

Aerobiological studies and low allergenicity of Date-Palm pollen in the UAE

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Abstract

Date-Palm trees (*Phoenix dactylifera* L.) are the most abundant crop in the United Arab Emirates (UAE). The aim of this work was to conduct aerobiological studies on Date-Palm pollens and correlate that to allergenicity. An aerobiological survey was performed at three Date-Palm farms. Radioallergosorbent test (RAST) and total IgE were performed on 477 airborne allergic patients. Small mass bioactive constituents were fractionated and isolated by HPLC. Aerobiological studies demonstrate the short distance traveled by the Date-Palm pollens. Pollen counts were about 800 counts/m³ within the Date-Palm farms and decreased by about 80% just 100 meters away from the farm area and almost diminished beyond 200 meters. Scanning electron micrograph of the pollen grain showed a uniform smooth texture with an oval shape. Out of 477 airborne allergic patients having high total IgE counts, only 2.3% gave positive RAST for Date-Palm pollen. HPLC chromatogram separated the non-protein content of Date-Palm pollen into four distinct peak fractions. The present study revealed that Date-Palm pollens have a low allergic effect on airborne allergic people. The short distance traveled by the pollen, the smooth texture, the short pollination period and low molecular weight biomolecule content may be the main factors behind the low allergenicity.

Keywords: *Date-Palm pollen*, *Phoenix dactylifera* L., *pollen allergy*, *aerobiology*

Introduction

The most widely spread tree in the United Arab Emirates is the Date-Palm and pollen formation of Date-Palm trees (the pollination period) is during the months of January–March. Pollen is one of the most common causes of allergic response involving the production of immunoglobulins and hence the release of histamine and other chemicals. The biochemicals responsible for such allergic responses are called allergens (Leuschner 1993). The chemical nature and biochemical activities of certain allergens has been studied (Gonzalez et al. 2001), but the precise composition and biochemical activities of many pollen allergens remains undefined. Allergens are usually proteins or glycoproteins present in pollen which stimulate IgE immunoglobulin production (type I hypersensitivity reactions) and consequently cause the release of histamines. This results in inflammation of

the respiratory tract or other organs. The allergenic molecules generally are water-soluble and can be easily leached from the airborne pollens. They react with IgE antibodies (immunoglobulin IgE) attached to the mast cells, initiating a series of pathologic steps that result in the release of chemical mediators (one of which is histamine) and allergic symptoms occur. The presence of allergic responses (allergens) can be tested by a skin prick test followed by *in vitro* immunoassays such as radioallergosorbent test (RAST) and enzyme-linked immunoassay (ELISA), that measure the presence of antigen-specific IgE antibody in the serum or plasma. The RAST concept assay has been modified to avoid the use of radioactive material and the use of fluorescent- and enzyme-labeled anti-IgE. In this study, an enzyme immunofluorescence test (RAST) assay on the available human serum of allergic patients was performed.

Pollens exert allergic symptoms and illness usually when they fulfill several criteria (Patterson et al. 1997): (i) if produced in large quantity by a plant that is common in that specific area; (ii) if the plant pollens depend primarily on the wind for their dispersal; (iii) the shape feature of the pollen itself is antigenic; (iv) contain proteins and glycoproteins that are allergenic antigens. Few, if any, aerobiological surveys have been done on Date-Palm pollens, particularly in the United Arab Emirates. The closest study was done on *Borossus flabellifer*, Palmyra Palm (Chakrabotry et al. 1998) and *Phoenix sylvestris* (Chakrabotry et al. 1999) in India.

In this study aerobiological sampling was undertaken to identify and quantify the Date-Palm pollen particles in Al-Ain ambient atmosphere, both within the Date-Palm tree cultivation and the city areas. The method of survey used was impaction samplers with adhesive coated small fast intermittently rotating rods. The survey extended during the Date-Palm tree pollination period and beyond. Furthermore, the possible reasons for the very low number of patients allergic to Date-Palm pollen was further investigated. In addition, the content of Date-Palm pollen were further investigated for bioactive molecules that might be involved in the prevention or reduction of type I hypersensitivity allergic reactions as antiallergic activities (Middleton & Drzewiecki 1985; Inoue et al. 2002).

Methods

Aerobiological studies

Date-Palm pollen was identified under the scanning electron microscope at the Central Laboratory Unit (CLU) at UAE University. This step was necessary to distinguish between Date-Palm pollens and other types of pollens (grasses and other trees) collected on the sample rods. To accomplish this, pure Date-Palm pollen grains were examined under the microscope along with other types of pollen that could be present in the atmosphere.

