Studii şi Cercetări Ştiinţifice 2017, **18** (2), pp. 191 – 200
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

RESEARCH REGARDING THE POTENTIAL ACTIVITY OF SOME HETEROCYCLIC COMPOUNDS ON PLANTS GROWTH AND DEVELOPMENT

Oana-Irina Patriciu[1], Ştefan Ciobanu[2], Daniela Nicuţă[2], Luminiţa Grosu[1], Mirela Suceveanu[1], Irina-Claudia Alexa[1*], Adriana Luminiţa Fînaru[1*]

"Vasile Alecsandri" University of Bacău, 157, Calea Marasesti, 600115, Bacău, Romania
[1]Faculty of Engineering, Department of Chemical and Food Engineering
[2]Faculty of Science, Department of Biology, Ecology and Environmental Protection

*Corresponding authors: irinaalexa@ub.ro, adrianaf@ub.ro

Received: May, 15, 2017
Accepted: June, 28, 2017

Abstract: It is well known that growth and morphogenesis of plant tissue cultures can be improved by small amounts of some organic compounds. Heterocyclic compounds such as chromanones and thiazoles derivatives, valuable because of their potential biological activities, have also been reported as pesticides, herbicides and plant-growth regulators.
In the present study, different concentrations of chromanones and thiazoles derivatives were employed to evaluate their effects on plantlets growth of *Ocimum basilicum* L. and *Echinacea purpurea* L. The studied compounds were proved to be growth inhibitors at high concentrations. A growth stimulation effect was registered at low concentration.

Keywords: *biomass accumulation, chromanones, Echinacea purpurea L., morphogenetic reaction, Ocimum basilicum L., thiazoles*

INTRUDUCTION

According to the literature, chromanones and thiazoles derivatives are involved in a variety of biological activities. The synthesis of chromanones is of great interest in the field of organic chemistry because they exhibit a wide variety of well-documented antiviral [1], antifungal [2], insecticidal [3] activities. Meanwhile, thiazoles and their derivatives have received continuous attention over the years because of their various biological activities such as antibacterial, antifungal, anti-inflammatory effects [4, 5]. Some are used widely as agrochemicals, such as pesticides, fungicides, and herbicides [6].
The current tendency is the elaboration of new effective ecologically safe plant growth stimulants. The advantage of application of these heterocyclic compounds is based on their high physiological effect at very low concentrations, broad specificity of action on different agricultural crops and lack of toxicity for plant, animal and human health [7, 8].
Numerous studies confirm that some low molecular weight heterocyclic compounds derivatives considerably stimulate plant shoot organogenesis *in vitro* conditions [7].
Medicinal and aromatic plants are good choices for researches concerning the study of growth and morphogenesis of plant tissue cultures by adding different amounts of some organic compounds.
Ocimum basilicum L., usually named common basil or sweet basil have many applications in food, pharmaceutical and cosmetic industries, and more recently as corrosion inhibitors etc. [9]. Antiviral and antimicrobial activities of this plant have been reported [10, 11].
Echinacea purpurea is a medicinal plant well known for its therapeutic effects that plays an important role in traditional medicine [12]. Existing literature suggests that Echinacea have an antibacterial and antiviral activity through the immune system modulation [13] and was found to be a very potent antioxidant [14].
In this perspective, as part of our research program and suite of the collaboration between ours Laboratory of Organic Chemistry and the Department of Biology [15 – 18], the impact of some synthetic heterocyclic compounds on plants growth and development, in laboratory conditions, was studied. This research was carried out on *in vitro* culture medium of *Ocimum basilicum* L. and *Echinacea purpurea* L.

MATERIALS AND METHODS

Plant material

The research was conducted using as biologic material the selected seeds of *Ocimum basilicum* L. and *Echinacea purpurea* L. provided by Agricultural Research and Development resort from Secuieni – Neamţ (Romania).

