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Palynomorphological Data of Pollen Grains of *Lamium garganicum*

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***Abstract*—**This study shows palynomorphological description of pollen grains of *Lamium garganicum*, species of the family *Labiatae*. Fresh material of this plant is taken in Mount Llogara, in Albania. By comparison made between palinomorphological characteristics of pollen grains of *Lamium garganicum* with those of *Lamium maculatum* and *Lamium purpureum*, showed that granules have similarities in the number of furrows. The pollen grains of *Lamium garganicum* were larger in length and width than those of *Lamium maculatum* and almost equal with those of *Lamium purpureum.* Furrows are longer than those of pollen grains in *Lamium maculatum* and shorter than those of *Lamium purpureum*. The layer of exine of *Lamium garganicum* was thinner than that of two others. The sculpture of exine was fine reticulate, where reticulas were uniform whereas in *Lamium purpureum* was verrucate, with small verrucae; in *Lamium maculatum* was reticulate.

Keywords*—Lamium garganicum*, pollen grains, Llogara, Albania.

I. Introduction

REFERING to the literature [8], there are 5 species plants of *Lamium* Genus, Labiatae family. *Lamium garganicum* is one of them. Pollen grains of this plant were collected in fresh conditions in its habitat of Llogara on 22th of June 2010. The palynomorphological features of *Lamium garganicum* plant were identified for the first time by this palynological study in our country.

In foreign palynological literature are shown a series of studies for the influence of ecological factors and laboratory processing method in the size of spores and pollen grains [2], [10]. Size of pollen grains, furrows and pores, shows large variations not only within the different species, but also within the same species. This is the object of this study.

This study aims to provide:

- The palynomorphological description of pollen grains of *Lamium garganicum*, Labiatae family;
- The identification of similarities and differences based on the palynomorphological feature of this plant against of *Lamium maculatum* and *Lamium purpureum*, compared with the data of literature [5], [4].

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II. Material and Methods

Pollen grains of *Lamium garganicum* were collected in fresh condition to the Llogara area, in Albania. Morphological characteristics of pollen grains were studied by using three analytical methods as follows:

- Acetolysis of Erdtman method [3];
- Acetolysis of Avetisjan method [1];
- Basic fuchsine of Smoljaninova & Gollubkova method [9].

The first two methods of acetolysis were used to get the best results of the study of sporoderma elements. The method of fuchsine was used to study the shape, size of aperture which in some cases enabled us to identify the sculpture elements of exine.

There were prepared 3-5 microscope slide for each plant by different methods and they were studied by the Digital Microscope/Camera Software, Motic Images Plus 2.0 ML, B_1 Series. (This microscop saves images in JPG, BMP, MIG and TIFF and process the image with a variety of filters). There were presented the microscopic photos of pollen grains of the plant studied in polar and equatorial view with magnification 400x and 1000x, taken by KALLAJXHIU Nikoleta.

A. The Method of Acetolysis According to Erdtman

The flower or leaf-bud was elaborated in ethanol with the aim to separate the other parts of the flower which could be separated inside distillated water. The pollen grains with its granules were dried in a thermostat, and then wetted with an acetolysis mixture (anhydrite acetic and sulfate acid concentration, with pure chemicals in a 9:1 ratio), which was done every time in a repeating way. The test-tubes together with granules and acetolysis mixture were placed in bathroom at a temperature 70-80°C. The length-time of granules staying in bathroom varied from one kind to another (from 5 to 20 seconds). Then, the test-tubes were centrifuged, whereas the granules were cleaned several times with distillated water. Granules were placed on slide and were observed with a microscope by dropping a drop from glycerin solution and water in a ratio 1:1. Right after the granules were darken enough, the material was separated in a test-tube by adding 1-2 sodium chloride and 1-2 concentration sulfuric acid drops till the material became lighter. Then, the second shower with distillated water was done. The material taken through separation and centrifugation was ready to be used as a preparation.

