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Effect of pre-sowing electromagnetic field treatment on growth and oleic acid content of cardoon (*Cynara cardunculus* L.)

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ABSTRACT

*The cardoon plant (Cynara cardunculus L.) is one of the most promising plants suitable for cultivation in the reclaimed desert lands in the Kingdom of Saudi Arabia (KSA). Here we report for the first time the cultivation of cardoon in the KSA, in particular at Alkharj Governorate. A field experiment was carried out during two successive seasons to study the effect of magnetic fields (MF) on cardoon growth and its oleic acid content. A 75 mT (*millitesla*) MF was used as follows: MF0 (control) not exposed to the magnetic field, three different MF exposure durations studied as MF1 (T1): 15 min, MF2 (T2): 30 min, or MF3 (T3): 45 min, considered for both non-wetted/dry (D) and wetted seeds (W). It was found that the MF had significant effects on most of agronomic parameters studied. Plant height (cm), number of leaves, number of offshoots, width and length of the fifth leaf (cm), and length of the longest leaf (cm) were all influenced by an increase in MF exposure time, with significant differences between dry and wet seeds. Of the various MF exposure durations, MF2D and MF3W exhibited the best growth attributes for dry and soaked seeds, respectively. Additionally, the oleic acid (C18:1) content in produced seeds was significantly influenced by 15 min of exposure to the MF, in the case of both dry and soaked seeds. These findings may open new prospects for the industrial use of MF and the cardoon plant, and offer the potential to increase growers' incomes.*

Keywords:cardoon;*Cynara cardunculus*;magnetic field; oleic acid; seeds.

INTRODUCTION

The application of electricity, magnetism, monochrome light and sound can each help to stimulate the growth of plants. This little-recognized technology, called Electro-culture, can accelerate growth rates, increase yields, and improve crop quality. Electro-culture can protect plants from diseases, insects, and frost. This method can also reduce the demands for fertilizers or pesticides, and growers can produce higher yields of better quality crops in less time, with less effort, and at a lower cost. Electro-culture can be applied to the seeds, plants, soil, or the water and nutrients [1,2,3].

Notable increases of plant growth and productivity have been claimed in response to magnetic fields (MFs). Several countries have developed magnetic technologies that are environmentally friendly, non-polluting to the soil, and affordable to growers [4,5]. Applying MF to seeds is a safe and inexpensive physical method to improve plant growth, vigour and yields [6,7,8,9,10,11].

Recently in many countries, *C. cardunculus* has been considered a biomass crop for energy and paper pulp production, and as green forage for ruminants in winter seasons [12,13,14]. An awareness of the bioactive chemical composition of cultivated cardoon is vital to increase its economic value, and subsequently to domesticate its production in the Kingdom of Saudi Arabia (KSA). Oleic acid (in its triglyceride form) is contained within a normal human diet as part of animal fats and vegetable oils. Oleic acid has industrial uses in biofuels, bioplastics, biolubricants, and paints [15], while the sodium salt in oleic acid is a major component of soap as an emulsifying agent, and is also used as an emollient [16]. In this context, it is important that the oil from cardoon seeds possesses a high oleic acid content. The aim of this research was to study the effect of MF treatments at the pre-sowing stage on cardoon plant growth during the vegetative and generative stages, and to evaluate the oleic acid content of the final seed yield.

EXPERIMENTAL SECTION

2.1. Plant material and field conditions

Cardoon seeds were supplied by Jelitto GmbH, Germany. Seeds of uniform size and shape were exposed to a controlled magnetic field in collaboration with the Department of Physics and were sown in the experimental farm station of Sara bint Rached bin Ghonaim, Research Chair for Cultivating non-Traditional Medicinal and Aromatic plants, Alkharj (24° 04′ N, 47° 08′ E), KSA. Monthly temperature and rainfall values were recorded during the two growing seasons and are presented in Table 1.