Seed sterilization and germination
The seeds were sterilized by immersion in a 5 % solution of commercial chloramine T trihydrate (Merck, Germany) and rinsed three times in sterile deionized water. Then, under sterile conditions, for obtaining sterile plantlets, the seeds were inoculated on

basal medium Murashige and Skoog (MS) [19, 20] without hormones or other stimulating growth substances.
All chemicals used for nutrients Murashige-Skoog medium culture were purchased from Merck (Germany) and Fluka (Switzerland).
The seeds were maintained in the growth chamber (LEEC, model PL2, Great Britain) at 20 °C in the dark for 2 weeks. After this period, the pots were moved into another growth chamber (SANYO, model MLR 351, Japan) at 23 ± 1 °C (photoperiod - 16 h, light intensity - 2500 lux), for obtaining sterile vitroplants.

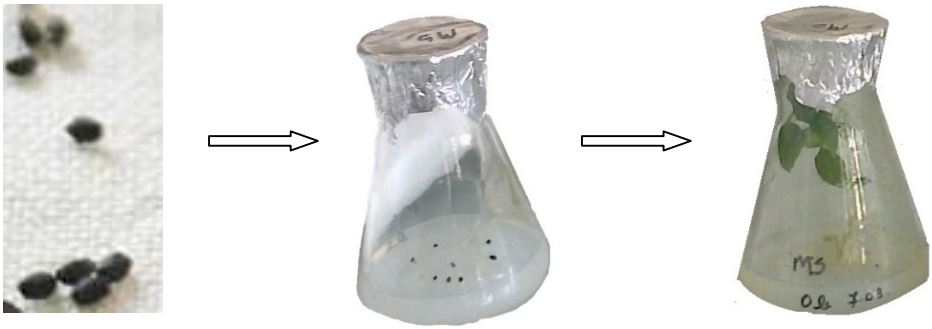

Figure 1. *Steps of inoculation and germination process of Ocimum basilicum L.*

Explants inoculation and culture conditions
After another 2 weeks, under sterile conditions, the explants taken from sterile vitroplants were transferred to Erlenmeyer flasks containing basal medium MS supplemented with different concentrations of studied heterocyclic compounds in order to evaluate their morphogenetic reaction at 30-days interval.
Apex and nodes were used as explants source in the case of *Ocimum basilicum*. For *Echinacea purpurea*, leaves and shoots were used as explants source.
These flasks were placed in a temperature-controlled room (SANYO) at 23 ± 1 °C with 16/8 h light/dark cycle. The light intensity was maintained at 2500 lux throughout the experiments.
All experiments were performed in three replicates.

Tested heterocyclic compounds

The effects of two heterocyclic compounds (Figure 2) on growth and development plants were tested:
- 3-isothiocyanato-3-morpholin-4-yl-methyl-chroman-4-one (molecular formula: $C_{15}H_{14}Cl_2N_2O_3S$; molecular weight: 373.26 $g \cdot mol^{-1}$) - **A** (mentioned as **BPIC** in figures);
- 4-[4-(3,5-dichloro-phenyl)-thiazol-2-yl]-morpholine (molecular formula: $C_{13}H_{12}Cl_2N_2OS$; molecular weight: 315.22 $g \cdot mol^{-1}$) - **B** (mentioned as **BADA** in figures).

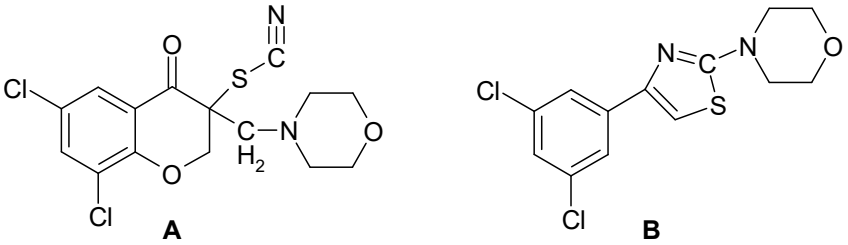

Figure 2. *Structures of tested heterocyclic compounds **A** and **B***

The synthesis of these heterocyclic compounds was already described [15 – 17].
The stock solutions of these two compounds were prepared (100 mg compounds in 100 mL distilled water). Different concentrations (0.1, 0.2, 1, 2 and 4 $mg \cdot L^{-1}$ respectively) of **A** and **B** were added in **MS** medium according to Table 1.

