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RESEARCH ARTICLE

Effect the Magnetic Water on Physiological Traits of Callus the Fenugreek (*Trigonella foenum graecum* L.)

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Abstract

The experiment was conducted in the tissue culture lab. Biology Dept., Babylon University. Callus induction was achieved by culturing the seeds of (Fenugreek) *Trigonella foenum-graecum* (L.) on MS medium supplemented with 1.0 mg/l^{-1} 2, 4-D and BA which combination with all study treatments'. The magnetic water effected by improving the growth of callus, physiological and biochemical characteristics of callus. This study aimed to investigate the effects of magnetic water on some characteristics of growth and chemical constituent in Fenugreek. To this end callus formation was allowed to take place for 45 days in the dark at 25±2 °C from Fenugreek seeds were grown as two experimental groups. A group of seeds were irrigated with distilled water and in parallel the other group with magnetic water at three gausses 1000, 2000 and 3000 gauss .Results revealed that in comparison to control plants, irrigating with magnetic water increases the growth parameters. The treatments 1000 and 2000 gauss showed significant increase in fresh and dry weight, but decline was at 3000 gauss overcome of all treatments including control (Distilled water). From the results obtained it can be said that Physiological Traits outperformed on control by gave the highest values of CAT and SOD by using of magnetic water.

Keywords: *Fenugreek (Trigonella), Tissue cultures, Magnetic water.*

Introduction

Plants, like other living organisms on Earth, are under the influence of the Earth's geomagnetic field (GMF). External application of a magnetic field (MF) or an electromagnetic field (EMF), which differs from GMF, alters the growth and development of plants both under ex vitro and in vitro conditions.

Practical application and use of MF and EMF treatments on seed germination, seedling development and yields of different species, such as field, fodder and industrial crops, herbs and medicinal plants, different vegetables and fruits, grasses, ornamentals, and model crops, have been extensively studied during the last 80 to 90 years and summarized elsewhere [1] together with their physiological and biochemical influences and possible physiological mechanisms. In vitro growth and development of a wide range of species, including herbs and medicinal plants [2, 3] were studied when exposed to external MFs (from super-weak to high MFs) or EMFs. Experiments conducted under an in vitro milieu proved that MFs and EMFs affected the growth and development of cultured cells, tissues and organs, and stimulated both axillary and adventitious organogenesis.

The effects of MFs on in vitro plant growth and development depend on the exact properties of MFs, such as polarity, intensity, exposure time, and magnet type. Since the observed effects were always genotype-dependent, all MFs should be tested individually before application to a given genotype. Unlike experiments at ex vitro, in vitro systems have the advantage of a standard and controlled environment and easy, fast and reliable reproducibility of

experiments; moreover, such systems need minimal space and material [4, 5]. Therefore they are very suitable as model systems or tools for studying different physiological, biochemical, or molecular changes and processes induced by environmental effects, such as MFs or EMFs. The water treated by the magnetic field or pass through a magnetic device called magnetized water. Magnetic treatment of water has been reported to change some of the physical and chemical properties of water, mainly hydrogen bonding, polarity, surface tension, conductivity, pH and solubility of salts.

These changes in water properties may be capable of affecting the growth of plants [6]. Most of these studies, however, employed a static (i.e., stationary) magnet [see images in 5] [7]. Observed that irrigation of common bean plants with magnetic water increased significantly the growth characteristics, potassium, gibberellin, kinetin, nucleic acids (RNA and DNA), photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoid), photosynthetic activity and translocation efficiency of photo assimilates as compared with control plants [8]. Found that magnetic field pretreatment of mung bean seeds could improve the elongation, fresh weight and dry weight, concentration of sprouts protein, soluble sugar, vitamin C and anthocyanin. Pre-sowing magnetic treatments enhanced the growth and development of tomato plants and improved their fruit yield and other yield variables [9]. In general, the literature review shows that there are possibly some advantageous impacts of magnetized water on plant growth, yield and productivity. However the available references of this technology are very limited. In this study, therefore, we investigate the effects of magnetic water on fenugreek yield and some physiological traits.

Materials and Methods

Magnetic Water

To provide magnetized water, the magnetic water generation device produced by the research and technology company was used as in Figure (1), which supplied three power of field strength including: 1000, 2000 and 3000 gausses.

