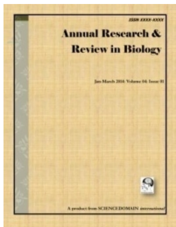

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Variable Magnetic Field Effects on Seed Germination of Broccoli (*Brassica oleracea* L.)

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Authors' contributions

This research was carried out in collaboration between all authors. Authors FRM, GPP, ADP and CHA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FRM and GPP managed the analyses of the study. Author EMO managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

This study was conducted to determine the effect of variable magnetic fields (VMF) on broccoli seeds (*Brassica oleracea* L.) pre-imbibed for 1h to improve their germination and early growth. The experiment was conducted at IPN - ESIME Zacatenco, Mexico DF, between October and November 2013.Seeds were treated with variable magnetic fieldat17, 24and 55mT for 1, 5, 7.5 and 10min.Final germination percentage, germination velocity index, fresh and dry weight and average hypocotyl length were evaluated. The magnetic field treatment of 17mT for 7.5min significantly increased fresh and dry weight by 5.7 and 8% respectively, and got on final germination percentage and germination velocity index, a positive effect but without significant differences compared to control and other treatments. However, at 55mT for 5min fresh and dry weight significantly diminished to 11.7% and 8.2% respectively, getting the lowest variables of growth compared to control and other treatments. At 17mT for 7.5min, fresh and dry weight obtained were the highest. Meanwhile, the stimulation with 55mT for 5 min showed the highest inhibition, in fresh and dry weight. It can be concluded that variable magnetic field treatment can be a suitable option to increase some germination and early growth variables of broccoli seeds.

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Keywords: Broccoli; variable magnetic field; germination; pre-sowing stimulation.

ABBREVIATIONS

Units

μT	:*micro Tesla*
cm	:*centimeter*
d	:*day*
h	:*hour*
Hz	:*Hertz*
μmol m^{-2} s^{-1}	:*PAR*
m	:*meter*
m^2	:*square meter*
min	:*minute*
mm	:*millimeter*
mT	:*milli Tesla*
s	:*second*

1. INTRODUCTION

Due to world population rise and climate change, it is the necessity to increase agricultural food production 50% by the year 2050 to avoid world famine [1], thus it is essential to improve crop quantity and quality [2].

Chemical methods can improve crop production; however, besides being expensive, they cause environmental pollution: land, water, food and humans [2]. Physical methods were shown to improve seed germination and early growth [3-6], which makes them more suitable alternative to replace chemicals [7]. Among them, a technique which has proved environment friendly, easy to use and economic is the pre-sowing treatment with magnetic field (MF) [8-10].

Constant magnetic fields (permanent magnets and coils) and variable magnetic fields (coils) have been applied as seed treatments in pre-sowing [11]. In magnetic stimulation, variable magnetic fields (VMF) are the most widely used in agronomy [12,10,13,14] and its generation is a function of the alternating current supplied (normal or rectified), and its frequency.

One of the four attributes of seeds that affect its productivity is their physiological quality, which is evaluated by the germination test [15]. Many researchers have shown the positive effects on seed germination in response to pre-germination exposure to variable magnetic fields. For example, Hernández et al. [14] found that VMF of 60Hz, 100mT for 7.5min can increase the vigor of hybrid maize seed (*Zea mays* L.) CL-11 x CL-12, while Domínguez et al. [9] reported similar results in the same variety of maize (*Zea mays* L.) stimulated with 560mT to 60Hz for 30min, increasing its emergency velocity, emergency percentage and dry weight of seedling. Furthermore, at 425MHz and 100mW for 1h, improved mung bean growth (*Vigna radiata* L.) [16]. VMF generated by a rectified signal of 100mTfor 3min can significantly increase seedling marigold germination (*Tagetes patula* L.) [17]. In pea seeds (*Pisum sativum* L.), growth, length, fresh and dry weight of stem and root exposed to 120mT (15min) and 180mT (5, 10 and 15min) at 50Hz were enhanced [18], however, exposure to 5, 15, 25 and 100mT, at 50Hz for 15, 15, 15 and 2min, respectively, gave the best mushroom yields (*Pleurotus spp.*) [19].

Broccoli (*Brassica oleracea* L.), is a healthy vegetable for human consumption known to combat obesity [20], a disease that has grown worldwide [21], as well as acting as anticancer agent [22-25]. For these reasons it is important to develop methods to increase the production of this plant species.

