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ALTITUDINAL VARIATIONS OF MORPHOLOGY, DISTRIBUTION AND SECRETION OF GLANDULAR HAIRS IN *ORIGANUM VULGARE* L. LEAVES

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ABSTRACT. The study analyses the morphology and distribution of secretory hairs, the quantity and quality of volatile oil produced by leaves of plants like *Origanum vulgare*, collected at different altitudes. The purpose of this study is to establish the variation of these micromorphologic and biochemical characters depending on altitude, emphasizing the value of an aromatic plant.

Keywords: *Origanum vulgare*, altitude, secretory hairs, morphology, morphometry, volatile oil, chemical composition

INTRODUCTION

In *Origanum vulgare* L., an aromatic and medicinal plant, the density of glandular hairs presents altitudinal and seasonal variations. The altitudinal gradient is associated with alterations in a number of environmental factors, such as air, temperature, water precipitation, etc. On the other hand, the seasonal gradient during the growing period is also associated with alterations in environmental parameters, such as photoperiod, air temperature and water availability. The combination of all these factors exerts a pressure on plants, which becomes expressed as changes in their morphology, anatomy, physiology and productivity (Kofidis et al., 2003).

Essential oils are well known to have toxic and repellent effects on insects and to inhibit hatching of insects eggs (Levin, 1973; Bestmann et al., 1987; Sharaby, 1988) (cf. Kofidis et al., 2003). They are also known to have strong antimicrobial properties (Vokou et al., 1984) (cf. Kofidis et al., 2003).

The glandular hairs are considered as the exclusive sites of essential oil biosynthesis and thus their number is linearly correlated to the amount of the plant-derived essential oil (McCaskill and Croteau, 1995) (cf. Kofidis et al., 2003).

From economically point of view, aromatic plants are important because of the essential oils they produce. Thus, many publications are dealing especially with the biochemical characteristics of the essential oils.

The present paper analyzes the morphology and distribution of glandular hairs and the quantitative and qualitative characteristics of the essentials oils they produce in the leaves at *Origanum vulgare* spontaneous plants grown in Romania, in different altitude (between 350 m and 700 m). The major aim of this study was to determine the aromatic value of the oregano leaves depending on altitude, by using the correlations between previous noted micro-morphological and biochemical features.

MATERIALS AND METHODS

Plant material

Origanum vulgare, spontaneous plants grown in regions with different altitude from Romania country (350m - Bâtca Doamnei, Neamţ County; 450 m - Gârcina, Neamţ; 700 m - Bicaz Chei, Neamţ County) were collected during the flowering stage of development.

Microscopic investigations (light and scanning electron microscopy)

For light microscopy, leaves from different stem levels were fixed and preserved in 70% ethylic alcohol. Semi-thin sections were performed using a razor blade. The sections were coloured with iodine green and ruthenium red. The photos were taken with an Olympus E-330 photo camera, using an Olympus BX51 research microscope.

For scanning electron microscopy, the 1 cm 2 samples were fixed using glutaraldehyde and osmium tetraoxyde and dehydrated using ethylic alcohol (35%, 50%, 70%, 90%, 95%), followed by anhydric acetone. The coating of the dehydrated samples has been carried out using gold in a 30-60A thick layer. The coated samples have been observed in the SEM vacuum chamber.

Morphometry and statistic analysis

The density of glandular hairs on both faces of the leaves was determined using leaf paradermal sections.The obtained data were statistical analyzed using the Anova test.

Volatile oil assay

To identify and measure the amount of the various sample components of volatile oil, Gas chromatography – mass spectroscopy (GCMS) and Thin layer chromatography (TLC) analyses were performed.

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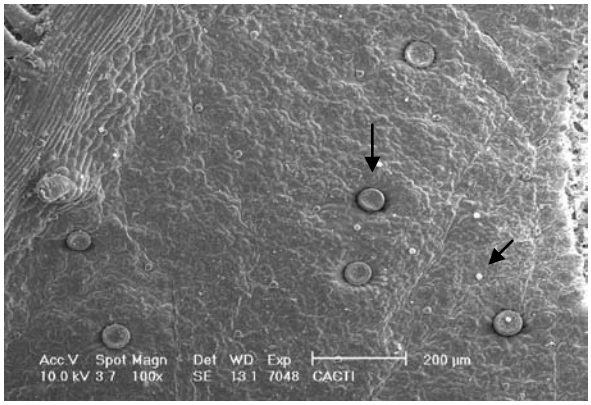

Fig. 1 Scanning electron micrograph of *Origanum vulgare* lower leaf surface. Two major types of glandular hairs are distinguished.

