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FACULTY OF BIOLOGY
DOCTORAL SCHOOL OF BIOLOGY

DOCTORAL THESIS SUMMARY

Biological effects induced by treatments with *Colchicum autumnale* L. extracts on taxa of the genus *Ocimum* L., under experimental cultivation conditions

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DOCTORAL THESIS CONTENT

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Introduction

Allelopathy is an ecological phenomenon by which plants provide themselves with a competitive advantage through biochemical pathways, in which the secondary metabolites they produce influence the growth and development of other organisms in an inhibitory or stimulatory manner, properties that give them a special potential in solving problems in agriculture (Macías et al., 2007; Duke, 2015; Amist, Li and Bai, 2019).

Environmental contamination through the excessive use of synthetic herbicides and pesticides in agriculture, which is constantly increasing due to the acquired resistance of weeds to synthetic compounds that are supposedly toxic or inhibitory, and the destruction of crop plants by pests or the inhibition of their development through competition with invasive plants are just some of the current problems in agriculture (Amist, Li and Bai, 2019). Among the possible solutions to these problems are allelopathic compounds of plant origin which, unlike synthetic compounds, are water-soluble and less toxic molecules; in addition, they have a relatively short half-life and are therefore safer for the environment (Ma et al., 2006).

To date, allelopathy has been studied from the point of view of classical applications based on the management of weeds in crops to obtain natural herbicides and pests and pathogens to obtain natural pesticides (Farooq et al., 2011). However, allelopathy may also find application as a means of stimulating the resistance of crop plants, by capitalizing on the properties of some secondary metabolites (Ali et al., 2019).

Allelopathic compounds can influence the composition, structure and dynamics of native and cultivated plant communities by exerting biological effects on plants, inhibiting or stimulating certain physiological and biochemical processes, such as germination rate, photosynthesis, respiration, enzymatic activity (Inderjit, Weston and Duke, 2005).

There are allelopathic secondary metabolites with effects on microtubules, which are also considered anti-mitotic compounds because they induce cell cycle deregulation. One such compound is colchicine, usually extracted from *Colchicum autumnale* L. and used in research and plant breeding to alter the ploidy level in plant organisms, owing to its property of inhibiting tubulin polymerization, division spindle formation, and, implicitly, cell division (Paul, Halder and Jha, 2013). The use of this compound or extracts containing this compound

in experimental studies of allelopathy could provide a better perspective on this biological phenomenon.

Knowledge of the mode of action or effects of a compound is of particular importance in allelopathy studies, because the allelopathic properties of a compound can be experimentally demonstrated by developing and applying a methodology to monitor a parameter specifically associated with the mode of action of the compound in a dose-dependent manner (Duke, 2015).

Furthermore, the use of allelopathic compounds such as colchicine in agriculture could have a positive impact on test plants, manifested by increased biomass, resistance to pests, interspecific competition, adverse environmental or stress conditions, and/or the production of secondary metabolites (Iannicelli et al., 2020; Manzoor et al., 2019).

In the context of what has been discussed, the present work proposes an experimental study that includes the exploration of different approaches regarding the applicability of allelopathy in plant cultivation, by developing an experimental methodology for studying the allelopathic effects of using different treatment methods with alcoholic and aqueous plant extracts of *Colchicum autumnale* L., capitalizing on the anti-mitotic properties of some allelopathic compounds in the extracts (colchicine and its derivative compounds) and of exploratory methods for observing and analyzing the biological effects induced by these compounds in test plants, represented by two cultivars of *Ocimum basilicum* L. This study also aimed to analyze the quantitative variations in the content of secondary metabolites with biological activity in different organs of *C. autumnale* L. species during the flowering, growth, and fruiting stages to provide a perspective on the defensive mechanisms and adaptation of a species with allelopathic potential during its ontogenetic cycle to environmental conditions.

Aim and objectives

The aim of this study was to analyze and evaluate the biological effects of plant extracts prepared from the organs of *Colchicum autumnale* L. on some taxa of the genus *Ocimum* L., to determine their level of phytotoxicity and possible allelopathic potential, and their application as biological treatments in the agriculture of plants of interest.

The objectives that were pursued in the research are:

1. Characterization of alcoholic and aqueous extracts with possible allelopathic potential prepared from vegetative and reproductive organs of *C. autumnale* L. plants harvested from the spontaneous flora of Romania.

2. Testing of treatment options (alcoholic and aqueous extracts prepared from vegetative and reproductive organs of *C. autumnale* L. plants) and of the methods of their application to test plant organisms (taxa of the genus *Ocimum* L.), to determine their level of phytotoxicity.

3. Analysis of morphological, anatomical, biochemical and physiological parameters of test plant organisms (taxa of the genus *Ocimum* L.) subjected to treatments with alcoholic and aqueous extracts of *C. autumnale* L., to determine the stimulatory or inhibitory effects induced by these extracts.

4. Comparison of the results regarding the responses of test plant organisms to treatments with alcoholic and aqueous extracts obtained from vegetative and reproductive organs of *C. autumnale* L. plants.

5. Determination of the possible allelopathic potential of extracts obtained from different organs of *C. autumnale* L. plants and their application as biological treatments in organic agriculture to plants of economic interest (ornamental, medicinal, and culinary).

Current state of knowledge of the phenomenon of allelopathy

Experimental studies on allelopathy begin with establishing the allelopathic potential of a species by determining the secondary metabolites it produces and their effects on target organisms (Duke, 2015). Researchers have developed various experimental variants over time to study this phenomenon, such as growing seeds of the recipient species on plant tissue residues from the donor plant or treating seeds of the recipient plant with extracts from the donor plant (Keech et al., 2005; Lau et al., 2008; Wurst et al., 2010), growing donor and recipient plants simultaneously (Fujii et al., 2007; Dayan et al., 2009), or growing the recipient plant in the soil in which the donor plant was cultivated (Duke, 2015).

Allelopathy research in the last 10 years has demonstrated that exposure of a plant organism to allelopathic compounds induces oxidative stress through the accumulation of reactive oxygen species in cells (Gniazdowska et al., 2015), which can result in apoptosis through the activation of proteases, a mechanism by which the plant sacrifices its affected tissues so that healthy tissues can survive, processes that gradually lead to morphological and anatomical changes, manifested by the inhibition of growth and development (Araniti et al., 2018; Šoln, Klemenčič and Dolenc Koce, 2022). Thus, morphometric, morphological, and anatomical parameters are important to monitor in allelopathy studies because they can provide clues regarding the mode of action and effects of the allelopathic compounds.

Allelopathic compounds can affect plant physiological processes by altering the proteins involved in photosynthesis and chloroplast structure, which can lead to reduced photosynthetic pigment content, decreased electron transfer rate, stomatal conductance, and transpiration rate (Yu et al., 2003; Wu et al., 2004; Yu et al., 2006; Bakhshayeshan-Agdam et al., 2020). Therefore, it is important to monitor variations in the assimilatory pigment content when studying the effects of allelopathic compounds on plants. The decrease in chlorophyll and carotenoid content in leaves in response to allelopathic stress may result from an imbalance in cellular homeostasis (Singh and Sunaina, 2014).

Another important parameter to monitor for determining the effects of allelopathic compounds on test plants is the amount of polyphenols, which are important biochemical indices for determining the level of stress in the studied plant organism, functioning by inhibiting reactive oxygen species (Agati and Tattini, 2010; Li et al., 2014). Flavonoids, a class of polyphenols involved in seed germination, growth, and development, protect plants

from oxidative stress (Pollastri and Tattini, 2011; Agati et al., 2012). Thus, an increase in the phenolic compound content may indicate an increased level of allelopathic stress in the studied plant organism.

The use of extracts from the organs of a species with allelopathic potential on the seeds and meristems of the test species plantlets represents a strategy to amplify the allelopathic effects of the donor species, so that they are observable and quantifiable in the recipient species, with short-term treatments being applied, according to the recommendations of researchers who have studied allelopathy in depth (Ciniglia et al., 2015; Tucker Serniak, 2016; Yan et al., 2016; Araniti et al., 2018; Šoln, Klemenčič and Dolenc Koce, 2022).

Seeds and plantlets are more sensitive than mature plants to environmental changes or stress factors (Dolenc Koce and Šoln, 2018) and represent ideal test organisms when studying allelopathic effects because they present quantifiable responses by calculating germination and growth indices. Meristems represent tissues that are not yet differentiated, with intense metabolic activity, but are also very sensitive to stress conditions or the action of compounds with biological activity, such as colchicine.

However, potential antagonistic or synergistic interactions with other soil compounds, the growth stage and physiological state of the recipient plant, the soil microbiota (especially the rhizosphere), soil moisture, temperature, and other factors (or variables) complicate the identification of the allelopathic capacities of plants (Duke, 2015).

The planting density of the recipient species can also influence the outcome in terms of determining the presence of allelopathy in the donor species. The effect of an allelopathic compound may diminish if the planting density of the recipient species increases because there is less phytotoxin per recipient plant. Another limitation of allelopathy studies is that the plant producing the putative allelopathic compound can only produce enough of it in the presence of a specific target plant species (Duke, 2015).

Descriptive aspects of the plant material used

Colchicum autumnale L. (family Colchicaceae), although a toxic plant species, is used in research and medicine as a source of colchicine. It also contains other secondary metabolites with allelopathic properties, such as polyphenols.

Colchicum autumnale L. was studied to determine its possible allelopathic potential towards plant species of economic interest (in this case, ornamental, culinary, and medicinal) applicable in biological treatments in organic agriculture. It presents an unusual ontogenetic cycle, the knowledge of which is important for establishing the moment of collection of plant material from the field intended for analysis. The cycle begins with the flowering stage, which occurs in autumn (September-October), followed by the winter period, in which the fruit is withdrawn into the soil by contractile roots, thus being protected from low temperatures. During the spring period (March-May), the plant is most photosynthetically active, and the fruits begin to appear above the ground, surrounded by leaves. This is followed by the fruit ripening period in July, and then the dormant period (August) (Jung et al., 2011).

Davoodi et al. (2021) identified the presence of alkaloids, phenolic compounds, tannins, flavonoids, coumarins, saponins, terpenoids, steroids and glycosides in the content of alcoholic extracts of *C. autumnale* L.

Colchicine, used in research and plant breeding to increase the amount of genetic material in plant organisms because of its known anti-mitotic properties (Paul, Halder and Jha, 2013), can also be considered an allelopathic compound because it induces effects at the cellular level, such as chromosomal abnormalities (chromosome agglomeration, condensation, and adhesiveness) (Udofia et al., 2024), but also at the morphological and physiological levels. These effects can be realized by inhibiting the germination and growth of plantlets of some species exposed to certain doses for a given period of time (Mangena, 2021; Zhuo et al., 2021; Manzoor et al., 2023; Zeinullina et al., 2023) or by increasing the biomass or size of some organs in other species (Niazian and Nalousi, 2020). Colchicine can indirectly affect the physiological processes of plants, such as the water regime or photosynthetic capacity, by inducing morphological changes, such as increasing the size of stomata on the leaf surface, simultaneously with reducing their density (Grouh et al., 2011; Sadhukhan et al., 2014; Gao et al., 2017). However, this compound can stimulate the

biosynthesis of secondary metabolites of pharmaceutical interest in plants, such as alkaloids, phenols, and anthocyanin compounds (Dewitte, Van Laere and Van Huylenbroeck, 2012; Mostafa and Alhamd, 2016; Al-Jubouri and Alqaisi, 2023).

Ocimum basilicum L. (basil), chosen as the test species, is an erect, annual herbaceous plant with a tetra-lobed stem and is taxonomically classified in the Lamiaceae family.

The 'Italiano Classico' cultivar, also marketed under the name 'Genovese', is a cultivar of sweet basil with oval leaves that fold slightly inward and is highly aromatic. It can be grown in greenhouses, pots, or open fields. Sowing is recommended between March and September, and mature plants can be harvested from May to December. In closed spaces, the supplier (Unisem S.A. Iași) recommends sowing between November and February, and harvesting plants can be performed from January to June, depending on their growth stage.

The 'Aromat de Buzău' cultivar is a Romanian basil cultivar obtained by the research team at SCDL Buzău, which, according to the supplier's specifications, is a semi-late cultivar that branches strongly, with slightly toothed leaf edges, a specific mentholated aroma, and resistance to specific diseases and pests. It is recommended that plants belonging to this cultivar be grown in a greenhouse, solarium, or pots placed indoors if year-round cultivation is desired, or in the field when establishing crops through plantlets in March-April.

Materials and methods

1. Preliminary research

Treatment of test plant material with colchicine

In this study, different treatment methods were tested with a predetermined range of colchicine concentrations in the form of aqueous solutions (Table 1), and the biological effects induced in the plants subjected to the treatments were determined to better understand the influence of colchicine on plant cells *in vivo*.

Schematization of treatments with aqueous solutions of colchicine

Colchicine treatment of test plants was performed using four methods designed according to the specialized literature (Omidbaigi et al., 2010; Manzoor et al., 2019; Iannicelli et al., 2020). The test plant material used in this study was represented by two cultivars of the species *Ocimum basilicum* L.: 'Aromat de Buzău' and 'Italiano Classico.'

1. The first method of applying colchicine treatment consisted of immersing 30 seeds in triplicate (90 seeds per extract treatment variant) in different concentrations of colchicine (Table 1), after which they were distributed in Petri dishes (Figure 1) and maintained in a thermostat at 20°C or 25°C under a lighting regime of 17 h per day, with a light intensity of 50 µmol m^{-2} s^{-1}. The germinated seeds were counted daily, at 24-hour intervals, and the germination indices were calculated (according to the formulas presented in Table 2). The resulting plantlets were measured to determine their vigor indices.

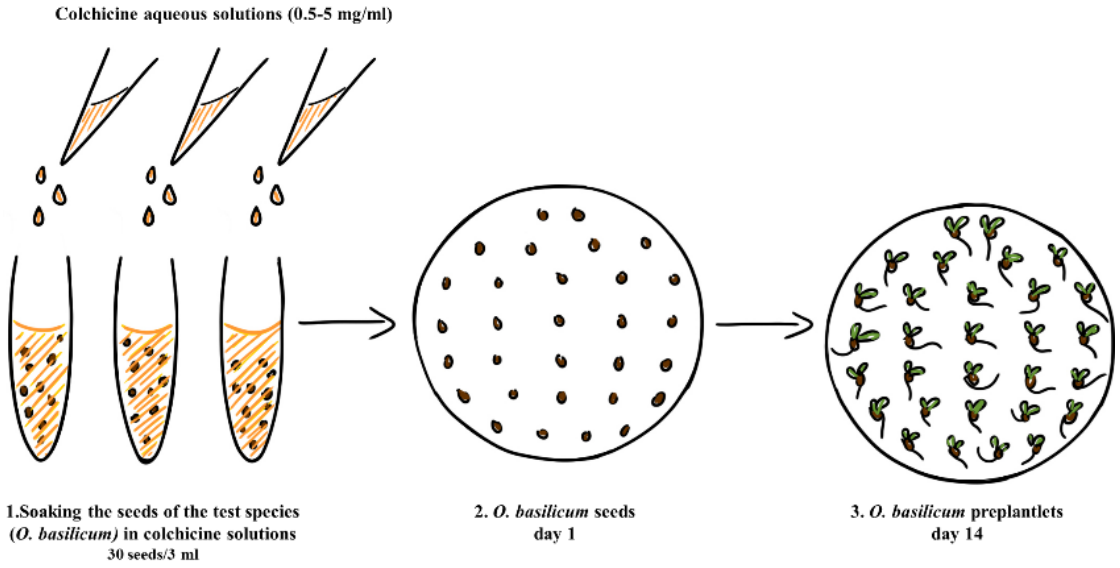

Figure 1. Schematic of the treatment with aqueous solutions of colchicine (0.5 – 5 mg/ml) on *O. basilicum* L. seeds.

2. Method no. 2 of applying colchicine treatment consisted of dripping colchicine solutions of different concentrations (according to Table 1) on *O. basilicum* L. seedlings distributed in Petri dishes (Figure 2), each plate containing 30 seedlings in triplicate (three Petri dishes for each experimental variant). After 6 and 12 h, the seedlings were rinsed and redistributed in Petri dishes according to the initial distribution and kept in a thermostat at 20℃ or 25℃ under a lighting regime of 17 h per day, with a light intensity of 50 µmol m^{-2} s^{-1}.

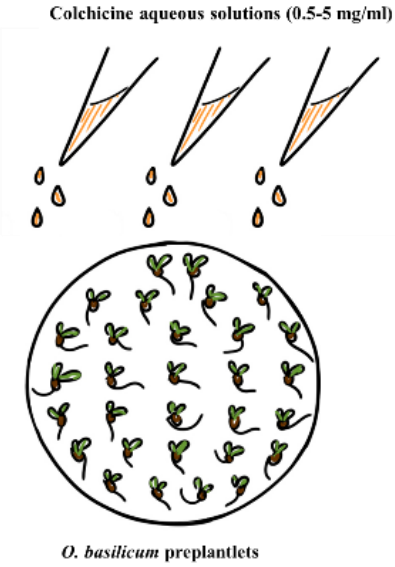

Figure 2. Schematic of the treatment with aqueous solutions of colchicine (0.5 – 5 mg/ml) on *O. basilicum* L. seedlings

3. Method 3 of applying colchicine treatment consisted of immersing the radicles of O. *basilicum* L. seedlings distributed in Petri dishes (Figure 3) in aqueous solutions of colchicine at different concentrations (Table 1), each plate containing 30 seedlings in triplicate (three Petri dishes for each experimental variant). After 6 and 12 h, the seedlings were rinsed and redistributed in Petri dishes according to the initial distribution and kept in a thermostat at 20 or 25°C under a lighting regime of 17 h per day, with a light intensity of 50 µmol m^{-2} s^{-1}.

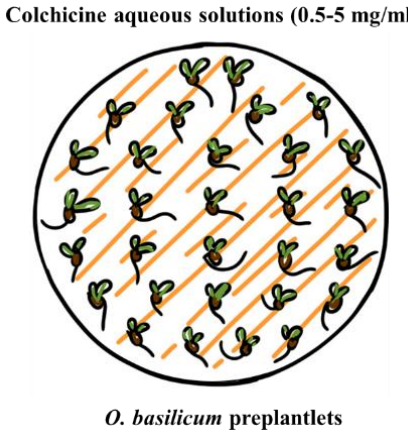

Figure 3. Schematic of the treatment with aqueous solutions of colchicine (0.5 – 5 mg/ml) on *O. basilicum* L. seedlings

4. Treatment method no. 4 consisted of placing cotton balls (approximately 2 mm in diameter) on the shoot apexes of test plantlets previously grown in pots in Compo Sana soil (peat and perlite), starting on day 15 from planting and soaking them with 100µl of aqueous colchicine solutions of different concentrations (according to Table 1) per day for three consecutive days (Figure 4). The plantlets were grown under a lighting regime of 17 h per day, with a light intensity of 50 µmol m^{-2} s^{-1}. Analyses of morphological, anatomical, physiological, and biochemical indices of the treated plantlets were carried out 93 days after planting (75 days from the time of application of the treatment).

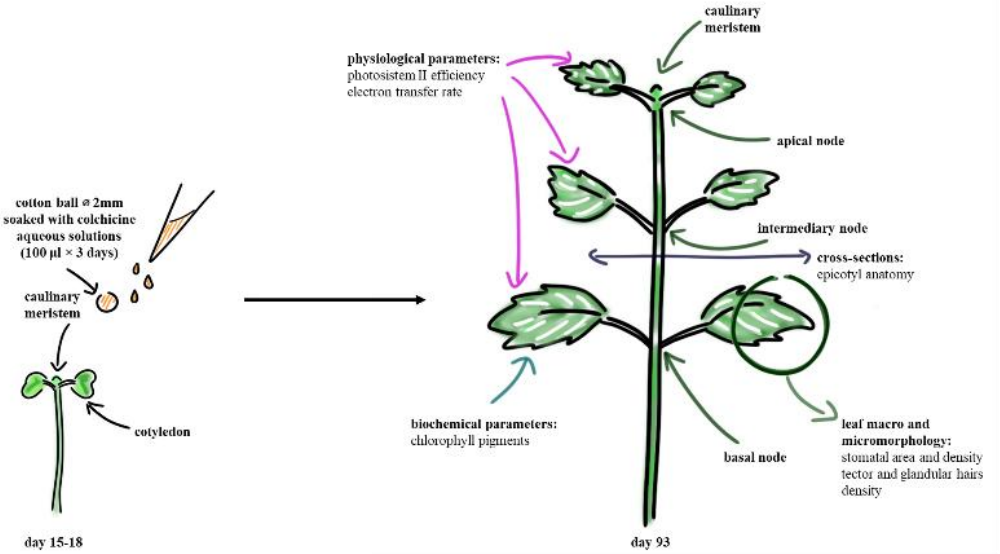

Figure 4. Schematic of the treatment with aqueous solutions of colchicine (0.5 – 5 mg/ml) on *O. basilicum* L. plantlets

Table 1. Colchicine treatment methods applied to different organs and tissues of *O. basilicum* L. cultivars 'Italiano Classico' and 'Aromat de Buzău' individuals

Treatment method		1	2	3	4
		imbibition	dripping	soaking	imbibition
Tissue/Organ		seeds	caulinary apex	radicular apex	caulinary apex
No. of seeds/seedlings/ plantlets		30 x 3	10 x 3	10 x 3	15
Total no. of seeds/seedlings/plantlets		540	150	150	90
Colchicine concentrations (mg/ml)		0,5; 1; 2; 3; 5			
Time of exposure (h)		24	6; 12	6; 12	72
Volume of solution (µl)		2000 (per experimental variant)	100 (per seedling)	2000 (per Petri dish)	100 x 3days (per cotton ball/plantlet)
Moment of treatment application (day)		0	6	6	18
Growth conditions	Light	17 h/day; 50 µmol m^{-2} s^{-1}			
	Temperature (°C)	20; 25	20±2		
Reference method		Omidbaigi et al., 2010			

Evaluation of the effects of colchicine on the germination of seeds of the test species (O. basilicum L.)

According to the method of applying the treatment of aqueous solutions of colchicine on seeds of *O. basilicum* L. cultivars 'Aromat de Buzău' and 'Italiano Classico', germinated seeds were counted daily, at 24-hour intervals, and germination indices were calculated using the formulas presented in Table 2.

From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Table 2. Formulas used in the calculation of germination indices for seeds of *O. basilicum* L. cultivars, after treatment with aqueous solutions of colchicine

Germination Index	Formula	Reference
FGP (Final Germination Percentage)	$FGP = \frac{n}{N} \times 100$	Bezini et al., 2019
GI (Germination Index)	$GI = \sum(n/d)$	Islam &i Kato-Noguchi, 2014; Bezini et al., 2019, according to Salehzade et al., 2009
SE (Seedling Emergence)	$SE = \frac{n_i \times 100}{n_f}$	Islam & Kato-Noguchi, 2014, according to Islam et al., 2009
MGT (Mean Germination Time)	$MGT = \frac{\sum(n \times d)}{n_f}$	Bezini et al., 2019, according to Akinci & Akinci, 2010
MDG (Mean Daily Germination)	$MDG = \frac{n_f}{D}$	Bezini et al., 2019, according to Almaghrabi et al., 2014
GE (Germinative Energy)	$GE = \frac{\%FG}{n_f}$	Islam & Kato-Noguchi, 2014, according to Ruan, Xue & Tylkowska, 2002
SVI (Seedling Vigor Index)	$SVI = \frac{L\ (mm) \times FGP}{100}$	Islam & Kato-Noguchi, 2014, according to Islam, Anuar & Yaakob, 2009

Legend: n = no. of germinated seeds on day x (n_i = initial germination; n_f = last or final germination); d = day x; N = total no. of seeds; D = total number of days; %FG = First Germination percentage; L (mm) = plantlets length at the end of the germination test.

Evaluation of the effects of colchicine on the seedlings of the test species (O. basilicum L.)

The test species seedlings (*O. basilicum* L.) treated by dripping and immersion with aqueous solutions of colchicine of different concentrations (0.5 – 5 mg/ml) that survived the treatments were transferred to pots with Compo Sana soil (peat and perlite) and maintained under a lighting regime of 17 h per day, with a light intensity of 50 µmol m^{-2} s^{-1}. However, it was found that the seedlings treated by dripping died shortly after transplantation, as was the case with those treated by immersion, most of which showed necrosis at the level of the radicles and cotyledons.

