THE INFLUENCE OF FERTILISATION ON THE ORNAMENTAL CHARACTERS AND ON THE ALANTOLACTONE AND TRITERPENIC ACIDS PROFILES IN *ECHINACEA PURPUREA*

INFLUENȚA FERTILIZĂRII ASUPRA CARACTERELOR ORNAMENTALE ŞI PROFILULUI ALANTOLACTONEI ŞI ACIZILOR TRITERPENICI LA *ECHINACEA PURPUREA*

MIRCEA (ARSENE) Cristina Cerasela[1], CIOANCĂ Oana[2], IVĂNESCU Bianca[2] , DRAGHIA Lucia[1], HĂNCIANU Monica[2]
e-mail: cris_arsene@yahoo.com

Abstract. *This paper presents the results regarding the ornamental characters and the contents in alantolactone and triterpenic acids of Echinacea purpurea plants fertilized with Osmocote. The fertilisation is positively correlated with the stem ramification, the growth of vegetative mass and the number of inflorescences. Qualitative and semiquantitative analyses for the identification and quantification of alantolactone and for qualitative estimates for triterpenic acids were performed. The general aspect of TLC chromatograms indicated the presence of alantolactone. To confirm the TLC results and to perform a semiquantitative estimate, the samples were analysed through HPLC. The analysis confirmed alantolactone presence. Qualitative analysis also aimed to highlight the profile of certain triterpenic acids (ursolic acid, oleanolic acid), important through the potential farmacodynamic action. Oleanolic acid is present in open inflorescences of Echinacea.*
Key words*: alantolactone, control released fertiliser, HPLC, morphology, TLC, triterpenic acids.*

Rezumat. *Lucrarea prezintă rezultate privind caracterele ornamentale şi conținutul în alantolactonă şi acizi triterpenici la plantele de Echinacea purpurea fertilizate cu Osmocote. Fertilizarea a fost corelată pozitiv cu gradul de ramificare a tulpinilor, creşterea masei vegetative şi cu numărul de inflorescențe pe plantă. Pentru identificarea şi cuantificarea alantolactonei şi pentru estimarea calitativă a unor acizi triterpenici s-au efectuat analize calitative şi semicantitative. Aspectul general al cromatogramelor CSS a indicat prezența alantolactonei. Pentru confirmarea rezultatelor cromatografiei în strat subțire şi efectuarea unei estimări semicantitative, probele au fost analizate prin metoda HPLC. Analiza a confirmat prezența alantolactonei la nivelul unor valori mici, ceea ce avertizează asupra potențialului alergenic al plantelor. Prin analiză calitativă s-a urmărit şi evidențierea profilului unor acizi triterpenici (acid ursolic, acid oleanolic), importanți prin acțiunea farmacodinamică potențială. Acidul oleanolic este prezent în inflorescențele deschise de Echinacea.*

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2] "*Grigore T. Popa*" University of Medicine and Pharmacy Iasi, Romania

***Cuvinte cheie**: alantolactonă, acizi triterpenici, CSS, HPLC, îngrăşământ cu eliberare controlată, morfologie.*

INTRODUCTION

Echinacea purpurea (L.) Moench is a hardy perennial plant which belongs to the *Asteraceae* family and is one of the most common species of the genus, used as ornamentals and commercially treated as medicinal plants due to their antiviral, antibacterial and immunostimulatory activities to humans (Chen *et al*., 2008; Draghia, 2004; Hăncianu, 2014; Stanciu, 2008). The aerial parts of the plant contain flavonoids, polyphenolic acids, essential oils, alcohols, pseudoguaianolide, sesquiterpenes, monoterpenes, sesquiterpene oxides, polyacetylenes, alkylamides and other chemical constituents which have been analysed by applying various chromatographic techniques (Diraz *et al*., 2012; Hăncianu, 2014).

Information regarding the effects of genetic diversity, growing climates and cultivation practices on active constituents (secondary metabolites) and biomass production of *Echinacea* is still very limited (Chen *et al*., 2008). It is known that biosynthesis and concentration of secondary metabolites of plants depend on growing sites, climate conditions, cultural practices, vegetation phases and on cultivar and specific organs of the plant (Mircea (Arsene) *et al*., 2015; Chen *et al*., 2008; Druțu *et al*., 2010).