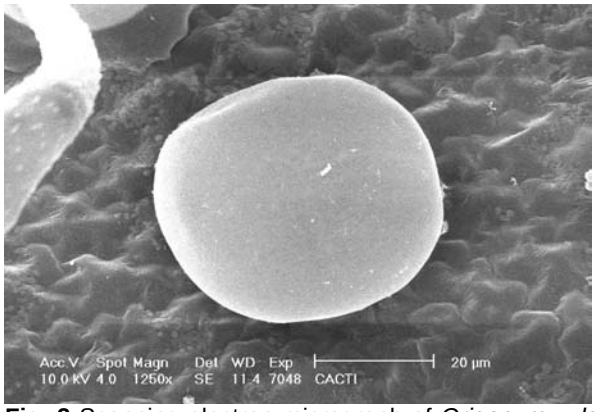

Fig. 2 Scanning electron micrograph of *Origanum vulgare* upper leaf surface. A capitate glandular hair is distinguished.

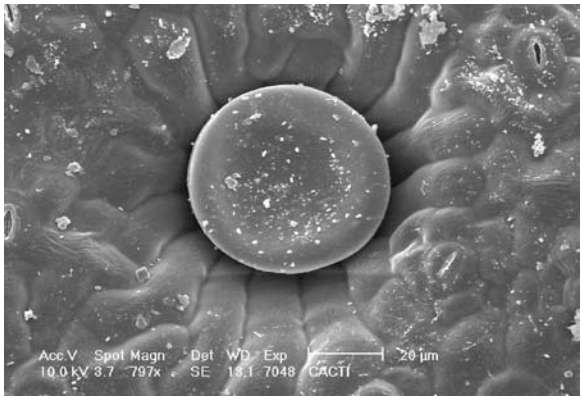

Fig. 3 Scanning electron micrograph of *Origanum vulgare* lower leaf surface. A peltate glandular hair is distinguished.

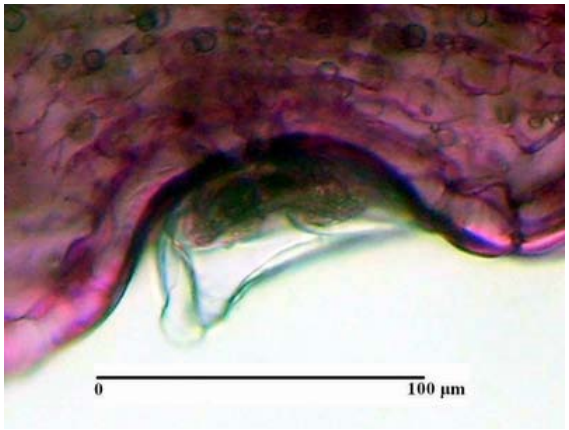

Fig. 4 Light micrograph of cross-section through *Origanum vulgare* leaf. A peltate glandular hair is distinguished in a depression of lower epidermis.

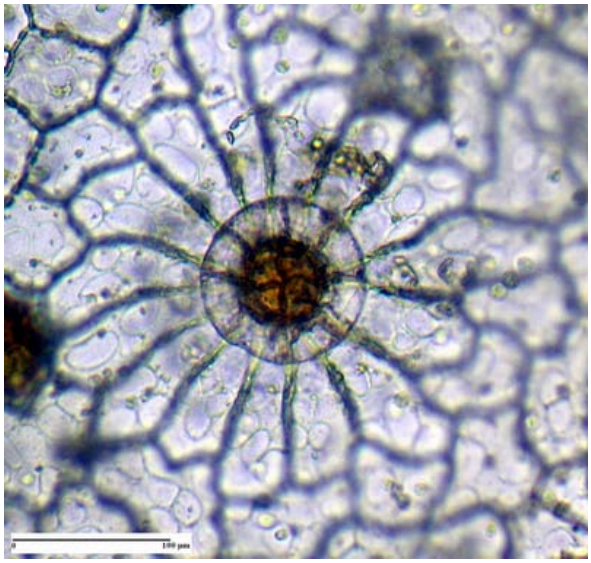

Fig. 5 Light micrograph of *Origanum vulgare* lower leaf surface. A peltate glandular hair is distinguished.