Evaluation of the effects of colchicine on the plantlets of the test species (O. basilicum L.) treated on the stem apex

Evaluation at the morphological level

a. Morphology

The morphological aspects and the differences observed between the individuals of *Ocimum basilicum* L. subjected to treatment were recorded by taking pictures with a Xiaomi Mi A1 camera (12 MP, f/2.2, 26 mm (wide), ½.9", 1.25µm, PDAF). The photographs of the plantlets from the experimental treatment variants were compared with those of the individuals from the control variant.

b. Micromorphology

To observe and photograph the micrometric elements, two leaves inserted at the basal node from three different individuals from each experimental variant were randomly chosen, and fragments were selected from these and inserted with tweezers into Eppendorf tubes. The plant material was immersed in successive acetone baths. The material was dried at critical point with CO_2 and subsequently metallized with Au particles in a metallizer. Finally, the material was analyzed under a microscope (Vega Tescan II SBH) from the Faculty of Biology of the "Alexandru Ioan Cuza" University of Iași, using the VegaTC Software program, and photographs were taken of both leaf surfaces (adaxial and abaxial).

The images obtained were analyzed visually, and the measurements for the following indices were taken: stomatal area, stomatal density, tector, and secretory hairs per mm^2 of leaf surface. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Evaluation of the effects of colchicine on the plantlets of the test species (O. basilicum L.) at the anatomical level

Fresh epicotyl fragments harvested from three individuals of each test variant were selected and immersed in 70% ethanol to obtain sections and examine them under an optical microscope.

The plant material was sectioned transversely using a hand microtome and a botanical razor. The sections were then stained using the double-staining method with iodine

green and ruthenium red. The stained sections were placed on a slide on a drop of preheated glycerol-gelatin, over which a coverslip was placed and allowed to dry. Sections of epicotyls of *O. basilicum* L. were observed under a Euromex bScope optical microscope and photographed with the microscope camera and the Xiaomi Mi A1 phone camera (12 MP, f/2.2, 26 mm (wide), ½.9", 1.25μm, PDAF), using the 10x eyepiece of the microscope. Photographs of sections through the epicotyls of plantlets from the experimental treatment variants were compared with photographs of sections of epicotyls of plantlets from the control variant.

Evaluation of the effects of colchicine on plantlets of the test species (O. basilicum L.) at the physiological level

Photosystem II efficiency (YPSII) and electron transport rate (ETR) were measured using a Hansatech Ltd. PAM Fluorometer (www.hansatech-instruments.com) for five leaves from five different individuals from each experimental variant, 93 days after planting (75 days from the last day of treatment application), after exposing the plantlets for 30 min to darkness. The data were saved in the memory of Hansatech Ltd. The PAM Fluorometer data were imported into the Parview32 program and analyzed using GraphPad Prism 9.5.1. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Evaluation of the effects of colchicine on the plantlets of the test species (O. basilicum L.) at the biochemical level

For biochemical analyses regarding the content of assimilatory pigments, it is necessary to prepare vegetable leaf extracts in triplicate for each experimental variant of *Ocimum basilicum* L. taken into the study. Thus, vegetable material from three different individuals from each treatment variant was selected and weighed on an electronic balance. After weighing, the material was ground with quartz sand, dissolved in 95% ethanol, and centrifuged.

The assimilatory pigments were analyzed according to the method of Sumanta et al. (2014), which involves measuring the absorbance of chlorophyll pigment solutions at three different wavelengths: 646, 664, and 470 nm, using a Shimadzu UV mini-1240

spectrophotometer (www.shimadzu.eu). The amounts of chlorophyll a (Ch-a), chlorophyll b (Ch-b), and carotenoid pigments (Cx+c) were quantified according to the formulas in the reference article, presented below:

$$Ch - a = 13.36 \times A663 - 5.19 \times A646;$$

$$Ch - b = 27.43 \times A646 - 8.12 \times A663;$$

$$\mathrm{Cx + c} = {(1000 \times A470 - 2.13 \times Ch - a - 97.63 \times Ch - b)}/{209};$$

$$Ch - a\ (mg/g) = Ch - a/m.$$

From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Statistical analyses

Statistical analyses were performed using GraphPad Prism version 9.5.1, using two-factor ANOVA (considering the cultivar as a qualitative factor) and Dunnett and Tukey post-hoc comparison test for morphological indices: stomata size, stomata density, tector and secretory hair density (on both leaf surfaces of plantlets treated with aqueous solutions of colchicine), biochemical indices: chlorophyll a, b, and carotenoid content, and physiological indices: photosystem II efficiency and electron transport rate. For germination indices, two-factor ANOVA (considering the cultivar as a qualitative factor) and Tukey's multiple comparison test were applied. The values represented in the graphs and tables are the means ± standard errors of the mean, and the results of the Dunnett and Tukey tests are marked with different letters.

Treatment of test species cultivars with methanolic extracts of C. autumnale L.

To determine the potential allelopathic effect induced by biologically active compounds present in the organs of *Colchicum autumnale* L. plants on other plant organisms, two alcoholic extracts were prepared from the bulbs and flowers of meadow saffron, and two cultivars of basil, *Ocimum basilicum* L., were chosen: *O. basilicum* 'Italiano Classico' and 'Aromat de Buzău' as test cultivars for the analysis of the effects thus induced by the treatments with extracts.

Preparation of methanolic extracts from the collected plant material (plants of the species C. autumnale L.)

The plant material consisting of the bulbs and flowers of *C. autumnale* L. was dried in an oven at a temperature of 65℃, then further dried in the dark for 7 days, and ground in an electric grinder until a powder was obtained, which was used for extraction.

Extraction was performed in a Soxhlet apparatus in absolute methanol, using 5 g of powder from each organ, according to the method described by Franz and Koehler (1992) (Alali et al., 2004). The extraction was performed until the solvent in the extraction chamber was clear (5–6 cycles or 1–1.5 cycles/h for bulbs and 17-18 cycles or 2–3 cycles/h for flowers). Methanol was then evaporated in a rotary evaporator (IKA RV3 Eco, Germany) and redissolved in 50 ml of 70% methanol to reduce the toxicity level of methanol.

Dosage, separation and quantification of the content and determination of the antioxidant activity of the extracts from the collected plant material (plants of the species C. autumnale L.)

Colchicine quantification in the extracts was performed using RP-HPLC, according to the method described by Alali et al. (2004).

Schematization of treatments with methanolic extracts from collected plant material (plants of the species C. autumnale L.)

The two extracts were used in treatments, applied as such or diluted with distilled water (1:1) on seeds (treatment method 1 presented in Table 1) and on the shoot apexes of plantlets (treatment method 4 presented in Table 1) of *O. basilicum* L. cultivars 'Italiano Classico' and 'Aromat de Buzău' grown in pots. Three control variants were prepared (C_0 = distilled H_2O, C_1 = MeOH 35%, C_2 = MeOH 70%) depending on the methanol concentration in the extracts and dilutions.

1. The first treatment method involved applying the extracts to *O. basilicum* L. seeds distributed in Petri dishes. The treatment was applied by soaking the filter paper with 2 ml of undiluted or diluted extract 1:1 (20 seeds/Petri plate × 3 replicates for each treatment

variant) (Figure 5). The seeds were incubated in a thermostat at a temperature of 20±1 °C, with a lighting regime of 17 h per day, at a light intensity of 50 µmol $m^{-2}s^{-1}$, for 14 days, and watered every other day with distilled water.

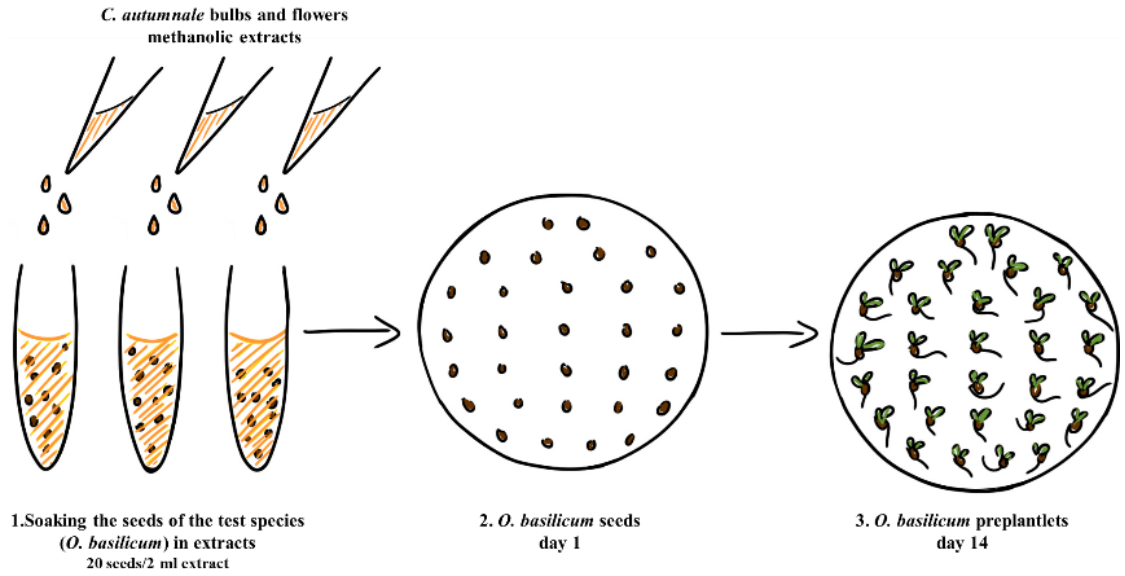

Figure 5. Schematic of the treatment with methanolic extracts from bulbs or flowers of *C. autumnale* L. on *O. basilicum* L. seeds.

2. The second method was performed by applying the treatment to basil plantlets on day 18 after planting in pots by soaking cotton balls applied to the shoot apexes with 100 µl of extract per day for three consecutive days (Figure 6). The plantlets were kept in pots with Compo Sana soil (peat + perlite) at 21±2 °C and maintained under a lighting regime of 17 h per day, with a light intensity of 50 µmol m^{-2} s^{-1}, and were watered once every three days with distilled water. The experiment lasted 93 days (75 days of monitoring plantlet development), and the treated plantlets were analyzed at the end of the experiment.

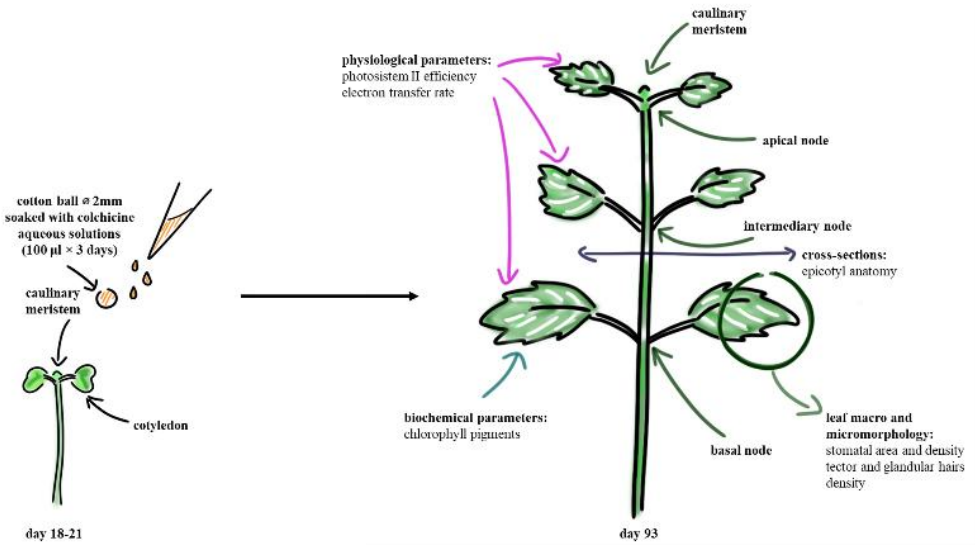

Figure 6. Schematic of the treatment with methanolic extracts from bulbs or flowers of *C. autumnale* L. on *O. basilicum* L. plantlets

Evaluation of the effects of methanolic extracts from collected plant material (plants of the species C. autumnale L.) on the germination of seeds of the test species (O. basilicum L.)

According to the method of applying aqueous colchicine solutions to seeds of *O. basilicum* L. cultivars 'Aromat de Buzău' and 'Italiano Classico, germinated seeds were counted daily at 24-hour intervals.

The final germination percentage index (FGP) was calculated according to the formula presented by Bezini et al. (2019): FGP = n/N × 100, where n represents the number of germinated seeds at the end of the test, and N is the total number of seeds used for each experimental variant.

Evaluation of the effects of methanolic extracts from the collected plant material (plants of the species C. autumnale L.) on the plantlets of the test species (O. basilicum L.) treated on the shoot apex

Evaluation of the effects of the extracts on the plantlets of the test species (O. basilicum L.) at the morphological level

a. Morphology

Morphological aspects and differences observed between the *Ocimum basilicum* L. individuals subjected to treatment were recorded by taking pictures with a Xiaomi Mi A1 camera (12 MP, f/2.2, 26 mm (wide), ½.9", 1.25µm, PDAF). The photographs of the plantlets from the experimental treatment variants were compared with those of the individuals from the control variant.

b. Micromorphology

To observe and photograph the micrometric elements, the method described in subchapter Evaluation of the effects of colchicine on the plantlets of the test species (*O. basilicum* L.) treated on the stem apex was used - Evaluation of the effects of colchicine on the plantlets of the test species (*O. basilicum* L.) at the morphological level - b. Micromorphology.

The images obtained were analyzed visually, and measurements were taken for the monitored indices: stomatal area, stomatal density, and tector and secretory hairs per mm2 of leaf surface. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Evaluation of the effects of the extracts on the plantlets of the test species (O. basilicum L.) at the anatomical level

Sections through the epicotyls of *O. basilicum* L. plantlets treated with methanolic extracts from bulbs or flowers of *C. autumnale* L. on the shoot apex were prepared according to the method described in the subchapter Evaluation of the effects of colchicine on the plantlets of the test species (*O. basilicum* L.) at the anatomical level.

Sections of epicotyls of *O. basilicum* L. treated with extracts of *C. autumnale* L. were observed under the Euromex bScope optical microscope and photographed with the microscope camera and the Xiaomi Mi A1 phone camera (12 MP, f/2.2, 26 mm (wide), ½.9", 1.25µm, PDAF). Photographs of sections through the epicotyls of plantlets from the experimental treatment variants were compared with photographs of sections of epicotyls of plantlets from the control variant.

Evaluation of the effects of the extracts on plantlets of the test species (O. basilicum L.) at the physiological level

The efficiency of photosystem II (YPSII) and electron transport rate (ETR) were measured using a Hansatech Ltd. PAM Fluorometer (www.hansatech-instruments.com) according to the method described in the subchapter: Evaluation of the effects of colchicine on plantlets of the test species (*O. basilicum* L.) at the physiological level. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Evaluation of the effects of colchicine on the plantlets of the test species (O. basilicum L.) at the biochemical level

The content of assimilatory pigments was determined according to the method described by Sumanta et al. (2014) in the subchapter Evaluation of the effects of colchicine on the plantlets of the test species (*O. basilicum* L.) at the biochemical level.

From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Statistical analyses

Statistical analyses were performed using GraphPad Prism version 9.5.1, using two-way ANOVA (considering cultivar as a qualitative factor) and Tukey's multiple comparison test for seed germination indices and morphological (stomatal size, stomatal density, tector and secretory hair density on both leaf surfaces), biochemical (chlorophyll a, b, and carotenoid content), and physiological (photosystem II efficiency and electron transport rate) indices of *O. basilicum* L. plantlets treated with methanolic extracts from *C. autumnale* L. bulbs or flowers. The values represented in the graphs and tables are the means ± standard errors of the mean, and the results of the Tukey multiple comparison test are marked with different letters.

2. Further research

Collection and preparation of plant material (organs of C. autumnale L.) for extraction

For the preparation of plant extracts, plant material (vegetative and reproductive organs) harvested from *C. autumnale* L. plants from a meadow in the Voroneț area, Suceava County, in three phases of the ontogenetic cycle was used: bulbs, leaves, and fruits in the growth or vegetative phase corresponding to the spring period, leaves and fruits in the fruiting phase during the summer, and bulbs and flowers in the flowering phase corresponding to the autumn period (Figure 7). For each period in which the plant material used to obtain the extracts was collected, an individual of *C. autumnale* L. was deposited as a voucher at the Herbarium of the Faculty of Biology of the "Alexandru Ioan Cuza" University in Iași. The collected plant material was dried in an oven at 65 °C for 12 h to stop enzymatic reactions, then further dried in the dark at room temperature (23±2 °C) for 7 days, after which it was ground until a fine powder was obtained.

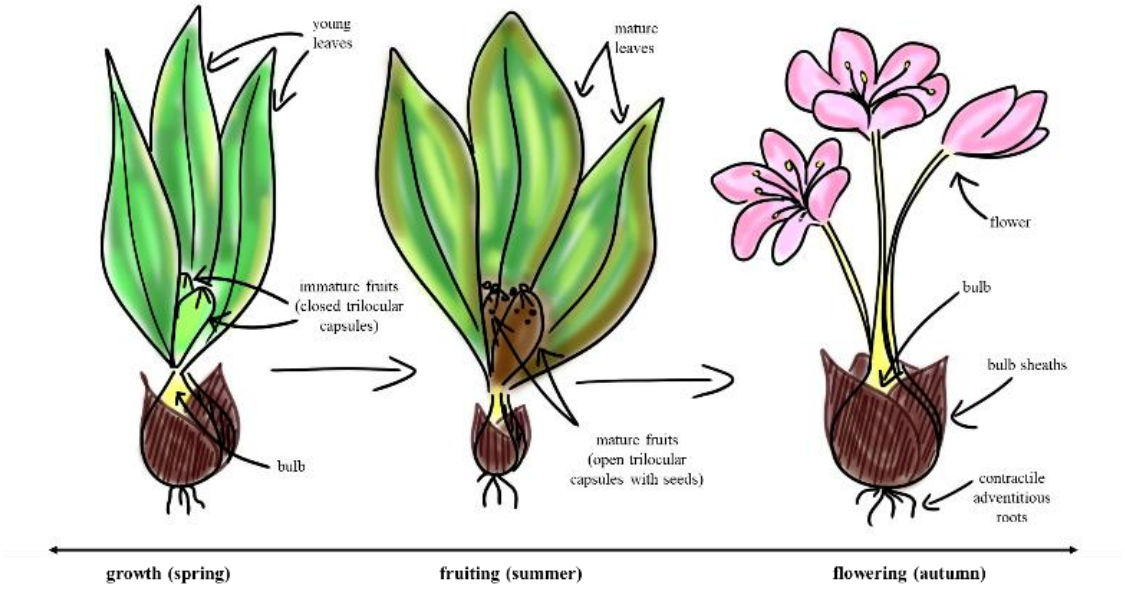

Figure 7. Schematic representation of the ontogenetic cycle of plants of the species *C. autumnale* L. (original)

Preparation of alcoholic and aqueous extracts from C. autumnale L. organs.

The alcoholic extracts were prepared using a Soxhlet apparatus by extracting 5 g of plant material from each collected organ in absolute methanol (method adapted from

Rocchetti et al., 2019). The solvent was then evaporated to dryness using a rotary evaporator, and the dry extract was redissolved in 70% ethanol.

Aqueous extracts were prepared by infusing 5 g of plant material from each collected organ in distilled water for 24 h at 25℃, after which they were filtered through filter paper. The solvent was then evaporated to dryness using a lyophilizer, and each dry extract obtained was redissolved in 50 ml of distilled water.

Finally, eight alcoholic and eight aqueous extracts were obtained, corresponding to the *C. autumnale* L. organs from which the extraction was performed.

Detection and quantification of allelopathic compounds in alcoholic and aqueous extracts prepared from organs of C. autumnale L.

The compounds and quantification of their concentration in *the C. autumnale* L. extracts were identified and quantified using reversed-phase high-performance liquid chromatography (RP-HPLC), as described by Alali et al. (2004). The concentrations of colchicine, colchicine, demecolcine, and apigenin in the alcoholic and aqueous extracts of *C. autumnale* L. were quantified based on the area of the detected peaks and calculated according to the standard curves.

The total polyphenol and flavonoid content and antioxidant activity of alcoholic and aqueous extracts prepared from C. autumnale L. organs were determined using the methods described by Herald et al. (2012).

Test species plant material (O. basilicum L.)

Ocimum basilicum L. seeds, cultivars 'Italiano Classico' (lot no. 0318-00) and 'Aromat de Buzău' (lot no. 9BZ1321-9BZ01/0BZ01A) were procured from commercial sources (Unisem S.A. Iași, respectively, S.C.D.L. Buzău), and 100 seeds from each lot were deposited at the Herbarium of the Faculty of Biology of the "Alexandru Ioan Cuza" University of Iași.

Genetic and physiological tests on the test species cultivars (O. basilicum L.)

To anticipate possible differences in the responses of test basil cultivars to treatments with *C. autumnale* L. extracts, analyses were performed on the chromosome number of *O. basilicum* L. plants, cultivars 'Italiano Classico' and 'Aromat de Buzău, by testing two treatment solutions: colchicine at a concentration of 0.2% (according to the method used by Căpraru and Băra, 2007) and 8-hydroxyquinoline 0.002 M (according to the method used by Truță and Zamfirache, 2013), with different exposure times. The preparations were made using the squash method, washed in successive baths of ethanol and xylene, and fixed with Canada balsam. The final permanent preparations were observed under a Nikon Eclipse E600 optical microscope and photographed with a Nikon E950 camera, at a 100x objective, using immersion oil.

To determine the toxicity level of ethanol (used in the preparation of alcoholic extracts of *C. autumnale* L.) on basil seed germination, a viability test was performed, according to the method described by Boldor, Raianu and Trifu (1983).

To determine the amount of extract imbibed in the seeds, an imbibition test was performed using the gravimetric method.

Schematization of treatments with alcoholic and aqueous extracts from vegetative and reproductive organs of plants of the species C. autumnale L.

The application of treatment with extracts from organs of *C. autumnale* L. on test plants was carried out by two methods designed according to the specialized literature, which presents methods of treatment with colchicine on plant species at different stages of development (Omidbaigi et al., 2010; Manzoor et al., 2019; Iannicelli et al., 2020).

The test seeds and plantlets used in this study were two cultivars of the species *Ocimum basilicum* L.: 'Aromat de Buzău' and 'Italiano Classico.'

1a. The first method of treatment application with alcoholic extracts consisted of preparing three dilutions for each extract (1:1, 1:2, and 1:3) with distilled water. A total of 90 seeds per treatment variant were soaked in the diluted extracts for 30 min, after which they were distributed in Petri dishes (Figure 8).

1b. The method of applying the treatment with aqueous extracts consisted of treating *O. basilicum* L. seeds with undiluted and diluted (1:1 and 1:2) extracts mixed with distilled water. A total of 90 seeds per treatment variant were soaked in undiluted or diluted extracts for 24 h, after which they were distributed in Petri dishes (Figure 8).

After applying the treatment, the seeds were monitored for 14 days, maintained at 25°C under a lighting regime of 18 h per day, with a light intensity of 50 µmol m-2 s-1. Germinated seeds were counted daily at 24 h intervals, and at the end of the experiment, germination indices were calculated and the resulting plantlets were measured to determine their vigor index (SVI).

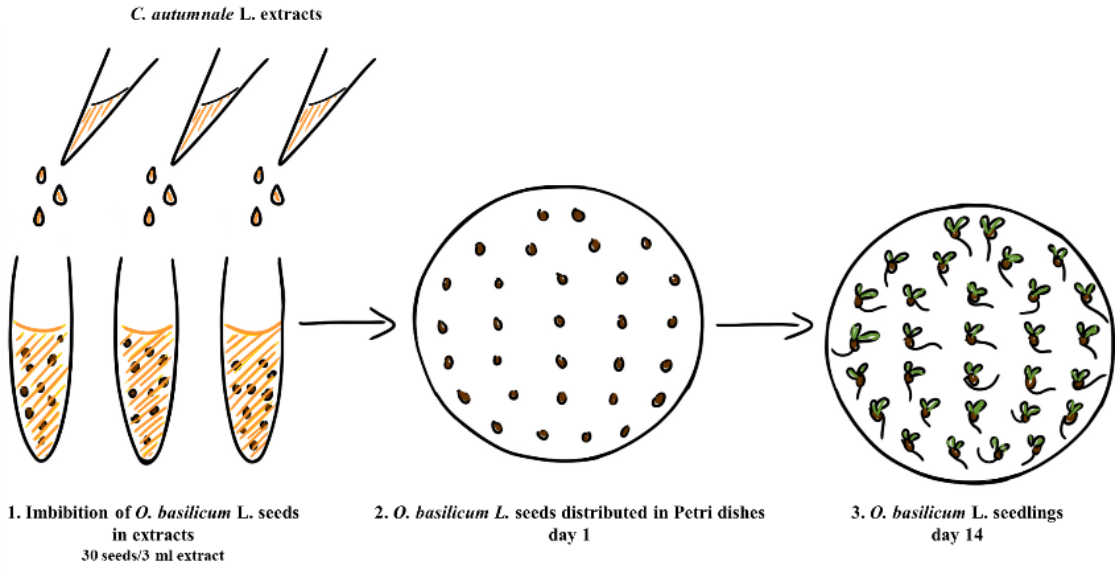

Figure 8. Schematic representation of the treatment with alcoholic and aqueous extracts from *C. autumnale* L. organs on *O. basilicum* L. seeds ('Italiano Classico' and 'Aromat de Buzău' cultivars) (original)

2. The second treatment method consisted of placing cotton balls (approximately 2 mm in diameter) on the shoot apexes of test plantlets previously grown in pots, starting on day 15 from planting, and soaking them with 100 µL of extract (alcoholic or aqueous) per day for three consecutive days (Figure 9).

