SYNTHETIC ACTIVITY REPORT 2018

for the implementation of the Postdoctoral Project

PN-III-P1-1.1-PD-2016-0484

"*Assessing the influence of nonsteroidal anti-inflammatory drugs on physiological characteristics and secondary metabolites in autochthonous vegetables*"

Stage 1. Growth of the autochthonous vegetables under stress conditions induced by NSAIDs; evaluation of their influences on certain photosynthetic parameters and emission of the volatile organic compounds

(May – December 2018)

Summary of Stage 1

Content of the scientific and technical report (RST)

Understanding the effects of many essential non-steroidal anti-inflammatory drugs (NSAIDs) on plants is still limited, especially at environmentally realistic concentrations. Thus, the first stage of the project (May – December 2018) included research activities regarding the influence of three most frequently used NSAIDs (diclofenac, ibuprofen and naproxen) at environmentally realistic concentrations on the autochthonous green leafy vegetables orache (*Atriplex patula* L.), spinach (*Spinacia oleracea* L.) and lettuce (*Lactuca sativa* L.). The research activities were focused on the determination of the photosynthetic parameters, the emission rate of volatile organic compounds (VOCs), and the ultrastructure evaluation of the studied vegetables after exposure to abiotic stress-induced by environmental pollutants, namely NSAIDs. Regarding the study of the effects of NSAIDs on photosynthesis, three photosynthetic parameters were analyzed the net assimilation rate (A), the stomatal conductance to water vapor (gs), and the intercellular CO_2 concentration (Ci). In order to assess the influence of the selected NSAIDs on the cellular organelles of green leafy vegetables, the ultrastructural analyses of the photosynthetic organelles (chloroplasts), energy supply organelles (mitochondria) and a cellular metabolism coordinator (nucleus), were performed.

Seeds of the selected vegetables were sown at a depth of 1 cm in plastic pots containing commercial garden soil. The selected green leafy vegetables were grown under controlled light conditions (for 12 h from 24 h) and a day/night temperature cycle of 25/18 °C. The abiotic stress to which the selected green leafy vegetables were subjected consisted of watering them once every two days with aqueous solutions of diclofenac, ibuprofen, and naproxen, respectively, at concentrations of 0.1 mg L^{-1}, 0.5 mg L^{-1} and 1 mg L^{-1}. Control vegetables were untreated plants that were watered using the same volume of distilled water. The experimental measurements were performed at two different times of the experiments: four weeks (set I) and eight weeks (set II) from the emergence of the green leafy vegetables.

The determination of photosynthetic parameters was carried out using a portable gas exchange system GFS-3000 (Walz). This system is provided with a cuvette that has a window with an area of 8 cm^2. The parameters that have been set for all measurements were: airflow in the cuvette (750 μmol s^{-1}), CO_2 (400 ppm), PARtop light mode (1000 mol m^{-2} s^{-1}), temperature (25 °C) and relative humidity (65%). The vegetable leaf was placed in the cuvette so that the entire surface of the cuvette window was covered. After the closure of the cuvette, the leaves were

subjected to light intensity, and within a few minutes, the stomata were opened, resulting in stable values of the A, gs, and Ci.

To get a complete insight into the impact of the three chosen NSAIDs on the selected vegetables, VOCs emission was also analyzed. Under stress conditions induced by the three NSAIDs, the selected vegetables emitted the oxidation product under the action of lipoxygenase (LOX: 3-hexenal) and the monoterpenes α-pinene, limonene, and β-ocimene. The sampling of VOCs was performed in parallel with the photosynthetic parameter measurements. Once stable photosynthetic parameters have been obtained from the cuvette of the system, a 4 L volume of air was passed into a steel cartridge containing an adsorbent optimized for quantitative analysis of LOX and monoterpenes. The VOCs that were captured in the steel cartridges were desorbed using a thermo-desorption system (Shimadzu TD20) and injected into a gas chromatograph coupled with a mass spectrometer (Shimadzu 2010 plus, GCMSTQ8040).