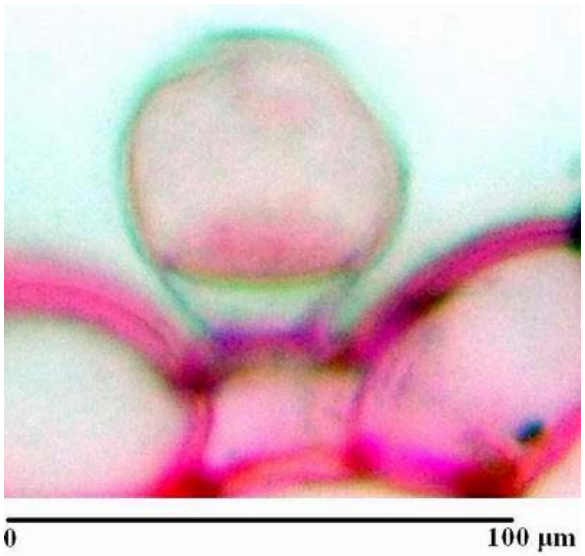

Fig. 6 Light micrograph of cross-section through *Origanum vulgare* leaf. A capitate glandular hair with unicellular secretory head is distinguished.

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RESULTS AND DISCUSSIONS

Morphology and distribution of glandular hairs on Origanum vulgare leaves

Two major types of glandular hairs (capitate and peltate) were distinguished on both leaf faces (Figs. 1-6). The glandular hairs consist of one or two epidermic basal cell, a unicellular stalk and a uni- or multicellular secretory head. The distinct peltate hairs were hiding in some depressions of the leaf surface (Fig. 4).

In all investigated *Origanum vulgare* plants, the density of glandular hairs was found to be greater on lower epidermis than on the upper one (Fig. 7). On both leaf surfaces of upland (700 m) plants, glandular hairs were more numerous compared with those from mid- (450 m) and lowland (350 m) (Anova Single Factor, F>F crit.).

In a recent study (2003), Kofidis et al. investigates the structural variations of *Origanum vulgare* leaves during growing season at native plants from different altitude (200 m, 950 m and 1760 m). The glandular hairs are more numerous on the lower leaf surface than on the upper one at 200 m altitude. In the leaves of lowland (200 m) plants, glandular hairs are denser compared with those of the mid- and upland (950 m, 1760 m) plants, for both the upper and lower surface. These conclusions can be explained by the fact that the attacks by insects have been found to be more frequent in low-altitude habitats than in high-altitude ones, where low temperature and short growing period reduce the activity of leaf beetles (Begon et al., 1990, Suzuki, 1998) (cf. Kofidis et al., 2003).