Analyses of morphological and anatomical indices of treated plantlets were performed 90 days after planting (72 days from the time of treatment application), except for epicotyl height, which was measured 60, 75, and 90 days from planting (42, 57, and 72 days from treatment). Biochemical and physiological analyses were performed 75 days after planting (57 days after treatment application).

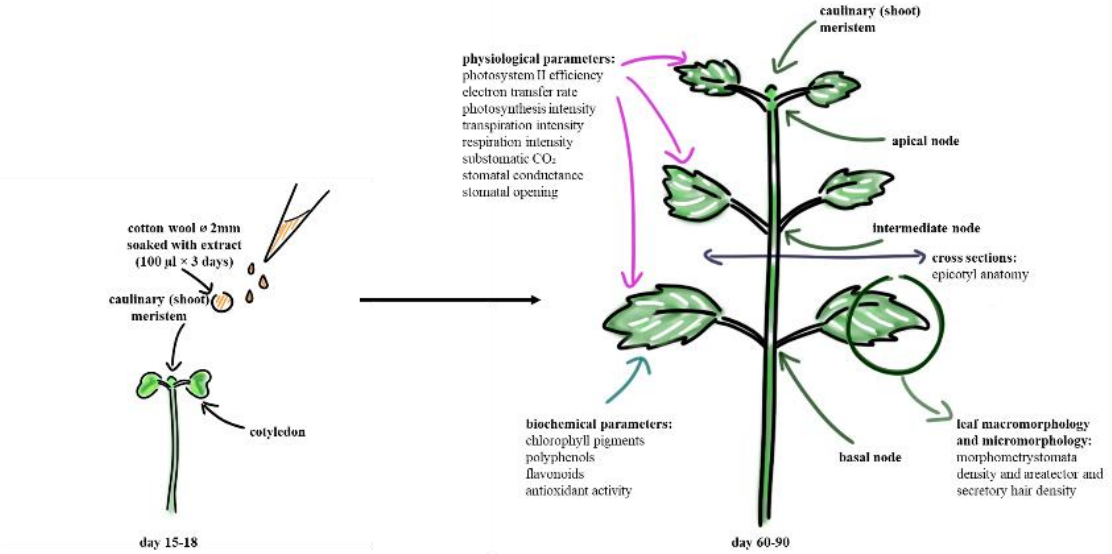

Figure 9. Schematic representation of the treatment with alcoholic and aqueous extracts from *C. autumnale* L. organs on the shoot apexes of *O. basilicum* L. plantlets ('Italiano Classico' and 'Aromat de Buzău' cultivars) (original)

Evaluation of the effects induced by treatments in test seeds (O. basilicum L.)

According to the method of applying the treatment with *C. autumnale* L. extracts on the *O. basilicum* L. seeds, the cultivars 'Aromat de Buzău' and 'Italiano Classico germinated seeds were counted daily, at 24-hour intervals, and the germination indices were calculated using the formulas presented in Table 2. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Evaluation of the effects induced by treatments in test plantlets (O. basilicum L.)

During studies on basil plantlets, contamination with pests occurred due to the soil used for the cultivation of these plantlets. It was tried as much as possible to select and use apparently healthy plantlets in the morphological, anatomical, physiological and biochemical analyses, but even these plantlets, after observations of leaf morphology and epicotyl anatomy under the electron microscope and optical microscope, respectively, proved to be affected by pests. The pests that affected *O. basilicum* L. plantlets were identified as species of thrips (Tizanoptera) and fungi (Ascomycetae).

Evaluation of the effects induced by C. autumnale L. extracts in test plantlets of O. basilicum L. treated on the stem apex at the morphological level

a. Morphology

Morphological aspects and differences observed between *Ocimum basilicum* L. individuals subjected to treatment were recorded by taking pictures with a Motorola One Zoom phone camera (48 MP, f/1.7, 1/2.0", 0.8µm, PDAF, OIS).

The epicotyl length of all plantlets was measured at three stages of development of *O. basilicum* L. plantlets (time 0 - time of treatment application, time 1 - 42 days after treatment application, time 2 - 57 days after treatment application, and time 3 - 72 days after treatment application) using a ruler. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Leaf area was measured on photographs using the ImageJ program on the leaves of *O. basilicum* L. plantlets, at 90 days of planting (72 days from the last day of application of the treatment) for the leaves from each leaf level (lower, middle or intermediate, upper or apical) measuring the leaf surface area for five leaves from each leaf level (from five plantlets from each experimental variant). From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

b. Micromorphology

To observe and photograph micrometric elements, the method described in the subchapter Evaluation of the effects of colchicine on the plantlets of the test species (*O. basilicum* L.) treated on the stem apex was applied - Evaluation of the effects of colchicine on the plantlets of the test species (*O. basilicum* L.) at the morphological level - b. Micromorphology.

The material was analyzed using the Vega Tescan II SBH scanning electron microscope from the Faculty of Biology of the "Alexandru Ioan Cuza" University of Iași, using the VegaTC Software program, taking photographs of both leaf surfaces (adaxial and abaxial).

The images obtained were analyzed visually, and the measurements for the following indices: stomatal area, stomatal density, tector and secretory hairs per mm^2 of leaf surface, as well as the degree of stomatal opening (sum of ostiole areas per mm^2 of leaf surface), were performed using ImageJ. The values obtained for the last morphological index analyzed

(degree of stomatal opening) were correlated with the values of the corresponding physiological indices determined: the intensity of photosynthesis, transpiration, respiration, substomatic CO_2 content, and stomatal conductance. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Evaluation of the effects induced by C. autumnale L. extracts in test plantlets of O. basilicum L. treated on the shoot apex at the anatomical level

Sections through the epicotyls of *O. basilicum* L. plantlets treated with alcoholic and aqueous extracts from vegetative and reproductive organs of *C. autumnale* L. plants on the shoot apex were made according to the method described in the subchapter Evaluation of the effects of colchicine on plantlets of the test species (*O. basilicum* L.) at the anatomical level.

The stained sections were placed on a slide on a drop of preheated glycerol-gelatin, over which a coverslip was placed and allowed to dry.

Sections of epicotyls of *O. basilicum* L. treated with aqueous and alcoholic extracts from vegetative and reproductive organs of *C. autumnale* L. plants were observed under the Euromex bScope optical microscope and photographed with the microscope camera and the Motorola One Zoom phone camera (48 MP, f/1.7, 1/2.0", 0.8µm, PDAF, OIS), using the 5x, 10x, 20x and 40x eyepieces of the microscope.

Evaluation of the effects induced by C. autumnale L. extracts in test plantlets of O. basilicum L. treated on the shoot apex at the physiological level

Following the application of the treatment on the shoot apex of basil plantlets, the degree of stomatal opening was determined for each leaf surface analyzed by calculating the total area of open ostioles per 1 mm^2, simultaneously with determining the percentage of open stomata in the analyzed field, on the photographs of the plant material obtained with the scanning electron microscope. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

The following physiological indices were also analyzed: the intensity of the photosynthesis, respiration, and transpiration processes, stomatal conductance, and the

amount of substomatic CO_2 with the ADC Bioscientific LCi device – compact portable photosynthesis unit (www.adc.co.uk). To determine the intensity of the respiration process, the standard method of determining the intensity of photosynthesis with the ADC Bioscientific LCi device was modified by covering the working chamber during the measurements with a dark material, which prevented the penetration of light to the leaf surface.

The efficiency of photosystem II (YPSII) and electron transport rate (ETR) were measured using a Hansatech Ltd. device. PAM Fluorometer (www.hansatech-instruments.com), according to the method detailed in the subchapter Evaluation of the effects of colchicine on the test species (*O. basilicum* L.) plantlets at the physiological level.

From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Evaluation of the effects induced by C. autumnale L. extracts in the test plantlets of O. basilicum L. treated on the shoot apex at the biochemical level

Biochemical analyses were performed 75 days after planting (57 days after treatment) to identify possible variations in the content of assimilatory pigments, polyphenols, flavonoids, and antioxidant activity of *Ocimum basilicum* L. plantlets induced by treatment with alcoholic and aqueous extracts of *Colchicum autumnale* L.

Assimilatory pigment content

The content of assimilatory pigments was determined according to the method of Sumanta et al. (2014), described in the subchapter Evaluation of the effects of colchicine on plantlets of the test species (*O. basilicum* L.) at the biochemical level. From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Polyphenols content

The method described by Herald et al. (2012) was used to determine the total polyphenol content, according to the method described by Singleton and Rossi (1965), with adaptations described in the subchapter Dosage, separation, ausing quantification of the content and determination of the antioxidant activ (2012) extracts from the collected plant material (plants of the species *C. autumnale* L.). From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Flavonoids content

The flavonoid content was determined by the colorimetric method based on aluminum chloride used by Herald et al., 2012, according to Zhishen, Mengcheng and Jianming (1999), with adaptations, detailed in the subchapter Dosage, separation and quantification of the content and determination of the antioxidant activity of extracts from the collected plant material (plants of the species *C. autumnale* L.). From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Antioxidant activity

The antioxidant activity of extracts from the leaves of *O. basilicum* L. plantlets after treatment with *C. autumnale* L. extracts was determined by the method used by Herald et al. (2012), after Thaipong et al. (2006), and Brand-Williams, Cuvelier, and Berset (1995), with adaptations, detailed in the subchapter Dosage, separation and quantification of the content and determination of the antioxidant activity of extracts from the collected plant material (plants of the species *C. autumnale* L.). From the obtained values, the means ± standard errors of the means were calculated for each experimental variant.

Statistical analyses

To interpret the results obtained from the analysis of germination indices, physiological, and biochemical indices, the two-factor ANOVA statistical test (considering the cultivar as a qualitative factor), Tukey test for multiple comparisons (post-hoc), and Pearson correlation coefficient in the GraphPad Prism 9.5.1 program were applied.

The Pearson correlation coefficient was determined for comparing the results of biochemical analyses of the extracts (comparing alcoholic and aqueous extracts), comparing the responses of the two basil cultivars to treatments (the responses of plantlets from different cultivars treated with the same extract), and by comparing the effects of alcoholic extracts with the effects of aqueous extracts on each basil cultivar studied, except for germination indices because the extracts used in the seed treatments had different dilutions (the germination indices of basil plantlets from the two studied cultivars were compared to the treatment with the same alcoholic or aqueous extract).

Results and discussion

1. Preliminary results

Preliminary studies focused on monitoring the effects of colchicine, the main allelopathic compound extracted from plants of the species *Colchicum autumnale* L. Different methods of colchicine treatment were used on seeds, seedlings, and plantlets of two cultivars of *Ocimum basilicum* L.: 'Italiano Classico' and 'Aromat de Buzău', to determine and compare the biological effects induced on test plants. In preliminary studies, different treatment methods were tested with a predetermined range of concentrations of 0.005-0.05 mg/ml colchicine in the form of aqueous solutions, and the biological effects induced in the plants subjected to the treatments were determined to better understand the influence of colchicine on plant cells in vivo (Moroșan et al., 2023).

The treatment methods that were successfully applied were those on seeds, by imbibition in aqueous solutions of colchicine, and those applied to the cauline apexes of plantlets, while treatments applied by dripping and by immersion of plantlets in colchicine solutions resulted in tissue necrosis or death of the treated plantlets. The results showed that colchicine treatment did not significantly affect seed germination parameters, except for the vigor index, which inhibited plantlet growth (Figure 10).

In addition, colchicine treatment applied to the shoot apexes of basil plantlets did not significantly affect photosynthetic efficiency and the content of assimilatory pigments, but it exerts a significant impact on some morpho-anatomical indices, manifested by the deviation of the veins and edges of the leaf blade (Figure 11), the appearance of heteromorphic cells and weak lignification of the xylem in the epicotyl structure (Figure 12), the appearance of stomata and heteromorphic epidermis cells, and the elongation of tector hairs on the examined leaf surfaces, observations that can be interpreted as possible consequences of the chemical stress induced by colchicine treatment.

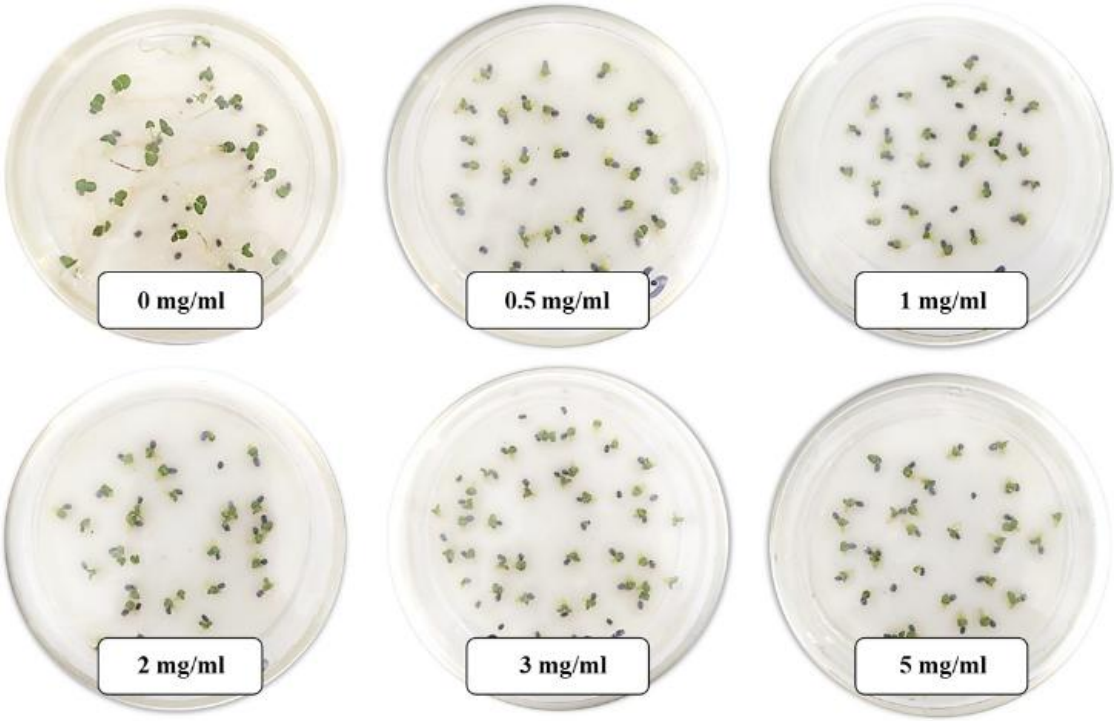

Figure 10. Appearance of *Ocimum basilicum* L. plantlets 14 days after treatment with different concentrations of colchicine

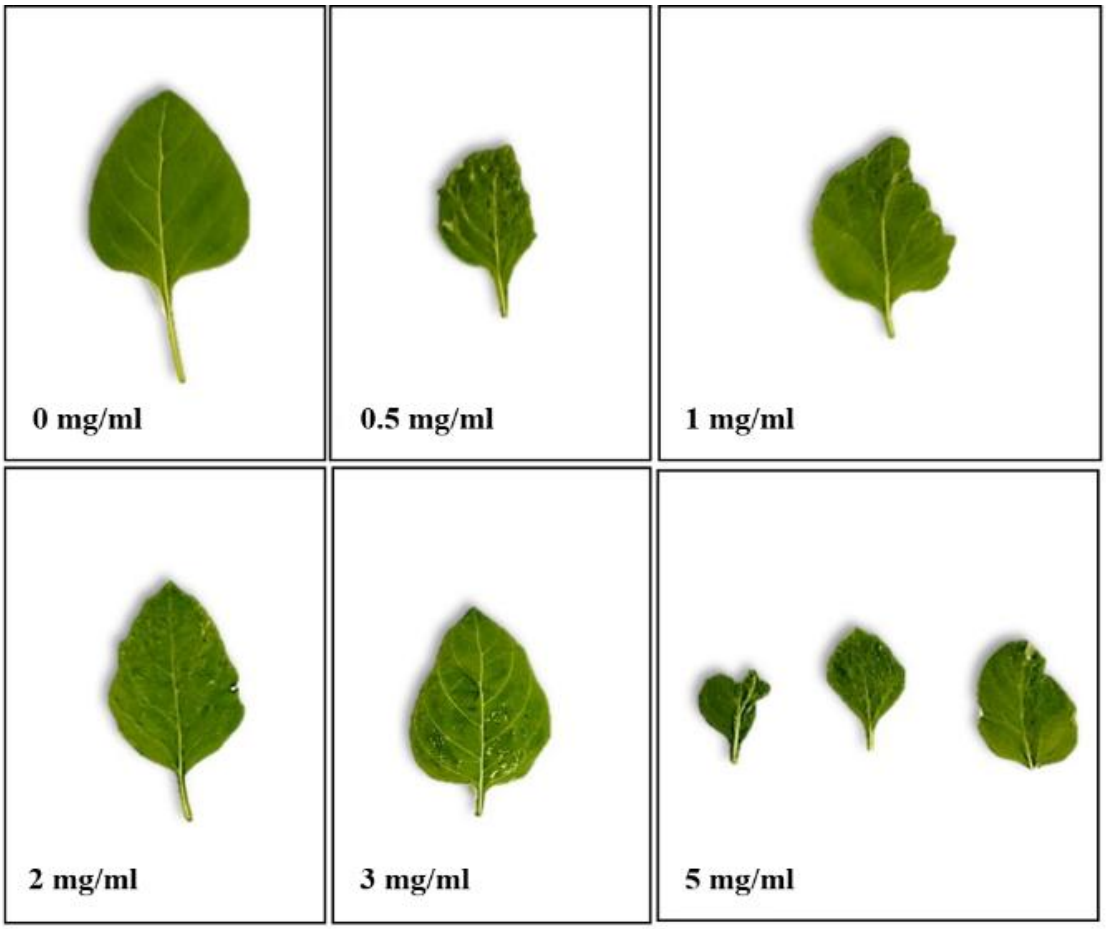

Figure 11. Variations in leaf morphology in *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with colchicine on the shoot apex

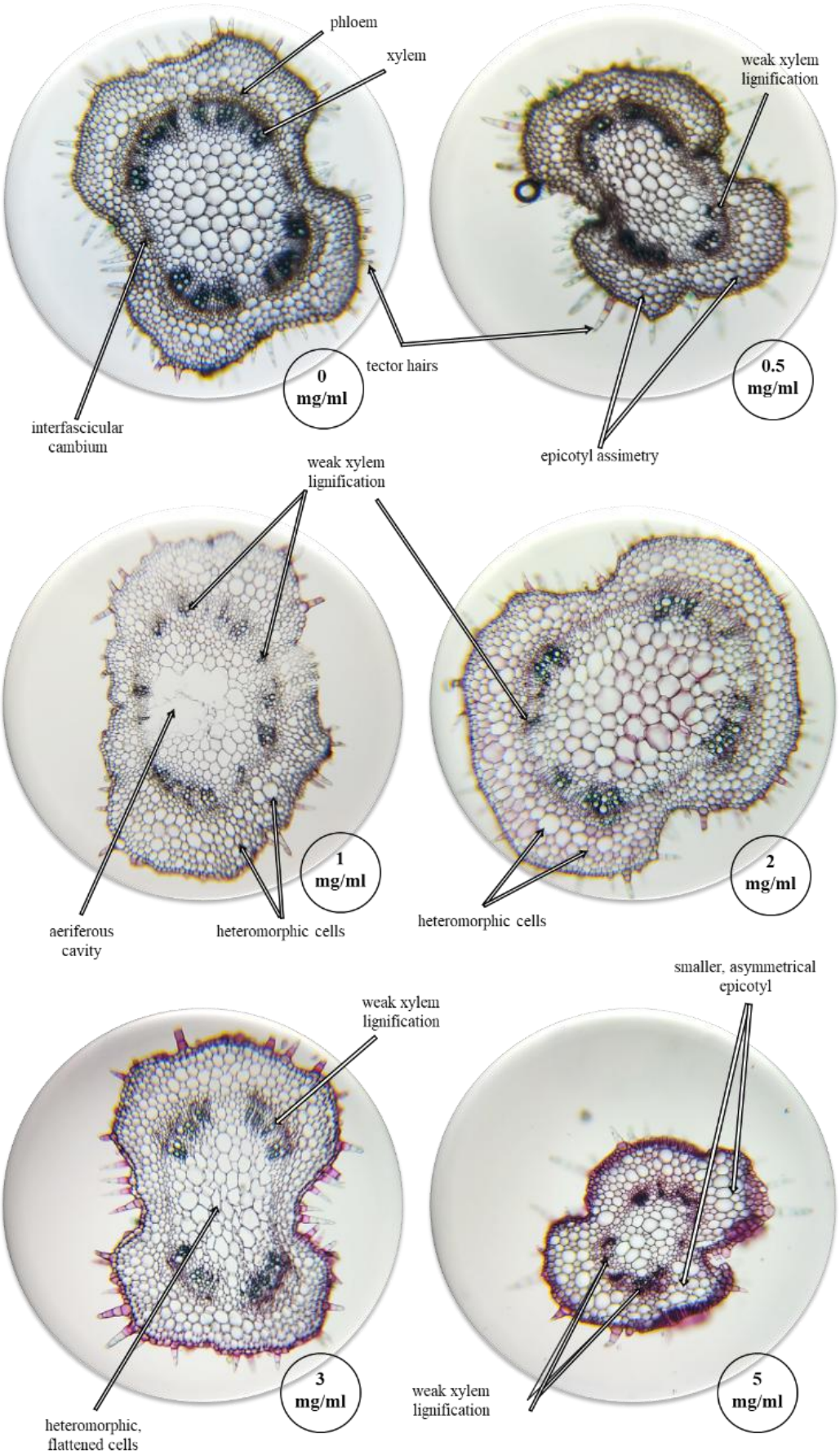

Figure 12. Transverse sections through epicotyls of *O. basilicum* L. cultivar 'Aromat de Buzău' treated with colchicine at the shoot apex

To determine the potential allelopathic effect induced by biologically active compounds present in the plant organs of *Colchicum autumnale* L. on test plants, two methanolic extracts from the bulbs and flowers of meadow saffron were prepared, and the same basil cultivars as in the previous experiment in which aqueous solutions of colchicine were used were used as test plants. Colchicine quantification in the extracts was performed by HPLC, according to the method of Alali et al. (2004), which has also been used in subsequent studies.

The results of a preliminary study on the allelopathic effects of methanolic extracts from the bulbs and flowers of *C. autumnale* L. (Moroșan et al., 2022) indicated that both undiluted extracts tested completely inhibited the germination of basil seeds from both cultivars, an effect that can be attributed to the toxicity of methanol used as a solvent in the extraction. In contrast, the diluted extract from the flowers partially inhibited seed germination, whereas the diluted extract from the bulbs of *C. autumnale* L. completely inhibited it (Table 3).

Table 3. Final germination percentage of *O. basilicum* L. seeds treated with *C. autumnale* L. alcoholic extracts (EB 1:1 = diluted bulb extract (1:1); EB = undiluted bulb extract; EF 1:1 - diluted flower extract (1:1); EF- undiluted flower extract, statistically significant differences are marked with different letters)

	'Italiano Classico'	**'Aromat de Buzău'**
C_0 (H_2O)	$80\pm1,92^{a}$	$68.89\pm7,78^{b}$
C_1 (MeOH 35%)	$70\pm11,55^{b}$	$23,33\pm4,41^{c}$
1:1 EB	0 ± 0^{d}	0 ± 0^{d}
1:1 EF	$40\pm18,93^{c}$	$33,33\pm6,01^{c}$
C_2 (MeOH 70%)	0 ± 0^{d}	0 ± 0^{d}
EB	0 ± 0^{d}	0 ± 0^{d}
EF	0 ± 0^{d}	0 ± 0^{d}

Regarding the responses of *O. basilicum* L. plantlets treated on the shoot apex with these extracts, observations showed that the treatments did not significantly affect the physiological processes of the plantlets or the content of assimilatory pigments but induced vein deviation and leaf shape anomalies (Figure 13), as in the case of treatments with aqueous solutions with different concentrations of colchicine.