Table 1: The weather parameters throughout the experimental period at the experimental farm station

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Year 2011												
Max *T*º (ºC)[1]	23	26	30	32	37	39	41	44	41	33	29	21
Min *T*º (ºC)[1]	10	13	16	21	27	33	32	32	27	22	10	6
Rainfall (mm)[2]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Year 2012												
Max *T*º (ºC)	22	26	29	36	41	42	40	40	37	34	27	34
Min *T*º (ºC)	7	12	13	20	32	33	36	29	29	20	14	12
Rainfall (mm)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[1]: mean temperature, [2]: sum.

Cardoon seeds were direct seeded in the field according to a Complete Randomized Block Design (CRBD) with three replicates. The properties of the soil used for growing cardoon are as presented in Table 2.

Table 2: Properties of the soil used for growing cardoon

Clay (%)	Silt (%)	Sand (%)	$C_{org.}$ (%)	OM[1] (%)	pH	EC (dSm^{-1})	N[2] (ppm)	P[3] (ppm)	K[3] (ppm)
17.28	8	74.72	0.34	0.58	7.83	1.51	16.8	14.6	154.33

[1] – organic matter, [2] – total, [3] – available.

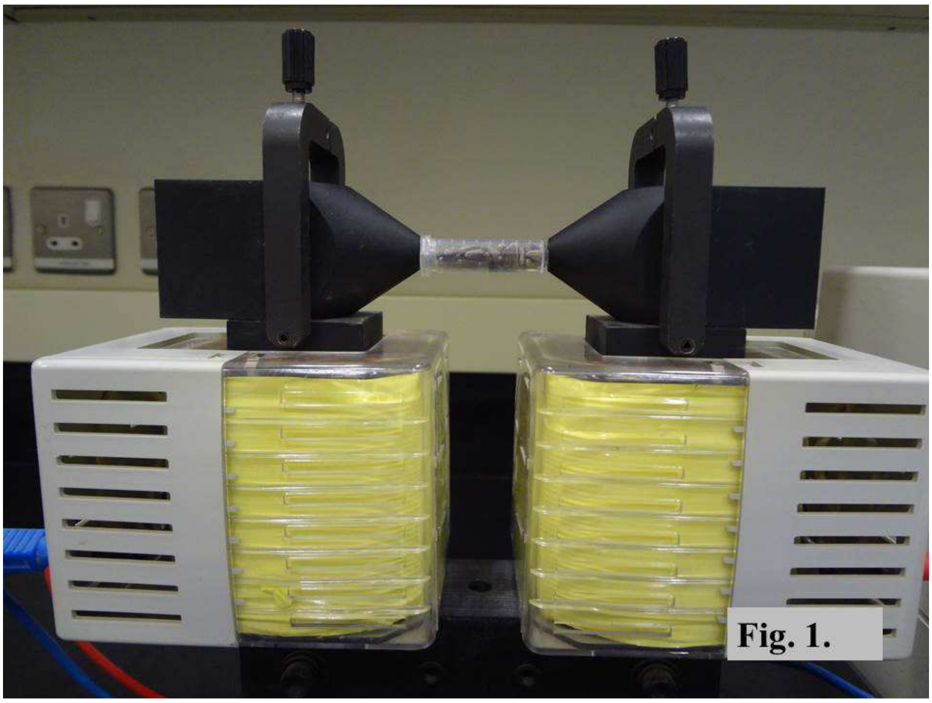
The field plot (basic unit area of 14.40 m^2) consisted of fifteen plants in total for each plot (with a planting density of 10415 plants ha^{-1}, and inter and intra-row distances of 0.80 and 1.20 m, respectively). The sowing date was the first week of November for two successive seasons. Farming practices were conducted under reduced energy inputs. Manual weeding was carried out two times a year over the study period. Minimal fertilization was conducted when the plants exhibited six true leaves (100 kg $ha^{-1}$of urea). Manual harvesting of the cardoon heads was undertaken at the end of each of the two growing seasons. Crop water requirements were fulfilled by dripping irrigation.