International Science Index, Bioengineering and Life Sciences Vol:8, No:5, 2014 waset.org/Publication/9999207

International Science Index, Bioengineering and Life Sciences Vol:8, No:5, 2014 waset.org/Publication/9999207

B. The Simplified Method of Acetolysis According to Avetisjan

Granules were placed on slide. Some drops of ethanol were dropped on the slide composite. All fat substances of granules, created after the ethanol actions were cleaned with blotting-paper. The mixture of acetolysis was prepared every time frequently. The microscope slide composite was treated with 1-2 acetolysis solution drops and later on it was warmed up in a thermostat or on the alcoholic lamp flames. The composite was continuously controlled over the warming phase by the microscope, thus it could not get darker then it was allowed. Right after the desired color was reached, a wash-up with ethyl alcohol (70%) was done. The composite was cleaned up from all residues and fixed with glycerin gelatin which was prepared according to Kisser method [3], [8].

C. The Colored Method of Basic Fuchsine According to Smoljaninova & Gollubkova

Some ethanol concentrated drops were added to the granules placed on the microscope slide. In cases of quick evaporation of ethanol, some extra drops were added. It was observed that the fat composite of granules were spread from alcohol towards the slide edges. The fat composite was taken away from the slide with blotting paper. After the slide was washed away from residues, the colored solution of basic fuchsine which was prepared according to two variants listed here-below, was added:

A. Basic Fuchsine, Alcohol 75% and Phenol in the Ratio of 1:700:100
B. Basic Fuchsine, Ethyl Alcohol 96% And Xylol in the Ratio of 1:600:800

Phenol and xylol were used in the transparency growth of markers and were necessary as antiseptic. The color materials were fixed with gelatin glycerin prepared according to Kisser method.

D. Fixture of Prepared Composites

The fixture of prepared composites was realised by using the method of glue-preparations through gel-glycerine. The gel-glycerine was prepared based on the Kisser method by using 50 gr of gel, 175 ml of distilate water, 150 gr glycerine and 7 gr phenol. Once the distilate water was heated up to 50°C, the gel was droped into it. It was mixed up several times till melted properly. Then, the glycerine and the composite were added and boiled till the liquid became thicker and viscose. After the phenol was added to the mixture, a uniform melted composition was taken. The prevention of air bubbles that might emerge during the process of composite preparation was made by warming up in advance all equipment used over the process. The final composite was isolated to the edges of microscope slide with spray or paraffin and after 3 days it was ready to be used and stored.

III. RESULTS

The study area belongs palynology. Below is showing the morphological description of pollen grains.

Family: Labiatae

Genus: *Lamium* L.

Lamium garganicum L.

It is a hemychriptophyta and perennial herbaceous plant, 20-60 cm high that is grown up in highland forests rocks. Calyx was 7,5-18 mm. Corolla was 25-40 mm with pink color, rare white color. This plant blooms in the months from April to August and it is a northern Mediterranean plant [7]. Pollen grains of *Lamium garganicum* were collected in fresh condition to the Llogara area, a mountain of Albania, on 22th of June 2010.

Pollen grains were tricolpate with spheroidal to oblate spheroidal contours. In equatorial view, pollen grains had spheroidal to prolate frame whereas in polar view they had circular triangular frame. The furrows were long, with smooth membrane and they weren't long enough to go to the pole. The length of furrows varied from 15.5-17.34 (13.21) µ whereas apocolpium varied from 8.56-11.83 (10.85) µ. Mesocolpium was 20.19-25.09 (23.34) µ. The exine had doubled thin layers with fine reticulate sculpture, where reticulas were uniform. The layers of exine were equal. The thickness of exine varied from 1.02-1.22 (1.05) µ. The length of pollen grains varied from 32.23-36.31 (33.49) µ while its width varied from 30.19-33.25 (31.13) µ.



(a) (b)



(c)

Fig. 1 (a) *Lamium garganicum*; (b), (c) pollen grains in equatorial view. (Digital Microscope/Camera Software, MO-magnification 400x, Photo: Kallajxhiu, Nikoleta)



(a) (b)



(c)

Fig. 2, (a), (c) pollen grains in polar view; (b) pollen grain in equatorial view (Digital Microscope/Camera Software, MO-magnification 10^3x, Photo: Kallajxhiu, Nikoleta)

IV. Discussion

By comparing the observed palynological data of *Lamium garganicum* L., with pollen grains of *Lamium maculatum* L. and *Lamium purpureum* L., refer to the literature [4],[6] many similarities and differences between them were identified. They are shown in the Table I, here below.

