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RESEARCH ARTICLE

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Infraspecific trichomes variations in *Acinos graveolens* (M.B.) Link

Seyed Mehdi Talebi* and Ali Reza Shayestehfar

Department of Biology, Faculty of Sciences, Arak University, Arak 38156-8-8349, Iran

ABSTRACT

Acinos Miller is the genus of Lamiaceae family comprises of 10 species. Some species of this genus are employed in folk medicine. Acinos graveolens (M.B.) Link is one of them which naturally grows in Iran and has a wide range of distribution, so that finds in different regions of western, north-western and central parts of the country. In this study, to compare the effects of various ecological factors on epidermal hairs, trichomes micromorphology of three different populations of this species were examined with the both scanning electron microscope and light microscope. On the leaf and stem surfaces of the studied population's, two main types of hairs were found: glandular and non-glandular. Glandular trichomes were present as sessile or stalked. Sessile hairs were seen as one or two-celled. Stalked trichomes were found in the shapes of peltate, digitiform and capitate. Digitiform trichomes presented as one to bi-celled. Capitate hairs were found as short-stalked and long-stalked in the studied populations. The length of their basal cell as well as the shape of apical cells varied between samples. Non-glandular trichomes were found as: one to four-celled or triangle. These types of trichomes were dominant and covered all epidermal surfaces of the aerial organs of plants. The wall thickness varied between these hairs. Some non-glandular trichomes had thin wall, but the others were thick-walled hairs. These ornaments were seen in the shapes of fusiform or linear. Types and frequency of trichomes as well as the kinds of dominant trichomes varied between these populations. This confirms the effect of various ecological factors on trichomes plasticity in this species.

Key words: *Acinos graveolens*, glandular and non-glandular trichomes, population.

INTRODUCTION

Plant hairs (trichomes) are of great interest to descriptive and experimental botanists and data on these and indumenta are routinely included in many types of studies. As simple morphological tools, trichomes are useful due to the ease with which they are examined and their almost universal occurrence, particularly among the ferns and flowering plants. Beyond their purely descriptive use, comparative data may be important for the study of evolution, relationships and for the roles of hairs in various aspects of physiological and ecological adaptation [1].Many authors, such as Behnke [2], Güemes et al. [3], Servettaz et al. [4] and Bini Maleci et al. [5] emphasize the great value of trichomes in modern taxonomy. The morphology of hair (glandular and non-glandular) has proved essential in taxonomic and ecological studies of Lamiaceae family [6, 7, 8]. There are two main types of glandular hairs, peltate and capitate, carrying out different metabolic processes.

Genus *Acinos* Miller is the members of Labiatae family comprises of 10 species that distributed over Mediterranean regions toward Central Asia [9] and has two species in Iran [10].Some species of the genus *Acinos* are employed in folk medicine as antiseptic, stimulant, tonic and antispasmodic due to their beneficial effects on melancholy, coughs, toothache, sciatica, neuralgia and gastrointestinal disorders. The essential oil of this species was shown to possess anti-*Candida* activity. With using of this species essential oil, a significant reduction in *Candida albicans* growth was observed. The measured activity was comparable with the conventionally used antifungal nystatin [11]. *Acinos graveolens* (M.B.) Link is one of the both which naturally find in Iran and has a wide range of distribution so that grows in different regions of western, north-western and central parts of the country [10].

Like most members of the family Labiatae, there were different types of trichomes on the epidermis surface of *A. graveolens*. In present study, in order to compare the effects of different ecological factors on epidermal hairs, trichomes micromorphology of three populations of the species were investigated with the both scanning electron microscope and light microscope. As far as our literature survey could ascertain, the trichomes micromorphology of this species has not been published previously.

MATERIALS AND METHODS

The plant specimens were collected from three different localities in Iran; Markazi province, Zarandiyeh, Vardeh village, 35° 15′ 22.2″ N, 50° 17′ 18.6″ E, 1566 m, Tehran province, Evin, Darakeh No.1, 35° 48′ 06.3″ N, 51° 23′ 02.3″ E, 2054 m, Tehran province, Evin, Darakeh No.2, 35° 48′ 51.0″ N, 51° 23′ 17.6″ E, 2376 m. For Light microscopy (LM), the embedded materials were used for microscopic investigation. Transverse hand sections of the lamina and stem were made from the mid-part of fully-grown leaves and stems. Embedded materials were prepared as follows: adult plant samples were fixed with F.A.A. (formalin 5%: acetic acid 5% and 90% ethanol) for 48- 72 hours, then dehydrated in a graded ethanol series and embedded in ethanol 90. For scanning electron microscopy (SEM), the samples were transfered directly to double-sided tape affixed stubs and vacuum-coated with gold in the Biorad E5200 auto sputter coater and photographed with the Camscan MV2300 scanning electron microscope at 10kV.

