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The influence of electromagnetic field on bitter melon callus (*Momordica charantia* L.)

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Abstract

Momordica charantia is a plant known for its antioxidant, antibacterial, antiviral properties, being used successfully in treating various diseases (HIV, some types of cancer, type II diabetes).
In vitro cultivation of selected plant tissues for proliferative and biosynthetic aptitudes is a rapid way of obtaining metabolites of interest.
The research aim of this paper is to study the influence of exposure time and intensity electromagnetic radiation on bitter melon callus grown in different *in vitro* systems, in order to select tissue lines with proliferative aptitude and producing secondary metabolites of interest.
The results obtained showed that the exposure of the *Momordica charantia* callus to moderate intensity of electromagnetic radiation has a favorable influence on the cell division, ensuring the increase of tissue biomass. The best results were obtained by using the system of cultivation in liquid medium.

Key words callus, *Momordica charantia*, electromagnetic radiation, polyphenols

Momordica charantia L. is a medicinal plant, herbaceous, rich in saponin, polysaccharide, protein (MAP 30), alkaloids, flavonoids, amino and fatty acids, vitamins A, B, C, but low in carbohydrates, fats and calories (3). This plant has been successfully used in the treatment of various diseases (HIV, different types of cancer, diabetes type II), demonstrating his antibacterial, antiviral and antiparasitic effects (5).
The research carried out by different authors, related to the effect of the magnetic field response in plants has been achieved at a low level of magnetic radiation intensities (4). The growth of plants has been observed, which can be accelerated both in the presence of static and dynamic magnetic fields (7, 8, 2).
The purpose of the present research is the influence of exposure time and intensity electromagnetic radiation on bitter melon callus in order to select tissue lines with proliferative aptitude and producing secondary metabolites of interest.

Material and Methods

The biological material is represented by fresh callus cultivated on solid and liquid MS (6) medium supplemented with hormonal balance 1.5mg / l NAA + 1 mg / l BAP, which has been exposed to electromagnetic radiation in the period of time and at different intensities, compared to the control represented by the callus unexposed (Table 1). The system of callus exposure was made at the Politehnica University from Timisoara.
Callus samples subjected to the electromagnetic radiation and unexposed control were grown at a temperature of 24 ° C with a photoperiod of 16 hours light and 8 hours dark in the growth room for 7 days. At the end of the 7 days period, the weight of the exposed callus was measured being compared to the control weight.
The callus of *Momordica charantia,* cultivated on the solid and liquid medium, exposed or not exposed to electromagnetic radiation was analyzed using the Folin-Ciocalteu method (1), to measure the concentration of polyphenols. So, one milliliter (ml) of the sample (alcoholic extract from bitter melon callus) diluted adequate was mixed with 5 ml of water and 1 ml of Folin-Ciocalteu reagent and were added 3 ml of sodium carbonate 15% in water to reach a final volume of 10 ml. The samples were incubated for 2 hours in the dark at room temperature before measuring the absorbance at 750 nm using UV-VIS spectrophotometer (Specord 205 Analytical Jena). The calibration curve was prepared using gallic acid equivalents of 0.05-0.5 mM · L-1 (GAE). The results were expressed in ppm (parts per million).

Table 1

Exposure time and intensity of the electromagnetic field of experimental variants

Nr. ctr.	Experimental variant	Exposure time (h)	Intensity of electromagnetic radiation (mG)
1.	Martor	0	0
2.	Exposed callus	1	10
3.			20
4.		3	10
5.			20
6.		7	10
7.			20
8.		24	10
9.			20
10.		72	10
11.			20
12.		168	10
13.			20

Results and Discussions

The influence of the duration and intensity of the electromagnetic radiation on the bitter melon callus growth compared to the control non-exposed is shown in Table 2. The results of the weight measurement reveals important differences between the control callus which has not been exposed and the exposed samples to electromagnetic radiation.

The callus exposed for 1h at the 10 mG (milligauss) intensity showed a smaller increase than the control (1.41g). At the same exposure time and radiation intensity of 20 mG was observed a callus growth value of 1.42g. Extending the callus exposure to 3h at 10mG resulted a slight increase in weight of the tissue (1.44g). An increase in electromagnetic radiation intensity to 20 mG with a 3 hour exposure time of callus tissue lead to a drastic decrease in tissue proliferation capacity, the value obtained being of 0.73g.