The objectives of the present study were to simultaneously investigate the alantolactone, ursolic and oleanolic acids profiles in the aerial parts of *Echinacea* and to evaluate macromorphology of the plants in relation to local conditions and nutrients availability. A controlled released fertilizer (CRF) was selected in the study due to its long term soil availability, low cost in terms of labour and good environment protection.

MATERIAL AND METHOD

Local conditions

The research was conducted at the Floricultural Department of the University of Agricultural Sciences and Veterinary Medicine of Iasi, in the 2013-2015 cultivation years. Soil parameters and climatic conditions were evaluated.

Cultivation trial

The experiment was performed as plot design based on randomised complete blocks with three replications. *Echinacea purpurea* plants were studied related to their macromorphology and terpenoid profile (alantolactone, ursolic acid and oleanolic acids), in two nutritional statuses: local (V1) and enriched soil conditions (V2).

Seedlings of echinacea were planted in the field at the beginning of May, at a density rate of 6 plants m^{-2}. The chosen fertilizer was osmocote-type, ecofriendly and 5-6 months availability; NPK (Mg): 15-10-12(2), microelements B, Cu, Fe, Mn, Zn, supporting ornamental growth and development with easy maintenance. The product was administered in 75g m^{-2} just before planting in the first year and at the start of vegetative growth in the next years. Biometric parameters measured were: height and width of plant, number of principal ramifications per plant, number and diameter of inflorescences per plant. Ten plants were randomly selected from each plot for all

parameters evaluated and the average was calculated in both nutritional statuses. Statistical significance between mean values was assessed through classic statistical calculations: significance of differences between the variants using LSD test. The variants were compared with their average. Stems and leaves (together) and inflorescences were studied for aforementioned chemical compounds content in two phenophases (budding stage and full flowering stage), in both nutritional statuses.

Chemicals and phytochemical analysis

Chemicals - All chemicals used in the present research were purchased from Sigma Aldrich (Germany). Stock solutions were prepared in HPLC grade methanol and stored at 4ºC until the finalization of the tests. All solvents were of analytical grade.

Extraction methods- For TLC evaluation dried plant material was extracted twice with ethanol 70% for 30 minutes under reflux (DER=1:10 g/mL) on a thermostatic water bath. An aliquot of 50 mL from each extract was concentrated using a rotary evaporator (Buchi R 210, Switzerland) to remove the solvent. The residues were dried and stored at 4°C for analysis. For HPLC alantolactone evaluation, the test solutions were obtained in acetonitrile R (1,0 g powdered drug in 3mL solvent, three times, sonicated for 15 minutes). The filtrates were reunited and brought to 10mL in a volumetric flask.

Vegetal samples were noted as follow:

V1f1 – buds from unfertilized plants (budding stage);
V2f1 – buds from fertilized plants (budding stage);
V1f2 – inflorescences from unfertilized plants (full flowering stage);
V2f2 – inflorescences from fertilized plants (full flowering stage);
V1-1 – leaves and stems from unfertilized plants (budding stage);
V2-1 – leaves and stems from fertilized plants (budding stage);
V1-2 – leaves and stems from unfertilized plants (full flowering stage stage);
V2-2 – leaves and stems from fertilized plants (full flowering stage stage).

Standards used are noted in TLC chromatograms with: **Ala** – alantolactone; **O** – oleanolic acid; **U** – ursolic acid.

Terpenoids pattern evaluation by TLC

In the present study were used:

a) for sesquiterpene lactones - Standard compound: alantolactone, 12 µL; Solvents: hexane - ether (25:75, v/v); Detection: Zimmermann reagent, VIS evaluation;

b) for triterpenes - Standard compounds: ursolic acid, oleanolic acid, 12 µL; Solvents: chlorophorm - glacial acetic acid - methanol - water (60:32:12:8, v/v); Detection: anisaldehyde - sulphuric acid reagent, VIS evaluation;

In both tests, the TLC plates were sprayed with 10mL of the reagent, heated at at 100°C for 5 min, and then evaluated in VIS.