RESULTS

Trichomes micromorphology of three populations of *A. graveolens* were examined with the SEM and LM. On the both leaf and stem surfaces of the studied population's, two main types of hairs were found: glandular and non-glandular. Glandular trichomes were seen as sessile or stalked. Sessile hairs were seen as one to two-celled (Fig.1 A, B). One-celled trichome was found on the stem surface of Vardeh, Darakeh No.1 and Darakeh No.2 populations, in addition two-celled one occurred on the stem surface of Darakeh No. 2 and Vardeh and the leaf surface of Vardeh and Darakeh No.1 populations. One-celled hair was dominant type of glandular trichome on the leaf surface of Darakeh No.2 and the stem surface of Vardeh populations. Stalked trichomes were found in the shapes of peltate, digitiform and capitate.

Peltate trichome was found on the leaf and stem surfaces of Vardeh and Darakeh No.1 samples as well as the leaf surface of Darakeh No.2 samples. It was abundant type of glandular trichome on the leaf surface of Darakeh No.1, 2 specimens. Digitiform trichomes presented as one-celled (leaf and stem of Vardeh and Darakeh 2 populations) bi-celled (as seen in Darakeh No.1, 2 and Vardeh samples).

Capitate hairs found as short-stalked and long-stalked in the studied populations. The length of their basal cell as well as the shape of apical cells varied between samples. Long-stalked capitate trichome had a large basic cell, short-necked and spherical or elongate-celled head which found on the stem epidermal surfaces of Vardeh and Darakeh No.2 and also on the leaf surface of Darakeh No.1. But, long-stalked trichome with elongate-celled head was seen only on the leaf surface of Vardeh, Darakeh No.2 and stem of Darakeh No.1 samples (Fig.1 C, D, Fig.2 A, B). This was dominant kind of glandular trichome on the stem surface of Darakeh No.1, 2 and leaf of Vardeh populations. Short-stalked capitate trichome consists, of a short stem cell with spherical head, was seen in stem surface of Darakeh No.1 and Vardeh with leaf surface of Darakeh No.2 populations (Fig.1 E, F, G).

Non-glandular trichomes were found as: one to four-celled or triangle. Triangular trichomes found in the all studied samples with except of stem surface of Darakeh No.1 samples (Fig.1 I). One to four-celled types of these trichomes were dominant and covered all epidermal surfaces of the aerial organs of the plant (Fig.2, C). In the all studied populations, one-celled (Fig.1 H), two-celled (Fig.1 J, K, L) and three-celled (Fig.1 M) trichomes were found, but four-celled hairs (Fig.1, N, Fig.2, D) occurred on the stem and leaf of Vardeh, leaf of Darakeh No.2 and stem of Darakeh No.1.

The wall thickness varied between different types of these hairs. Some non-glandular trichomes had thin wall, but the others were thick-walled. Non-glandular hairs had fine structures on their surfaces. These ornaments were seen in the shape of fusiform or linear (Fig.2, E, F).

DISCUSSION

In this study, trichomes morphology of three populations of *A. graveolens* were examined. In taxonomy, *A. graveolens* is related to the west Mediterranean and North African taxa of Labiatae family [11]. The Lamiaceae is a large family that rich in aromatic species used as culinary herbs, folk medicines, fragrances and etc. Many species of

this family possess essential oils secreted by glandular trichomes [12], as secondary metabolites as part of their normal physiological function of growth, ecological function (interaction with the environment), development, or in response to pathogen attack or stress [13]. In the studied samples, two main types of trichomes, glandular and non-glandular, were found on the epidermal surfaces of the stem and leaf. Glandular trichomes were seen in the forms of digitiform, peltate, capitate and sessile. Among these, the capitate and peltate hairs were very important in accumulation and secretion of essential oil. In Labiatae family, two distinct types of glandular hairs are distinguished, the peltate and capitate hairs, which mainly differ in the volume of their secretory head. The density of these epidermal structures on the leaves has been found to be positively correlated with the essential oil content of the plants [14].

