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EFFECTS OF MAGNETIC FIELD IRRADIATION ON BROCCOLI SEED WITH ACCELERATED AGING

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Abstract. In order to improve the physiological quality of aged broccoli seed (*Brassica oleracea* var. Waltham29), in this study a variable magnetic field (VMF) of 3.6 mT, 60 Hz by 5min was used as pre-sowing treatment. The accelerated aging treatment was applied for 0, 48 and 72 h (AA0h, AA48h and AA72h) and then the VMF treatment, under a randomised complete block design with 4 replications of 30 seeds. For seeds aged for 72 h a final germination of 20% was obtained, whereas without magnetic stimulation it was null. However, the magnetic bio-stimulation in 48 h aged seeds was significantly negative, reducing the final germination by 45.83% compared to the treatment without magnetic field, while the VMF effects in unaged seed were zero. The variable magnetic field effects depended on the seed aging time, resulting in positive, negative and null outcomes (responses) in vigour variables.

Keywords: Broccoli, variable magnetic field, physiological quality, bio-stimulation

INTRODUCTION

Physical methods like magnetic field treatments (MF) can improve seed quality (Tahir and Karim, 2010), and they are classified as constant (permanent magnets and electromagnets) and variable magnetic fields (electromagnets) (Pietruszewski and Kania 2010). Several pre-sowing variable magnetic fields (VMF) studies have reported a significant increase in root oregano seed formation (*Origanum*) compared to those obtained using hormones and the combination of hormones and MF (Bilalis *et al.* 2012); in broad bean seeds (*Vicia faba* L.) treated with 30 and 85 mT by 15 s, accelerated plant emergence by 2-3 days was observed (Podleśny *et al.*

2004); likewise Pietruszewski and Kania (2010) reported an improvement in germination and yield of wheat (*Triticum*) treated with 60 mT, 8 s and 30 mT by 3 d, observing also that different seed varieties react differently to the same magnetic field and time exposure (Pietruszewski *et al.* 2013, Zepeda *et al,* 2010); the use of VMF also modified maize seed physical characteristics (*Zea mays* L.) with 480 mT (Gutiérrez *et al.* 2011, Zepeda *et al.* 2011).

VMF is a function of the current frequency that generates it, classified as low (0-300 Hz), medium (300 Hz to 10 MHz) and high (10 MHz-300 GHz) frequency, the low frequency VMF being the most used pre-sowing treatment. This has shown increase in germination of wheat (*Triticum* L.), finding the best results in seeds sown 20 d after treatment with 5 mT, 16 Hz by 2 h (Rochalska *et al*. 2011), in sunflower seeds (*Helianthus annuus* L.) treated with 30 mT, 50 Hz by 15 and 30 s, increased seedling weight and height (Matwijczuk *et al*. 2012); likewise, Dominguez *et al*. (2010) reported an increase in the emergence rate, establishment percentage and dry weight of maize (*Zea mays* L.) with 3.6 mT, 60Hz and 30 min.

VMF produced by rectified alternating current improved germination, vigour and starch metabolism compared to control in marigold seeds (*Tagetes patula* L.), stimulated with 100 mT by 3 min (Afzal *et al*. 2012), while other authors reported significant increase ($P < 0.05$) in fresh and dry weight of mushroom (*Pleurotus* spp.) with 76.43 and 38.26%, respectively (Jamil *et al.* 2012). On the other hand, vegetable seeds such as pea (*Pisum sativum* L.), treated with 120mT, 50Hz, 15min and 180 mT, 10 min, increased significantly their growth variables such as length, fresh and dry weight of shoot and root (Iqbal *et al.* 2012).