Table 1. *Media variants for in vitro explants development*

Concentration of tested heterocyclic compounds [$mg \cdot L^{-1}$]	**Media variants**	
	BPIC variants	**BADA variants**
4	BPIC I (**A1**)	BADA I (**B1**)
2	BPIC II (**A2**)	BADA II (**B2**)
1	BPIC III (**A3**)	BADA III (**B3**)
0.1	BPIC IV(**A4**)	BADA IV (**B4**)
0.2	BPIC V (**A5**)	BADA V (**B5**)
0	**MS** (control)	

Growth parameters and analysis

After 1 month, the morphogenetic reaction was evaluated for all samples and compared with the control sample (**MS** medium without addition of tested heterocyclic compounds).
After removal of culture medium, the parameters measured for evaluating the potential activity of two tested compounds on the growth and development of plants were: the number of plantlets, the plant height (growth rate) and the fresh weight (biomass accumulation). The biomass accumulation reflects the complexity of metabolic process which it can be influence by the addition of tested heterocyclic compounds (stimulation or inhibition effect).

RESULTS AND DISCUSSION

Ocimum basilicum L. analysis

Morphogenetic reaction of Ocimum basilicum L.

On variants containing the compounds **A** and **B** in high concentrations (**A1**, **A2**, **B1**, **B2** and **B3**), after a small development of plantlets, a degeneration and necrosis were observed (Figure 3). Only in the case of **A3** medium, further necrosis has not occurred.

a)  ***b)*** 

Figure 3. *The morphogenetic reaction of Ocimum basilicum on **B3** medium: a) development; b) necrosis*

The growth and development of plants were visibly positive in the case of samples with low concentrations (**A4**, **A5** and **B4**, **B5**) comparative with the control sample and / or with the samples with high concentrations (Figure 4).

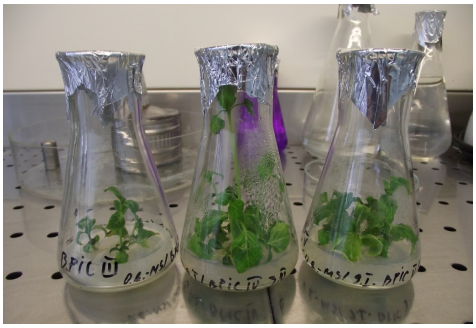

Figure 4. *The comparative morphogenetic reaction of Ocimum basilicum on **A3, A4** and **A5** medium*

As can be seen in Figure 4, on **A4** medium (MS + 0.1 mg·L^{-1} chromanone) the plantlets are vigorous, the leaves present an intense green color without a chlorophyll deficit. The morphogenetic reaction on **A5** medium (supplemented with 0.2 mg·L^{-1} chromanone) was less intensive that one of **A4** medium.
Compound **B** at low concentrations presents a similar influence as compound **A**, a stimulating effect in the development of plantlets and the roots being distinguished. The leaves proportion and size were comparable and the color is lighter than in the case of **A** medium (Figure 5).

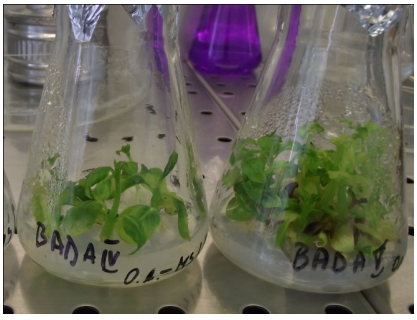

Figure 5. *The comparative morphogenetic reaction of Ocimum basilicum L. on **B4** and **B5** medium*

Biometric measurements in the case of Ocimum basilicum L.

The biometric measurements concerning the explants of *Ocimum basilicum* L. are presented in Table 2.

