Synthetic scientific report

regarding the project implementation during the period 2011 -2014

1. The growth of plants in microwave field; determination of the optimal methods for extraction of the bioactive compounds

1.1. Preparation of the system for microwave irradiation of the plants considered for the study

In order to study the effects of microwave irradiation on aromatic plants were selected the following plants: celery (*Apium graveolens*), parsley (*Petroselinum crispum*) and dill (*Anethum graveolens*). In this stage were bought seeds of these plants, from Kotanyi Company, and were seeded into the ground. Three weeks after sowing, the plants were placed into four identical anechoic chambers, two reference chambers and two chambers used for microwaves action in two domains: **a)** GSM, using a generator in the domain 860 – 910 MHz, output total power 30 dBm (1000 mWatt) **(M1)**; **b) WLAN** (wireless internet connection) using a generator router 802.11g, with operating frequency in the domain 2.4 – 2.49 GHz, with main operating channel at 2.42 GHz, output total power 18 dBm ($10^{1.8}$ mWatt) **(M2)** (Fig. 1).

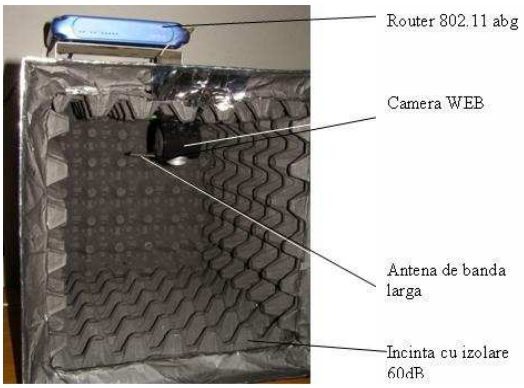


Fig. 1. Treatment enclosure in microwave field

The cubic enclosure (37x37x37cm^3) with walls lined with tall pyramid structure of 4 cm, were placed in the same conditions of temperature/humidity and completely closed. The enclosures showed a radiofrequency isolation of 60dB between the interior and exterior. Temperature and humidity between the reference and irradiated chambers were recorded continuously and there was observed a good correlation between them; the increasing temperature conducted to the decrease of the humidity with the same percentage in both chambers, so the growth conditions were nearly identical. In these enclosures, the plants will be maintained for 3 weeks and will be analyzed in the next stages.

1.2. Selection of the extraction methods for bioactive compounds analyzed from the aromatic plants considered for the study

1.2.1. Obtaining and characterization of vitamin C extracts obtained from the reference plants using new methods of extraction and various solvents

In this activity was followed the extraction of vitamin C using three methods: grinding/centrifugation (M), sonication (UAE) and extraction in microwave field (MAE), using as extraction solvent water and

various aqueous solutions of 8% concentration of the following acids: trichloroacetic acid (TCA), metaphosphoric acid (MPA), and acetic acid (CA).

1. Grinding / centrifugation. An amount of 1 g of fresh plant was powdered in a mortar and pestle at 20°C with approximately 1 g of sand and 5 mL of solvent (8% aqueous solution of acid), to obtain a thin homogeneous paste. Further, the solid paste is transferred into a tube and centrifuged (8000 rpm) at 20°C for 5 minutes, and the clarified extract obtained was decanted. The mortar was rinsed with another 5 mL of solvent which was transferred back into the centrifuge tube over the initial solid mixture obtained after decantation, and the centrifugation process was repeated for 5 minutes. The two extracts obtained were transferred into a 10 mL volumetric flask and adjusted to the volume (brought to volume with 8% acid solution). The extracts were stored in dark bottles at 4°C.

2. Sonication. The grounded plant material was extracted at the temperature of 20°C with 5 mL of solvent (8% aqueous solution of acid) using an ultrasonic bath. It was used 1 g of plant material which was extracted with 5 mL of solvent for 30 minutes, the extract was decanted, and then the remaining plant material was extracted the second time with ultrasounds, with an additional 5 ml of solvent for 30 minutes. The total time of the extraction by sonication was 1 hour. The final volume obtained by combining the two extracts was adjusted to 10 mL.

3. *Extraction in microwaves field.* The powdered plant material was used for the extraction of vitamin C at 30°C with 10 mL solvent, in microwave continuous field, using a Monowave 300 system, Anton Paar. For reaching the 30°C temperature was necessary a time of 1 minute, and the cooling of the system to room temperature, also was reached in 1 minute. During the irradiation process, the samples were stirred with a speed of 250 rpm. The microwave field was applied for 4 minutes, and the clarified extract obtained was filtered. The final volume obtained was adjusted by combining the two extracts and brought to 10 mL using a volumetric flask.

The obtained extracts were analyzed by HPLC-MS and electrochemically.

1.2.2. Obtaining and characterization of essential oils from reference plant extracts, using various new methods of extraction and various solvents

In these activities of establishing the extraction method, the studies were conducted on reference plants. For extraction of volatile oils were used dried plants. After the maceration of the dried leaves, powder portions of 0.5 g were subjected to extraction with various solvents, using various techniques. The following solvents and solvent mixtures were used for extraction of the essential oils: E1 - diethyl ether - ethanol (1:1, v/v); E2 - ethanol; E3 - hexane; E4 - diethyl ether; E5 - diethyl ether - hexane (1:1, v/v).

The extraction techniques used were: maceration, extraction by sonication with solvent and extraction with solvent in a power microwave field. Each extraction process was optimized taking into account the main factors (time, temperature etc.). All the extractions were performed at 30°C.

The maceration (M) was performed in 14 days, with 4 mL extraction solvent (E1 - E6). The maceration was followed by filtration and washing of the plant residue, the final volume being 4 mL.

The extraction by sonication with solvent (UAE) was carried out in two stages, using an ultrasonic bath Transsonic T 310 at 35 kHz, and installed power of 95W. After 30 min of sonication in 4 mL solvent, the extract was separated. Finally, the sample was filtered and the residue washed. The extracts were combined and the final volume was 4 mL.

Solvent extraction in power microwave field (MAE) was performed using a device built in INCDTIM Cluj-Napoca. With this device was possible to control the following parameters: operating time, temperature and filling coefficient. The sample (0.5 g dried and powdered plant) with solvent extraction (4 mL) was introduced into the cell extraction. Taking into account the specific material (plant), were selected

the following work parameters: action time of 1 minute, 40% filling coefficient at a power of 900W. Depending on the absorption capacity of the solvent, the total extraction was longer than 1 minute because the cell should not exceed 30°C. The longest duration of extraction was registered in diethyl ether + ethanol mixture, but did not exceed 30 min. Because the operating temperature was small, the solvent systems used din not boiled, and so the extraction was conducted at atmospheric pressure. The samples obtained were filtered, washed and the final volume was 4 mL.