In a previous study [6], we investigated the effects of variable magnetic field of 3.6mT for 5 min in aged broccoli seeds (accelerated aging for 48 and 72h), finding to 72h, a significant improvement in final germination percentage, germination velocity index, fresh and dry weight and average hypocotyl length, with respect to control. Based on that investigation, the objective of the current study was to determine the effects of variable magnetic field at three different exposurelevels17, 24 and 55mT at 60 Hz and exposure duration of 1, 5, 7.5 and 10 min, on broccoli seeds, in order to improve their germination and growth.

2. MATERIALS AND METHODS

2.1 Biological Material

In this research broccoli seeds variety Waltham 29 of Itsco® brand were homogenized with a 1.2±0.25mm mesh; the screen was sized with a vernier of Stariett®, 125MEB model. The average weight of 150 seeds was 4.49mg, measured with a Velab® electronic balance, VE-1000 model. The experiment was conducted at Superior School of Mechanical and Electrical Engineering (ESIME) of National Polytechnic Institute (IPN), Mexico DF.

2.2 Experimental Design and Analysis

The experiment was conducted on laboratory conditions between October and November of 2013, with 16 days duration, an average temperature and humidity of 20ºC and 31%, respectively.

Thirteen treatments with four replicates of 30 seeds including a control were evaluated, under a randomized complete block experimental design. Data were subjected to analysis of variance, using the GLM procedure of SAS (SAS Institute, 2002). Comparison of means was based on the multiple comparison procedure using least significance difference (LSD) to separate the means at a significance level of 0.05.

2.3 Variable Magnetic Field Treatment

After being imbibed for 1hin purified water, the seeds were treated with three levels of magnetic field: VMF1 (17mT), VMF2 (24mT) and VMF3 (55mT), measured with FWBell® Gauss/Teslameter, 5070 model, and four exposure times: T1 (1min), T2 (5min), T3 (7.5min) and T4 (10min), in addition to the non-treated control.

2.4 Germination Test

The seeds were sown in 9cm Petri dishes fitted with a moistened layer of Whatman No.1 filter paper, purified water by electrolysis, Electropura® brand was used. The dishes were immediately covered and distributed on foil coated shelves, lighted with LED emergency lamps (Sanelec®, SE-2165-90L model), modified to provide 12h photoperiods, with a PAR (photosyntethically active radiation band) of 9.45 μmol m^{-2} s^{-1}; the average temperature throughout the test was 20ºC.

The treatments were subjected to a stress condition by lack of water, because 3 ml purified water was added per plate until the sixth day after sowing, knowing that the broccoli germination test lasts 10 days [26]; then 1ml of water was added per plate the 10, 13 and 15 days after sowing. Daily germination (G) was evaluated, considering seeds with a radicle length equal to or greater than 2mm. At the last day of the test, final germination (FG) was determined, considering normal seedlings (NS) with a minimum length of 8mm. The hypocotyl average length (HAL) and fresh weight (FW) were measured using a Velab®VE1000 balance, then the seedlings were dried into a Riossa® oven E-51 at 65ºC for 72 h, and the dry weight (DW)was determined, using an Ohaus® balance, Adventurer model.

Using data obtained, germination velocity index (GVI) was calculated based on equation 1 [27,28] and the final germination percentage (FGP) using equation 2 [9].

$$\text{GVI} = \frac{\text{Germinated seeds in first count}}{\text{Days of first count}} + \ldots\ldots + \frac{\text{Germinated seeds in "n" count}}{\text{Days of "n" count}} \quad \ldots \quad \text{(Eqn.1)}$$

$$\text{FGP} = \frac{\text{Total Normal seedlings}}{\text{Total planted seeds}} \text{x } 100 \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{(Eqn. 2)}$$

3. RESULTS AND DISCUSSION

3.1 Fresh and Dry Weight

The ANOVA results, for two seed performance parameters, revealed significant ($P<0.05$) differences among treatments. Table 1 shows the comparison of means analysis of the variables obtained: final germination percentage, fresh weight, dry weight, average length of hypocotyl, and germination velocity index. The pre-sowing stimulation of broccoli seeds with variable magnetic field (VMF) at 17mT for 7.5 min (MF1-T3) significantly increased ($P<0.05$) fresh weight (FW) and dry weight (DW), by 5.7% and 8% respectively, compared to the control. Iqbal et al. [18] obtained similar results in pea seeds (*Pisum sativum* L.) stimulated with rectified magnetic field at 180mT for 15 min and 10 min, which significantly increased fresh and dry weight of shoots, compared to the control. However, in this research also was noted that broccoli seeds exposed at 55mT for 5 min (MF3-T2), significantly reduced ($P<0.05$), fresh weight 11.7% and dry weight by 8.2%; confirming that positive or negative effects of magnetic stimulation depend on the magnetic field intensity, exposure time and seed variety used [29].