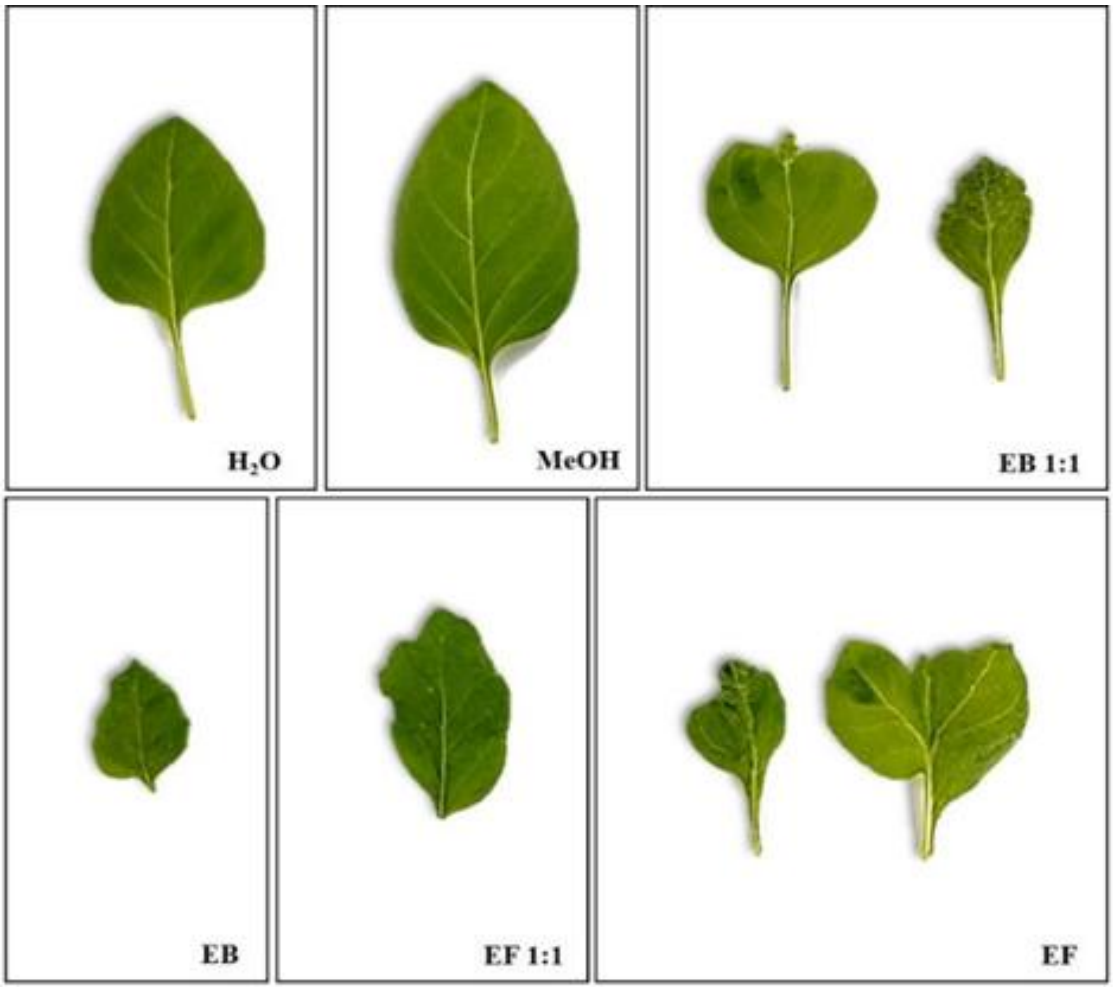

Figure 13. Morphology of leaves of *O. basilicum* L. cultivar 'Aromat de Buzău' from the basal node of plantlets treated with alcoholic extracts of C. autumnale L. (EB 1:1 = diluted bulb extract (1:1); EB = undiluted bulb extract; EF 1:1 - diluted flower extract (1:1); EF- undiluted flower extract)

Moreover, treatment with methanolic extracts of *C. autumnale* L. bulbs and flowers induced on the leaf surfaces of basil plantlets of both cultivars treated on the shoot apex similar effects to those observed in the case of those treated with aqueous colchicine solutions: increased number of tector hairs and their elongation, heteromorphic cells, and weak lignification of the xylem (Figure 14).

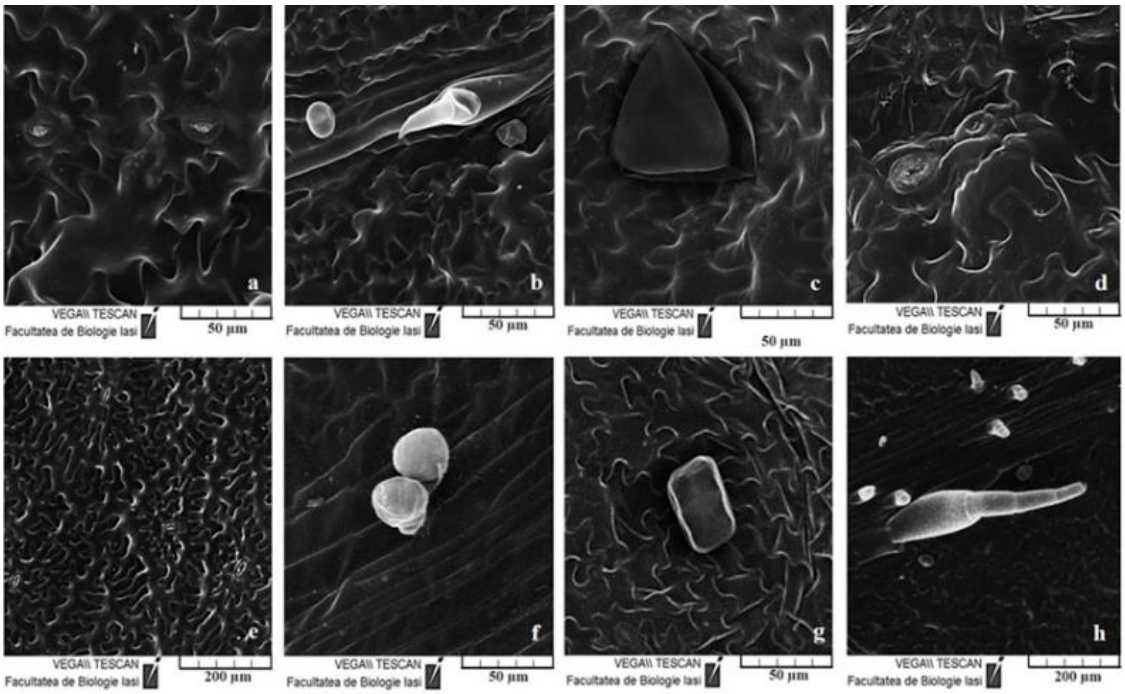

Figure 14. Anomalies observed on the leaf surfaces of *O. basilicum* L. cultivars 'Italiano Classico' and 'Aromat de Buzău' after application of treatment with alcoholic extracts of *C. autumnale* L. on the stem apex (a – typical appearance of the abaxial surface; b – typical appearance of the adaxial surface; c – glandular hair with altered shape; d – twin stomata; e – epidermal cells with very wavy lateral walls; f – twin glandular hairs; g – glandular hair with modified shape; h – morphogenesis of tector hairs and an elongated multicellular tector hair)

After treatment with methanolic extracts of *C. autumnale* L., dehydration of the epidermis and glandular hairs with altered shape were observed on the leaf surfaces of basil plantlets of both cultivars, and the transverse contour of the examined epicotyls showed a pronounced alteration, explained by the appearance of heteromorphic cells in their structure (Figure 15).

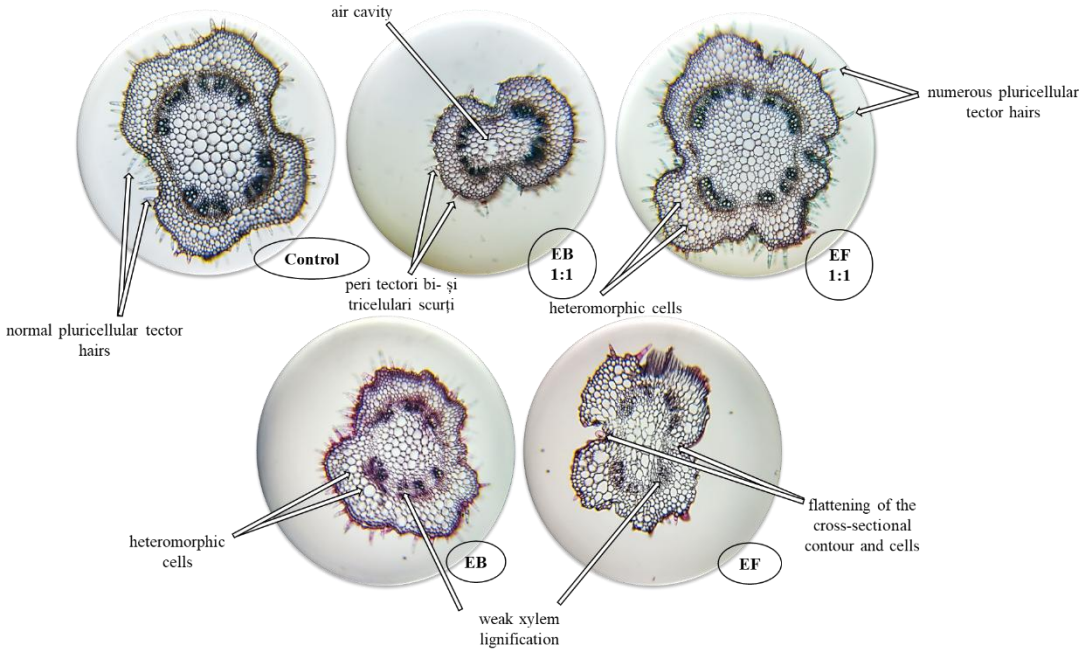

Figure 15. Differences identified in the epicotyl structure of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets, after treatment with alcoholic extracts of *C. autumnale* L., compared to the control (EB 1:1 = diluted bulb extract (1:1); EB = undiluted bulb extract;
EF 1:1 - diluted flower extract (1:1); EF- undiluted flower extract)

Thus, additional research is required to elucidate the level of toxicity and allelopathic potential of secondary metabolites produced by plants belonging to the species *C. autumnale* L. towards other plants, as well as to determine how this species can affect the dynamics of plant populations in its vicinity and whether it would be beneficial to be introduced in a controlled manner into edible or medicinal plant crops as a protective agent against diseases and pests without qualitatively affecting the respective crops. Thus, it was proposed to modify the working protocol, replace the solvent in the extracts (methanol was replaced by ethanol), and expand and diversify the analyses at the morpho-anatomical, physiological, and biochemical levels.

2. Furter results

Characterization of alcoholic and aqueous extracts from vegetative and reproductive organs of C. autumnale L.

In extracts prepared from *C. autumnale* L. plant organs, the content of detected bioactive compounds was generally higher in alcoholic extracts than in aqueous extracts prepared from the same plant material (using the same mass of powdered plant material in

the preparation of the extracts, namely 5 g), except for the extract from bulbs collected during the flowering period.

The highest content of colchicine, the main bioactive compound, was detected in alcoholic and aqueous extracts prepared from flowers (8), and the lowest was detected in extracts from bulb sheaths (7). Colchicine, like many secondary metabolites, contributes to plant survival through its toxic effects on herbivores and pathogens (Ghosh and Jha, 2008), a phenomenon that may explain the higher colchicine content in the above-ground organs of meadow saffron compared to that in the underground organs.

Compared to the results of previously conducted research, included in the preliminary results, in which extracts were prepared from plant material (bulbs and flowers) collected from the same meadow (in October 2019), with colchicine concentrations detected by the same method (0.119±0.007 mg/ml in the bulb extract and 0.286±0.015 mg/ml in the flower extract, respectively), the colchicine concentration in the extracts obtained from the material collected in 2021 was two times lower in the bulbs and approximately three times higher in the flower extract. These differences may be due to the environmental conditions in which the plants grew, the time of collection, the total biomass of the harvested plant material, the solvent used in the extraction, and the extraction time.

The differences highlighted by comparing the results of the present study with the preliminary ones may be due to the fact that the plant material was harvested in different years, but also to the fact that the method was subsequently adapted after observing that methanol (in a ratio of 70:30 with H_2O) is very toxic to the seeds and plantlets of *O. basilicum* L., and ethanol (in a ratio of 70:30 with H_2O) was chosen as the final solvent with lower toxicity for the use of the extracts in treatments applied to the test basil cultivars.

In the present study, the colchicine content in the bulbs was 1.328±0.003 mg per gram of plant material collected during the growing period (spring) and 0.617±0.002 mg per gram of bulbs collected during the flowering period (autumn), using a 70:30 ethanol-water ratio. Comparing the results obtained by Davoodi et al. (2021), who obtained a content of 4.4592 ± 0.0109 mg of colchicine per gram of plant material represented by bulbs using methanol-water in a ratio of 80:20 as the final solvent, indicates that methanol could be a more suitable solvent for colchicine and this compound has a better solubility in a higher concentration of methanol. In this situation, it is essential to consider several factors that may influence the colchicine content of the extracts, including the environmental conditions

in which the *C. autumnale* L. plants were grown, the neighboring plant species that compete with the meadow saffron for nutrient resources, the availability of nutrients in the soil, the time of collection of the plant material, and the extraction method used to obtain the extracts.

Colchicine is considered the main degradation product of colchicine (in addition to lumicolchicine) (Kurek and Barczyński, 2016), and is used for quality control of plant extracts. Colchiceine was identified at low concentrations in the alcoholic and aqueous extracts of young leaves (2) and in the alcoholic and aqueous extracts prepared from fruits (3E, 5E, and 5A) and was very low in the alcoholic and aqueous extracts of bulbs (1 and 6), alcoholic extract of mature leaves (4E), and in the alcoholic and aqueous extracts of flowers (8). This compound was not detected in the aqueous or ethanolic extracts of the bulb sheaths. The content of this compound increases in *C. autumnal*e L. fruits during ripening, as indicated by the higher concentration of colchicine in alcoholic and aqueous extracts of mature fruits (5) compared to that in extracts from immature fruits (3).

Demecolcine, although reported in several studies on the biochemical composition of *C. autumnale* L. (Malichová et al., 1979; Yoneda et al., 1984; Herbert, Kattah and Knagg, 1990; Davoodi et al., 2021), was not detected in the extracts prepared in the present study. This may indicate that the substrate in the area from which the plant material was collected and the environmental or stress conditions experienced by *C. autumnale* L. individuals before collection influenced the chemical composition of these plants, as determined by the analysis of alcoholic and aqueous extracts.

Apigenin was identified in the alcoholic and aqueous extracts of flowers (8), aqueous extract of mature leaves (4A), and alcoholic extract of young leaves (2E). This compound is a flavone with antioxidant properties that helps protect plant cells from oxidative stress, a crucial property for preventing damage caused by reactive oxygen species (Madunić et al., 2018; Azeem et al., 2024) and UV-B rays (Righini et al., 2018). As a secondary metabolite, apigenin contributes to plant defense mechanisms against pathogens and environmental stressors (Mushtaq et al., 2023; Azeem et al., 2024). Comparing these results with previously obtained results and with data from the literature (Burzo et al., 2005), it was confirmed that apigenin is found in the flowers of *C. autumnale* L. and was also identified in very small amounts in leaf extracts.

Salicylic acid was identified at the highest concentration in the alcoholic and aqueous extracts of flowers (8) and leaves (2 and 4). This compound was also identified in the

alcoholic extracts of other organs, such as bulbs collected in spring (1E), immature and mature fruits (3E and 5E), and bulb sheaths (7E). The quantification of this compound in the alcoholic extracts of leaves (2E and 4E) and fruits (3E and 5E) suggests an increase in its content during the maturation of these organs. Salicylic acid is a compound involved in the flowering process, plays an important role in the protection of plants against biotic and abiotic stress factors (Wani et al., 2017), and contributes to the regulation of physiological and biochemical processes throughout the plant life cycle, influencing their growth and development (Vicente and Plasencia, 2011; Mo et al., 2020). However, given the very low content of this compound in the extracts, it may not exert visible biological effects on the test seeds and plantlets in the short term.

Analyses of plant material (vegetative and reproductive organs of *C. autumnale* L. plants) showed that during the vegetative stage, the highest polyphenol content was found in the aboveground organs, especially in leaves (2, 4) and flowers (8), and the polyphenol content determined was higher in bulbs collected during spring (1) than in those collected in autumn (6). In addition, the analysis of alcoholic extracts suggested that the accumulation of polyphenols in fruits (3E and 5E) would occur during ripening; however, in aqueous extracts, this content remained approximately constant.

Similarly, in the analysis of the polyphenol content in alcoholic and aqueous extracts from plant organs of *C. autumnale* L., it was found that during the growth stage (spring period), the highest flavonoid content was found in the aboveground organs, especially in leaves (2, 4), flowers (8), and fruits (3, 5). Flavonoids are phenolic compounds that regulate plant development and contribute to the pigmentation of flowers and fruits, which are essential for attracting pollinators (Mierziak, Kostyn and Kulma, 2014; Mathesius, 2018). This may explain their abundant presence in these organs. Moreover, these compounds accumulate in the epidermis and protect plants from harmful solar radiation (Shah and Smith, 2020; Ferreyra, Serra and Casati, 2021).

Flavonoids exhibit strong antioxidant properties, helping to eliminate reactive oxygen species (ROS) and thus play a significant role in plant stress tolerance (Khalid, Bilal, & Huang, 2019; Dias, Pinto, & Silva, 2021; Shomali et al., 2022), but also against pathogens and herbivores, increasing their resistance to biotic stress (Mathesius, 2018; Alseekh et al., 2020; Shah and Smith, 2020). Moreover, flavonoids are involved in cell signaling processes, such as root-rhizosphere interactions, in which they can stimulate or inhibit microbial activity, affect nutrient uptake, and mediate, influence, or even determine allelopathic

interactions (Hassan and Mathesius, 2012; Shah and Smith, 2020). This explains their presence, even in lower quantities, in underground organs such as tubers.

The antioxidant activity was considerably higher in alcoholic extracts prepared from the vegetative and reproductive organs of *C. autumnale* L. plants than in aqueous extracts. All meadow saffron organs exhibited high antioxidant activity, except for the spring-collected bulb extract. In the case of aqueous extracts, the detected antioxidant activity was weak, and no significant variations were observed between the extracts.

The highest antioxidant activity was detected in alcoholic extracts from fruits (3E and 5E), bulbs collected in autumn (6E), and flowers (8E), without significant variations, but also in leaves (2E, 4E, and 7E). Polyphenols and flavonoids were detected in extracts from flowers (8E), leaves (2E and 4E, but not 7E), and fruits (3E and 5E). It is possible that flavonoids are the main compounds that contribute to the antioxidant activity in the aboveground organs of meadow saffron, although there are significant differences in flavonoid content between the alcoholic extracts prepared from these organs.

The very low antioxidant activity in the case of aqueous extracts prepared from the vegetative and reproductive organs of *C. autumnale* L. plants may indicate the presence of compounds with higher solubility in alcohol or insoluble/less soluble in water. In addition, the polyphenol and flavonoid content of alcoholic extracts from bulbs collected in autumn (6E) was very low, providing clues regarding the presence of other compounds in this extract that contribute to increased antioxidant activity.

Preliminary tests on O. basilicum L. cultivars

Following the application of the squash method and the observation of chromosomes under an optical microscope, it was established that the optimal method of treating plantlets to clearly visualize the chromosomes for the cultivars ‘Italiano Classico’ and ‘Aromat de Buzău’ of the species *O. basilicum* L. is with 8-hydroxyquinoline 0.002 M (according to the method used by Truță and Zamfirache, 2013) for 240 min at room temperature (Figures 16, 17). The metaphase study indicated that the ‘Italiano Classico’ and ‘Aromat de Buzău’ cultivars of *O. basilicum* L. each presented the same number of chromosomes (2n = 48, x = 12), an observation that is consistent with the information in the literature regarding the number of chromosomes present in this species (Pushpangadan and Bradu, 1995; Khosla,

1995; Archna et al., 2013), but there is also variability in the number of chromosomes of other cultivars belonging to this species (Paton and Putievsky, 1996; Mukherjee et al., 2005). Therefore, the potential differences in the responses of basil plants to treatment with *C. autumnale* L. extracts may be due to morphogenetic and metabolic factors specific to each of the studied cultivars.

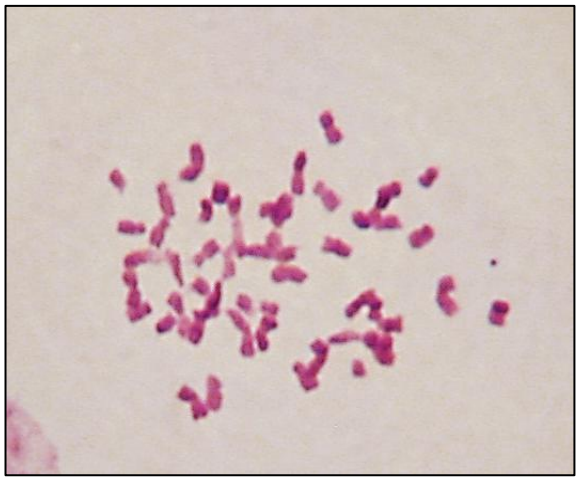

Figure 16. Metaphase chromosomes of *O. basilicum* L. seedlings - 'Italiano Classico' cultivar - treated with 8-hydroxyquinoline 0.002 M for 4 hours (2n = 48)

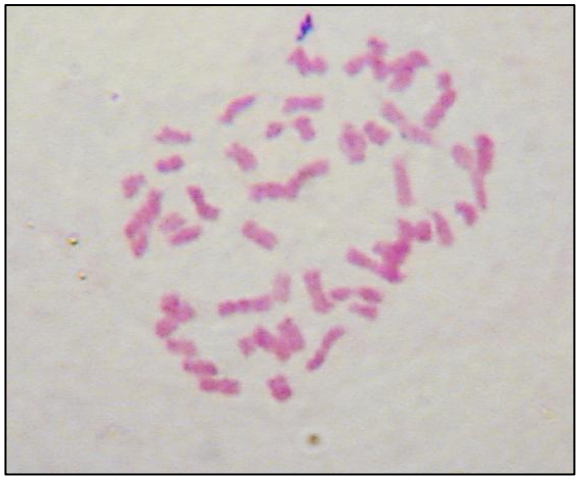

Figure 17. Metaphase chromosomes of *O. basilicum* L. seedlings - 'Aromat de Buzău' cultivar - treated with 8-hydroxyquinoline 0.002 M for 4 hours (2n = 48)

After performing the viability test according to the method described by Boldor, Raianu and Trifu (1983), we found that a concentration of 70% ethanol had a significant impact on the viability of the embryo of *O. basilicum* L. seeds in the case of both cultivars studied, causing necrosis of the embryonic tissues after only 30 min of exposure. Similarly, a concentration of 35% ethanol completely inhibited the viability of the seeds of *O. basilicum* L. cultivar 'Italiano Classico' and partially of those of the cultivar 'Aromat de Buzău', this cultivar appearing to be more resistant to the chemical stress induced by ethanol. Simultaneously, a lower concentration (23.33%) of ethanol significantly affected only the viability of the embryos of the seeds of the cultivar 'Italiano Classico.' The lowest ethanol concentration tested (17.5%) did not significantly affect the viability of basil seed embryos of the 'Aromat de Buzău' cultivar compared to the control variant (H_2O treatment), but only affected the viability of basil seed embryos of the 'Italiano Classico' cultivar (Table 4).

Table 4. Percentage of viability (%) of seed embryos of *O. basilicum* L. cultivars 'Italiano Classico' and 'Aromat de Buzău' after 30 minutes of imbibition with different concentrations of ethanol (statistically significant differences are marked with different letters)

Treatment	***O. basilicum* L. 'Italiano Classico'**	***O. basilicum* L. 'Aromat de Buzău'**
H_2O	93.33±7.14 [a]	86.67±7.69 [b]
EtOH 17.5%	80±14.43 [b]	86.67±7.69 [b]
EtOH 23.33%	40±28.87 [c]	86.67±15.38 [b]
EtOH 35%	0±0 [d]	26.67±25 [d]
EtOH 70%	0±0 [d]	0±0 [d]

The imbibition test performed on *O. basilicum* L. seeds with alcoholic and aqueous extracts from the vegetative and reproductive organs of *C. autumnale* L. indicated that the mass of extract absorbed by imbibition per seed was inversely proportional to the extract concentration, but this was lower in the case of extracts than in the corresponding control.

