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Favourable and Unfavourable Effect of Homogeneous Static Magnetic Field on Germination of *Zea mays* L. (Maize) Seeds

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Abstract

The effect of homogeneous static magnetic stimulation on *Zea mays* L. (maize) seeds and its potential utility as a tool in biotechnological development for the improvement of maize seeds was studied. The values of magnetic flux density that influenced the biological development of some plant species of the Poaceae family were determined from a literature review. ICA V-305 variety corn seeds were exposed to seven values of magnetic flux density between 50.0 mT and 250.0 mT, with homogeneity of 98.4% and at (1.0, 3.0, 5.0 and 7.0) min exposure times. The mean germination time (MGT), index of germination speed (V_{Ger}) and germination rate (G_{max}) were evaluated as responses. The magnetic flux density of 50.0 mT with a one-minute exposure time recorded the largest reduction (12.4%) in the MGT while the germination rate for the same treatment increased by 17.4% with respect to the control. No significant effects of the magnetic treatment were recorded for the $G_{máx}$. The magnetic treatment of seeds with homogeneous static fields does not have as favourable a response as the treatments with fields with magnetic gradients, that is to say, using toroidal magnets.

Keywords: magnetic flux density, magnetic field gradient, germination rate, average germination time, seed magnetic treatment

1. Introduction

Advances in the knowledge of the evolution of living beings under the presence of the geomagnetic field has generated interest in the study of magnetosensitivity of various organisms. Regarding plants, Belyavskaya presents an interesting review on the effect of the geomagnetic field in the biochemistry, physiology and biology of plants (Belyavskaya, 2004). Although there is a large number of reports, mainly in the area of agriculture, which indicate that plant systems respond when treated with magnetic field (Galland & Pazur, 2005; Maffei, 2014; Pietruszewski & Martinez, 2015; Teixeira da Silva & Dobránszki, 2015; De Sousa et al., 2016), magnetic stimulation of plant systems can be considered as a technique still in the research stage. In this sense, works can be found with barley, wheat, and oats, among others, and for maize seeds, effects are reported which are presented in Table 1. Seeds have gone through magnetic fields with values from microtesla to hundreds of millitesla, although there are studies of the response to stimuli in units of tesla. For this purpose, passive magnetic sources (permanent magnets) or active sources have been used: Helmholtz coils, electromagnets and/or solenoids (Table 1), in which the values of B, selected by the experimenters, and the values that generated favourable responses (B_{fav}) in the study variables are also recorded.

The results reported raise several levels of discussion, of which two can be taken into consideration. The first level corresponds to the need to determine which of the physical factors are determinant in this technique and how they should be controlled during exposure. Taking into account what was stated before, it is established that the biological effect of magnetic fields is dependent on factors such as the polarity of the field and the value of B it generates (Van, Teixeira da Silva, Ham, & Tanaka, 2011). Nevertheless, it is notorious that in the methodology of exposure reports selected in Table 1, few of them presented the values of homogeneity or gradient of the magnetic field.

The second level is aimed at identifying and explaining the cause of the biological effects observed from the processes activated at the biophysical and biochemical level, since the interpretation of these affectations can be contributed to the understanding of the mechanisms that are unchained in the plant under the effect of the magnetic treatment. The literature reports influence on enzymatic activation, imbibition (Vashisth & Nagarajan, 2010; Shine, Guruprasad, & Anand, 2011), enhanced reactive oxygen species content (Shine et al., 2017), variations in ionic currents (Socorro & García, 2012), modifications in water adsorption processes (Torres, Socorro, & Hincapie, 2018) and changes in the cellular membrane characteristics and RNA quantification (Goodman, Greenebaum, & Marron, 1995).

For all the above mentioned reasons, the effect of homogeneous and intense static magnetic flux density on the germination of maize seeds are studied in the present work, unifying criteria that have been proposed in Valberg (1995), Kaune (1995), and Lee (1996) reports, where studies regarding to the physical variables involved through characterization of the magnetic field sources used in the experiments are presented.