RP-LC-DAD analysis of sesquiterpene lactones (alantolactone)

A Thermo-Fischer UltiMate 3000 system coupled with DAD detector was used to assess the profile sesquiterpene lactones. The working conditions were: Accucore XL-C18 column (4.6 x 150 mm, 4 µm); column temperature: 34°C; detection wavelength was set at 225 nm and the flow rate was 1,2 mL/min. The mobile phase consisting of two eluents as A (water) and B (methanol) used the following linear gradient elution: 0-3 min 38%B; 3-20 min 45%B; 20-30 min 45% B; 30-55 min 55%B; 55-57 min 100%B; 70 min 100%B; 90 min 38%B. As standard, alantolactone was used in amount of 20 µL of a 5mg/mL solution. Samples UV spectra registered at 225nm were automatically compared by Chromeleon 7.2 software and the concentration was expressed as % of the standard's aria/concentration.

RESULTS AND DISCUSSION

The fertilization increases the plant height, number of principle ramifications and number of inflorescences per plant, and diameter of inflorescences but distinctly positive differences were recorded only in three parameters (tab. 1).

Table 1

***Echinacea purpurea:* the influence of fertilisation on morphological parameters**

Treatment	Plant height (cm)	Plant width (cm)	Number of principle ramifications/ plant	Number of inflorescences/ plant	Diameter of inflorescences (cm)
V_1	75,00^{00}	50,00^{ns}	6,00^{00}	45,00^{00}	7,10^{ns}
V_2	80,00^{xx}	48,00^{ns}	10,00^{xx}	59,00^{xx}	8,00^{ns}
Average	**77,50**	**49,00**	**8,00**	**52,00**	**7,55**
LSD 5%	0,43	7,17	0,66	4,97	0,66
LSD 1%	0,99	16,55	1,52	11,47	1,52
LSD 0,1%	3,16	52,67	4,83	36,49	4,83

Note: **00/xx** = distinctly negative/positive significance; **ns** = not significant

The TLC general overview indicated that alantolactone (violet grey zones, Rf 0,74) is present in closed inflorescences of young plants (budding stage) and in stems and leaves of mature plants (especially fertilized ones).

The HPLC analysis confirmed the presence of alantolactone (Fig. 1). Comparative results obtained (TLC/HPLC) are tabulated in table 2.

Semiquantitative quantification showed that alantolactone is present at low levels (some micrograms at 100 gr dried plant). Moreover, the unfertilised plants contain only traces of alantolactone compared to the fertilised samples.

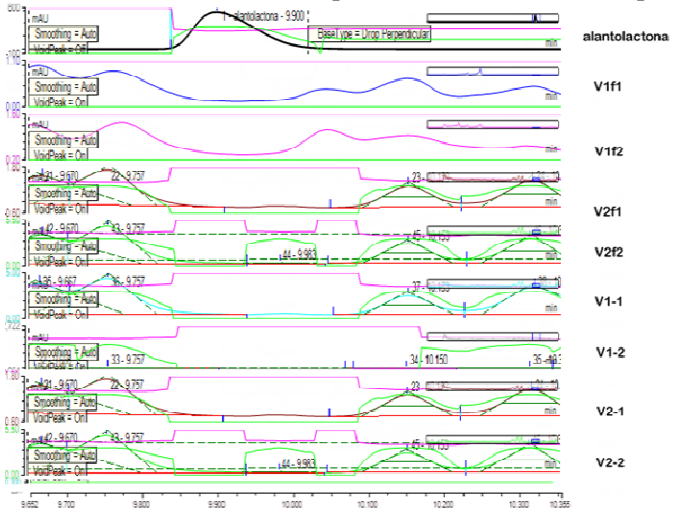

Fig. 1 - HPLC alantolactone identification in *Echinacea purpurea* samples

Table 2

Qualitative and semiquantitative identification of alantolactone in *Echinacea purpurea*

Method	Buds / Open inflorescences				Stems and leaves			
	V1f1	V1f2	V2f1	V2f2	V1-1	V1-2	V2-1	V2-2
TLC	u	–	u	–	–	u	–	+
HPLC	0,0763	0,1010	0,3649	0,0898	0,0135	0,1639	0,0213	0,3065

Note: **u** = traces; **+** = present; **–** = not identifiable.

Alantolactone represents a proven allergen and its presence in plants demonstrate that echinacea is potentially allergenic, especially through leaves and stems of fertilised mature plants.

