UNIVERSITY OF AGRONOMIC SCIENCES AND VETERINARY MEDICINE FROM BUCHAREST

FACULTY OF MANAGEMENT, ECONOMIC ENGINEERING IN AGRICULTURE AND RURAL DEVELOPMENT

DOCTORAL THESIS

RESEARCH ON THE MANAGEMENT OF TECHNOLOGY AND THE USE OF THE SPECIES „*ARNICA MONTANA L.*”

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SUMMARY

Arnica montana L., known under the popular name of arnica or fairies' chariot, is found in our country on the pastures and the grasslands of the mountain and subalpine areas, particularly in the Apuseni Mountains.

Due to the fact that *Arnica montana L.* is a medicinal plant rich in active principles with complex action (antiseptic, anti-inflammatory, bacteriostatic and fungicide, coleretical, diuretic, hypotensive, etc.), the plant of the spontaneous flora is used at the maximum, this resulting, in the end, to the extinction of the species.

The social and economic importance of the *Arnica montana L.* species has led to agro-technical and agro-biological measures concerning the increase of production, as well as to the continuous increase in the content of active substances and in the purity of the raw material through the introduction into the culture of the species from the spontaneous flora.

From a phytotherapeutic perspective, *Arnica montana L.* owes its therapeutic action to the presence of the volatile oil, as the inflorescences (*Arnicae flos*) contain volatile oil ranging between 0.05 and 3.8%, depending on the biological material and the conditions of vegetation, as well as triterpene alcohols, carotenoids, yellow pigments and other compounds. The use of volatile oils has developed a new sub-branch of phytotherapy, namely, the aromatherapy.

Given the medicinal importance, and not only, of the *Arnica montana L.* species and the orientation of the population to the natural medicine, it is necessary to carry out complex studies on the introduction of the plant into the culture.

By the research carried out in the framework of the Ph.D. thesis on the management of the Arnica *montana L.*species, we believe that we will be able to make a small contribution to the knowledge of the plant and to the creation of new cultures under conditions similar to those examined in the Corbeni commune, Argeș County. Moreover, the research on the *in vitro* multiplication, the germination on solid substrate, the introduction into the culture of the species and the analysis carried out on the spontaneous flora of the Gârda de Sus commune, Alba County, will offer more information to those studying the plant *Arnica montana L.*

The research carried out for the purpose of preparing the Ph.D thesis entitled: ”Research on the management of technology and the use of the *Arnica montana L.*species” has been carried out on the experimental field from the Corbeni commune, Argeș County and on the spontaneous flora of the Gârda de Sus commune, Alba County. The research carried out under laboratory

conditions has been carried out in two stages, the first stage concerned the comparative analysis of the effectiveness of obtaining *in vivo* rooted plants through the normal germination of seeds on different substrates, compared with the introduction and the stimulation of *in vitro* seed germination and the use of aseptically germinated plantlets as a source of explants for in vitro multiplication at optimized levels, and the assessment of the *in vitro* growth and regeneration of plants compared with *in vivo* ones, with a view to using them for the initiation of the culture. The experiments have been carried out in the Laboratory of Plant Cytobiology within the Institute of Biology, Bucharest. And the second stage has concerned the determination of the percentage of humidity of the floral heads and their drying, the preparation of the samples for the purpose of extracting the oils, the determination of the active principles, the analysis of the oils extracted. The experiments have been carried out in the Laboratory for physical and chemical analysis within the National Institute for Agricultural Machines.

Among the reasons which have determined this research are the following:

- The need to know the most effective *in vitro* multiplication of the plant, obtaining the most effective regeneration and the more genetically stable plants;

- The need to identify the parameters required for the germination and the development of the plants sown in different substrates;

- The need to know the culture conditions and the response of the species in culture;

- The need to know the diseases and the pests and their influence on the production and its quality;

- The need for a study on the research concerning the development of the species in the spontaneous flora;

- The need for a study on the active principles contained in the *Arnica montana L.* inflorescences, obtained by *in vitro* multiplication, sown on different substrates or collected from the spontaneous flora.

The purpose of the Ph.D thesis research has been the learning of the management of the *Arnica Montana L.* species by comparing two development mediums of the plant, the natural environment of the Gârda de Sus area, Alba County and the culture obtained, in the Corbeni area, Argeș County, from plants obtained under laboratory conditions.