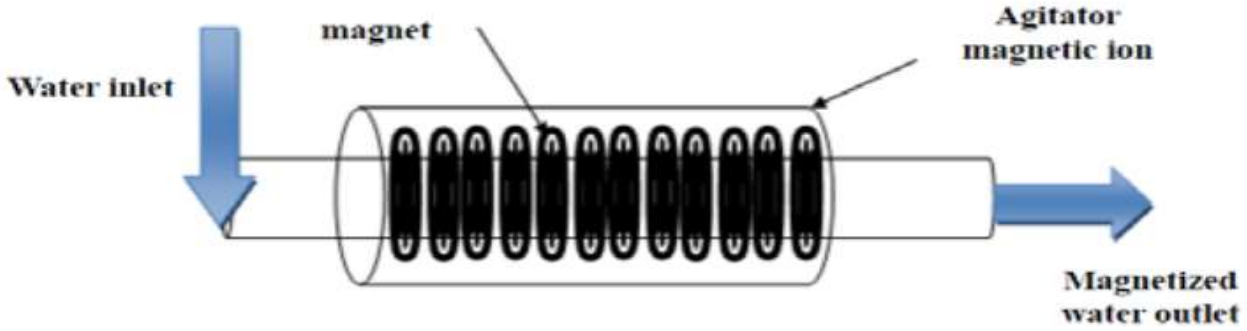

Figure 1: Explain the magnetic water generating device

Callusing

For studying the effect of magnetic water on the callus of fenugreek, seeds as explants were chosen from local markets in the Babylon. Seed divided into two groups of samples including control and treated samples, the antiseptic seeds were transferred to MS media that combination of plant growth regulator in concentration 1mg/l of 2,4-D and BA inside the laminar air flow cabinet, then were placed in the incubator chamber at 25°C ± 1 such as figure (2). All conditions of callusing, the samples were similar and watered with distilled water as (control sample) except the magnetic water samples only treated.

At three week interval, the tissues were subculture for callus induction, maintenance and extraction.

Growth Parameters

Each of the sterilized explants was cut into 2-3 mm pieces using sterile scalpel. Then, it was inoculated in vial containing sterile culture MS medium combinations with growth regulators for regeneration the tissue as in Figure (3). The cali was initially weighed for fresh weight and then weighed as a powder, this is done in a fashion similar to that of callus dried for dry weight.

Antioxidant Activity

The antioxidant constituents were analyzed and determined (CAT and SOD) enzyme using ELISA KIT produced with Responsibility by PARS BIOCHEMTM. **Preparation of callus crude extract:** biochemical analysis were performed under laboratory conditions, after the fresh callus was cleaned, it was weighted and icy ground using a mortar and pestle with alcohol (methanol).

Statistical Analysis

Data were analyzed by SPSS statistical package software, that were used CRD and compared the averages by using least significant difference L.S.D to show the statistical differences among coefficients and the probability level of 0.05.

Figure 2: shows the conditions of callusing, the samples watered similarly with distilled water only

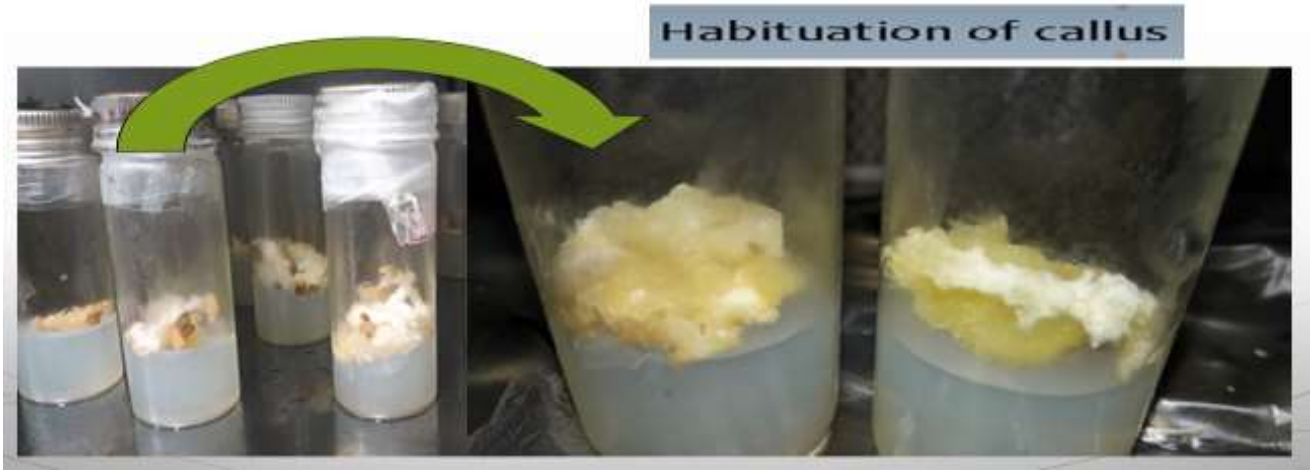

Figure 3: explain the regeneration of callus maintenance, the samples treated with magnetic water by subculture at three week interval