2. Materials and Methods

2.1 Plant Material

Commercial Maize seeds (*Zea mays* L. cv. ICAV305, Semillas del Pacífico, Cartago, Colombia), fit between 1.000 and 2.000 MASL were used. The seeds without visible damage and with uniform morphology were pre-selected prior to the magnetic treatment. The preselected seeds were first sieved by passing them through a (8.0 × 8.0) mm mesh sieve, and later sieved using a (6.0 × 6.0) mm mesh sieve to homogenize the sample size separating seeds into large, medium and small sizes in order to use the medium size seeds which have 0.3878±0.0002 g average mass and 0.356±0.008 cm^3 average volume.

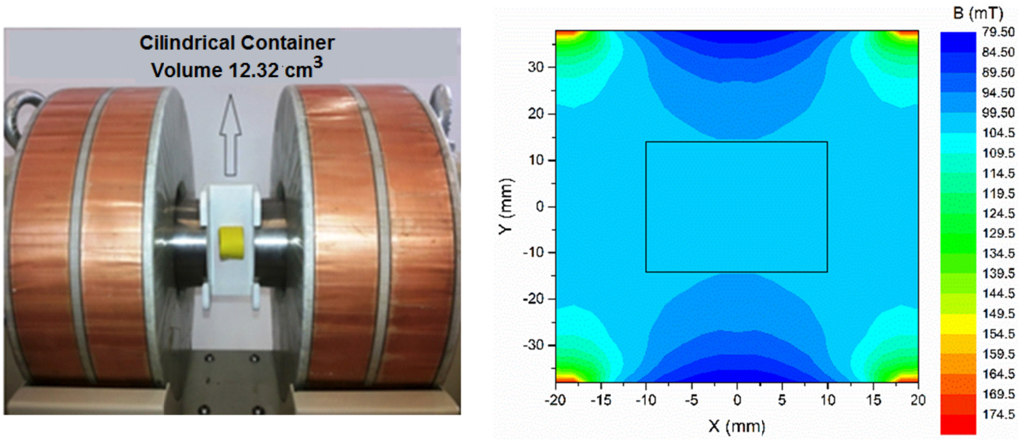
Table 1. Maize seeds magnetic treatment experiments characteristics. N.I, no information

Variety	B_{fav} (mT)	f (Hz)	t_{exp} (min)	Source	B Variation (%)	Improvement	Reference
CL-11, CL-12	160, 560	0	30, 60	Coil	N.I.	In germination processes	Dominguez, et al., 2010
N.I.	150	0	10	N.I	N.I.	In germination processes and establishment of seedling	Aladjadjiyan, 2002
N.I.	50	0	Continuous	Magnet	N.I.	In the first ontogenetic states	Rácuciu & Creanga, 2006
Ramda	125, 250	0	1, 10, 20, 60, 1.440	Magnet	N.I.	In germination processes and establishment of seedling	Florez, Carbonell, & Martínez, 2007
Ganga Safed-2	100, 200	0	60, 120	Electromagnet	0.6% horizontal axis, 1.6% vertical axis	In germination processes, root characteristics and establishment of seedling	Vashisth & Nagarajan, 2008
Ganga Safed-2	100, 200	0	60, 120	Electromagnet	0.6% horizontal axis, 1.6% vertical axis	In the viability of stored seeds	Vashisth & Nagarajan, 2009
Ganga Safed-2	100, 200	0	60	Electromagnet	0.6% horizontal axis, 1.6% vertical axis	In germination processes and vigour of seedlings, improvement in water absorption in phases II and II of germination	Vashisth & Nagarajan, 2010
HQPM-1	200	0	60	Electromagnet	0.8% horizontal axis, 1.5% vertical axis	In seedling characteristics and chlorophyll content	Vashisth & Joshi, 2016
San Jeronimo, San Jose, San Juan	480	0	3, 6, 9, 112, 15	Solenoid	N.I.	In the establishment of the seedlings	Zepeda et al., 2010
AS722, HS2, CAZ	480	0	5, 10, 15	Solenoid	N.I.	Changes in the characteristics of exposed seed	Zepeda et al., 2011
San Jose	4	0	3	Solenoid	N.I.	In the establishment of the seedlings	Isaac, Hernández, Domínguez, & Cruz, 2011
CL-11, CL-12, CL-13, CL-1, CL-4	60	60	7.5	Electromagnet	N.I.	In the vigour of the plant. The response depends on the genotype of the seed	Hernandez et al., 2009
HQPM.1	100, 200	0	60, 120	Electromagnet	0.6% horizontal axis, 1.6% vertical axis	In germination processes	Shine, Kataria, Guruprasad, & Anand, 2017
N.I	10	50	60	Helmholtz	N.I.	Mitotic index increase	Rácuciu, 2011

