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Article

The Pollen Status of Allergic *Dysphania botrys* (L.) Mosyakin & Clemants in Eskişehir, Turkey: A Case Study of Amaranthaceae Pollen

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Abstract: In this study, the pollen morphology of the allergic *Dysphania botrys* (L.) Mosyakin & Clemants (Amaranthaceae) taxon, which has a natural distribution within the borders of Eskişehir province, was investigated in terms of pollen morphology and allergic properties. Pollen grains of *D. botrys* are periporatae and sphaeroidal shaped. As a result of microscopic examinations, A/B=1.06 μm (W), A (polar axis): 4.08 μm; B (Equatorial axis): measured at 25.04 μm. The thickness of the Exine is 1.73 μm.

Keywords: *Dysphania botrys*; Amaranthaceae; Pollen; Allergy; Eskişehir; Türkiye

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1. Introduction

Palynology is a branch of botanical science that studies pollen and spores. This term was first coined in 1945 by H. A. Hyde and D. A. Williams. The term "palynology" comes from the Latin word “Paluno” which means to scatter or distribute. The branch of palynology that examines airborne pollen and spores is called aeropalynology. In England, the allergic effects of pollen were first demonstrated by Blackley (1873), who conducted skin tests with *Lolium italicum* (Italian rye grass) pollen, which caused his own hay fever (Pehlivan, 1995). Subsequently, various researchers have shown through their studies that pollen is responsible for allergic reactions (Yurdukoru, 1978; Pehlivan, 1984; Anderson, 1985).

With the arrival of spring, nature comes to life, and pollen emerges. However, pollen is an allergen for sensitive individuals. Pollen allergy manifests itself as allergic rhinitis, eye allergies, and asthma. The symptoms of these patients worsen as spring arrives. Common symptoms include frequent sneezing, nasal congestion, watery and itchy eyes, coughing, wheezing, and even shortness of breath. Treatment options include antihistamines, corticosteroid nasal sprays, or medications taken through the respiratory tract, as well as bronchodilators for breathing relief. In severe cases, immunotherapy may be administered. Pollen, also known as flower pollen, is composed of protein substances that facilitate the reproduction of flowering plants. Pollen grains are so small that thousands of them can be found in a single speck of pollen, and they can be easily carried to distant places by the wind (Majad and Ghanati, 1995; Potoğlu Erkara, 2005).

Every year, different types of plants release their pollen into the environment during seasons that have suitable temperature and humidity levels for them. For example, most tree pollen is abundant in late winter and early spring, while grass and cereal pollen are prevalent in spring and early summer. Weed pollen, on the other hand, tends to be more concentrated in late summer and autumn. The pollen seasons and intensities can vary from city to city depending on factors such as the region’s vegetation, precipitation patterns, and temperature (Aytuğ et al., 1990).

Through aeropalynological studies conducted for specific regions of each country, pollen and spore calendars specific to those regions are created on an annual, monthly, weekly, daily, and even hourly basis. These results are communicated to the public through radio and newspapers (Pehlivan, 1995). In recent years, studies related to this topic have gained importance in our country. The allergenic effects of airborne pollen and the exposure of the local population to these effects have led to research in the field of allergies. Allergy is the reaction and response of our bodies' antibodies to substances that may be harmless to others. These substances can include dust, food, airborne particles, pollen, mites, and animal dander. Allergies can occur in all age groups. While they are often considered to be genetic, allergies can also develop at different ages due to environmental factors. Smoking and environmental pollution have been identified as major causes of allergies. Allergy symptoms can vary from person to person, and the severity of the allergy can impact the symptoms experienced. Itching, nasal congestion, frequent sneezing, chronic cough, shortness of breath or wheezing, and skin rashes or swelling are among the common signs of allergies (Potoğlu Erkara et al., 2009).

Our aeropalynological study conducted in the atmosphere of Eskişehir province aimed to identify the presence of Amaranthaceae pollen and investigate their distribution and quantities throughout the months. By comparing the pollen data with meteorological conditions, the study aimed to shed light on plant systematics and provide assistance to physicians in the diagnosis and treatment of allergic diseases, as well as to individuals suffering from allergies.

2. Materials and Methods

The research material consists of pollen samples collected from the atmosphere of Eskişehir province between 2021-2022.