Alantolactone is positive correlated with biomass, especially with ramification and number of inflorescences. These findings are in accordance with Lerdau *et al*. (1997) and Ormeño *et al*. (2008) which have found a positive correlation between the soil and leaves nutrient concentration (especially N) and the concentration of terpenoides from leaves. Gershenzon (1994) stated that nutrient-terpenoid relation has species specificity.

Triterpenic profile confirmed the presence of traces of ursolic acid (Rf 0,40) in open inflorescences while oleanolic acid (Rf 0,58) was confirmed in whole plant. TLC chromatograms are illustrated in figure 2 and the interpretation of results is presented in table 3.

Table 3

Triterpenoides in *Echinacea purpurea* samples

Triterpenic acid	Organ					
	Buds / Open inflorescences				Stems and leaves	
	V1f1	*V2f1*	*V1f2*	*V2f2*	*V1-2*	*V2-2*
Ursolic acid	-	-	u	u	-	-
Oleanolic acid	u	+	u	u	u	+

Note: **u** = traces; **+** = present; **–** = not identifiable.

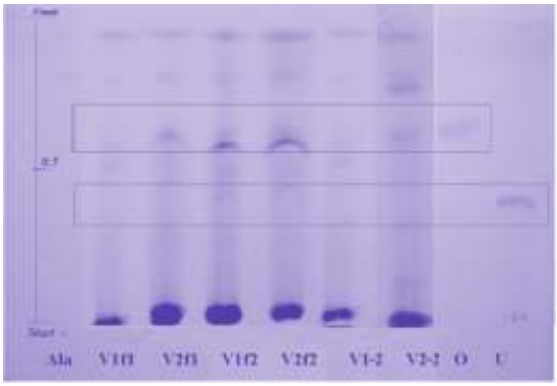

Fig. 2 - Triterpenoids in *Echinacea purpurea* samples (TLC)

CONCLUSIONS

Fertilisation with controlled release fertilizer intensifies plant growth and ramification, also increases the number of inflorescences per plant.

The alantolactone content is positive correlate with biomass of the plants and the concentration of mentioned sesquiterpenlactone is greater in leaves and stems of fertilised mature plants comparative with unfertilized ones.

The TLC triterpenic acid profile in *Echinacea purpurea* confirms the presence of oleanolic acid in all organs of plant while ursolic acid is present only in traces, in open inflorescences.

Acknowledgments: *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. Chen C.L., Zhang S.C., Sung J.M., 2008 - *Biomass and caffeoyl phenols production of Echinacea purpurea grown in Taiwan*. Experimental Agriculture, vol. 44, nr. 4, pp. 497-507.

2. Diraz E., Karaman S., Koca N., 2012 - *Fatty Acid and Essential Oil Composition of Echinacea Purpurea* (L.) *Moench, Growing in Kahramanmaras-Turkey*. ICEBS'2012 December 21-22, Bangkok (Thailand).

3. Draghia L., 2004 - *Floricultura*. Editura „Ion Ionescu de la Brad", Iaşi.

4. Druțu C.A., Gille E., Axinte M., 2010 - *Influența îngrășămintelor chimice cu azot și fosfor asupra producției și calității la Echinacea purpurea* (L.) *Moench*. AN. I.N.C.D.A. Fundulea LXXVIII (2).

5. Gershenzon J., 1994 - *Metabolic costs of terpenoid accumulation in higher plants*. J. Chem. Ecol., 20(6), pp. 1281-1328.

6. Hăncianu M., 2014 - *Echinacea spp.* in: Stănescu U. (ed.). *Plante medicinale de la A la Z*. Editura Polirom Iași, pp. 238-243.

7. Lerdau M., Guenther A., Monson R., 1997 - *Plant production and emission of volatile organic compounds*, Bioscience, 47(6), pp. 373-383.

8. Mircea (Arsene) C., Cioancă O., Draghia L., Hăncianu M., 2015 - *Morphological caracteristics and polyphenol variations in Rudbeckia hirta* L. Rom Biotechnol. Lett, vol. 20, nr. 4, pp. 10688-10695.

9. Ormeño E., Fernandez C., 2012 - *Effect of Soil Nutrient on Production and Diversity of Volatile Terpenoids from Plants*. Current Bioactive Compounds, nr.8, pp.71-79.

10. Stanciu M., 2008 - *Studii și cercetări privind biologia, tehnologia producerii și valorificarea speciilor de Echinacea*. Teză de Doctorat, USAMV București, România.