2.2 Magnetic Stimulation

The generation of the magnetic field was conducted with a GMW electromagnet with 7.0 cm diameter circular cores, and a 4.0 cm spacing between them, with an operating range between 0 and 1.300 mT (Figure 1a), fed with an N5768A Agilent Technologies® direct current (DC) source. B measurements were performed with an FW Bell 5180 teslameter with transverse probe and 0.01 mT resolution in ranges up to 30.00 mT, and 0.1 mT for ranges up to 3,000.0 mT. The spatial characterization of the electromagnet allowed defining the distribution of B and thus to relating the volume of the cylindrical container used to place the seeds with the homogeneity value of B (h_B). The volume of the cylindrical container for 25 maize seeds is 12.3 cm^3 which corresponds to h_B of 98.4%. The box in Figure 1b represents a cross-section of the yellow cylinder in Figure 1a, whose axis is collinear with that of the cores. The uniformity of the magnetic parameters h_B and B in the seeds exposure of the complete experiment is guaranteed by the design and elaboration of a support that positions the cylindrical container (Figure 1a) and with the use of a high stability current source. In addition, the temperature in the electromagnet coils and the seeds being exposed was monitored thus ensuring that it was not higher than 2.0 °C above room temperature.

Magnetic stimulation was performed for 29 doses (D) plus control, exposing seeds to values between 50.0 mT and 250.0 mT and exposure times (t_{exp}) of (1.0, 3.0, 5.0 and 7.0) min (Table 2). Four replications for each treatment were developed.



a b

Figure 1. (a) System for magnetic stimulation; (b) Graph of the characterization of B, in z = 0, with 40.0 mm separation between the cores of the electromagnet, operating with a nominal B of 100.0 mT

Table 2. Values of magnetic stimulation doses relating the magnetic flux density and the time of exposure

B (mT)	**t_{exp} (min)**			
	1.0	**3.0**	**5.0**	**7.0**
50.0	D11	D21	D31	D41
100.0	D12	D22	D32	D42
120.0	D13	D23	D33	D43
150.0	D14	D24	D34	D44
160.0	D15	D25	D35	D45
200.0	D16	D26	D36	D46
250.0	D17	D27	D37	D47

2.3 Sowing

Sowing was done as established by the International Seed Testing Association (ISTA). The parameters of temperature (T) and volume of water (V_{H_2O}) were determined in a previous germination test in which V_{H_2O} responses of (8.0, 12.0, 16.0 and 20.0) ml and T of (24.0, 27.0 and 30.0) °C (data not shown) were evaluated. The best response was obtained with 12.0 ml mL of V_{H_2O} and 30.0 °C.

After receiving the magnetic treatment, the seeds were planted in (100 × 15) mm Petri dishes with absorbent paper moistened with 12.0 ml of distilled water as germination matrix. The seeds were kept in an Incucell 222 l incubator without light. The incubation temperature was 30.10±0.14 °C and the humidity inside the incubator was 59.0 ± 3.39%.

Additionally, a characterisation of the magnetic flux density inside the incubator was conducted every 5.0 cm for the vertical and horizontal directions. Resulting values were obtained in a range between a B_{min} = 0.02 mT and a B_{max} = 0.14 mT from these measurements and a particularly high B generated by a magnet that triggers the closure of the internal door of the incubator B_P = 4.19 mT.

The distribution of the Petri dishes in the incubator was randomly defined, ensuring that plates were not positioned within 10.0 cm of the magnet located in the internal door of the incubator (Figure 2). This procedure is required since there are reports that present responses with significant differences, depending on the variables employed for magnetically treated seeds. For instance, in the case of DC, values close to 4.0 mT have been reported in Cakmak, Dumlupinar, and Erdal (2010), and Majd, Shabrangi, Bahar, and Abdi (2009).

