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Synergism of Magnetized Water Technology with Tissue Culture in the Production of Bioactive Compounds from Fenugreek Seed

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Abstract

This study aims to increase the content of some types of alkaloids and saponins, especially the steroidal type, as well as phenolic acids. Medicinal plants form an essential part of the local pharmaceutical system and are used in many traditional medicinal treatments, including fenugreek (*Trigonella foenum-graecum* (L.)), which belongs to (Fam. Fabaceae, Fenugreek). This study summarizes the germination and formation of callus by planting the fenugreek seeds that were selected for the study on tissue culture medium type MS, which contained a concentration of (1.0 mg.L^{-1}) of both 2,4-D and BA as stimulating factors for the emergence of callus, taking into account its preservation. This process took approximately 45 days under standard conditions of sterilization and complete darkness at a temperature of $25 \pm 1^\circ\text{C}$. Finally, a measured weight of the callus formed was collected to be tested using the high-performance liquid chromatography (HPLC) technology. The methanolic extract of callus showed a statistically higher rate of concentrations of bioactive compounds when compared with the seeds of the control sample. In order to maximize the amounts of secondary metabolic compounds in the current study, magnetic water technology was introduced at three levels (1000, 2000, and 3000 gauss). The best results appeared in the MS callus growth medium, which had been previously treated with magnetic water through its use in preparing and processing the growth medium, especially at the level of 3000 gauss, which gave a noticeable significant increase in the production of diosgenin, trigonelline, and phenolic acids, reaching (102.462, 45.729 $\mu\text{g/ml}$ and 173.070 mg/l) per gram of dry weight of callus used in the analysis, respectively. From a practical standpoint, Iranian fenugreek seeds were a good choice over the local fenugreek seeds chosen for the study to provide the raw materials needed to produce the studied metabolites, given that Iran is a major source of the natural biodiversity of the medicinal fenugreek plant. The statistically significant differences between all study results obtained were observed using SPSS version 21.0 and the Least Significant Difference test (L.S.D) at the $p \leq 0.05$ level.

Keywords: Magnetized Water, Tissue Culture, Magnetic Water, Bioactive Compounds, HPLC Technology

Introduction

Plant-based foods represent the primary source of the essential elements, such as proteins, vitamins, and fibers in human diets ^[1-3]. Therefore, efforts are focused nowadays on enhancing both the quantity (mass production) and quality of the plant-based foods. Hence, increasing crop production and developing new plant-sourced foods with high nutritional value became a predominant goal in agriculture, which all countries are paying great attention to ^[3]. The recent developments in the production of functional foods and pharmaceutical products using different types of nutritional plants that contain high concentrations of natural antioxidants, such as phenolic acids, have significantly minimized the usage of synthetic drugs ^[4]. Therefore, a great deal of research is devoted nowadays to using natural antioxidants, from plants, as a replacement for synthetic drugs. For instance, many researchers investigated the possibility of using edible seeds and grains in the production of pharmaceutical products by selecting and breeding varieties, species, and strains depending on their nutritional value, phytochemical properties, and mineral contents ^[5]. In addition, the nutritional values and phytochemical characteristics of plants have been widely studied ^[6]. The literature proved that applying effective breeding programs has effectively increased the nutritional values of many crops, such as wheat and corn ^[7].

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Generally, the key factors that are used to select the proper plant-based foods include the content of protein, carbohydrates, antioxidant compounds, vitamins, unsaturated fats, and crude fibers. Fenugreek (*Trigonella L. spp.*) is an essential legume crop that is widely cultivated worldwide for food purposes [8]. These plants are called legumes because they produce protein-rich seeds and are known as effective medicinal plants, which are widely cultivated worldwide. The biological and therapeutic influences of fenugreek are largely linked to the diversity of its bioactive chemical compounds. Fenugreek is also used to manufacture many hormonal and therapeutic drugs [9, 10]. Its seeds are characterized by the presence of diverse natural compounds, including flavonoids, alkaloids (such as trigonelline), steroidal saponins (such as diosgenin), tannins, and phenols [7, 10-14]. Fenugreek seeds also include a significant amount of key nutrients, vitamins, plant gum, protein, and fats [7, 15].

In view of the nutritional and medicinal importance of the fenugreek plant, this study was conducted to evaluate the content of trigonelline and diosgenin, as well as phenols, in one of the Iranian fenugreek plants and compare it with the approved and most common local fenugreek plant, under ideal and fairly uniform agricultural conditions, to determine which type is the most appropriate for the use food and pharmaceutical industries at the local level. The process of stimulating and increasing the production of some economically important secondary metabolic compounds from the callus seeds of the fenugreek plant was carried out using the laboratory magnetic water addition technique, which was gained by flowing water through a permanent magnet or an electromagnet fitted in or on the feed water pipeline prepared for laboratory preparations.

