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Original article

Effect of electromagnetic field on okra (*Hibiscus esculentus* L.) developmental stages and the effect of okra extract on breast cancer cells

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Abstract

Background and objectives: Electric, magnetic and electromagnetic fields can act as stress factors with different effects on biological systems. Due to the nutritional and medicinal values, and the increasing electromagnetic radiations, the present study was performed to investigate the effects of the electromagnetic field on the developmental stages and cytotoxic properties of okra. **Methods:** Both dry and wet seeds were exposed to electromagnetic field with the intensities of 2 and 4 mT for 60 minutes. MTT assay was applied to evaluate the potential cytotoxic effects of okra extract on MCF-7 cell line. The anatomical structure of leaves in both treated and untreated (control) plants were examined. **Results:** The results showed that a field intensity of 4 mT increased the speed of germination of wet treated seeds and the stem length of dry treated seeds. MTT assay revealed no cytotoxicity of the aqueous extracts of okra pods up to the concentration of 100 µg/mL from either the treated or the control plants towards the MCF-7 cell line. **Conclusion:** The results suggest that the electromagnetic fields would be able to increase the speed of germination without effects on percentage of germination.

Keywords: electromagnetic field, MCF-7 cell line, MTT assay, okra

Introduction

Okra is native to Africa and is used in almost every kitchen around the world. The plant is very useful for the digestive system due to its high contents of polysaccharides and micronutrients [1,2]. In addition, the fruit contains flavonoids, polyphenolic compounds, vitamins E and C and antioxidants [3].

Breast cancer is one of the most common diseases in women and its prevalence in the world between 1980 and 2010 has increased to 2.25%. In a study performed by Ren and Chen [4], okra polysaccharides were extracted and used for the treatment of 4 types of human cancers: ovarian cancer, breast cancer, cervical cancer and gastric cancer, and the collective data demonstrated growth inhibition of the cancer

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cells. The lectin found in okra with its anti-tumor effects has been shown to induce apoptosis in breast cancer cells [5].

In the past century, high frequency electromagnetic fields with high intensities have been introduced and could easily be generated. Due to their vast applications in physics and communications, these fields have been well investigated by different research groups. Electromagnetic (Em) fields are generated by electrically charged objects. They affect the behavior of charged objects in the field domain. EM fields can be generated by EM sources such as magnetrons, clystrons, solid state sources and etc. The generated field can be then radiated using antennas and propagated in the surrounding environment. EM fields can penetrate different objects such as plants. Low frequency and high intensity EM fields show a greater penetration [6]. It is now well known that electromagnetic fields have notable effects on plant growth. Various research groups have studied such effects on different plants. For example a study on the speed of germination and growth rate in potato has shown that EM field can cause a reduction in germination speed. It was also shown that the anatomy of plants is changed due to EM field illumination [7].

Due to the lack of information on the effect of electromagnetic field on okra seeds, this study was designed to investigate the effect of EM field on okra seed germination. In addition, the cytotoxic effects of both EM field-treated and untreated okra fruits were examined against breast cancer cell lines.

Experimental

EM field treatment

To treat seeds with an electromagnetic field, they were placed in petri dishes containing a wet filter paper for 12 hours, while others were placed in petri dishes with a dry filter paper. Five wet and five dry treated petri dishes were placed in electromagnetic fields with intensities of 2 and 4 mT (total of 20 dishes) for 60 minutes. Treated and control (five untreated seeds) samples were then planted in identical conditions of light, temperature and water in a garden at Seed and Plant Improvement Institute in Mohammadshahr, Karaj, Iran until they reached the desired growth level (ie., 3 months) to perform the studies. A number of seeds were also kept in petri dishes to examine speed and percentage of germination.

Speed and percentage of germination

Percentage of germination is the ratio of the germinated seeds to the total amount of seeds. In order to calculate the germination speed, it is required to precisely record the germination time. In each group, the germination time was defined as the time that 50% of seeds were germinated. Germination time was calculated for five different groups including a control and four treatments.

Seperation of fruits and leaves

The fruits and the leaves were separated for each sample. There were five groups of samples and in each, there were five different plants.

Microscopic examination

Okra leaf samples were placed in equal proportions of glycerin and alcohol between polystyrene and cut manually with a razor into slices. These slices were kept in 5% bleach for 15-30 min before being washed in distilled water for 3-5 min. In order to neutralize the pH of the bleach, slices were kept in 3% acetic acid for 3-5 min and re-washed again in distilled water. Slices were stained using the blue metal and carmine zocchi method. Slices were kept in the carmine zocchi for 15-20 min in order for pectocellulosic walls to turn red. Then, after washing with distilled water for about 2 min, they were kept in 3% blue metal for the wooden walls to turn blue. They were then washed in distilled water to remove extra stain. Finally, the stained samples were placed on microscope slides for microscopic examination under a ZEISS optical microscope (Germany).