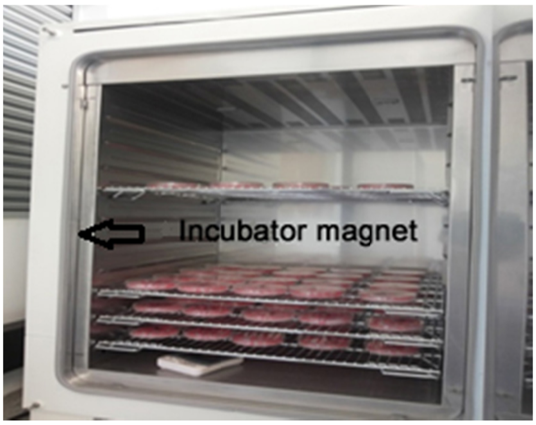


Figure 2. Image of seed sowing system

2.4 Germination Tests

Germination was reviewed 16 hours after sowing every four hours until the 68th hour. Corn seeds are assumed to germinate when the radicle reaches a length equal to or greater than 1 mm (Florez, Carbonell, & Martínez, 2007).

In order to evaluate the effect of the treatments on germination, the mean germination time (MGT) (Equation 1) according to the formula of (Maguire, 1962), and the rate or percentage of germination (G_{max}) was evaluated.

$$MGT = \frac{\sum_{i=1}^{n} N_i \times t_i}{\sum_{i=1}^{n} Ni} \tag{1}$$

$$V_{Ger} = \sum_{i=1}^{n} \frac{Ni}{ti} \tag{2}$$

Where, Ni is the number of seeds germinated in the i^{th} time; ti is the i^{th} time elapsed.

2.5 Data Analysis

The choice of the statistical test for the analysis of the MGT, V_{Ger} and G_{max} was made with the evaluation of two completely randomized design assumptions: normal distribution of errors (Shapiro-Wilks) and homoscedasticity (Bartlett). Given the parametric nature of the data, a one-way ANOVA test was used with the R program. Pairwise comparisons were performed using the Least Significant Difference (LSD) test.

3. Results and Discussion

For V-305 ICA maize seeds variety, the data obtained from the effect of the treatment with homogeneous static magnetic field on the germinative development behaviour show an effect on MGT and V_{Ger} that has favourable and unfavourable effects. Five doses that had significant differences with the control for MGT are presented in Figure 5 and in Table 3. Doses D11, D26, D32, D37 and D44 presented statistical differences for MGT in relation to the control (Table 3). With D11 a reduction of 12.4% (3.4 h) was obtained, whereas with D37, D32, D26, D44 significant increase between 5.2% (1.4 h) and 12.0% (3.3 h) was observed.

Table 3. MGT of the magnetic treatments studied

Treatment	MGT	Treatment	MGT	Treatment	MGT	Treatment	MGT
Control	27.386±0.979						
D11	23.997±1.510****	D21	27.033±2.131	D31	28.084±1.198	D41	26.606±0.296
D12	26.771±0.615	D22	26.659±1.309	D32	29.748±1.743***	D42	26.379±0.964
D13	26.933±1.166	D23	28.688±2.028	D33	26.901±1.838	D43	27.875±2.850
D14	26.860±1.868	D24	27.536±1.436	D34	26.933±1.064	D44	28.800±1.492*
D15	26.737±0.750	D25	26.441±0.904	D35	28.657±2.238	D45	26.841±1.963
D16	28.282±0.942	D26	28.875±1.252*	D36	26.203±0.909	D46	27.431±0.930
D17	27.189±1.944	D27	26.873±1.341	D37	30.660±1.397****	D47	26.767±2.271

Note. The table shows the average value and the standard deviation for each of the treatments. The asterisk indicates differences with the control: **** ($P < 0.001$) very strongly significant, *** ($0.001 < P < 0.01$) strongly significant, ** ($0.01 < P < 0.05$) significant and * ($0.05 < P < 0.1$) differences.