In the observed capitate trichome, the stem cell size and also head cell shape differed and on the basis of these conditions, two types of capitate trichomes, long-stalked and short-stalked, were found. Long-stalked capitate trichome was dominant kind of glandular trichome on the stem and leaf surface of some populations. Other studies confirmed this subject, for example Ascensˇao et al., [15] showed that capitate hairs of different Lamiaceae species vary in stalk and head shape, depending on the size of the subcuticular space. These structures produce a unicellular base, a unicellular stalk, and a 12-celled head in *Satureja thymbra* (eight-celled in *Mentha piperita*), and then commence filling the subcuticular oil storage cavity [16, 17]. Other form of glandular trichomes was peltate which occurred rarely on the leaf or stem surfaces of some populations, but it was abundant type of glandular hairs on the leaf surface of some populations, for example Darakeh No.1.

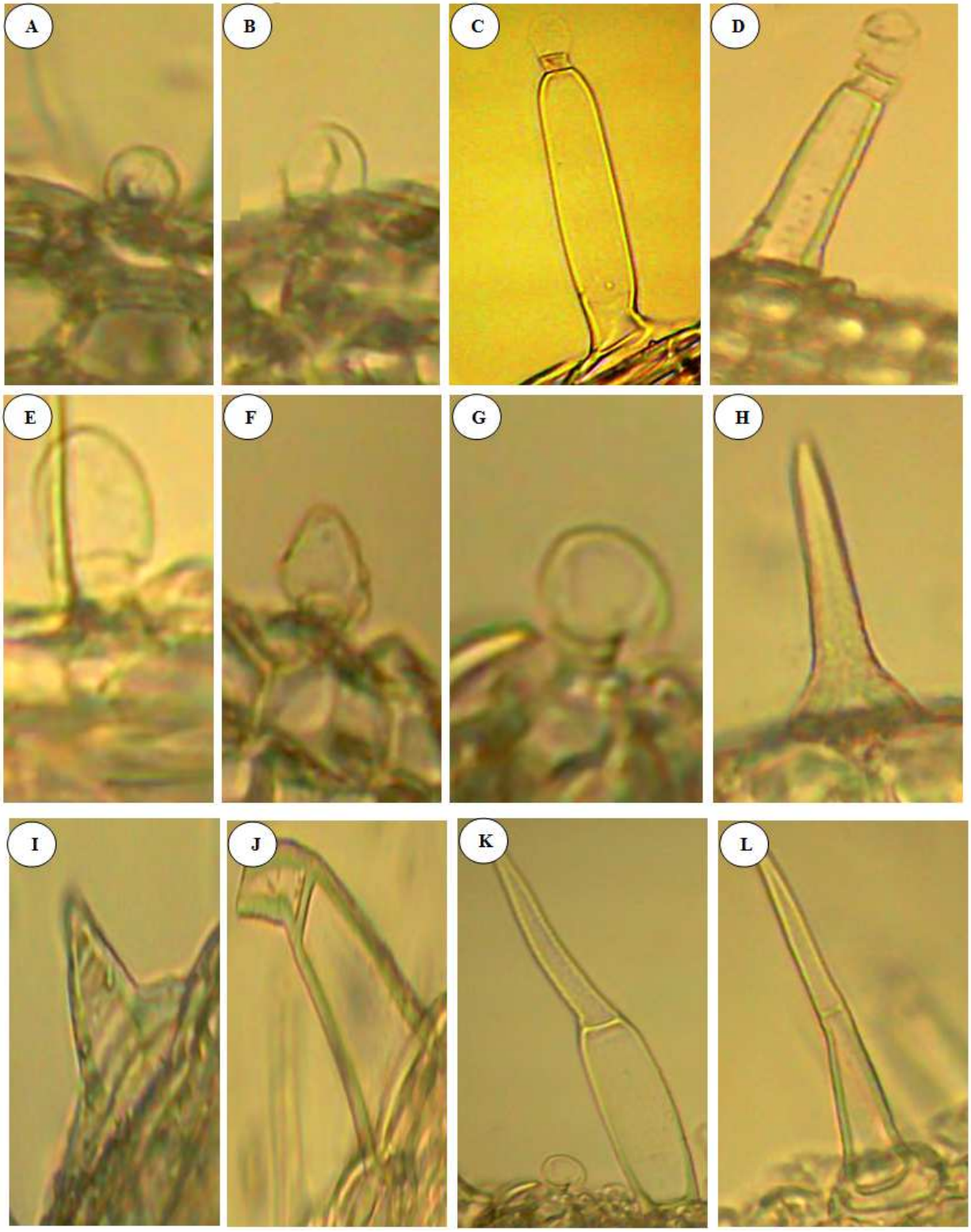
Studies showed that the oil content of the aerial parts of *A. graveolens* was 0.06 ml (0.06%, v/w). Compared with other *Acinos* species whose oil yields were between 0.01-2.3 percent, it seems that *A. graveolens* belongs to a species with a rather low oil yield [11], while the individuals of this species give out a scent and their aromas are feel in natural habitat. The reason may be related to the kinds of their prominent glandular trichomes. Because the secreted compounds of capitates glandular trichomes mostly excreted to the surrounding environment, apparently through pores in the cuticle of the head cell(s), but on contrast, in peltate glandular trichomes the secretions accumulate in a capacious subcuticular space formed by the separation of the head cell walls from the cuticular dome that encloses them, and remain there until the cuticle is physically ruptured. Thus, peltate glandular trichomes function as repositories for the specialized phytochemicals that they secrete [18].

