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Research Article

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Biological study of *Lagochilus maracanthus* Fish. et Mey endemic species of Iran

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ABSTRACT

Lagochilus macracanthus was the endemic species of Labiatae family in Iran. In this study the morphological and micromorphological structure of vegetative and reproductive organs, nutlet, pollen and anatomical structure of leaves and trichomes micromorphology of stems, leaves, calyx and spines were survived with light microscope and scanning electron microscope. Variations in leaf shape in this species were existing. In the tip of leaves and calyx small spine could be seen. Pollen surface was reticulate with pores which produced by tectate exine structure. The seed of this species was elliptic nutlet that its dorsal surface was convex with outstanding decorations and ventral surface V shape with reticulate structure associated by micro pores on it. Leaf cross-section structure was isobilateral and the main vein vascular system was crescent shape. Nine different types of trichomes in vegetative and reproductive organs could be seen, which divided into two main groups of glandular and non-glandular (simplified) trichomes. Glandular trichomes were seen as: peltate, capitates and digitated and non-glandular hairs were uni or bi-celled with thin or thick wall.

Key words: *L. macracanthus*, Morphology, Seed micromorphology, Palynology, Anatomical structure.

INTRODUCTION

Lagochilus is the genus of Lamiaceae family and has five species which naturally grows in different regions of Iran [1]. The species of this genus are used as a local medicine in treating the skin diseases, helping to control the bleeding, and creating peace for nervous disorders. Lagochilin is a diterpene that forms a grey crystalline solid. It was found in various species of the genus *Lagochilus*, most notably *Lagochilus inebrians*, and is thought to be responsible for the sedative, hypotensive and hemostatic effects of this plant [2,3]. These plants are rich sources of Iridoid polysaccharides which are used by human due to its medical importance [4]. *Lagochilus macracanthus* Fish. et Mey is the endemic plant species of Iran and is regarded as the endangered species which may be made extinct if it is not protected[5]. Due to limited geographical distribution of this species and lack of comprehensive biosystematics study on the species and the necessity of understanding the exclusive species of Iran, the morphological characters of vegetative and reproductive organs, micromorphological characters of seed and pollen grains, anatomical features of leaf and trichomes micromorphology of stems, leaves, calyx and spines will be studied for first time.

EXPERIMENTAL SECTION

Plant samples:

The natural population of *L. macracanthus* was collected from the 20-kilometer of Saveh-Hamedan road on (Samavak village) May 2010, and was recognized based on the Flora Iranica [1]. and was dried according to the common principles in plant taxonomy.

Morphological study:
Several individuals were selected from the mentioned population and some important morphological characters of vegetative and reproductive organs such as: the shape and dimensions of basal and flower leaves and its marginal forms, the base and tip of leaves, the shape and size of corolla and calyx were studied. The quantitative features and were described based on the Stern terminology [6].

- **Palynologycal study**:
The Pollens were obtained from the mature buds and used for light microscopy (LM) and scanning electron microscopy (SEM) by the prolonged acetolysis method of [7]. For LM, the pollens were mounted in glycerin jelly and sealed with paraffin. The Polar (P), equatorial (E) and colpus lengths, and sizes of pollen grains were measured under the light microscope and P/E ratios were calculated. For SEM, the pollen grains were transferred directly to double-sided tape affixed stubs and were vacuum-coated with gold in Biorad E5200 auto sputter coater and photographed with a Camscan MV2300 scanning electron microscope. The terminology in this paper is based on Moore *et al*. (1991) work [8].

- *Seed micromorphology:* some healthy and ripe seeds were taken from the ovary of this plant, and images were taken using the Stereomicroscope and scanning electron microscope. For SEM, selected seed were directly mounted onto aluminum stubs with glue, were vacuum-coated with gold in Biorad E5200 auto sputter coater and photographed with a Camscan MV2300 scanning electron microscope. Some of nutlet characteristics such as, nutlet size and its shape, the shape of nutlet tip and base and nature of surface sculpturing and its size were survived.