Increasing the exposure time at 7 hours, it was observed and recorded a weight of callus growth higher than the control not exposed, at both intensities with values 1.36 g to 10 mG and 1.41 g to 20 mG. The callus exposed to 24 hours also recorded an increase compared to the control, reaching values of 1.33 g and 1.46 g to 10 mG to 20 mG. The callus exposure for 72 h resulted in a decrease of the tissue proliferative capacity at both intensities studied (0.89 g and 0.86 g). Extending the exposure time up to 168 hours lead to a growth of callus weight, recording the best results of weight measurement, with values of 1.97 g at 10 mG and 2.15 g at 20 mG (Fig.1).

Table 2

Influence of exposure time and intensity of electromagnetic radiation on tissue growth at *Momordica charantia*

Nr. ctr.	Experimental variant	Exposure time (h)	Intensity of electromagnetic radiation (mG)	Initial weight (g)	Measured weight (g)	Difference (in growth, g)
1.	Control	0	0	0.5	1.45	0,95
3.	Exposed callus	1	10	0.5	1.41	0,91
4.			20	0.5	1.42	0,92
5.		3	10	0.5	1.44	0,94
6.			20	0.5	0.73	0,23
7.		7	10	0.5	1.86	1,36
8.			20	0.5	1.91	1,41
9.		24	10	0.5	1.83	1,33
10.			20	0.5	1.96	1,46
11.		72	10	0.5	1.39	0,89
12.			20	0.5	1.36	0,86
13.		168	10	0.5	2.47	1,97
14.			20	0.5	2.65	2,15

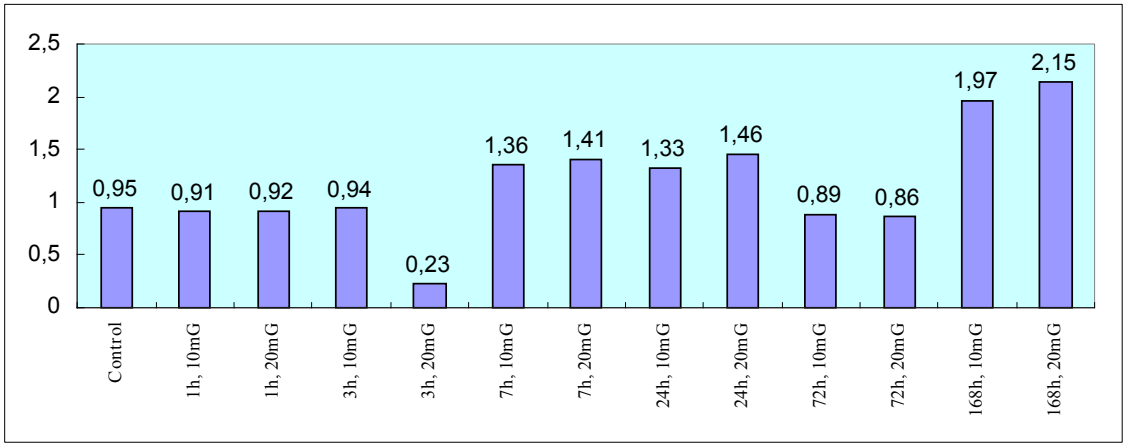

Fig. 1 Growth of bitter melon callus cultivated on solid medium exposed and not exposed to electromagnetic radiation

In conclusion, we can say that the exposure of the *Momordica charantia* fresh callus to moderate intensity of electromagnetic radiation has a favorable influence on the cell division, ensuring the increase of tissue biomass.

The results regarding the concentration of total polyphenols tested in *Momordica charantia* callus exposed to the different intensities of electromagnetic radiation are shown in Table 3.

The callus exposed for 1h at the intensity of 10 mG and 20 mG recorded concentrations of polyphenols lower than the control, respectively 179,8 ppm and 198,6 ppm. Extension of exposure time at 3h led to an increase in the concentration of polyphenols in the tissue, but did not exceed the value of the control (216,6 ppm). The best result (424,8 ppm) was obtained when the callus was exposed for a period of 7 hours at a radiation intensity of 20 mG. The exposure of callus to 10 mG resulted in a decrease in the total polyphenols concentration in samples submitted to a 1, 3, 72 and 168h.

Table 3

The influence of exposure time and different intensities of electromagnetic radiation on the concentration of total polyphenols in *Momordica charantia* callus

Nr. Ctr.	Experimental variant	Exposure time (h)	Intensity of electromagnetic radiation (mG)	Concentration of total polyphenols (ppm)
1.	Control	0	0	369
2.	Exposed callus	1	10	179,8
3.			20	198,6
4.		3	10	216,6
5.		7	10	236,6
6.			20	424,8
7.		24	10	252,2
8.			20	210,2
9.		72	10	171,4
10.			20	186
11.		168	10	154,48
12.			20	319,6

