

SUMMARY

The pharmaceutical importance of the *Digitalis lanata* Ehrh. species was established in the early twentieth century, when it was found that its leaves were richer in cardiac heterozide than those of *Digitalis purpurea*, currently being the raw material for extracting the digitalis glycosides (Ciulei, 1993).

The little woolly finger leaves (*Digitalis lanata*) are not used as such, in fresh or dried forms, but its served as raw material for extraction of active principles, which are used in medicines for the heart failure, having a toning effect on the myocardium.

The lanatoglicozidele are 3-5 times more active than the purpureaglicozidele, obtained from *Digitalis purpurea*, their effect being of shorter length, they are able to eliminate from the body faster.

Currently, all cardiac drugs in our country are obtained from the *Digitalis lanata* leaves, only our industry produces a wide range of such products like Lanatozid C, Nidactil (acetyl digitoxin), digoxin (desacetyl lanatozid C) and others, consisting of their derivatives.

The wide range of cardiac drugs is necessary not only to have at hand preparations that are acting faster or slower, with short or long term effects, but also to continue the treatment in cases of intolerance.

The *Digital* leaves have a diuretic action due to the presence of the saponozids and the flavanols in their composition; the cardiotonic and the diuretic effects persisting for nearly two weeks after preparation.

The fresh leaves, harvested before flowering, are used in homeopathic treatments: first as a cardiotonic remedy, but also for urinary tract diseases, for the water retention in the body, for the nervous depression, sleep disorders, headaches, hepatitis, jaundice and prostates.

From the little finger leaves (*Folium Digitalis*) is prepared the tincture, the dry extract and the infusion, the preparations of the therapeutic activity happening due to the complex of thecardiotonic glycosides, digitoxin, gitoxin and gitaloxin. In literature there are

numerous methods of glycosides extraction, formalized by the Romanian Pharmacopoeia or from other countries.

The PhD thesis called “**Contributions to the Study of the Chemical and Physical Mutagens Effects on Some Morpho-Physiological and Biochemical Characters in the Little Woolly Finger (*Digitalis Lanata*)**” is extended to 186 pages and contains, in accordance with the current legal stipulations, two main parts and comprises V chapters, plus the introduction, the summary, bibliography, tables, figures and graphs.

The first part is a summary of the bibliographical data on the theme of the PhD thesis, on the nature, on the climatic conditions in the experimentation years, on the research material and methods, and the second part presents the results of own research on the subject of the PhD thesis.

After the documentation, I found that foreign literature is quite rich in terms of systematic research, growing technology and chemical composition of this plant.

In Romania, the research on improving the *Digitalis lanata Ehrh* species is very modest, though.

Given the importance of the *Digitalis lanata Ehrh* species., we intend to induce a bigger variability of the main morpho-physiological and biochemical characters, by using chemical mutagens, by seed treatments in the vegetation period on the apical meristems level, in different concentrations and exposure times.

In addition to increasing the quantity of leaves / plant (drug production), the main selection criterion to obtain new forms, will be the chemical one, the amount of cardiac glycosides.

Following the treatment with chemical mutagens that have been studied, their effects, depending on the concentrations used on some morpho-physiological and biochemical characters: the emergence of morphological changes of little finger plants (the size and the shape of the leaves, the number of the leaves in the rosette to the plants in their first year of vegetation, the fresh leaf weight and the weight of them after drying), biochemical indices (the free amino nitrogen, total sugars, reducing sugars and isozymes) and physiological indices (the catalase activity, the vegetation period, the diseases and pests resistance etc.).

During 2007-2010, investigations and observations were conducted and they followed:

- The *Digitalis lanata Ehrh.* species increased variability by the mutations induction by chemicals;
- The behavior of mutant forms to identify the value of biological forms, in terms of productivity and high content of active principles;

- Determining the phases of plant growth in the pedo-climatic conditions from Moldavia.

- The evaluation of the effect induced by chemicals, on the morpho-physiological and biochemical particularities in *Digitalis lanata Ehrh* species.

The biological material was represented by the little woolly finger variety *Lanata L*, created by Silva, F., (1974), at the SCPMA Fundulea. The variety was approved in 1974 and in 1999 it was registered once again in the Official Catalogue of Crops Varieties of Romania.

The *Lanata I*. variety seeds came from the "Biofarm nature naturans" Company from Baiculesti - Curtea de Arges.

The *Lanata I*. variety is characterized by an emphasized polymorphism in terms of morpho-physiological characters, it produces around 1,600 kg / ha of dry leaves, with an average of 0.21% lanatozidă C content, exceeding by almost 55% the average content in this active principle, the local populations of little woolly finger.