The results for V_{Ger} present a similar behaviour as shown in Table 4 and in Figure 6. The treatments D11, D23, D26, D31, D32, D35, and D37 registered significant differences with the control (Table 4), and D11 showed an increase of this index of speed of 17.4% (0.16 seeds/h). In contrast, doses D37, D31, D32, D26, D35 and D23 had a decrease between 7.3% (0.07 seeds/h) and 16.1% (0.14 seeds/h).

It was not possible to establish significant differences for the germination rate, but it must be made clear that the control seeds showed a germination rate of 91.0%, which was reached by the seeds that received the magnetic treatments.

In analysing these results, it is important to highlight two distinctive features: first, the number of treatments with unfavourable results (five) was higher for the variables V_{Ger} and MGT, which presented statistical differences between significant and highly significant (Tables 3 and 4). The second shows that for exposure with homogeneous static magnetic field, low dose treatments improve germination and high dose treatments decrease germination in MGT and V_{Ger}.

Table 4. Speed of germination of the studied magnetic treatments

Treatment	V_{Ger} (sem/h)	Treatment	V_{Ger} (sem/h)	Treatment	V_{Ger} (sem/h)	Treatment	V_{Ger} (sem/h)
Control	0.895±0.058						
D11	1.051±0.059****	D21	0.914±0.027	D31	0.793±0.074***	D41	0.941 ±0.052
D12	0.884±0.072	D22	0.869±0.111	D32	0.795±0.060***	D42	0.940±0.073
D13	0.907±0.071	D23	0.830±0.051*	D33	0.895±0.068	D43	0.917±0,054
D14	0.913±0.064	D24	0.838±0.072	D34	0.884±0.078	D44	0.953±0.089
D15	0.926±0.039	D25	0.883±0.130	D35	0.818±0.014**	D45	0.928±0.060
D16	0.950±0.070	D26	0.809±0.077**	D36	0.884±0.034	D46	0.909±0.032
D17	0.888±0.066	D27	0.935±0.031	D37	0.751±0.106****	D47	0.907±0.110

Note. The table shows the average value and the standard deviation for each of the treatments. The asterisk indicates differences with the control: **** ($P < 0.001$) very strongly significant, *** ($0.001 < P < 0.01$) strongly significant, ** ($0.01 < P < 0.05$) significant and * ($0.05 < P < 0.1$) differences.

When analysing these results, the positive and negative behaviour suggests that the characteristics of the magnetic fields, such as homogeneity or gradients, are probably influencing alterations in metabolism and/or the transport of phytohormones involved in the germination process. This can be interpreted as if the effect of the homogeneous and intense static magnetic field has a low positive response on the germinative processes in maize seeds.

On the other hand, seeking to contrast this behaviour with others obtained with magnetically treated maize, in the literature it is found that this type of seed has been studied in different varieties for which there was an increase in response to the attack of a pathogen, when the seeds were treated with a electromagnetic field at 60 (Zepeda et al., 2014) length and mass of the root and plant (Aladjadjiyan, 2002; Vashisth & Nagarajan, 2009), vigour indexes (Kataria, Baghel, & Guruprasad, 2015; Vashisth & Nagarajan, 2009; Isaac, Hernández, Domínguez & Cruz, 2011) and establishment (Zepeda et al., 2010). Variables that express effects on germination improvement are reported in: G_{max} between 10% and 16% (Hernandez et al., 2009; Dominguez et al., 2010; Zepeda et al., 2011; Isaac, Hernández, Domínguez,, & Cruz, 2011); V_{Ger} (Dominguez et al., 2010; Shine et al., 2017; Zepeda et al., 2010); MGT and the time to germinate 10%, 25%, and 75% of the seeds have presented significant decrease up to 21% for the MGT (Florez, Carbonell, & Martínez, 2007; Martinez, Florez, & Carbonell, 2017) regarding the control.

However, by reviewing each of the references presented in detail and identifying the magnetic source and the factors related to the characteristics of the magnetic field with which the exposure was conducted, it was found that for works mentioned in Table 1 sources that show magnetic fields with spatial characteristics and parameters different between them and different from those used in this experiment have been used. In addition, in the methodology of exposure few presented homogeneity values or gradients.