In order to determine the modifications induced in the leaves of the green leafy vegetables treated with the three selected NSAIDs during the growth period, light microscopy (LM), scanning and transmission electron microscopy (S/TEM) analyses were performed. The leaves collected at the same moment had approximately the same size and maturity. The microscopic analyses were performed with samples of the selected green leafy vegetables treated with the three NSAIDs (1 mg L^{-1}) and control samples (untreated). A number of 564 photos were recorded, of which 187 were most suggestive for highlighting the similarities and/or differences between the green leafy vegetables treated with NSAIDs and the controls (untreated).

The experimental results obtained during this stage of the project showed that the key environmental pollutants, NSAIDs, affect the photosynthesis and influence the metabolic pathways of the green leafy vegetables *A. patula*, *S. oleracea,* and *L. sativa*. The VOCs emission increase with increasing the concentration of NSAIDs and increasing the volume of NSAIDs solution used for watering the vegetables. VOCs emission rates were the most sensitive indicator of the stress induced by the NSAIDs to green leafy vegetables (Fig. 1). These increases can be employed as a stress signal of the plants or as a biomarker.

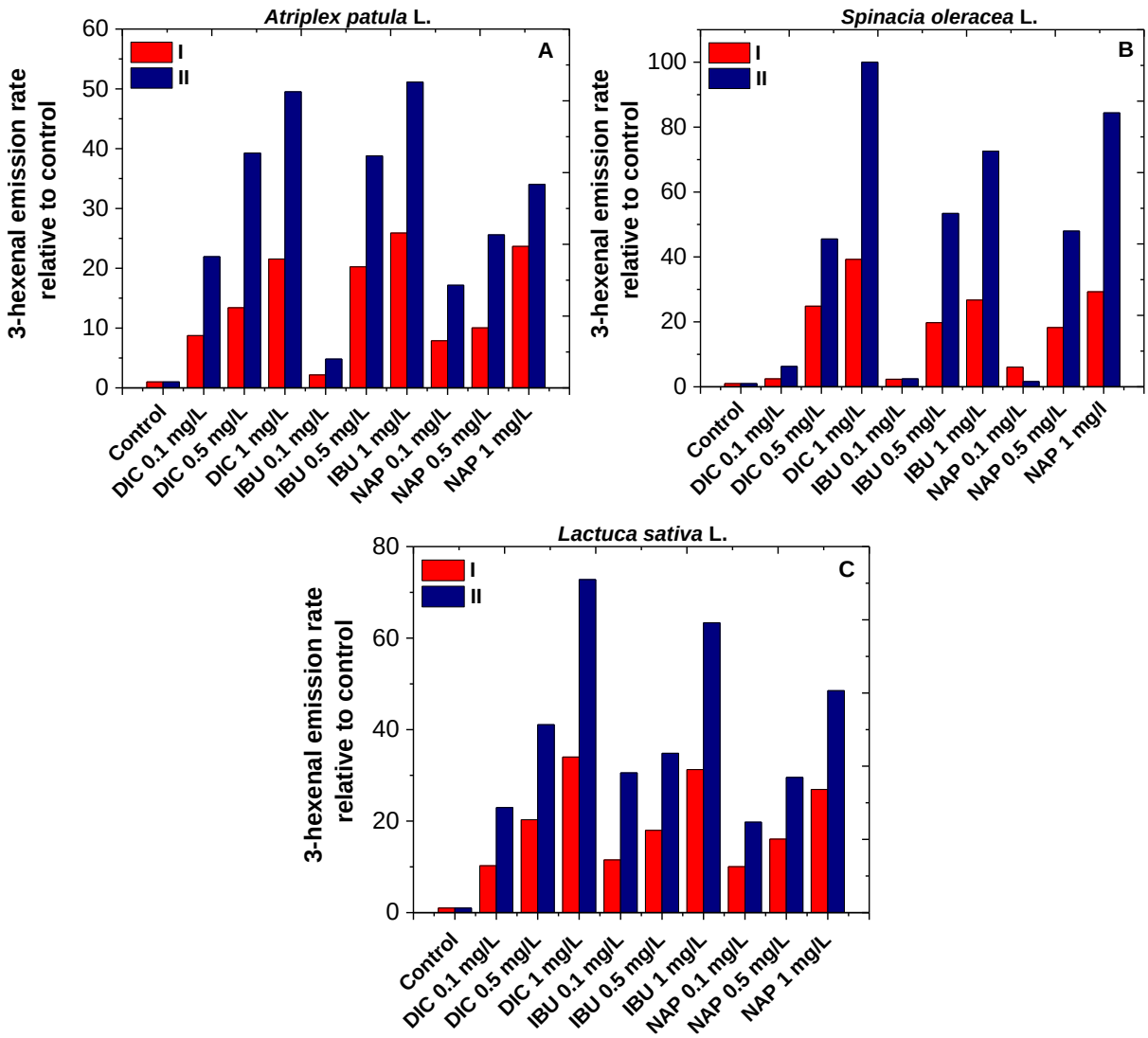

Fig. 1. Emissions of lipoxygenase pathway product (3-hexenal) from *A. patula* (A), *S. oleracea* (B), and *L. sativa* (C) green leafy vegetables (four and eight weeks old) treated with solutions of diclofenac (DIC), ibuprofen (IBU), and naproxen (NAP), concentration of 0.1, 0.5, and 1 mg L^{-1}.

Also, structural changes were observed in the vegetables treated with NSAIDs. Microscopic analysis showed that *A. patula* leaves were most affected by naproxen, and *L. sativa* and *S. oleracea* by ibuprofen, and none of them showed significant modifications induced by diclofenac. All leaves had the chloroplasts affected, which may suggest that these drugs induce a harmful effect on the vegetables. The experimental results obtained contribute to the understanding of the influence of drugs on plants/vegetables with nutritional importance in the nutrition of humans.

The results obtained during this stage of the project are:

