
ABSTRACT

The deep knowledge of medicinal plants, including the *Calendula*, the broadening of their use has taken a fairly large scale, especially in the second half of last century, for their cultivation being interested more and more people, because of the exceptional therapeutic qualities they possess and which are based on biologically active complex.

For the first time the *Calendula officinalis* L. species is introduced in the taxonomic hierarchy by Carl Linnaeus in his „Species Plantarum”, published in 1753 (Brânzilă, 2005).

Currently, the *Calendula officinalis* L. is among the ten most grown herbs in Europe (Hoppe, 1997).

The special therapeutic properties of calendula are determined by the complex of biologically active substances, contained in the material: carotenoids (provitamin A), sterine, triterpenoids, flavonoids, flavone glycosides, volatile oil, coumarins, minerals and bitter substances, gumirins, mucilages, vitamin C, cholesterol esters, protein substances (Britton, 1996). In the countries where this species is cultivated, the work is to achieve by improvement works, big, abundant, orange inflorescence (Craciun, 1977).

The Ph. D thesis entitled „***Research on the effect of mutagenic agents on morpho-physiological and biochemical characters in Calendula officinalis L.***” is extended on 255 pages and is made in accordance with current legal provisions, with two main parts and comprises eight chapters, plus introduction, summary, bibliography, tables, figures and graphs.

The first part is a summary of bibliographic data on Ph. D. thesis theme, the natural climatic conditions in the years of experimentation, research material and methods, and the second part presents the results of the own research, on the Ph. D. thesis topic.

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

In Romania, the research on improving the *Calendula officinalis* L. species are quite modest.

The experiments conducted between 2005-2008, under field conditions, at the Ezareni farm of the Teaching Science Station, and in the laboratory had as main purpose to obtain improved lines in *Calendula officinalis* L. in the production of inflorescences, rich in active principles, important for the pharmaceutical industry.

This involved the assessment of genetic, biochemical and morpho-physiological features of different test lines of *Calendula officinalis* L. in the absence and presence of treatment with chemical mutagens.

During 2005-2008, investigations and comments were made, that followed:

- The increase of the variability of the *Calendula officinalis* L. species by induction of mutations using chemicals;
- The behavior of mutants for identification of the valuable biological forms in terms of productivity and high content of active principles;
- The determination of the phases of plant growth in pedo-climatic conditions of Iași.
- The assessment of the effect induced by chemicals on morpho-physiological and biochemical peculiarities in the *Calendula officinalis* L. species.

As biological material for research there have been used seeds of *Calendula officinalis* L., the Petrana variety created at the University of Agricultural Sciences and Veterinary Medicine of Bucharest, in 1990.

The substances used in our research had varying concentrations: 0.01% 0.02% 0.03% and 0.04%.

Some of the treatments were applied to seeds, for 3 and 6 hours before they were sown in the field, and some treatments were made in the field after the plants from untreated seed, reached the growing stage of 4-6 leaflets.

The chemical sensitivity has been studied in the laboratory, by conducting some tests on the seed germination capacity.

The cytogenetic investigations were performed on root meristems obtained by seed germination and microscopic preparations were obtained using the Feulgen method developed in 1924 (Țirdea, 2003).

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

The qualitative and quantitative chemical study of the inflorescences was done on methanol and dichloromethane extracts by TLC and HPLC, in the nine selected lines after selection in the M₂ and M₃ generation at the INCDSB / CCB,, Stejarul” Piatra Neamt.

The qualitative chemical study used the TLC for three groups of active pharmacologically important principles: flavonoids, phenolic acids, triterpenoid compounds

The determination of carbohydrate biosynthetic process from leaves followed the highlight of the adaptability to environment capacity and the differences between treatment variants from the action of the mutagenic chemicals.

The determination of leaf carbohydrates, has been made using the Bertrand method, combined with Borel method in the biochemistry laboratory of the Faculty of Biology, from the „Al. I. Cuza" University of Iasi.

The determination of pigments in the vegetation period was carried out using the spectrophotometric method in the oenological research laboratory of UASVM Iasi.

For the enzyme research, the determination of the catalase activity in leaves and flowers was done by using the gasometric method, with an improved device (modified) from the Plant Physiology Laboratory of UASVM Iasi.

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

The data derived from observations and determinations made were statistically processed according to established models, mentioned in the literature.

The M1 generation, the sensitivity to mutagens of the species *Calendula officinalis* L. was determined in three repetitions, by calculating the percentage of germination energy, growth rate of the roots and strains and calculating the correlation coefficient between the intensity of forming the floral buds and the substance concentration, namely the frequency and spectrum of mutations in mitosis.