Aerobiological studies for Date-Palm pollen from the atmosphere were established by collecting pollen grains using Rotorod Sampler Model 40 (Sampling Technology, Inc.). The rotorod sampler recovers particles suspended in the air on two rapidly spinning polystyrene rods. One surface of each rod is coated with silicone grease to capture and retain impacted particles. The rotorod sampler was set on a stand such that collection rods were at a height of about 180 cm from the ground. This height was selected to resemble the height of an average individual so that the concentration reflected what a person would breathe in. In each case a sampling duration of three hours was performed with a 10% duty cycle (one minute on and 9 minutes off). Date palm pollens were counted using a light microscope. The counts on both

rods were added up. The number was then used to calculate Date-Palm pollen concentration using the following formula:

$$\text{Pollen concentration (count/m}^3\text{)} = \frac{\text{Count on both rods}}{6.24\text{m}^3 \times \frac{3\text{hrs}}{24\text{hrs}}}$$

where 6.24m^3 represents the volume of atmosphere sampled in 24 h on a 10% duty cycle (Fiorina et al. 1999).

Preliminary sampling at sites away from a Date-Palm farm showed that there were no Date-Palm pollen grains in such locations. It was decided then that sampling locations would be restricted to Date-Palm farms. Three Date-Palm farms were selected as sampling locations: Al Muwaiji, Od Al Tobba, and Al Tawiya. These three farms are located within the city of Al-Ain and they are a few km away from each other. Od Al Tobba farm is located in the City Center while the other farms are located in less populated areas. Sampling devices were set in fixed places in almost the middle of the farms when samples were collected.

To investigate spatial variations in Date-Palm pollen concentration, sampling was conducted at various distances (ranges from 0–210m) from the edge of Al Tawiya farm. This sampling was conducted on the same day. All samples were collected during the months of February–April 2003. Counts were determined immediately after sample collection.

IgE-specific RAST tests

The measurement of circulating allergen specific IgE in human serum or plasma by an enzyme immunofluorescence test (RAST) (Wide et al. 1967) was performed on the UniCAP 100 machine. The sample is incubated with a solid phase (Immuno CAP) coat and tested for a specific allergen. Enzyme-labeled antibodies against IgE were added to form an immune complex. Then a developing agent was added. The reaction was stopped and the fluorescence of the eluate measured, which is proportional to the amount of specific IgE present in the specimen. Serum (10 ml) of whole blood was collected in a plain tube and centrifuged for 10 min at 3000 rpm. Samples were stored at $2-8^{\circ}\text{C}$ for up to 7 days, otherwise aliquots were kept at -20°C .

The specific IgE calibrators were run in duplicate to obtain a full calibration curve. This can be stored and subsequent assays may be evaluated against it using two curve controls run singly. The calibration curve is valid for one month or until a new lot number of UniCAP Specific IgE conjugate is introduced, or until curve controls are outside specific limits. All reagents are brought to room temperature before running the samples on the UniCAP 100 instrument.

Isolation and detection of flavonoids

Pollen grains were collected from male *Phoenix dactylifera* L. palm trees during the pollination season from January–March. Extraction was performed according to the methods of Batanero et al. (1994) with some modification. The extract was centrifuged at $16,000 \times g$ for 60 minutes and the supernatant was filtered through $0.22 \mu\text{m}$ filter, lyophilized then stored in sterile vials at -20°C .

The concentrated crude extract was analyzed on C-18 reverse-phase high performance liquid chromatography (HPLC). A sample volume of $20 \mu\text{L}$ was applied using a manual injection system. Aqueous acetonitrile mobile phase gradient (5%–95%) containing 0.1%

trifluoroacetic acid (TFA) was used, at a flow rate of 1.0mL/min., and 360 nm spectrophotometric detection (Mechref & Novotny 1998).

Results

Seasonal variations of Date-Palm pollen in the atmosphere around Al-Ain area showed that the peak concentration in pollen count occurred at different dates for the different farms, but the highest number of Date-Palm pollen grains in the air was from 10–20 March. Obviously, there were some Date-Palm pollens in the atmosphere before 28 February, but this was not captured due to late sampling. Figure 1 illustrates the variations in pollen concentration over the sampling period in the three sampling locations. Furthermore, the peak value varied among the different farms with Al Tawiya farm being the highest. Figure 1 also demonstrates that date pollen concentration drops significantly in the last week of March and there was no Date-Palm pollen within the farms after the first week of April.

