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Research Article
Study of the Expected Impact of Palm Pollen on Human Respiratory Tract Allergy

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Abstract
Background and Objective: Date-Palm trees (*Phoenix dactylifera* L.) are the most abundant crop in Saudi Arabia. Date Palm Pollen (DPP) are considered an important allergens. The reasons for the increase in susceptibility to allergy are not clear. This investigation aimed to link the size, shape, moisture contents and biogenic contents of the collected pollens to susceptibility. **Materials and Methods:** Pollens were collected from three different regions in the Kingdom. Date palm pollen sizes were determined in wet and dry states by Microtrac. A microscope with a camera was used to image the forms of date palm pollens. The biogenic amines were determined after extraction using HPLC. Data were analyzed by one-way analysis of variance (ANOVA), using SPSS version 16.0. **Results:** There was a variation in the sizes of pollens from different regions ranging between 3.3-704 µm. Most pollen grains are spherical. The concentrations of six biogenic amines were detected in all samples but with different concentrations. They included B-phenyl ethylamine, Putrescine, Cadaverine, Histamine, Tyramine and Spermidine. **Conclusion:** The shape and size of the pollen grains studied were variable and will not facilitate deep penetration into the respiratory system but their biogenic contents were very high and suggested to cause allergy.

Key words: Date-palm, biogenic amines, allergy, pollen size, HPLC, putrescine, histamine, spermidine

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Date-Palm trees (*Phoenix dactylifera* L.) are the most abundant and popular crops in Saudi Arabia. It is the leading fruit crop of the Kingdom. Qassim is regarded as one of the most cultivated areas in the Kingdom of Saudi Arabia. Besides their economic importance, palm trees are also used for decoration and as the ornament of streets and houses. In Saudi Arabia, as in other regions, male seedlings are used for pollination. These male seedlings are highly different, especially in many aspects such as growth, size, pollen quality, strength and sheathing characteristics[1,2]. The production cycle of the date palm depends on the pollen which appears in the male palm and then becomes volatile in the surrounding air. Many studies have reported that there is a high risk resulting from short-term exposure to airborne contaminants[3-6]. It was found that plant pollen is regarded as one of the most important particulate matter in the air; however, there is variation in size between plant pollens. Radwan *et al.*[7] reported that DPP should be considered as an important allergen in countries where palm trees grow and should be included in Skin Prick Testing (SPT). One of the most widespread types of allergies is related to the presence of allergenic pollen in the air. Its seasonal outbreaks cause a quick rise in symptoms and an increase in the consumption of antihistamines. The reasons for the increase in susceptibility to allergens, in particular to pollen allergens, remains elusive; however, environmental and lifestyle factors appear to be the driving forces. Evidence has shown that chemical air pollutants and anthropogenic aerosols can alter the impact of allergenic pollen by changing the amount and features of allergens, thereby simultaneously increasing human susceptibility to them.

WHO[5] reported that exposure to 150-200 µg m^{-3} of ne particles for 120 min could affect the respiratory system. Solman and Al-Obeed[2] using scanning electron microscopy images reported that the size of date palm pollen ranges between 7-10 µm, while Martinez *et al.*[8] found that a single grain of date palm pollen could have a mean diameter between 22-55 micrometer. Nesiem *et al.*[9] reported that date palm pollen diameters were significantly different when the type and source of pollen varies. The main route of entry for airborne particulates to the human body is through the respiratory system. Most particles are smaller than 10 microns can reach the human lungs. The effect of airborne particulates on the respiratory system depends on particle size, shape and density in addition to chemical composition. Pope *et al.*[10] reported that exposure to high concentrations of particulate matter is connected with adverse health effects and ne particles have a bigger health impact[11] because these particles can reach deeper parts of the respiratory tract[12].

The importance of Date Palm (DP, *Phoenix dactylifera*) pollen and pollens from other members of the palm family, as potent inhalant allergens, has been reported in different areas of the world where these palms grow[13-15]. The prevalence of skin sensitization to the Pollen of Date Palm in Marrakesh, Morocco was studied[16].