- **Anatomical study**: the leaf of collected samples were fixed in the F.A.A (formaldehyde acid acetic ethanol solution) solution and transferred to 70% alcohol for cutting. Several cuttings were done in the middle of the leaf and they were colored by carmine and methylen blue .Then, they were studied by the Olympus microscope and some images were provided with different magnifications.

- **Trichomes micromorphology**: to study the trichomes of *L. macracanthus*, some cut-outs were provided from the stem, leaf, sepal and thorns of the stem according to mentioned method in above anatomical study. Then they were studied by optical and electronic san microscopes.

RESULTS

Morphology:
Leaf: the length of leaves varied between20 to 24 mm and these widths were 10 to 12 mm. in *L. macracanthus*, different shapes of leaf margins were seen in the leaves such as: palmate, laciniate and entire. The entire leaf often was rectangle or semi- rectangle and had three main veins. Its venation was palmated. The palmate leaf had three lobs and palmate veins. In the laciniate leaf, the leaves had lots of branching. In the tip of all leaves, there was a spine, its length was 1mm. No petiole was seen in the leaves and all of them were sessile. (Figure 1.A)

Thorn: at the axile of all leaves, there were 4 thorns on the nod of stem. Its length was varied between15 to 22 mm.

Calyx: calyx was like a tube and had five lobes. Its dimension was 17-22 ×2-3.5mm. The size and shapes of these lobs were equal, 8-12 ×1.5-2.5 mm and their end had a spine, and its length was 0.5-1 mm. There were some veins on its lobs. (Figure 1.C)

Corolla: was cream and had three lobs at the top and two lobs at the bottom. There were 4 stems in this plant. The two upper stems were longer than the two lower ones. The style was long and had two branches in the end. The length of corolla was more than the calyx and it exits from it. (Figure 1.D)

Pollen micromorphology:
Pollen shapes in polar view was circular and in Equatorial view was elliptic. Pollen size: in this species in order to measure the pollen size, polar and equatorial axis of the pollen grains was investigated. Diameter of the equatorial axis was40.24 µm and its polar axis was24.51 µm andP/E ratio was 0.6

Aperture: an aperture is any thin or missing parts of the exine which is independent of the exine pattern. There were two groups of aperture named pori and colpi [6]. pollen grains in the *L. macracanthus* was divided into trizonocolpate group which has three long and boat shaped groove with pointed ends and many small pores, these colpus being situated in equatorial zone of the pollen. The length of pollen colpi in studied species was 38.06 µm. the pori were distributed in the surface of pollen and its diameter was 0.25 µm.

Sculpturing: decorating exine surfaces in the studied sample of this species was reticulated. The reticulated structures were pentagonal and the length of each was about 0.88 µm. (Figure 2)

