ABSTRACT

Keywords: toxicity, allergenicity, flowering species, *Asteraceae*, secondary metabolites, sescviterpenelactones, alantolactone, polyphenols, fertilisation, biosynthesis, CSS, HPLC.

The research that is the subject of the PhD thesis aims to study the allergenicity and toxicity of flowering species correlated with the valorification of these plants and their potential health risks. Six ornamental species from the *Asteraceae* family (*Calendula officinalis, Carthamus tinctorius, Echinacea purpurea, Rudbeckia hirta, Tagetes erecta, Chrysanthemum indicum*), with multiple uses (alimentary, medicinal, cosmetic, etc.) were evaluated in order to identify their allergenic potential, determined by the content of metilate sescviterpenelactones (expressed as alantolactone). The valorification possibilities of vegetal extracts were investigated through the research of the content of bioactive compounds from the polyphenolic and terpenoid classes. Biosynthetic capacity for the researched secondary metabolites was analysed in relation to plant phenology, plant parts and fertilisation applied.

To achieve the purpose and the objectives formulated by the PhD thesis, the following general aims were established:

1. the identification of toxic and allergenic compounds in flowering species, of the pathogenic mechanisms and of the potential health risks;

2. the study of natural setting conditions in which the research unfolded;

3. the study of the ornamental characters of the studied species in the conditions of fertilisation with control-released fertiliser (CRF);

4. the research of allergenic potential of the studied species and of the variability of the biosynthesis capacity for sescviterpenelactones (expressed as alantolactone) and for triterpenic acids;

5. the evaluation of the content of antioxidant compounds from the polyphenolic class (polyphenol flavones and polyphenolcarboxilic acids) and the determination of total phenolic content in the vegetal extracts from the studied plants;

6. the evaluation of some methods of reducing the risks in the use and commercialisation of potentially allergenic and toxic ornamental plants.

The PhD thesis titled "**Research regarding the use and valorification of ornamental plants with allergenic and toxic properties**" is structured in two parts and includes nine chapters. The first part of the paper contains the "Introduction" and two chapters referring to the current stage of knowing the issues addressed, while the second part includes own research, presented in seven chapters to which are added the "Conclusions and recommendations".

In chapter 1 – "**Toxic plants and biosynthesised toxic compounds**" – are defined specific terms, are described secondary vegetal metabolites with the presentation of general data about their synthesis and dynamics, the role in the plant's life, the economic role as well as the taxonomic and systematic importance. Moreover, the effects of secondary metabolic compounds on the human organism are described, at the same time as the evaluation of the health risks. The current stage of research regarding the classification of toxic compounds in plants is synthesised in the summary of this chapter, ending with the presentation of legislative and commercial regulations regarding the use and valorification of toxic and allergenic plants, as well as the labelling of ornamental species with a health risk potential.

Chapter 2 – "**Phytochemical aspects of the *Asteraceae* plants**" presents general aspects of secondary metabolites synthesised by plant belonging to the *Asteraceae* family, with the description of sescviterpenelactones (particularly alantolactone) and polyphenols. The allergenicity of the plants in this family is analysed through the prism of the metilate sescviterpenelactone content. The chapter ends with the listing of morphological and phytochemical particularities of the species included in this study, as well as with the presentation of the valorification possibilities and of the potential health risks in using them.

The "**purpose and objectives of the research**" as well as "**the material and method of research**" are detailed in chapter 3. General and specific objectives are listed in parallel with the specific research activities, the methods and research techniques and the estimated realisation indicators.