Seeds of *O. basilicum* L. cultivar 'Aromat de Buzău' showed a greater capacity to absorb and retain a greater quantity of alcoholic and aqueous extracts than the 'Italiano Classico' cultivar.

Effects of extracts prepared from the vegetative and reproductive organs of C. autumnale L. plants on O. basilicum L. seeds

I. During growth

Alcoholic and aqueous extracts prepared from the vegetative and reproductive organs of *C. autumnale* L. plants collected during the growing period (spring) affect the germination of seeds of the tested cultivars differently. Alcoholic and aqueous extracts from bulbs (1) inhibited plantlet growth, as highlighted by the significant reduction in the seedling vigor index (SVI). Leaf and fruit extracts exert their effects in different ways, depending on the concentration of their biologically active compounds and the solvent used for extraction. Compared to seeds treated with distilled H_2O (C_0), the alcoholic extract from meadow saffron bulbs inhibited all seed germination indices of both basil cultivars, and the aqueous extract inhibited the seedling index (SE) of the 'Aromat de Buzău' cultivar.

The aqueous leaf extract (2), with a high colchicine content (higher than that of the bulbs but lower than that of the alcoholic leaf extract), inhibited seedling growth (SVI), while the alcoholic extract stimulated seedling emergence (SE) and daily germination time (MDG)

of the seeds of both basil cultivars. However, there were also specific responses of the seeds of each cultivar to the treatment with the alcoholic leaf extract: the germination of the seeds of the cultivar ‘Italiano Classico’ was stimulated in terms of final germination percentage (FGP) and seedling vigor index (SVI), while the germination of the seeds of the cultivar ‘Aromat de Buzău’ (GI) was stimulated in terms of germination energy (GI) and germination index. Compared to seeds treated with distilled H_2O (C_0), the alcoholic leaf extract inhibited the final germination percentage (FGP) of both cultivars, as well as the germination indices (GI) and seedling vigor in the case of seeds of the ‘Italiano Classico’ cultivar, but these inhibitory effects can actually be attributed to the ethanol extract.

The aqueous extract of the fruit (3) inhibited both seedling growth (SVI) and the seedling emergence index (SE), whereas the alcoholic extract stimulated seed germination in terms of final germination percentage (FGP) and emergence index (SE), compared to the treatment with 70% ethanol, in both tested cultivars. Moreover, the alcoholic extract of the fruit stimulated daily germination time (MDG) and seedling growth (SVI) in the ‘Italiano Classico’ cultivar. It seems that a low concentration of the compounds in the prepared extracts negatively affects the germination of basil seeds, while a high concentration shows stimulatory effects. In contrast, compared to seeds treated with distilled H_2O (C_0), the alcoholic fruit extract inhibited the final germination percentage (FGP), germination index (GI), and seedling vigor (SVI) in both cultivars.

II. During fruiting

The aqueous leaf extract (4) inhibited the seedling emergence index (SE) of both basil cultivars and the germination energy (GE) and seedling growth (SVI) of the ‘Aromat de Buzău’ cultivar. In contrast, the alcoholic extract prepared from the same plant material stimulated the seedling emergence index (SE) of the ‘Aromat de Buzău’ cultivar, compared to the ethanol treatment. Compared to seeds treated with distilled H_2O (C_0), the alcoholic leaf extract inhibited all germination indices of seeds of both cultivars.

The aqueous fruit extract (5), although not containing high levels of colchicine, inhibited the growth of plantlets (SVI) of both basil cultivars, while the alcoholic extract stimulated the seedling emergence index (SE) and germination energy (GE) compared to ethanol treatment. These observations support the theory that a lower concentration of the compounds causes inhibitory effects in the recipient plants, while a high concentration (in alcoholic extracts) could induce a beneficial stress that stimulates seed germination in both

O. basilicum cultivars. Compared to seeds treated with distilled H_2O (C_0), the alcoholic fruit extract inhibited most of the germination indices of both cultivars.

III. During flowering

The aqueous extract from bulbs (6) collected during flowering inhibited seed germination of both basil cultivars in terms of seedling emergence index (SE), germination energy (GE), and seedling growth (SVI). The alcoholic extract prepared from the same plant material inhibited mean germination time (MGT) and seedling growth (SVI) compared to treatment with 70% ethanol, as well as all germination indices, compared to seeds treated with distilled H_2O. It seems that this organ (bulb), even if it does not contain a high concentration of colchicine, exhibits inhibitory effects and fulfills the role of storing allelopathic compounds, ensuring the protection of the plant in the underground zone.

The aqueous extract from the bulb sheaths (7) did not exert significant effects on the germination process of the seeds of the tested basil cultivars, and the alcoholic extract showed stimulating effects on germination compared to the treatment with ethanol (but was inhibitory compared to the seeds treated with distilled H_2O). However, the cultivars responded differently to the treatments with this extract. The alcoholic extract from the bulb sheaths stimulated the final percentage germination (FGP), average daily germination time (MDG), and seedling vigor index (SVI) of the 'Italiano Classico' cultivar, as well as the emergence index (SE) and germination energy (GE) of the seedlings of the 'Aromat de Buzău' cultivar. If these results had been analyzed strictly in relation to those of the control variant C_0 (with H_2O), it could be said that this organ shows strictly inhibitory effects. In this context, we consider that the bulb sheaths, being the remains of the leaves that protected the plant throughout its life cycle, have lost their allelopathic properties at low concentrations but present stimulatory potential at high concentrations.

Aqueous and alcoholic extracts of flowers (8) inhibited the growth of plantlets (SVI) belonging to both basil cultivars studied, compared to the ethanol treatment, and the aqueous extract of flowers inhibited the emergence of plantlets (SE) of the 'Aromat de Buzău' cultivar. Compared to seeds treated with distilled H_2O (C_0), the alcoholic extract of flowers (with a high concentration of colchicine) inhibited all germination indices of seeds of both basil cultivars. This organ is the allelopathic core of the plant in the aboveground area, protecting it not only from oxidative stress, pests, and herbivores, but also from competition

with other plant species in the vicinity, fulfilling a special role in the perpetuation of *C. autumnale* L. species by protecting its reproductive structures.

Effects of extracts prepared from vegetative and reproductive organs of C. autumnale L. plants on O. basilicum L. plantlets

I. During growth

The aqueous extract from the bulbs (1) stimulated the growth of the epicotyl of the plantlets belonging to both basil cultivars (Figure 40) and increased the leaf area at the basal node of the plantlets of the 'Aromat de Buzău' cultivar. However, the alcoholic extract prepared from the same plant material caused a decrease in leaf area at the basal and intermediate nodes of the 'Italiano Classico' cultivar plantlets (Figure 41). Both types of extracts caused morphogenetic anomalies, such as heteromorphism of the epidermal cells and stomata or the appearance on the leaf surface of some areas of morphogenesis interspersed with areas of developed tissue (Figure 18), anomalies that are most likely determined by colchicine. This extract did not influence the micromorphological modification of the density of the tector and secretory hairs but induced an increase in the stomatal area and changes in their density, depending on the examined leaf surface and the basil cultivar. Cell heteromorphism can also be observed in anatomical sections and a weaker lignification of the conducting vessels (Figure 19), which, according to the literature, is due to colchicine. From a physiological perspective, the alcoholic and aqueous extracts prepared from the bulbs resulted in an increase in the amount of substomatic CO_2, a decrease in the chlorophyll a content in the leaves (Figure 43), and an increase in the antioxidant activity in both basil cultivars (Figure 44). These extracts also decreased the polyphenol content in the leaves of the 'Italiano Classico' cultivar plantlets, and the aqueous extract decreased the flavonoid content in the leaves of both cultivars. Although the plantlets treated with alcoholic and aqueous extracts of *C. autumnale* L. bulbs suffered less from pest attack than those treated with ethanol, the analyzed indices clearly indicated the consequences of the treatments applied.

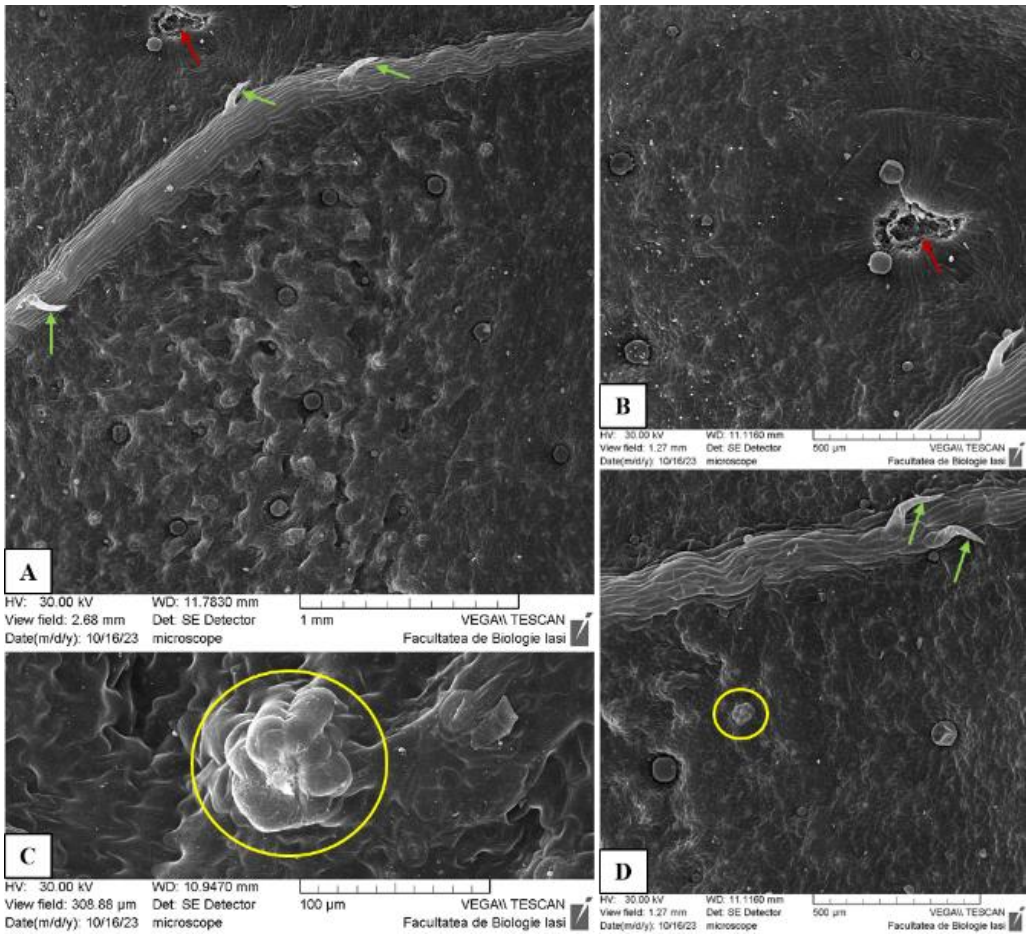

Figure 18. Abaxial leaf surface of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. bulbs (1A) collected in spring (green arrows indicate the appearance of tector hairs; red arrows indicate a scarred area following insect attack; yellow circles frame morphogenesis areas)

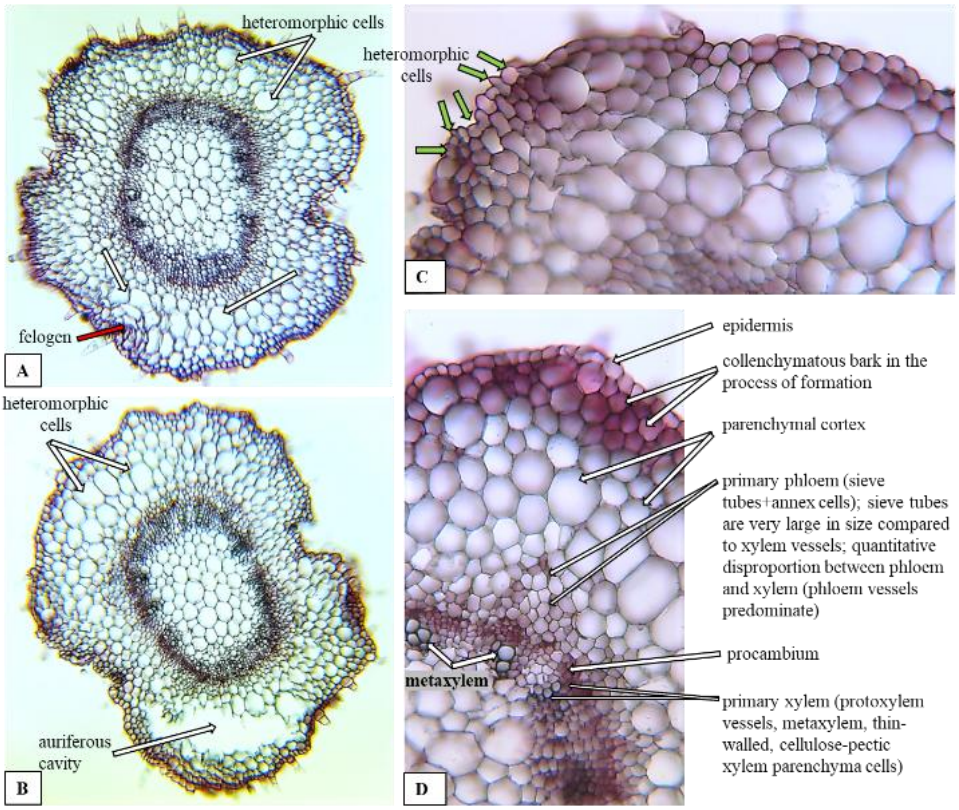

Figure 19. Epicotyl anatomy of *O. basilicum* L. cultivar 'Italiano Classico' plantlets treated with alcoholic extract from *C. autumnale* L. bulbs collected in spring (1E)

The aqueous leaf extract (2) stimulated the growth of the epicotyl of plantlets belonging to both basil cultivars (Figure 40) and of the leaf area at the intermediate node of plantlets of the 'Aromat de Buzău' cultivar (Figure 41). The alcoholic extract prepared from the same plant material stimulated the growth of the epicotyl and leaf area at the intermediate node of plantlets of the 'Aromat de Buzău' cultivar but inhibited leaf growth at the basal and intermediate nodes of plantlets of the 'Italiano Classico' cultivar (Figure 41). Both extracts induced morphogenetic anomalies on the examined leaf surfaces of both cultivars (Figure 20), as well as the appearance of tector hairs on the abaxial surface of the leaves (Figure 20), an area where they do not usually appear. These changes indicate a response of the test plantlets to the chemical stress induced by the treatment. Treatment with these extracts increased the stomatal area on the leaf surfaces of both basil cultivars and altered stomatal density, while the density of trichomes decreased on the analyzed adaxial surfaces. The aqueous extract induced an increase in the density of secretory hairs, which would provide the plantlets with better resistance to the attack of pests and herbivores. However, the test plantlets did not acquire this resistance, as deduced from the observations made on the photographs of the leaf surfaces taken under an electron microscope. Both extracts altered the anatomical structures of the epicotyls of the test plantlets, which acquired a rectangular

outline (Figure 21), a character that represents a slight deviation from the usual square outline, specific to species of the Lamiaceae family. The alcoholic leaf extract caused an increase in the electron transfer rate, an increase in the amount of substomatic CO_2, a reduction in the chlorophyll a content (Figure 43), and an increase in the antioxidant activity in the plantlets of both treated basil cultivars (Figure 44). The aqueous extract caused a decrease in the electron transfer rate (Figure 42) and carotenoid pigment content in the plantlets of the 'Italiano Classico' cultivar and an increase in the content of chlorophyll b (Figure 43), flavonoids, and antioxidant activity in the plantlets of the 'Aromat de Buzău' cultivar (Figure 44). The aqueous extract increased the intensity of transpiration and decreased the content of substomatic CO_2 in the leaves of the plantlets of both basil cultivars. Considering the above, we can consider that young leaves of *C. autumnale* L. represent organs with a certain allelopathic potential, which affect in different proportions various morpho-anatomical-physiological parameters in recipient plantlets and induce chemical and oxidative stress.

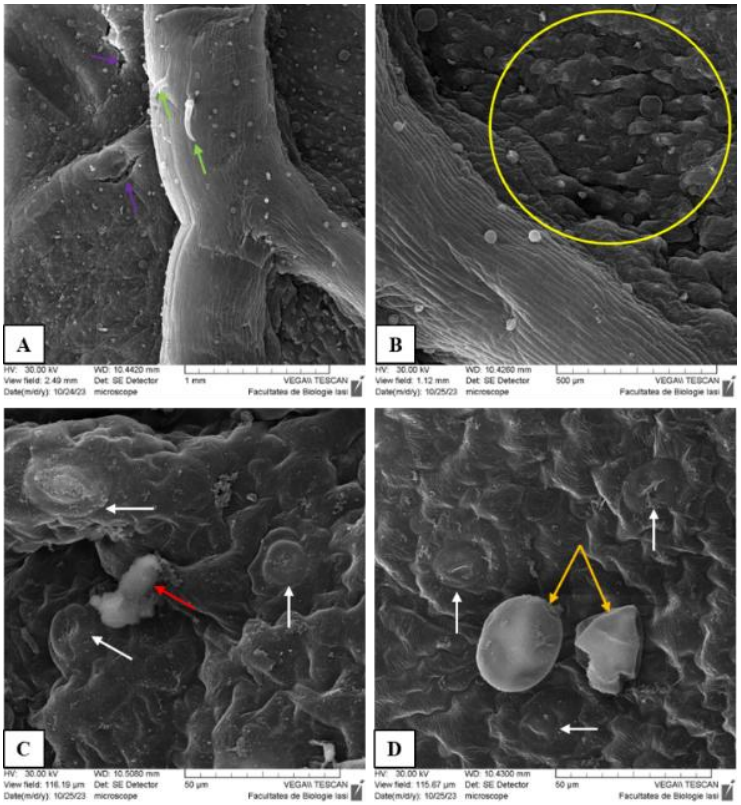

Figure 20. Abaxial leaf surface of *O. basilicum* L. cultivar 'Italiano Classico' plantlets treated with aqueous extract from *C. autumnale* L. leaves (2A) collected in spring (purple arrows indicate epidermal breaks; yellow circle encloses a morphogenesis zone; white arrows indicate heteromorphic stomata; red arrow indicates a foreign formation deposited by insects; orange arrows indicate twin secretory hairs)

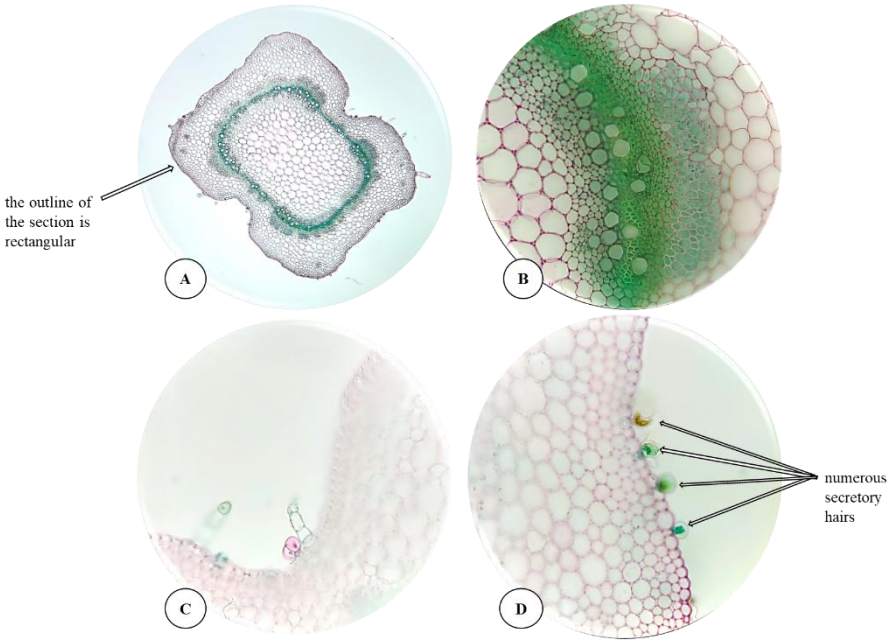

Figure 21. Epicotyl anatomy of *O. basilicum* L. cultivar 'Italiano Classico' plantlets treated with aqueous extract from *C. autumnale* L. leaves (2A) collected in spring

The aqueous extract from the fruit (3) affected the plantlets of the two basil cultivars differently. In the 'Italiano Classico' cultivar, this extract caused a reduction in leaf area at all three nodes (Figure 41), stimulated the elongation of tector hairs on the adaxial leaf surface, simultaneously with a decrease in their density, increased stomatal area on the abaxial surface, decreased electron transfer rate (Figure 42), substomatic CO_2 content, and chlorophyll b content (Figure 43), but also increased antioxidant activity (Figure 44). In the plantlets of the 'Aromat de Buzău' cultivar, this extract stimulated epicotyl growth (Figure 40) and stomatal area on both leaf surfaces, caused variations in stomatal density, and decreased polyphenol and flavonoid content. In contrast, the alcoholic extract of the fruit, similar to the aqueous one, caused a reduction in the leaf area at all three nodes of the plantlets of the 'Italiano Classico' cultivar, but stimulated an increase in the leaf area at the basal node of the plantlets of the 'Aromat de Buzău' cultivar (Figure 41). This extract caused the appearance of morphogenesis anomalies on the leaf surfaces of the plantlets belonging to both basil cultivars, as well as the appearance of tector hairs on the abaxial surface (Figure 22) (chemical stress), an increase in the stomatal area on both leaf surfaces, structural asymmetry of the epicotyl (Figure 23), and an increase in the electron transfer rate (Figure 42). The fruits of plants belonging to the species *C. autumnale* L., although immature and incompletely developed, have the capacity to induce chemical stress in the recipient plants and to affect various parameters, depending on the cultivar, inducing instability in both

anatomical and morphological structures, as well as in physiological and biochemical processes, thus sensitizing them to pest attack, as was also observed in the photographs taken of the leaf surfaces examined under the electron microscope. It can be said that these extracts, in addition to those prepared from the bulbs collected during the flowering period of *C. autumnale* L. plants, as well as from their leaves collected during the fruiting period, showed the most harmful effects on the recipient plantlets belonging to the two cultivars of the test species, *O. basilicum* L.

