the percentage of germination was given by the relationship that exists between the number of seed sown and the germinated seeds. To determine this the following formula was used:

$$\text{Percentage of germination} = \frac{\text{\# germinated seeds}}{\text{\# Seed sown}} * 100 \quad (1)$$

### 2.4 Measurement of parameters of growth and development

During a production of sunflower, the following morpho-physiological characteristics were measured: a) Germination percentage given by the ratio between the number of seeds sown and germinated. To determine the percentage, the following formulate was used. Germination percentage = (sprouts / total number of seeds in a test) X 100 (Rosso et al, 2015; b). Plant height (cm) was measured using a measuring tape (or square) from the stalk base to the tip of the last completely formed leaf; c) Leaf Area ($cm^2$): three plants for each treatment (each one independent) were used. For these plants were has marked on a bond paper (belonging at same ream of paper) the silhouette of all leaves of high photosynthetic activity. The silhouettes were cut out and the weight of each unit was determined by the analytical balance. Besides, a (1) $cm^2$ of type of paper used was weighed, and by the rule of three, the leaf area of each plant for each treatment 386 was determined; d) Fresh matter (g): the fresh weight of three plants was determined with an analytical balance for each of treatments. This measurement was carried out within fifteen (15) days following the start of germination and thirty days after the first measurement; e) Dry matter (g) was determined according to the procedures (Cortés-Castillo et al., 2010); f) Growth rates and development. According to physiological indices determined by (Suarez et al., 2016 and Ortiz et al., 2015).

### 2.5 Content of chlorophyll and carotenoids in sunflower plants.

Spectrophotometry was used to determine the chlorophyll pigments (A, B and Totals). For the realization of the readings an extraction by maceration of the leaf part of the plant is carried out (approximately 2 gr in 10 ml of acetone to 80%). After extraction of the solutions the absorbance at 470, 647 and 663 nm (using a UV spectrophotometer) is read. Subsequently, the pigment contents (mg / L) was calculated with equations 2 to 5.

$$\text{Chlorophyll a} = (12.25 * A_{663nm}) - (2.79 * A_{647nm}) \quad (2)$$

$$\text{Chlorophyll b} = (21.5 * A_{647nm}) - (5.1 * A_{663nm}) \quad (3)$$

$$\text{Total Chlorophyll} = (7.15 * A_{663nm}) - (18.7 * A_{647nm}) \quad (4)$$

$$\text{Carotenoids} = \frac{(1000 * A_{470nm}) - (1.82 * \text{chlorophyll A}) - (85.02 * \text{chlorophyll B})}{198} \quad (5)$$

These equations take into account the molar absorptivity coefficients of these pigments when all three are present in the same 80% acetone solution.

### 2.6 Context of the experimental design.

The experiment was developed under a bifactorial design (induced field intensity x field induction time), the basis of the experimental design lies in the assembling of 8 germination trays with 200 seeds per treatment and its later effect in 200 plants for each treatment, exposed to two magnetic field intensities ($A_1$ = 14 μT and $A_2$ = 422 μT) and four exposure times ($B_1$ = 60min, $B_2$ = 180min, $B_3$ = 300min and $B_4$ = 15 days); applied to the seeds under study (*Helianthus annuus* L.).

### 2.7 Statistical analysis

A single analysis of variance (ANOVA) between the averages of the samples by treatment at a significance level of 95% ($\alpha$ = 0.05) was carried out to establish whether any differences exist for the variables under evaluation (Suárez, 2011). If there was no significant difference between the samples, a multiple range test was performed using the statistical package Statgraphics Centurion.

## 3. Results and discussion

### 3.1 Percentage of germination

It were can see, that the seeds began their germination process from day 4 of exposure to the magnetic fields, presenting percentages of germination in a range of 0 to 2.5% found in treatments C2T3 (300 minutes - 422μT) and C1T4 (15 days - 14 μT); (C2T1 - 422μT and C1T1 - 14μT), 180 minutes (C2T2 - 422μT and C1T2 - 14μT), 300 minutes (C1T3 - 14μT), permanent treatments (C2T4 - 422μT) and control, which indicates that the time factor of exposure of seeds to magnetic fields they presented a better incidence on the germination time of the seedlings, compared to the control, showing that the longer exposure time to the magnetic fields (300 minutes and permanent) increase percentage of germination of the seeds, independent of magnetic field intensity.