Skin sensitization to the date palm pollen was reported in five studies in Spain. It was found that the prevalence of skin sensitization was between 6.1 and 29.41 in patients with allergy symptoms[17].

Allergy is an Immunoglobulin E-mediated type 1 hypersensitive disorder and is caused by specific proteins called allergens affecting >25% of the world's population[18]. When allergens interact with the immune system, mast cells and basophils are activated, causing them to release pro-inflammatory molecules like histamine, leukotrienes and cytokines which create the allergic phenotype. Pollen grains harbor a few such proteins which constitute a major part of the aeroallergen load. Susceptible individuals exposed to such pollen grains often suffer from seasonal allergic rhinitis, dermatitis, rhinoconjunctivitis and bronchial asthma.

The WHO estimates that around 235 million people currently suffer from asthma worldwide. It is the most prevalent chronic childhood disease. Asthma can be caused by many factors, including poor air quality and the presence of strong airborne allergens. Asthma costs Europe an estimated 17.7 billion per year, including the cost of lost productivity estimated at €10 billion per year. According to recent estimates, the prevalence of palm pollen allergy ranges from 13% in the United Arab Emirates and to over 44% in India and Saudi Arabia[19,20].

Date Palm Pollen (DPP) has been identified as a potent allergen source[21] with the sensitization rates among respiratory patients ranging from 25% in Saudi Arabia to 18.9% in Spain and with a higher incidence among residents of rural than of urban communities.

The main route of entry for airborne particulates into the human body is the respiratory system. This is because most particles smaller than 10 microns can reach the human lungs. The effect of airborne particulates on the respiratory system depends on particle size, shape and density, in addition to chemical composition. Pope *et al.*[10] reported that exposure to high concentrations of particulate matter is associated with

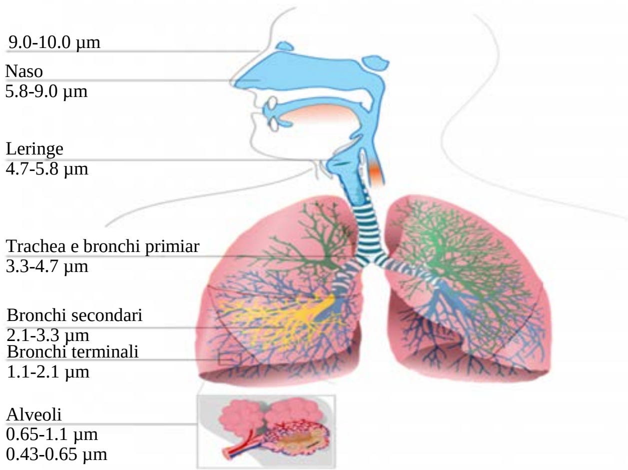

Fig. 1: Particulate matter diagram
Source: (https://www.sccpre.cat/maxp/iwhRRiR/)

adverse health effects and ne particles have a bigger health impact[11] because these particles can reach deeper parts of the respiratory tract[12,22]. On the other hand, many studies have reported the risk resulting from short-term exposure to airborne contaminants[3,4]. However, plant pollen is regarded as one of the most important particulate matter in the air, with varying sizes between plant pollens (Fig. 1). One of the causes of common allergies is the presence of pollen allergens in the atmosphere. An increase in exposure rapidly causes a seasonal spread of antihistamines.

The present study aimed to determine indicators of the health impact of date palm pollen on the human respiratory system by:

- Measuring the size of the pollen as well as its general physical forms
- Determination of biogenic amines in the pollen that is responsible for most of the symptoms of allergy to humans

MATERIALS AND METHODS

Study area: This study was carried out in the Qassim region, Saudi Arabia during the period extending from July, 2018-February, 2019.

Pollen grains collection: Three samples of palm pollen were collected from each farm of Wattania (A), Awqaf (B) and Hanakyia (C). The samples were dried in a drying oven at 60°C for three days.