Extraction
Fresh Fruits were sliced and extracted with 500 mL water for 24 h with contious shaking. The extract was filtered and freeze dried. The freez dried extract was kept in refrigerator for further experiments

MTT assay
The cells were seeded in 96-well flat bottom tissue culture plates at a density of 8500 cells/well and incubated for 24 h at 37 °C. Fresh medium containing different concentrations, 100, 50, 25, 12.5, 6.25, 3.125 mg/mL, of the extracts were then added and the plates were reincubated for 72 h. The supernatant was then removed and MTT solution (0.5 mg/mL) was added to each well. Following a 4-hour incubation, the resultant formazan crystals were dissolved in DMSO (200 µL) and their absorbance was recorded by a microplate reader at 570 nm. Viability of the cells was assessed according to the following equation:

$$\% \text{ cell viability} = (A \text{ samples}/A \text{ control}) \times 100$$

Where A samples was the absorbance of wells containing the extracts and A control was the absorbance of wells in absence of extracts [8,9].

Results and Discussion
The results of the germination speed and percentage for the control and the four treatments showed an insignificant (P>0.05) effect on the percentage of germination (figure 1). Figure 2 shows that the greatest index of germination speed belonged to the wet seeds at 4 mT, indicating that wet treatment at 4 mT required less time to have 50% of seeds germinated. These results are in line with those obtained on the effect of electromagnetic field on wheat [10] and lentil [11] seeds. Histologically the following existed in a leaf tissue: the epidermis, the leaf mesophyll and vascular tissue.
Epidermis appeared as a row of cells. Mesophilic parenchyma was below the epidermis. There were gaps between spongy parenchyma cells which they were irregular. Xylem and phloem

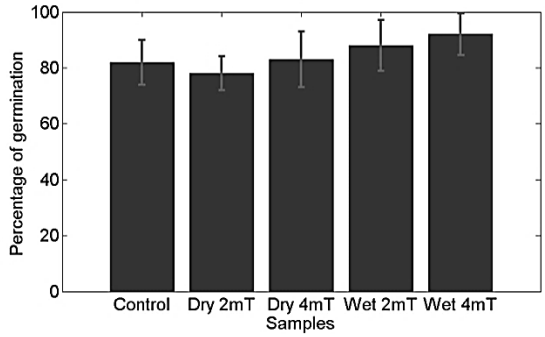

Figure 1. The percentage of germination of okra seeds; Dry 2mT: dry seeds treated with an electromagnetic field of 2 mT. Dry4 mT: dry seeds treated with an electromagnetic field of 4 mT. Wet 2mT: wet seeds treated with an electromagnetic field of 2 mT. Wet 4mT: wet seeds treated with an electromagnetic field of 4 mT

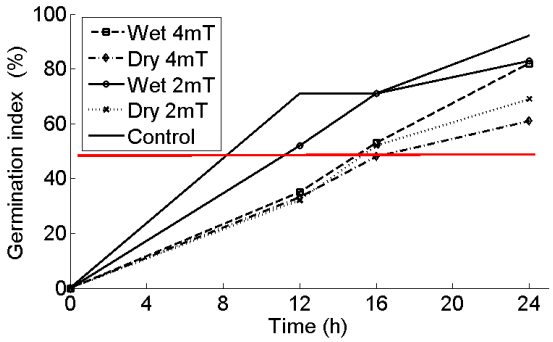

Figure 2. Germination speed for control and the four treatments in okra seeds. Dry 2mT: dry seeds treated with an electromagnetic field of 2 mT. Dry 4mT: dry seeds treated with an electromagnetic field of 4 mT. Wet 2mT: wet seeds treated with an electromagnetic field of 2 mT. Wet 4mT: wet seeds treated with an electromagnetic field of 4 mT

were located in vascular veins (figure 3).In the leaves of plants grown from treated seeds, in particular the dry seeds at 4 mT, an increase in the number of xylem and pheloem rows were detected. Also, the size of spongy parenchyma cells was larger with greater intercellular spaces. It seems that applying the electromagnetic field to the okra leaves increased the number of cell division, resulting in an increase of xylem and phloem rows in leaf. In the leaves of both wet and dry treated samples a further increase in the number of collenchyma cells below the epidermis was observed.