2.1. Gravimetric Method

The gravimetric method was employed in this study, with the Durham sampler being the instrument of choice. This device was initially developed by Durham in 1946 (Pehlivan, 1984). The method determines the amount of pollen deposited per unit area based on the effect of gravity. The Durham sampler consists of two disks, approximately 22.7 cm in diameter, placed at a distance of 8-10 cm from each other (Figure 1). The lower disk contains a 2.5 cm-high microscope slide holder, while the upper disk protects the rod from weather conditions such as sunlight, rain, and wind. A cylindrical stem, approximately 1 m in length, is located beneath the lower disk to secure the device firmly onto the ground.

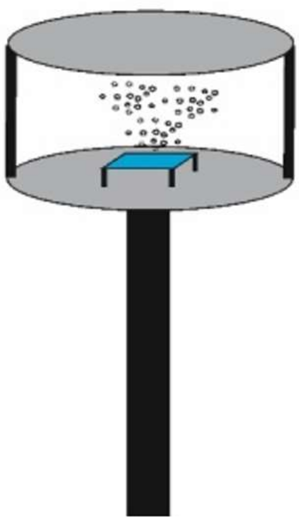

Figure 1. Durham device.

The Durham sampler was positioned in an open area at a height of 1.75 m above ground level on the Eskişehir Osmangazi University, Meşelik Campus in Eskişehir. Thin strips of basic fuchsin-glycerin jelly were applied onto the slides placed in the instrument. The slides were collected daily from January 1, 2021, to December 31, 2022. A previously prepared amount of glycerin jelly, approximately 1-2 mm^3, was placed on the slide obtained from the sampler. The slide was heated, and a 22x22 mm coverglass was applied after melting the basic fuchsin-glycerin jelly on a heating plate.

The coordinates and elevations of the research site were measured using a GARMİN GPS 12 CX (Global Positioning System) device (Table 1).

Table 1. Coordinates and altitudes from sea level of the locality

Station	Coordinates		Altitude
	(m)		
	North	East	
Eskişehir Osmangazi University Meşelik Campus	39°48' 758''	30°32'252''	845

2.2. Preparation of Basic Fuchsin-Glycerin Jelly

Gelatin plates were soaked in distilled water for 2-3 hours and then heated. The heated gelatin was divided into two parts, with the third part being mixed with glycerin. Desired amounts of basic fuchsin and 2.3% phenol acid were added to the mixture. The mixture was placed in a water bath until it reached 80 °C and then allowed to cool to solidify.

To assist in the identification of pollen collected with the gravimetric method, reference preparations were prepared from dried herbarium samples of plants collected from the research area where the Durham sampler was placed and its vicinity, following the "Wodehouse Method".

2.3. Wodehouse Method

Pollen grains extracted from anthers were placed on a clean slide, and 2-3 drops of 96% alcohol were added for the melting of resins and oils. The slide was heated on a heating stage between 30-40 °C for the evaporation of alcohol. A small amount (1-2 mm^3) of basic fuchsin-glycerin jelly was placed on the identified pollen grains on the slide and melted by heating. The pollen grains were evenly distributed on the slide using a clean needle, and 22x22 mm^2 cover slips were applied without leaving any air bubbles (Aytuğ et al., 1971). The preparations were left upside down at room temperature for 1-2 days to ensure solidification. Labeled slides were then placed in boxes for storage purposes.

2.4. Examination of Preparations under the Microscope

The identification and counting of pollen were conducted using a Prior binocular microscope. For counting, an x10 ocular, x10 and x40 plan objectives were employed, while an x100 plan oil-immersion objective was used for identification. Each interval on the ocular micrometer measures 0.98 μm. Microphotographs were taken using an Olympus microscope equipped with a Spot In-SIGHT Color Digital camera located in the Biology Department of the Faculty of Sciences at Eskişehir Osmangazi University. The magnification of the photographs is x1000.

The monthly distribution of pollen per cm^2 was presented using graphs. To improve the accuracy of pollen counting, a small square was placed within the eyepiece, and the length of the square's sides was calculated separately for each magnification of the microscope. The square, whose length was determined, was placed in the upper-left corner of the slide. The pollen grains within the square were counted. Subsequently, the square was moved along the edge of the slide to the right, and all pollen grains were counted. The average values were calculated as the number of pollen grains per cm^2 within the scanned area of 4.84 cm^2, expressed as the number of pollen grains per cm^2 within the slide area.