Table 2. *Biometric measurements on Ocimum basilicum L. samples*

Samples	Media variants	Number of shoots / sample	Fresh weight [g]	Fresh weight / Shoot number ratio	Shoot length [cm]
A	A3	5	0.98	0.196	min. 0.2 max. 3.1
	A4	8	4.21	0.526	min. 2.5 max. 12.5
	A5	6	2.82	0.470	min. 1 max. 8.5
B	B3	necrosis			
	B4	6	2.59	0.431	min. 1.5 max. 6.5
	B5	8	4.04	0.505	min. 3 max. 10.5
Control sample	MS	7	3.32	0.474	min. 2.5 max.7.5

It can be noticed that the best results concerning the fresh weight / shoot number ratio were obtained for the samples supplemented with low concentrations of heterocyclic compounds (the case of **A4** medium followed by **B5** medium - highlighted in Table 2). In these cases, the biomass accumulations are superior to control sample (**MS**).

The Figure 6 presents the difference of shoot length between the samples with addition and without addition of tested heterocyclic compounds.

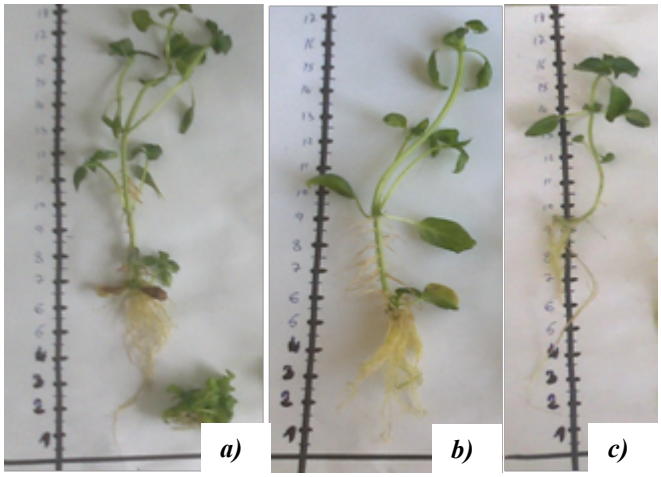

Figure 6. *Comparative biometric measurements of Ocimum basilicum L. between the samples with addition of chromanone **A** (**A4** medium – a), of thiazole B (**B5** medium – b) and without addition of tested heterocyclic compounds (**MS** medium – c)*

Echinacea purpurea L. analysis

Morphogenetic reaction of Echinacea purpurea L.

Also in the case of *Echinacea purpurea* L., the mediums with high concentrations of heterocyclic compounds reveal not to be favorables for plantlets development. For example, after 3 weeks, on **A1** and **A2** medium there has been a process of stagnation in leaves development followed by necrosis (Figure 7). The **B1** variant as well as variant **B2** are not good environments for the development of plant material.

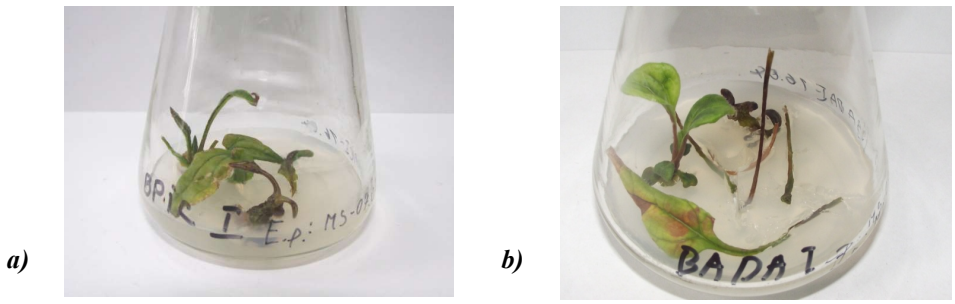

Figure 7. *The morphogenetic reaction of Echinacea purpurea* L. *on **A1** (a) and **B1** (b) medium*

In the case of **A3** medium, as can be seen in Figure 8, the explants are better developed than those of the control sample **MS**. A stimulating effect in the development of plantlets and the roots is notable in the case of **A4** medium. As in the case of basil samples, the plantlets are vigorous and the leaves present an intense green color. The morphogenetic reaction on **A5** variant was comparable with the one of **A4** variant.