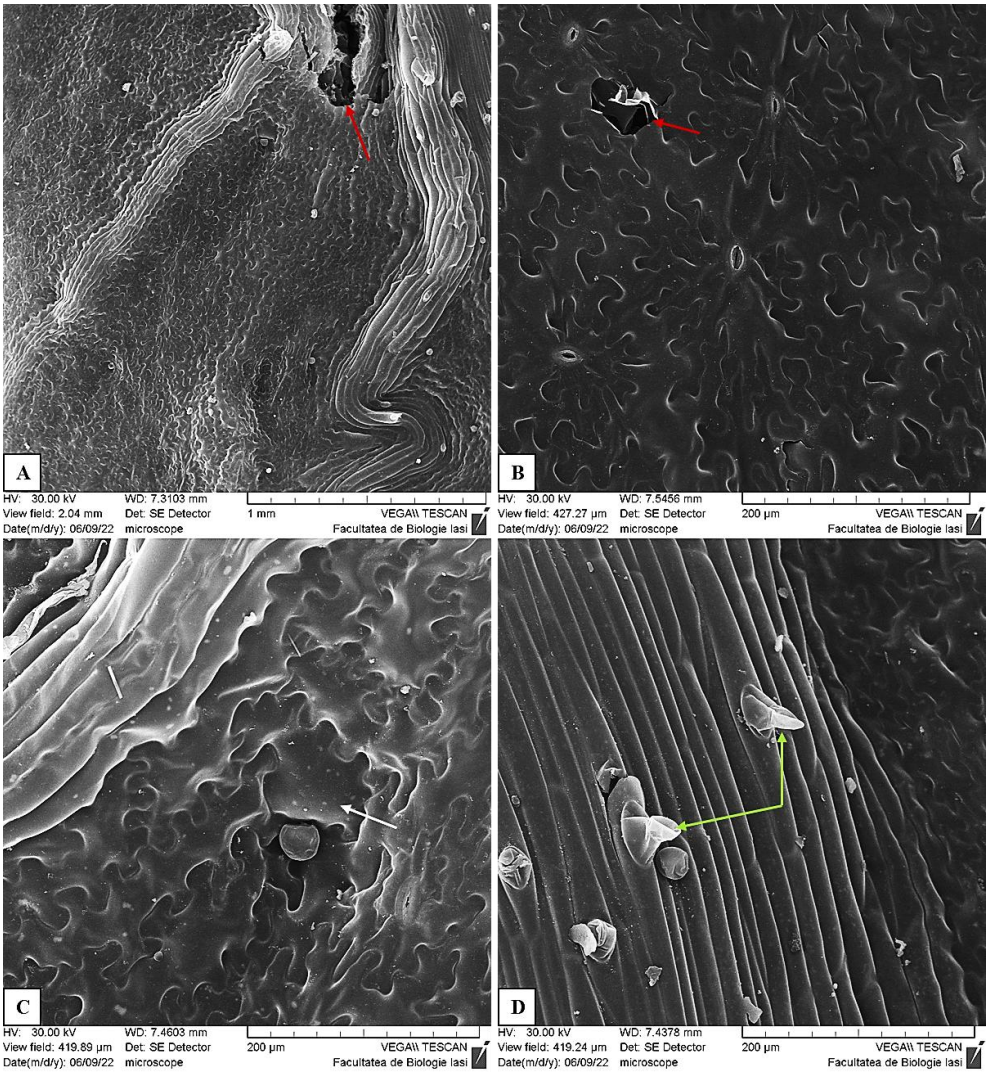

Figure 22. Abaxial leaf surface of *O. basilicum* 'L. Aromat de Buzău' plantlets treated with alcoholic extract from *C. autumnale* L. (3E) fruits collected in spring (red arrows indicate scarring of the epidermis following insect attack; white arrow indicates an epidermal cell with altered shape and large dimensions; green arrows indicate the appearance of tector hairs)

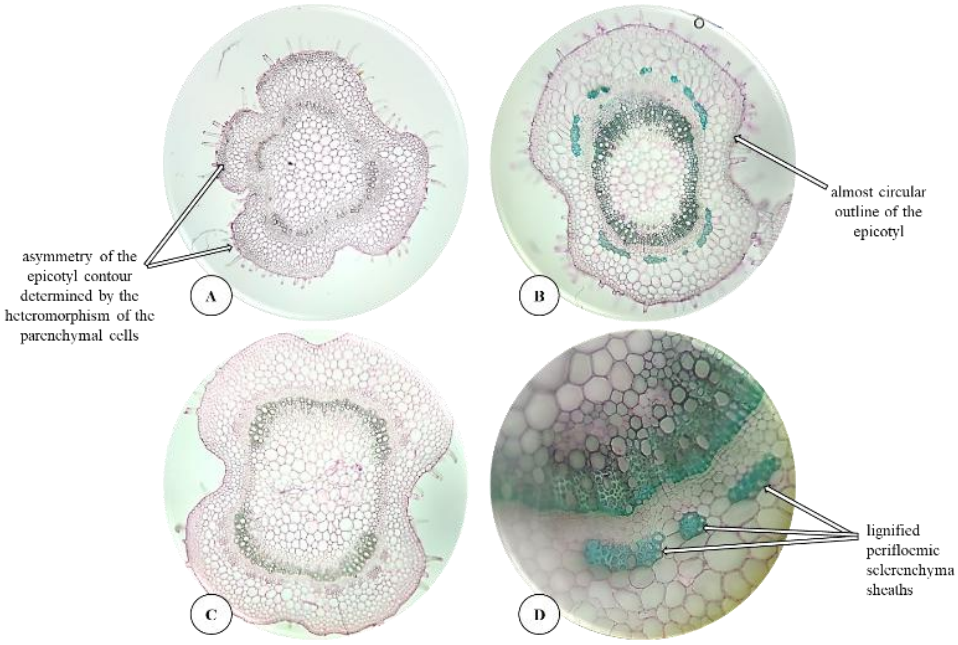

Figure 23. Epicotyl anatomy of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L fruits: (3A) collected in spring

II. During fruiting

Alcoholic and aqueous extracts prepared from the leaves (4) of *C. autumnale* L. plants stimulated the growth of epicotyls of basil plantlets of the 'Aromat de Buzău' cultivar. The aqueous extract from the leaves caused a decrease in the leaf area at all three nodes of the plantlets of the 'Italiano Classico' cultivar and at the intermediate node of the plantlets of the 'Aromat de Buzău' cultivar (Figure 41), an increase in the stomatal area on the abaxial surface of the leaves of the plantlets of the 'Italiano Classico' cultivar and on both leaf surfaces of the plantlets of the 'Aromat de Buzău' cultivar, as well as variations in the density of stomata, tector, and secretory hairs. The aqueous leaf extract from *C. autumnale* L. plants caused a decrease in the electron transfer rate (Figure 42), a decrease in the substomatic CO_2 and polyphenol content, and an increase in antioxidant activity in the plantlets of the 'Italiano Classico' cultivar (Figure 44) and an increase in the substomatic CO_2 and chlorophyll a content (Figure 43) in the plantlets of the 'Aromat de Buzău' cultivar. Simultaneously, this extract also caused a decrease in flavonoid content in the leaves of plantlets belonging to both basil cultivars. The alcoholic extract from the leaves increased the leaf area at the basal node of the plantlets of the 'Aromat de Buzău' cultivar (Figure 41) and variations in the density of stomata, tector, and secretory hairs. Both types of extracts caused morphogenetic abnormalities on the leaf surfaces of the plantlets belonging to the two studied cultivars (including the appearance of tector hairs on the abaxial leaf surface) (Figures 24 and 25), but

also in the epicotyl structure (Figure 26). The alcoholic extract increased the intensity of leaf transpiration. The aqueous extract appeared to be more harmful to the recipient plantlets than the alcoholic extract prepared from the same plant material, affecting various morpho-anatomical-physiological parameters and sensitizing them to pest attack, as was also observed by examining the micromorphological images of their leaf surfaces under an electron microscope.

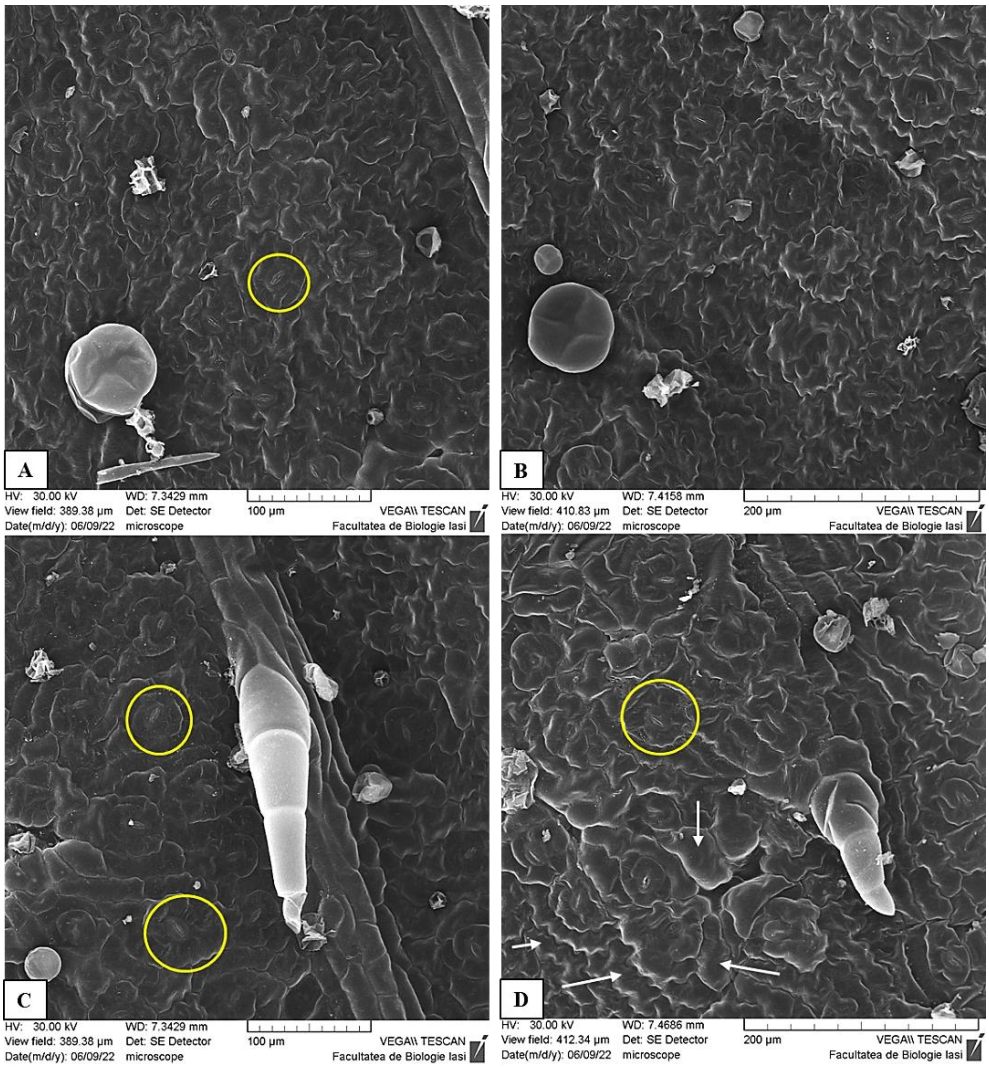

Figure 24. Adaxial leaf surface of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with alcoholic extract from *C. autumnale* L. leaves (4E) collected in summer (white arrows indicate heteromorphic cells; yellow circles enclose areas of stomatogenesis)

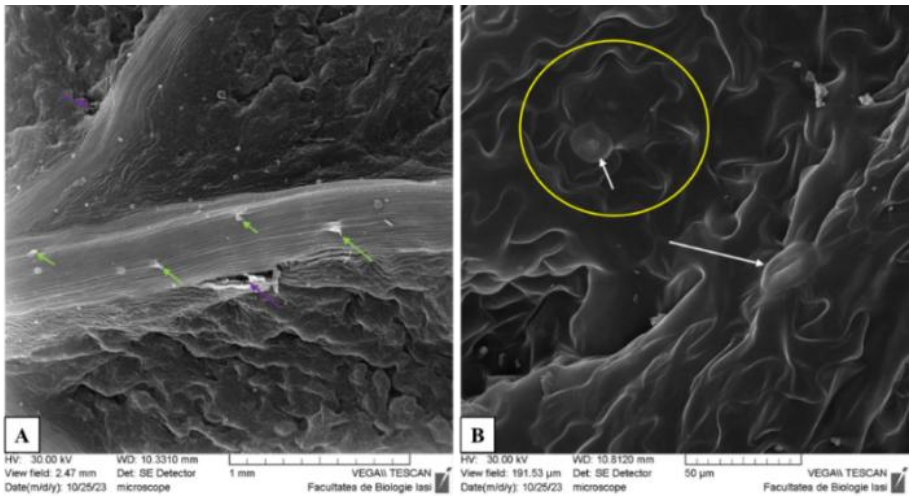

Figure 25. Abaxial leaf surface of *O. basilicum* L. cultivar 'Italiano Classico' plantlets treated with aqueous extract from *C. autumnale* L. leaves (4A) collected in summer (purple arrows indicate breaks or bumps in the epidermis; green arrows indicate the appearance of tector hairs; white arrows indicate heteromorphic stomata; yellow circle frames a stomata in during morphogenesis)

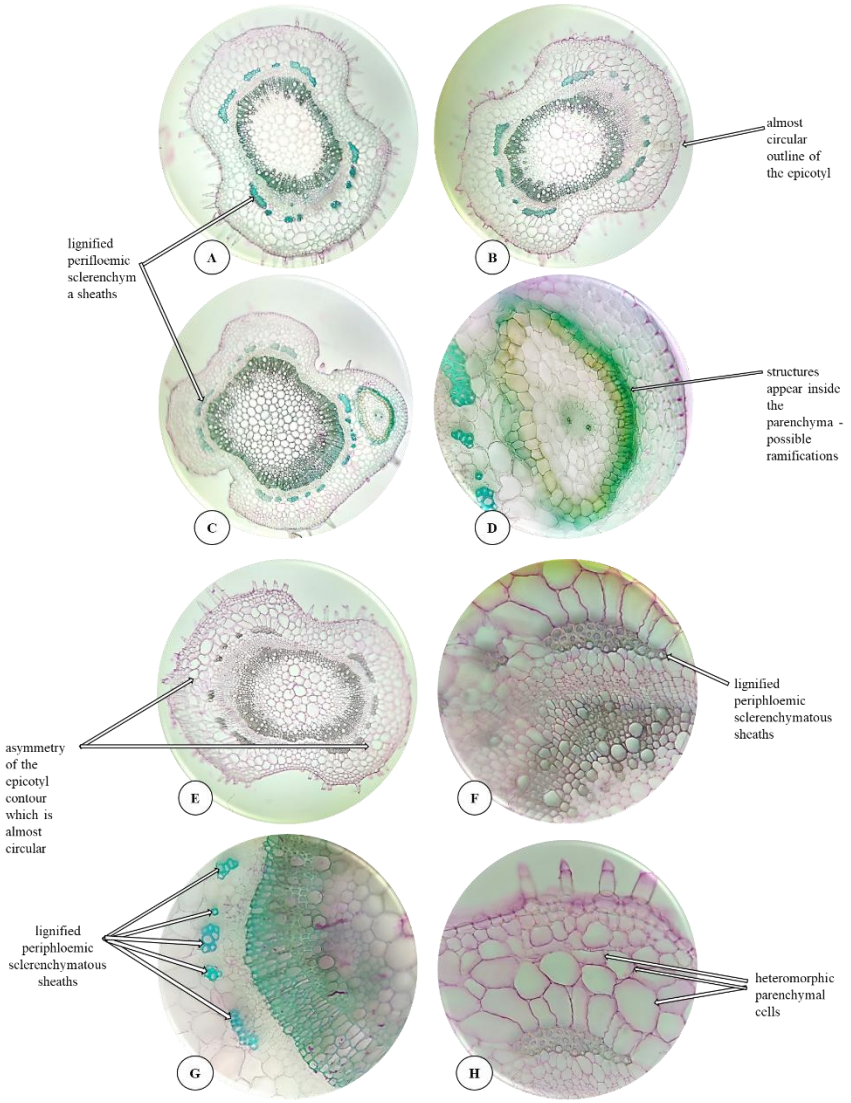

Figure 26. Epicotyl anatomy of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. leaves (4A) collected in summer

The aqueous fruit extract (5) of *C. autumnale* L. stimulated the growth of the epicotyl of plantlets of the 'Italiano Classico' cultivar (Figure 40), inhibited the growth of leaf area at the basal and intermediate nodes of plantlets of the 'Aromat de Buzău' cultivar (Figure 41), induced variations in micromorphological and anatomical indices on the leaf surfaces of both cultivars, decreased the electron transfer rate (Figure 42), the substomatic CO_2 content, polyphenols, and flavonoids in both studied cultivars, and decreased the antioxidant activity in the leaves of plantlets of the 'Aromat de Buzău' cultivar (Figure 44).

The alcoholic extract prepared from the same plant material induced a reduction in leaf area at the intermediate and apical nodes of plantlets of the 'Italiano Classico' cultivar and an increase in leaf area at the intermediate node of plantlets of the 'Aromat de Buzău' cultivar (Figure 41). This extract caused the appearance of signs of fragility and premature senescence in the structure of the examined epicotyls (Figure 29), most likely caused by the growth hormones present in the composition of the fruits of *C. autumnale* L. from which the extract was prepared. Simultaneously, we noted that the discussed extract intensified the respiration process in the plantlets of both cultivars and decreased the chlorophyll b content in the leaves of the 'Aromat de Buzău' cultivar (Figure 43).

Both types of fruit extracts caused morphogenetic abnormalities in the leaves (Figure 27), including the appearance of tector hairs on the abaxial leaf surface (Figure 28) and abnormalities in the epicotyl structures (Figure 29) in the plantlets of the two basil cultivars. However, these extracts did not have such a harmful effect compared to the other extracts on the test plantlets, which, as a result of the treatment received, proved to be more resistant to pest attack or were attacked less than the test plantlets from other experimental variants.

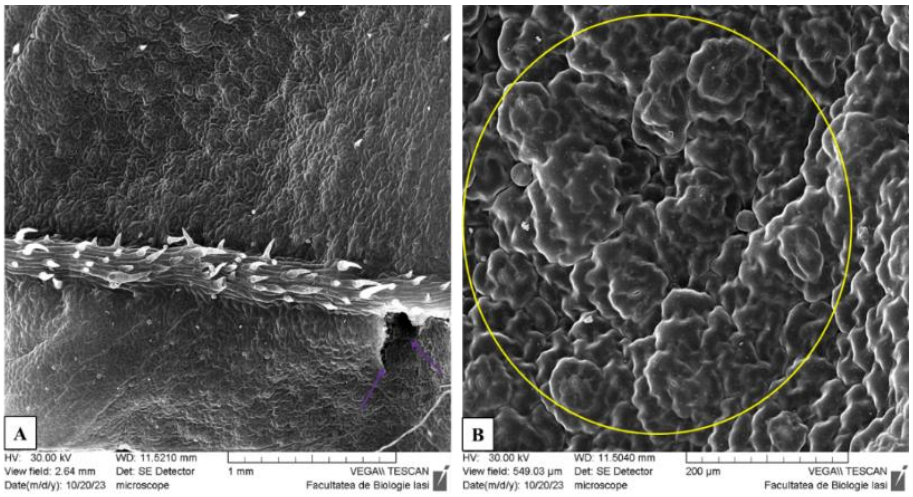

Figure 27. Adaxial leaf surface of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. fruits (5A) collected in summer (purple arrows indicate epidermal breaks; yellow circle frames areas of stomatogenesis)

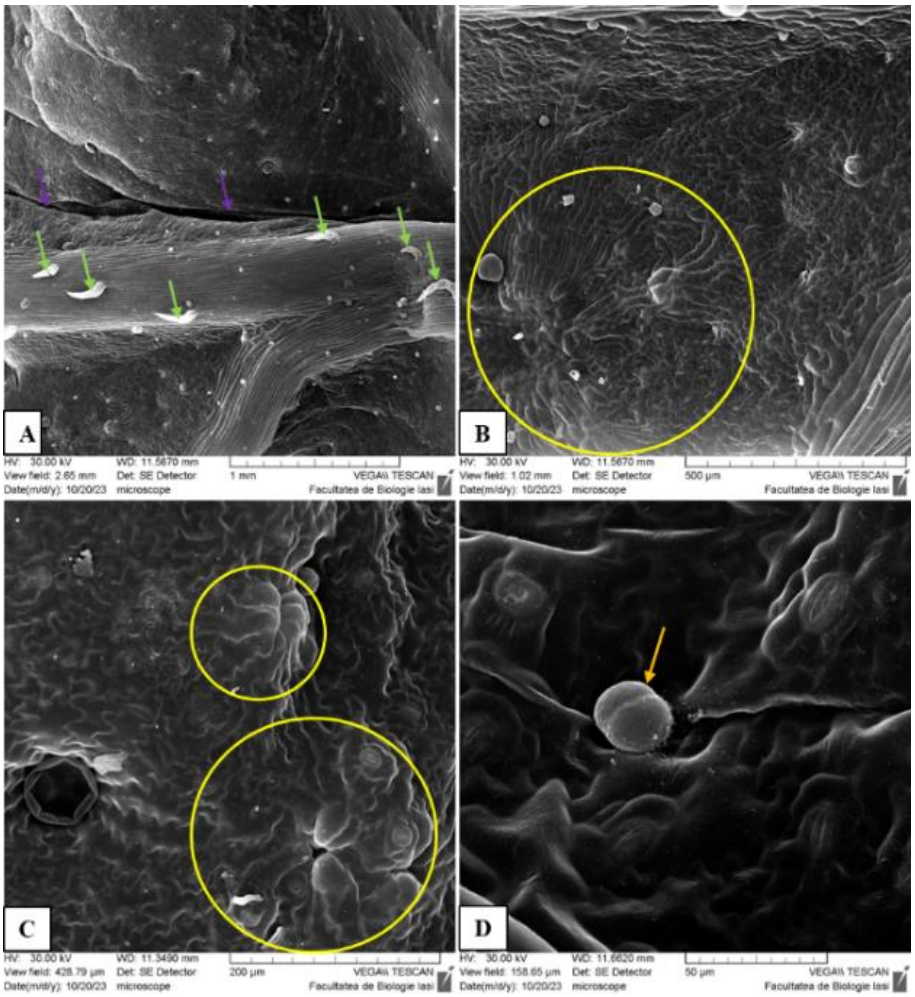

Figure 28. Abaxial leaf surface of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. fruits (5A) collected in summer (purple arrows indicate folds, bumps or discontinuities of the epidermis; green arrows indicate the appearance of tector hairs; yellow circles frame areas of morphogenesis or disorganization of the epidermis; orange arrow indicates a secretory hair with a bicellular secretory head)

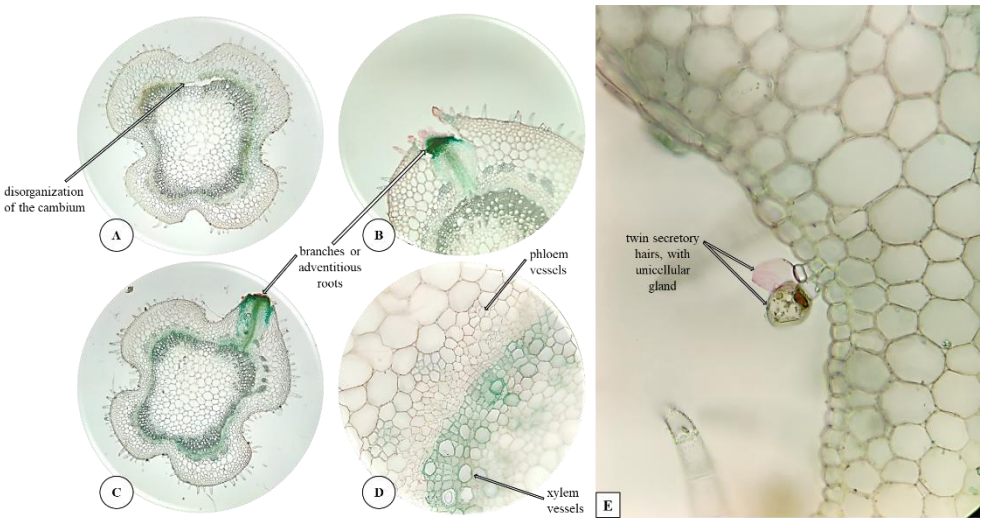

Figure 29. Epicotyl anatomy of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. fruits (5A) collected in summer

III. During flowering

The aqueous extract from bulbs (6) collected during flowering stimulated epicotyl growth of plantlets of the 'Aromat de Buzău' cultivar, inhibited leaf area growth at all three nodes of plantlets of the 'Italiano Classico' cultivar (Figure 41), increased stomatal area on the abaxial surface of leaves of plantlets of the 'Italiano Classico' cultivar and on both leaf surfaces of the 'Aromat de Buzău' cultivar, as well as increased stomatal density and decreased tector hair density on the adaxial surface of leaves of plantlets of the 'Italiano Classico' cultivar. This extract induced a decrease in the electron transfer rate (Figure 42), substomatic CO_2 content, and flavonoid content in the leaves of plantlets of both basil cultivars. The alcoholic extract prepared from the same plant material induced a decrease in the leaf area at the basal and intermediate nodes of the 'Italiano Classico' cultivar plantlets, and this parameter was stimulated by the treatment with the extract in question applied to the 'Aromat de Buzău' cultivar plantlets (Figure 41). The stomatal area decreased, as an effect of the same treatment, on the abaxial surface of the 'Italiano Classico' cultivar plantlets and increased on both leaf surfaces of the 'Aromat de Buzău' cultivar plantlets. In addition, the extract induced an increase in the density of tector hairs on the adaxial surface of the 'Aromat de Buzău' cultivar plantlets and secretory hairs on the upper (adaxial) surface of the leaves of the 'Italiano Classico' cultivar plantlets. This extract induced an increase in the amount of substomatic CO_2, a decrease in the chlorophyll a content (Figure 43), and an increase in antioxidant activity (Figure 44) in the leaves of plantlets belonging to both studied cultivars, increased the electron transfer rate in plantlets of the 'Aromat de Buzău' cultivar

(Figure 42), and the chlorophyll b content in the leaves of plantlets of the 'Italiano Classico' cultivar (Figure 43). According to what has been discussed, we can consider that the 'Aromat de Buzău' cultivar seems to be more resistant than the 'Italiano Classico' cultivar to the allelopathic compounds present in the composition of this extract, specifying, at the same time, the fact that both basil cultivars presented morphogenesis anomalies (Figures 30 and 31), as an effect of applying treatments with alcoholic and aqueous extracts prepared from *C. autumnale* L. bulbs.