The atmospheric and reference pollen preparations are preserved in pollen storage cabinets in the Biology Department of the Faculty of Sciences at Eskişehir Osmangazi University.

Various fundamental palynological literatures were used for the identification of pollen grains collected with the Durham sampler in the research area (Wodehouse, 1935; Erdtman, 1966; 1969; Erdtman et al., 1954; Pokrovskaia, 1958; Kuprianova, 1967; Aytuğ et al., 1971; Charpin et al., 1974; Faegri and Iversen, 1975; Yurdukoru, 1978; Moore et al., 1991; Pehlivan, 1995).

3. Results

Amaranthaceae pollen is most commonly observed from May to December during the years 2021-2022. The peak density occurs in the month of August, with a gradual decrease in pollen concentration from late November to early December.

Pollen Type: Periporatae
Pollen Shape: Sphaeroidae, A/B=1.06 (A=24.08 µm; B=25.04 µm)
Exine: Average thickness: 1.73 µm.
Apertures: Regular arrangement of 34-36 porus with distinct operculum composed of granules. Porus: pa=pb (2.16 µm).
Structure: Tectatae
Sculpture: Granules. Coarse granulation with occasional anastomosis.
Intine: Very thin. Difficult to observe. Ex/int = 2/1.

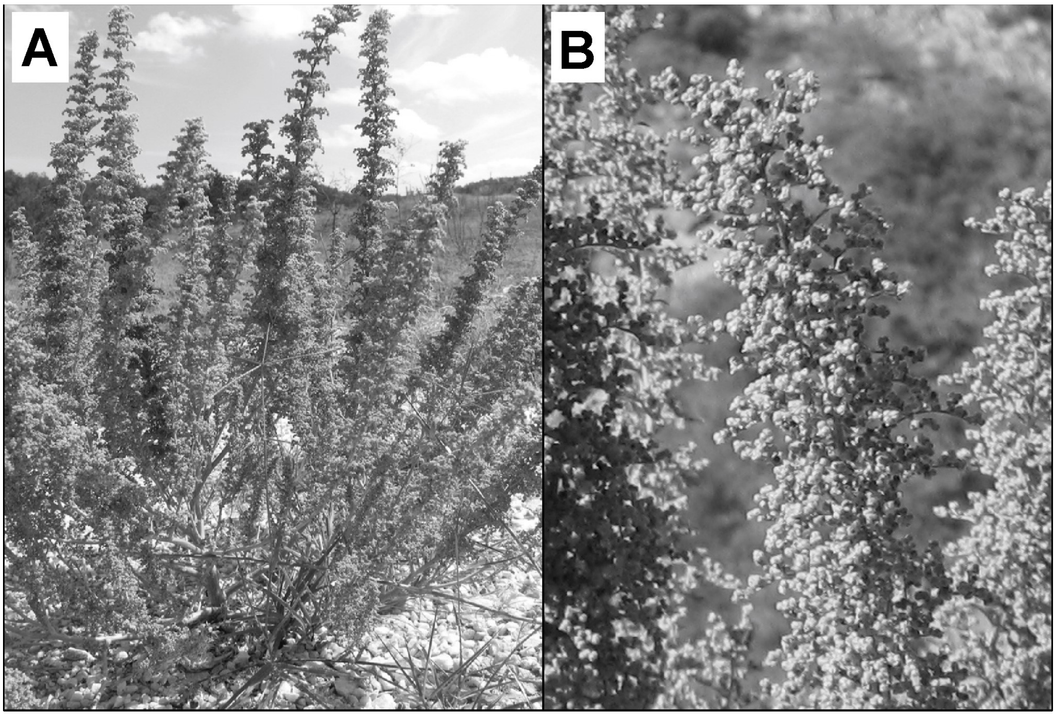

Figure 2. *Dysphania botrys* a. General appearance of the plant, b: Close-up view of the flower.