2.2. Magnetic exposure conditions

The magnetic field (MF) was generated using coils powered from a high current power supply (220-240 V, 50/60 Hz), with the strength of the field being measured by a teslameter in conjunction with a tangential B-probe (Leybold Didactic GmbH, Huerth, Germany). Presowing magnetic field treatments were applied as described by De Souza et al. (2006)[17] with various modifications as shown in Figure 1.

Cardoon seeds were loaded into an Eppendorf tube (2 mL) without any medium or support, and the tube was then placed in the ring of the electromagnet. The seeds were exposed to a 75 mT MF for varying time intervals: MF1 (T1): 15 min, MF2 (T2): 30 min and MF3 (T3): 45 min. MF0 (control) was not exposed to the magnetic field at all. In each case results were considered for both non-wetted/dry (D) and wetted seeds (W). The required working strength of the MF (75 mT) was attained by changing the voltage applied to the coil unit.

Figure 1. The experimental electromagnet field setup. An Eppendorf tube is placed in the air gap between the two iron poles to expose the seeds to magnetic fields (MF)



2.3. Evaluation of the oleic acid content in collectedseeds
2.3.1. Sample preparation andextraction procedure
At the end of the growing seasons, matured cardoon seeds were collected from each treatment. The seeds were processed following the method of Szczepanik et al. (2012) [18]. In summary, 80 g of the matured seeds were cut into small pieces and ground. The ground plant material was then placed in a 2 L round flask, one third filled with distilled water before being subjected to hydrodistillation for 2 h, using a Deryng apparatus to obtain a volatile fraction in 1 mL of cyclohexane. This process was repeated two times and about 5 mg of the volatile fraction were obtained. The fractions were collected in 2.5 mL and 5 mL vials and stored in a refrigerator at +4°C in readiness for the GC-MS analyses.

2.3.2. GC-MS analysis
Gas chromatography-mass spectrometry analysis (GC-MS) is an advanced technique to isolate, identify and quantify the main components in a sample substance. In this study, a Saturn 2000 MS Varian Chrompack mass spectrometer was used, with a DB-5 (5% phenyl methylpolysiloxane) 30 m x 0.25 mm ID x 0.25μm film column and with the ion-trap analyser set at 1508, and the electron voltage at 1350 V for all analyses. One scan per second was performed in the range of 39–400 m/z using electron impact ionisation at 70 eV, and 1 mL of the sample was analysed. Helium, with a flow rate of 1.0 mL min^{-1} in a split ratio of 1:20, was used as the carrier gas; the injector temperature was 200°C, while the detector temperature was 300°C. The column temperature used the following programme: from an initial temperature of 80°C the temperature was increased at a rate of 5°C a minute until it reached 200°C; the temperature was then further increased by 25°C a minute until it reached 300°C. It was held at this final temperature for 6 min. Three different analytical methods were used to identify the compounds: 1) Kovats indices (KI), 2) GC-MS retention indices (authentic chemicals), and 3) mass spectra (authentic chemicals and the NIST05 spectral library collection). Identification was considered to be tentative when it was based only on mass spectral data.

2.4. Statistical analysis
All the experimental data was statistically analysed using CoStat Version 3.03, an interactive statistics program for computers. F-test and the least significant difference (LSD) used for the comparison between treatment means at the 5% probability level.

RESULTS AND DISCUSSION

The growth parameters of cardoon plants were compared with control plants 156 days after sowing (i.e., during the early part of the flowering stage). It was found that MF treatment in the pre-sowing stage significantly enhanced the growth characteristics of plant height, number of leaves and number of offshoots per plant, fifth leaf width and length, and longest leaf length. The oleic acid content in the collected yield of seeds was also found to have significantly increased at the 95% confidence interval of the mean. Table 3 represents the mean values of plant height and shows the 95% confidence interval of mean. Significant differences between both magnetically treated and untreated seeds were observed for all the applied exposure durations, compared to the control group, but the strongest effects were evident for MF2D followed by the MF3W treatments.

