

SYNTHETIC ACTIVITY REPORT 2019

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 2. Evaluation the influence of the NSAIDs on non-volatile secondary metabolites and the antioxidant capacity of the selected vegetables

(January – December 2019)

Summary of Stage 2

Content of the scientific and technical report (RST)

Introduction

1. Quantitative evaluation of the photosynthetic pigments (chlorophylls and carotenoids), polyphenolic compounds (total flavonoids and total polyphenols), vitamin C, and determination of the antioxidant capacity of the studied vegetables

1.1. Extraction and analysis of photosynthetic pigments (chlorophylls and carotenoids) from the control and treated vegetables using HPLC-DAD/MS

1.2. Extraction and analysis of the total flavonoids and polyphenols from the control and treated vegetables using UV-Vis spectroscopy

1.3. Extraction and analysis of vitamin C from the control and treated vegetables using HPLC-DAD/MS

2. Data analysis and results dissemination

*2.1. Influence of NSAIDs on *Atriplex patula* L.*

*2.2. Influence of NSAIDs on *Spinacia oleracea* L.*

*2.3. Influence of NSAIDs on *Lactuca sativa* L.*

3. Conclusions

The global presence of pharmaceuticals in the environment has been mainly considered a concerning problem with unknown consequences. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs in the world, and as a result, they are commonly found in different environmental compartments.

The second stage of the project (January – December 2019) included research activities regarding the influence of abiotic stress resulted from diverse NSAIDs (diclofenac, ibuprofen and naproxen) on the composition of orache (*Atriplex patula* L.) spinach (*Spinacia oleracea* L.), and lettuce (*Lactuca sativa* L.), green leafy vegetables with important nutritional values. In this stage of the project was presented the content of the chlorophylls (*a + b*) and carotenoids (zeaxanthin, lutein, and *B*-carotene), total polyphenol and total flavonoid contents, and the antioxidant capacity of the selected green leafy vegetables exposed to NSAIDs.

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 stress factors were applied through watering of the selected green leafy vegetables with solutions of three the most used NSAIDs at different concentrations (0.1 mg L⁻¹, 0.5 mg L⁻¹ and 1 mg L⁻¹), corresponding to moderately high ones found in the environment. 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. We expected that NSAIDs to reduce the pigment and total polyphenol contents, the antioxidant capacity of the green leafy vegetables in a dose-dependent manner.

The pigments (chlorophylls and carotenoids) were extracted in ice-cold 100% acetone and analyzed using a high-performance liquid chromatograph (HPLC) equipped with a photodiode array detector (PAD) and mass spectrometer detector (MS). The total polyphenols and flavonoids were extracted in ethanol : ultrapure water (60 : 40, v/v) and spectrophotometrically analyzed. The antioxidant capacity was determined using DPPH (2,2'-diphenyl – picrylhydrazyl) method.

Exposure of the green leafy vegetables to NSAIDs resulted in a reduction of chlorophylls, carotenoids, polyphenols, flavonoids in a dose-dependent manner. From all photosynthetic pigments analyzed during this stage of the project, the chlorophyll content of the green leafy vegetables was the most affected by the selected drugs. In general, the chlorophylls content

decreased with the increase of the concentration of the drug used in the treatment of green leafy vegetables (Fig. 1). In the *A. patula* L. vegetables, the chlorophyll content decreased up to 57%, in *S. oleracea* up to 34%, and in *L. sativa* up to 42%, in comparison with the control ones. The lowest chlorophyll contents were recorded for ibuprofen and naproxen treatments.