In order to establish the optimum extraction conditions (solvent extraction and method), the experiments were performed on reference plants. In the stages that followed, the best method was applied on plants irradiated with microwaves. The quality of the extracts was determined by chromatographic methods (TLC, GC-FID).

All the determinations were carried out using the samples in triplicate.

1.2.3. The selection of the method for the extraction of vitamin C and essential oils, from studied aromatic plants

The samples obtained by the methods described above were compared in order to select the optimal method of vitamin C extraction, essential oils respectively.

The selection of the method for the extraction of vitamin C was performed using both chromatographic (HPLC) and electrochemical method; the results obtained by the two methods are in concordance.

After performing the analysis it was found that 8% MPA presented the greatest properties of extraction using sonication extraction method.

The essential oil analysis was performed by GC-FID using a Shimadzu 2010 gas chromatograph with flame ionization detection (GC-FID). Capillary column used was TA-5 type (30m), and the analyses were carried out in helium at a flow rate of 4 mL/min. The recording of chromatograms was started at 50°C for 2 min and heating was continued with a rate of 8°C/min up to 250°C and maintained for 15 min. The injection temperature was 250°C. To establish the best methods for essential oils extraction were overlaid the chromatograms obtained and compared the size of the peaks (Fig. 2).

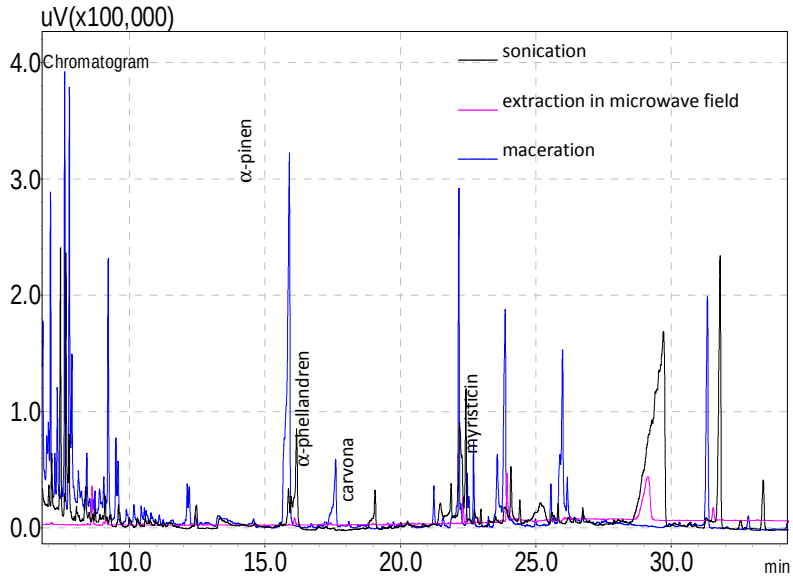


Fig. 2. The chromatograms of dill extracts obtained with solvents mixture E1 using three extraction techniques

Based on the chromatograms recorded, it was observed that the maceration was the best method of essential oils extraction, followed by sonication. The best solvent extraction has proven to be the ethyl ether: ethanol (1:1, v/v) mixture followed by hexane.

2. The investigation of microwave effects on the content of vitamin C from the studied plants

2.1. The monitoring of vitamin C variation in the irradiated plant in comparison with the reference plants

The studied plants (parsley, celery and dill) were grown in the conditions mentioned at 1.1.1, after which leaves were excised, were obtained the extracts and analyzed both by HPLC and electrochemical method.

In order to determine vitamin C was used an aqueous solution of 8%r acetic acid for the extraction from celery, parsley and dill. 1 g of plant material was extracted with 5 mL of solvent by sonication for 30 minutes. The extract was decanted and then the remaining plant material was extracted the second time by sonication, with an additional 5 ml of solvent for 30 minutes. The total time of the extraction by sonication was 1 h and the temperature 20°C. The final volume obtained by combining the two extracts was adjusted to 10 mL.

The analysis of the vitamin C extracts from studied plants was performed using HPLC method with HPLC Shimadzu 2010 system and as a stationary phase was used a column Alltima, C18, 3µ, 100 x 3 mm. The elution was performed with a gradient using a mobile phase which was consisted of: 15 mM phosphate buffer at pH = 2.7 (A) and methanol (B), with a flow rate of 0.4 mL/min. The gradient was performed as follows: 0 min: 10% B, 5 min: 20% B, 10 min: 10% B. The column temperature was 30°C.

Comparing the amount of vitamin C determined in parsley, dill and celery it was found that in the case of the reference plant, the parsley contained the highest amount of vitamin C (264 mg AA/100 g fresh weight), followed by dill with 121 mg AA/100 g fresh weight and celery with 103 mg AA/100 g fresh weight (Fig. 3).

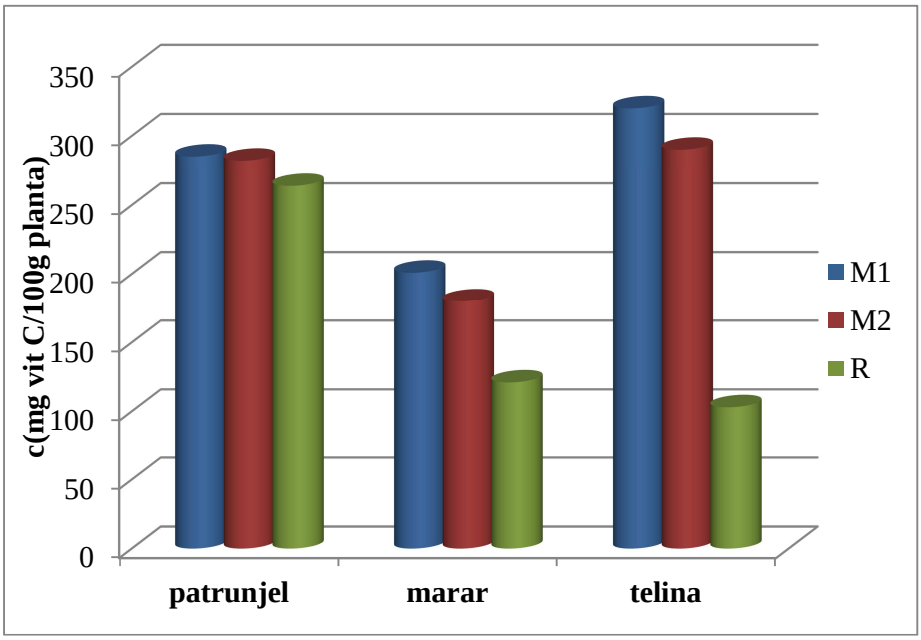


Fig. 3. The vitamin C content determined by HPLC, in the studied plants gown in different conditions

2.1.1. The quantitative determination of vitamin C from irradiated and non- irradiated plant, by electrochemical method

The electrochemical chronoamperometry experiments were performed with a potentiostatic device assisted by a computer (Autolab- PGSTAT 302N, EcoChemie, Utrecht, aNeederland). Chronoamperometry involves the study of variation of the current system response time in potentiostatic control.

