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Biological effects of some *Colchicum autumnale* L. extracts on tissue development of two varieties of *Ocimum basilicum* L.

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Abstract: *Colchicum autumnale* L. is a perennial herb from the Colchicaceae family with an unusual life cycle, and it is characterized by an underground corm and hypogynous flowers that appear in autumn. Its medicinal importance is represented by its primary alkaloid, colchicine, which has been studied for its anti-inflammatory and antimitotic properties and used in the treatment of some diseases and artificial polyploidy induction in plants. This study aims to determine and evaluate the biological effects induced by treatment with *C. autumnale* extracts on tissue development in test plants, represented by two *Ocimum basilicum* L. varieties: 'Italiano Classico' and 'Aromat de Buzău'. Morpho-anatomical observations and measurements and photosynthetic pigments analyses were employed. Results show unusual shapes of leaves, differences in stomata size and density, and heteromorphic cells in leaves and epicotyl's structure in both studied varieties of basil treated with *C. autumnale* extracts.

1. Introduction

Colchicum autumnale L., generally known as meadow saffron, naked lady, or wild saffron (Nagesh *et al*., 2011), is a colchicine-containing species widespread throughout Europe. It belongs to the Colchicaceae family, which comprises perennial herbs characterized by an underground corm and hypogynous flowers with six tepals (Bowles, 1924; Nordenstam, 1998, in Jung *et al*., 2011). The biochemical profile of this species reveals the presence of alkaloids, phenolics, terpenoids, glycosides, and other bioactive compounds (Davoodi *et al*., 2021; Hailu *et al*., 2021). The alkaloid concentration in this species varies depending on the plant organ; it was reported that it varies in seeds (0.5-1.2%), fresh flowers (1.2-2%), fresh leaves (0.15-0.4%), and fresh bulbs (0.1-0.6%); among all alkaloids, colchicine constitutes 50-70% of the total alkaloid content, followed by some small amounts of colchicoside, demecolcine, and other tropolone derivatives (Kupper *et al*., 2010). These variations are due to the fact that

secondary metabolites biosynthesis and accumulation are influenced by genetic, morphogenetic, and environmental factors (Yang *et al*., 2018).

Despite its toxicity, colchicine has been used in the treatment of gout, Familial Mediterranean Fever (FMF), and other diseases (Nagesh *et al*., 2011), and its antimitotic properties have been exploited in plant breeding for the production of polyploid crops (Roberts and Wink, 1998, in Jung *et al.*, 2011) and ornamental species (Manzoor *et al*., 2019).

To extract colchicine from the plant source, the most commonly used method is Soxhlet, and the best choice of solvent for this method has been reported to be methanol for *C. autumnale* and other species of the Colchicaceae family (Finnie and Van Staden, 1991; Pandey and Banik, 2012, in Çankaya *et al*., 2019). Colchicine binds to tubulin dimers and prevents microtubule assembly, inducing microtubule depolymerization and preventing mitotic spindle formation (Caperta *et al*., 2006). It was shown that colchicine treatment affects specific morphological (stomatal size), physiological (photosynthetic rate), and biochemical (chlorophyll content) indices in various plant species (Cao *et al*., 2018; Trojak-Goluch *et al*., 2021), but the effects of colchicine derivatives or *C. autumnale* extracts in plants are unknown.

This study aims to evaluate the effects of *C. autumnale* methanolic extracts on seed germination and plantlet tissue development by measuring several morpho-anatomical and photosynthetic indices to determine the allelopathic effect and the impact of the extracts of this plant species on other plants. For this purpose, *Ocimum basilicum* was chosen as a test species for its culinary, ornamental, medicinal, and economic importance and specific morpho-anatomical characteristics: four-edged stem (rectangular-quadrangular shaped) with four ribs and ovate-lanceolate opposite leaves with attenuate serrate edges, uniseriate pluricellular tector hairs, peltate (four-celled head) and capitate (one or two-celled head) glandular hairs that appear on the surface of the stem and leaves (Zamfirache *et al*., 2008; Nassar *et al.*, 2014), that make potential abnormalities easy to spot.

2. Materials and Methods

Plant material

The seeds of *O. basilicum* 'Italiano Classico' and 'Aromat de Buzău' were purchased from commercial sources (Unisem S.A., Iași, and S.C.D.L. Buzău). A total of 32 individual plants of *C. autumnale* were collected from a pasture in Voroneț, Suceava County, lat. 47.58889° N, long. 25.90861°E, alt. 576.68 m, in October 2019. The colchicine standard was provided by the Institute of Biological Research, Iași, Romania.