Date palm pollen sizes were determined in wet and dry states by Microtrac (Microtrac Icn., USA) which utilizes the phenomenon of scattered light from multiple laser beams projected through a stream of pollen. The amount and direction of the light scattered by date pollen were measured using an optical detector array and then analyzed by Microtrac software. Date palm pollen samples may be delivered in a wet state and to scan an image for palm pollen, a microscope was used with a camera to image the forms of date palm pollen.

Determination of biogenic amines: The biogenic amines Tryptamine, B-phenyl ethyl amine, Putrescine, Cadaverine, Histamine, Tyramine and Spermidine were extracted and determined according to[23-25] with some modifications.

Reagents:

Dansyl chloride solution: About 500 mg of Dansyl chloride (5-{Dimethylamino} naphthalene-1-sulfonyl chloride) was dissolved in 100 mL acetone.

Standard solutions: Stock standard solutions of the tested amines: 25 mg of each standard was dissolved in 25 mL distilled water, individually.

Extraction of pollen grains: In this study, 20 mL of 5% Trichloroacetic acid was added to 1 g of ground pollen grain in a 50 mL centrifuge. The sample was mixed and sonicated for 5 min (Diagger ultrasonic processor 750 Watts) Daigger Scientific, Inc Hamilton, NJ 08691. The cups were centrifuged at 4500 rpm for 5 min. Thereafter, 10 mL of the supernatant was transferred into a culture tube with 4 g NaCl and 1 mL of 50% Na OH, shaken and extracted three times by 5 mL n-butanol/chloroform (1:1 v v^{-1}) stoppered and shaken vigorously for 3.0 min. Centrifugation was done for 5.0 min at 4500 rpm and the upper layer was transferred to a 50 mL separating funnel using a disposable Pasteur pipette. To the combined organic extracts (upper layer), 15 mL of n-heptane was added and extracted three times with 1.0 mL portions of 0.2 N HCl, the HCl layers were collected in a glass stoppered tube. The solution was evaporated just to dry, using a water bath at 95°C with the aid of a gentle current of air.

Formation of dansyl amines: In this study, 100 µL of each stock (standard) solution was transferred to a 50 mL vial and dried. About 0.5 mL of saturated $NaHCO_3$ solution was added to the residue of the sample extract (or the standard); stoppered and carefully mixed to prevent loss-due to

Table 1: Gradient program for injection of prepared pollen extracts in HPLC

Time (min)	Flow rate ($mL\ min^{-1}$)	Solvent Methanol (%)	Acetonitrile (%)	Acetic acid 0.02 N
0	1	20	20	60
10	1	40	40	20
15	1	35	50	15
20	1	55	40	5
25	1	30	40	30
30	1	20	20	60
35	1	20	20	60

spattering. Carefully, a 1.0 mL dansyl chloride solution was added and mixed thoroughly using a vortex mixer (Fisherbrands™ Analog Vortex Mixer).

The reaction mixture was incubated at 55°C for 45 min. About 10 mL of distilled water was added to the reaction mixture, stoppered and shaken vigorously using a vortex mixer. The extraction of dansylated biogenic amines was carried out using three times 5.0 mL portions of diethyl ether, stoppered, shaken carefully for 1.0 min and the ether layers were collected in a culture tube using a disposable Pasteur pipette. Fisherbrand™ Disposable Borosilicate Glass Pasteur Pipets.

The combined ether extracts were carefully evaporated at 35°C in a dry bath with the aid of the current air. The obtained dry film was dissolved in 1 mL methanol, then 10 µL was injected in HPLC using a gradient program as follows (Table 1).

Apparatus: High-Performance Liquid Chromatography (HPLC) was used for the determination of dansyl amines using an Agilent 1100 system (Agilent Technologies) equipped with a quaternary pump model G1311A, UV detector model G1314A (Marshall Scientific, USA) set at 254 nm wavelength, autosampler model G1329A. Agilent Zorbax Eclipse XDB C18 4.6×150 mm, a 5 m column was used for biogenic amines separation. Data were integrated and recorded using the Chemstation Software program.