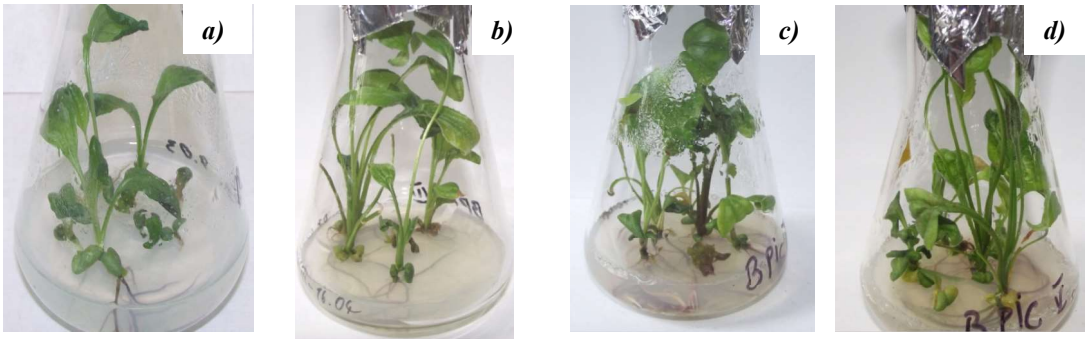

Figure 8. *The comparative morphogenetic reaction of Echinacea purpurea* L. *on **MS** medium (a) with **A3** (b), **A4** (c), and **A5** (d) medium*

In Figures 9 a) and b), it can be observed that on the **B3** medium (supplemented with 1 mg·L^{-1} thiazole) after a small development, the explants dry out over time.
The growth and development of plants were also evidently positive in the case of samples with compound **B** in low concentrations (**B4** and **B5** variants). On these variants, the plantlets present large leaves with thick petiole - Figures 9 c) and d).

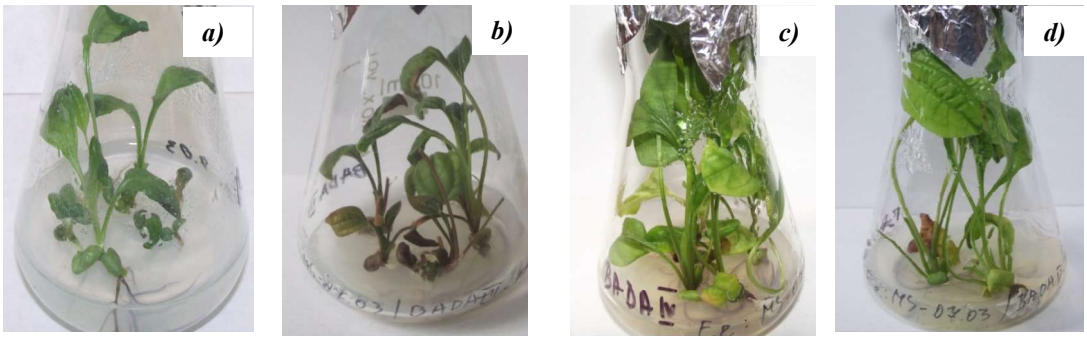

Figure 9. *The comparative morphogenetic reaction of Echinacea purpurea* L. *on* ***MS*** *medium (a) with* ***B3*** *(b),* ***B4*** *(c) and* ***B5*** *(d) medium*

Biometric measurements in the case of Echinacea purpurea L.

The biometric measurements concerning the explants of *Echinacea purpurea L.* are presented in Table 3.