Results

Table 1: shows the effect of magnetic water on growth parameters

Water quality		Callus weight (means) gm.	
		Fresh	Dry
Distilled water (control)		2.684	0.288
Magnetic water (guess)	1000	3.271	0.351
	2000	5.117	0.549
	3000	1.053	0.113
L.S.D.(0.05) =		2.131	0.145

The changes of growth criteria (weight) of fenugreek callus irrigated with magnetic water are shown in the results data of table (1). Callus watered with magnetic water exhibited highly significant increases in regeneration rate at subcultures and their fresh weigh (2.684 and 3.271 g). A result Table (1) indicates that the averages dry weight of callus treated with magnetized water has increased comparison to the control value (0.288 g) that exposure callus to magnetic field has a favorable effect and consider an important factor on the development in the early stage

Table 2: explain the effect of magnetic water on antioxidant constituents using ELISA KIT biochemical analysis

Water quality		Con. antioxidant enzymes (means) u/ml	
		CAT	SOD
Distilled water (control)		0.772	2.826
Magnetic water (guess)	1000	0.942	3.444
	2000	1.473	5.388
	3000	0.303	1.108
L.S.D.(0.05) =		0.504	2.302

Results table (2) provided a clear indication of the presence of free radical scavenging compounds in the methanolic extracts derived from callus. All extracts displayed antioxidant agents with variable of their different chemical nature as CAT and SOD enzymes. Both treatments (1000, 2000) revealed more antioxidant at magnetic water whereas, that of 3000 showed less antioxidant ones (0.303 and 1.108*) u/ml. However, the highest scavenging activity was obtained from callus (1.473, 5.388) u/ml followed by (0.942, 3.444) u/ml to CAT and SOD respectively

Discussion

Water is a diamagnetic molecule which can be affected by magnetic fields. Since water is the most important constitutive element of living cells, so all cellular biochemical reactions take place in water. Due to molecular characteristic of water, it is assumed that a part of these effects happened under magnetic field treatment.

The treatments with magnetic water exhibited marked significant increase in the fresh and also dry weight over the control as table (1) which explains in Figure (4). These results may be due to the effect of magnetic field on alteration the key of cellular processes such as gene transcription which play an important role in altering cellular processes, it also may be due to the increase in growth promoters [10].

Figure 4: show the comparison between magnetic treatments and control sample of biomass callus

The simulative effect of magnetized water on biomass may be attributed to increasing absorption and assimilation of nutrients, induction of cell metabolism and mitosis, increasing auxin, total phenol and protein biosynthesis [10, 11]. Similarly, [12] reported that irrigation with magnetically treated water increased RWC of jojoba plants, he was noticed that, irrigation with magnetically treated water lead to an increase in all elements such as calcium, magnesium and potassium content except sodium. Thus, magnetized water treatment increases plant metabolism in terms of water uptake [13]

Proposed that RWC was better measure or plant's water status than thermodynamic state variable (water potential, turgor potential and solute potential). RWC is closely related with cell volume, it may more closely reflect the balance between water supply and transpiration rate. In the present study positive effect of magnetized water on RWC may be attributed to increasing callus growth and water absorption. Our results are in agreement with those obtained by [9] who found significance increase in the growth and rate of water absorption with the increase of magnetic force.

In the current study positive effects of magnetic water on callus production may be also ascribed to the increase in improvement RWC. Alike to our research others also found that magnetized water increased biomass in chickpea, lentil and tomato [10, 11, 14].The research aimed to study antioxidant activity of methanolic extracts of callus the fenugreek

seeds, free radical scavenging activity was evaluated by ELISA KIT method. *T. foenum-graecum* were accompanied by an increasing in free radical scavenging activity suggesting that callus formation enhanced the antioxidative secondary metabolites production that show in Table (2). Similar responses were observed for in vitro cultures of *Salvia officinalis* and *Rosmarinus officinalis* where callus demonstrated higher antioxidant activity than the mother plant [15, 16]. Have been mentioned that the phenolic compounds are widely distributed in plants that have been reported to exert multiple biological effects including antioxidant activity, free radical scavenging, anti-inflammatory, and ant carcinogenic. *T. foenum-graecum* categorized as one of Asian vegetables which contain very high antioxidant activity might be due to their high phenolic contents. Several strategies have been adopted for the enhancement of bioactive metabolite production in in vitro cultures, one of them is using growth regulators which are often a crucial factor, the type and concentration of auxin or cytokinin or the auxin/cytokinin ratio may alter dramatically both the growth and the product formation in cultured plant cells. Therefore, it is of great interest to evaluate the antioxidant activity of callus fenugreek seeds.

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