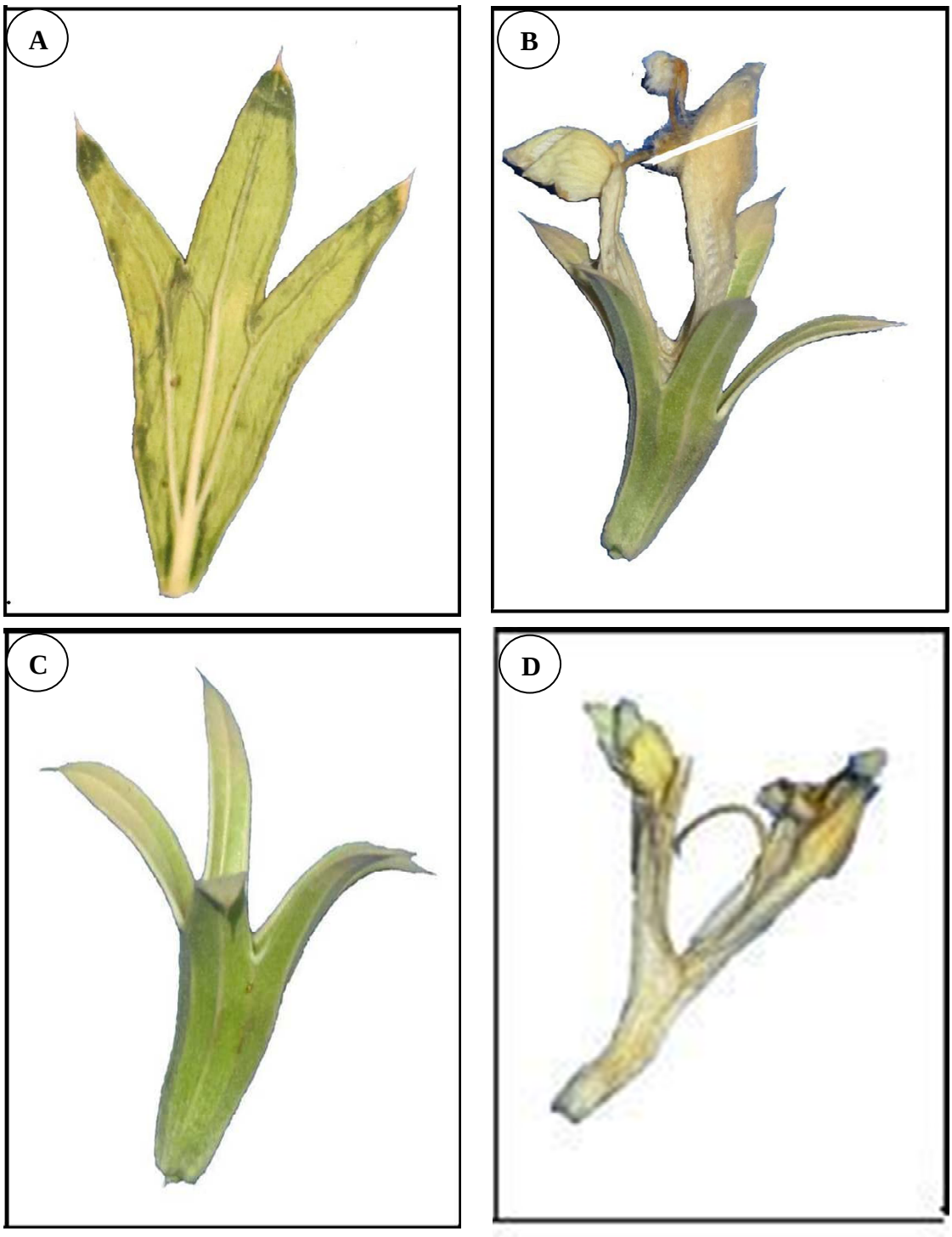

Fig.1.The shape of leaf, calyx and corolla

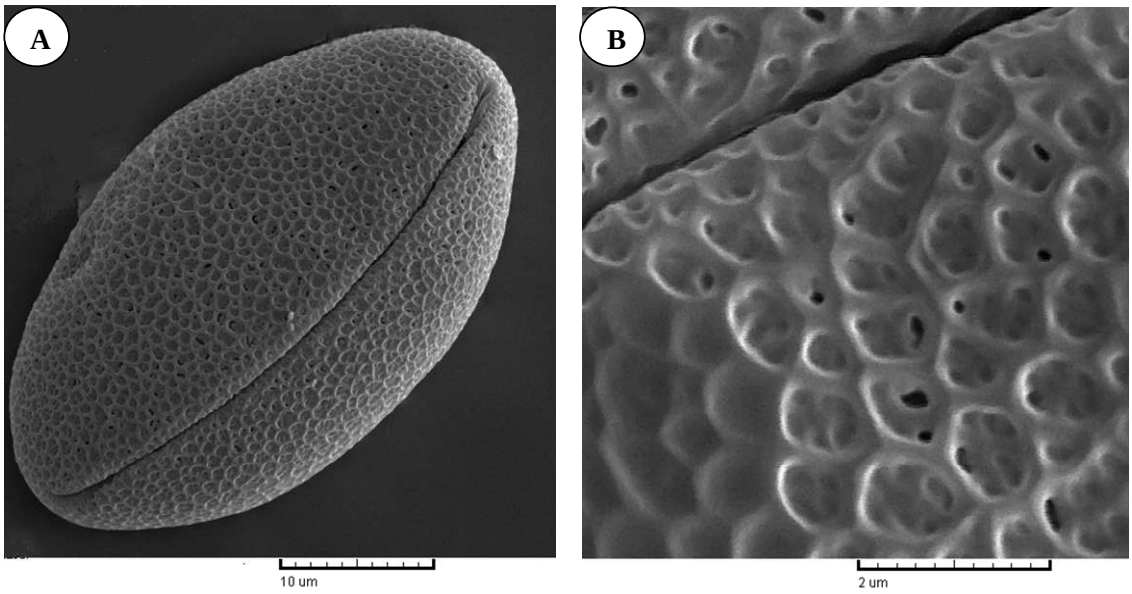

Fig.2.Pollen micrograph of *L. macracanthus*

- *Seed micromorphology:*

The nutlet of this species is long and has dorsiventral surfaces and these surfaces were different in surface sculpturing. The dorsal length was 5.20 mm and its width was 2.79mm. The end part of the nutlet is boride and flat, and its tip is thin-edged. The ventral surface of the seed is convex and some raised structures were observed. Its length and width were 5.33 and 3.10 mm respectively. The ventral surface of the seed was V-shape and continues across the ventral surface of the seed. The structures of this surface are reticular and numerous holes are seen in this part. (Figure 3)

Anatomical structure of leaf's cross-section:

In this species the anatomical structure of leaf was isobilateral [9]. The epidermis consists of a layer of long cell. In the outer surface of the epidermis, a relatively thick layer of cuticle was seen. There were some stomata between the epidermis cells, particularly the lower epidermis. The mesophyll texture of the leaf consists of palisade and spongy parenchyma. The cells of palisade parenchyma were seen in the cross of leaf, from dorsal epidermis to ventral surface. The spongy parenchyma of was placed in the middle part of leaf between upper and lower palisade. Its cells had different shapes and there were lots of intercellular spaces. The cells of palisade in each dorsal and ventral surface were seen as two extended layers across the ventral surface of the leaf, which were densely placed next to each other. (Figure 4.A)

Vascular tissue: in the central area of leaf, the vascular tissue was extended like a crescent. The phloem cells were placed on the bottom surface and the xylem cells were in the upper surface. Among the xylem cells, there are some fiber cells relevant to the sclerenchyma. Around the vascular tissue, there was a cell layer of vascular belt made of parenchyma. In the upper and lower surface of vascular belt, there were two and three layers of collenchyma tissues, respectively. The epidermis tissue in the middle vein of leaves formed of the spherical cell on which there was a relatively thick cuticle layer. (Figure 4.B)