In addition to glandular trichomes, non-glandular hairs with different shape and size were present on the epidermal surfaces of the studied plant. All observed non-glandular trichomes were unbranched and had one to four cells with thin or thick cell wall. The non-glandular trichomes are diverse in morphology, anatomy and microstructure. Basically, they are classified according to their morphology. They may be unicellular or multicellular, and both types can be unbranched and branched. The variability in coverage, by both non-glandular and glandular trichomes and different proportions of these types, between species and even between the two sides of organs, such as leaves, bracts, sepals and petals, is observed. They may appear on one side only or on both sides, equally or unequally [19].

The studied populations selected from three different habitats, therefore ecological features of habitat varied, therefore the studied samples confronted with various biotic and abiotic agents. Different populations of same species have local adaptations for fitness with ecological conditions that ruling in each habitat. These adaptations may be lead to creation of plasticity in plant phenotype [20, 21]. Populations Darakeh No.1 and 2 selected from the same habitat with different elevation. Difference in elevation influences the ecological conditions of habitat and this subject impresses the plant structures. In the some cases, trichomes types and abundant varied between these populations, for example, one-celled trichome was dominant type of glandular trichome on the leaf surface of Darakeh No.2. Triangular as well as one-celled digitiform trichomes were absent on the stem surface of Darakeh No.1 samples. Ecological conditions of the Darakeh population's habitat differed from habitat of Vardeh population in some factors such as elevation, longitude and altitude. Therefore it was clear that Darakeh populations differed from Vardeh population in kind of prominent trichome. For example, contrary to Vardeh population, peltate trichome was abundant in Darakeh habitat No.1, 2 populations. Similar studies on the trichomes morphology of some Labiatae species such as *Stachys lavandulifolia* vahl. [22] and *Ziziphora tenuior* L. [23] showed that ecological and edaphical conditions had strong effects on the composition, dispersion and diversity of trichomes, therefore it lead to interpopulation variations in the trichomes morphology between different populations of the same species.

It seems that glandular trichomes, in addition to secretion and accumulation of essential oil, have some important rules in plant. For example, in some desert species, the principal role of glandular trichomes is to secrete large quantities of exudate, which form a continuous layer on the plant surface, increasing light reflectance, thereby reducing leaf temperature [24, 25]. In the studied populations, long-stalked trichome was dominant kind of glandular trichome on the stem of Darakeh No.1, 2 and leaf of Vardeh populations. Histochemical tests showed that capitate

trichomes secrete varying amounts of polysaccharides [7], in addition to essential oils; these components were consumed by different pollinator insects that visit the plants. Because of the kind and size of existing pollinators, due to different ecological conditions, differed between populations, in order to adaptation with pollinators, the location of capitate trichomes varied between different populations.