The seed aging effects are reduction of germination and vigour, causing low yield, being a problem in the seed industry (Vaz *et al*., 2013). Low frequency VMF stimulation has been used in wheat seeds (*Triticum*) with low percent germination (50%), where the application of 30 mT, 50 Hz by 15min, significantly increased (28.25%) rooted seeds compared to control (Aksyonov *et al*., 2001); similar effects were obtained with low vigour beet seeds (*Beta vulgaris* L.) treated with 5 mT, 16 Hz by 2 h, showing a 25% improvement in germination, proving that magnetic stimulation provides greater benefits in low vigour seeds (Rochalska *et al*. 2008). These researches show that VMF could be a viable option to improve the physiological quality of aged seeds.

The aim of this investigation was to determine the effects of variable magnetic field of 3.6 mT by 5 min, produced with a low frequency (60 Hz) sinusoidal signal, in Broccoli seeds (*Brassica oleracea* L.) treated with accelerated aging (AA). To improve aged seed (storage) physiological quality, the development of methods to increase production of this vegetable is needed, especially in view of its importance to maintaining health, fighting and preventing obesity (FAO, 2006) as well as some types of cancer (Herr and Buchler 2010, Li *et al.* 2010).

MATERIAL AND METHOD

Biological material. In this research project, Itsco® broccoli seeds (*Brassica oleracea* L.) variety Waltham 29 were used, homogenised with a 1.2 mm ± 0.25 mm screen, sized with a Stariett®, 125MEB vernier. The average weight of 150 seeds was 4.49 mg, measured with a Velab® electronic balance, VE-1000 model. The experiment was conducted at IPN – ESIME Zacatenco University, Mexico DF, (19˚29'56 "N 99˚08'06" W).

Experimental Design. The experiment was conducted in spring of 2013, with a day average temperature and humidity of 20.48°C and 31.71%, respectively. Six treatments with four replications of 30 seed were evaluated, under a randomised complete block experimental design. Seed conditions were: 1) unaged seeds (AA0h), 2) 48 h accelerated aging (AA48h) and 3) 72 h AA (AA72h), each one with two treatments: no exposure (T1) and with exposure (T2) to variable magnetic field.

Accelerated aging test. Accelerated aging was performed on two sets of 120 seeds each (AA48h and AA72h), previously homogenised, placed on a wire mesh screen and suspended 1 cm over 28 ml of purified water, inside plastic boxes (90 x 65 x 35 mm). Finally, the boxes were hermetically sealed and deposited into a container (260 x 155 x 60 mm) with 500 ml of purified water, to be placed inside a Riossa® oven E-51, maintained at 50˚C by 48 and 72 h, with a relative humidity of 100%, modified from Moncaleano *et al.* (2013). Seeds without aging treatment were kept at 6°C into a Mabe® cooler RMT1951Z, until sowing.

Variable magnetic field treatment. After being aged and hydrated through 1min, each group was treated with 3.6 mT VMF by 5min field produced by a solenoid powered with a sinusoidal signal of 60 Hz. Seeds were placed inside the solenoid where the largest and most homogeneous magnetic induction exists.

Germination test. Each sowing treatment was performed in 9 cm diameter Petri dishes with 0.4 mm thick filter paper, previously moistened with 3 ml of purified water, which were distributed under a randomised complete block experimental design, into two foil coated shelves. The shelves lighting was provided with four LED emergency lamps (Sanelec®, SE-2165-90L model), modified to complete 12 h alternating cycles of light and dark (photoperiod), generating 700 lux illumination, measured with a Steren® light meter model HER-410.

Through the germination period, at 3, 5, 6 and 7th day, 1ml of purified water was added at all Petri dishes; at the 7th day, the boxes were uncovered to allow free growth of seedlings, thereby at 8 and 9th day the supply of water increased to

2 ml. Daily germination (G) was evaluated every 24 h, considering seeds with a radicle length equal to or greater than 2 mm. At the 10th day, final germination (FG) was determined, seedlings with at least 8 mm of length and having all their parts (root, hypocotyl and cotyledons) was considered as normal (ISTA, 2011); also hypocotyl length (HL) and fresh weight (FW) were measured, using an electronic bascule (Velab®, VE-1000). Finally, seedlings were dried in a Riossa® oven E-51 at 65°C for 72 h, obtaining their dry weight (DW) with an Ohaus® bascule, Adventurer model.

