Evaluation of Total Chlorophyll Content in Microwave-Irradiated *Ocimum basilicum* L.

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Abstract

Microwave stress is an important cause of stress today. This study was carried out to investigate the effect of microwave radiation derived from a wireless router on chlorophyll pigments from *Ocimum basilicum* L. Treatments included reference (no microwave), microwave-stressed plants and control plants (growth in normal condition). The extracts were analyzed using a Shimadzu UV-160A spectrophotometer for chlorophyll content determination. The content of chlorophylls a and b from plants subjected to microwaves was smaller than in the reference plants.

Introduction

Chlorophyll pigments are very important in the process of photosynthesis and chlorophyll concentration is closely related to the stress factors, this fact being shown by many researchers over time (Merzlyak and Gitelson, 1995; Penuelas and Filella, 1998).

Chlorophyll is one of the most important and most abundant photosynthetic pigments. The amount of chlorophyll is correlated with productivity and plant health (Dash and Curran, 2007). Chlorophyll content of leaves is also an indicator of stress, giving us vital information regarding plant responses to climate change events. When a plant is under stress, low chlorophyll content changes are evident in the initial stages. As stress increases, chlorophyll content decreases faster than other pigments (Bannari et al., 2007). These changes of chlorophyll content indicate the stress to which the plant was subjected (Radu et al., 1981). The chlorophyll content is, therefore, a plant stress response.

The adaptability of organisms to extreme conditions of life, environmental stress response mechanisms is an area of great current research. Nowadays, has become more

demanding the use of mobile telephony and wireless devices generating an exponentially increased level of electromagnetic radiations. Thus, there is a new stress factor - electromagnetic field, especially from the microwave area (Vashisth and Nagarajan, 2008).

The present study aimed to determine the effect of microwave field on chlorophyll pigments content from *Ocimum basilicum* L., a very important culinary spice and medicinal plant from *Lamiaceae* family.

Material and methods

In this paper the microwave influence on chlorophyll content from *Ocimum basilicum* L. was studied. The plants were grown in the laboratory, using seeds purchased from ARO Company (Romania). For this experiment were used plants grown in normal conditions and plants exposed to microwaves. For microwaves exposure, two identical anechoic chambers were used: a reference chamber and a microwave chamber.

At three weeks after seeding, vessels with sprung plants were placed in two identical anechoic chambers (E. Surducan et al., 2012), one being the reference chamber and the other with the active microwave field (Figure 1).

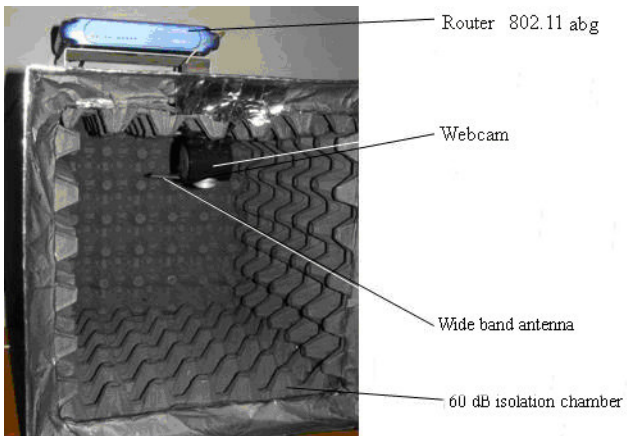

Figure 1. Installation to stimulate the plant development by applying a low power electromagnetic field.

The installation to stimulate the plant development by applying low power electromagnetic field consists in a closed chamber which contains the artificial light sources, humidity sensor, temperature and humidity sensors, video camera and a microwave generator that emits by means of an electromagnetic field antenna on the plants culture at a power level between $10W/m^2$ - $10mW/m^2$. The power is measured by a power meter with the probe located in the area where the plants are subjected to stimulation, and which is connected to a microcontroller and process computer which can interconnect a network of such premises (E.

