ABSTRACT

of doctoral thesis

"Contributions on producing milk thistle (Silybum marianum [L.] Gaertn.) lines, using chemical mutagenic agents"

The importance of the research of this species derives from the phyto-therapeutic qualities of active principles present in achenes of the milk thistle, known as silymarine highlighted by numerous medical and pharmaceutical studies, making milk thistle (*Silybum marianum* [L.] Gaertn.) one of the most important species of medicinal plants. The spreading area of culture and the achievement of new biological forms is a necessity as the silymarine-based pharmaceutical preparations extracted from achenes of milk thistle with hepatho-protector effects are highly sought on the home market.

For the development of the work and the interpretation of the results obtained, a bibliography was consulted on the issues addressed, appealing to the last news in the field.

The paper is presented on 240 pages, having attached a list which includes a number of 224 titles.

The thesis itself is organized into nine chapters, conclusions and recommendations, comprising 54 tables and 84 figures, plus 10 annexes and 2 drawings attached at the end.

The paper has two distinct parts, the former is a summary of bibliographical data regarding the theme of doctoral thesis, of research on *Silybum marianum* [L.] Gaertn. species in the natural, the climate conditions from the experimental years, the material and methods of research. This part covers 69 pages. In the latter the results of my own research regarding the theme of research are presented 48 tables 79 figures and photo, 10 appendixes, 2 drawings.

Chapter I contains information regarding the importance of medicinal and aromatic plants, worldwide and on national plan(home and abroad), traditions regarding the use of medicinal plants in Romania, the therapeutic potential of medicinal plants.

Chapter II refers to the species taken in study, the origin, spread, importance and utilization of the milk thistle.

In Chapter III morphology, biology and technology for plant growing are presented in reference to the harvest way and the optimal harvest time taking into account the staggered maturation. In preparing this chapter we have synthesized the results of research in Romania and worldwide, regarding some aspects of the biology of species, some technological links but also some index of quality of achenes, to be kept a long period without affecting the content in active principles, as well as research of very recent date regarding the medicinal benefits of preparations

from this plant.

Chapter IV contains data reffering to the current state of research regarding the content of active principles in the plant *Silybum marianum* [L.] Gaertn. and their therapeutic implications, with reference to the bioactive substances present in fruits of the plant, chemical affiliation of active principles, the action way of silymarine in its role of protecting and liver cell regeneration. In this chapter the studies performed both in the country and the world to improve milk thistle are presented.

In Chapter V the purpose, objectives, material and methods of the research are presented. Research objectives include: the induction of variability by using chemical mutagenic agents in the local population De Prahova, the study of the effect of mutagenic chemical substances on the morphological and physiological characters,the selection and retention of valuable elites of M_2 generation, the selection and retention of valuable lines of M_3 generation, the analysis and characterization of lines M_3 retained in the selection field regarding some morphological, physiological characters and biochemical analysis, the establishment of some correlations between some characters of the lines M_3 promoted in the field of control. The ultimate aim of the research is the obtaining through selection of new lines of milk thistle.

The seed used in the experiences of local people De Prahova comes from SCDA Secuieni and was subjected to, before being sown, for 6, 12 and 24 hours, the action of three mutagenic chemical agents in three different concentrations: colchicine (0,1%; 0,15%; 0,2%); methilic ester of methane sulphonic acid (0,0125%; 0,025%; 0,05%); hydroxylamine (0,005%; 0,01%; 0,1%).
The experimental lot was placed as randomized blocks method in three rehearsals together with the untreated witness.

In M_1 and M_2 generations, have made observations regarding the influence of mutagenic chemical agents on the following characters: *morphological characters*: the degree of springing, the degree of survival of plants in the field after 30 days from springing,the plant height, the degree of ramification of plants ;the capitulum number per plant, the achenes number per capitulum; achenes weight per plant; *physiological characters:* duration of vegetation period, duration of flowering period, resistance to shaking, resistance to diseases and pests. Observations and biometric measurements were conducted at plants located in various pheno-phase and at full maturity, achene samples for biochemical analysis have been taken.
Meanings of differences between treated variants and witness were determined by the method of differences limit (DL 5%, 1% and 0.1%). The results of observations were statistically interpreted using the analysis of the variance and the coefficient of variability. In the M_3 generation were

determined average values of quantitative characters (x) and coefficients of variability (s%), and the meanings of differences between treated variants and witness have been determined by the method of differences limit (DL).

In the laboratory was studied the effect of mutagenic agents on: the ability of seed germination; the length of small roots, the small strain growth rate, the mitotic index, the frequency of chromosome aberrations in mitosis.

The biochemical tests were performed to determine the content of: gross fats, gross protein, silymarine, calcium, phosphorus, magnesium.

Chapter VI includes the characterization of natural and ecological conditions of experiments in terms of the medicinal plants requirements and especially *Silybum marianum* [L.] Gaertn., the climate conditions from the year of the experiments were favorable to growth and development of the species.

The experiments have been conducted on nearby the town of Bacau, on the terrace I of Siret river, upon a tipically chernozem , moderate leachate, medium clayey soil. This type of soil is developed normally, slightly acid, medium supplied with the main nutrients: nitrogen, phosphorus and potassium, with a good structure, good solubility with fluid. The average temperatures in the months of March to September in the period of the research development, was 18.18 Celsius degrees, which means good favorability for the growth and development of the milk thistle. The heat is positively correlated with the development in good conditions of flowering, fructification and maturation of seeds.