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA), followed by an assessment of differences by Tukey's *post hoc* test. All statistical calculations were performed using SPSS version 16.0. The results were considered statistically significant at $p<0.05$.

The data were expressed as mean values with their standard deviation. The effects of the source (A, B, C) and status (fresh, dry) were analyzed using a General Linear Model (GLM), followed by an assessment of differences by Tukey's *post hoc* test. All statistical calculations were performed using SPSS version 16.0. The results were considered statistically significant at $p\leq0.05$.

RESULTS

The moisture content of pollen grains in the three regions is presented in Table 2. There was no much difference in the moisture contents of pollens from the three farms. However, the water content (%) in pollen from Wattania (A), Awqaf (B) and Hanakyia (C) were 3.17, 2.45 and 2.98%.

Images of palm pollen samples: Using microscopy images, the shapes of pollen grains from Wattania (a), Awqaf (b) and Hanakyia (c) are presented in Fig. 2. It shows that most pollen grains are spherical.

Size of palm pollen samples: The data in Table 3 presents the percentage of pollen size as an indicator of the differences between the wet and dry weights of pollens. Generally, there was a difference in the percentage of pollen size in all samples and the range of pollen sizes was between 3.3-704 µm, but the results showed that there was a little percentage of pollen sizes around 10 µm in both wet and dry samples and the only sample from Awqaf (B) gave a result of small size under 9 µm in both the wet and dry samples, by 2.12 and 2.46%, respectively. Sizes of less than 26 µm were more than 50% in wet/dry Awqaf (B) samples, but around 30% in other samples.

The cumulative distribution for the percentage of pollen sizes in wet and dry samples is presented in Fig. 3-4. This shows that differences result in the cumulative percentage of pollen sizes, while under dry and wet conditions, the percentage of small pollen sizes in the Awqaf (B) samples were highest compared to the Wattania (A) and Hanakyia (C) samples. Also, 80% of the Awqaf (B) samples were less than 100 µm compared to 55% obtained from Wattania (A) and Hanakyia (C) samples in both wet and dry samples.

Concentration of biogenic amines in pollen: The data in Table 4 shows the concentration of biogenic amines in fresh and dry pollen grains collected from three different farms. Six biogenic amines were detected in all samples but at different concentrations. The biogenic amines detected

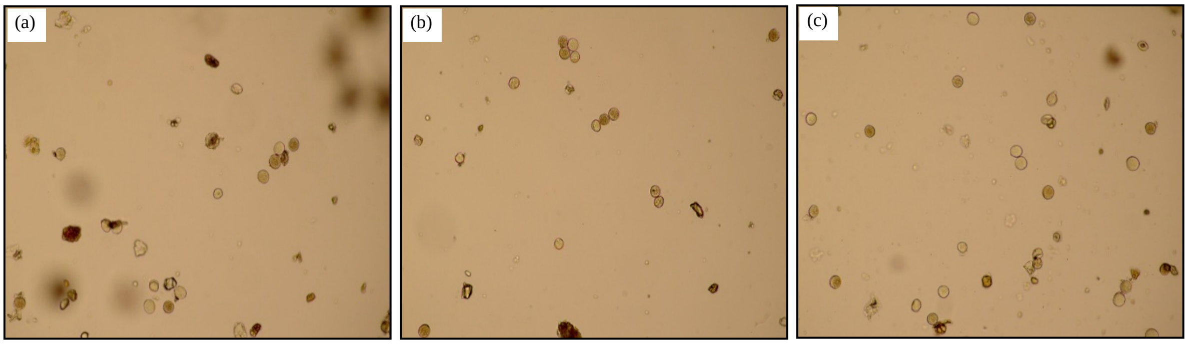

Fig. 2(a-c): Scanning microscopy image of palm pollen samples from (a) Wattania, (b) Awqaf and (c) Hanakyia