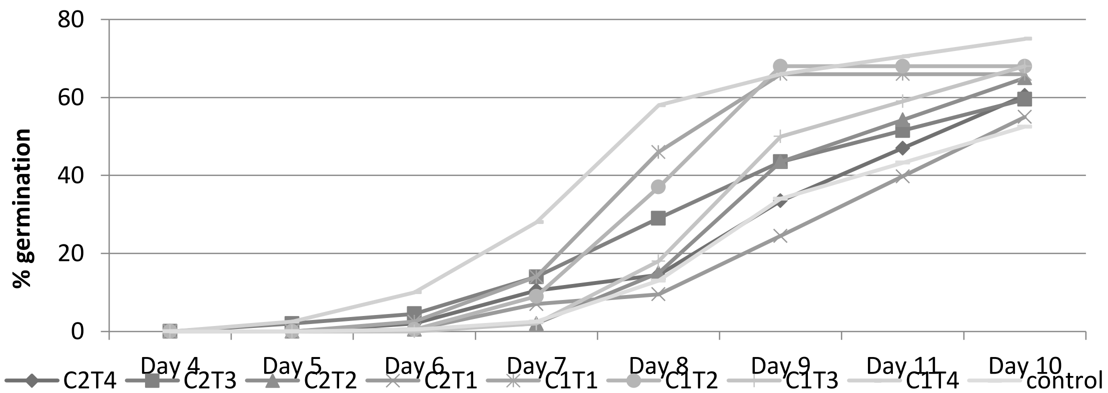


*Figure 1. Behavior of the percentage of germination according to each treatment.*

The results are consistent with those of Carbonell et al. (2004), where they concluded that the application of magnetic fields leads to a reduction in the time needed to obtain a certain number of germinated seeds, obtaining better results with chronic treatments. Likewise, (Isaac, 2011), indicates that the germination process in maize is favored between 2 and 3% with an induction of 4 μT for 3 minutes. On the other hand, Ahamed et al. (2013) was concluded that magnetically-treated pepper seeds (*Capsicum annuum* L.) germinated 24 h before control. At the end of the time of exposure of the seeds to the magnetic fields (day 11), it was shown that the treatment C1T4 (14μT - permanent) reached an increase in the germination and growth rate of sunflower seeds (*Helianthus annuus* L.) with a germination percentage of 75%. It was concluded that the lower intensity of the magnetic fields to a longer exposure time had a higher incidence in the percentage of germination compared to the other treatments and the control. After, day 11, stage I treatments including control reached a percentage of germination in a range of 52.5 to 75%, the highest percentage being exposed to an exposure intensity of 14 μT and the lowest percentage control.

### 3.2 Evaluation of morpho-physiological parameters of plants

3.2.1.Number of leaves in plants

It was evidenced that the lowest intensity of the field (14μT) had a greater incidence in the growth of leaf number during the 4 weeks where this parameter was measured, since week 1 was the one that had the largest number of leaves and, obtained a number of 10 leaves per plant. Those indicate that, with a less intensity of magnetic field to a greater time of exposure, favors to the growth of number of leaves of the plants of sunflower (*Helianthus annuus* L.). Anaya et al. (2011) found similar results by applying fields in fungi and yeasts, obtaining variable responses for different treatments, with stimulatory, inhibitory or no effect effects; this is possibly due to the nonlinear dynamics of biological systems, as well as to the intensity and frequency of the electromagnetic field, the number of pulses, the characteristics of the system to be treated, the conditions of the culture and some intrinsic factors of the system in question.

3.2.2 Behavior of leaf area (LA) in plants.

The Initial Leaf Area (A-Li) was performed before the transplant (stage I) and the Final Leaf Area (A-Lf) was performed 15 days after the transplant (stage II). This evidence that the treatments with a higher A-Li were C2T4 (15 days - 422μT), C2T3 (300 min - 422μT) and C1T4 (15 days - 14μT) with values of 0.5300 $cm^2$, 0.5453 $cm^2$ and 0, 5045 $cm^2$ respectively, the other treatments presented a value in the similar leaf area in a range of 0.4036 $cm^2$ to 0.4650 $cm^2$, being the lowest value for the C1T2 treatment (180 min - 14 μT). This indicates that the magnetic fields did not present a higher incidence in the A-Li of the plants since the treatments that obtained the higher values did not exceed the treatments with the lowest value and the control. According to the results, the sunflower (*Helianthus annuus* L.) plants showed a favorable response to the low exposure times to magnetic fields (60 min), above the control, the other treatments exposed at different times. Besides, the two intensities they presented a homogeneous behavior for the A-Lf of the plants under study.