Between 2004 – 2008, the wettest year was 2005 when a rainfall amount of 840.9 mm (with max. 24 h of 86.4 mm in July) was recorded.

Chapter VII contains the results of the research on the creation of lines of milk thistle, *Silybum marianum* (L.) Gaertn. through the use of some mutagenic chemical agents. The first phase of the research is an assessment of the initial biological material, the local population, De Prahova on some characters and correlations between these characters. Following the evaluation it was found that the population that is currently in culture, has low resistance to shaking, and the production capacity is below the species potential. The obtaining of the new lines and the use of the appropriate technology would lead to the production of stable and superior quality crops .

To test the strength of the biological forms taken in the study and to cause a higher variability within, the seeds have been treated with the three chemical mutagenic substances, each substance having three doses and three times of action on the seeds: 6 hours, 12 hours, 24 hours.

The mutagenic agents used, increased the frequency of chromosome restructuring, and

determined the diminishing of values of morphological characters analyzed.

In our research we proposed the selection and highlighting of the best mutant lines, in terms of quantity and quality.

We have studied the changes resulted from treatments with chemical substances with mutagenic effect on the following characteristics: the ability of seeds germination, small rooth length, the rate of growth of the small strains, mitotic index. Values of these characters have been reduced by the action of chemicals substances used, the decrease being more pronounced with the increasing of concentration and time of exposure. The frequency of chromosome aberrations in mitosis has been increasing. Were identified and photographed chromosome aberrations in meta-phase, ana-phase, telo-phase.

In M_1 and M_2 generations we have studied the effects of mutagenic chemicals substances on morphological characters: the plants sprout degree, the survival degree of plants in the field, variability of plant height, variability of plants ramification degree, variability in the number of achene in capitulum, variability of achenes weight per plant, on some physiological characters: length of vegetation period, time of flowering, resistance to shaking, resistance to diseases and pests.

It's followed the selection of those individuals who manifest superiority over the witness for the characters pursued. Data obtained from observations and calculations performed, have been statistically processed according to models, referred to in literature. As a result of selection, in M_2 generation had been retained a number of 103 elites, the greatest number of elites was obtained after treatments with hydroxylamine of 2.31% of total elites retained by 5,89%.

In Chapter VIII morphological, physiological characters, the production capacity and as well as some qualitative characters for descendents M_3 are presented. In this generation, were studied 81 lines resulted from treatment with the three chemical substances to which were verified the stability of characteristics, their variability, to identify and retain valuable M_3 lines. For that the variability of morphological characters has been followed: plant height, the total number of ramifications per plant, achene number per capitulum and per plant, weight of achenes; variability of physiological characters: length of vegetation period, duration of flowering, shaking resistance, resistance to disease and pests, and variability of qualitative characters: silymarine content, silybine content, the fats content. Biometric measurementsand biochemical analysis for the individual of each variant of work have been conducted , values of variability coefficient (s%) compared with untreated witness for all the characters studied have been analyzed. From the 81 lines M_3, only 9 lines cumulated lower values of coefficients of variability compared with the untreated witness have been analyzed, thus showing to be stable as regards the useful micro-mutations induced by the treatment with

chemical mutagenic agents. These lines are: P-c-3-9.3-07 and P-c-3-5.2-07, resulted from treatments with colchicine; P-h-2-1.6-07, P-h-2-1.9-07, P-h-3-5.6-07 and P-h-3-8.7-07, retained after the treatments with hydroxylamine and three lines after the treatments with methylic ester of methane sulphonic acid (EMAM): P-e-2-7.4-07, P-e-3-9.5-07, P-e-3-6.2-07. The morphological, physiological, biochemical investigations performed in our experiments, have helped us in the selection of the best lines in order to obtain a new variety.

The results support the idea that the use of chemical substances in low doses, and for an exposure time of 8 hours or 12 hours, followed by a rigorous selection in mutant generations, may be an effective method of obtaining of new genotypes, higher to the untreated witness.

From the analysis of results obtained in the research conducted between 2004-2008, which had as main purpose the creation of valuable genotypes by induced muta-genesys, it was found that the application of different doses of mutagenic substances used, has led to the pheno-typical manifestation of some important changes of character studied at the plants of *S. marianum*, the local population De Prahova and confirmed for the 9 lines retained, a real superiority over the untreated witness expressed by differences, from distinctly significant to very significant.

Therefore, the results obtained give us the right to say that in this species, the treatment with chemical mutagenic substances is an effective method to increase the genetic variability, to obtain new biological forms.

Chapter IX refers to correlations between some morphological and quality characters in descendents of generation M_3, resulted from the treatments with the three chemicals substances mentioned, and correlations between 78 pairs of characters at lines M_3 promoted in the control field. The purpose of this calculation was the highlightening of effects of mutagenic substances on the content of silymarine and main mineral elements that are in achenes and to highlight the links between morphological and quality characters, which will help us to guide the selection process of useful forms according to the proposed objectives.

As a result of the treatments applied to seeds, the plants of the milk thistle, belonging to the lines promoted in the field of control, have been registered to the analyzed characters, values of the coefficients of correlation modified by the dose and time of application of mutagenic substance. The results obtained show that the treatment with chemical mutagenic substances is an effective method to increase genetic variability. Based on the results obtained during the experiments, some conclusions and proposals to extend the area of crop of species *Silybum marianum* (L.) Gaertn., have been made, to continue the research on other biochemical compounds present in achenes as well as in the vegetal material of plant too.