

ABSTRACT

Two species of the family *Lamiaceae*, respectively, *Ocimum basilicum* L., *Dracocephalum moldavica* L., and two *Asteraceae* family, respectively *Calendula officinalis* L., *Cynara scolymus* L., were selected for the study. **The purpose** of the research is to determine the influence of ecological foliar fertilizers on the production and quality of the studied species in the pedoclimatic conditions in Iasi.

The objectives for achieving the proposed goal were:

1. Specifying the influence of organic foliar fertilizers on the production of the studied species
2. Determination of the influence of ecological foliar fertilization on the physiological processes in the studied species
3. Highlighting the influence of organic products used on some metabolites in the studied species
4. Determining the influence of ecological fertilization on volatile oils or other compounds of studied species.

The thesis is structured in **two parts** and **seven chapters**.

The first part of the thesis contains **two chapters** referring to the current state of the research regarding ecological technologies, by comparison to the conventional ones, as well as research on the use of organic fertilizers in the cultivation of medicinal plants. The last chapter of the first part and **the third chapter** of this thesis make some references to the ecological cultivation technologies of the studied species: Basil (*Ocimum basilicum* L.), *Dracocephalum moldavica* L., *Calendula officinalis* L., artichoke (*Cynara scolymus* L.).

The second part of the thesis, structured in **four chapters**, contains details of the own contributions made in the research.

Then, **the fourth chapter** of the thesis specifies the purpose, objectives and importance of the researches undertaken. The biological material used consisted of seeds of the studied species, and for foliar fertilization water was used for control plants and four foliar fertilizers: Fylo®, Geolino Plants & Flowers®, Cropmax®, Fitokondi®.

The method of settling experiments in the experimental field was in the form of subdivided plots, and the setting up of experiments was carried out at calendar dates specific to each studied species.

The fertilization was applied in different phenophases for each species: for basil, marrow and marigolds, at the beginning of vegetation (2-3 pairs of leaves), at the appearance of the flowering rod, during flowering, and for artichokes at the beginning of the vegetation with 2-3 pairs of leaves) and during vegetative development (6-8 leaves).

The experience was bifactorial, and the factors investigated in the studied species were:

- **Factor A - Organic fertilization with 5 graduations:**
 - a1 - Control (sprinkled with water)
 - a2 – Fylo
 - a3 - Geolino Plants & Flowers
 - a4- Cropmax
 - a5-Fitokondi
- **Factor B - Number of applications, at each fertilization, with 2 graduations:**
 - b1 - full dose applied once
 - b2 - a half dose, applied twice, during the same phenophase, at 7 days

In the same chapter, there are also detailed methods of research performed in the laboratory, namely: **the physiological methods** used to determine the chlorophyll fluorescence, the intensity of the photosynthesis processes, the quantity of assimilable pigments, **biochemical methods (determination of phenol content, flavonoids, antioxidant activity)**, **extraction methods and quantitative and qualitative analysis of essential oils (gravimetric method, GC MS) (SilicaGel, Sephadex, TLC, HPLC, LC-MS, CCS, SemiPrep, PrepChrom)**, **the chromatographic methods for the isolation of**

new compounds or the analysis of some compounds from the studied plants under the influence of ecological fertilization, and **statistical methods of processing the results**.

The fifth chapter describes the natural and institutional framework where the researches from 2014-2017, respectively, took place within the Experimental Field of the Faculty of Physiotechnics belonging to the "Vasile Adamachi" Didactic Station, and the analyses in three laboratories: Laboratory of biomonitoring and environmental quality remediation CERNESIM - "Alexandru Ioan Cuza" University of Iasi, Laboratory of Chemistry of Natural Products and Analytical Chemistry - MAICh, Chania, Crete, Department of Biology Pharmacy, Christian-Albrecht University of Kiel, Germany.

The sixth chapter includes the **results of the research** conducted for each species on the influence of organic foliar fertilizers on **production, physiological processes, antioxidant activity and polyphenols content, on the amount and/or composition of the essential oil as well as some of the main compounds of plant material**.

In *Ocimum basilicum* L., the application of organic foliar fertilizers has resulted in an increase in production on average over two years of experience, ranging from 21% to 40%, in all fertilized variants, yielding very significant positive controls compared to the control. The fractional dose had, on average, over the course of two years, a very significant negative influence, with the largest production being recorded on variants where the full fertilizer dose (recommended by the producer).