Scanning electron microscopy was done on Date-Palm pollen grains at a magnification $\times 4000$. As shown in Figure 2, the pollen grains appeared relatively uniform, oval-shaped with a longitudinal groove (monocolpate), having a size of ca. $24 \times 11 \mu\text{m}$ and a mass of ca. 47 ng. This natural longitudinal groove (monocolpate) feature may play an important role in recognition by the stigma of the plant. The smooth texture surface and high mass, relative to most known pollen grains, might be a factor that participates to a high extent in preventing the pollen grain being carried long distances by the wind (Figure 3). This short distance traveled is an indication that this type of plant does not utilize the wind method of dispersal. This explains the traditional hand-held method of pollinating the female Date-Palm trees.

Figure 3 shows spatial variations in the date pollen concentration away from the edge of Al Tawiya farm. All the samples were collected on the same day (16 March) within 4 h. Obviously, pollen concentration drops significantly as the distance away from the farm

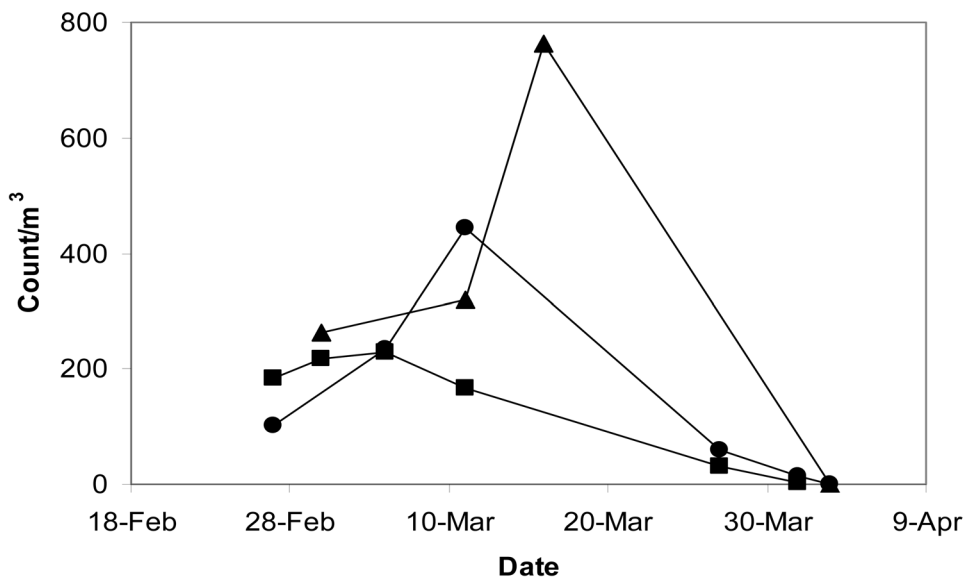


Figure 1. Variation of Date-Palm pollen concentration at three farms in Al Ain. Rectangles for Al Muwaiji farm, circles for Od Al Tobba farm, and triangles for Al Tawiya farm.