Table 3: The effect of magnetic field (MF) on cardoon growth and oleic acid content of the first season

Treatments		Plant height (cm)	Number of leaves / plant	Number of offshoots / plant	5th leaf (cm)		Longest leaf length (cm)	Oleic acid (%)
					Width	Length		
Control	Dry seeds	31.5e	9.00d	1.00d	27.50d	67.25c	68.66e	32.22c
MF1D		47.83b	12.91c	1.88bc	34.50b	72.66bc	76.33d	36.60a
MF2D		53.30a	17.39a	2.94a	39.47a	79.56ab	84.52b	35.99ab
MF3D		43.14c	10.05d	1.50cd	31.91bc	73.66bc	78.00cd	36.94a
MF1W	Wet seeds	34.08de	12.25c	1.39cd	29.41cd	68.33c	75.41d	36.51a
MF2W		37.41d	15.25b	1.66cd	29.75cd	71.91bc	80.33c	35.86ab
MF3W		49.08b	17.25a	2.47ab	33.58b	84.91a	101.83a	33.51bc
LSD (5%)		3.70	1.70	0.74	3.11	7.27	2.72	2.61

Control: not exposed to the MF, three different MF exposure durations as MF1 (T1): 15 min, MF2 (T2): 30 min, or MF3 (T3): 45 min, D: non-wetted/dry seeds, W: wetted seeds. Values followed by the same letter within a column are not significantly different (P = 0.05).

The mean number of leaves per plant of treated sample was 17.39 for MF2D followed by MF2W (15.25), while that of control was just 9.00. The number of offshoots per plant was also found to be remarkably significant ($P<0.05$); with pre-treatment increasing the number of offshoots per plant compared with untreated samples. The maximum value of offshoot numbers per plant was 2.94 compared to a control value of 1.00 (Table 3). This suggests that lateral growth was greatly increased after pre-treatment with MF.

The mean values of cardoon plant fifth leaf width were 39.47 cm (MF2D), 34.50 cm (MF1D), 33.58 cm (MF3W), 31.91 cm (MF3D), 29.75 cm (MF2W) and 29.41 (MF1W). The mean fifth leaf width of three replicates of the untreated samples, meanwhile, was 27.50 cm. Table 3 sets out the mean values of fifth leaf length; showing the 95% confidence interval of mean. The greatest differences between treated and untreated samples were obtained for the exposure durations of MF3W and MF2D. Significant differences compared to the control were also obtained, however, for all other applied exposure durations.

The mean values of the longest leaf length of the cardoon plants 156 days post sowing were 101.83 cm (MF3W), 84.52 cm (MF2D), 80.33 cm (MF2W), 76.33 cm (MF1D), 75.41 (MF1W) and 78.00 cm (MF3D), while measurements from three replicates of the untreated samples had a longest leaf length of 68.66 cm. Table 3 demonstrates these mean values of longest leaf length, showing the 95% confidence interval of mean. Here, the greatest differences between treated and untreated samples were obtained for exposure durations of MF3W and MF1W. Significant differences compared to the control were also obtained, however, for all other applied exposure durations. Finally, in respect to the oleic acid content of the collected yield of seeds, MF pre-treatment enhanced the oleic acid content compared to the untreated samples. The maximum value of oleic acid content was 36.60% compared to a control value of 32.22% (Table 3). The same trends for all parameters shown in table 3 were observed in the second season, but with slight differences in values as shown in Table 4.

Table 4: The effect of magnetic field (MF) on cardoon growth and oleic acid content of the second season

Treatments		Plant height (cm)	Number of leaves / plant	Number of offshoots / plant	5th leaf (cm)		Longest leaf length (cm)	Oleic acid (%)
					Width	Length		
Control	Dry seeds	32.7e	9.90d	1.13d	28.50d	68.15c	69.27e	32.93c
MF1D		49.03b	13.82c	2.02bc	35.20b	73.57bc	76.93d	37.31a
MF2D		54.51a	18.29a	3.07a	40.17a	80.46ab	85.13b	36.71ab
MF3D		44.34c	10.96d	1.63cd	32.62bc	74.57bc	78.60cd	37.65a
MF1W	Wet seeds	35.28de	13.15c	1.52cd	30.12cd	69.23c	76.02d	37.22a
MF2W		38.62d	16.15b	1.80cd	30.45cd	72.82bc	80.93c	36.57ab
MF3W		50.28b	18.15a	2.60ab	34.28b	85.82a	102.43a	34.22bc
LSD (5%)		3.71	1.71	0.75	3.12	7.27	2.72	2.61