The bulbs and flowers of *C. autumnale* were kept in an oven at 65°C for 12 hours to stop enzymatic reactions and then dried at room temperature (21±1°C) for 7 days, away from any source of light. Subsequently, the dry material was ground in an electric grinder, placed in glass jars wrapped in aluminum foil, and stored in the refrigerator until used in extract preparation.

Extraction and quantification of colchicine content in extracts

The extraction was processed in a Soxhlet apparatus in methanol (Sigma Aldrich, Germany), using 5 grams of powder from each organ (bulb and flower), according to the method of Franz and Koehler (1992, in Alali *et al*., 2004). The extraction of colchicine from the dried plant material was performed until the solvent in the extraction chamber was clear, in the following way: 5-6 cycles (1-1.5 cycles/hour) for bulbs and 17-18 cycles (2-3 cycles/hour) for flowers. After extraction, the methanol was evaporated in a rotary evaporator (IKA RV3 Eco, Germany). Each dry extract was weighed and then dissolved in 50 ml of 70% methanol. Two colchicine-containing extracts were obtained, one from the bulbs and abbreviated BE (bulb extract) and one from flowers - abbreviated FE (flower extract).

The quantification of the colchicine in the extracts was performed by RP-HPLC, according to Alali *et al*. (2004). The separation was performed on a Shimadzu Prominence HPLC system (2x LC20AD pumps, SIL20AC autosampler, CT20AC oven, SPD M20A DAD detector) using a Zorbax Eclipse XDB - C18 (250 mm length, 3 micron particle size) column, with acetonitrile as mobile phase A, and 3% acetic acid (Sigma Aldrich, Germany) as mobile phase B. Elution was performed at a flow rate of 1 ml/min using the following program: 0-3 min 90% B isocratic, 3-11 min 90-40% B gradient, 11-12 min 40% B isocratic, 12-13 min 40-90%B gradient, 13-20 min 90% B isocratic. A volume of 20µl of colchicine standard and 5 µl of each extract were injected. Colchicine was detected at 245 nm and eluted at 13.4±0.08 minutes. Chromatographic data were acquired using the Shimadzu LC solution Software and manually inter-

preted. For calibration, a range of 0.2 to 5 µg colchicine was used.

Experimental design

The two extracts were used in treatments, pure or diluted with distilled water (1:1) on the seeds and on the cauline apexes of *O. basilicum* 'Italiano Classico' and 'Aromat de Buzău' potted plantlets (Fig. 1). Three controls (C_0 = distilled H_2O, C_1 = 35% MeOH, C_2 = 70% MeOH) were prepared according to the methanol concentration in extracts and dilutions.

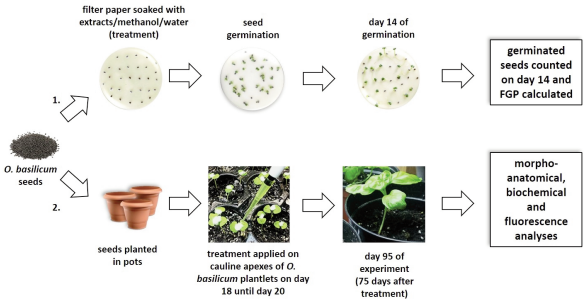

Fig. 1 - Treatment application strategies.

The method of treatment consisted of applying extracts on *O. basilicum* seeds distributed in Petri dishes. The seeds were previously sterilized with 3% H_2O_2 (5 minutes) and 5% NaClO (5 minutes). The treatment was applied by soaking the filter paper with 2 ml of pure or diluted extracts (20 seeds/Petri dish x 3 replicates for each variant of treatment). The seeds were kept in a thermostat at a temperature of 20±1°C and a 17h/7h light-dark cycle per day for 14 days and were watered once every two days. The experiment was performed in triplicate. Final Germination Percentage (FGP) was calculated according to the formula by Bezini *et al*. (2019):

$$FGP = n/N \times 100$$

where n represents the number of germinated seeds at the end of the germination test and N is the total number of seeds.

The other treatment application strategy was based on some methods used for polyploidy induction by colchicine treatment (Suzuki *et al*., 2005; Ye *et al*., 2010; Kushwah *et al*., 2018) and was carried out by applying the treatment on basil plantlets on day 18 after planting, by soaking cotton balls applied on the cauline apexes with 100 µl of extract per day for three consecutive days. The plantlets were kept in pots with Compo Sana soil (peat + perlite) at 21±2°C and 17 hours of light per day and were watered once every three days. The experiment lasted for 95 days (18 days of pre-cultivation and 75 days of monitoring of plant development) and the treated plantlets were analyzed at the end of the experiment (day 75 after treatment).

Morphological observations of treated plantlets

The macromorphological differences in leaves' shape and stem development that were observed between variants of treated *O. basilicum* plantlets of both varieties were photographed.

