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AN ALLERGOPALYNOLOGIC COMPARATIVE STUDY OF THE *HUMULUS LUPULUS*' POLLEN GRAINS (CANNABACEAE) IN DIFFERENT HABITATS

ABSTRACT

Pollen allergy is a human disease that is rapidly growing among the Albanian population. There are many plants in Albania that cause this disease and Humulus lupulus (Cannabaceae) is one of them. In this study is presented an allergopalynologic description of pollen grains of this plant. They were taken in the Elbasan region of Albania and are studied for the first time by the local researchers. The slides were prepared using two methods: acetolysis and basic fuchsine method. The results have shown that the pollen grains of H. lupulus were triporate. Also, is done the comparison of the size of pollen grains for the specie studied with others from foreign literature that belong to different habitats. The study showed the pollen grains of H. lupulus taken in Elbasan area have similarities to the morphological features with H. lupulus' pollen according to the foreign literature, but they differ from their size. By our study, it turns out that the pollen grains of H. hupulus from Elbasan habitat have smaller sizes than those obtained from the literature. We conclude that the change in the size of pollen grains caused by environmental factors and the chosen method during processing laboratory.

Keywords: *Humulus lupulus, allergopalinology, pollen grains, exine, pori.*

INTRODUCTION

The *Cannabaceae* family in Albania comprises two genera, each with a single species, *Humulus lupulus* L. and *Cannabis sativa* L. (Paparisto *et al*., 1988).

Humulus lupulus is a native European plant growing as a climber often in alder fen-woods, but also in hedges and thickets. There are pollen records from peat of the Holocene period (Godwin, 1975) and from Pleistocene cores (Zagwijn, 1973).

There are only three species: European hop, *Humulus lupulus*, found throughout Europe; Japanese hop, *Humulus scandens* (syn. *Humulu sjaponicus*), found in Japan and throughout most of China and *Humulus yunnanensis*, native to Yunnan province (Polunin, 1969; Horak and Jager, 1979; Ye *et al*., 2004; Weber, 2008). Both, *Humulus lupulus* and *H. scandens* are now found throughout other parts of the world, including the eastern United States and Canada west to Manitoba (Wodehouse, 1971; Lewis *et al*., 1983).

Hop is dioecious, with separate male and female plants. Female flowers is entirely wind-pollinated and a large amount of pollen is produced (Wodehouse, 1971).

Both genera on the Cannabaceae family, have long histories of use by humans. Cannabis has been documented for millennia as an important economic plants for textile, food oil, medicine and as recreational/religious drug (Merlin, 2003; Clarke and Merlin, 2013).

There have been many research palynological studies by foreign authors for these species (Moore and Webb, 1978; Nilsson and Persson, 1981; Punt and Malotaux, 1984; Pehlivan, 1987; Faegri and Iversen, 1989; Ceglińska, 2008).

Also, from foreign literature, it results that pollen grains of *Humulus lupulus* cause allergies to people susceptible to allergy (Ciampolini and Cresti, 1981).

The symptoms of pollen allergy confirm a correlation with the air born pollen (Solomon, 1969; Botelli *et al*, 1982; Spieksma, 1991). The sensitivity of patients to allergic pollens has many elements of genetic origin but the spread of this disease is also related to urban development, pollution and the environment in which the population lives (Ducker and Knox, 1986; Obtulowicz *et al*., 1991; Lekli *et al*., 2008;). The city of Elbasan where the material is taken is one of the most polluted cities in Albania (Hoxha and Avdolli, 2007; Gega and Shehu, 2007).

By the palynological study of *Humulus lupulus* we complete over 80% of the list of allergic plants studied in our country or about 90% of them for the Elbasan region. In the Elbasan region, are estimated approximately 120 species that cause allergy (Kallajxhiu, 2011). The palynological data of this plant, accompanied by a morphological description of the vegetation causing this allergy, the habitat where they live, with the elements of their phenomena, constitute an aiding key in the hands of the allergist doctor to prevent and cure allergic patients.

MATERIALS AND METHODS

Humulus lupulus is a herbaceous plant with long rhizomes, 3-9 m high. The leaves are 10-15 cm long, parallel. Male flowers are with 5-part circles; female flowers with circular shapes that surround the ovar. Grows in fresh areas and is cultivated. Bloom: June-September. Female flowers are used in the industry of beer, in medicine to fight anemia and lack of appetite; they are also used in perfumery (Paparisto *et al.,* 1988). It is an anemophylous plant.

The material was picked fresh in a region of Elbasan from different individuals of

Fig. 1. *Humulus lupulus* (male flowers).

the same habitat. Flora of Albania is used to identify the species (Paparisto *et al*., 1988).