For chronoamperometry measurements was used an electrochemical cell equipped with three electrodes: the counter electrode from plate or wire of platinum, the reference electrode $Ag/AgCl/KCl_{sat}$ and working electrode, carbon paste electrode CPE. The support electrolyte of pH = 6.8 was 0.1M phosphate buffer, stirred with 500 rpm and the applied potential was +600 mV vs $Ag/AgCl/KCl_{sat}$.

The concentrations of ascorbic acid from the samples obtained by extraction from studied irradiated and non-irradiated plants were calculated by interpolating the signal (current) in the linear portion of the calibration curve (Fig. 4).

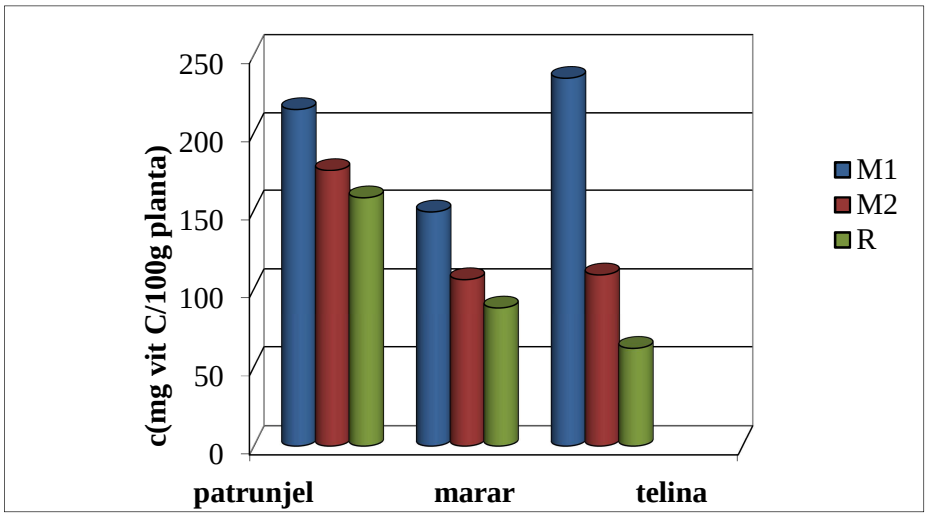


Fig. 4. The vitamin C content electrochemical determined in the studied plants gowned in different conditions

2.1.2. The comparison of the results obtained by these two methods. The comparison of the results obtained for the irradiated and reference plants

The results obtained by HPLC and electrochemical methods are in close correlation, the variation in vitamin C content follows the same trend, except that in the chromatographic method, the amount of vitamin C was higher than the amount electrochemically determined. This aspect may be due to matrix effect that occurs in the electrochemical determinations with acid solution used for obtaining of the extracts.

Taking into account both analysis methods used, it was found that in the case of plants grown in a microwave field increased the amount of vitamin C. The highest amount of vitamin C was obtained in the case of irradiation with a generator working in the GSM domain, followed by the amount vitamin C found in plants grown in microwave field in the WLAN operating frequency (Fig. 5).

After all the determinations performed, it was observed that in the case of celery irradiated with GSM microwaves the amount of vitamin C increased (211% compared to reference). This aspect could be due to the fact that the surfaces of the leaves are the largest from all the plants studied, so it can absorb a greatest amount of electromagnetic radiation. The lowest increasing of the vitamin C amount was observed it the case of parsley irradiated with WLAN frequency microwave (8% compared to reference).

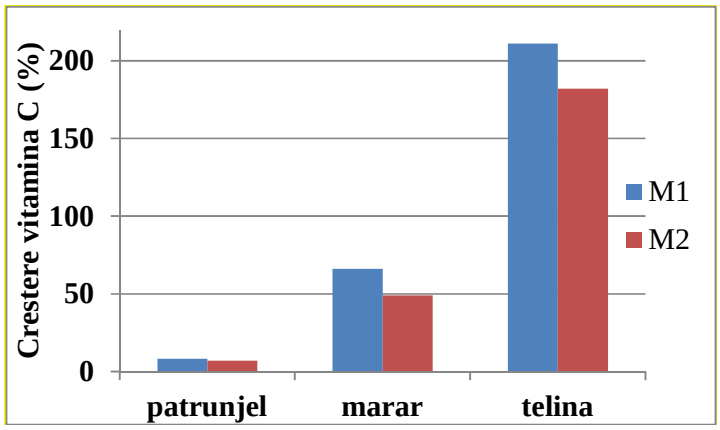


Fig. 5. The increasing amounts of vitamin C in the irradiated plants compared to those of references

2.1.3. Ultrastructural and morphological analysis of studied plants

The investigations were performed in an electronic microscope of transmission type TEM, Jeol 1010, equipped with a CCD Camera. The analysis was performed on samples of the studied plants, properly processed by fixation in glutaraldehyde and osmic acid, permeated and included in an epoxy resin type Epon 812, cut with a diamond cutter type Leica UC6 at one ultramicrotome, sections which were contrasted with U and Pb atoms (solution of uranyl acetate and lead citrate). All procedures were performed in accordance with conventional techniques and classic methodologies used for transmission electron microscopy analyzes.

Experimental biological material consisted of plant parsley, celery and dill grown, in parallel, in two different rooms, reference plants, untreated plants, and plants subjected to irradiation. The irradiation was performed on separate batch, neither with microwave equivalent to GSM installations, or wireless microwave. For the analysis were excised plant leaves.

The experimental batch:

- R - reference parsley leaves grown in the room with the same environmental conditions (PR).

- M1 - plants irradiated with GSM microwaves (PM1);

- M2 - plants irradiated with wireless microwaves (PM2).

The same experimental model was applied also for celery (TR and TM1, TM2) and dill (MR and MM1).