The significances of differences between treated and control variants were determined by the limit differences method (DL 5%, 1% and 0.1%). Quantitative characters were interpreted statistically using analysis of variance and coefficient of variability.

For the statistic interpretation of experimental data on the effect of mutagenic agents on morpho-physiological and biochemical characteristics were used standard functions of Microsoft Excel statistical component of Microsoft Office 10 program group for Windows XP, by calculating the correlation coefficient.

Making selection in the experimental field was carried out after the half-seed method (Munteanu, 2000), typical for the cross-fertilized plants as is *Calendula officinalis* L.

To determine the chemical mutagen action on the *Calendula officinalis* L. plants were used several tests recommended by the literature. So, have determined the changes resulting from chemical treatments on some biometric parameters namely: the number of inflorescences per plant, the number of buds per plant, the number of leaves on the plant,

leaves weight per plant, number of flowers to fruition, number of flowers fruited, the number of branches per plant, stem and branch weight.

It has been established a link between the concentrations of chemicals used for treatment and their ability to stimulate the formation of inflorescences, this having, of course, impact on production.

For the experimental years 2007 and 2008, the biometric measurements were made only for selected forms from the selection of plants considered to be mutant. Selection was made by morphological, biochemical, physiological and cytogenetic criteria and thus were retained the forms that met superior characteristics to the untreated control.

Because of the very large number of lines resulting from the treatment from the M₂ generation, there have been detained nine lines, considered valuable for quantitative characters in the production of inflorescences of each variant of treatment carried out both at the seed and the growth vegetative peak.

The biometric elements analyzed showed variability under the influence of the treatments compared to the untreated contro, with to small differences statistically insignificant.

Catalase values from the treatments carried out on the vegetative peak for all variants have an increased activity compared with the catalase activity when the treatment was applied at seeds for 3 and 6 hours.

Regarding the *induced effect of the chemical treatment on the carbohydrate content of leaves* from *Calendula officinalis* L., at all concentrations of the three substances used in experience, **the amount of di carbohydrates** recorded highest values for seed treatment applied for 6 hours.

The time for action and increased concentrations of substances contributed to the reduction of the acumulation process of **soluble poli carbohydrates** for the 2.4 D acid and ethidium bromide.

Appreciable amounts of **insoluble poli carbohydrates** were recorded for seed treatment carried out for 6 hours, followed by the one aplied on the vegetative peak and then by the seed treatments carried out for 3 hours.

The colchicine has favorable influenced the **photosynthesis** and the concentrations applied had an incentive role to the photosynthetic process.

For the ethidium bromide (treatment performed on growth vegetative peak) there is a reversal of values recorded for treatments with colchicine manifested by a lower foliar pigments, especially photosynthetic in all the phases of vegetation.

At the experiences made with 2,4 D, it can be noticed that in both absorption bands for the treatments made on the growth vegetative peak, the absorbance values decrease with the growing of the concentration of the substance. This behavior can be considered as a compensation reaction in the metabolic, biochemical and physiological activity, to ensure optimal photosynthesis.

The chlorophylls concentration is negatively correlated with the one of the substances in all phases of vegetation in which the determinations were made. This suggests that at low concentrations of substance the toxic effects are less specific, while at high concentrations those depend on it.

The production values recorded for the 36 lines obtained in late 2006, following chemical treatments, constituted another selection criteria for the years 2007 and 2008 of the most productive variants.

For 2008, for the 2.4 D acid, the production differences were positive for the vegetative peak treatment variant and negative for the seed treatments applied for 3 and 6 hours, compared to the control variant.

The analysis of polyphenol content of plant material (inflorescences) for 2007 and 2008, revealed the presence of active principles (polyphenolic acids and flavonoids) in accordance with the standards used. Cumulative analysis showed that phenolic acids had low values compared with the untreated control, just only when it was treated with colchicine for 3 hours.

The average of the two years revealed for the colchicine treatments carried out for 6 hours, the accumulation of the largest amounts of flavones (117.31 mg/100 g dry plant material).

Colchicine has stimulated the synthesis of phenolic acids in the treatments applied on the growth vegetative peak, and also for the time of action of 6 hours.

The ethidium bromide has stimulated the accumulation in the inflorescences of these substances for the 3 hours treatments, after which it could be noticed a reduction in the biosynthesis process as the action time of the substance was increased (6 hours).

In conclusion, the climatic conditions of the Iasi area meet the full requirements of Calendula for heat and it can be said that from this point of view, the species may carry the full cycle of vegetation, and the applied treatments did not affect the growth phases, each being carried out on all variants simultaneously.