Regarding the fertilization interaction x dose, on average over the course of two experimental years, the majority of the variants recorded positive differences very significantly in comparison to the control. Higher yields obtained from the Fitokondi fertilizer (on average 31489.3 kg / ha) are probably due to the biohumus in its composition along with the other micro and macroelements.

The intensity of photosynthesis of basil plants was positively influenced differentiated in 2015 by Fylo and Geolino fertilizers and in 2016 by Cropmax and Fitokondi, probably due to different climate conditions during the two years of experimentation, as well as the moments of readings and of the time intervals from the application of the treatments. As for the influence of the chemical composition of fertilizers on the photosynthesis process, we could say that the higher photosynthesis intensity in Fylo plants is probably due to the presence of macroelements, organic carbon and many vitamins. Fitokondi contains more potassium, which could underline the fact that basil is influenced by the transport of assimilates. Cropmax contains a significant percentage of magnesium, iron, manganese and zinc, which affects photosynthesis as a whole, even if there are no differences at the time of determinations.

Chlorophyll fluorescence values in basil culture were generally not significantly influenced by organic foliar fertilizer treatments, which did not exert considerable effects on the effectiveness of the photosynthetic apparatus. Generally, the content of phenolic and flavonoid compounds was stimulated in the culture of basil fertilized compared to the control for two consecutive years, with an increase of 24% to 47%.

The antioxidant potential of basil extract generally showed higher values in plants treated with organic fertilizers, although the differences were not statistically significant. During both years, the treatment with Fitokondi obtained higher values, followed by the one with Geolino and Fylo, compared to control plants.

Both in 2015 and in 2016, the essential oil content of the dried flowers and leaves was influenced by the application of organic foliar fertilizers. The essential oil content ranged between 0.2% and 0.42% (for basil leaves) and between 0.52% and 0.69% (for basil blossoms).

GC-MS analysis indicated the presence of 41 compounds representing 98.54-99.26% of the oils. Our study showed that those predominant compounds in the essential oil of all the samples that were analyzed were linalool (37.44-49.46%), α -muurolol (11.26 - 19.26%), methyl chavicol (2.87-10.39%), eugenol (3.65-8.98%), γ -cadinene (3.13-4.79%), α -bergamotene (3.02-4.53%), eucalyptol (1.82-4.48%), germacrene D (1.66-3.29%), cubenol (1.41-4.48%). Other compounds were also present in less than 2%. The application of organic foliar fertilizers induced the change of the individual content of oil compounds. In addition, the essential oils extracted from the treated basil leaves showed a series of compounds that were not detected in the control.

The percentage variation of certain constituents of the analyzed oils can be attributed to macronutrient supply, which is recognized for playing a major role in the biosynthesis of terpenoids (Sell, 2003). The foliar fertilizers used in this research also contain macroelements: N, P and K, which are known for positively influencing the growth of medicinal plants and the synthesis of essential oils.

According to the LC-MS' analysis, in *Ocimum basilicum* L. revealed the influence of ecological fertilization which, in some treatment variants, produced increases but also decreases in the concentration of quercitrin, rosmarinic acid and rutin, present in leaves or flowers.

In the case of *Dracocephalum moldavica* L., on average over the period of two years, under experimental conditions in Iasi, three fertilized variants produced positive results significantly over the control and one positive distinctly significant production. The effectiveness of all organic foliar fertilizers was highlighted by the increase in production compared to the control having values between 33% and 13%. Existing plant growth stimulants in the composition have probably led to an increase in Cropmax fertilized plants (14823.4 kg / ha) compared to other treatment variants.

Regarding the application of the fertilizers, in the year 2015 and in 2016, the highest yields of 16616.7 kg/ha (2015) and 17790.0 kg/ha (2016) were obtained in the variants where the whole fertilizer dose (control) was applied. Regarding the intensity of photosynthesis and fluorescence of the plants of rosacea, in the years 2015-2016, higher values were recorded for fertilizers with higher nitrogen content, but the differences between the results were not statistically significant.

In the period of two years, the application of organic foliar fertilizers produced the highest value of chlorophyll pigment content in plants treated with Cropmax, and the lowest in Fylo, the differences being not statistically significant.

Synthesis of phenolic compounds in rosacea plants during two years of cultivation has been enhanced by ecological foliar fertilization. In 2015, increases in flavonoid content ranging between 21.5% (Geolino) and 56% (Fitokondi), and in 2016 the same content was higher by 13.3% (Fylo) and 66, 8% (Fitokondi). The total content of phenols was increased by the use of foliar fertilizers by 34% (Fitokondi) and 37% (Fylo) in 2015, while in 2016 the increases varied between 30.2% (Geolino) and 50% (Cropmax).