TABLE I
DIMENSIONS OF POLLEN GRAINS OF *LAMIUM GARGANICUM* COMPARED WITH *LAMIUM MACULATUM* AND *LAMIUM PURPUREUM*

The palynological features	Average Dimension of *Lamium garganicum*	Average Dimension of *Lamium maculatum*	Average Dimension of *Lamium purpureum*
The length of pollen grains	33.49 μ	23.77 μ	31.5 μ
The width of pollen grains	31.13 μ	25.16 μ	31.6 μ
The length of furrow	13.21 μ	25.16 μ	31.6 μ
The width of furrow	10.85 μ	4.65 μ	6.5 μ
Mesocolpium	23.34 μ	18.66 μ	17.8 μ

Based on the palynological features, as indicated in Fig. 3, it was noted that pollen grains of *Lamium garganicum* are longer and wider than those of *Lamium maculatum* and almost equal to those of *Lamium purpureum.* Furrows of pollen grains of *Lamium garganicum* are shorter than those of *Lamium purpureum* and longer than those of *Lamium maculatum.* Furrows of *Lamium garganicum* were wider than the two other species. The distance between two furrows (mesocolpium) in *Lamium garganicum* is bigger than the mesocolpium of *Lamium purpureum* and *Lamium maculatum.*

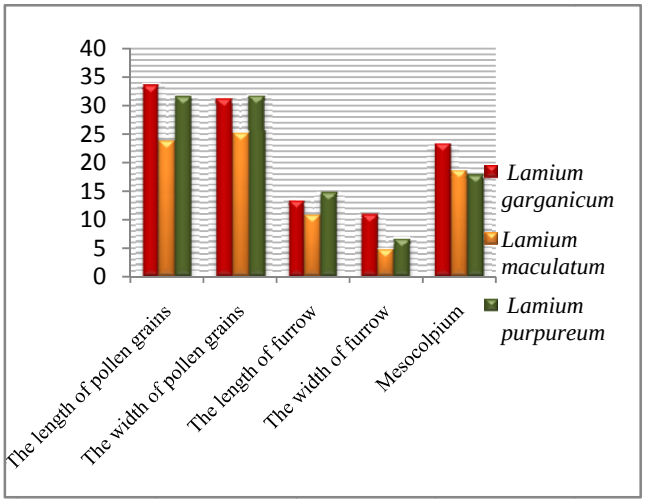


Fig. 3 Chart of average dimensions of pollen grains of *Lamium garganicum*, *Lamium maculatum* and *Lamium purpureum*

The sculptures of exine of pollen grains of *Lamium garganicum* was fine reticulate, where reticulas were uniform whereas at *Lamium maculatum* the exine was reticulate and at *Lamium purpureum* was verrucate, with small verrucae.

TABLE II
DIMENSIONS OF EXINE OF *LAMIUM GARGANICUM* COMPARED WITH *LAMIUM MACULATUM* AND *LAMIUM PURPUREUM*

The palynological features	*Lamium garganicum*	*Lamium maculatum*	*Lamium purpureum*
The thickness of exine	1.05 μ	1.34 μ	1.5 μ
The sculpture of exine	Fine reticulate sculpture, where reticulas were uniform	Reticulate sculpture	Verrucate sculpture with small verrucae

As noted by the data of Table II and Fig. 4, the layer of exine of pollen grains at *Lamium garganicum* is thinner than that of two other species.

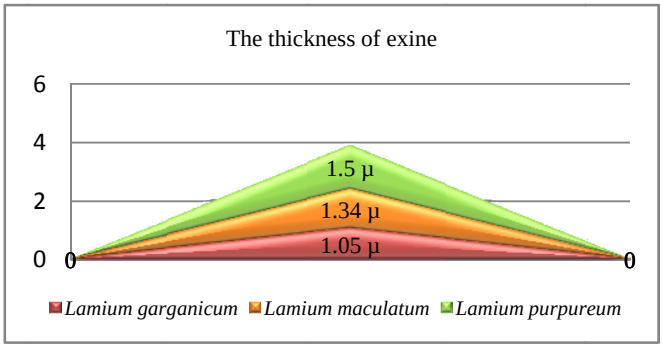


Fig. 4 Chart of average dimensions of exine at *Lamium garganicum*, *Lamium maculatum* and *Lamium purpureum*

V. Conclusions

The palynological study of *Lamium garganicum* showed that there were many changes and many similar in palynomorphological features with 2 other species.

International Science Index, Bioengineering and Life Sciences Vol:8, No:5, 2014 waset.org/Publication/9999207

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