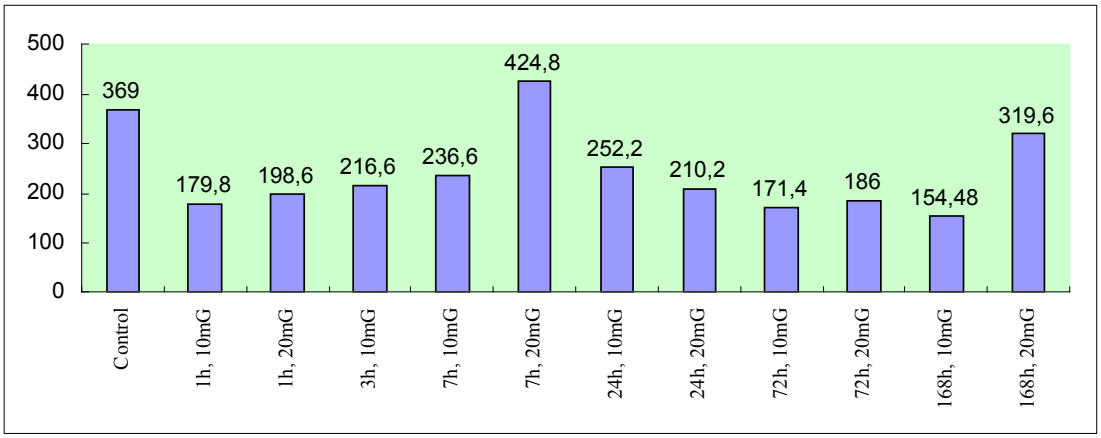

Fig. 2. The total polyphenol concentration of bitter melon callus grown on solid medium

Our experimental results show the influence of electromagnetic radiation on the polyphenol content of the exposed tissue. The increase in total polyphenol content occurs when the bitter melon callus is exposed to an intensity of 20 mG over a period of 7h.

The *Momordica charantia* callus was also cultivated on the liquid MS medium to determine the influence of the cultivation system on the growth of tissue mass and polyphenolic content following exposure of this tissue to electromagnetic radiation. The results are shown in Table4.

At a 1 hour exposure time, the callus recorded a lower weight than the control for both intensity of the electromagnetic field, respectively 0.65 g for 10 mG and 0.70 g for 20 mG..

The prolongation of callus exposure up to 7 h resulted in an increase of the proliferative capacity, reaching the values of 0.90 g at 10 mG and 0.92 g at 20 mG, while at 24h exposure time the values recorded were 0.97 g at 10 mG and 1.03 g at 20 mG.

The best results in weight of tissue growth were obtained at an exposure of 168 hours, recording values of 1.30 g at 10 mG and 1.45 g at 20 mG.

Following the results we can say that the exposure time at electromagnetic radiation of tissue has a positive influence on the growth of bitter melon callus on liquid medium (Fig. 3).

Table 4

The influence of the exposure time and electromagnetic field intensity on the growth of *Momordica charantia* callus cultivated in liquid medium

Nr. ctr.	Experimental variant	Exposure time (h)	Intensity of electromagnetic radiation (mG)	Initial weight (g)	Measured weight (g)	Difference (in growth, g)
1.	Control	0	0	1	1.75	0,75
2.	Exposed callus	1	10	1	1.65	0,65
3.			20	1	1.70	0,70
5.		7	10	1	1.90	0,90
6.			20	1	1.92	0,92
7.		24	10	1	1.97	0,97
8.			20	1	2.03	1,03
11.		168	10	1	2.30	1,30
12.			20	1	2.45	1,45