The Fig. 1 shows the effects in dry weight at three magnetic field intensities (17, 24 and 55mT) with four exposure times (1, 5, 7.5 and 10 min), compared with the control.

3.2 Final Germination Percentage

Moreover, it was found that the final germination percentage (FGP) did not show significant differences, but a trend of increase using MF1-T3 treatment (17mT for 7.5 min), compared to control. Similar results were reported in amaranth (*Amaranthus* L.) where no significant effects were observed but a trend of increase applying variable magnetic field (VMF) at

30mT for 30s [30]; however, at 100mT on marigold (*Tagetes patula* L.) [17] and at 128µT for 10 min, on medicinal plants (*Medicago radiata, Medicago polymorpha* and *Medicago scutellata*) [31] significantly positive effects were observed, phenomenon that suggests, seeds can resonate when treated with the appropriate magnetic field, absorbing its energy [8,17], exciting the proteins, carbohydrates and inside water of the seeds [32].

Table 1. Comparison of means analysis for seeds treated with three intensities of variable magnetic fields and four exposure times

Treatment	B(mT)	t(min)	FGP (%)	FW (g)	DW (g)	AHL (mm)	GVI
VMF1-T1	17	1	90.83a	0.85ab	0.058ab	27.56a	23.58a
VMF1-T2	17	5	90.83a	0.83 ab	0.061ab	27.36a	22.64a
VMF1-T3	17	7.5	95.83a	0.92a	0.066a	27.37a	23.75a
VMF1-T4	17	10	90.00a	0.8ab	0.060ab	26.96a	21.88a
VMF2-T1	24	1	87.50a	0.78ab	0.057ab	26.73a	21.13a
VMF2-T2	24	5	92.50a	0.89ab	0.061ab	26.83a	22.40a
VMF2-T3	24	7.5	94.16a	0.86ab	0.062ab	27.08a	22.20a
VMF2-T4	24	10	94.16a	0.82ab	0.058ab	27.11 a	22.44a
VMF3-T1	55	1	90.00a	0.86ab	0.062ab	26.35a	22.05a
VMF3-T2	55	5	85.00a	0.76b	0.056b	26.84a	22.91a
VMF3-T3	55	7.5	93.33a	0.86ab	0.062ab	26.97a	22.95a
VMF3-T4	55	10	93.33a	0.88ab	0.064ab	27.94a	22.83a
Control	0	0	94.16a	0.87ab	0.061ab	27.33a	23.39a

Means with the same letter in a column are statistically equal (Tukey, 0.05). VMF – variable magnetic field, B - magnetic field intensity, t -exposure time, FGP - final germination percentage, FW - fresh weight, DW - dry weight, AHL - average length of hypocotyl, GVI - germination velocity index.

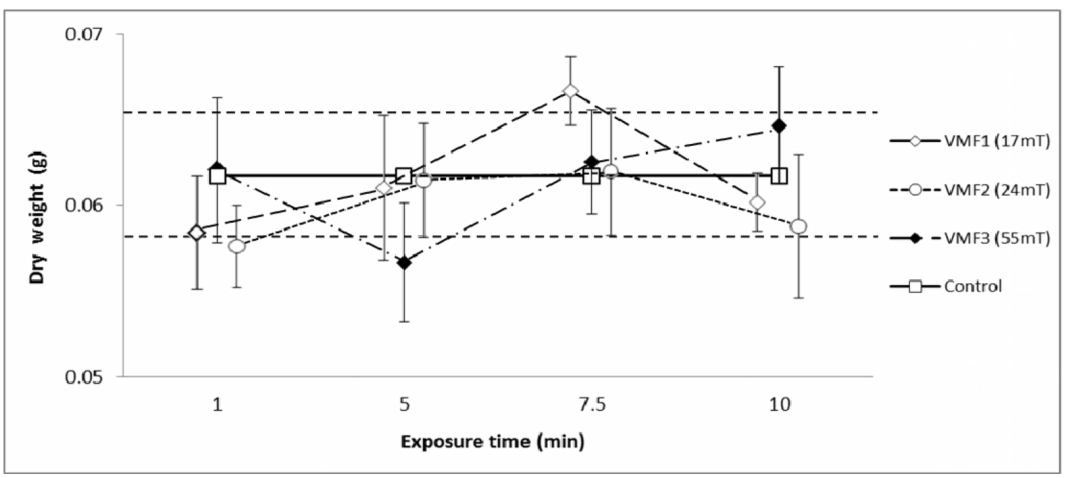

Fig. 1. Dry weight at three variable magnetic field intensities and four exposure times on broccoli seeds (*Brassica oleracea* L.)