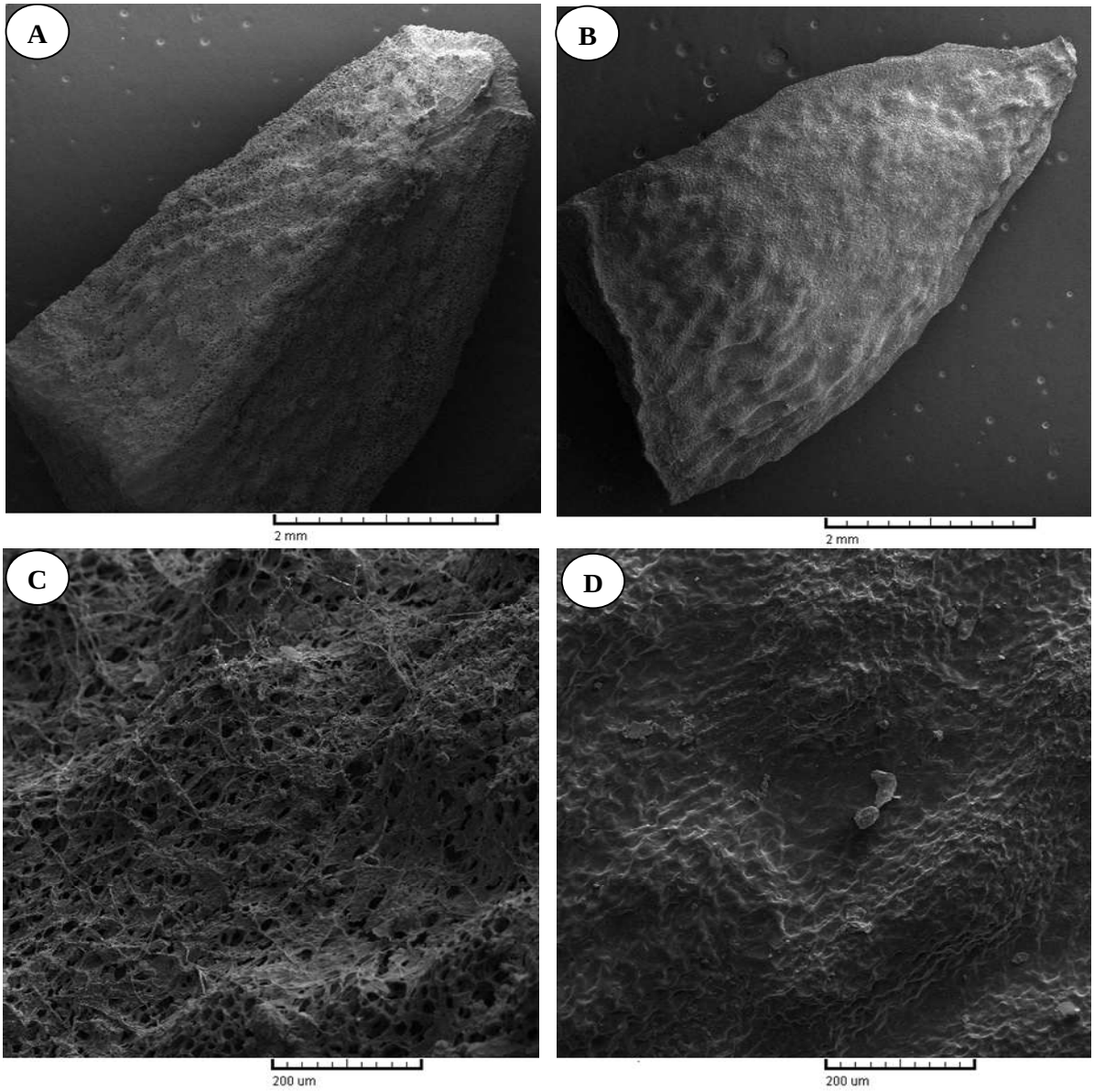

Fig.3.Nutlet micrograph of *L. macracanthus*

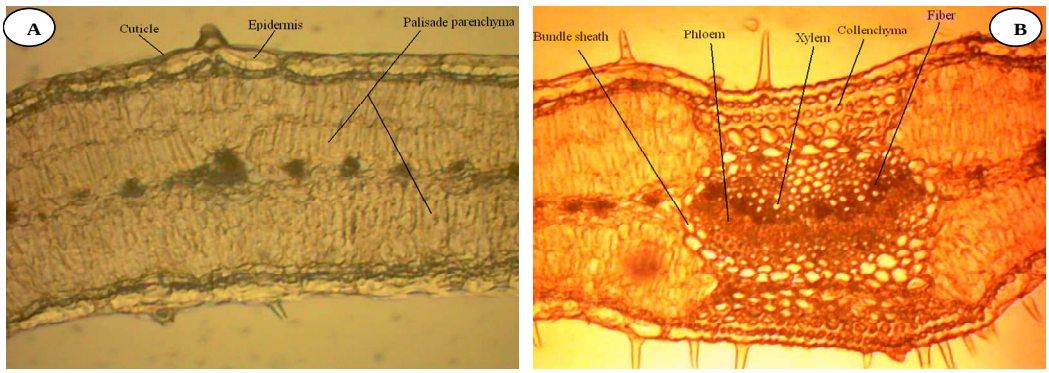

Fig.4.Trans section of leaf of *L. macracanthus*

- Trichomes micromorphology: the trichomes of stem, sepal, thorn, and the dorsiventral surfaces of the leaf were studied with scanning electron microscope (SEM). Nine different types of trichomes were observed which were classified as glandular and non- glandular trichomes. The glandular trichomes were observed as peltate, digitated and capitated. The peltate trichomes had a basic and a small stem cell and a head area with eight cells (Figure 5E, 6K). The capitate trichomes had a basic and two apical cells (Figure 6 G) which abounded at the ventral surface of the leaf. The digital trichomes were in two types of monocellular and bicellular in the leaf and stem (Figure 5 B & F). The non-granular trichomes had three main types of bicellular, monocellular with a thick wall, and rough and the pyramidal trichomes of monocellular with thick wall and some small bulges. (Figure 5)