Control: not exposed to the MF, three different MF exposure durations as MF1 (T1): 15 min, MF2 (T2): 30 min, or MF3 (T3): 45 min, D: non-wetted/dry seeds, W: wetted seeds. Values followed by the same letter within a column are not significantly different (P = 0.05).

It is significant to note that the growth parameters of cardoon plants treated with MF were found to be significantly better than those of the control plants: the MF treatments improved plant height, number of leaves and offshoots per plant, fifth leaf width and length, longest leaf length, and oleic acid contents of the collected yield of seeds. Our findings are in agreement with those of other researchers across a range of different crops. Florez et al. (2007) [19] and Vashisth and Nagarajan (2008, 2010) [20,21] each noted improvements in the growth of maize, chickpea and sunflower seeds when treated magnetically. Likewise, Fischer et al. (2004)[22] observed improved growth of sunflower plants compared to the growth exhibited by untreated seed samples. Marks and Szecówka (2010) [23], meanwhile, reported that the surface parts of the potato plant exhibited more vigorous growth when subjected to variable pre-sowing MF stimulation, and Florez et al. (2004, 2007)[24,19] reported improvements in rice germination when exposed to 125/250 mT MF for specific time intervals.

The literature clearly shows that the most beneficial effects occur when there is an appropriate combination of optimal exposure doses and optimal exposure durations. This study shows that appropriate MF treatment for specific exposure durations can accelerate the growth and enhance the oleic acid content of the collected seeds significantly in the cardoon plant. Our results in respect to the growth parameters of cardoon plants are in agreement with Yinan et al. (2005)[25] for cucumber and Podleśny et al. (2004)[26] for magnetically treated wheat, barley and many bean cultivar seeds.

Despite this extensive body of research showing that MF has a significant positive effect on seed germination and plant growth parameters, how it has this effect is not yet well known. Labes (1966) [27] suggested that the effect was due to biochemical changes or altered enzyme activities. It has also been suggested that MF treatment serves to increase the permeability of the cell membrane, affecting the metabolic pathways by increasing the ion transport in the ion channels [28]. An enzymatic route of action, meanwhile, is suggested by the fact that those enzymes necessary for the specific stages of seed germination were found to be higher in magnetically treated seeds [17].

An increase in water uptake rate due to MF treatment has been found, which may be responsible for increased cardoon plant growth. [27,26,29]. The fact that our results show that the improvement in cardoon plant growth parameters is correlated with the oleic acid content suggests that these improvements might be attributed to higher α-amylase, dehydrogenase, and protease activities [21]. α-amylase, in particular, is responsible for depleting the nutrient reserves of the seedling during germination, therefore more active α-amylase may be a factor in the increased growth in magnetically treated seeds compared with the unexposed control.

One model that has been suggested to explain how MF interact with biological systems is the radical pair mechanism. In this model magnetic fields are seen as modulating the single/triplet inter-conversion rates of a radical pair. The MF increases the average concentration of free radicals by prolonging their lifetime and thus increasing the probability of a radical reaction with cellular components. When the exposure duration of MF is increased, the effects of that field on growth parameters change because of the increased peroxide activity [30].

CONCLUSION

This study has shown that the pre-sowing magnetic field (MF) treatments significantly enhanced cardoon (*Cynara cardunculus* L.) plant growth parameters in comparison with a control sample. In addition, MF treatments have a significant positive effect on the oleic acid content of cardoon seeds. Together these results indicate that magnetically treated seeds can be used improve growth rates in agricultural settings, leading to enhanced biomass and oleic acid content. These findings may open new prospects for the industrial use of MF and the cardoon plant, and offer the potential to increase growers' incomes.

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