Table 2: Average moisture content in palm pollen

	Wet sample	Dry sample	Moisture	Moisture (%)
A	14.02	13.57	0.44	3.17
B	14.01	13.67	0.34	2.45
C	14.02	13.60	0.42	2.98

Wattania (A), Awqaf (B) and Hanakyia (C)

Table 3: Percentage of pollen sizes in samples

	Dry (%) of sample			Wet (%) of sample		
Size (µm)	A	B	C	A	B	C
3.3	0	0.4	0	0	0.38	0
3.9	0	0.42	0	0	0.49	0
4.6	0	0.41	0	0	0.52	0
5.5	0	0.33	0	0	0.4	0
6.5	0	0.3	0	0	0.33	0
7.8	0	0.26	0	0	0.34	0
9.3	0.55	0.51	0.45	0.56	0.58	0.42
11	1.28	1.45	1.12	1.34	1.45	1.02
13.1	2.85	4.03	2.88	3.13	4.05	2.59
15.6	5.01	9.08	5.87	5.74	8.89	5.31
18.5	6.39	12.56	8.02	7.38	12.59	7.39
22	6.11	11.91	7.97	7.22	12.23	7.49
26.2	4.91	8.84	6.18	5.75	9.19	5.89
31.1	3.74	6.13	4.43	4.33	6.43	4.28
37	2.97	4.48	3.27	3.41	4.71	3.21
44	2.57	3.58	2.63	2.94	3.77	2.66
52.3	2.42	3.08	2.32	2.78	3.25	2.44
62.2	2.48	2.78	2.22	2.83	2.94	2.45
74	2.7	2.6	2.28	3.04	2.74	2.62
88	3.09	2.5	2.48	3.37	2.64	2.93
104.7	3.6	2.52	2.82	3.8	2.64	3.39
124.5	4.11	2.64	3.48	4.3	2.71	3.99
148	4.48	2.8	3.79	4.87	2.78	4.71
176	4.79	2.73	4.39	5.08	2.75	5.5
209.3	5.32	2.97	4.73	6.43	2.59	6.31
248.9	6.33	2.82	5.72	6.94	2.3	6.91
296	7.33	2.52	5.76	6.24	1.91	6.75
352	7.34	2.02	5.23	4.27	1.67	5.42
418.6	5.22	1.41	4.61	2.23	1.12	3.41
497.8	2.64	0.9	3.62	1.01	0.66	1.74
592	1.25	0.65	2.53	0.51	0.56	0.85
704	0.52	0.37	1.2	0.5	0.39	0.32
Total (%)	100	100	100	100	100	100

Table 4: Concentrations of biogenic amines (μg g^{-1}) in fresh and dry pollen grains collected from three different regions

Samples	Tryptamine	B-phenyl ethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine
AW	ND	ND	30.89±4.7[a]	ND	1.86±0.5[ab]	ND	92.23±16.5[a]
AD	ND	ND	27.73±4.5[a]	0.82±1.4[bc]	1.11±0.3[ab]	ND	24.50±2.7[b]
BW	ND	ND	29.27±3.4[a]	2.92±0.3[a]	2.06±0.2[a]	ND	36.80±6.1[b]
BD	ND	0.76±0.3[a]	23.74±2.4[a]	2.25±0.4[ab]	1.40±0.4[ab]	0.26±0.2	15.70±9.7[b]
CW	ND	ND	33.27±6.2[a]	0.89±0.8[bc]	0.90±0.2[b]	ND	113.16±18.9[a]
CD	ND	0.85±1.5[a]	26.95±7.9[a]	0.21±0.4[c]	0.92±0.6[b]	ND	93.01±36.2[a]

AW: Wattania wet, AD: Wattania dry, BW: Awqaf wet BD: Awqaf dry, CW: Hanakyia wet, CD: Hanakyia dry. Means on the same column with different letters are significantly different at p<0.05