Table 3. *Biometric measurements on Echinacea purpurea L. samples*

Samples	Media variants	Number of shoots / sample	Fresh weight [g]	Fresh weight / Shoot number ratio	Shoot length [cm]
A	A3	5	1.97	0.394	min. 1.5 max. 12.5
	A4	9	4.14	0.460	min. 2.5 max. 13
	A5	10	4.57	0.457	min. 2.5 max. 14
B	B3	5*	1.75*	0.350	min. 1.5 max. 7
	B4	13	6.15	0.473	min. 4 max. 16.5
	B5	10	4.45	0.445	min. 4 max. 17
Control sample	MS	5	1.87	0.374	min. 2 max. 9.5

*partial necrosis

The biometric indexes of samples treated with tested heterocyclic compounds used in concentrations ranging from 0.1 to 1 mg·L^{-1} were higher than the biometric indexes of plantlets grown on basal medium (**MS**).

The highest growth stimulating activity for *Echinacea purpurea* was found when 0.1 mg·L^{-1} of thiazole **B** was added, followed narrowly by the sample treated with chromanone **A** in the same concentration (highlighted in Table 3).

In contrast to *Ocimum basilicum* plants, the concentration of 1 mg·L^{-1} had a stimulating growth effect for *Echinacea purpurea*.

CONCLUSION

In the case of investigated species *Ocimum basilicum* L. and *Echinacea purpurea* L., growth and development were affected by the tested concentrations of two heterocyclic compounds.

The chromanone **A** and the thiazole **B** were proved to be growth inhibitors at high concentrations (2 and 4 $mg \cdot L^{-1}$). Generally, a growth stimulation effect was registered at low concentration (0.1 and 0.2 $mg \cdot L^{-1}$).

Slightly different behaviors between the tested chromanone and thiazole derivatives were found. Thus, the best results concerning the ratio of the amount of biomass accumulated compared with control sample were obtained in the case of chromanone **A** for *Ocimum basilicum* (variant **A4** supplemented with 0.1 $mg \cdot L^{-1}$) and thiazole **B** for *Echinacea purpurea* (variant **B4** supplemented with 0.1 $mg \cdot L^{-1}$).

It was found that in the case of *Echinacea purpurea* all tested heterocyclic compounds used in low concentrations significantly accelerate plant growth and development of plantlets as compared to control plants.

The preliminary qualitative analysis of metabolites accumulated in plantlets by UV-Vis spectroscopy analysis of the alcoholic extracts is underway and will be the subject to a future publication.

REFERENCES

1. Xu, Z.Q., Buckheit, R.W., Stup, T.L., Flavin, M.T., Khilevich, A., Rizzo, J.D., Lin, L., Zembower, D.E., Thaa, U.: In vitro anti-human immunodeficiency virus (HIV) activity of chromanone derivative, 12-oxocalanolide A, a novel NNRTI, *Bioorganic Medicinal Chemistry Letters*, **1998**, **16**, 2179-2184;
2. Zhao, P.L., Li, J., Yang, G.F.; Synthesis and insecticidal activity of chromanone and chromone analogues of dicylhydrazines, *Bioorganic & Medicinal Chemistry*, **2007**, **15**, 1888-1895;
3. Ayati, A., Falahati, M., Irannejad, H., Emami, S.: Synthesis, in vitro antifungal evaluation in silico study of 3-azolyl-4-chromanone phenylhydrazones, *DARU Journal of Pharmaceutical Sciences*, **2012**, **20** (46), doi: 10.1186/2008-2231-20-46;
4. Wilson, K.J., Utig, C.R., Subhasinghe, N., Hofmann, J.B., Rudolph, N.J., Molloy, C.J., Bone, R., Green, D., Randall, J.: Synthesis of thiophene-2-carboxamidines containing 2-amino-thiazoles and their biological evaluation as urokinase inhibitors, *Bioorganic Medicinal Chemistry Letters,* **2001**, **11**, 915-918;
5. Jung, K.Y., Kim, S.K., Gao, Z.G., Gross, A.S., Melman, N., Jacobson, K.A., Kim, Y.C.: Structure–activity relationships of thiazole and thiadiazole derivatives as potent and selective human adenosine A(3) receptor antagonists, *Bioorganic & Medicinal Chemistry*, **2004**, **12**, 613-623;
6. Dayan, F.E., Vincent, A.C., Romagni, J.G.: Amino-and urea-substituted thiazoles inhibit photosynthetic electron transfer, *Journal of Agricultural and Food Chemistry*, **2004**, **48**, 3689-3693;
7. Tsygankova, V., Andrusevich, Y., Shtompel, O., Hurenko, A., Solomyannyj, R., Mrug, G. Frasinyuk, M., Brovarets, V.: Stimulating effect of five and six-membered heterocyclic compounds on seed germination and vegetative growth of maize *(Zea mays* L.), *International Journal of Biology Research*, **2016**, **1** (4), 01-04;
8. Chattha, F.A., Munawar, M.A., Nagra, S.A., Ashraf, M., Khan M.A., Kosur, S.: Synthesis of 3-aryl-1H-indazoles and their effects on plant growth, *Journal Plant Growth Regulators*, **2013**, **32** (2), 291-297;