For micromorphology analysis, the plant material consisting of the first leaves was prepared in the following way: for each variant 2 first node leaves from 3 different plants were cut (1 cm^2) and transferred in successive acetone baths, critical-point dried with CO_2, and covered with a 10 nm gold (Au) layer using an EMS 550x sputter coater. Leaf samples of each surface were analyzed using scanning electron microscopy (Tescan Vega II SBH electron microscope from the Faculty of Biology, ''Alexandru Ioan Cuza" University of Iași, Romania) with VegaTC software. Stomata size (on both leaf surfaces) measurements were made using the ImageJ software for 5 stomata on 6 leaves. Stomata, tector, and glandular hairs densities were determined by counting their occurrences on 1 mm^2 of leaf surface on 6 leaves.

Anatomy analysis of treated plantlets

Epicotyl fragments from 3 plantlets of each treatment were selected for sectioning and examination. The plant material, previously kept in 70% ethanol was sectioned using a hand microtome and botanical razor. The sections were stained through the double staining method (with ruthenium red and iodine green), then placed on slides, observed through an optical microscope (Euromex bScope BS.1153-Pli) using a 10x (0.25) lens, and photographed using Xiaomi Mi A1 camera (12 MP, f/2.2, 26 mm (wide), ½.9", 1.25 µm, PDAF). Epicotyl circumference was measured using ImageJ and compared to control variants.

Photosynthetic pigments content assay of treated plantlets

The content of photosynthetic pigments in both *O. basilicum* varieties was analyzed according to Sumanta *et al.* (2014) using ethanol (Chemical Company S.A., Iași, Romania) as a solvent for leaf extracts (fresh leaves from 3 plants of each variant of treatment were weighted and milled with quartz sand, then dissolved in ethanol and filtered). The contents of chlorophyll a, chlorophyll b, and

carotenoid pigments were calculated with formulas given in the reference article. The data was processed in GraphPad Prism version 9.3.0.

Chlorophyll fluorescence measurement of treated plantlets

For the measurement and evaluation of chlorophyll fluorescence (indirect measurement of photochemical efficiency of photosystem II = ΦPSII and electron transport rate = ETR), 3 leaves were selected from different individuals from each treatment (from plantlets that were pre-exposed to dark conditions), and were analyzed using the Hansatech Ltd. PAM Fluorometer. The data was imported from the data system of the Hansatech device to Parview32 software and analyzed in GraphPad Prism version 9.3.0.

Statistical analyses

Statistical calculations and comparations were performed in GraphPad Prism version 9.3.0., using Two-way ANOVA and Tukey's multiple comparisons test for all morphological: stomata size (n= 3), stomata, tector and glandular hairs density (n= 3) on both leaf surfaces, anatomical: epicotyl circumference (n= 3), biochemical: chlorophyll a, b and carotenoids content (n= 3), and physiological indices: final germination percentage for treated seeds (n= 20) and photosystem II efficiency and electron transport rate (n = 3) for treated plantlets. Values in graphs were represented as means ± SEM, and for Tukey's multiple comparison tests, statistical significance is marked on graphs in the following way: **** = p<0.0001, *** = p<0.001, ** = p<0.01, * = p<0.05. Where no asterisk is present, results are not statistically significant.

3. Results

Colchicine content quantification in extracts

The detected colchicine (Fig. 2a) concentrations were 0.119±0.007 mg/ml colchicine in the bulb extract (Fig. 2b) and 0.286±0.015 mg/ml colchicine in the flower extract (Fig. 2c).

Germination test

The final germination percentage recorded on seeds treated with extracts revealed that the bulb extract and a concentration of 70% methanol impede the germination of all seeds, regardless of the variety of basil. *O. basilicum* 'Italiano Classico' seeds had a higher germination percentage than the 'Aromat de Buzău' cultivar under treatment, but the latter