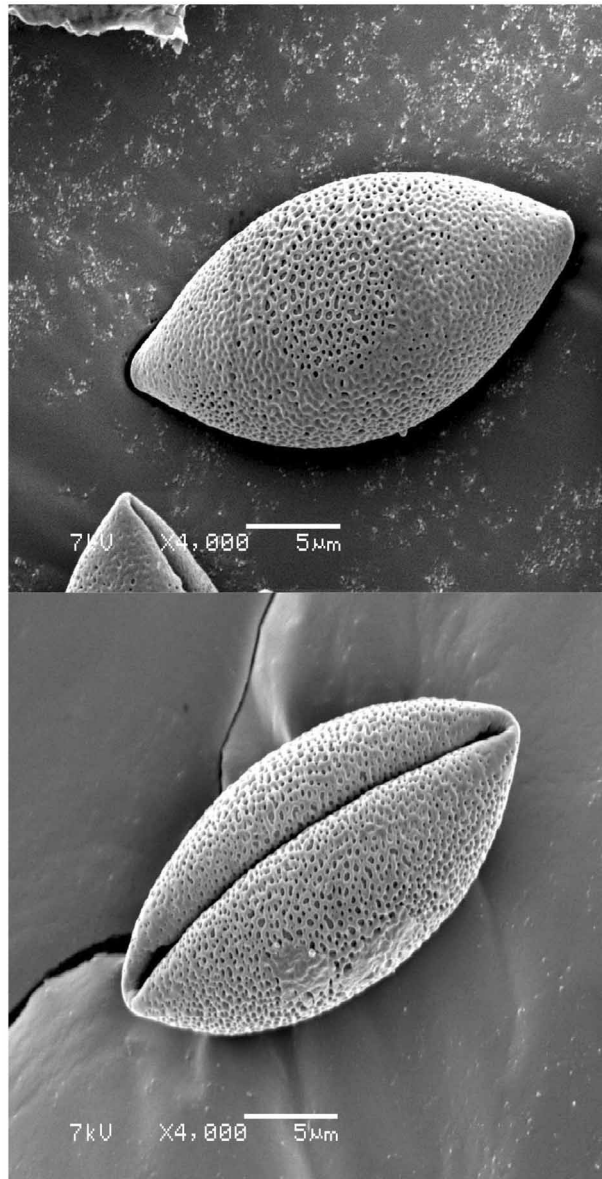


Figure 2. Scanning electron micrograph of Date-Palm pollens (x 4000).

increases. It should be noticed that the concentration of pollen in the atmosphere within the farm was about $800/\text{m}^3$ on this day (Figure 1). The level reduces almost 80% just 100 meters outside the farm area and it reaches zero beyond 200 m from the farm.

Enzyme immunofluorescence anti-IgE (RAST) assay showed that all positive RAST patients had high total IgE counts, and not all high total IgE count patients gave positive RAST. Out of 477 airborne allergic patients diagnosed at Tawam hospital (at one of the leading allergy clinics in the country), Al-Ain, UAE, during the year 2003, that were assayed for RAST (at least 5 different specific anti-IgE assay per sample), only 22 had high total IgE

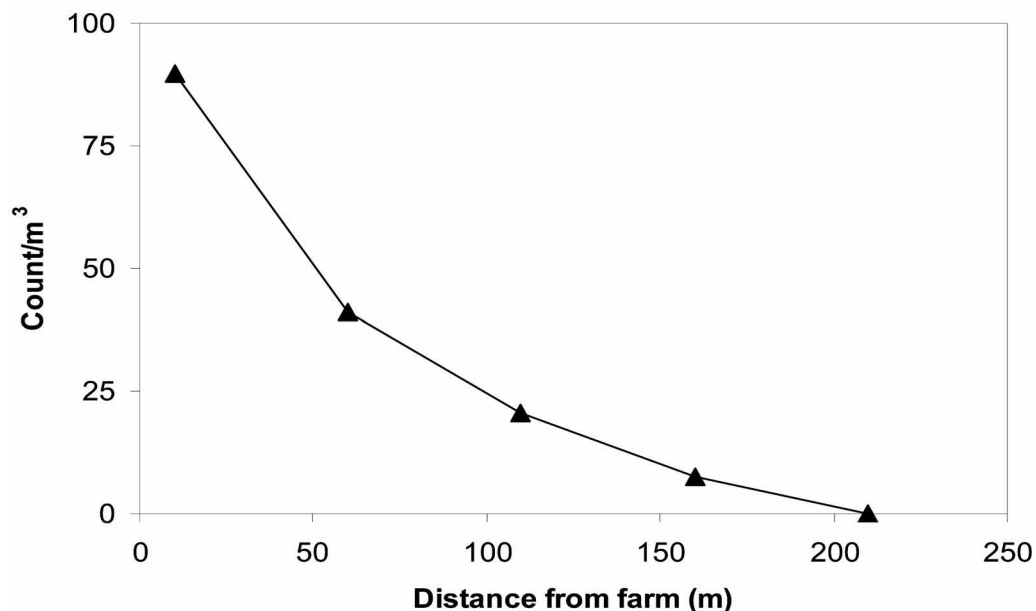


Figure 3. Spatial variation of Date-Palm pollen concentration from Al Tawiya farm.

counts for Date-Palm pollen allergy, and only 11 samples had positive RAST tests (2.3% only). On the other hand, RAST tests towards grass pollens showed 16.1% and Mesquite tree pollens showed 12.4% positive RAST tests.