9. Hiltunen, R., Holm, Y.: *Basil: The Genus Ocimum,* Harwood Academic Publishers, Taylor & Francis Library, Amsterdam, **2005**;
10. Bozin, B., Mimica-Dukic, N., Simin, N., Anackov, G.: Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils, *Journal of Agricultural and Food Chemistry,* **2006**, **54** (5), 1822-1828;
11. Chiang, L.-C., Ng, L.-T., Cheng, P.-W., Chiang, W., Lin, C.-C.: Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*, *Clinical and Experimental Pharmacology and Physiology*, **2005**, **32**, 811-816;
12. Kumar, K.M., Ramaiah, S.: Pharmacological importance of *Echinacea purpurea, International Journal of Pharma and Bio Sciences*, **2011**, **2** (4), 304-314;
13. Kurkin, V.A., Dubishchev, A.V., Ezhkov, V.N., Titova, I.N., Avdeeva, E.V.: Antidepressant activity of some phytopharmaceuticals and phenylpropanoids, *Pharmaceutical Chemistry Journal*, **2006**, **40** (11), 614-619;
14. Dalby-Brown, L., Barsett, H., Landbo, A.K., Meyer, A.S., Molgaard, P.: Synergistic antioxidative effects of alkamides, caffeic acid derivatives, and polysaccharide fractions from *Echinacea purpurea* on *in* vitro oxidation of human low-density lipoprotein, *Journal of Agricultural and Food Chemistry,* **2005**, **53** (24), 9413-9423;
15. Cascaval, A., Finaru, A., Prisecaru, M.: Synthesis of some 2-substituted 4-chromanones using ortho-hydroxyacetophenones, *Revue Roumaine de Chimie*, **1998**, **43** (8), 747-751;
16. Finaru, A.; Cascaval, A.; Prisecaru, M.: Mannich-Werner mono-bases of 4-chromanones, possible plant growing regulators, *Revista de Chimie*, **2003**, **54** (10), 837-840;
17. Lazar, S., Soukri, M., Leger, J.M., Jarry, C., Akssira, M., Chirita, R., Grig-Alexa, I.C., Finaru, A., Guillaumet, G.: Efficient synthesis of 2- and 3-substituted-2,3-dihydro [1,4]dioxino[2,3-b]pyridine derivatives, *Tetrahedron*, **2004**, **60** (31), 6461-6473;
18. Alexa, I.-C., Pavăl, P., Nicuţă, D. Bran, P., Patriciu, O.-I., Grosu, L., Fînaru, A.L.: Effects of hormones addition for in vitro plant development of *Calendulla officinalis*, *Scientific Study & Research - Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2015**, **16** (2), 75-79;
19. Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiologia Plantarum*, **1962**, **15** (3), 473-497;
20. Jain, S.M., Saxena, P.K. (Editors): *Protocols for In Vitro Cultures and Secondary Metabolite Analysis of Aromatic and Medicinal*, Series: Methods in Molecular Biology, vol. 547, Humana Press (Springer), New York, **2009**.