3.3 Germination Velocity Index

The germination velocity index (GVI) did not show significant differences, but presented a positive trend with the same magnetic stimulation (17mT for 7.5min) compared to the control. Meanwhile at 30mT, 50 Hz for 30s were obtained a significant increase on alfalfa seed (*Medicago sativa* L.) of 12.8% compared to control [33]. Fig. 2, showed the behavior of the germination velocity index (GVI) at 17mT for 7.5 min (MF1-T3) and 55mT for 5 min (MF3-T2) treatments, which showed statistically significant differences in fresh and dry weight, compared to control.

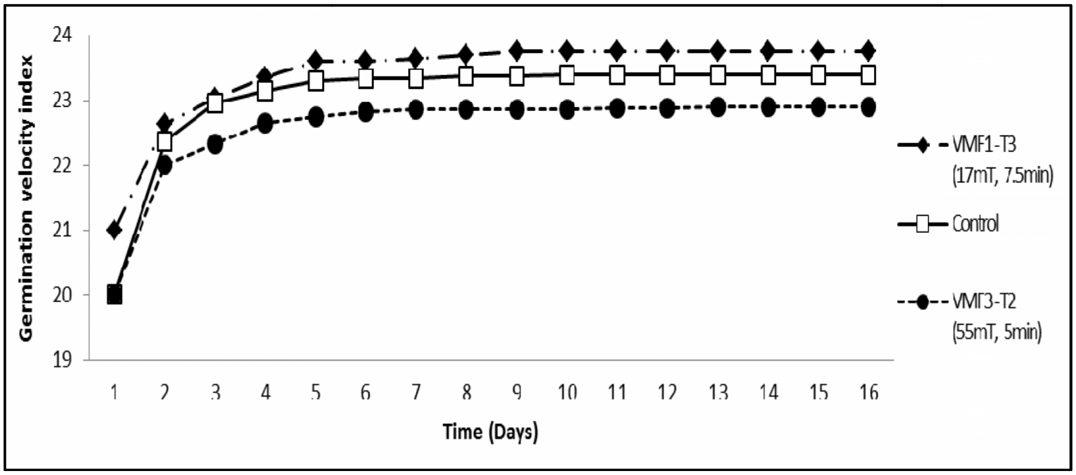

Fig. 2. Germination velocity index of the two most significant magnetic field treatments on broccoli seed (*Brassica oleracea*), compared to control

3.4 Average Hypocotyl Length

With respect to average hypocotyl length (AHL), an improving trend was also observed, although no significant differences, mainly with treatment of 55mT for 10min (MF3-T4); while in potato tubers (*Solanum tuberosum* var. Vineta) stimulated with VMF at 80mT for 1h, significant statistical differences were observed [34].

This research showed that the variable magnetic field of 17mT for 7.5min (MF1-T3) can be used to increase fresh and dry weight of broccoli seedlings, so, further researches are needed to find specific intensities and exposure times that positively toughen other variables such as germination percentage, germination velocity index, among other, providing a benefit to agriculture in both field and controlled environment.

4. CONCLUSIONS

The stimulatory effects of variable magnetic field on broccoli seeds fresh and dry weight were observed to be a function of magnetic field intensity and exposure time. The treatment with magnetic field of 17mT for 7.5 min resulted in the best response on fresh and dry weight. Final germination percentage and germination velocity index were not significantly affected. Exposure to 55mT for 5 min caused the highest inhibition to fresh and dry weight. The variable magnetic field treatment can be a suitable option to increase germination and early growth variables of broccoli seeds. More research is needed in broccoli and other vegetables at different magnetic field treatments to improve germination and early growth in order to obtain higher yield under different stress conditions. Negative effects of high doses of magnetic fields may be exploited for controlling the germination and growth of undesirable plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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