Using the measured data, germination velocity index (GVI) used by Sinefu *et al.,* (2011), final germination percentage (FGP) (Moreno, 1996), and average hypocotyl length (AHL) (AOSA, 1983) were determined.

$$\mathrm{GVI} = \frac{\mathrm{G1}}{\mathrm{N1}} + \frac{\mathrm{G2}}{\mathrm{N2}} + \ldots\ldots + \frac{\mathrm{Gn}}{\mathrm{Nn}} \quad \ldots. \qquad (1)$$

where: GVI = germination velocity index,

G1, G2…Gn = number of germinated seeds in first, second… last count,

N1, N2…Nn = number of sowing days at the first, second… last count.

$$\mathrm{FGP} = \frac{\text{Total Normal seedlings}}{\text{Total planted seeds}} \qquad (2)$$

$$\mathrm{AHL} = \frac{\mathrm{L1} + \mathrm{L2} + \ldots + \mathrm{Ln}}{\mathrm{t}} \qquad (3)$$

where: AHL = average hypocotyl length,

L1, L2…Ln = hypocotyl lengths,

t = total seeds used for the calculation.

Statistical analysis. Data obtained were subjected to variance analysis using the GLM procedure of SAS (SAS, 1998 version). Variables with significant differences ($P \leq 0.05$) were subjected to the Tukey procedure of multiple comparison of means (HSD, Highly significant differences), with an alpha level of 0.05.

RESULTS

Based on the evaluated variables: daily germination (G), final germination percentage (FGP), germination velocity index (GVI), fresh (FW) and dry weight (DW) and average hypocotyl length (AHL), it was shown that the effect of the magnetic field on the physiological quality of broccoli seed depends on its level of aging. The means comparison shows that the VMF treatment on unaged seeds (AA0h) presented no significant effects in all the evaluated variables (Tab. 1).

Table 1. Comparison of means of seeds aged for 0, 48 and 72 h with and without magnetic field

	G24	G48	G240	FG	FGP	GVI-24	GVI-48	GVI-240	FW	DW	AHL
Accelerated aging for 0 hours, "AA0h" (T1 Vs. T2)											
T1	0.00 a	19.25 a	27.2 a	26.5 a	88.3 a	0.00 a	9.62 a	11.8 a	1.05 a	.087 a	3.93 a
T2	0.25 a	19.75 a	28.2 a	27.5 a	91.6 a	0.25 a	10.0 a	12.4 a	0.96 a	.087 a	3.77 a
Accelerated aging for 48 hours, "AA48h" (T1 Vs. T2)											
	G96	G120	G240	FG	FGP	GVI-96	GVI-120	GVI-240	FW	DW	AHL
T1	4.25 a	8.0 a	17.0 a	15.0 a	50.00 a	1.062 a	1.812 a	3.118 a	0.615 a	0.052 a	3.42 a
T2	0.00 b	0.25 b	1.50 b	1.25 b	4.167 b	0.00 b	0.05 b	0.207 b	0.035 b	0.004 b	1.22 a
Accelerated aging for 72 hours, "AA72h" (T1 Vs. T2)											
	G144	G168	G240	FG	FGP	GVI-144	GVI-168	GVI-240	FW	DW	AHL
T1	0.00 a	0.0 b	0.00 b	0.0 b	0.00 b	0.00 a	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
T2	2.00 a	3.5 a	6.75 a	6.0 a	20.0 a	0.333 a	0.547 a	0.943 a	0.160 a	0.020 a	2.196 a

Means with the same letter in a column are statistically equal (Tukey, 0.05). G24 = germination at 24 h, G48 = germination at 48 h, G96 = germination at 96 h, G120 = germination at 120 h, G144 = germination at 144 h, G169 = germination at 168 h, G240 = germination at 240 h. FG = final germination, FGP = final germination percentage; GVI-24 = germination velocity index at 24 h, GVI-48 = germination velocity index at 48 h, GVI-96 = germination velocity index at 96 h, GVI-120 = germination velocity index at 120 h, GVI-144 = germination velocity index at 144 h, GVI-169 = germination velocity index at 168 h, GVI-240 = germination velocity index at 240 h, FW = fresh weight, DW = dry weight, AHL = average length of hypocotyl.