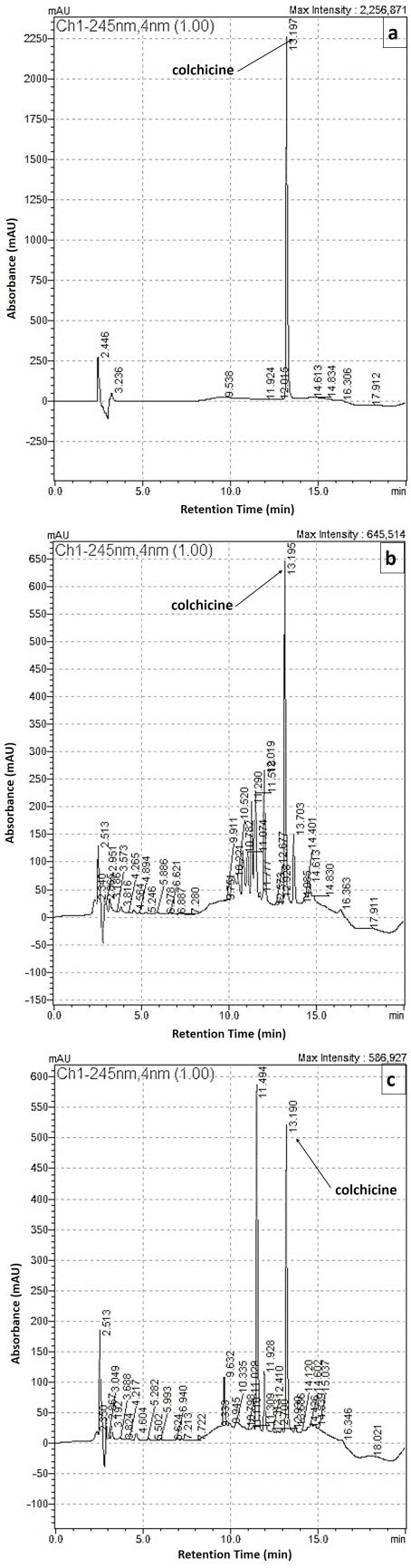

Fig. 2 - Chromatograms for colchicine standard (a) and for bulb (b) and flower (c) extracts.

showed a better germination percentage than its control variant when treated with diluted flower extracts (Table 1). The inhibitory effects of treatments (with extracts and methanol) effects were statistically significant (**** = p<0.0001) compared to C_0. Effects of *C. autumnale* extracts were also significant (p<0.0001), compared to their corresponding methanol control (1:1 BEt and 1:1 FEt to C_1; BEt and FEt to C_2). Other statistical comparisons are presented in supplementary materials (SM) Tables S1-S6.

Morphological observations of treated plantlets

Plantlet morphology. In 'Italiano Classico' basil plantlets, the epicotyl's growth was inhibited; the plantlets remained short (mainly when treated with bulb extracts), and their epicotyls had very short internodes (Fig. 3). The upper leaves were darker in color compared to the control and displayed unusual shapes: elongated or bifurcate, with straight or incomplete margins and diverted nervures (Fig. 4 c, d, e).

Treated 'Aromat de Buzău' plantlets in different stages of growth and development, with various stem heights and ramifications are reported in figure 5. In some plantlets, the main epicotyl's growth was either inhibited or necrotic, which strongly stimulated the ramifications, mostly when treated with pure

Table 1 - Final germination percentage of *O. basilicum* seeds under treatment with *C. autumnale* extracts

Test variant	Final germination percentage (%)	
	'Italiano Classico'	'Aromat de Buzău'
C_0 (H_2O)	80±1.92	68.89±7.78
C_1 (MeOH 35%)	70±11.55 ****	23.33±4.41 ****
1:1 BEt	0±0 ****	0±0 ****
1:1 FEt	40±18.93 ****	33.33±6.01 ****
C_2 (MeOH 70%)	0±0 ****	0±0 ****
BEt	0±0 ****	0±0 ****
FEt	0±0 ****	0±0 ****

BE= bulb extract; FE= flower extract; t= treatment; ****= p <0.0001.

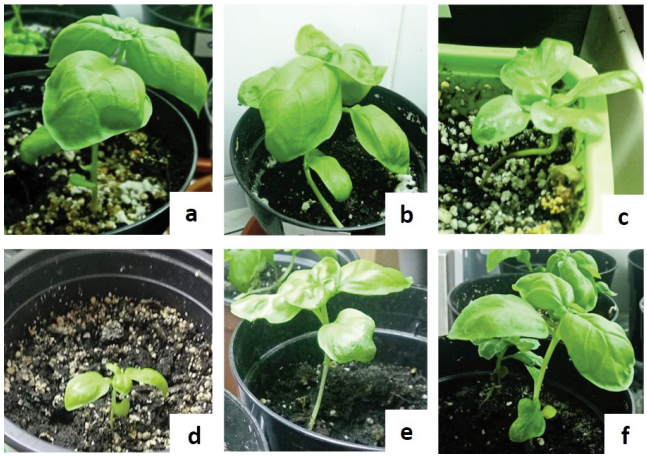

Fig. 3 - Morphology of *O. basilicum* 'Italiano Classico' plantlets treated with *C. autumnale* extracts. a) H_2O Control; b) MeOH; c) diluted (1:1) bulb extract; d) pure bulb extract; e) diluted (1:1) flower extract; f) flower extract.