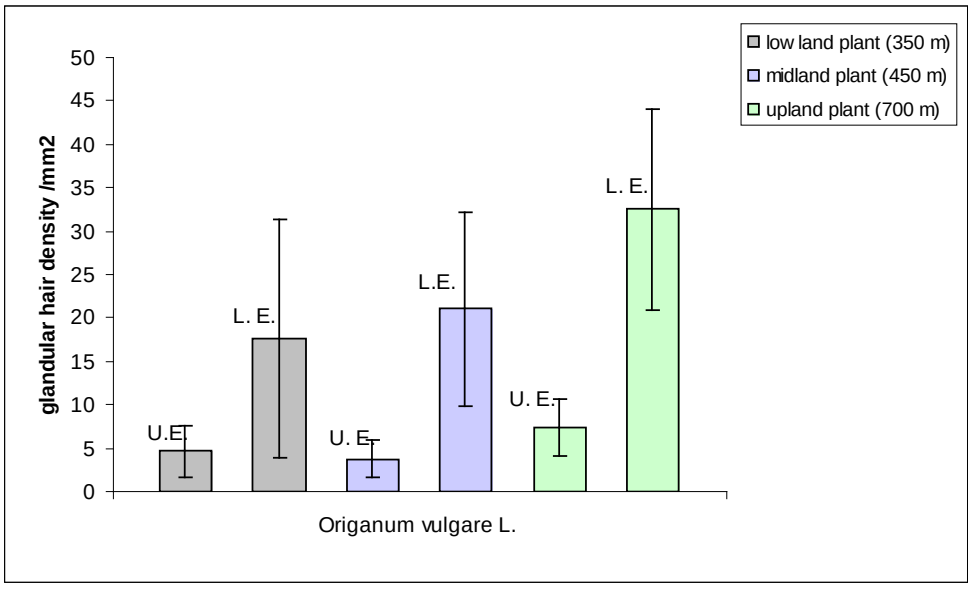

Fig. 7 Altitudinal variations of glandular hairs density on the upper (U.E.) and lower (L.E.) leaf surfaces in *Origanum vulgare* L. plants (±s. d., n=10).

Quantitative and qualitative features of *Origanum vulgare* volatile oils

The biggest amount of volatile oil was determined at upland plants (700 m). This quantity (2.850 ml %) is corresponding to European Pharmacopoeia standards.

In all investigated *Origanum vulgare* plants, several similar components of volatile oil (Sabinene (10.5% - 15.8%), γ-Terpinene (3.6% - 11.7%), 1,8-Cineole (9.4% -11.5%), β-trans-Ocimene (5.4 - 8.3%), β-cis-Ocimene (9.0 - 10.4%), Anetol (1.5 - 15.3%), Thymol (max. 1.73%), Carvacrol (0.15 - 2.44%), trans-β-Caryophyllene (4 - 9.2%), Germacrene D (3 - 9%) were identified using both GCMS and TLC analyses (Fig. 8).

Our data are in concordance with those from the literature on the field (Figueredo G. et al., 2006; Viuda-Martos M. et al., 2007), according to which the same types of components were identified in *Origanum vulgare* ssp. *vulgare* volatile oil, differing only in their quantity.

CONCLUSIONS

The aromatic value of a plant is given by the morphological types and number of glandular hairs on unit surface of the organ and the quantity and quality of volatile oils they produce.

In our investigated *Origanum vulgare* plants, two morphological types of glandular hairs are determined on leaves surfaces. Their density on organ unit surface and the quantity of volatile oils varies with the altitude the plants grow.

Correlating these obtained data, the most aromatic *Origanum* plants grow at 700 m altitude compared with those from 350 and 450 m altitude.

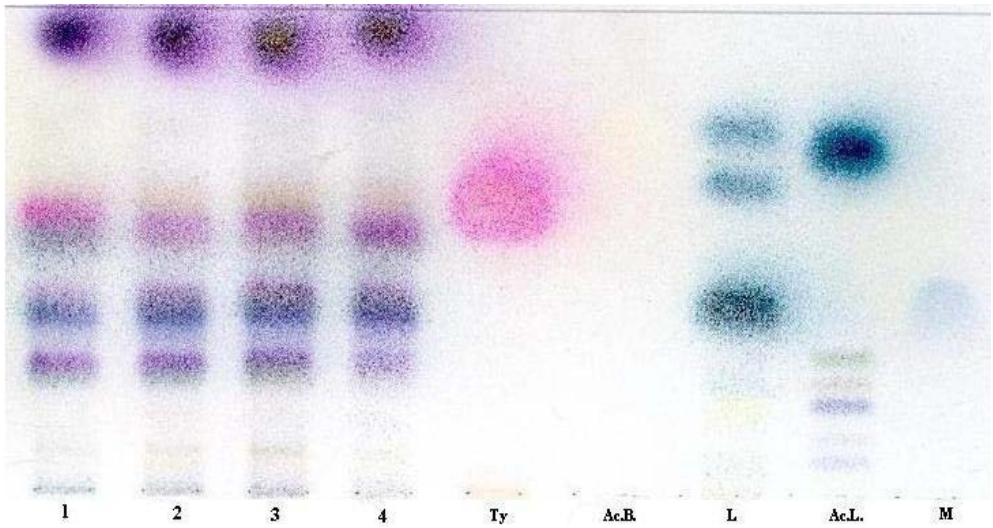

Fig. 8 Thin layer chromatography of *Origanum vulgare* volatile oils. 1 - Upland plants (700 m); 2 – lowland plants (350 m); 3 – midland plants (450 m). Etalons: Ty=Thymol, Ac.B.= Bornil acetate; L=Linalool; Ac. L.= Linalool acetate; M=Menthol

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