In this context, the possibility of producing bioactive compounds from Fenugreek seeds using the synergism of magnetized water technology with tissue culture was investigated in this study.

Materials and Methods

Two sets of seeds were obtained, one of which is a type of fenugreek plant commonly used in the Horticultural Research Station of the University of Tehran, Iran, and the other type is of local fenugreek seeds that are approved and most common in agriculture. The experiment was conducted in a completely randomised block design (CRBD) with 3 replications for each treatment. This approach was applied in this study due to the unidirectional variations in the experimental materials and to provide more accuracy. It was according to the following stages:

1. Seed collection and sterilization: The dried and mature seeds of the fenugreek plant were collected and prepared for study. An appropriate amount of them was placed in a bottle containing 70% ethanol for 25 seconds with good manual shaking for the purpose of sterilization. Then they were transferred to a 2% sodium hypochlorite solution for 600 seconds. After that, the seeds were rinsed several times using sterilised water. Later, the studied samples were deposited in a scientific herbarium to be preserved under standard conditions
2. Callus stimulation and preservation: After sterilizing the outer surface of the seeds, they are planted directly

on the MS basal germination medium (Murashige and Skoog, 1962), which has been prepared in advance and is supplemented with 1 mg.L⁻¹ of 2,4-D and BA. Then the growth media is incubated in the special growth chamber at a temperature of 25±0.1 °C, taking into account ideal sterilization conditions during all stages of the work.

3. Increasing the development of secondary metabolites in callus: 150 mg of the formed callus was replanted in a new MS growth medium prepared using magnetized water at three different levels (1000-3000 guess) instead of using distilled water, which was used as the control sample in this study.

Extraction and identification of diosgenin, trigonelline, and phenolic compounds using HPLC

Samples were prepared for diosgenin extraction first following the method of Herrera *et al.*, [16]. The extraction of trigonelline was entrusted to Campa *et al.*, [17]. The phenolic compounds were also extracted using the method adapted by Riasat *et al.*, [18] with some minor modifications. Compound analysis was carried out using HPLC technology, where water:acetonitrile ratio of 10:90 (v/v) was applied as mobile phases for diosgenin and trigonelline, while the mobile phase for phenolic compounds was methanol with 1% formic acid. The analysis was conducted using calibration curves for standard solutions of diosgenin and trigonelline. Finally, the calibration curve was drawn after meeting the requirements of the standard examination, and all the resulting data were subjected to the concentration calculation equation [19], which is:

Sample concentration = standard concentration X (sample area/standard area) X dilution factor (1)

Data analysis

All experiments in this investigation were repeated 3 times, and the data were analysed using SPSS-21.0 software. One-way analysis of variance was used to test for significant differences, and a post hoc test was conducted using the Least Significant Difference test (L.S.D) at $p < 0.05$, based on calculating means and standard deviations in all tests.

Results

Table 1 shows the average content of trigonelline in both types of fenugreek seeds that were chosen for the study. Samples of imported fenugreek seeds that were treated with magnetized water at three levels (1000, 2000, and 3000 guess) gave the highest average content of trigonelline, reaching (24.284, 32.612, 45.729 µg/ml per 1 gram of dry callus weight) respectively, while the average content of trigonelline ranged between (17.966, 29.462, 38.838 µg/ml) according to the same treatments in local fenugreek seeds. The results of table 2 indicate the average diosgenin content, which shows a direct relationship between the type of seeds used and the magnetic treatment technique. It is noted that the Iranian imported type of seeds outperforms the local type when responding to treatment with magnetic water, especially at level 3000 guess, where the highest percentages of average diosgenin content are recorded, which reached (102.462, 88.243 µg/ml) respectively.

Table 1: Showing the quantitative analysis of the average amount of Trigonelline ($\mu\text{g/ml}$ per 1 gram of dry callus weight) using HPLC.

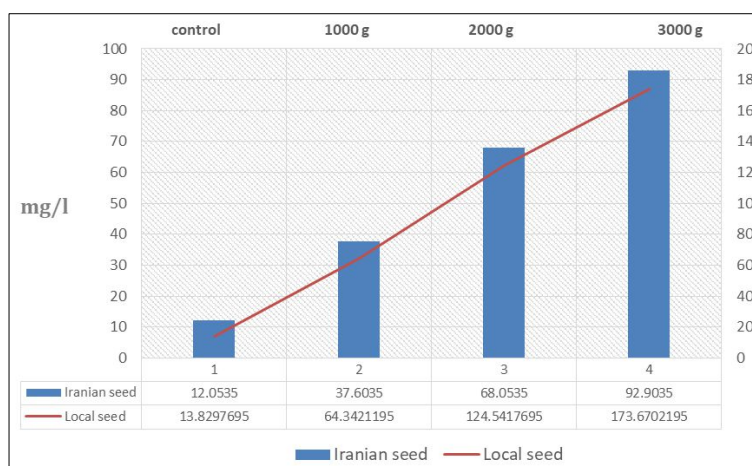
Treatment water type		Type of studied seeds	
		Iranian seed	Local seed
Distilled water (control)		13.516	8.323
Magnetic water (guess)	1000	24.284	17.966
	2000	32.612	29.462
	3000	45.729	38.838
L.S.D.(0.05) = 5.352			

Table 2: Showing the quantitative analysis of the average amount of Diosgenin ($\mu\text{g/ml}$ per 1 gram of dry callus weight) using HPLC.