In regard to the execution of the experiences, six *Asteraceae* species were chosen, three annual and three perennial, chosen for their ecological and utilitarian plasticity, as well as for their tradtitional presence in Romanian crops: *Chrysanthemum indicum, Rudbeckia hirta,*

Echinacea purpurea, Tagetes erecta, Carthamus tinctorius, Calendula officinalis. The research followed two stages. In the first stage, the ornamental characteristics of the plants growen in the experimental conditions in the didactic field of Faculty of Horticulture, Department of Floriculture, were evaluated. In the second stage, samples from aerial parts of studied species were fitochemical analysed (separation/concentration, qualitative, semiquantitative and/or quantitative identification) within the laboratory of the Pharmacognosy of the Faculty of Pharmacy, the University of Medicine and Pharmacy "Gr. T. Popa", Iași. Preliminary stages of the study were realised both within the aforementioned laboratory as well as in the Center of Horticultural Research of the Horticulture Faculty, The University of Agricultural Sciences and Veterinary Medicine Iași.

The chosen experimental scheme was of the randomised block type with three repetitions and it followed the determination of the influence of fertilisation with control released fertiliser NPK(Mg)/15:10:12(2) on the morphology and phenology of plants as well as on the profile of phenolic compounds such as flavonoids and polyphenolcarboxilic acids, on the concentration of total polyphenols and on the profile and concentration of alantolactone, in the samples collected from the aerial part of the biological material from the crops.

The general research methods included bibliographical study, experiments, observation, as well as analysis, synthesis and comparison . In order to fulfill the objectives of the thesis, the research included specific work methods and techniques: biometry techniques, qualitative analysis techniques (thin layer chromatography, CSS), quantitative analysis techniques (spectrophotometric determination), semiquantitative analysis techniques (high performance liquid chromatography, HPLC) and methods of statistical data processing.

The results of biometric measurement are expressed as the media of crop years. The quantitative and semiquantitative analyses were performed three times and expressed as an average ± standard deviation (n=3). Averages and standard deviations were calculated with Microsoft Office Excel. The significant data differences were calculated using the method of limit differences (Ardelean *et al.*, 2007). The experiemental variants were compared with their average. The qualitative analysis was recorded three times and the resulting chromatograms (CSS) were compared with the database (Wagner and Bladt, 2001).

In chapter 4 are described "**the natural environmental conditions**" specific to the research experimental area, along with "**the organisational and institutional framework**" of the research. General data include topographical, pedological, hydrographical, hydrological and climate data. Climate parameters are centralised – temperature, precipitations – registered in the

research years 2012-2015, compared with normal values statistically registered. The analytical characterisation of the soil from the research field is also presented, obtained prior to the establishment of the crops.

Chapter 5 presents "**the contributions**" of the author to "**the characterisation of the allergenic and toxic profile of flowering species**". The study of flowering plant toxicity aimed to identify and phytochemically classify allergenic and toxic compounds in these plants, to specify the main pathogenic mechanisms, to evaluate the risks to human health and to create a database with the most common toxic and allergenic ornamental plants in our country. The chapter contains classifications of the constituents with toxic potential in ornamentals, as well as allergenic and irritative constituents present in these plants, along with a synthesised description of 70 species commonly met in Romania.

Chapter 6 presents "**the results and discussions** regarding **the influence of fertilisation on the morphological characters and on the dynamic of the phenophases in the studied species**". The fertilisation with Osmocote (NPC 15:10:12) is positively correlated with the stem ramification, the growth of vegetative mass and of the number of inflorescences in the studied species. In the same time, it determines the reduction of the inflorescences diameter in *Chrysanthemum* and its growth in *Rudbeckia*. In the fertilised versions a downgrade of the triggering of phenophases can be observed, spanning from 2 to 7 days.

Chapter 7 – "**Results and discussions regarding the alantolactone and triterpenic acids profiles in the studied species**" includes the results obtained from performing qualitative and semiquantitative analyses for the identification and quantification of alantolactone. The general aspect of chromatograms indicated the presence of alantolactone (R_f 0,74) but also of other sescviterpenelactones which migrated in $R_f > 0,74$ for which no standards were available. To confirm the CSS result and to perform a semiquantitative estimate, the samples were analysed through HPLC. The analysis confirmed alantolactone at low levels in plant materials. In unfertilised plants, the richest content in this compound was identified in the mature leaves and stems of *Calendula* (6,0045µg/100g), in *Calendula* buds (0,9472µg/100g) and in *Chrysanthemum* open inflorescences (1,2751µg/100g). The presence of alantolactone draws the attention on the allergenic potential of plants containing this metabolite, proven to be involved in producing contact dermatitis.