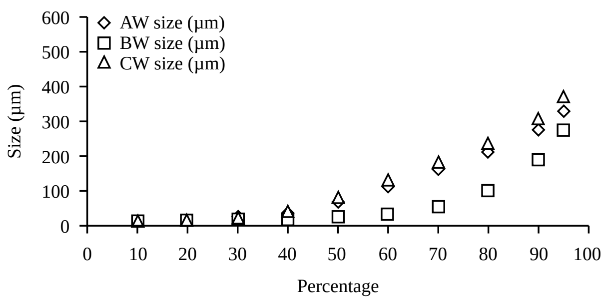

Fig. 3: Cumulative distribution for the percentage of palm pollen size (micrometer) for all wet samples

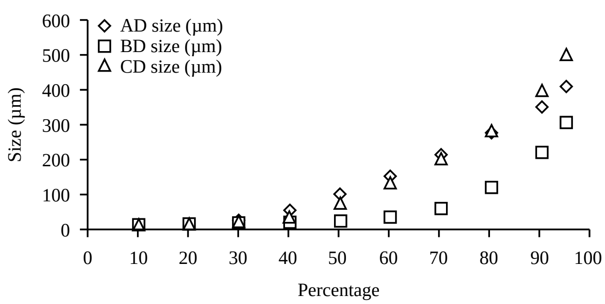

Fig. 4: Cumulative distribution for the percentage of palm pollen size (micrometer) for all dry samples

included B-phenyl ethylamine, Putrescine, Cadaverine, Histamine, Tyramine and Spermidine. Generally, it can be noted that dry pollen samples from the three areas contained less biogenic amines when compared with the wet samples. Putrescine, Histamine and Spermidine were the most abundant and highly concentrated biogenic amines in both wet and dry samples collected from all studied areas. There was no significant difference in the concentration of Putrescine collected from Wattania, Awqaf and Hanakyia. Histamine concentration varied from one area to another and the lowest concentration was detected in samples collected from Hanakyia while the concentration of Spermidine was the highest when compared with the other biogenic amines investigated.

DISCUSSION

Date palm is regarded as the most important plant and has many beneficial uses for many people around the world, especially in the Arabian Peninsula and North Africa. Dates are of high importance commercially and have high calorific value. Date palm usually flowers between February and April and pollen production is high. One male palm produces pollen sufficient to pollinate between 50-100 trees. When produced, pollen usually contains less moisture. In the present study, the moisture contents of pollen grains collected from three farms are presented in Table 2 and it can be seen that there were no many differences between them. However, the water content (%) in pollen from Wattania, Awqaf and Hanakyia were 3.17, 2.45 and 2.98%, respectively. The shapes of pollen grains from the three farms Wattania (A), Awqaf (B) and Hanakyia (C) are presented in Fig. 1-3. This indicates that most pollen grains are spherical, while Solman and Al-Obeed[2] reported that palm pollen grains are oval with sharp limbs. This indicates a variation in palm pollen grains shapes. The shape described by Solman and Al-Obeed[2] will be more harmful when inhaled. Pollen grains come in a wide variety of shapes, sizes and surface markings characteristic of the species. Most, but certainly not all, are spherical. Pollens from different plants have different shapes and sizes because different flowers have different requirements for pollination.

In the present study, the sizes of the pollen grains ranged between 3.3704 μm (Table 3). However, the results showed that there was a little percentage of pollen sizes around 10 μm in both wet and dry samples and only the samples from Awqaf (B) gave sizes under 9 μm in both wet and dry samples which represent 2.12 and 2.46%, respectively. Sizes less than 26 μm were found to represent more than 50% in wet/dry Awqaf (B) samples while in samples from Wattania and Hanakyia, these represent about 30%. This means that pollen grains from Awqaf are potentially more harmful.

Further confirmation of the above assumption was reached when the cumulative distribution for the percentage of pollen sizes in wet and dry samples was studied. As presented in Fig. 3-4 showed differences in the cumulative percentage of pollen sizes under dry and wet conditions. It was found that the percentage of small-sized pollens in the Awqaf (B) sample was highest compared to the Wattania (A) and Hanakyia (C) samples. It was found that 80% of Awqaf (B) samples had sizes less than 100 micrometers compared to 55%, only from Wattania (A) and Hanakyia (C) samples in both the wet and dry states.