Gravimetric methods and GS-MS analyses have highlighted the influence of organic foliar fertilizers on the oil content, the concentration of the main compounds of essential oil extracted from the leaves and flowers of *Dracocephalum moldavica* L. Thus, the oil content of the leaves increased significantly, in the case of two fertilizers, namely Cropmax (0.20%) and Fitokondi (0.23%).

The percentage change in the production of oil and some of the essential oil of *Dracocephalum moldavica* L. can be attributed to the composition of fertilizers, but also to pedoclimatic conditions, agricultural therapies or ecological factors; taking into account all these factors, it can be said that the oil of rosacea can change its composition, the main compounds being geranyl acetate, geranial, neral, followed by geraniol and neril acetate. The difference in composition is revealed by other studies that highlighted other main compounds such as citral and linalool.

According to the LC-MS' analysis, *Dracocephalum moldavica* L. leaves revealed how ecological foliar fertilization produced a decrease in the concentration of specific compounds such as apigenin, luteolin. Also, in the flowers of *Dracocephalum moldavica* L., the compounds under the influence of ecological fertilization were retinal, apigenin, komarovinone A, having lower concentrations in the fertilized Fylo, Geolino and Cropmax samples compared to the control. Fitokondi increased the concentrations of the compounds compared to the control.

By thin-layer chromatography and high-performance liquid chromatography, the isolation of dried herb DPA from *Dracocephalum moldavica* L. was started. The data obtained so far reveals the possible identification of three compounds; for the other compounds not enough substance could be isolated for structure elucidation so that research could continue.

On average, in the period between 2015-2016, in the production of fresh inflorescences of *Calendula officinalis* L., there were percentage increases in foliar fertilized variants ranging from 18% to 29% compared to the control.

During two years of experimental field research, the application of the whole fertilizer dose recorded an average yield of 7884.7 kg /ha, fresh inflorescences being higher than the fractional dose; dose interaction x fertilizer recorded mean significant positive differences in half the treatment variants

In order to ensure maximum productivity, it is advisable to harvest 2-3 days in high temperature periods, reaching 4-5 days depending on climatic conditions. The staggering and the dynamics of production highlight in 2015 a maximum of production that was obtained between August 15 and 31, and in 2016, during July 1-15.

Although physiological processes (photosynthesis, fluorescence, chlorophyll pigments) varied in marigold plants, they did not induce negative changes in primary metabolism (growth, development, production), but the changes were more pronounced in secondary metabolism, so the amount of phenols, flavonoids, and antioxidant activity, increased the amount of oil in some treatments to the control.

For example, in the first year, the highest total phenol content (+ 51%), total flavonoids (+ 84%) and antioxidant activity (+ 43%) were obtained in Fitokondi fertilized plants, compared to control . In the second year, monthly measurements showed that the highest total flavonoid content (36.4 mg quercetin / g dry weight) and highest free radical removal capacity (83%) were recorded in September for treatment with Geolino while the total content of maximum phenols (6.7 mg gallic acid / g dry weight) was recorded in July. Fertilization, in some months, resulted in an increase in the total content of flavonoids (up to 53%) and phenols (by 60%) under Cropmax treatment, while antioxidant activity was improved mainly by Fitokondi, with a 100% increase compared to the control.

In *Cynara scolymus* L., the conditions of fertilization and irrigation, the cutting mode, the harvesting period and the climate conditions in the two years of experimentation have led to the production of average yields of about 35-40 t/ha; the percentage increases in fertilized plants compared to the control were between 14% and 39%.

In terms of fertilizer application, on average, in the two years of experiment, the fractional dose had a very significant negative effect, with the highest yields being recorded on fertilized full-grain fertilizer plants (the one recommended by the producer).

Generally, artichoke plants had optimal growth conditions and variations in physiological processes were within normal range, lower values, indicating that fertilizers did not induce stress in plants.

On average, over the two years of 2015-2016, organic foliar fertilizers generally favored a higher content of flavonoids and phenols in artichoke plants.

Organic fertilization has increased the concentration of specific compounds in artichoke leaves, cynarine, luteolin-7-o-glucoside, chlorogenic acid, compounds recognized as having a major role in the control of liver disease or cancer.

The general conclusions of the thesis expressed in the seventh chapter highlight the achievement of the aim and objectives of the researches carried out as well as the opening of new perspectives of approach or the continuation of practical investigations for other studies or comparative researches.