For the study of palynomorphological features are analyzed 31 pollen grains. The treatment of material is made with acetolysis method and basic fuchsine. The fixing of pollen grains is made with glycerin gelatin. The study and photos of pollen grains in polar and equatorial views were presented with magnification of 400x and 1000x, taken by using the Digital Microscope/Camera Software, Motic Images Plus 2.0 ML, B_1 Series. In this study is presented the comparison of the size of pollen grains of *Humulus lupulus* taken in Elbasan area with those obtained from foreign literature (Ciampolini and Cresti, 1981; Apsa Member*, 2007; Bucher and Kofler, 2004).

The used terminology was taken by Faegri (1957) and Erdtman (1969; 1972).

The acetolysismethodology according to Erdtman

The flower buds were kept in ethanol. Anthers were soaked in glacial acetic acid, centrifuged and in sequence submitted to the acetolysis mixture (concentrated acetic anhydrite and sulfuric acid in a 9:1 ratio). The mixture was placed in water bath at a temperature of 70-80ºC for 5 to 20 seconds. The sediment obtained was washed three to four times with distilled water. Pollen grains were observed under a microscope after adding a drop of a 1:1 glycerin-water mixture. Very dark pollen

grains were submitted again in another test-tube by adding 1-2 drops of sodium chloride and 1-2 drops of concentrate sulfuric acid till the material became lighter. After centrifugation the sediment was left for a half hour in 1:1 glycerin-water mixture and centrifuged again. Glycerin-gelatin, prepared according to Kisser was used to prepare the pollen slides, and sealed with paraffin (SMOLJANINOVA and GOLLUBKOVA, 1953; ERDTMAN, 1960; SLADKOV, 1967).

The basic fuchsine methodology according to Smoljaninova & Gollubkova

Solution of basic fuchsine were prepared according to two variants:

- Basic fuchsine, alcohol 75% and phenol in the ratio of 1:700:100,
- Basic fuchsine, ethyl alcohol 96% and xylol in the ratio 1:600:800.

A few drops of 100% ethanol were added to the pollen grains placed on a microscope slide. In case of quick ethanol evaporation, extra drops can be added. Oil spread from the pollen grains was removed with blotting paper from the slide. Then drops of the staining solution were added. Phenol and xylol were used to obtain a better transparency. Glycerin-gelatin was used to prepare the pollen slides also.

The best result was obtained using the acetolysis methodology to study the pollen grains sporoderm. Pollen grains shape, size of apertures and, in some cases, the observation of sculpture elements of the exine could be best analyzed when fuchsine stain was used (SMOLJANINOVA and GOLLUBKOVA, 1953).

RESULTS AND DISCUSSION

Morphological and palynological description

Family: **Cannabaceae**

Genus: ***Humulus* L.**

Species: ***Humulus lupulus* L.**

The pollen grains of *Humulus lupulus* are triporate, with an oblate – suboblate shape in equatorial view and circular when it is in polar view,isopolare (Fig. 2). Equatorial axis of pollen grains varies from 17 – 21.8 (19.72) μm, while polar diameter varies from 12.92 – 19.72 (15.70) μm. Based on the results obtained (the large size), we find that the pollen grains of *Humulus lupulus* are moderately small (MANDRIOLI *et al.*, 1984). Pori with a subtle annulus. The distance between the pores (apocolpium) is about 6.8 μm. The sculpture of exine is scabrate granulate and it is associated with elements more or less isodiametric.

The pollen grains of this plant are classificated as allergicall (CIAMPOLINI and CRESTI, 1981; NILSSON and PERSSON, 1981).

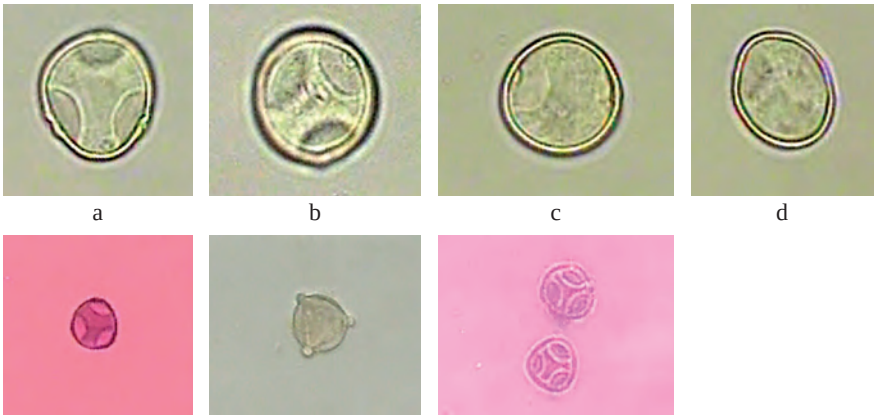

Fig. 2. *Humulus lupulus* pollen grains. a, b, c Polar view, acetolysed; d. Equatorial view, acetolysed (obtained at 1000x magnification); e, f, g Polar view, fuchsine stained (obtained at 400x magnification); photo: Kallajxhiu N.