The objectives of the thesis research consisted in:

- To obtain plant material by *in vitro, in vivo* multiplication and direct sowing into the substrate;

- Initiation of the culture from the plants obtained in the laboratory;

- Observations made on the pastures constituting the habitats for the Arnica *montana L.* species, from the Gârda de Sus area, Alba County;

- Observations made on the experimental field from the Corbeni area, Argeș County;

- A description of the characteristic symptoms, frequent on the experimental field;

- Determination of the incidence and intensity of the attack and the calculation of the degree of attack on the experimental field;

- Quantitative estimates on the productivity of pastures with *Arnica montana L.*, compared with the productivity obtained on the experimental field;

- Determinations of the active principles, fat oils and volatile oils;

The biological material used in the research undertaken in the framework of the Ph.D thesis was represented by plants obtained by septical and aseptical germination of seeds of *Arnica montana L*. coming from Germany, from the Botanical Garden of the University of Göttingen.

The Ph.D thesis has 169 pages, is divided in 5 chapters, including 19 tables and 53 figures, of which 16 tables and 49 figures are original.

Chapter I – ”The current state of knowledge on the management of technology and the use of the *Arnica montana L.* species” - has been drawn up on the basis of large documents having as bibliographical sources manuals, books on medicinal plants, scientific articles and specialist publications in the field of medicinal plants and containing knowledge on the Arnica *montana L.* species, codex and current information from the internet websites, the Ministry of Agriculture and Rural Development, the 2015 Statistical Yearbook of Romania, etc. This chapter describes the main characteristics of the Arnica *montana L.* species, the situation of the culture in the world and in Romania, the current stage of knowledge on the processes of multiplication of the species at national and international level, the introduction of the plant into the culture, as well as the diseases and the pests detected in the culture, the importance of the culture, pharmacological data and use, legal regulations in the field of medicinal and aromatic plants.

Chapter II –”The natural environmental where the research has been carried out” - describes the organizational framework relating to the location of the research and the characteristics of the soil and climate conditions existing during the experimentation years.

Chapter III entitled ”Material and research method” includes the description and the organization of the experiences, the working methodology, the calculation formulae, the biological material used in the experiments and the equipment used for the determinations carried out.

Chapter IV entitled ”Results obtained” comprises the results obtained in respect of the initiation and the establishment of the *in vitro* cultures, the assessment of the growth of Arnica *montana L.* plantlets obtained in vitro, the initiation and the establishment of cultures in solid substrate, the assessment of the growth of plantlets obtained by sowing, the *ex-vitro* transfer of rooted plants, the assessment of the plants in culture, the description and the analysis of the attack of the pathogen *Phytophthora infestans*, the harvesting and the drying of the material obtained from the culture, the research on the spontaneous flora and the active principles determined from the flowers of *Arnica montana L.*

Chapter V –”Conclusions and recommendations” presents the conclusions and the recommendations resulting from the research undertaken, after the analysis and processing of the data, notes and observations made on the experimental field and in the laboratory, during the experimentation years.

The studies and the research on the management and the exploitation of the species *Arnica montana L.* have led to the following conclusions:

- An effective multiplication of the species *A. Montana* is carried out by the direct morphogenesis with the origin in the apical meristem of the plantlets obtained by the aseptic germination of the seed.

- The aseptic culture mediums have generated on average a seed germination rate of 47.76 %, obtaining a maximum of 82% in the case of the culture medium supplemented with 1000 mg/l polyvinyl pyrrolidone and a minimum of 18.10% in the case of the culture medium composed of 1 mg/l zeatin, 0.1 mg/l alpha-naphthyl acetic acid and 0.25 mg/l gibberellic acid;

- The best answer concerning the number of regenerants/ initial inoculum, were registered on the variant supplemented with 1000 mg/l polyvinyl pyrrolidone. 7 regenerants/ explant have been obtained after the first four weeks of culture, reaching 17 regenerants/ initial explant, and, after eight weeks, the average value was of 14.62 regenerants/ explant;

- The *ex-vitro* acclimatization of well-developed plants (height of 4-5 cm and roots of 7 -10 cm) has been carried out on perlite mixed soil substrate, at a temperature of 20^0C, lighting 2000 lux and humidity ranging between 95 and 75%, with a gradual drop to 75% in the third week;

- At the same time, the unsterilized seeds, which have been treated for two days with distilled water and 5mg/l gibberellic acid, were sown on 3 different solid substrates. The seeds germinated on solid substrate have generated plantlets, whose growth has been assessed in the 4^{th} and 8^{th} week;

- The location of the experimental field offers favourable conditions for the growth and development of *Arnica Montana L.*, both from a perspective of the climate and soil characteristics;