Therefore, in order to compare the results of this work with those mentioned above, not only the value of B should be taken into consideration. The first step is to identify the type of source, whether a passive (magnets) or active source (coils, electromagnet or solenoid) were used, and if magnets were used, identify if they were toroidal or cylindrical or square bar magnets. Given that it must be clear that there is always some degree of heterogeneity in the spatial distribution of the magnetic field, which, when categorized in percentage terms from highest to lowest, appears with higher percentage in toroidal magnets, followed by cylindrical rods, solenoids, electromagnets and in much lower percentage in Helmholtz coils. For example, when comparing the images presented in Figure 1b and Figure 4, it can be observed that there is a magnetic field with high homogeneity in the electromagnet, while in toroidal magnets magnetic gradient values are very high besides a variation in the polarity can be observed, which suggests very different experiments (Torres, Hincapie, & Gilart, 2018). But, in order to have a high homogeneity value in the electromagnet, the samples must be positioned in the central zone between the cores since, otherwise, this value decreases drastically.

If the magnetic source was active, it is necessary to determine whether it was fed by DC or AC, since the energy density of a magnetic field AC (ρ_{AC}) is twice that of DC (ρ_{DC})—Equations 3 and 4—as well as to consider the exposure doses (D) which result from operating the field energy density with exposure time (t_{exp})—Equation 5—as discussed in (Pietruszewski & Martinez, 2015).

$$\rho_{AC} = \frac{B^2}{\mu_0} \tag{3}$$

$$\rho_{DC} = \frac{B^2}{2\mu_0} \tag{4}$$

$$D = \rho t_{exp} \tag{5}$$

Thus, when comparing the results of this study with those of previous reports, care should be taken with the interpretation of the results with stimulation in AC and those of experiments that were done with toroidal magnets. Consequently, when comparing the favourable results of this work with other works, we started by discarding those who worked in AC only leaving reports such as: Isaac et al. (2011), with (2.0, 4.0 and 6.0) mT at 3.0 min and Zepeda et al. (2011), 480 mT (5.0, 10.0 and 15.0) min, which had exposure with static magnetic field generated with solenoids indicating lower homogeneity values, and the results of Vashisth and Nagarajan (2009) and (2009a), with treatments of 100 mT, two hour and 200 mT, one hour that used electromagnet, Shine et al. (2017) and Kataria et al. (2015), with similar magnetic flux densities but with higher magnetic field doses than those presented in this work, obtaining similar results to those presented by Flórez et al. (2007), that used toroidal magnets locating the seeds in the walls of the orifice of the toroidal magnet where the values of B change in magnitude, and direction and with a very low homogeneity (Figure 4), the latter having a very good germinative process improvement.

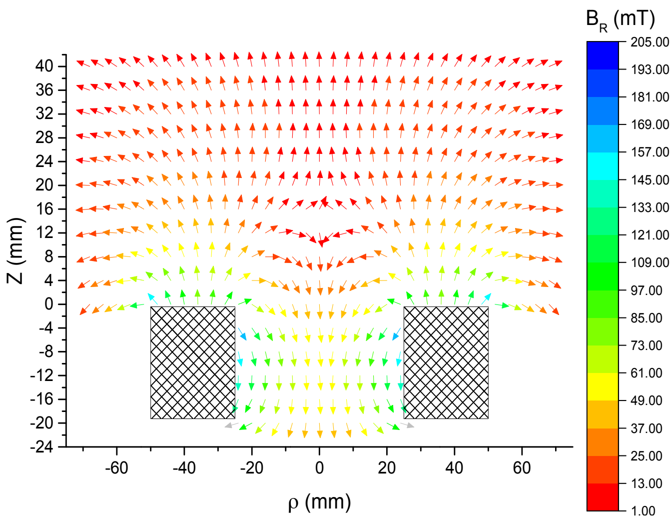


Figure 4. Distribution of the magnetic flux density ($\vec{B}$) for a transverse plane of a toroidal magnet with nominal *B* 100.0 mT, with external radius of 5.00 cm and internal radius of 2.50 cm

It can be considered that there are several factors of the magnetic field generating favourable and unfavourable effects in the germination. The analysis of this results in the experiment compared to those presented by the mentioned authors, concluded that of exposure to magnetic fields with gradients generated on the germination mechanisms have a better effect than the exposure with homogeneous static fields.