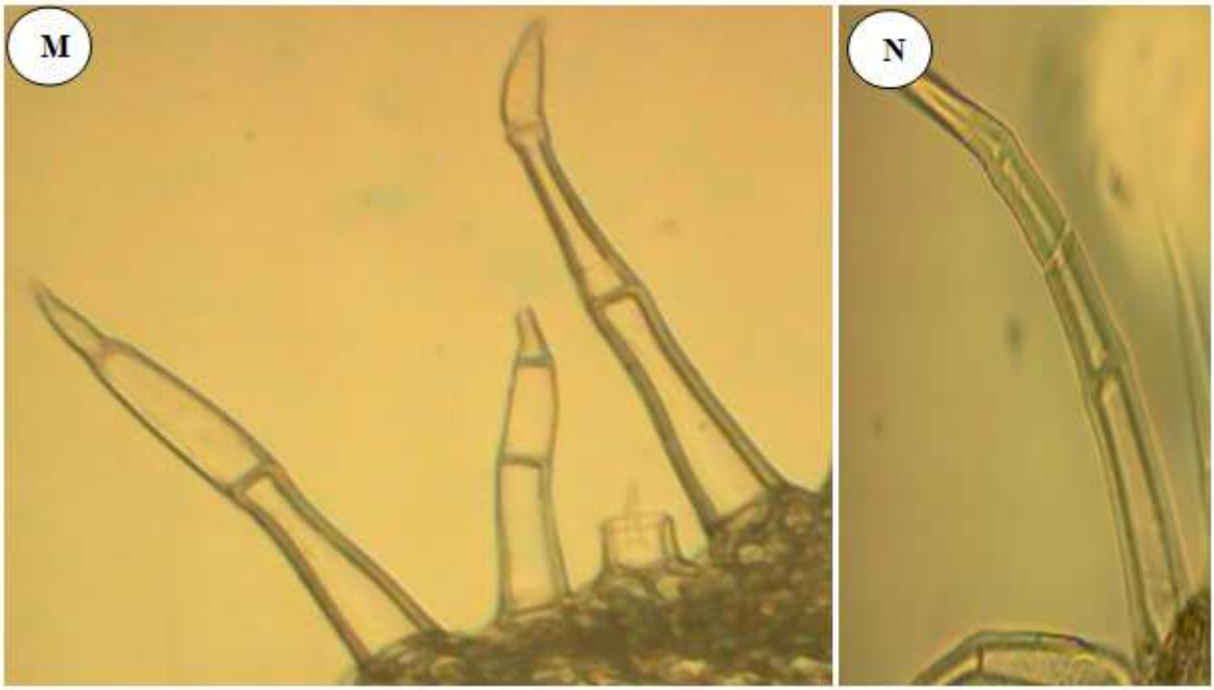
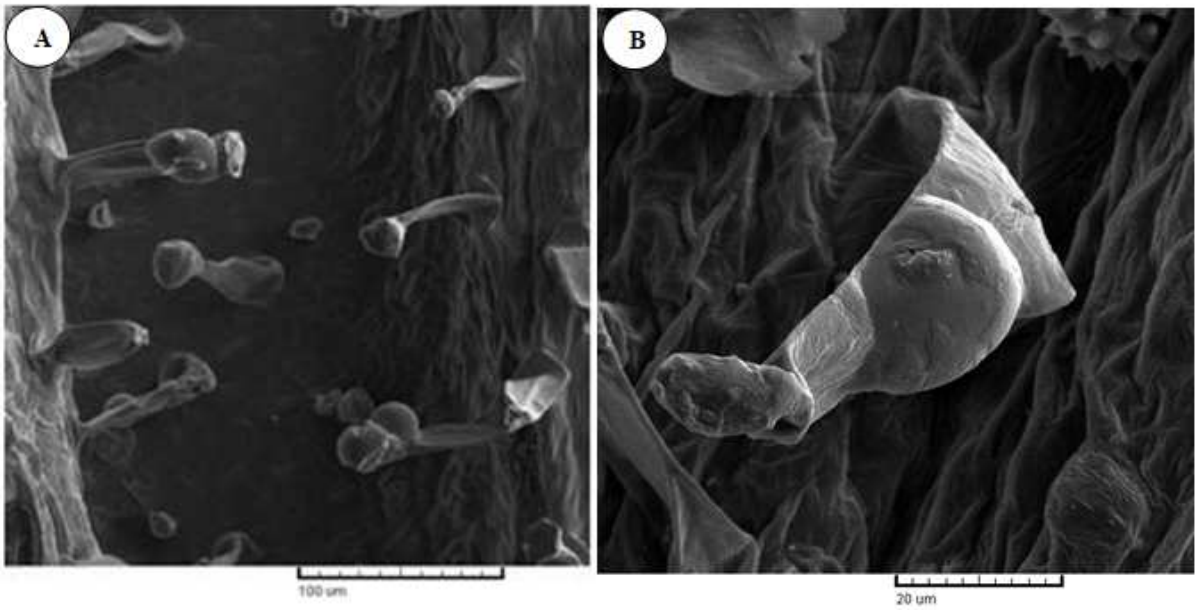


Fig.1. Light micrograph of trichomes morphology in the studied populations.