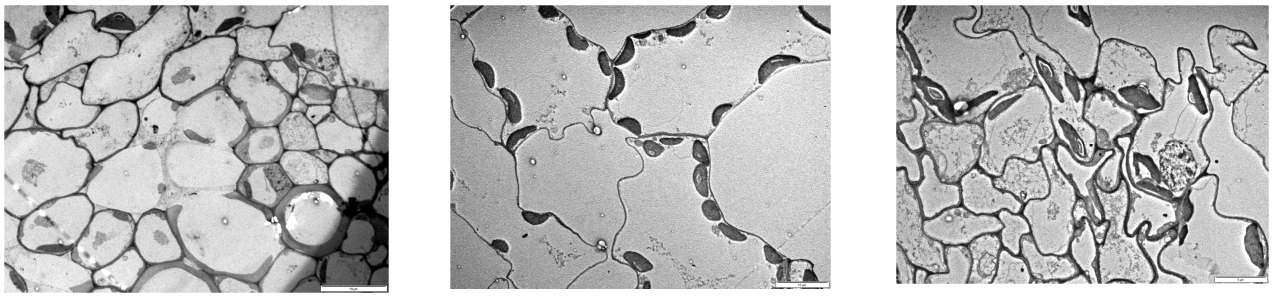
For ultrastructural analysis of leaves collected from all plants from experimental batch, it was intended to investigate the main components of the leaf lamina, respectively: upper epidermis covered with cuticle, palisade parenchyma cells where are present most of the chloroplasts, mesophilic tissue found in the middle of the leaf lamina, where liberian and woody vessels are stationed, lacunars tissue with cells thin arranged and having a few chloroplasts, and lower epidermis.

Among the cellular components the photosintetisating organelles were mainly aimed, chloroplasts, providing energy organelles, mitochondria and the nucleus as a coordinator of cellular metabolism.

Analysis of the images obtained from the plants leaves irradiated with GSM frequency and power compared with the reference batch images, presents the following ultrastructural characteristics:

- Upper epidermis is unchanged;

- Palisade parenchyma cells and mesophillic cells have cell walls (cellulose), slightly waved (Fig. 6b), compared with those of the reference, where they were straight (Fig. 6a), which means a slight alteration of the spatial arrangement of cells in the leaf lamina, signification of a slight decrease in turgidity of leaf lamina.



a b c

Fig. 6 The images of cell walls for a) PR; b) PM1; c) PM2

- Chloroplasts retained their ultrastructure and normal arrangement within cells, but the presence of starch grains is evident, suggesting a slight increase in their synthesis.

- Mitochondria have electron densified matrix and the mitochondrial cristae slightly rarefied, suggesting a slight decrease in their metabolic activity.

- The nuclei of most cells have normal structure, but with a greater presence of heterochromatin, or the outline is presenting waves. In some of the cases appeared the nuclei with altered structure.

In conclusion, the GSM microwaves with intensity used in the experiment induced relatively slight changes in the ultrastructure of leaf parsley, highlighted at the cell walls, mitochondria, chloroplasts and especially on the nuclei of cells, without significantly influence of the general metabolic activities of the leaf. Perhaps that the action of microwaves on a longer duration or higher intensities, the early negative effects observed above, will be enhanced and can induce higher alterations.

The analysis of the images obtained on the batch irradiated with microwave of WLAN intensity and power highlights that early negative effects presented in batch M1, now appeared intensified and with obvious deterioration as follows:

- The covering epidermis, both the upper and lower ends, thinned and became irregular;

- Because of the waves of the cell walls, palisade parenchyma cells lost the characteristic arrangement of cylindrical cells closely stitched with the rectilinear walls. The mesophillic cells also had waved walls, which means loss of cell turgidity, conducting to wilting of the leaves;

- The chloroplasts were fewer, most did not have the classic form of horn and all had one starch grains, suggesting the accumulation of energy reserves as a protection against stress caused by wireless microwave of high frequency;

- Mitochondria had the matrix and crystae rarefied, showing signs of deterioration.

- The nuclei had apparently normal structure, but with more heterochromatin like normal, and easy irregular contour. In some cells the nuclei were with altered structure, without an evidence distinction of eucromatine and heterochromatin of the cariolimph.

Similar results were obtained for all the plants studied.

3. The investigation of the microwaves effect on the essential oils present in the aromatic studied plants

3.1. The evaluation of essential oils composition and morphological changes from the irradiated plants in comparison with the references plants

3.1.1. The cultivation and growth of a new batch of plants

3.1.2. Obtaining the essential oil extracts from the irradiated and reference plants

In this stage, new batches of parsley, celery and dill were grown both in classic conditions, reference, and in microwaves field, as it was described in the previous reports. Three weeks after sowing, the plants were placed into four identical anechoic chambers, two reference chambers **(R)** and two chambers used for microwaves action in two domains: **1)** GSM, using a generator in the domain 860 – 910 MHz, output total power 30 dBm (1000 mWatt) **(M1)**; **2) WLAN** (wireless internet connection) using a generator router 802.11g, with operating frequency in the domain 2.4 – 2.49 GHz, with main operating channel at 2.42 GHz, output total power 18 dBm ($10^{1.8}$ mWatt) **(M2)**. In these enclosures, the plats were kept 3 weeks, after that the leaves being excised, obtained the extracts and analyzes both by HPLC and the electrochemical methods.

The first step was set the most effective method of essential oils extraction from studied plants. Thus, for the extraction of essential oils from both categories of plants, irradiated and non-irradiated plants, was used the sonication method of extraction, using a mixture of diethyl ether: n-hexane (1:1, v/v) as extraction solvent. The extraction by sonication with solvent (UAE) was carried out in two stages, using an ultrasonic bath Transsonic T 310 at 35 kHz and installed power of 95W. After 30 min of sonication in 4 mL solvent, the extract was separated. Finally, the sample was filtered and the residue washed. The extracts were combined and the final volume was 4 mL.

3.1.3. The qualitative determination of the essential oils from the obtained extracts by the analysis techniques GC and HPLC

The extracts were analyzed both by GC-MS and HPLC. The GC-MS analyses were performed with a Shimadzu QP2010 Plus chromatograph coupled with quadrupole mass spectrometer. The analysis conditions were: injector temperature: 215°C, initial oven temperature at 40°C was held for 1 min; ramped at 5°C min^{-1} up to 200°C, held at this temperature for 1 min; ramped at 10°C min^{-1} up to 200°C, held at this temperature 1 min, ramped at 10°C min^{-1} up to 220°C and held for further 5 min. Helium was employed as carrier gas with a constant flow rate of 1 mL/min. The mass spectrometer was operated in electron-impact mode (EI) at 70 eV, in the scan range *m/z* 30–400; the transfer line temperature was set at 240°C and ion-source temperature at 150°C.