The biological material, meaning the little woolly finger seeds, were treated with three chemicals in four concentrations each: the 2,4-D acid (0.01%, 0.02%, 0.03% and 0.04%), the ethidium bromide (0.01%, 0.02%, 0.03% and 0.04%) and the colchicine (0.01%, 0.02%, 0.03% and 0.04%).

On seeds there were applied 3, 6 and 12-hour treatments before they were sown in the field. The chemo-sensitivity had been studied in the laboratory by performing several tests on seed germination capacity.

The cytogenetic investigations were performed on the radicular meristems obtained through the seed germination and the microscopic preparations were obtained through the Feulgen method developed in 1924 (Tîrdea, 2003).

With the help of the cytogenetic methods, during the experiments, I determined the frequency and the aberrations spectrum in the ana-telophase mitosis and I have revealed the cell division intensity under the mutagenic substances action by calculating the mitotic index.

The cytogenetic research was made in the Genetics laboratory of USAMV Iasi.

The qualitative and quantitative chemical study of the leaves was performed on methanol and dichloromethan extracts, by TLC and HPLC, on the 9 lines retained after the selection in the INCDSB / CCB,, Oak" Piatra Neamt.

The qualitative chemical study used the TLC for three groups of active principles of pharmacologically importance: flavonoids phenolic acids triterpene compounds.

The determination of carbohydrate biosynthetic process in the leaves aimed at highlighting the adaptability to the environment and highlighted differences between the treatment variants after the mutagenic chemicals action.

The determination of the carbohydrates in the leaves and the content of pigments was done in the INCDSB / CCB,, Oak"Piatra Neamt.

On enzyme research, determining the catalase activity in leaves and flowers was done by the gas collector method in the Genetics Laboratory of USAMV Iasi.

The biochemical and cytogenetic analysis constituted a selection criterion of the best lines that could be proposed for approval and recommendation later in production.

The data from the observations and measurements made were statistically processed according to the established models mentioned in the specialized literature.

The *Digitalis lanata Ehrh.* species sensitivity to mutagens was determined in three repetitions by calculating the percentage of the germination energy and of the growth rate of the little roots and the little stains.

The significance of the differences between the treaty and control variants were determined by the differences limit method (DL 5%, 1% and 0.1).

For the statistically interpretation of the experimental data regarding the mutagens effect on morpho-physiological and biochemical characteristics there has been used the standard statistical functions of Microsoft Excel of Microsoft Office 10 program for Windows XP.

To determine the action of chemical mutagens on the *Digitalis lanata Ehrh* plants., there have been used several tests recommended by the literature. Thus, there have been determined the changes resulting from the chemical treatments on some biometric parameters such as: the number of leaves per plant, the weight of leaves per plant.

Due to the extremely large number of lines resulting from the treatment, there have been detained nine lines, considered valuable for quantitative characters in the production of leaves from each variant of the treatment carried out at the seed.

The analyzed biometrics elements showed variability under the influence of untreated control treatments applied with small differences statistically insignificant.

The catalase activity was stimulated by the three chemicals studied, in most cases being recorded an increase in this enzyme activity, expressed in U.C. with increasing concentrations.

In what concerns the induced effect by the chemicals treatment on the carbohydrate content of *Digitalis lanata Ehrh.* species leaves, at all concentrations of the three substances used in the experience, the amount of dicarbohydrates registered the highest values for the 6-hour treatments applied to seeds.

The action time and the increasing substances concentrations contributed to the decline in accumulation of soluble poliglucidelor for the 2,4-D acid and the ethidium bromide.

Appreciable quantities of insoluble poliglucide were recorded for the 6 and 12 hours seed treatment and then for the 3 hours seed treatments.

The colchicine had favorable influenced the photosynthesis and the applied concentrations had a stimulating role on the photosynthetic process.

The chlorophylls concentration is negatively correlated with the substances concentration. This suggests that at low concentrations of the substance, the toxic effects are less specific, while at high concentrations depend on it.

The analysis of polyphenol content of plant material (leaves) for the years 2007 and 2010, revealed the presence of active principles (polyphenolic acids and flavonoids) in accordance with the standards that have been used. The pooled analysis of the phenolic acids showed that there were lower values of this parameter compared with the untreated control only at 3 hours treatment with colchicine case.

The average of the two years revealed in the case of the 6 hours treatments with colchicine, the accumulation of the biggest amounts of digoxin.

The colchicine stimulated the synthesis of phenolic acid on the treatments applied to seed for 6 hours.

The ethidium bromide stimulated the accumulation in leaves of these substances on 3 hours treatments, after which we could see a decline in the biosynthesis process as the substance actin time was increased (6 hours).

In conclusion, the climatic conditions of Iasi area satisfy the little woolly finger`s requirements to heat and humidity, and can say that from this point of view, the species can carry out its full cycle of vegetation, and the treatments applied did not affect the phases of vegetation, they were accomplished at the same time to the all versions.