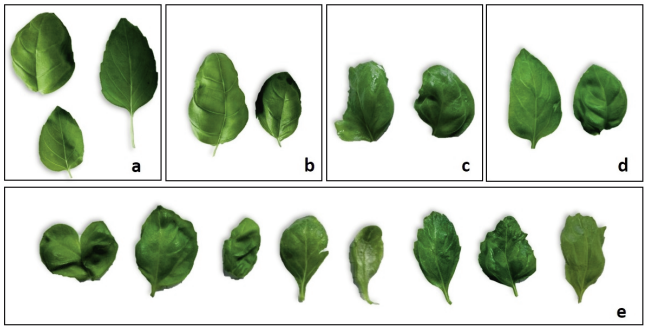

Fig. 4 - Morphology of *O. basilicum* 'Italiano Classico' leaves from the basal node of plantlets treated with *C. autumnale* extracts. a) H_2O Control; b) MeOH; c) diluted bulb extract; d) diluted flower extract; e) pure flower extract; leaves from plantlets treated with pure bulb extract could not be photographed because of insufficient plant material.

Fig. 5 - Morphology of *O. basilicum* 'Aromat de Buzău' plantlets treated with C. autumnale extracts. a) MeOH and H_2O Control; b) diluted (1:1) bulb extract; c) pure bulb extract; d) diluted (1:1) flower extract; e) pure flower extract.

flower extract. The upper leaves were much smaller than those from the control variant and displayed unusual shapes (Fig. 6c, d, e), with incomplete margins and deviated or bifurcated nervures (Fig. 6f).

Leaf micromorphology observations of treated plantlets. A typical basil leaf (of a plant treated with H_2O = C_0) observed through scanning electron microscopy has diacytic and anomocytic stomata, glandular and tector hairs (tector hairs appeared only

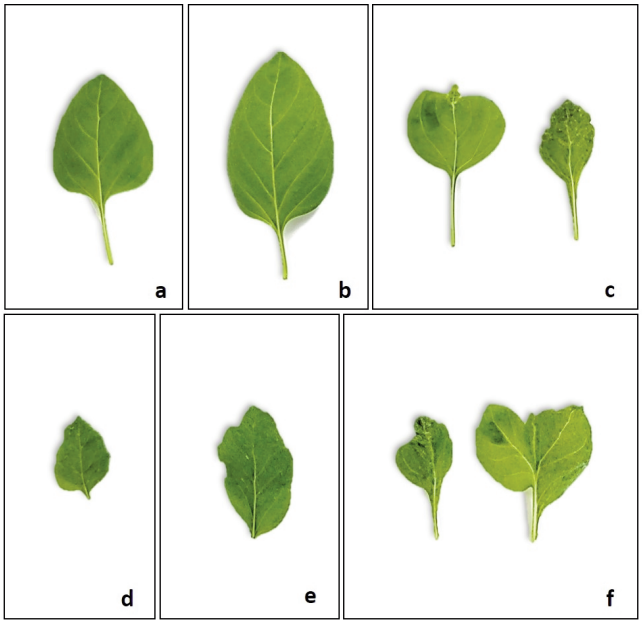

Fig. 6 - Morphology of *O. basilicum* 'Aromat de Buzău' leaves from the basal node of plantlets treated with *C. autumnale* extracts. a) H_2O Control; b) MeOH; c) diluted bulb extract; d) pure bulb extract; e) diluted flower extract; f) pure flower extract.

on the adaxial leaf surface), and epidermal cells with wavy sidewalls (Fig. 7a). The leaf epidermis of plantlets treated with methanol appears dehydrated and uneven (Fig. 7b), tector hair morphogenesis and elongation are stimulated, and glandular hairs with an altered shape of the glandular cells occur (Fig. 7c).

Leaves from 'Italiano Classico' plantlets treated with flower extracts showed heteromorphic or twin stomata (Fig. 7d), accentuated corrugations of the epidermal cell sidewalls (Fig. 7e), and abnormalities of the glandular hairs' shapes (Fig. 7f, g). Bulb extracts treatments only stimulated tector hairs morphogenesis.

The aspect of a typical leaf surface of 'Aromat de Buzău' basil plantlets is similar to 'Italiano Classico', but when treated with bulb extracts, epidermal cells appear elongated, the shape of the sidewalls is