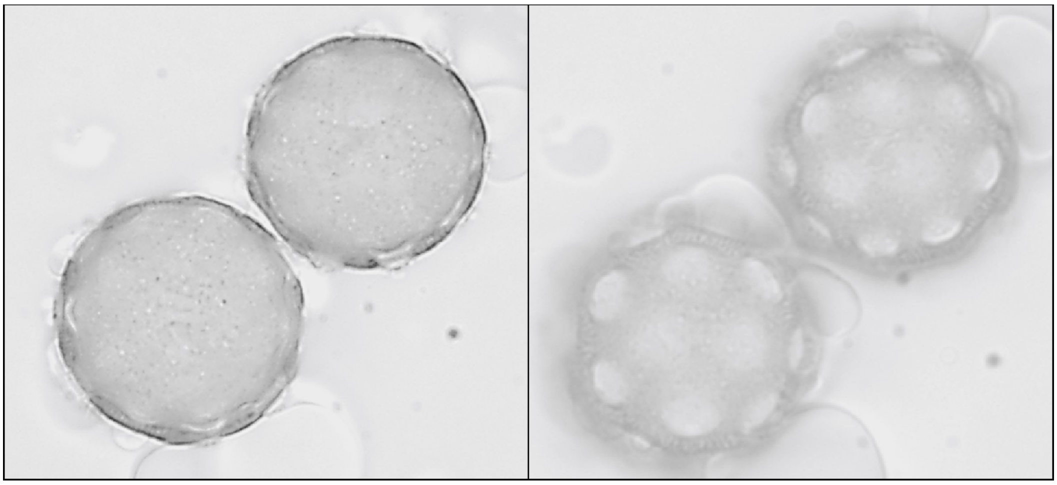

Figure 3. *Dysphania botrys* pollen grains (W) x100.

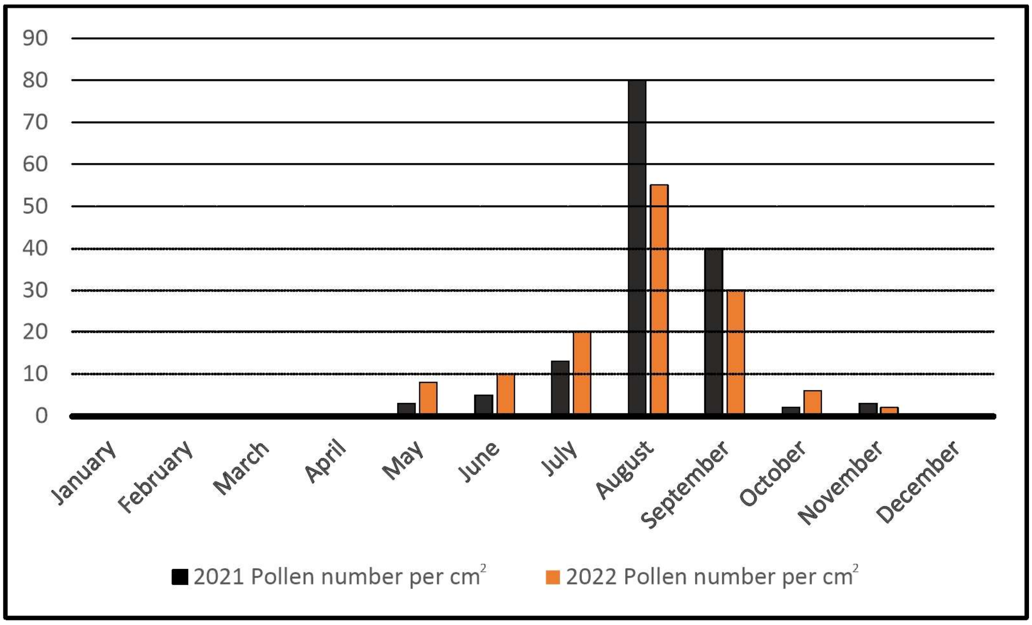

Figure 4. Distribution of *Dysphania botrys* pollen in Eskişehir by month for the years 2021-2022.