Based on the registered chromatograms, were detected 10 componds in *Petroselinum crispum*, 11 compounds in *Anethum graveolens* and 7 compounds in *Apium graveolens*. In all species, the monoterpenes constituted the most significant constituent of essential oils. In addition, several specific benzenoids were also dominating components of the oil apiol in parsley, myristicin and dillapiole in dill. Lipoxygenase pathway compounds were important constituents in essential oils in celery: 3-hexen-1-ol, myrcene, α-ocimene, γ-terpinene (Fig. 7).

In our study, it was considered that microwave irradiation by GSM-frequency generally increased the essential oil contents, while the effect of WLAN-frequency microwaves was less clear, varying from positive or negative for different compounds and species (Fig.7). Comparing the all three plants species selected for the study, the strongest effects of microwaves irradiations were observed in the case of essential oils from *Anethum graveolens*.

For individual compounds in *P. crispum*, the microwave irradiation produced by GSM generator statistically increased the hexen-1-ol, myrcene, α phellandrene, β -phellandrene, myristicin and apiole contents. Compared to the reference, the strongest increase in response to GSM-frequency irradiation was observed for apiole (more than seven times greater content).The WLAN-frequency microwaves statistically increased the content of α-pinene, β-phellandrene, myristicin and apiol in this species (Fig. 7a).

In *Anethum graveolens* irradiated with GSM microwaves, increased content was observed for β-pinene, α–phellandrene and dillapiole (Fig. 7b). However, WLAN-frequency microwaves reduced α-phellandrene, myristicin and dillapiole content, whereas the greatest reduction was observed for myristicin (approximately to the level 18% of that in reference plants, Fig. 7b).

In *Apium graveolens*, both types of microwaves used in this study increased 3-hexen-1-ol content (Fig. 7c). Irradiation by WLAN frequency microwaves reduced myrcene (19%) and α-ocimene (21%) contents (Fig. 7c).

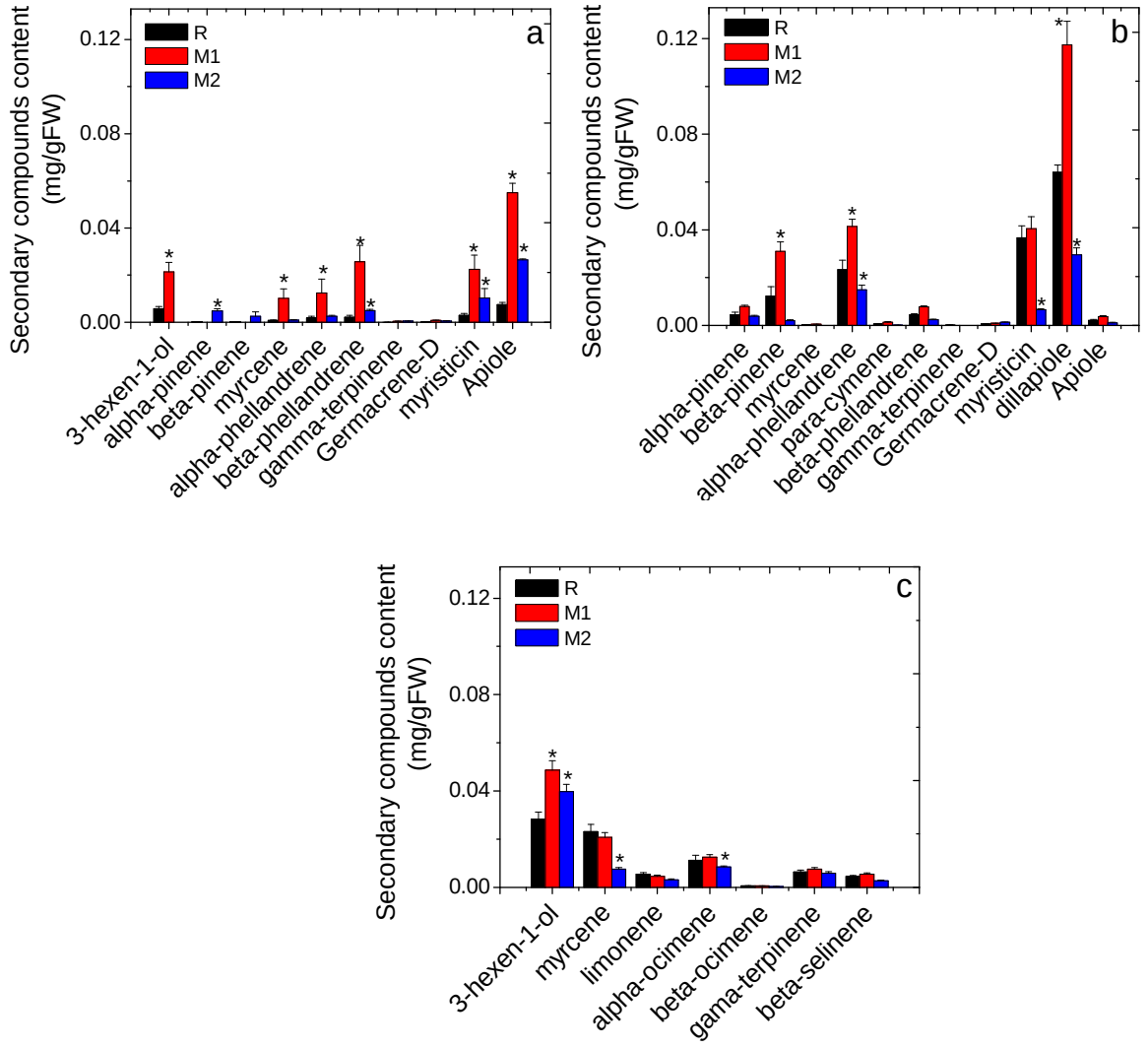


Fig. 7. Changes in terpene content (mg g^{-1} FW) *in Petroselinum crispum (a), Anethum graveolens subsp. hortorum* (b) and *Apium* graveolens (c) foliage in response to microwave irradiations in GSM and WLAN domain

The volatile oil analysis by HPLC method was performed with a Shimadzu chromatograph with DAD detector and LiChrosorb RP-18 (5µm, 25x0.4 cm) column thermostated at la 25°C. The eluents consisted of ultrapure water (A) and acetonitrile (B). The program of gradient elution started from 0 to 1 min with 100%

B and then the eluent B decreased in 15 min to 25%. The flow rate was set at 1 mL min^{-1}. The peaks corresponding to the studied compounds showed maximum absorption at 197 nm for linalool and 201 nm for myristicin. For these two compounds were drown the calibration curves and were determinate the limits of detection and quantification. The correlation coefficients were closed to 1 (0.9993 for myristicin and 0.9991 for linalool), the linearity being very good. The minimum concentration levels at which the compounds can be reliably detected (LOD) and quantified (LOQ) were found to be 32.68 and 64.57 µg mL^{-1}, respectively, for myristicin, and 6.33 and 12.50 µg mL^{-1}, respectively, for linalool.