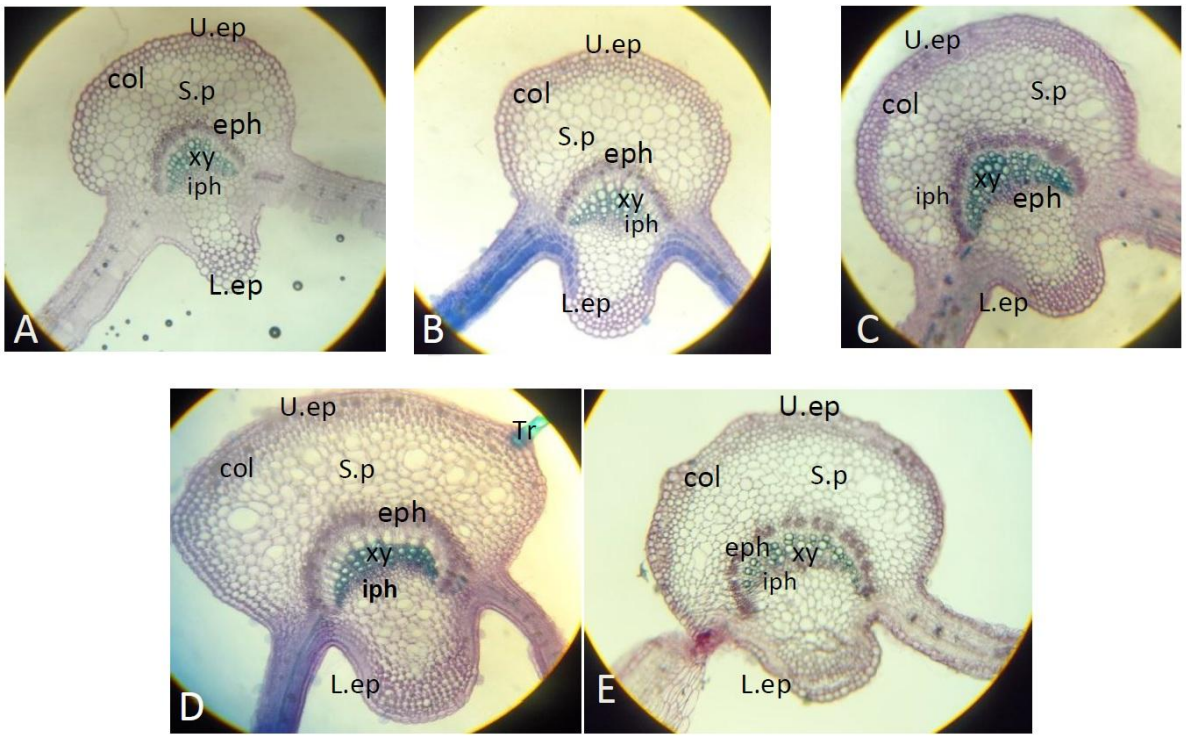

Figure 3. Cross sections of the main vein in leaves of okra. A: untreated control; B: wet 4mT EM field treated; C: dry 2mT EM field treated; D: dry 4 mT EM field treated; E: wet 2 mT EM field treated. Upper epidermis (U.ep), xylem (Xy), spongy parenchyma (S.p), external phloem (ePh), internal phloem (iPh), the lower epidermis (L.ep), colanchyma (Col), tricurdium (Tr)

However, the number and diameter of xylem vessels showed a more significant increase compared to the control samples, justifying the fact that under stress conditions, increasing the number of collenchyma cells under the epidermis reduces the rate of evaporation by reducing the volume of the vascular system [12]. compatibilities in the leaves under stress conditions are in line with our findings with respect to the increase in collenchyma, while the reduction in leaf vascular tissue is not in agreement.

The results of the present study about the cytotoxicity showed that the aqueous extract of okra to the concentration of 100 µg/mL showed no cytotoxic effect on MCF-7 cell line. These results are in agreement with those obtained for the aqueous extract of okra by Ilango [13] using acetone for precipitation. However, they contradict those of Ren and Chen [4] for the aqueous extract of okra using ethanol for precipitation. In studies by Ilango [13], the human cancer cell line showed no morphological changes and the cell viability was nearly 100%. Reduction of MTT by cells indicates mitochondrial activity, which may be interpreted as proof of cell viability. None of the okra polysaccharides induced cytotoxic effects at the used concentrations while in the study by Ren and Chen [4], one of the fractions had an obvious inhibition effect on MCF-7 cells with the lowest survival rates of 63.90%. The plant tissues and the extract solution used by this study differed from those used in the present study, which may explain the contradicting results obtained in the present investigation.

The fruit extract whose seeds were previously treated with the electromagnetic field intensities of 2 and 4 mT in our study, demonstrated no cytotoxic effect on MCF-7 cancer cell line; however, the extract obtained from plants directly

exposed to EM fields may produce different results.

The present study suggested that electromagnetic fields increase the speed of germination while they might have no effect on percentage of germination. They can increase the number of collenchyma cells which results in reduction of the rate of evaporation.

Acknowledgments

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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