In this study, the characteristics of Amaranthaceae pollen, which is one of the most commonly encountered allergenic pollens in the atmosphere of Eskişehir Province, have been determined. Pollen grains belonging to herbaceous plant taxa exhibited a significantly long pollination period in the Eskişehir atmosphere during 2021 and 2022. It was observed that Amaranthaceae taxa released a large amount of pollen into the atmosphere during the process starting in May. Herbaceous plant pollens were also extensively observed in the atmosphere from July to October. While herbaceous plant pollens decreased in July, they increased again in August, reaching their highest value. This is due to the fact that the period with the highest detection of Amaranthaceae pollen in both years falls in August. These taxa's pollen remained the most detected pollens in the atmosphere in September-October as well. Moreover, pollen from the Amaranthaceae families was seen in abundance in the atmosphere in October. In November and December, pollens from Amaranthaceae plants were also encountered.

Among the herbaceous plant pollens, Amaranthaceae family pollen was most frequently detected, accounting for 8% of the air samples between May and December. Furthermore, Eskişehir Province and its surroundings are areas where *Beta vulgaris* (sugar beet) cultivation is widespread, with a sugar factory present. Therefore, it was determined that the pollen of *B. vulgaris* reached its highest density in August in local areas where this plant is cultivated. This situation is also consistent with the ratios of Amaranthaceae family pollens in the atmosphere of Ankara, Sivas, Bursa, Burdur, Isparta, and Zonguldak (İnceoğlu et al., 1994; Pehlivan and Özler, 1995; Bıçakçı et al., 1996; 2000; Bıçakçı and Akyalçın, 2000). It has been determined that Amaranthaceae family plant pollens are highly allergenic. Studies conducted on 421 patients in Israel revealed that the most influential pollens were those of the Amaranthaceae family (Liebeskind, 1960). It has been noted that Amaranthaceae pollens are significant in terms of causing allergic diseases in America and Poland (Durham, 1935; Dabrowski, 1974). They have been found to contribute to allergic diseases such as asthma and hay fever in Oklahoma (Buck and Levetin, 1980). Moreover, the pollens of Amaranthaceae have been found to have a high allergic potential in India, Portugal, and Italy (D'Amato et al., 1992; Banik et al., 1992; Ceairo et al., 2007). It has also been emphasized that these family's pollens are clinically important (Tuchinda and Theptaranon, 1976). In Ankara, it was determined that the pollens of Amaranthaceae and Poaceae family plants are more important in terms of allergic diseases compared to other pollens in the atmosphere (Pehlivan and Bütev, 1994). We believe that the long and numerous observations of Amaranthaceae pollens in the air of Eskişehir Province may have significant allergic effects in this region.

Meteorological conditions have been proven to have a significant impact on the distribution of pollen in the atmosphere, as demonstrated by numerous studies. For instance, heavy rain leads to significant reductions in atmospheric pollen concentrations. Similarly, the long-distance transport of pollen by wind reveals important variations in pollen counts, particularly in individuals exposed to seasonal air currents (Berggren et al., 1995). It has been established that temperature and relative humidity also contribute to an increase in pollen counts (Pehlivan and Özler, 1995). In the Eskişehir, it is observed that higher temperatures and average humidity levels during certain months result in an increase in pollen concentration. These findings are consistent with the results presented by Pehlivan and Özler (1995). Considering the influence of meteorological conditions, the richness of vegetation cover, and changes in cultivation areas, it is evident that such studies should be conducted routinely in this region.

This study aimed to determine the atmospheric pollen in Eskişehir Province. During the research period, the Amaranthaceae pollen, which is highly allergenic and related to the region's flora, was detected and identified in the sample of allergenic *Dysphania botrys*. Furthermore, it was determined that the study sheds light on plant systematics and contributes significantly to the preparation of the pollen calendar for Eskişehir Province. Additionally, by determining the temporal quantities of pollen, considering their effects on human health, it is believed that this study will provide valuable insights for individuals sensitive to pollen and medical professionals in the region.

Conflict of Interest

The authors have no conflict of interest to declare.

Financial Disclosure

Authors declare no financial support.

Authors' Contributions

Collection and identification of plant specimens, O.S.; Preparation of plant specimens for palynomorphological investigation, İ.P.E., and O.S.; funding acquisition, İ.P.E.; investigation, İ.P.E., and O.S.; methodology, İ.P.E.; resources, İ.P.E, and O.S.; supervision, İ.P.E.; writing-original draft, İ.P.E., and O.S.; writing-review and editing, İ.P.E. All authors have read and agreed to the published version of the manuscript.

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