Treatment water type		Type of studied seeds	
		Iranian seed	Local seed
Distilled water (control)		60.849	45.258
Magnetic water (guess)	1000	78.533	53.291
	2000	86.079	62.379
	3000	102.462	88.243
L.S.D.(0.05) = 16.911			

The differences in phytochemical properties are due to the genotype of the plant as well as environmental factors. The study samples were analyzed using HPLC technology for the purpose of estimating the average content of some phenolic compounds present and formed during the experimental treatments. The results of the phenolic acids

obtained recorded a statistically significant increase in a gradual manner and in a parallel relationship for both types of fenugreek seeds used, as shown in Figure 1 when compared with the control sample, which has the lowest value and amounts to (12.0535 mg/l per 1 gram of dry callus weight).

**Fig 1:** Shows a comparison between the average total content of the main phenolic compounds identified in fenugreek species.

Discussion

The studied plant species grown in the same environmental conditions showed great diversity in the phytochemical properties of their biologically active compounds for the possibility of enriching or supplementing diets with essential nutrients. Both the local fenugreek plant and the imported (Iranian) fenugreek plant showed high percentages of protein, which indicates the possibility of using them safely in agricultural systems for human consumption. The two species also showed phytochemical properties, especially their richness in secondary metabolites, so they can be used in the pharmaceutical industry and at the local production level, considering fenugreek a good option for providing materials. The raw materials needed to produce steroid medicines and introduce them as species suitable for further exploitation in agricultural, food and pharmaceutical systems. Finally, from a practical standpoint, although the main factor affects the content of the nutritional and phytochemical compounds of the studied fenugreek species, the large variation in these compounds also depends on environmental factors. Therefore, cultivating the proposed

species in other regions with different climatic conditions may lead to a positive change in the content of their nutritional qualities and phytochemical compounds.

Alalwani and his group^[19] indicated that biotechnology methods can stimulate metabolic pathways in cultured plant tissue cells to produce the desired compound, resulting in a significant increase in the production of secondary metabolites. The results of Al-Alwani's study indicated that introducing magnetic water into the MS growth medium was more effective in producing secondary metabolites in fenugreek plants. Magnetic fields (MFs) can alter plant growth and development by improving their quantity and quality. One method of applying magnetic fields is magnetizing water, which produces magnetic water (MW), as used in this study. Also^[20] indicate that improving germination and early development can alter mineral content using MW technology, which depends on water quality, ionic content, and the type of magnetization, in addition to the plant species and genotype. Magnetic water is used either as an abiotic stress agent or as a growth stimulating or inhibitory agent. It is preferable to use it

against the use of permanent magnets with sustained magnetism because magnetic water may lose its magnetism over time as well as distance. The application of an external magnetic field causes changes in the atomic, molecular and electronic structure of the treated water, which leads to an increase in the polarization effects of water molecules, all of which were summarized in a research ^[21] where they indicated a decrease in the strength of the surface tension of magnetic water compared to untreated water, its repulsion to water due to the agglomeration structure, and an increase in the polarization effect of treated water. The same research also showed that magnetic water has some important properties, including effects and changes in the strength of surface tension.

Among the beneficial effects of magnetic treatment revealed by our current study is the occurrence of some changes at the biochemical level of the plant in addition to its potential effects at the cellular level. This is mainly due to increasing the type and quantity of secondary products by encouraging precursors to produce secondary metabolites. As shown in the results above, which were consistent with the findings of ^[22] found a noticeable increase in the rate of water absorption accompanied by an increase in magnetic force. This is due to the structural bioenergy that stimulates the pumping of cells as well as the stimulation of enzymes. Therefore, the effect of magnetic water appears in some plants at the level of chemical composition and nutritional elements, which causes an increase in some types of alkaloids, saponins and phenols in the callus, as is clear in the results of the current study, where the addition of magnetic water to the medium of growth and formation of the callus leads to the induction and increase in the production of these compounds, or perhaps due to the continuous sub-multiplication in the callus and the appearance of physical variation in the cells, which may cause an increase in the production of secondary metabolites, and this is according to the findings. There are some research papers, including ^[23, 24, 25].

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