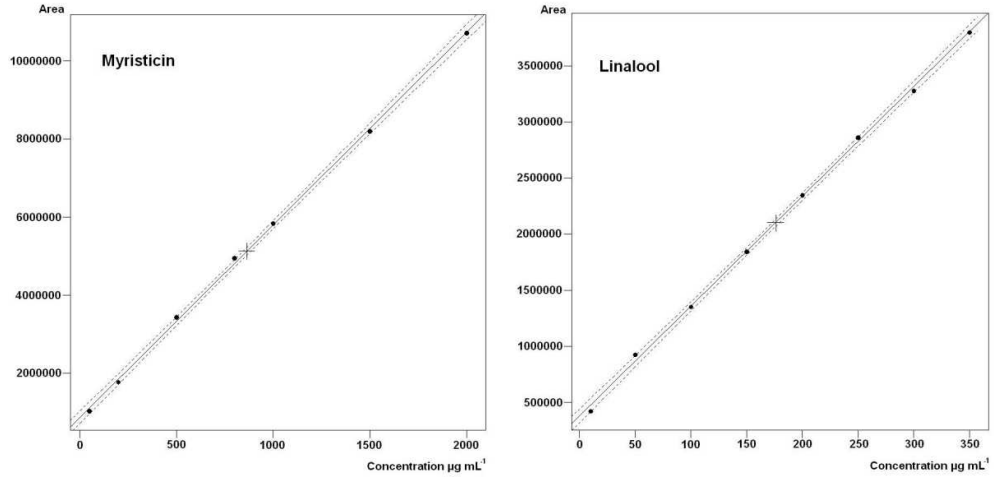


Fig. 8. The calibration curves for myristicin and linalool.

Myristicin was determined in two of the aromatic plants dill, and parsley. In parsley, the amount of myristicin decreased by 26% in the plants irradiated with GSM microwaves, and by 52% in the plants irradiated with WLAN microwaves, compared to control plants (Fig. 9).

Using the same HPLC method, linalool was determined in all three plants studied (Fig. 9). From a quantitative point of view the amount of linalool from plants was much higher than the amount of myristicin. In dill and celery plants, the amount of linalool suffered slight increases after applying microwave irradiation (up to 6%). Between the plants irradiated with GSM microwaves and those irradiated with WLAN microwaves did not appeared differences (Fig. 9).

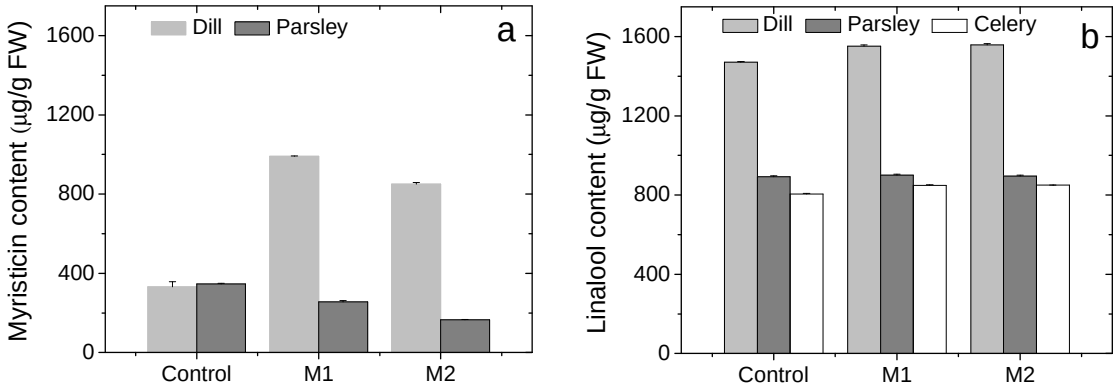


Fig. 9 Myristicin (a) and linalool (b) content (µg/g FW) in aromatic plants irradiated with GSM (M1) and WLAN (M2) microwaves

3.1.4. Ultrastructural and morphological analysis applied to a new batch of studied plants and the comparison of the obtained results

The results obtained in the last stage were in good concordance with those presented in the previous scientific report. The ultrastructural and morphological analyses suggested that the irradiation with WLAN microwaves conducted to a more powerful stress of the plants, compared to GSM microwaves,

but the effect of WLAN on volatile oils was inhibitory. There was an agreement between anatomical and chemical traits with anatomically most resistant species *Apium graveolens* being chemically least responsive. The data obtained from the ultrastructural analysis are presented in Table 1.

Table 1. Ultrastructural analysis of the studied leaves.

Growth condition	Cell wall thickness (µm)	Chloroplast length (µm)	Chloroplast area (μm^2)	Mitochondrion length (µm)	Ration of starch grain area to chloroplast aerea (%)
			Petroselinum crispum		
Control	0.300 ± 0.07	6.78 ± 0.12	13.31 ± 0.22	1.00 ± 0.27	8.93 ± 0.13
GSM	0.250 ± 0.06	6.76 ± 0.28	8.491 ± 0.06	0.90 ± 0.13	9.99 ± 0.12
WLAN	0.200 ± 0.05	6.50 ± 0.16	7.807 ± 0.11	0.70 ± 0.05	6.01 ± 0.08
			Anethum graveolens		
Control	0.187 ± 0.01	5.90 ± 0.13	8.43 ± 0.23	1.68 ± 0.13	5.21 ± 0.13
GSM	0.175 ± 0.01	5.20 ± 0.17	8.08 ± 0.29	1.00 ± 0.25	8.13 ± 0.08
WLAN	0.175 ± 0.01	4.85 ± 0.31	7.04 ± 0.22	0.80 ± 0.10	0
			Apium graveolens		
Control	0.160 ± 0.01	5.80 ± 0.20	7.68 ± 0.14	1.57 ± 0.08	5.34 ± 0.28
GSM	0.156 ± 0.01	4.33 ± 0.26	7.06 ± 0.35	0.55 ± 0.13	0
WLAN	0.136 ± 0.01	3.33 ± 0.26	6.68 ± 0.14	0.25 ± 0.13	0

±SE for 6 successive measurements

4. Investigation of microwave effect on BVOC captured from respiration of the studied plants; identification of the chemical changes from the stressed plants