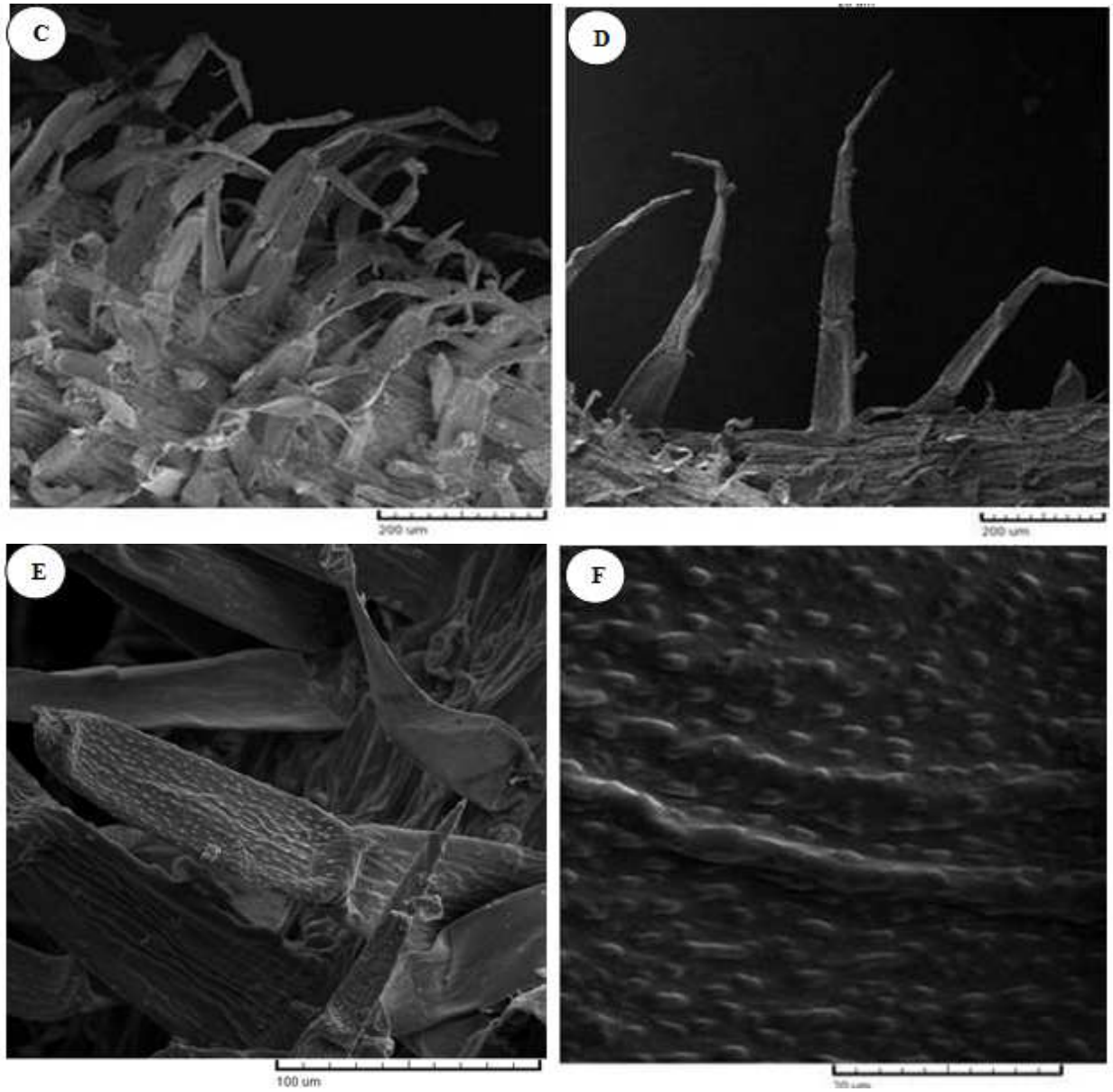


Fig.2. Electronic micrograph of trichomes morphology in the studied populations.

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