The isolated crude extract of Date-Palm (*Phoenix dactylifera* L.) pollen contains approximately 2.33 mg protein per one-gram pollen as was analyzed using Bradford total protein assay. HPLC chromatography separated the protein and glycoprotein content into several observable peaks (data not shown). The supernatant of precipitated and separated proteins showed four distinct peaks on HPLC chromatogram in the early fractions (Figure 4). Complete absorbance UV spectrum showed similar maximum absorption at 210 and 260 nm characteristic of ring structures. Further analysis of the fractions of the peaks from HPLC separation of deproteinated Date-Palm pollen extract using ion-trap mass spectrometry, showed the presence of distinct low molecular weight masses (data not shown). The resulted masses suggested the presence of flavonoids commonly found in plants. Explicit analysis, molecular weight assignments and glycosylation type of these flavonoids is under investigation.

Discussion

The implications of these results are that the general public who may develop allergies to Date-Palm pollens should not be concerned as long as they stay a distance from Date-Palm farms (greater than 200 meters). However, the high level of pollens within the farms could be problematic to allergic workers at the farms. The limited short period of pollination season is an advantage to the environment and to the people allergic to airborne particles. Two interesting features of Date-Palm pollen imply its lesser involvement in development of type I hypersensitivity allergic reactions towards pollen exposure. First, the smooth texture provides low wind resistance and the lowest lung airway irritation. Second, the short distant traveled

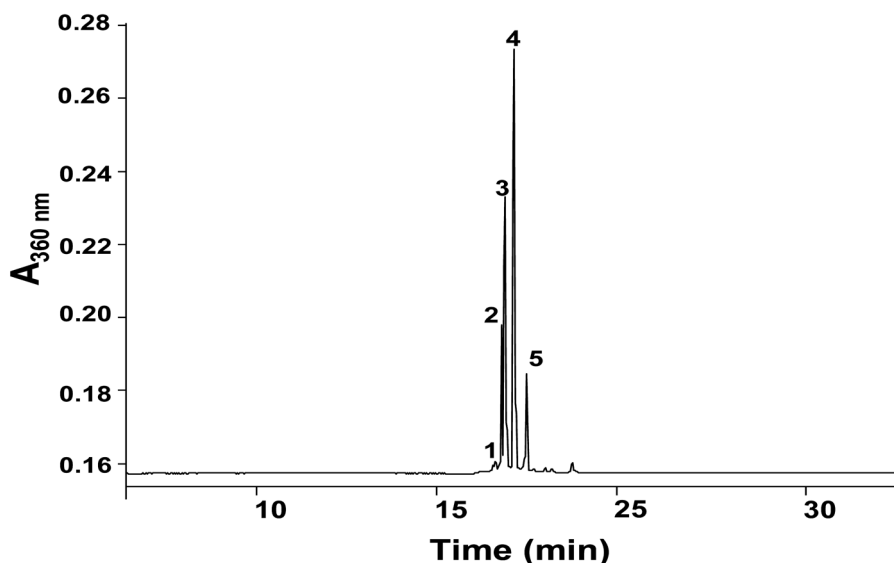


Figure 4. C-18 reverse-phase HPLC chromatogram of deproteinized crude extract of Date-Palm pollens [flow rate: 1.0mL/min., mobile phase: aqueous acetonitrile gradient (5%–95%) containing 0.1% trifluoroacetic acid].

and manual method of pollination minimizes the direct exposure to the pollens. In addition, the presence of the assumed antiallergic bioactive compounds allows allergic patients to have low allergic responses towards Date-Palm pollen. The findings of this study of low allergenicity towards Date-Palm pollens is in general agreement with the findings of several other research groups in different areas of the world. For instance, in Riyadh, Saudi Arabia, pollen of some species of Date-Palm did not have any allergic responses (Kwaasi et al. 1994). Another study in Al-Ain, UAE, by Bener et al. (2002) showed that among allergic responses to airborne pollens during the period 1996–1998, Date-Palm pollen showed the lowest percentage allergenicity among allergic patients relative to other pollens studied.

Conclusions

Although Date-Palm trees are widespread in UAE, the pollen produced has a minimal effect on allergic responses among the population. Exposure to Date-Palm pollen is limited mainly to the month of March. Workers on Date-Palm farms are subject to high concentrations of Date-Palm pollens, whereas the general public are not exposed to high pollen concentration in the atmosphere as the amount of Date-Palm pollen drops dramatically a short distance outside the farm area. The authors have proposed some reasons for the low allergenicity and encourage more detailed investigations on the bioactive constituents of the pollen.

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