Date pollen is very unique in morphology[21], some are oval while others are spherical. They also differ in size and it was noticed that pollen size varies greatly. Also, pollens with a size of less than 10 µm have a greater chance of entry into deeper parts of the respiratory tract and cause problems. Pope *et al.*[10] reported that fine particles have bigger health effects as can be depicted in Fig. 1.

Almehdi *et al.*[26] attributed the low allergic effect of date palm pollen on susceptible people in UAE to many factors including the smooth shape of the pollen, the low molecular weight of the contents and the short pollination period.

However, in the present study, the sizes of the pollen samples ranged from 3.3-704 µm (Table 3). The results showed that there was a little percentage of pollens with sizes around 10 µm in both wet and dry samples and only the samples from Awqaf (B) gave sizes under 9 µm in both wet and dry samples which represent 2.12 and 2.46%, respectively. When allergens interact with the immune system, it results in the release of pro-inflammatory molecules like histamine, leukotrienes and cytokines thereby creating the allergic phenotype.

Little information is available on the chemical composition of the pollen grains of date palm[27]. They contain various phytochemicals and nutrients and are particularly rich in phytosterols, flavonoids, triterpenes, saponins and carotenoids. Stanley and Linskens[28] reported that palm pollen contains carbohydrates, proteins, lipids, nucleic acids, free amino acids, lipid-soluble vitamins and different kinds of enzymes and cofactors.

It is well known that date pollen can cause asthma and allergic rhinitis. Despite the well known severe effects caused by date pollen, there are no comprehensive studies on date palm allergens. Kwaasi *et al.*[14] reported that peptides are potent allergens. Harb *et al.*[13] studied the allergenic components of date palm (*Phoenix dactylifera*) pollen and investigated their effects using skin test reactivity. The pollen was subjected to fractionation using polyacrylamide gel electrophoresis and 22 immunoreactive bands were reported. In the present study, various biogenic amines were found and their concentrations were determined. Seven biogenic amines including B-phenyl ethylamine, Putrescine, Cadaverine, Histamine, Tyramine and Spermidine were detected in date pollen collected from three different farms-Wattania, Awqaf and Hanakyia. Wind-pollination, hand pollination and insect pollination are usually the dominant means of pollination and as such result in the inhalation of pollens into the lungs of inhabitants leading to asthma and rhinitis. As the pollination process is seasonal, so the appearance of allergy is also seasonal and highly connected to pollination. However, the inhalation of pollens depends and is governed by the morphology and size of the pollen. Small pollens will penetrate deeper into the respiratory tract and the body will respond.

CONCLUSION

It can be concluded that there are no differences in the moisture contents in pollens from the three regions. Pollens reported in this study are spherical in shape and larger in size consequently their deep entry into the respiratory tract is not facilitated, nevertheless seasonal allergy and asthma are prevalent in this region probably due to the presence of six biogenic amines in date palm pollen mainly, Putrescine, Spermidine and Histamine while Tryptamine was not detected.

SIGNIFICANCE STATEMENT

This study revealed that date palm Pollen grains come in a wide variety of shapes, sizes and surface markings characteristic of the species. The pollen shape found in this study is spherical while other workers reported an oval shape. The sizes of the pollen grains in the present study are larger than the sizes reported by other workers. Little information is present about the chemical composition of pollen grain of date palm, however; six biogenic amines were detected in our study. The inhalation of pollens depends and is governed by the size and shape of the pollen. Small and smooth pollens will penetrate deeper into the respiratory tract. The Shapes and sizes reported in the present study will not facilitate the entry of pollens into deeper parts of the respiratory tract and cause problems. However, allergies caused by date palm pollens are widespread in the region and further studies are needed to study the link between this allergy and pollination and chemical air pollutants.

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