Whereas, the means comparison shows (Tab. 1) that VMF treatment in 48 h aged seeds (T2) inhibited significantly ($P<0.01$) daily germination from 96 h after sowing to the end of the test, and the final germination percentage (G96, G120, G240 and FGP) at 14.17, 25.83, 51.67 and 45.83%, respectively (Fig. 2), FW 94.31%, DW 91.88%, compared to its control (T1). The final germination velocity index showed a slowing from 3.11 to 0.207 for T1 and T2 respectively, as shown in Figure 1.

In contrast, 72 h aged seeds without magnetic stimulation (T1) completely lost their vigour, getting a zero final germination percentage; however, VMF treated seeds (T2) increased significantly ($P < 0.05$), according to the variance analysis and means comparison (Tab. 1), the evaluated variables: G168 to G240 with 11.67, 22.50%, FGP 20.0% (Fig. 2), FW 0.16 g, DW 0.0208 g, AHL 2.197 cm. An acceleration of 0.943 was observed in the germination velocity index, as shown in Figure 1.

With respect to the average hypocotyl length (AHL), seeds treated with VMF and unaged (AA0h), as well as 48 h aged, showed no significant differences compared to their own controls; however, for AA72h it increased significantly (P < 0.01), getting 2.19 and 0 cm for T2 and T1 respectively.

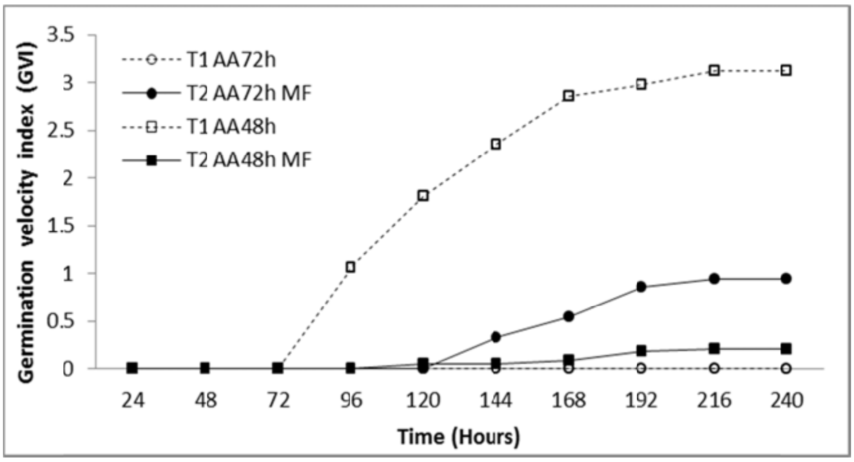

Fig. 1. Effects of magnetic field irradiation of broccoli seed (*Brassica oleracea*) with accelerated aging on the germination velocity index (**p** ≤ 0.05). a) T1 AA72h, b) T2 AA72h MF, c) T1 AA48h and d) T2 AA48h MF