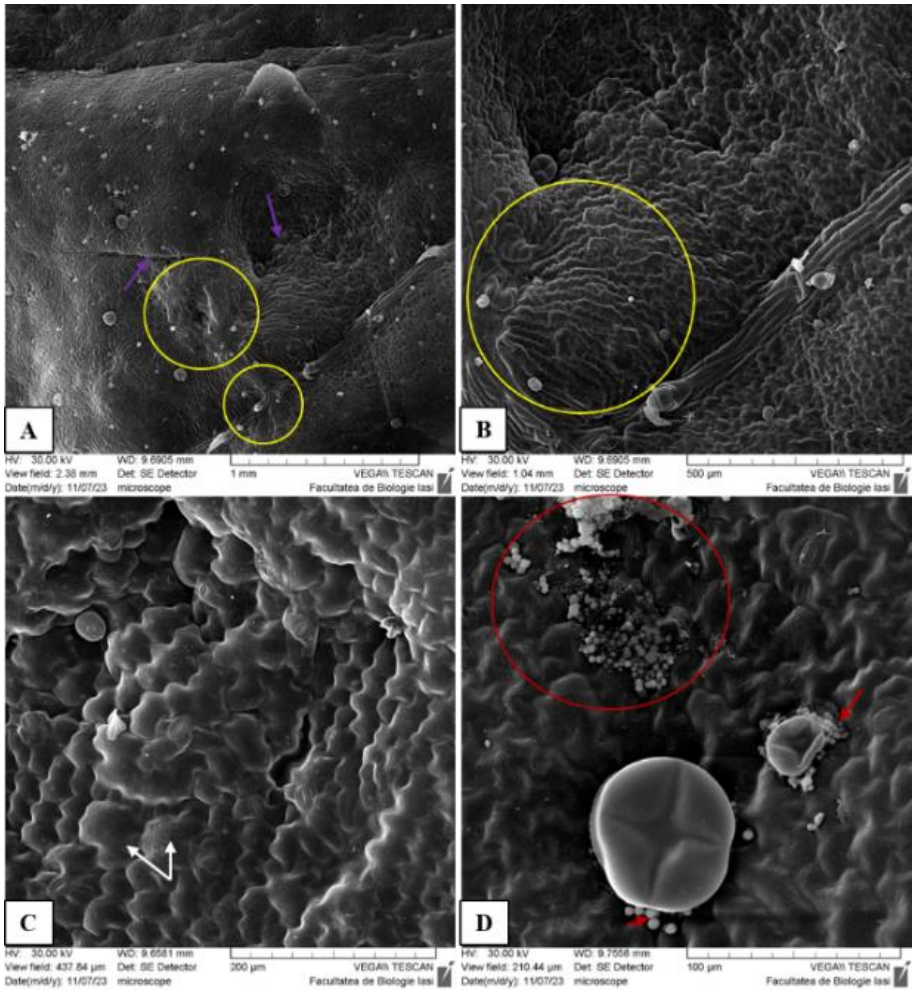

Figure 30. Adaxial leaf surface of *O. basilicum* L. cultivar 'Italiano Classico' plantlets treated with aqueous extract from *C. autumnale* L. bulbs (6A) collected in autumn (purple arrows indicate bumps and folds of the epidermal relief; white arrows indicate heteromorphic epidermal cells; red arrows and red circle indicate foreign formations; yellow circles enclose areas of morphogenesis)

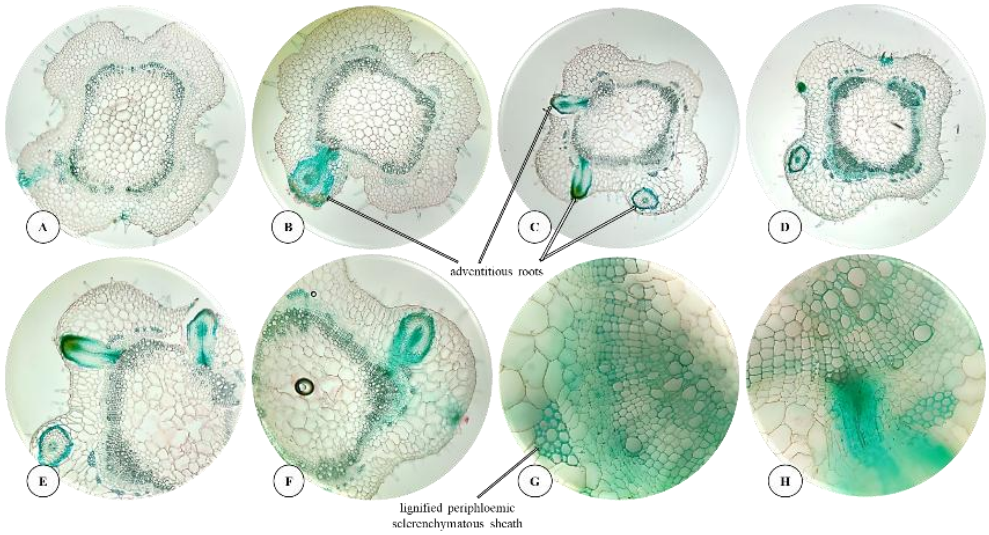

Figure 31. Epicotyl anatomy of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. bulbs (6A) collected in autumn

The aqueous extract from the bulb sheaths (7) stimulated the growth of the epicotyl of the plantlets of the 'Aromat de Buzău' cultivar (Figure 40), inhibited the growth of the leaf area at the basal and intermediate nodes of the plantlets of the 'Italiano Classico' cultivar (Figure 41), and reduced the electron transport rate (Figure 42) and polyphenol content in the leaves of the plants belonging to this cultivar. This extract induced an increase in the stomatal area on the abaxial leaf surfaces of both cultivars and a decrease in the substomatic CO_2 content and flavonoid levels. In the 'Aromat de Buzău' cultivar, the stomatal area also increased on the adaxial surface, and their density increased on the abaxial surface. Similarly, the alcoholic extract from the bulb sheaths stimulated epicotyl growth of 'Aromat de Buzău' plantlets (Figure 40) and increased leaf area at all three nodes of 'Italiano Classico' plantlets (Figure 41). This extract induced variations in the micromorphometric parameters of the leaf surfaces examined, especially in 'Italiano Classico' plantlets, in which the density of stomata and secretory hairs increased on both leaf surfaces and determined an increase in the electron transfer rate (Figure 42), substomatic CO_2 content, and antioxidant activity (Figure 44) of plantlets of both cultivars, but induced a decrease in chlorophyll a content (Figure 43). These extracts appeared to be the least toxic of the extracts tested for the test plantlets, although both types induced morphogenesis abnormalities (Figures 32, 33, and 34) and the appearance of tector hairs on the abaxial leaf surface of plantlets from both basil cultivars.

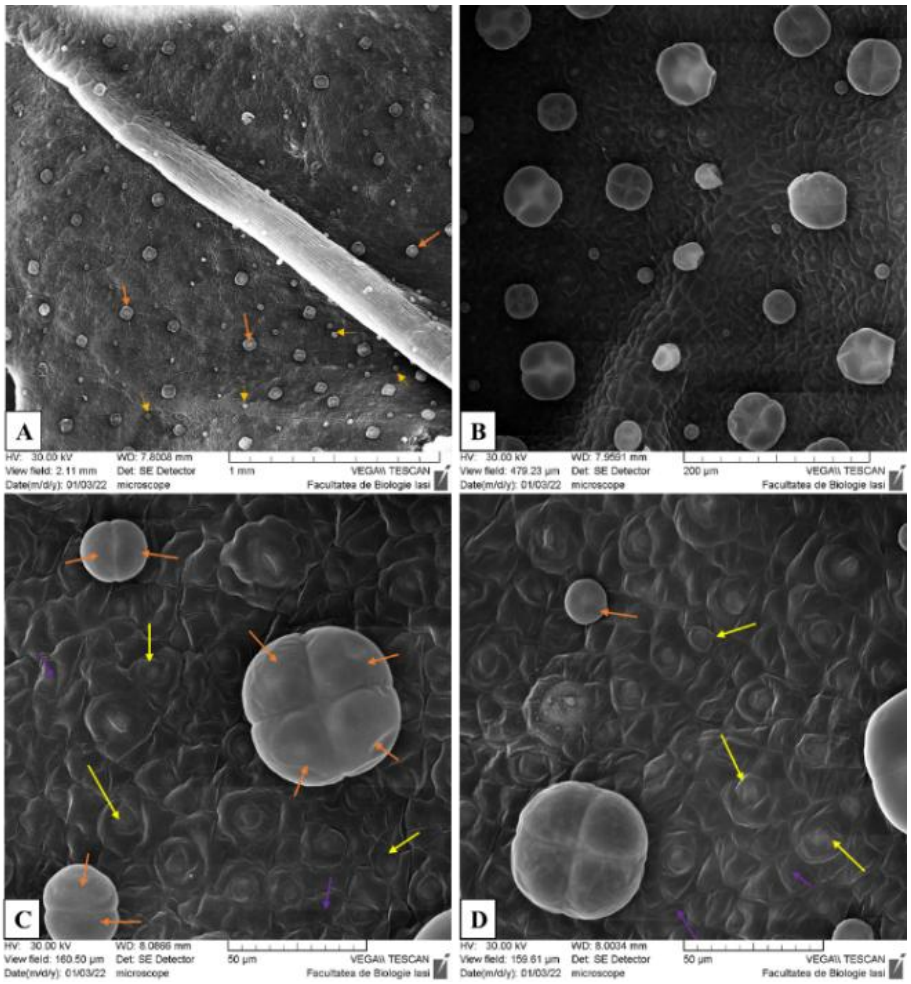

Figure 32. Abaxial leaf surface of *O. basilicum* L. cultivar 'Italiano Classico' plantlets treated with alcoholic extract from *C. autumnale* L. bulb sheaths (7E) (orange arrows indicate glandular hairs with different numbers of glandular cells; purple arrows indicate dehydration of the epidermis; yellow arrows indicate stomata during morphogenesis)

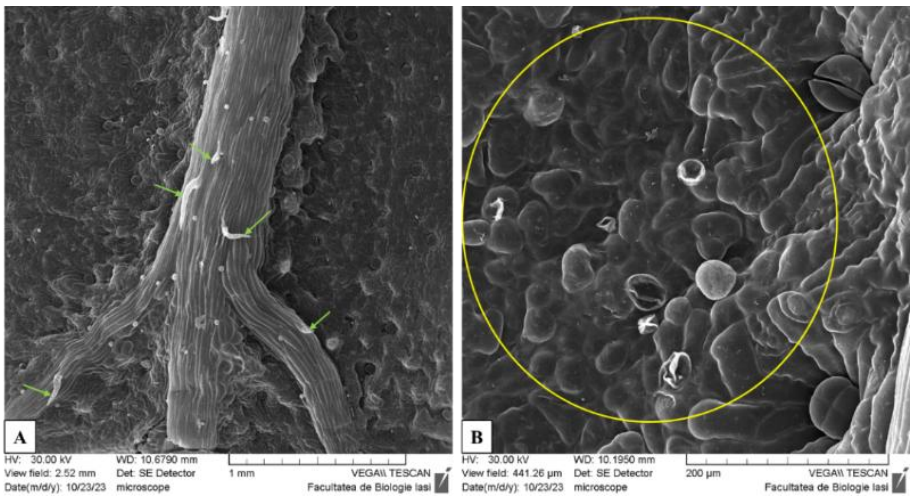

Figure 33. Abaxial leaf surface of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. bulb sheaths (7A) (green arrows indicate the appearance of tector hairs; yellow circle frames a morphogenesis area)

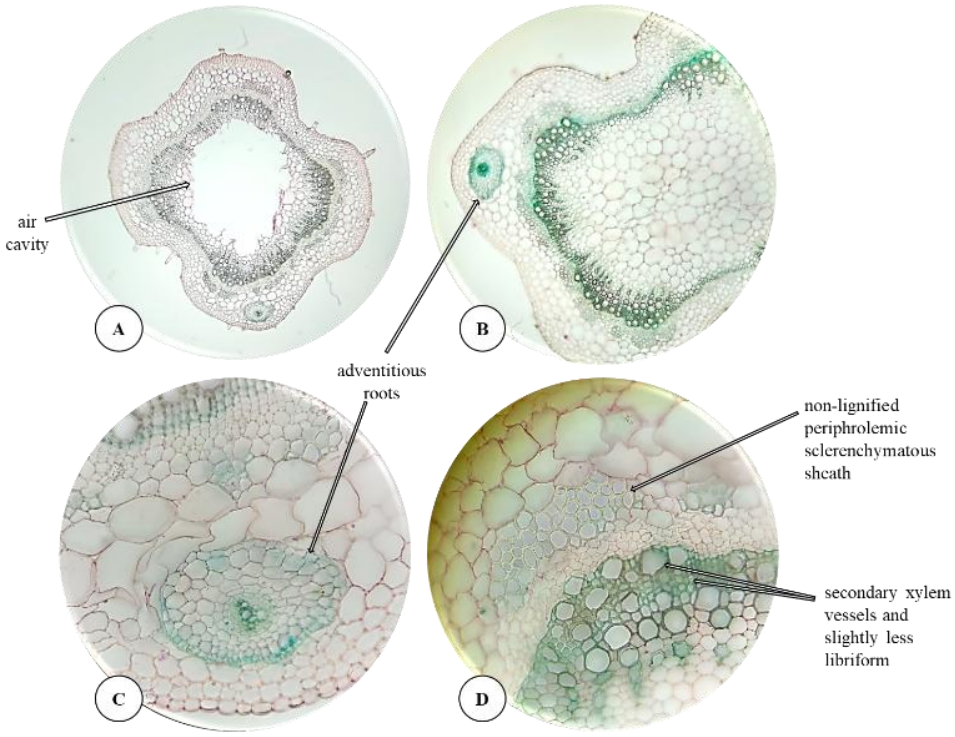

Figure 34. Epicotyl anatomy of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. bulb sheaths (7A)

The aqueous extract of flowers (8) induced epicotyl growth in plantlets of the 'Aromat de Buzău' cultivar (Figure 40), and in plantlets of the 'Italiano Classico' cultivar, it inhibited leaf growth at the basal and intermediate nodes (Figure 41). In plantlets of the 'Italiano Classico' cultivar, this extract induced an increase in the stomatal area on the abaxial surface, a decrease in the density of tector hairs on the adaxial surface, a decrease in the content of polyphenols and flavonoids, and an increase in antioxidant activity (Figure 44). In plantlets of the 'Aromat de Buzău' cultivar, the aqueous extract of *C. autumnale* L. flowers increased the stomatal area on both leaf surfaces. In plantlets of both cultivars this extract determined a decrease in the electron transfer rate (Figure 42) and in the substomatic CO_2 content. The alcoholic extract of the flowers inhibited the growth of the leaf area at the basal node of the plantlets of the 'Italiano Classico' cultivar and stimulated the growth of the leaf area at the intermediate node of the plantlets of the 'Aromat de Buzău' cultivar (Figure 41), in the latter cultivar also stimulating the increase in the density of stomata and secretory hairs on the adaxial surface. In the plantlets of both basil cultivars studied, this extract determined the increase in the stomatal area on both leaf surfaces, the increase in the substomatic CO_2 content and the antioxidant activity (Figure 44), as well as the decrease in the chlorophyll a content in the leaves (Figure 43). Both types of extracts prepared from the flowers induced in the treated plantlets anomalies of the structural elements of the leaves

(Figures 35, 36 and 37) and epicotyls (Figures 38 and 39), inhibiting lignification and accentuating the asymmetry through the heteromorphism of the epidermal cells and stomata. Moreover, the alcoholic extract from the flowers caused the appearance of a ring-type procambium in the epicotyl structure (Figure 32), which is uncharacteristic of the *O. basilicum* L. species. The morpho-structural and functional modifications highlighted in the test plantlets of the two basil cultivars subjected to treatments with aqueous and alcoholic extracts from *C. autumnale* L. flowers can be considered effects induced by the compounds with strong allelopathic potential contained (colchicine, polyphenols and flavonoids), dosed practically in maximum concentrations in the applied extracts.

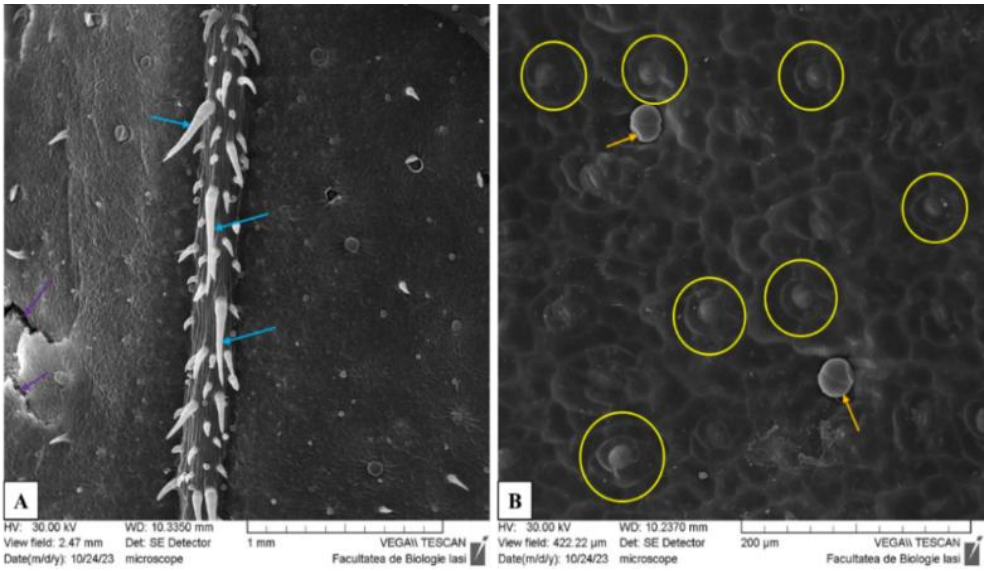

Figure 35. Adaxial leaf surface of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. flowers (8A) (blue arrows indicate elongation of tector hairs; orange arrows indicate secretory hairs with bicellular secretory head; yellow circles frame stomata during morphogenesis)

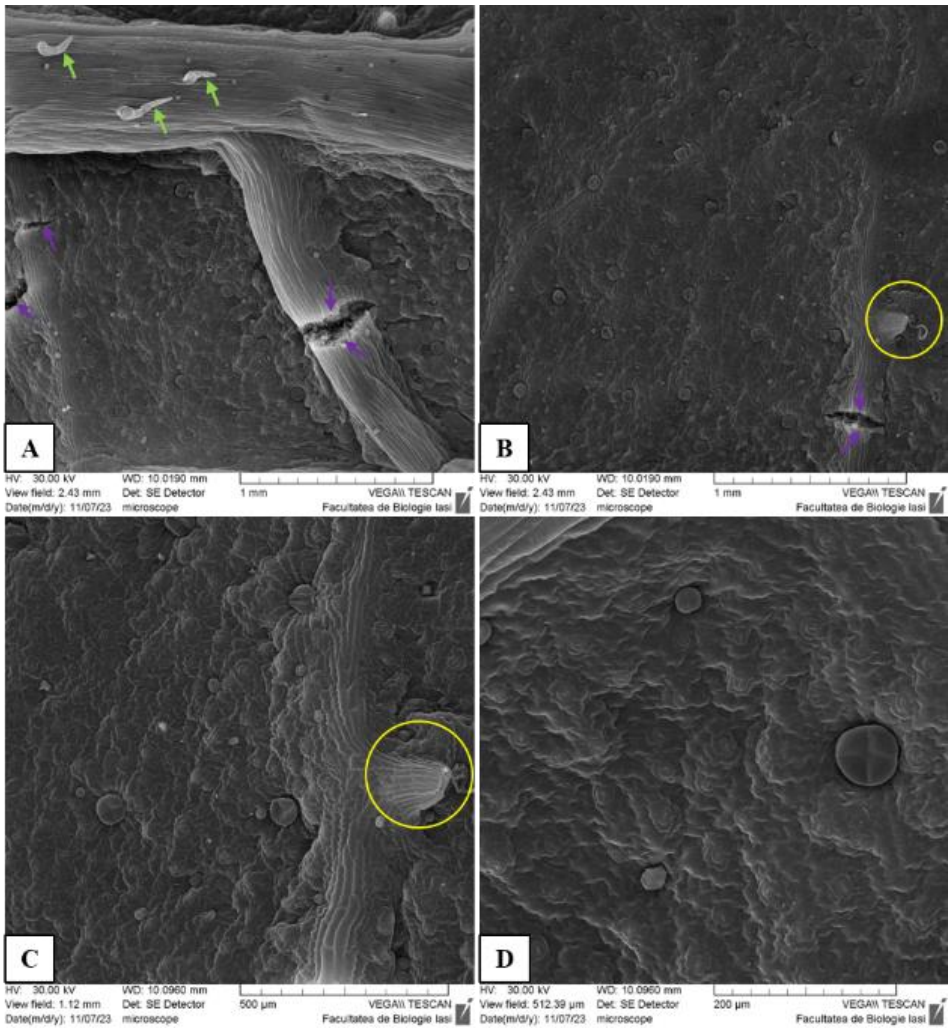

Figure 36. Abaxial leaf surface of *O. basilicum* L. cultivar 'Italiano Classico' plantlets treated with aqueous extract from *C. autumnale* L. flowers (8A) (purple arrows indicate epidermal folds; yellow circles indicate a zone of morphogenesis)

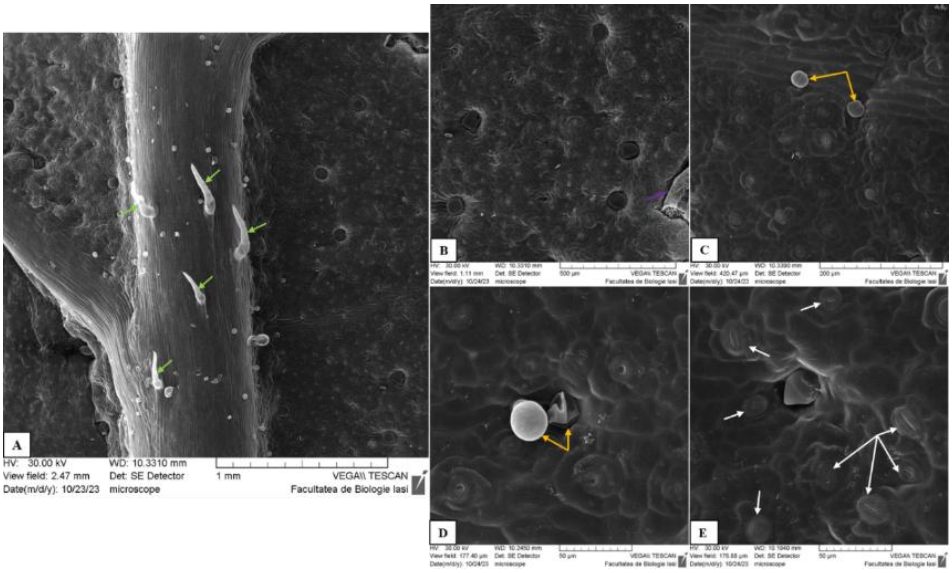

Figure 37. Abaxial leaf surface of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. flowers (8A) (green arrows indicate the appearance of tector hairs; orange arrows indicate secretory hairs with bicellular glandular heads; white arrows indicate heteromorphic stomata)