4.1. Investigating the changes in the composition of volatile organic compounds (BVOC) from plants stressed by a microwave field of different frequencies and powers

4.2. Monitoring the changes occurred in BVOC emitted from irradiated plants compared to reference plants

VOC sampling was performed using a portable gas exchange system GFS-3000 (Waltz GmbH, Effeltrich, Germany). The system has an environment-controlled cuvette with 8 cm2 window area and multiple leaves were enclosed in the cuvette to fill the whole cuvette window. A volume of 4 L of air exiting the cuvette was sampled in a multibed stainless steel cartridge (8.88 cm × 0.65 cm, Supelco, Bellefonte, PA, USA) filled with Carbopack adsorbents (C20/40 mesh, C 40/60 mesh, and X 20/40 mesh). The chamber air was sampled at a flow rate of 200 mL min^{-1} for 20 min using a 1003SKC constant flow sampling pump (SKC Inc., Houston, TX, USA) at room temperature. Background air samples were taken before and after the measurements using the same system without the leaves enclosed in the cuvette.

For VOC analysis, an automated cartridge desorber Shimadzu TD20 (Kyoto, Japan) and a Shimadzu QP2010 Plus gas chromatograph coupled with quadrupole mass spectrometer (GC–MS) (Kyoto, Japan) were used.

For the analysis of volatile organic compounds the initial oven temperature was set at 40°C and held for 1 min; the heating was performed in two stages: first with 5°C min^{-1} up to 200°C, held at this temperature for 1 min, and then with 10°C min^{-1} up to 200°C, held at this temperature 5 min. Helium was employed as carrier gas (purity 99,9999 %, Elmer Messer Gaas AS, Tallinn, Estonia), with a constant flow

rate of 1 mL min^{-1}. The mass spectrometer was operated in electron-impact mode (EI) at 70 eV, in the scan range *m/z* 30–400, the transfer line temperature was set at 240°C and ion-source temperature at 150°C.

The essential oils and VOC were identified by comparing the mass spectra of individual compounds with the spectra of GC purity external standards (Sigma–Aldrich, St. Louis, MO, USA) and with the spectra library.

Amount of VOC released from control and microwaves irradiated plants (parsley, dill and celery), were expressed as Φ (nmol/m^2 s), the number of nmol of volatile substance emitted on an area of 1 m^2 plant in interval of 1 second (s).

Our data demonstrate that the emissions observed did reflect a mixture of both storage emission consisting of compounds present in essential oils and de novo emissions. The blend of volatiles was very complex and, in all plant species, the non-stressed plants also emitted monoterpenes and benzenoids present in essential oils, in some cases even compounds not present in essential oils (Fig. 4).The number of compounds detected in the emissions was greater than in the essential oils, and characteristic de novo released stress volatiles were observed (Fig. 11). 16 compounds were detected in the emissions of *Petroselinum crispum*, 16 compounds in *Anethum graveolensand* 20 compounds in *Apium graveolens*.

There was evidence of similar enhancement of essential oils and emissions for several monoterpenes (Fig. 10), especially for GSM microwave treatments in *Petroselinum crispum and Anethum graveolens.* However, in these species, emissions were more strongly enhanced under WLAN microwave treatment, which appeared to have an inhibitory effect on the content of the same terpenoids, e.g. α -pinene and β -phellandrene (Fig. 10).

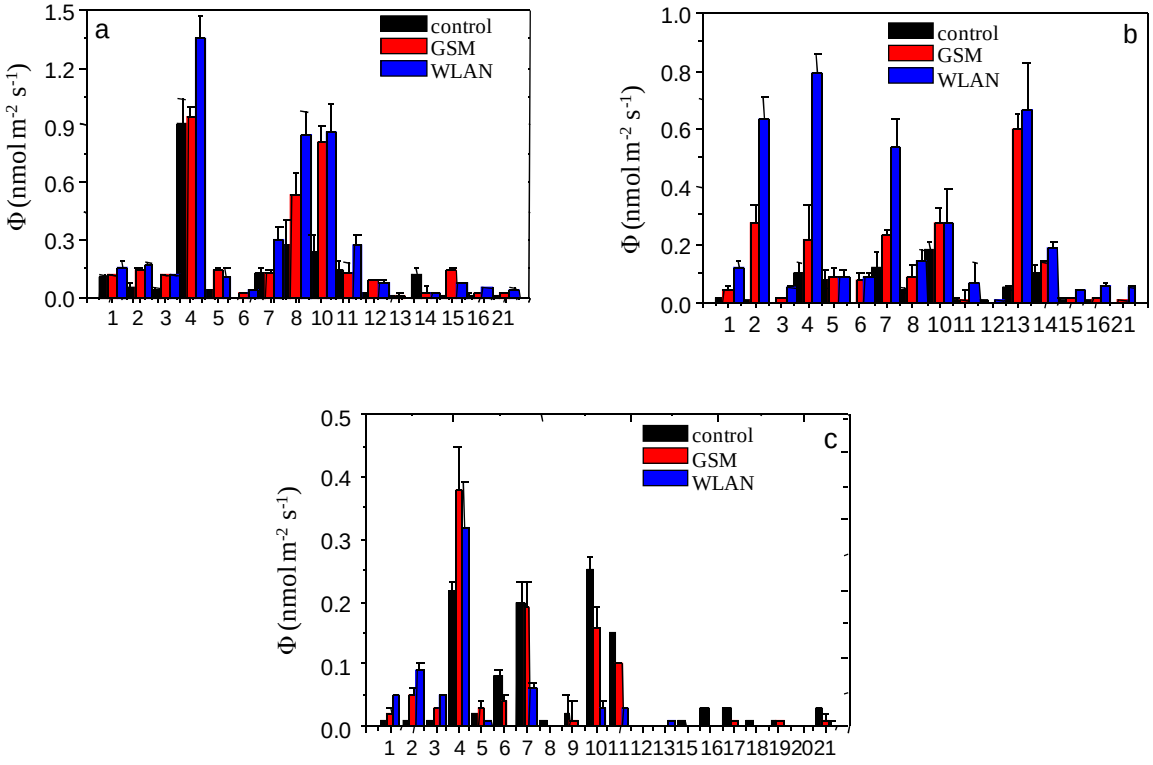


Fig. 10. Alteration of the emission of volatile organic compounds (nmol $m^{-2}s^{-1}$) from foliage of *Petroselinum crispum* (a), *Anethum graveolens subsp. hortorum* (b)and *Apium graveolens* (c) in response to microwave irradiations at bands corresponding to wireless router (WLAN) and mobile devices (GSM). Each number corresponds to a particular volatile compound as follows: **(1)** 1-hexanol; **(2)** (Z)-3-hexen-1-ol; **(3)**(E)-2-hexenal; **(4)** α-pinene; **(5)** camphene; **(6)** β-myrcene; **(7)** β-pinene; **(8)** α-phellandrene; **(9)** Δ-3-carene; **(10)** D-limonene; **(11)** para-cymene; **(12)** β-phellandrene; **(13)** (E)-β-ocimene; **(14)** 1,8-cineol; **(15)**

iso-bornyl acetate; **(16)** longicyclene; **(17)** caryophyllene oxide; **(18)** α-selinene; **(19)** (Z)-β-farnesene; **(20)** α-caryophyllene; **(21)** geranylacetone.