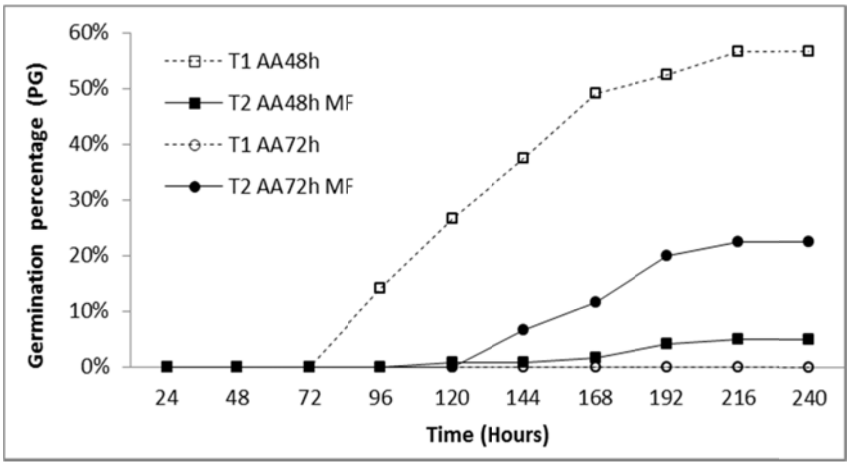

Fig. 2. Effects of magnetic field irradiation of broccoli seed (*Brassica oleracea*) with accelerated aging on the daily germination percentage (p ≤ 0.05). a) T1 AA48h, b) T2 AA48h MF, c) T1 AA72h and d)T2 AA72h MF

DISCUSSION

Based on the results obtained in this investigation it was observed that the magnetic field acts differently with relation to the seeds condition, with favourable results in the seed with the highest level of stress according to the accelerated aging test used (AA72h), while for the seed with a lower level of stress (AA48h) a negative effect to the same irradiation parameters applied (B = 3.6 mT, 5 min, 60 Hz) was obtained, and to the seed without accelerated aging (AA0h) the results were null. Similar results have been reported for corn seeds aged for 6d at 40^{o}C and VMF stimulated with 100 mT for 2 h and 200 mT for 1h, for which a partial improvement was obtained in the germination variables, such as stem length, dry weight and seed vigour (Vashisth and Nagarajan 2009).

Seed aging causes decreased vigour (Kibinza *et al*. 2011, Hussein *et al*. 2011). Even under controlled conditions of temperature and humidity, long period storage results in loss of viability due the metabolism of the seeds remaining active even at low levels of water, consuming its reserves, which is reflected in a marked decrease in their levels of starch and soluble proteins (Moncaleano *et al.* 2013), causing an increase in the germination time (Duran *et al.* 2011), because during this time, damaged tissues produced through storage are repaired (Puntarulo and Boveris 1990).

In the present research it was observed that VMF allowed the start of germination of aged seed 72 h (AA72h) at sixth day of sowing, while the seed without VMF presented no germination. However, in aged seeds AA48h, the VMF (T2) delayed germination by one day with respect to untreated seed (T1), as shown in Figure 1. In AA0h seeds, the pre-sowing treatment with VMF had no significant effect on daily germination. Other authors have also reported positive and negative effects of magnetic fields stimulation on germination, e.g. Gholami and Sharafi (2010) who, in unaged wheat seeds (*Omid and Backcross roshan*) and seeds treated with permanent magnets of 125 and 250 mT, found that the average germination time increased by nearly 3 hours compared to the control, being a negative effect of the magnetic field, while Podleśny *et al.* (2004) reported, for unaged broad bean seeds treated with 30 and 85 mT for 15 s, a uniform and earlier germination (2-3 days) than the control.

The results obtained in this investigation show that the response of daily germination (Fig. 2) and germination velocity index (Fig. 1) are closely related. Both variables in unaged seeds (AA0h) increased rapidly and stabilised, while for aged treatments (AA48h and AA72h) germination started later and the increases were gradual. Similar results were reported for sunflower aged seeds (Hussein *et al.* 2011) behaviour with relation to the time required for repair of damaged tissues during aging (Moncaleano *et al.* 2013).

VMF effects in aged seeds 48 h (AA48h) produced a significant decrease in final germination percentage (FGP) (Fig. 2) and germination velocity index (GVI) (Fig. 1), while in the 72h aged treatment (AA72h) VMF caused a significant increase in the FGP and GVI values. Hernandez *et al.* (2009) reported a similar behaviour for corn seeds (CL-4 X CL-1) without aging, treated with VMF of 60 T for 30 in, finding an increase in seedling emergence of 14% compared to the control, however with 7.5 min a decrease of 16.2% was obtained.