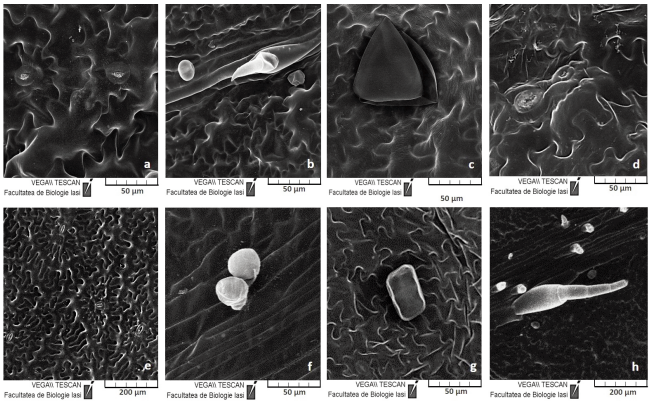

Fig. 7 - Abnormalities observed on *O. basilicum* leaf surfaces after treatment with methanol or *C. autumnale* extracts. a) typical aspect of an abaxial leaf surface; b) typical aspect of an adaxial leaf surface; c) glandular hair with an altered shape; d) twin stomata; e) epidermal cells with very wavy sidewalls; f) twin glandular hairs; g) glandular hair with a modified shape; h) tector hair morphogenesis and an elongated pluricellular tector hair.

altered, and the morphogenesis and elongation of tector hairs occur following the treatment with methanolic flower extracts (Fig. 7h).

Micromorphology measurements of treated plantlets. Stomata area and density. Stomata size significantly increased on both leaf surfaces of 'Italiano Classico' plantlets treated with diluted bulb extract ($p<0.05$ when compared to C_0, $p<0.0001$ when compared to C_1 on the inferior leaf surface, and $p<0.0001$ when compared to C_0 and C_1 on the superior leaf surface) and pure flower extract ($p<0.001$ when compared to C_0, $p<0.0001$ when compared to C_2 on the inferior leaf surface, and $p<0.0001$ when compared to C_0 and C_2 on the superior leaf surface) and only on the abaxial leaf surface when treated with diluted flower extract ($p<0.0001$ when compared to C_0 and C_1)(Fig. 8a), and their density increased in plantlets treated with pure bulb extract on the inferior leaf surface ($p<0.01$ when compared to C_0 and $p<0.05$ when compared to C_2) (Fig. 8c, d). The density of tector hairs increased significantly when plants were treated with 70% MeOH on the inferior leaf surface as a response to chemical stress.

Stomata size increased on both leaf surfaces of 'Aromat de Buzău' basil plantlets treated with pure bulb extract ($p<0.0001$ when compared to C_0 and C_2) and only on the adaxial leaf surface when the plantlets were treated with diluted flower extract ($p<0.0001$ when compared to C_0 and C_1) (Fig. 8a, b). Stomata density increased on both leaf surfaces of

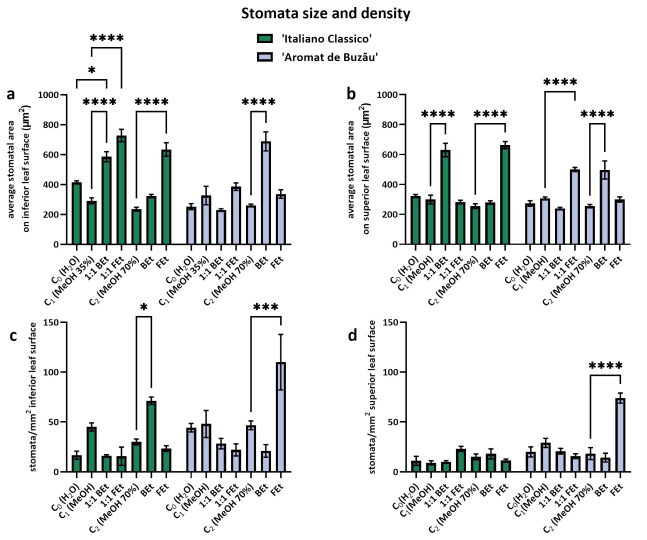

Fig. 8 - Average stomatal area on abaxial (a) and adaxial (b) leaf surfaces and density on abaxial (c) and adaxial (d) leaf surfaces of treated *O. basilicum* plantlets (BE = bulb extract; FE = flower extract; t = treatment; **** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.05).

plantlets treated with pure flower extract (p<0.001 when compared to C_0 and C_2) (Fig. 8c, d).

Glandular and tector hairs density. The density of tector hairs on the abaxial leaf surface increased in all 'Italiano Classico' basil plantlets, being highest in those treated with pure bulb extract and in the 'Aromat de Buzău' plantlets treated with flower extracts and 70% methanol, compared to C_0, where they did not appear. The treatment with extracts did not significantly impact either basil variety on the adaxial leaf surface. On the other hand, glandular hairs density did not change on either leaf surface of 'Italiano Classico' basil plantlets, but it was significantly higher on the adaxial leaf surface of 'Aromat de Buzău' plantlets treated with 70% methanol (SM - Fig. S1).

Epicotyl anatomy of treated plantlets

By examining the epicotyl anatomic structure of plantlets treated with *C. autumnale* extracts, some changes in the cross-section's outline, an uneven lignification of the xylem vessels, and heteromorphic cells in the cortical parenchyma and marrow, and an increase in the size and density of tector hairs were observed.