Surducan et al., 2012). The reference chambers do not contain the microwave generator. The temperature and humidity in both chambers were continuously registered and a good correlation between the two was observed.

The irradiation of plants was performed with microwaves modulated by a WLAN communications protocol, in the 2.412 – 2.48 GHz frequency band. Irradiation was performed over a period of two weeks, within the plants were watered with 10 mL of ultrapure water at an interval of 3 days. After the irradiation period, the leaves were manually excised and subjected to chlorophyll extraction.

The weighed plant samples (1 g) were homogenized with an Ultraturrax in the presence of 20 mL 90 % acetone in water and then left for two hours with stirring. The extraction was repeated with 10 mL 90 % acetone in water until bleaching of the plant material, and then the samples were filtered. Each extraction was performed in three parallel samples.

The quantitative analysis of chlorophyll a and b pigments content from the samples extract was performed using a Shimadzu UV-160A spectrophotometer. The calculation of chlorophyll pigments concentration was carried out by reading the absorbances at 663 and 645 nm wavelengths, according to the following equations (1), (2), (3) (Arnon, 1949):

$$\text{Chl a} = \frac{[12.7(\text{A663}) - 2.69(\text{A645})]\text{xV}}{(1000\text{xW})} \quad (1)$$

$$\text{Chl b} = \frac{[22.9(\text{A645}) - 4.68(\text{A663})]\text{xV}}{(1000\text{xW})} \quad (2)$$

$$\text{Chl total} = \frac{[20.2(\text{A645}) + 8.02(\text{A663})]\text{xV}}{(1000\text{xW})} \quad (3)$$

where: Chl a – chlorophyll a, Chl b – chlorophyll b, Chl total – total chlorophylls content, A663 – sample absorbance at 663 nm, A646 – sample absorbance at 646 nm, V – volume of solvent, W – fresh weight of tissue extracted.

Results and discussion

In most of the cases any of stress factors by a specific intensity can lead to decrease of chlorophyll concentration in the plant. This was observed in the case of our plants (Figure 2).

a) b)

Figure 2. *Ocimum basilicum* L. plants: a) microwave-irradiated and b) reference.

It can be observed that reference plants have a darker green color than those microwave-irradiated.

The obtained results concerning the concentration of chlorophyll pigments in basil extracts are presented in Figure 3.

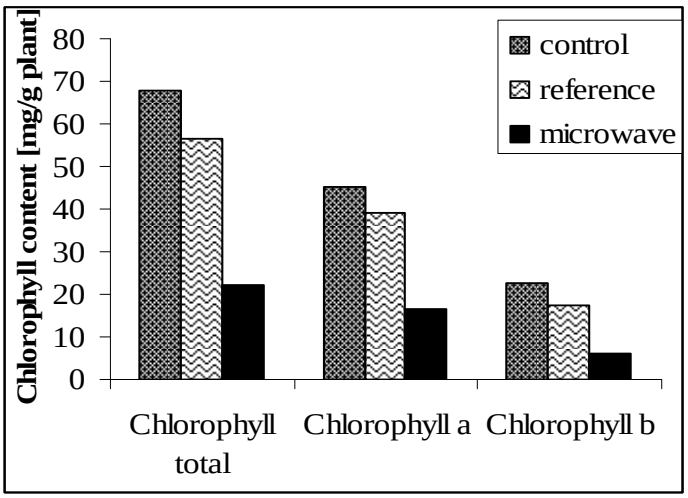

Figure 3. Content of chlorophylls in control, reference and microwave-irradiated plants of basil (*Ocimum basilicum* L.).

As can be seen in Figure 3, the chlorophyll content in control plants is higher than in the plants grown in the reference and microwave chambers. Also, chlorophylls a and b content from plants subjected to microwaves was lower than in non-irradiated plants.

The total chlorophyll content was 56.41 mg/g for the reference plants, and 22.26 mg/g for irradiated plants. Thus, the amount of total chlorophyll in irradiated plants is smaller by 60.54 % compared to reference plants.

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