- In 2014, the plants obtained *in vitro* had an average length of the leaves of 7 cm, in 2015, the average length of the leaves was of 13.5 cm, and in the third year an average length of the leaves of 17 cm has been noted in the culture;

- The plants obtained by germination in solid substrate composed of 50 % soil and 50 % sand, had an average length of the leaves of 6.5 cm in 2014, and, in 2015 and 2016, the average was of 14.4 cm, respectively, 17.9 cm;

- The plants germinated in solid substrate composed of 50 % soil and 50 % perlite, had an average length of the leaves of 5.7 cm in 2014, and, in 2015 and 2016, the average was of 11 cm, respectively, 15 cm;

- The plants obtained by germination in the substrate composed of 50 % soil, 25% perlite 25% peat, had generated an average length of the leaves of 4.1 cm in 2014, and, in 2015 and 2016, the average was of 6.8 cm, respectively, 11.4 cm;

- The rate of survival after the first year of culture of the plants has been noted in 2015, with a maximum of 90% obtained in the case of plants germinated on solid substrate composed of soil and sand, the minimum being of 25% in the case of plants germinated on solid substrate composed of soil, perlite and peat;

- In 2016, the highest survival rate has been of 100%. As in the previous year, the best response has been obtained in the case of plants germinated on a substrate made of soil and sand;

- In 2016, the first inflorescences in culture have been obtained, the highest average number of inflorescences on the plant was 21, obtained on the plants obtained *in vitro,* the lowest being of 12 inflorescences on the plant, obtained on plants germinated in a mix of soil, perlite and peat;

- The inflorescences have been assessed in respect of the diameter of the heads, the largest being of 7.8 cm obtained in the case of plants germinated on substrate composed of soil and sand;

- During the years of culture, 10.83 % of the plants have shown visible symptoms of the pathogen *Phytophthora infestans*;

- In 2015 the intensity of the fungi attack had higher values compared to the frequency of the attack on the plants studied, the highest intensity of the attack of the pathogen was of 40.83%, obtained in the case of plants germinated in a mix of soil, perlite and peat, the plants

germinated *in vitro* having an attack intensity of 35 %. The plants germinated in a substrate composed of sand mixed soil and perlite mixed soil have not shown symptoms of the pathogen;

- In 2016, the highest intensity of the attack of the pathogenic agent has been of 27.5 % in the case of plants germinated on a substrate of perlite mixed soil, the lowest being of 0.25 % in the case of plants produced *in vitro*. The plants germinated on a substrate composed of sand mixed soil and of a mix of soil, perlite and peat have not shown symptoms of the pathogen;

- In the 3 years of research, the development of the species has been monitored in 4 areas, 2 areas in the southern region (the siliceous region) and 2 areas in the northern region (the limestone region), which are considered representative for the development of the species *Arnica montana L.* from the region of the Gârda de Sus commune, Alba County;

- The active substances of the inflorescences harvested, respectively collected from the spontaneous flora have been identified by the TLC method (thin-layer chromatography).

- The content in sesquiterpene lactones, volatile oil and polyphenols of the three samples of inflorescences has been examined, namely: sample 1, represented by the inflorescences harvested from the culture, originating from the plants produced *in vitro*, had a content of 0.45% sesquiterpene lactones, 0.23 % thymol and 0.08% phenols; sample 2, represented by the inflorescences harvested from the culture, originating from the sowing in solid substrate, with a content of 0.86 % sesquiterpene lactones, 0.46 % thymol and 0.18% phenols and sample 3, represented by the inflorescences collected from the spontaneous flora, with a content of 0.93 % sesquiterpene lactones, 0.5 % thymol and 0.26% phenols.

The results of the research undertaken have been used in 2 articles published in specialist journals indexed in the international databases, B+ category. The bibliography of the Ph.D thesis includes 160 sources selected from the Romanian and international academic literature (articles from specialist journals, specialist books, Internet portals, scientific papers).

The thesis brings important and current contributions to the management of the species *Arnica montana L.*, through data on the multiplication and the introduction of the plant into the culture, in the context of the climatic conditions existing during the experimentation years and of the biological material researched. The research undertaken in the framework of the Ph.D thesis provides a broad research of the species *Arnica montana L.*, with good results of the culture, the determination of the active principles of the inflorescences, studies of the spontaneous flora, determinations concerning the biology of pathogens, the behaviour of the species under the conditions of the areas studied, results which bring new and interesting data in addition to the results of specialist fundamental research.

Key words: *Arnica montana L., multiplication, culture, active principles*