Among the de novo emissions, GLV, also called volatiles of lipoxygenase pathway (LOX volatiles) are released in plants in response to different stresses. GLVs are formed in the hydroperoxidelyase pathway of oxylipin metabolism from free octadecanoic fatty acids and consist usually of a mixture of C6 aldehydes and ketones.

In our study, all microwave-irradiated plants emit-ted the following GLVs: (E)-2-hexenal, (Z)-3-hexenol, 1-hexanol, while the emissions of GLVs were very low at the level of detection limit of our device in control plants (Fig. 10).

In general, in all plant species studied the emissions of GLV were greater for WLAN-frequency microwaves compared to GSM-frequency microwaves (Fig. 1, $P < 0.001$ for all). These results suggest greater stress in the case of WLAN microwave irradiation, and are in agreement with the more significant changes in anatomy of leaves induced by WLAN microwaves. Stronger GLV emissions under more severe stress have been shown for water [5], ozone [6], herbivory attack and temperature stresses.

The GLV emissions of the *Petroselinum crispum* and *Anethum graveolenswere* dominated by the 1-hexanol (Fig. 10), while in *Apium graveolensthe* main component was (Z)-3-hexenol that was also important constituent in the essential oil in this species (Fig. 10). The total GLV emission from *Petroselinum crispum* and *Anethum graveolens* was five times higher than from *Apium graveolens*.

The monoterpenes detected in the emissions were α-pinene, β-pinene, camphene, limonene, 3-carene, para-cymene, β-phellandrene, (E)-β-ocimene, eucalyptol and bornyl acetate. In *Petroselinum crispum*, emission of α-pinene, β-pinene and β-phellandrene were dominant and enhanced by microwave irradiation, especially in the case of WLAN-frequency microwave treatment (Fig. 10a).

Treatment effects on monoterpene emissions were similar for *Apium graveolens* and *Anethum graveolens*, but the main components are at some extent different (Figs. 10b and 10a). Monoterpene emissions from *Anethum graveolens* were dominated by α-pinene, β-phellandrene and limonene, and these emissions were enhanced by microwave irradiation (Fig. 4b).

In *Apium graveolens*, the emissions were almost four times lower than in the other species and were dominated by α-pinene, β-pinene and limonene (Fig. 10c). The emission of terpenes was inhibited by microwave irradiation similarly to the content of essential oils (Fig. 10).

Overall, these emitted monoterpenes are characteristic plant-released compounds and are not specific to stress-induced missions. However, the emission rates of these typical monoterpenes is also often enhanced in stress conditions, implying that induced and constitutive emission are often difficult to separate.

Among the characteristic induced monoterpenes, emissions of (E)-β-ocimene and 1,8-cineole were strongly enhanced by microwave-irradiation in *Anethum graveolens* (Fig. 10). In addition, both *Petroselinum crispum* and *Anethum graveolens*, emitted in low amounts longicyclene, a stress induced sesquiterpene, under WLAN-frequency irradiation.

4.3. Conclusions regarding the chemical and structural changes appeared in the irradiated plants

The project main objective was to determine the effect of microwave in GSM frequency (mobile), respectively, WLAN (wireless), on the volatile oils, vitamin C and secondary metabolites contained in three aromatic plants belonging to the family *Apiaceae: Petroselinum crispum* (parsley), *Anethum graveolens* (dill), and *Apium graveolens* (celery).

After the performed studies were established the following conclusions:

• Depending on the solvent and the extraction technique used, were extracted different classes of bioactive compounds in different quantities;

• The most effective method for volatile oils extraction from parsley, dill and celery was found to be sonication with the solvent system n-hexane - diethyl ether (1: 1, v/v);

• The extraction efficiency of L-ascorbic acid was carried out by sonication with an aqueous solution of 8% acetic acid;

These experimental conditions were subsequently used to analyze the irradiated and non-irradiated plants with microwaves.

• It was determined that changing the conditions of plant growth by introducing them in a microwave field with different intensities (GSM and wireless), influenced both plant development and content of biologically active compounds investigated.

• Based on HPLC analysis, it was found that the highest increase t of L-ascorbic acid amount was recorded in the case of celery irradiated with GSM microwaves (211%), and the smallest increase in the amount of the same compound was registered in the case of parsley irradiated with WLAN microwaves (6.8%).

• Analysis of volatile oils, performed by GC-MS, showed that their content increased in plants irradiated with microwaves from GSM frequency, while WLAN frequency microwave effect was variable, depending on the plant analyzed. The strongest effects of the microwaves were observed in essential oils from dill.

• The total amount of volatile organic compounds (captured from plant respiration) released by parsley and dill due as a results of microwaves irradiation, was considerably higher than in celery, because as it was observed, in the case of this plant volatile oils, celery was less sensitive to microwaves than parsley and dill.

• All three plants species irradiated with microwaves emitted volatile compunds E)-2-hexenal, (Z)-3-hexenol, 1-hexanol in important cantities, while the control plants had lower level of emitted volatile organic compounds. It was established that the emissions of (E)-β-ocimene and 1,8-cineole were strongly enhanced by microwave-irradiation in dill. Both parsley and dill plants emitted in low amounts longicyclene, a stress induced sesquiterpene, under WLAN-frequency irradiation.

• Ultrastructural analysis (TEM) of the plants leaves from the experimental batch was aimed to investigate the main components of the leaf lamina (upper epidermis, palisade parenchyma tissue mesophilic, lacunar tissue, lower epidermis), and the main component of cellular phototintetisating organelles (chloroplasts), providing energy organelles (mitochondria) and coordinator of cell metabolism (nucleus). Comparing the effects of the two types of microwave actions on plant leaf cells ultrastructure, it was concluded that WLAN microwaves induced negative changes significantly higher than those founded when were applied GSM microwaves. Celery was the most anatomically resistant species to microwaves, while dill was the most affected species.