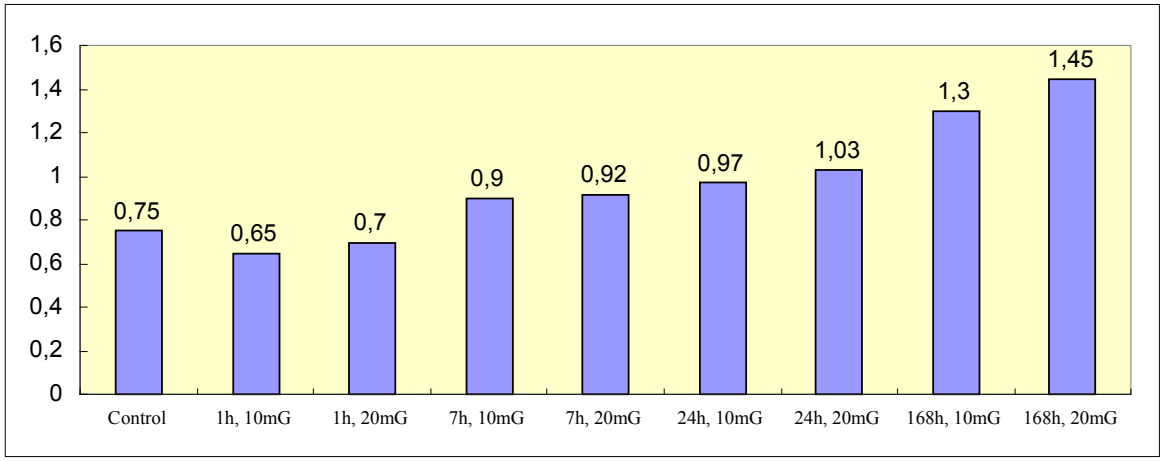

Fig. 3. The influence of the exposure duration and the electromagnetic field intensity on the growth of the *Momordica charantia* callus cultivated in liquid medium

The callus grown in liquid medium and exposed to electromagnetic radiation was tested for the evaluation of the content of polyphenols. The results are shown in Table 5.

The results obtained from biochemical analyzes show an increase in the polyphenol content in the bitter melon callus at all experimental variants, surpassing the control. The control sample and the exposed callus are represented by the tissue line - fresh callus, grown on MS medium supplemented with 1.5mg / l NAA + 1 mg / l BAP. The callus exposed for 24 hours at 10 mG recorded the highest polyphenol content (1327,24 ppm) and at the intensity of 20 mG recorded the value of 361,78 ppm. Extending the exposure time to 168 hours led to lower values on the polyphenol content of 388,28 ppm to 10 mG and 559,5 ppm to 20 mG..

Table 5

The influence of exposure time and magnetic field intensity on the concentration of polyphenols in *Momordica charantia* callus grown in liquid medium

Nr. Ctr.	Experimental variant	Exposure time (h)	Intensity of electromagnetic radiation (mG)	Concentration of total polyphenols (ppm)
1.	Control	0	0	320,76
2.	Exposed callus	24	10	1327,24
3.			20	361,78
4.		168	10	388,28
5.			20	559,5

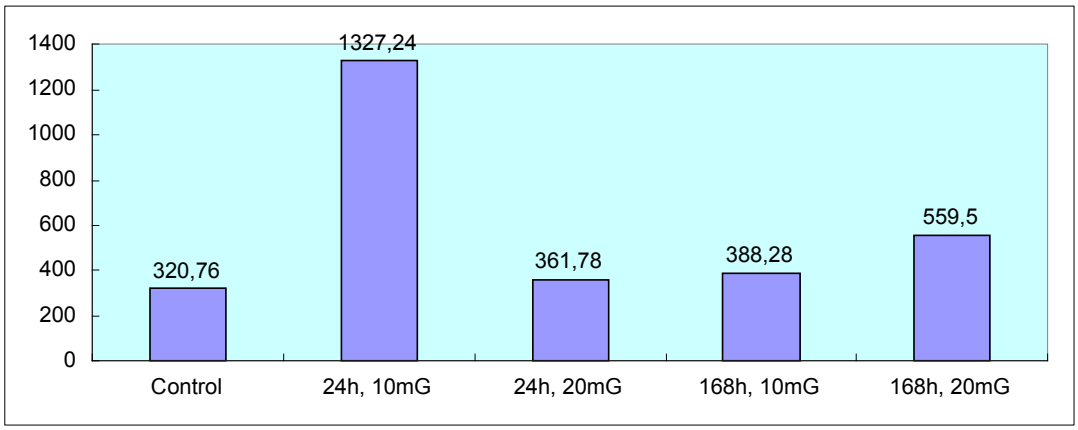

Fig. 4. The influence of the magnetic field duration and intensity on the polyphenols concentration into callus grown in liquid medium

The results obtained demonstrate the random influence of the electromagnetic field intensity and the duration of exposure on the total polyphenol synthesis capacity in the callus grown in the liquid medium (Fig.4).

Conclusions

The duration of exposure, the intensity of the electromagnetic field and the cultivation system used have some influence on the increase in callus and total polyphenol content.
Exposure of bitter melon callus to these moderate intensities of electromagnetic radiation has a favorable influence on cell division, providing increased tissue biomass.
The increase in total polyphenol content occurs when the bitter melon callus was exposed to a intensity of 20 mG for a period of 7 hours.
The use of the liquid culture system and exposure to electromagnetic radiation at 20 mG intensity influences the growth of the callus, the best results were obtained at an exposure time of 168 h (1.45 g) followed by the one at 10 mG (1,30g).
As for the content of polyphenols in callus exposed at electromagnetic radiation, grown on the liquid medium, the best results were recorded at the exposed callus for 24 hours at an intensity of 10 mG, the value obtained being 1327,24 ppm followed by the one at 168h, the intensity of radiation was 20 mG (559,5 ppm).

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