Table 1. Minimum dimensions of pollen grains of *Humulus lupulus* in different habitats

Pollen grains dimensions	Minimum *Humulus lupulus* (present data)	Minimum *Humulus lupulus* (Ciampolini and Cresti, 1981)	Minimum *Humulus lupulus* (Apsa Member*, 2007)	Minimum *Humulus lupulus* (Bucher and Kofler, 2004)
Polar diameter (µm)	12.92 µm	16.0 µm	22.8 µm	17.0 µm
Equatorial diameter (µm)	17.0 µm	19.0 µm	28.2 µm	20.0 µm

Table 2. Maximum dimensions of pollen grains of *Humulus lupulus* in different habitats

Pollen grains dimensions	Maximum *Humulus lupulus* (present data)	Maximum *Humulus lupulus* (Ciampolini and Cresti, 1981)	Maximum *Humulus lupulus* (Apsa Member*, 2007)	Maximum *Humulus lupulus* (Bucher and Kofler, 2004)
Polar diameter (µm)	19.72 µm	19.0 µm	28.6µm	21.0 µm
Equatorial diameter (µm)	21.8 µm	23.0 µm	33.5 µm	23.0 µm

Comparing our results for pollen grains morphology of *Humulus lupulus* taken in Elbasan area and *Humulus lupulus* taken from literature, similarities of morphological features were observed, but changes in size were identified.

Based on the palynological features, as indicated in Tab. 1, Tab. 2, pollen grains of *Humulus lupulus* of Elbasan area were smaller in minimum and maximum dimensions than those studied by other authors, taken from literature.

Also, it can clearly seen in Fig. 3 and Fig. 4 that the pollen grains of *Humulus lupulus* taken in Elbasan area, have average polar and equatorial dimensions smaller than the others.

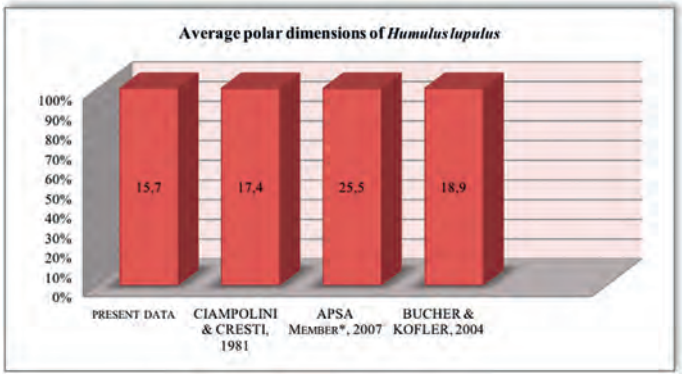

Fig. 3. Chart of average dimensions of pollen grains of *Humulus lupulus* for polar diameter (μm).

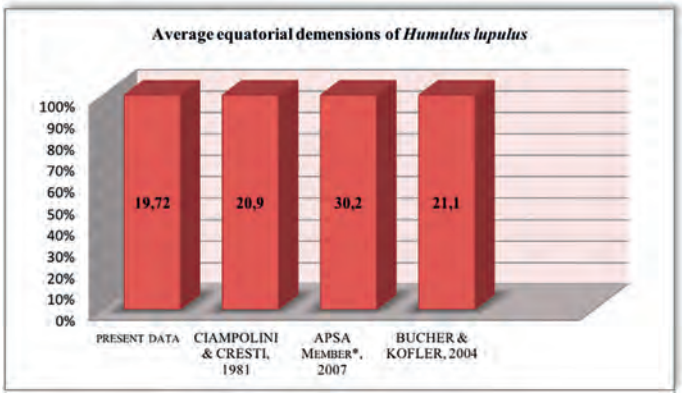

Fig. 4. Chart of average dimensions of pollen grains of *Humulus lupulus* for equatorial diameter (μm).

CONCLUSION

From the data presented, it was conlcuded that:

The pollen grains of *Humulus lupulus* taken in Elbasan area of Albania have great similarity with the pollen grains of *H. lupulus* taken by the literature regarding the morphological features, in terms of:

Pollen grain has an oblate – suboblate shape in equatorial view and a circular shape when it is in polar view.

Pollen grains were triporate.

The sculpture of exine was scabrata granulata.

The differences of the dimensions between the pollen grains are only in their dimensions. The pollen grains of *H. lupulus* studied were smaller in all dimensions than those of pollen grains taken from literature.

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