The contour of the cross-section through the epicotyl of a typical basil plantlet (control) is square, with 4 prominent ribs, which is a characteristic aspect of the species from the Lamiaceae family. The single-layered epidermis has numerous single-celled, long tector hairs and glandular hairs. The layers below the epidermis are differentiated into two subzones: the angular collenchyma and the parenchymal cortex, with 4-6 layers of rounded cells that leave small air gaps between them. The central cylinder comprises conductive tissues in which elements of the primary structure (generated from procambium) and the secondary structure (generated from cambium) are observed. Secondary xylem and libriform (sclerenchyma wood) fibers can be observed in approximately equal proportions. The marrow is parenchymal-meatic (Fig. 9, 10).

'Italiano Classico' basil plantlets treated with diluted bulb extract showed a weaker lignification of the xylem bundles and an elongated, rectangular contour of the epicotyl cross-section. The asymmetry of the epicotyl's shape in plantlets treated with flower extract is correlated with an increased number of cell layers on one side. Many cell layers indicate individuals with thicker epicotyls than the control. Also, more multicellular tector hairs were observed compared to the control (Fig. 9).

'Aromat de Buzău' basil plantlets treated with diluted bulb extract displayed a thinner epicotyl than the control variant, shorter tector hairs, and an aeriferous cavity in the pith, whereas the epicotyl of the plantlets treated with undiluted bulb extract displayed some heteromorphic cells in the parenchymal cortex, which led to an abnormal outline of the cross-

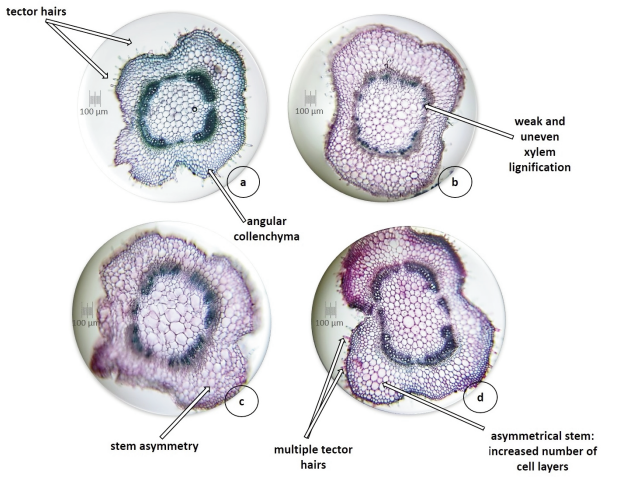

Fig. 9 - Epicotyl cross-section from *O. basilicum* 'Italiano Classico' plantlets, after treatment with *C. autumnale* diluted bulb (b), flower (c), and pure flower (d) extracts, compared to the control (a) (epicotyls of plantlets treated with pure bulb extract could not be analyzed because of insufficient plant material).

section. Similar modified characteristics were observed in 'Aromat de Buzău' basil plantlets treated with flower extract: when treated with a diluted flower extract, the epicotyl displayed an increase in the number of tector hairs, in contrast with undiluted flower extract treatment, where it decreased. In addition, a weak lignification of xylem vessels, heteromorphic parenchymal cells, and an overall outline thinning of the epicotyl were possible consequences of the treatment (Fig. 10).

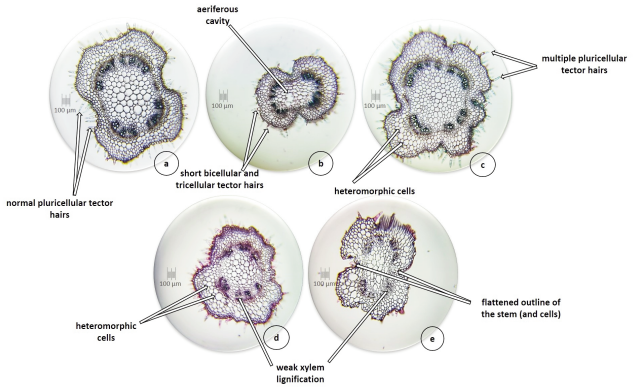

Fig. 10 - Epicotyl cross-section from *O. basilicum* 'Aromat de Buzău' plantlets, after treatment with *C. autumnale* diluted bulb (b), flower (c), and pure bulb (d) and flower (e) extracts, compared to the control (a).