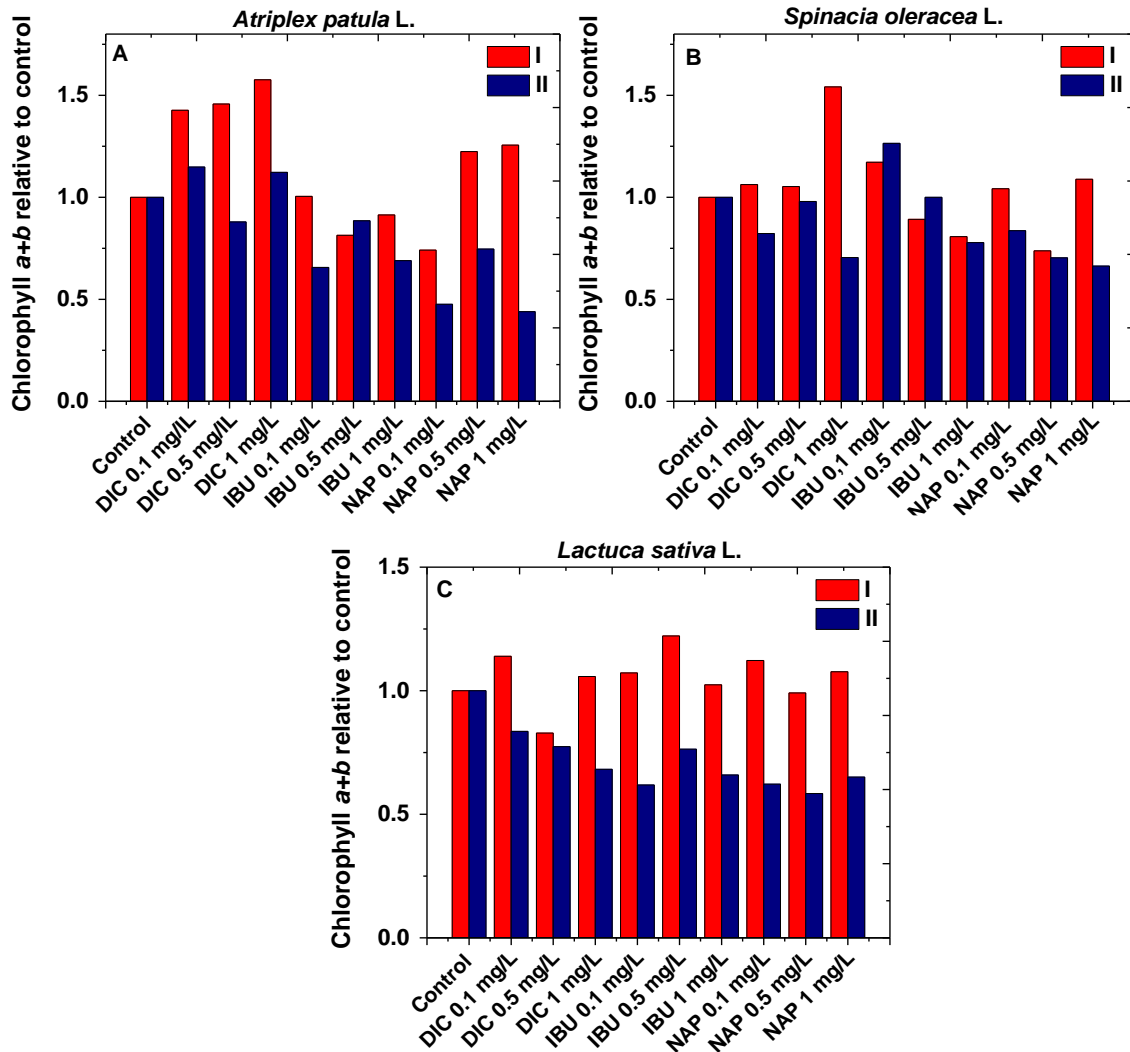


Fig. 1. The sum of chlorophyll *a* and *b* contents in *Atriplex patula* L. (A), *Spinacia oleracea* L. (B), and *Lactuca sativa* L. (C) green leafy vegetables (four and eight weeks old) treated with solutions of diclofenac (DIC), ibuprofen (IBU), and naproxen (NAP) of 0.1 mg L⁻¹, 0.5 mg L⁻¹, and 1 mg L⁻¹ concentration.

In the *A. patula* and *S. oleracea* vegetables treated with the selected drugs, lutein slightly decreased compared to the control. In the *A. patula* vegetables treated with the selected drugs, the

total polyphenol content decreased with increasing the concentration used for the treatments. The total flavonoid content of the *S. oleracea* and *L. sativa* vegetables was significantly lower than the control vegetables.

Excessive use of pharmaceutical products leads to their presence in the environment. Despite the beneficial properties and desired effects during therapeutic applications, the same properties of pharmaceuticals can be disadvantageous to the environment, causing adverse effects on microorganisms, plants, as well as potential risks to human health.

The results obtained during this stage of the project are:

- a) **study** regarding the impact of NSAIDs on non-volatile secondary metabolites from selected vegetable;
- b) **scientific and technical report (RST)**;
- c) **dissemination of the results**:
 - **update of the web page** (<http://www.itim-cj.ro/PNCIDI/ru12/index.html>) of the project;
 - **three papers presented at international conferences**:
 1. “*Changes in composition and ultrastructure of green leafy vegetables after exposure to non-steroidal anti-inflammatory drugs*” (Authors: **Ocsana OPRIȘ**, Alexandra CIORÎȚĂ, Maria Loredana SORAN, Ildikó LUNG, Lucian COPOLOVICI), 12th International Conference Processes in Isotopes and Molecules, September 25th–27th, 2019, Cluj-Napoca, Romania; poster presentation;
 2. “*Effects of non-steroidal anti-inflammatory drugs on the ultrastructure and composition of autochthonous vegetables*” (Authors: **Ocsana OPRIȘ**, Maria Loredana SORAN, Ildikó LUNG, Alexandra CIORÎȚĂ, Lucian COPOLOVICI), 4th Edition of Global Conference on Plant Science and Molecular Biology, September 19th–21st, 2019, London, United Kingdom; poster presentation;
 3. “*The influence of ibuprofen, diclofenac and naproxen on composition and ultrastructural characteristics of *Atriplex patula* and *Spinacia oleracea**” (Authors: **Ocsana OPRIȘ**, Ildikó LUNG, Maria Loredana SORAN, Alexandra CIORÎȚĂ, Lucian COPOLOVICI), ICABBBE 2019: XIII. International Conference on Agricultural, Biotechnology, Biological and Biosystems Engineering, October

21st-22nd, 2019, Athens, Grece; oral presentation for which it was obtained “**BEST PRESENTATION AWARD**”.

• **two scientific articles:**

1. “*Impact assessment of paracetamol on Phaseolus vulgaris L. and Triticum aestivum L. plants*” (Authors: **Ocsana OPRIȘ**, Ildikó LUNG, Maria-Loredana SORAN, Lucian COPOLOVICI, Dana Maria COPOLOVICI, Maria Clara COSTA); article accepted for publication in Revista de Chimie;
2. “*Investigating the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on the composition and ultrastructure of green leafy vegetables with important nutritional values*” (Authors: **Ocsana OPRIȘ**, Ildikó LUNG, Maria-Loredana SORAN, Alexandra CIORÎȚĂ, Lucian COPOLOVICI); article under review – Science of The Total Environment.

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