3.3.3 Behavior of the variables fresh mass (FM) and dry mass (DM) in plants.

This shows that the application of magnetic fields in sunflower plants (*Helianthus annuus* L.) had no greater incidence in the accumulation of fresh mass and dry mass in stage I of the experiment compared to the results obtained with control. On the other hand, it was possible to show that for the magnetic field of intensity 422 μT the treatments that obtained higher values were those exposed to intermediate times of exposure between (180 and 300 min), and the magnetic field of 14μT the greater value was for the treatment exposed to a time of 60 min.

Figure 2 shows the fresh and dry biomass for the sunflower (*Helianthus annuus* L.) plants in stage II of the experiment. This evidence that for the two FM and DM indicators there was a greater response for the treatment of C1T4 (15 days - 14μT) above the other treatments and the control that at this stage obtained the lower values for the two parameters. The C1T4 treatment obtained results superior to the control, demonstrating that the exposure to magnetic fields of 14μT presented higher incidence than the other treatments used on the sunflower plants, specifically in the accumulation of fresh matter of the same. For this variable, it is also evident that with magnetic fields of less intensity (14 μT) and a longer exposure time (15 days), the results showed better biomass accumulation, obtaining an FM and DM of 8.376 and 0.847 respectively. To determine which average of FM and DM are significantly different from others, taking the corresponding ANOVA as a reference, it was determined that the lowest fresh mass results at the second stage were presented by the control plants; while the best results of mass were presented in the plants under the C1T4 treatment.

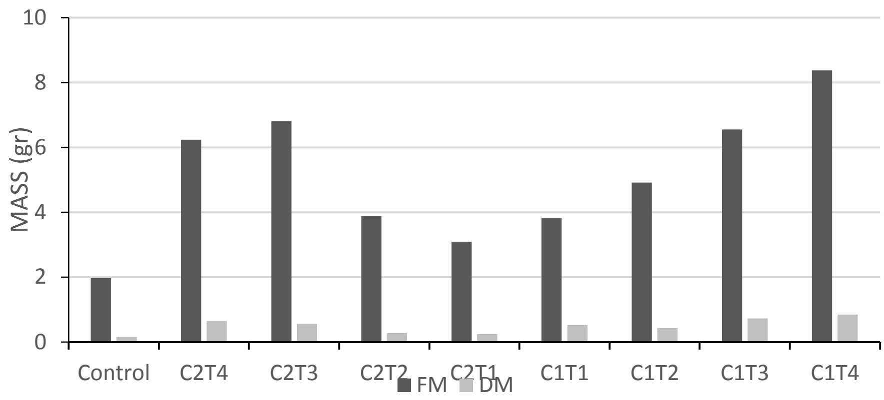


*Figure 2. Fresh mass and dry mass stage II in sunflower* (*Helianthus annuus* L.) *plants.*

3.3.4 Growth and development index

Table 2 evidence the results obtained for the growth and development indexes of the plants under study, with the values of accumulation of dry mass, leaf area and fresh mass of the plants in stage II of the experiment.

*Table 2. Growth and development index*

| Treatment | RGR g/(g day) | NAR g/(cm$^2$ day) | LAI | CGR g/(cm$^2$ day) | AGR g/ day | LAD cm$^2$/day | SLA cm$^2$/g |
|---|---|---|---|---|---|---|---|
| **Control** | 0,0535 | 0,0035 | 3,04 | 0,0012 | 0,0058 | 9,12 | 6,40 |
| **C2T4** | 0,1523 | 0,0570 | 9,14 | 0,0078 | 0,0389 | 27,42 | 7,24 |
| **C2T3** | 0,1293 | 0,0473 | 9,18 | 0,0064 | 0,0320 | 27,54 | 7,26 |
| **C2T2** | 0,0847 | 0,0120 | 5,16 | 0,0026 | 0,0132 | 15,49 | 6,87 |
| **C2T1** | 0,0088 | 0,0073 | 31,21 | 0,0004 | 0,0021 | 93,64 | 28,92 |
| **C1T1** | 0,0387 | 0,0536 | 30,39 | 0,0031 | 0,0154 | 91,18 | 13,0 |
| **C1T2** | 0,1473 | 0,0283 | 6,74 | 0,0052 | 0,0260 | 20,21 | 7,83 |
| **C1T3** | 0,1815 | 0,0605 | 8,57 | 0,0091 | 0,0455 | 25,70 | 7,83 |
| **C1T4** | 0,1806 | 0,0942 | 12,16 | 0,0105 | 0,0527 | 36,48 | 8,25 |