a) **experimental model** regarding the growth of the green leafy vegetables treated with NSAIDs;
b) **study** on the influence of NSAIDs on photosynthesis and emission of volatile organic compounds from selected vegetables;
c) **study** regarding the impact of NSAIDs on the ultrastructure of green leafy vegetables;
d) **scientific and technical report** (RST);
e) **dissemination of the results**:
   - the **web page** (http://www.itim-cj.ro/PNCDI/ru12/index.html) of the project;
   - **four papers presented at international conferences**:
     1. *"Changes in foliage photosynthetic characteristics and structure of autochthonous vegetables upon nonsteroidal anti-inflammatory drugs exposure"*; The 17th International Symposium – Prospects for the 3rd Millennium Agriculture, September 27th – 29th, 2018, Cluj-Napoca, Romania; Authors: **Ocsana OPRIŞ**, Maria L. SORAN, Ildikó LUNG, Alexandra CIORÎŢĂ, Lucian COPOLOVICI; poster presentation;
     2. "*The influence of nonsteroidal anti-inflammatory drugs on photosynthesis and leaf anatomy of Atriplex patula L.*"; The 17th International Symposium – Prospects for the 3rd Millennium Agriculture, September 27th – 29th, 2018, Cluj-Napoca, Romania; Authors: **Ocsana OPRIŞ**, Ildikó LUNG, Maria L. SORAN, Alexandra CIORÎŢĂ, Lucian COPOLOVICI; poster presentation;
     3. "*Toxic influence of environmental pollutants on green leafy vegetables*"; The 4th International Conference "Modern Technologies in the Food Industry" MTFI – 2018, October 18th – 20th, 2018, Chişinău, Republic of Moldova; Authors: **Ocsana OPRIŞ**, Maria L. SORAN, Ildikó LUNG, Alexandra CIORÎŢĂ, Lucian COPOLOVICI; poster presentation;
     4. "*Chromatographic determination of different drugs from environment"*; Separation Sciences in the RChS Centenary, October 26th, 2018, Cluj-Napoca, Romania; Authors: **Ocsana OPRIŞ**, Maria L. SORAN, Ildikó LUNG, Lucian COPOLOVICI; oral presentation;

- **a scientific article** *(*"*Evaluation of the photosynthetic parameters, emission of volatile organic compounds and ultrastructure of common green leafy vegetables after exposure to non-steroidal anti-inflammatory drugs (NSAIDs))"*; Authors: **Ocsana OPRIŞ**, Alexandra CIORÎŢĂ, Maria-Loredana SORAN, Ildikó LUNG, Dana COPOLOVICI, Lucian COPOLOVICI), under review at the Ecotoxicology journal.

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