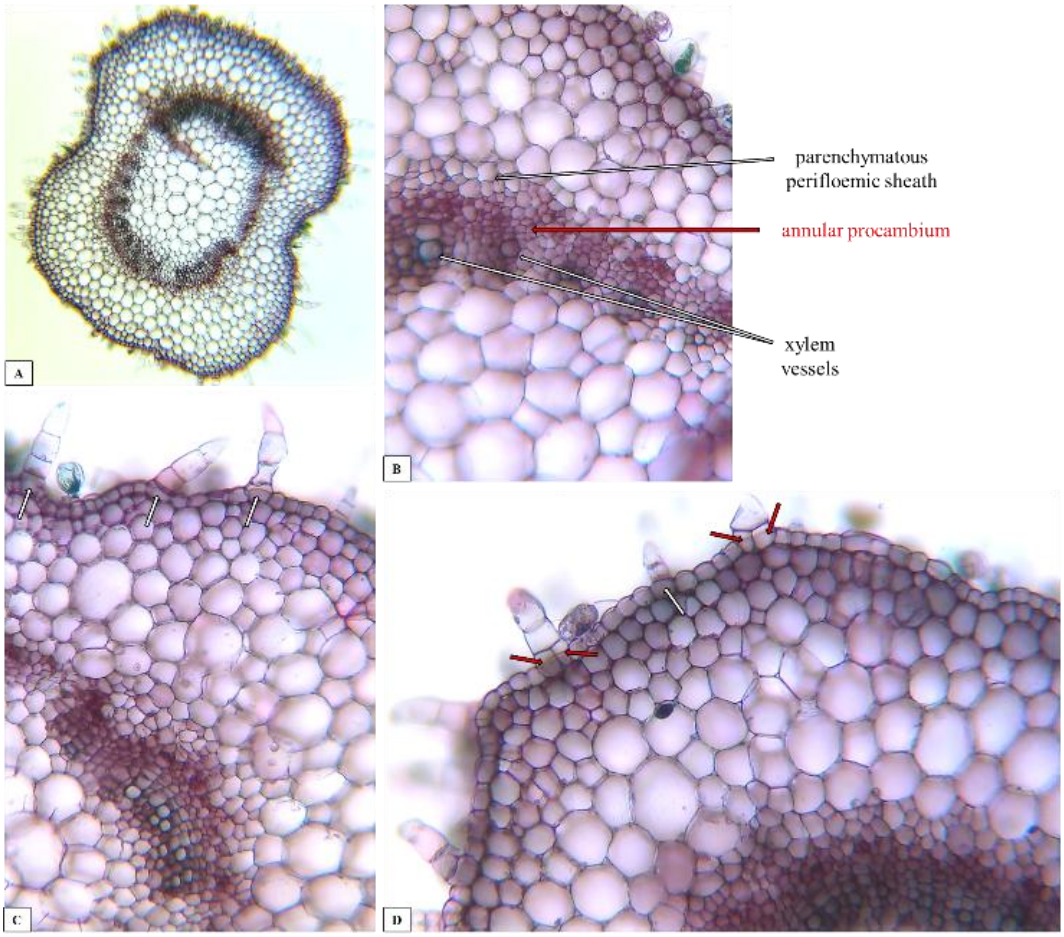

Figure 38. Epicotyl anatomy of *O. basilicum* L. cultivar 'Italiano Classico' plantlets treated with alcoholic extract from *C. autumnale* L. flowers (8E)

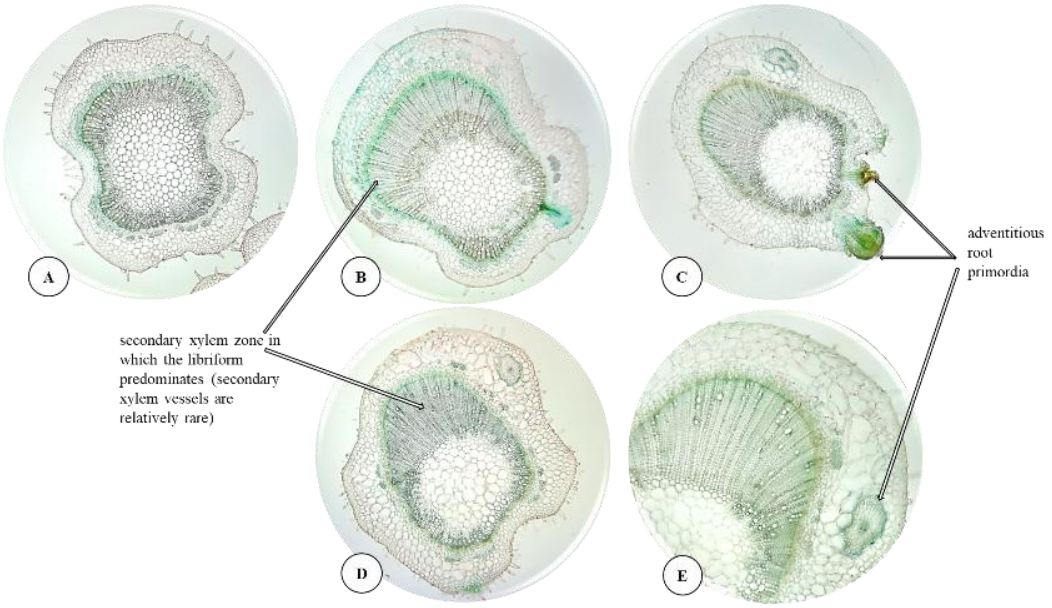

Figure 39. Epicotyl anatomy of *O. basilicum* L. cultivar 'Aromat de Buzău' plantlets treated with aqueous extract from *C. autumnale* L. flowers (8A)

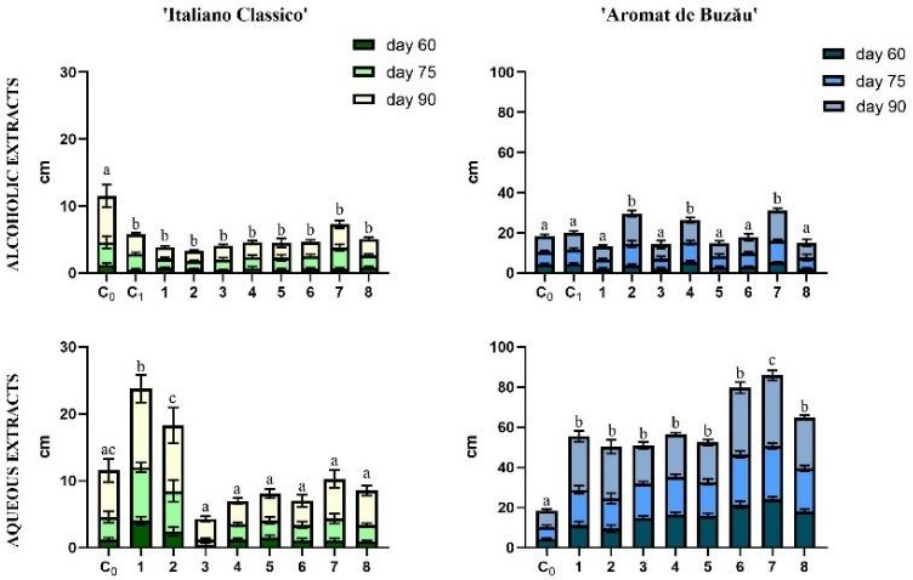

Figure 40. Average epicotyl height of *O. basilicum* L. plantlets, cultivars 'Italiano Classico' and 'Aromat de Buzău' treated with alcoholic and aqueous extracts of *C. autumnale* L. at 60, 75 and 90 days after planting

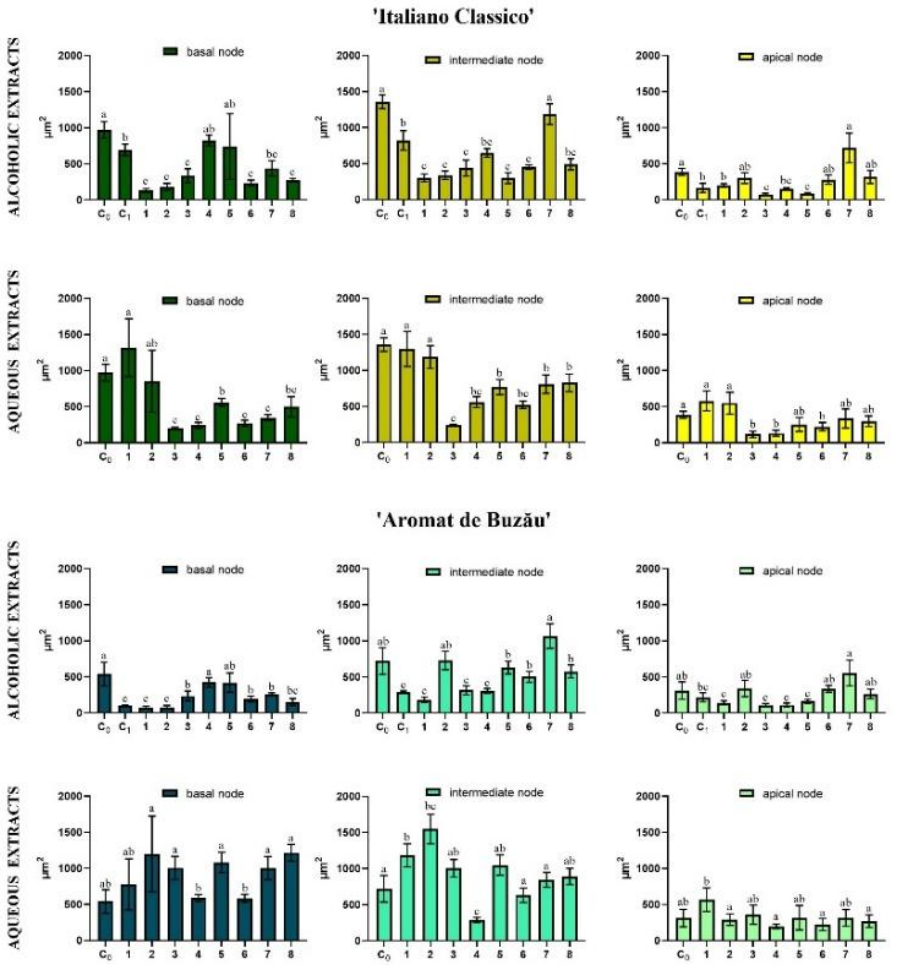

Figure 41. Average leaf area at the nodes of *O. basilicum* L. plantlets, cultivars 'Italiano Classico' and 'Aromat de Buzău' treated with alcoholic or aqueous extracts of *C. autumnale* L., 90 days after planting

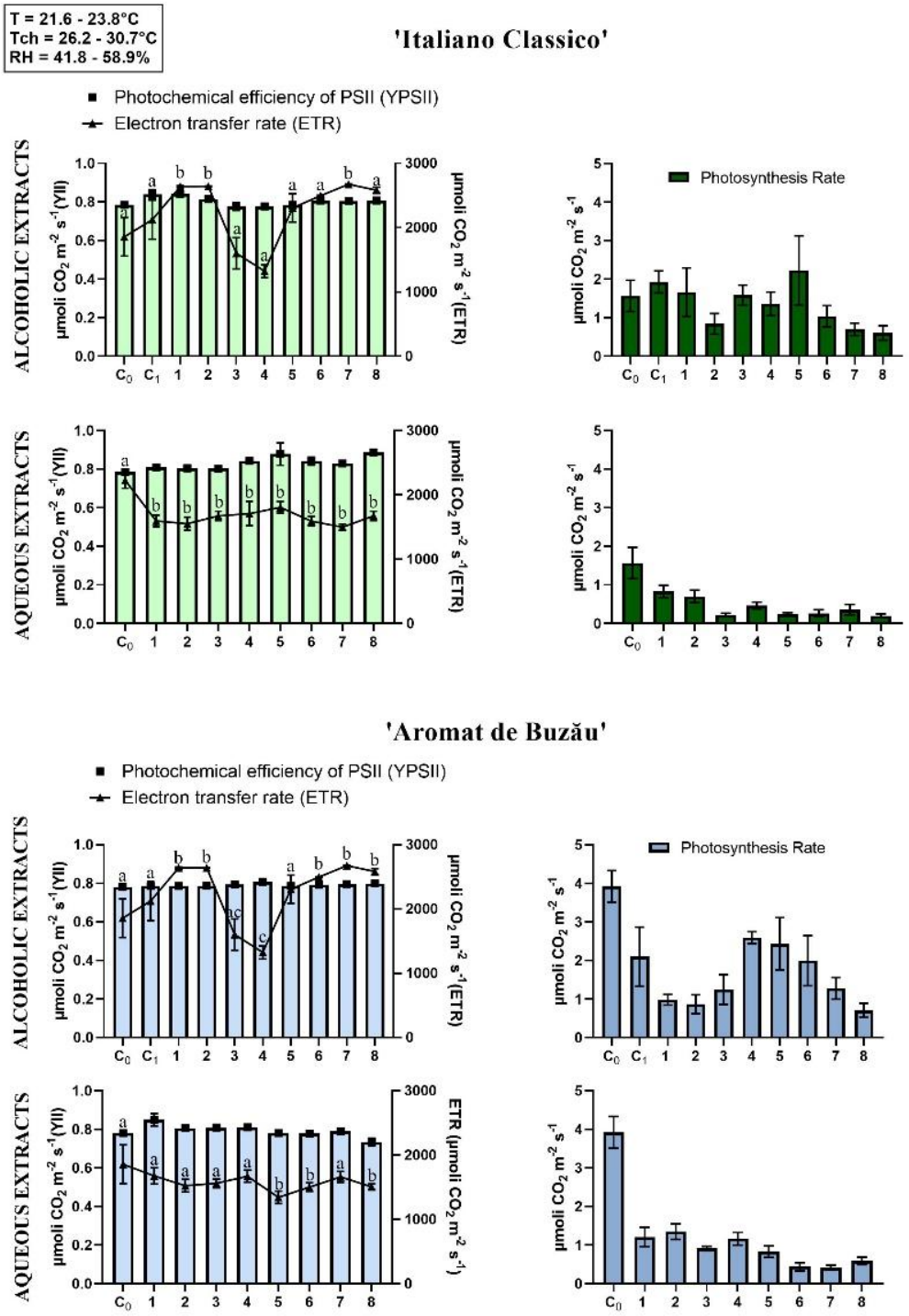

Figure 42. Indices of photosynthesis in *O. basilicum* L.plantlets of the 'Italiano Classico' and 'Aromat de Buzău' cultivars treated with alcoholic or aqueous extracts of *C. autumnale* L.

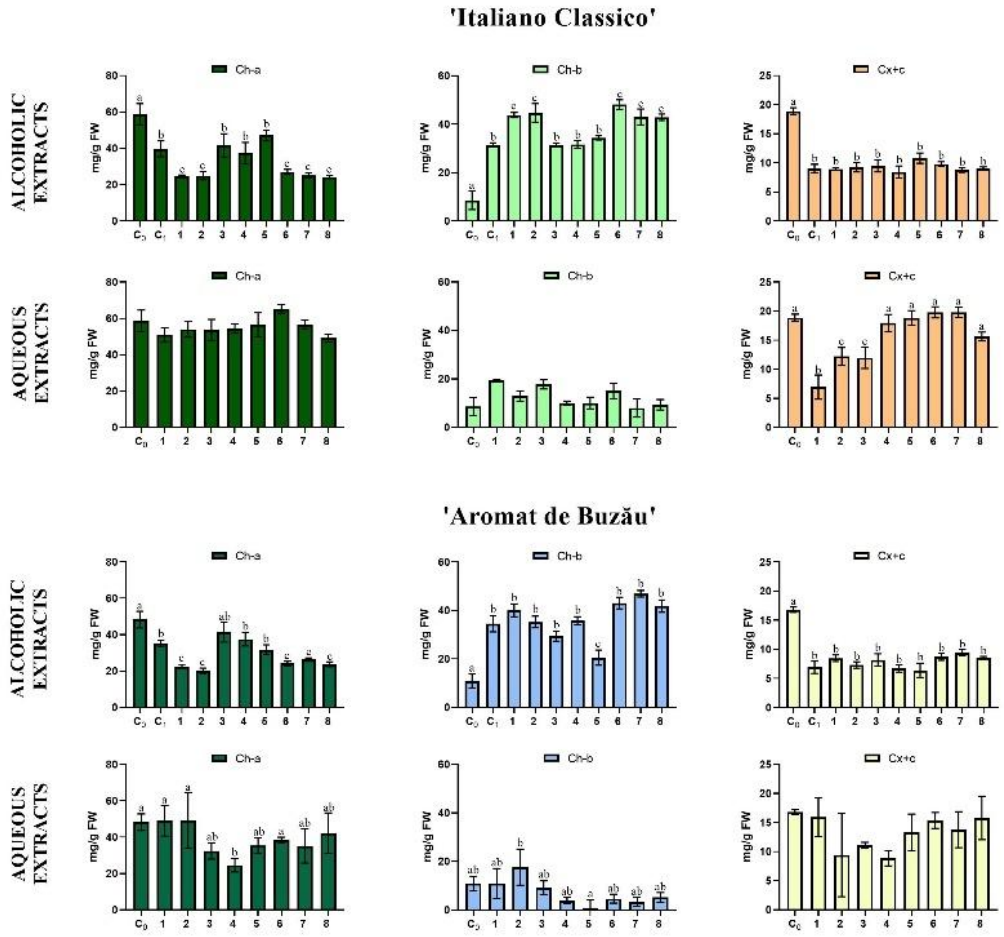

Figure 43. Variations in the content of assimilatory pigments in the leaves of *O. basilicum* L. cultivars 'Italiano Classico' and 'Aromat de Buzău' plantlets treated with alcoholic or aqueous extracts of *C. autumnale* L.

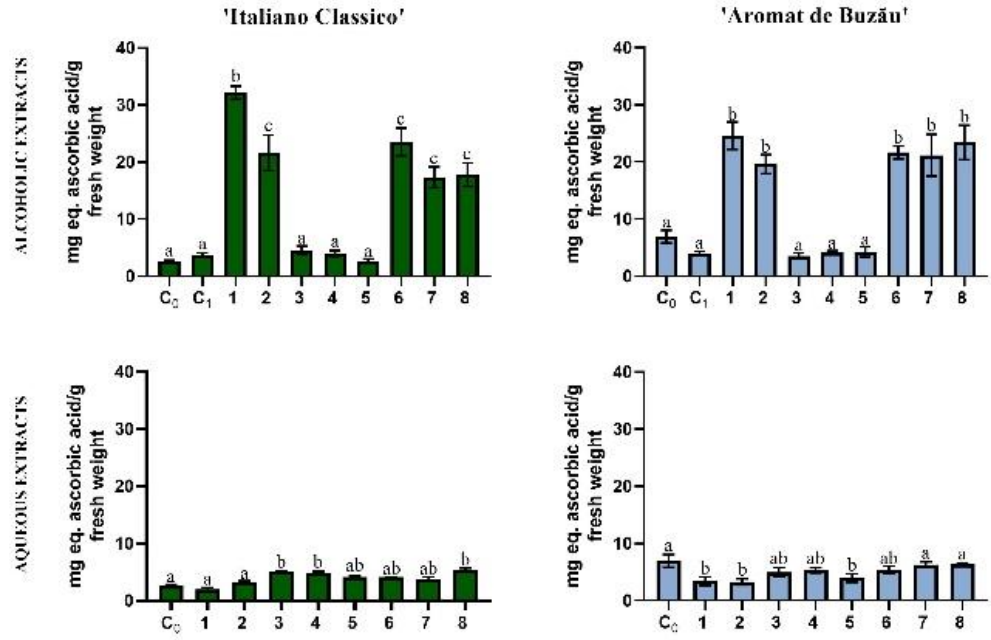

Figure 44. Antioxidant activity in the leaves of *O. basilicum* L. plantlets, cultivars 'Italiano Classico' and 'Aromat de Buzău', treated with *C. autumnale* L. extracts

Final considerations

Testing extracts from organs collected at different stages of the ontogenetic cycle of a species represents a new approach in allelopathy studies that can provide clues regarding the various adaptation mechanisms of plants by adjusting the release pathways, types and concentrations of secondary metabolites. At the same time, analyses of quantitative variations in the content of biologically active secondary metabolites in different organs of the species with allelopathic potential, *C. autumnale* L., at the flowering, growth and fruiting stages, provide insight into the mechanisms by which this species ensures its survival throughout its ontogenetic cycle.

It can be stated that plants of the species *C. autumnale* L. resort to different strategies for the synthesis and release of allelopathic secondary metabolites by adjusting sources and reserves during the ontogenetic cycle, being a perennial species, which can be differentiated as follows:

During the growth period, the secondary metabolites found in the underground bulb of meadow saffron show inhibitory effects on the growth of plantlets of other species from seeds, in this case, the two cultivars of *O. basilicum* L., 'Italiano Classico' and 'Aromat de Buzău'. On the other hand, the allelopathic compounds released from the above-ground organs, at low concentrations, induce inhibitory effects on the growth of test seedlings, and at high concentrations, induce stress in the recipient seedlings that determine the acceleration of the germination, growth and development processes, which can be beneficial for the recipient species. However, the effects of allelopathic compounds released by *C. autumnale* L. plants on plantlets whose meristems were previously treated with extracts containing these compounds manifest differently. Compounds released from the underground and aboveground organs of meadow saffron can stimulate the growth and development of recipient plantlets, or inhibit them, and affect certain physiological and biochemical processes, such as electron transfer rate, transpiration, chlorophyll a content and antioxidant activity. At the morpho-anatomical level, these compounds cause morphogenesis abnormalities, manifested by a heteromorphism of epidermal cells and stomata in the composition of leaves and epicotyls, or the appearance on the leaf surface of areas of morphogenesis interspersed with areas of developed tissue and weak lignification of conducting vessels, anomalies that are most likely determined by the action of colchicine,

but also the appearance of tector hairs on the abaxial surface, which indicates a response to the chemical stress induced by the treatment.

During the fruiting period, the leaves of the meadow saffron produce and release compounds that induce inhibitory effects on the recipient plant species, delaying their germination and growth, while the fruits may have stimulating effects on the germination of seeds of other species, an effect that may be due to the growth hormones present in abundance in the fruits. Similar effects at the morpho-anatomical, physiological and biochemical levels of the compounds released during the growth period were also observed after treating the shoot meristems of the test plantlets with extracts from leaves and fruits.

During the flowering period, the underground organs (bulbs) of meadow saffron produce secondary metabolites that inhibit the germination and growth of the seedlings of the recipient species, similarly to those released during the growth period, but during the flowering period the effect of delaying the germination of seeds of other species was also observed. On the other hand, the bulb sheaths, representing the remains of the leaves that fulfilled their functions before the flowering period, produce compounds that have neutral or even stimulating effects on the seedlings of the recipient species, which can be explained by the protective role they maintain even underground, by enveloping the bulb. The above-ground organs (flowers), having the highest concentration of colchicine, present the strongest inhibitory effects on seed germination and seedling growth of the test species. After treating the cauline meristems of the test plantlets, similar effects of the compounds released by the donor species during the growth and fruiting period were observed.

Secondary metabolites with allelopathic properties present in the vegetative and reproductive organs of plants belonging to the species *Colchicum autumnale* L., interpreted as stressors that most likely act synergistically, induce instability in the physiological and biochemical processes of the recipient plants depending on their nature and concentration, directly influenced by the stage of the ontogenetic cycle of the donor plant (in this case meadow saffron), by the stage of development of the plants of the recipient species (in this case basil seeds and plantlets), by the organ of the donor plant in which they were synthesized as well as their release route into the environment.

The results of the research conducted in this study indicated that all organs of *C. autumnale* L. plants present allelopathic potential, through the accumulation of secondary metabolites with allelopathic properties such as colchicine and polyphenolic compounds,

which protect it and give it an advantage in interspecific competition throughout its ontogenetic cycle.

The most important organs of meadow saffron involved in the phenomenon of allelopathy are the above-ground organs, especially the leaves and flowers, in the structure of which colchicine and phenols are found in higher concentration, compared to the other organs of plants belonging to this species.

The biological responses practically recorded in the test recipient organisms belonging to the two cultivars of *Ocimum basilicum* L. ('Italiano Classico' and 'Aromat de Buzău') during the research of the possible allelopathic effects induced by the application of plant extracts (alcoholic and aqueous) prepared from vegetative and reproductive organs of plants of the donor species (*Colchicum autumnale* L.) cover a varied palette of effects, such as:

- The alcoholic and aqueous plant extracts prepared from the sheaths of meadow saffron bulbs did not have negative effects on the test basil cultivars but, on the contrary, they stimulated the germination of their seeds.

- Both types of vegetable extracts prepared from meadow saffron flowers, individualized by the highest contents of compounds with strong allelopathic potential (colchicine, polyphenols and flavonoids) practically dosed, induced obvious biological changes, more or less beneficial to the test organisms (seeds and plantlets of the mentioned basil cultivars), detectable changes at the micromorphological, structural, biochemical and functional levels.

The results obtained in this study highlight the dynamic nature of the biochemical profile of the *C. autumnale* L. species throughout its ontogenetic cycle and the importance of considering these variations both in research and in practical applications, suggesting potential ecological implications and possible applications in weed biocontrol strategies by optimizing the extraction of colchicine and other bioactive compounds.

This study contributes to the understanding of the biochemical complexity of the *C. autumnale* L. species and provides a model for investigating the chemical composition and potential applications of other plants with similar (alelopathic) properties in weed biocontrol strategies by optimizing the extraction of bioactive secondary metabolites.

Future research on this topic may contribute to the adaptation and improvement of methods for preparing plant extracts from the vegetative and reproductive organs of *C. autumnale* L. plants, as well as to the standardization of their field application methods as biological treatments with stimulating or protective effects in organic agriculture for crops of economic interest, depending on the practical purpose pursued: increasing agricultural and horticultural production or restoring anthropized natural ecosystems in biological decline.

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