To obtain the Relative Growth Rate (RGR) results, were used the values obtained in the accumulation of MS for each treatment and control, the treatment exposed to (15 days, 300 min and 180 min) got the best results in intensities of (14 μT and 422 μT). The same form, for the results of Net Assimilation Rate (NAR) the accumulation values of DM of the plants were used, where it can be seen that the best result was the one obtained by the treatment C1T4 (15 days - 14 μT). In turn, the best values were those exposed to lower intensities of the magnetic fields (14 μT).

**3.3 Content of chlorophyll A, B, total and carotenoids of sunflower (*Helianthus annuus* L.) plants**

Table 3 shows the values obtained for chlorophyll A, chlorophyll B, total chlorophyll and carotenoids for sunflower (*Helianthus annuus* L.) plants in stage II, where it can be seen that the results are almost homogenous for all treatments. For chlorophyll A the highest values were in a range of 1.0 - 0.7 obtained in the treatments that were exposed to the lower magnetic field intensities 14 μT, values that were significantly higher than the control and the others Treatments, in the same way for the results of chlorophyll B where the highest values were those obtained by the treatments C1T1, C1T2 and C1T3. This is in agreement with Racuciu et al. (2006) that in magnetically treated seeds there are significant differences with respect to the chlorophyll content by carotenoid pigments, which yields more favorable results than with higher magnitudes (100, 150, 200 and 250? Mu T).

Exposure to less intense magnetic fields has a favorable impact on the contents of chlorophyll A and B in sunflower (*Helianthus annuus* L.) plants, regardless of the exposure time to which they are subjected, unlike treatments with intensity 422 μT and the control. This coincides with that published by Dhawi & Al-khari (2009), who observed that the magnetic fields positively affect the pigment content, obtaining better results with low magnetic fields (100 μT) due to a stimulation of the photosynthetic pigments, mainly Chlorophyll A and carotenoids. For the values obtained in total chlorophyll, a homogeneous response was observed for all treatments including control, for this result there was no significant difference of any of the treatments exposed to the magnetic fields. Regarding the carotenoids, the treatment C1T4 (15 days - 14 μT) obtained results of 0.5 and 0.4 significantly above the control and other treatments, with this it is concluded that the magnetic fields of low intensities exercise A favorable result for carotenoid contents in sunflower (*Helianthus annuus* L.) plants.

*Table 3. Content of chlorophyll and carotenoids*

| Treatment | Chlorophyll A | Chlorophyll B | Total Chlorophyll | Carotenoids |
|---|---|---|---|---|
| C2T4 | 0,4 | 0,45 | 0,9 | 0,1 |
| C2T3 | 0,4 | 0,45 | 0,85 | 0,1 |
| C2T2 | 0,35 | 0,45 | 0,75 | 0,1 |
| C2T1 | 0,4 | 0,45 | 0,85 | 0,1 |
| C1T1 | 0,55 | 0,6 | 1,15 | 0,15 |
| C1T2 | 0,75 | 0,9 | 1,7 | 0,2 |
| C1T3 | 0,7 | 0,8 | 1,5 | 0,15 |
| C1T4 | 0,8 | 0,3 | 1,05 | 0,45 |
| Control | 0,35 | 0,35 | 0,65 | 0,1 |

## 4. Conclusions

Given the application of the magnetic fields, it is concluded that there is an increase in the germination rate, where the exposure time had a positive effect on this variable. This time was shorter (4 days) in the seeds permanently exposed to an intensity of 14 μT, compared to the control that started its germination two days later. The magnetic fields induced on the sunflower plant (*Helianthus annuus* L.) constitute a potentiating method that stimulates the rates of growth and plant development of the same. Finding, that the exposure had a significant effect on stem length, number of leaves, leaf area and biomass produced by the plant in relation to the control (fresh and dry biomass increased 4 times the weight of the C1T4 treatment with respect to the control), being the plants treated to fields with less intensity (14 μT) the ones that better answered for these variables. By last, a stimulating effect was generated on plants in their early stages of development, duplicating the content of the pigments active (chlorophylls) with respect to the highest intensity and the Control, this behavior was similar for carotenoids.

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