With respect to the average hypocotyl length (AHL), VMF produced no significant differences in seeds with little aging stress (0 and 48 h). However, with more stress (AA72h), it increased significantly, compared to their own controls. A consequence of having aged seeds is their late germination, which generates shorter seedlings, affecting their photosynthesis (Hye Kim *et al.* 2004). Both effects of aging seed coincided with the results obtained with broad beans without aging, indicating an increase in initial growth with VMF of 235 μT, 60Hz and an inhibition with 19.33 μT, 50 Hz (Huang and Wang 2007, 2008).

The effect of VMF on fresh and dry weight improved significantly on aged seeds 72 h in this research. On the other hand, both variables decreased significantly with seeds aged for 48 h, but with 0h aged seeds the effect was null. Several studies have reported similar results: Hussein *et al.* (2012), in aged maize seeds (*Zea mays* L.), found a reduction in vigour and viability. Hernandez *et al.* (2009) found positive, negative and null bio-stimulation in dry weight when applying VMF, the results varying in dependence on the genotype of corn used. Positive effects on DW were also found by Vashisth and Nagarajan (2009), in aged maize seed, improving 24.5% under VMF treatment compared to control.

The results of this research, where dicotiledon seed was used, depended on the aging time of broccoli seeds (*Brassica oleracea*).

Future research could investigate the effects of magnetic field in stored seeds and check the potential benefits that this stimulation might provide to improve their physiological quality.

CONCLUSIONS

1. Positive, negative, and no effects were found with the use of the variable magnetic field (VMF), in the function of aging time of broccoli seeds (Brassica oleracea), for physiological variables: final germination percentage, germination velocity index, average hypocotyl length, fresh and dry weight, evaluated in this research.
2. Positive bio-stimulation was found in seeds aged for 72 h (AA72h), applying VMF stimulation of 3.6 mT by 5 min, finding a significant increase ($P < 0.05$) of 20% in the final germination percentage.

3. Negative bio-stimulation was found in seed aged for 48 h (AA48h), with VMF stimulation of 3.6 mT by 5 min, observing a significant effect of decrease ($P < 0.01$) in final germination percentage, by 45.83%, compared to treatment without VMF. Null results of VMF were found in unaged seed.

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WPŁYW DZIAŁANIA POLA MAGNETYCZNEGO NA PRZYSPIESZENIE STARZENIA SIĘ NASION BROKUŁA

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Streszczenie. W celu uzyskania poprawy właściwości fizjologicznych nasion brokuła (*Brassica oleracea* var. Waltham 29) poddanych przyspieszonemu starzeniu, zastosowano przedsiewne zmienne pole magnetyczne (VMF) o wartości 3,6 mT, 60 Hz przez 5 min. Proces przyspieszonego starzenia prowadzono przez 0, 48 and 72 h (AA0h, AA48h i AA72h), po czym nasiona poddawano działaniu VMF. Badanie prowadzono metodą bloków losowych w 4 powtórzeniach po 30 nasion. Przyspieszone starzenie przez 72 godziny spowodowało wzrost wskaźnika kiełkowania nasion o 20%, podczas gdy dla próby bez działania pola magnetycznego wynik był zerowy. W przypadku nasion poddanych starzeniu przez 48 godzin efekt stymulacji magnetycznej był negatywny, powodując spadek końcowego wskaźnika kiełkowania o 45,83% w porównaniu do wariantu bez pola magnetycznego. Dla nasion niepoddawanych starzeniu wpływ działania VMF był zerowy. Wpływ działania zmiennego pola magnetycznego na nasiona był uzależniony od czasu starzenia nasion, powodując pozytywne, negatywne lub zerowe zmiany w wartościach wskaźników ich wigoru.

Słowa kluczowe: brokuł, zmienne pole magnetyczne, jakość fizjologiczna, biostymulacja