Qualitative analysis also aimed to highlight the profile of certain terpenic acids (ursolic acid, oleanolic acid), important through the potential farmacodynamic action. Ursolic acid (R_f =

0,40) was highlighted in open *Tagetes* inflorescences, while oleanolic acid (R_f = 0,58) is present in *Carthamus* leaves and stems, open *Echinacea* inflorescences and all *Rudbeckia* organs.

"**The results and discussions regarding the phenolic profile and total polyphenolic content in the studied species**" are included in chapter 8. Research confirmed that the flavonoid and polyphenolcarboxilic acids spectre presents particularities depending on the species, organ, phenophase and applied fertiliser. The compounds which were highly present in the samples studied were rutoside, apigenol-7-glucoside, narcissine and isoramnetil-rutinosil-glucoside in *Calendula*; echinacoside and its derivatives, cinarine and cicoric acids in *Echinacea*; ferulic acid and a glycosidate derivative of luteine in *Tagetes*; cvarcetol-3-0-glucuronide in *Carthamus*; hyperoside in *Rudbeckia*; isoramnetol and luteoline derivatives in *Chrysanthemum*. The inflorescences contain a richer phenolic spectre than the leaves and stems in *Carthamus* and *Rudbeckia*, while in *Echinacea*, *Calendula* and *Tagetes* the highlighted compounds are intensely present in vegetative organs. The fertilisation can modify the spectre of polyphenolic compounds, the obtained chromatograms suggesting that the plants can lose or win specific metabolites, under the influence of administered nutrients. The study of total polyphenols content present in the samples harvested from the researched species, highlighted a different behaviour depending on the organ or fertilisation. Of the unfertilised plants, *Rudbeckia* inflorescences are the richest in overall polyphenols, followed by *Tagetes*, *Echinacea*, *Calendula*, *Chrysanthemum* and *Carthamus*. The ranking differs for leaves and stems, the descending order being *Tagetes*, *Rudbeckia*, *Carthamus*, *Calendula*, *Echinacea* and *Chrysanthemum*.

Total polyphenols have values between 1146mg% and 145mg% in inflorescences and 483mg% and 79,82mg% respectively, in unfertilised stems and leaves (expressed as mg GAE/100g dry vegetal substance). Under the influence of fertilisation, total polyphenols from the vegetative parts decrease in annual plants and increase in perennials, while they can have different dynamics in inflorescences.

The present research demonstrate that the studied *Asteraceae* plants are rich in flavonoid and polyphenolcarboxilic acids type compounds, being a potent source of antioxidant compounds. The simultaneous analysis of morphological and phytochemical parameters in relation to the applied nutrients highlighted particular aspects regarding the physiology and biochemistry of these species.

In chapter 9, titled "**Methods of reducing the risks in the use and commercialisation of potentially allergenic and toxic ornamental plants**" is presented in a plant labelling system



and of communicating the health risk, in the area of commercialisation and valorification of ornamental plants. Criteria for uses of toxic and allergenic plants in landscaping are formulated.

The content of the thesis finally contains the resulting **conclusions and recommendations** regarding the use and valorification of ornamental plants with allergenic and toxic properties. The paper, through the issues approached and the conclusions formulated, has a wide addressability both to experts from various areas (horticultural, landscaping, pharmaceutical, medical - including public health) and to individuals interested in environmental biosecurity.

The PhD thesis ends with the **bibliography**, which contains the list of documented materials from abroad and from the country consulted throughout the development of the thesis and which were cited within.