Epicotyl circumference increased in 'Italiano Classico' basil plantlets when treated with pure flower extract ($p<0.01$ when compared to C_0 and $p<0.0001$ when compared to C_2). The plantlets treated with the bulb extract could not be analyzed due to the fragility of the plant material and some anomalies of the epicotyl (very short internodes). In contrast, 'Aromat de Buzău' basil had a much lower epicotyl circumference when treated with diluted bulb extract ($p<0.0001$ when compared to C_1 but not significant when compared to C_0) but higher in plantlets treated with pure bulb extract ($p<0.0001$ when compared to C_0 and C_2) (Fig. 11).

Photosynthetic pigments content of treated plantlets

The applied treatments did not affect chlorophyll a and carotenoid pigments content in both basil varieties. Chlorophyll b content was highest in plantlets treated with 70% methanol (SM Fig. S2).

Photosystem II efficiency and electron transport rate of treated plantlets

The photosystem II efficiency of both varieties and the electron transport rate (ETR) of 'Aromat de Buzău' basil plantlets were not significantly affected by the applied treatment. In 'Italiano Classico' basil, the electron transport rate was very high when plantlets were treated with 70% MeOH (C_2) (SM Fig. S3).

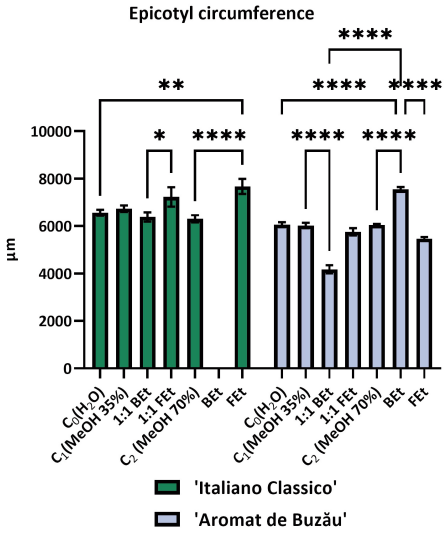

Fig. 11 - Epicotyl circumference of *O. basilicum* plantlets after treatment with *C. autumnale* extracts. BE= bulb extract; FE= flower extract; t= treatment; **** = $p<0.0001$; ** = $p<0.01$; * = $p<0.05$; epicotyls of plantlets treated with pure bulb extract could not be analyzed because of insufficient plant material.

4. Discussion and Conclusions

C. autumnale pure extracts inhibited the germination of seeds of both 'Italiano Classico' and 'Aromat de Buzău' basil varieties, probably because of the high concentration of methanol, but they could also have an inhibitory effect on germination and growth of weeds in plant cultures due to their allelopathic properties. More seeds of the 'Italiano Classico' basil variety germinated than 'Aromat de Buzău', but the latter withstood the treatment on cauline apex better than the Italian basil.

Morphological observations showed that the treatments have similar effects on leaf morphogenesis of both basil varieties, at macroscopical and microscopical levels, with some differences in the response of a particular basil variety to flower or bulb extract treatments. The epidermis dehydration, alterations in glandular hair shapes, and stimulated morphogenesis and elongation of tector hairs could appear because of the solvent used in treatments

rather than due to the applied treatments with *Colchicum autumnale* extracts. The increase in the number of tector hairs observed on the epicotyl epidermis, or their occurrence where they wouldn't usually appear (on the abaxial leaf surface), might indicate a defensive response to the chemical stress induced by methanol. In contrast, an increased density of secretory hairs on the leaf adaxial surface of 'Aromat de Buzău' plantlets treated with methanol might indicate a better capacity to protect themselves, as glandular hairs' main role is to protect the plant against external factors: herbivores and pathogens, extreme temperatures, excessive loss of water, and competing plants by secreting lipophilic substances that act as repellents or poisons (Hazzoumi *et al*., 2020). The observations on the anatomy of the plantlet epicotyl confirmed the information from the literature that colchicine affects xylem differentiation and thickening because of the disappearance of wall microtubules (Pickett-Heaps, 1967), which might lead to abnormal morphogenesis of the mature plants' stem that develops in a plagiotropic position. Another interesting observation is that heteromorphic cells induced by the treatment with *C. autumnale* extracts that appear in the leaf structure and in the epicotyl might indicate the presence of mixoploid tissues, which could also explain the abnormal leaf shapes and the epicotyl development anomalies.

The treatment with *C. autumnale* extracts did not affect biochemical and physiological indices of the photosynthetic apparatus of treated basil plantlets. Only treatment with methanol might influence photosynthesis due to its impact on chlorophyll b content and electron transport rate.

These indices might be suitable for potential polyploid evaluation and selection from plants subjected to similar treatments. If its toxicity levels are adequately managed and exploited, *Colchicum autumnale* could have a great potential for becoming a cost-effective source of allelopathic compounds for crop pest control or herbicide production, besides its importance as a source of anti-mitotic agents for artificial polyploidy induction in plants.

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