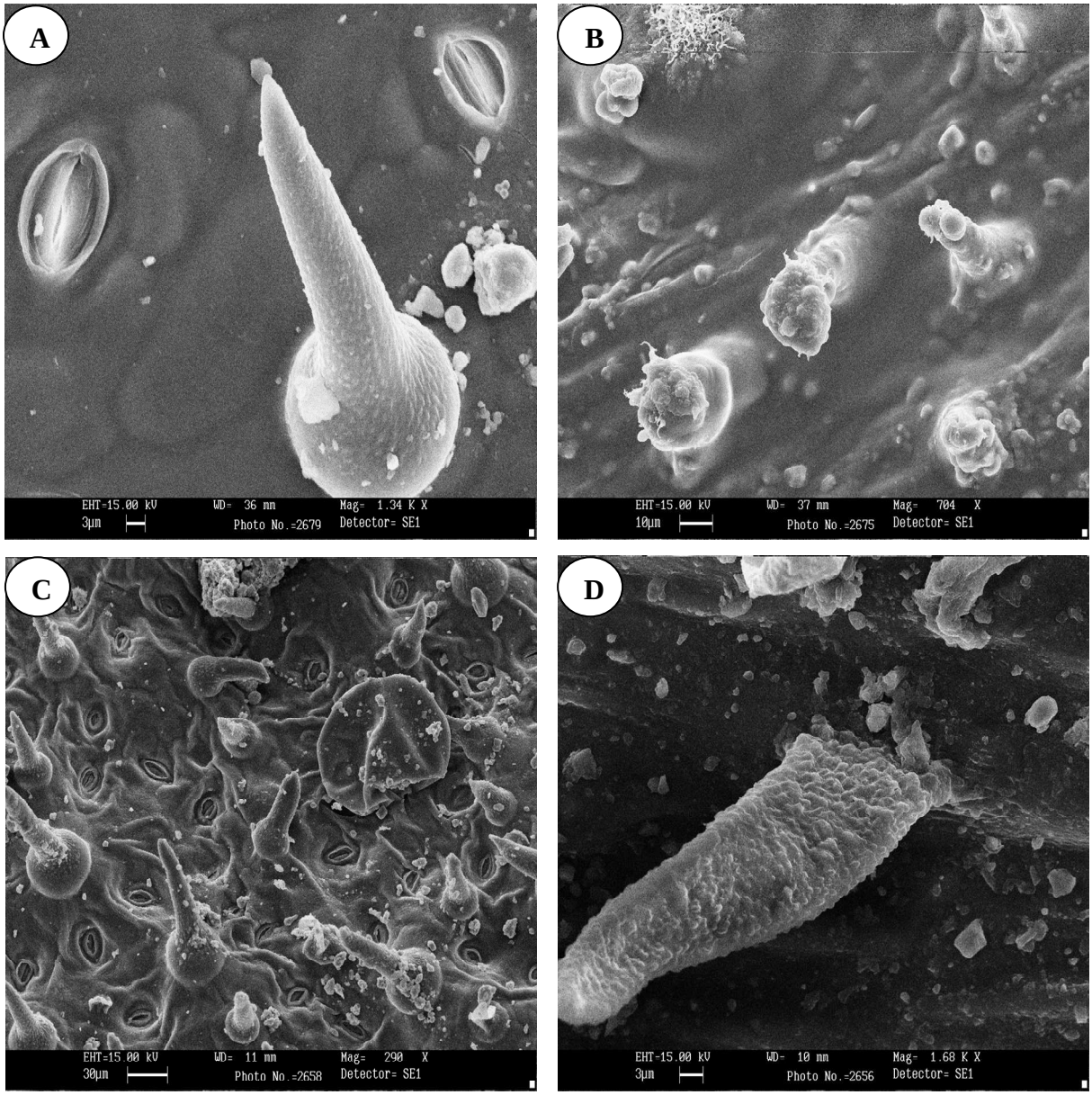

Fig.5.Trichomes micrograph of *L. macracanthus*

DISCUSSION

This species has been collected from a semi-arid region. So, the characteristics of a xerophytes plant could be observed in this sample. These features include the modified of plant stem's leaflets to thorns, reduction in the size of leaves, growth of sharp thorns in the end of leaves and sepals, a thick layer of cuticle on the epidermis, four compressed layers of palisade in the mesophyll, and non-granular trichomes in the outer surface of the vegetative and reproductive organs. Studies on nutlet morphology in the Labiatae were very useful at different taxonomical levels [10]. The extracts of *Lagochilus* spp were very useful in treatment of hemophilia. The main components

responsible for such activity of these species remedies were labdane diterpenoids, thirty of which have been isolated from these herbs [11]. There were two types of nutlet in Labiatae family: mucilage and non-mucilage (myxocarpic) nutlet. Myxocarpy is the phenomenon of producing mucilage when the nutlet becomes wet, has been a topic in the family Lamiaceae [12]. The seed in *Lagochilus* species is non-mucilage. Studies show that the absence of mucilage was correlated with special characteristics of the nutlet or is connected with the species habitats [13]. Plants which grow in moist habitats more often have non-mucilaginous nutlet than the ones from a drier habitat [14]. The whole of *Lycopus* species grow in low wet places and have non-mucilaginous nutlet, which agrees with his speculation, but this subject, was reverse in *Lagochilus macracanthus*.

There were two main pollen types in Labiatae family: tri- and hexacolpate, which were usually bi- and triangulate respectively. Pollen grains with four, eight, nine and ten colpi in the exine were also reported [15, 16]. The tetra- and octacolpate types being regarded as modifications in polyploid forms of species with tri- and hexacolpate pollen respectively. Like the other members of Labiatae family, the pollen of *Lagochilus macracanthus* was trizonocolpate. On its outer surface, there were some small holes

In this plant two main group of trichomes, granular and non-granular, were found. The main roles of glandular trichomes were producing essential oil and nectar in the plant. The essential oil and nectar were effective in attracting pollinate and causing secondary metabolites and eliminating herbivores. The essential oil produced by glandular trichomes is one of the characteristic features of the Lamiaceae family [17]. Volatile oil produced by glandular trichomes is important for pesticide, pharmaceutical, flavouring, fragrance and cosmetic industries [18]. It was found that essential oil extracted from leaves and flowers of *L. kotschyanus* exhibited interesting antibacterial activity [19].

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