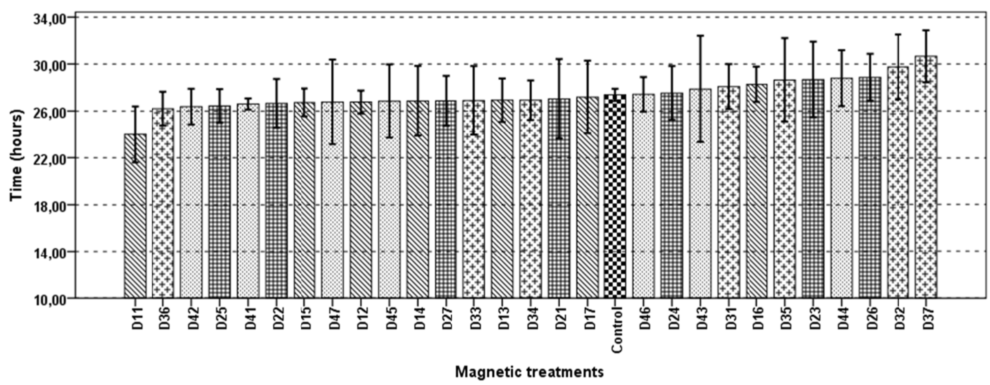


Figure 5. MGT (h) of the evaluated magnetic treatments and control

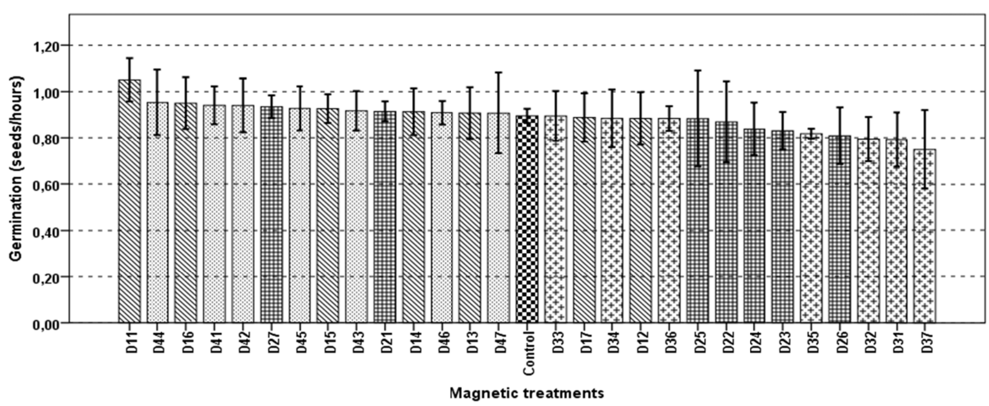


Figure 6. Index of speed of germination of the studied magnetic treatments and control

5. Conclusions

Results presented in this work suggest that the stimulation with homogeneous static magnetic field compromises biological structures and interferes in relevant processes during the germination of *Zea mays* seeds for the magnetic flux densities and exposure times.

The treatment responses may be affected by different parameters of the magnetic field such as the gradients or the homogeneity of the magnetic flux density produced by the generators. This phenomenon indicates that the treatment with static magnetic field is better when conducted with field gradients, different to the results obtained with homogeneous fields.

Further research with magnetic seed treatment is needed to consolidate a standard procedure that defines the stimulation criteria in such a way as to ensure that the doses involved in the biological processes of the stimulated seeds are secured and that the results are reproducible under established conditions.

In order for the magnetic seed treatment to be profiled as an alternative for their improvement at the agricultural level, a unified application methodology must be developed, which is the result of the verification of which field parameter affects each seed parameter in the seeds and that allows the verification of the results of the investigations in this field and therefore the experimental reproducibility.

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