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Reactivity to *Conocarpus* tree pollen in patients with respiratory allergic diseases in the south-western part of Iran

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Abstract

Aeroallergens are known as the major cause of allergic respiratory diseases. The present study aimed to investigate the skin prick test (SPT) reactivity to the pollen of *Conocarpus erectus* trees and to find the association between the indoor and outdoor aeroallergens in the patients. In the present case series multicentre study, 104 patients with respiratory allergies such as allergic rhinitis and asthma who were referred to the Bushehr University allergy clinic participated. The SPT was applied by using the extract of *Conocarpus erectus* pollen and the other allergens to determine the patients' reactivity. The frequency of positive sensitivity to *Conocarpus erectus* pollen was 61 (58.6%) and it was 62.5%, 55.7%, and 53.8% for the tree pollen allergens *Citrus X Sinensis*, *Morus rubra*, and *Phoenix dactylifera*, respectively. In addition, the maximum frequency of sensitivity to other aeroallergens was with house dust mite (HDM) (78.8%), *Salsola kali* (71.2%), and cockroach (71.2%). The maximum frequency of reactivity to food allergens was with egg yolk (37.6%), shrimp (37%), and beef (34.6%). This study concludes that the frequency of sensitivity to *Conocarpus erectus* pollen was high and similar to the pollen allergens of other trees that are widely cultivated in southern Iran. Green spaces are the key element of urban management, and the interaction between citizens and the environment, as well as promoting human health are very effective. Therefore, the lack of planning in urban populations in the choice of ornamental species can be a triggering factor, especially in patients with respiratory allergies.

Keywords: *Conocarpus erectus*; aeroallergen; skin prick test; allergic diseases

Airborne pollen plays an important role in triggering different types of allergic diseases such as allergic rhinitis (AR) and asthma in human beings (D'Amato Cecchi et al. 2007; Damialis et al. 2019). Recent studies indicated that allergic diseases especially respiratory condition are on the rise in Iran and other places in the world (Pazoki et al. 2018; Sophie Seit   et al. 2020). Importantly, the epidemiological

studies indicated that approximately 20% of the population in industrialised countries are affected by respiratory allergies (De Weerd et al. 2002). The allergenic content of the atmosphere changes according to geographical location (Fereidouni et al. 2009; Ghaffari 2012) and airborne pollen grains in the atmosphere depend on the extent of local vegetation and the nature and the period of pollination generally

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differs from one area to the other (D'amato & Liccardi 1994; Perveen et al. 2014).

The most important allergenic species belong to such taxa as Poaceae (grasses), weeds such as *Ambrosia* sp. (ragweed), and trees such as *Olea europaea* (olive) and *Betula* sp. (birches) (Kashef et al. 2003; Shakurnia et al. 2013; Fouladseresht 2014). In southern Asia, other plants are also considered an important sources of pollen allergens, e.g. *Salsola imbricata* (Al-Dowaisan et al. 2004) or *Conocarpus erectus* (Rad et al. 2019). In

Pakistan, *Conocarpus erectus* pollen was one of the most common pollen in the air (Perveen et al. 2015).

Originally, the *Conocarpus* tree is from America and it has been imported by Americans to the southern regions of Iran several years ago. *Conocarpus erectus* trees are categorised as amphiphilic species, which pollinate in the spring and autumn seasons and their very small pollen (9–13 μm) (Figure 1) is transported by both wind and insects (Namjoyan et al. 2020). In recent years, *Conocarpus* trees have

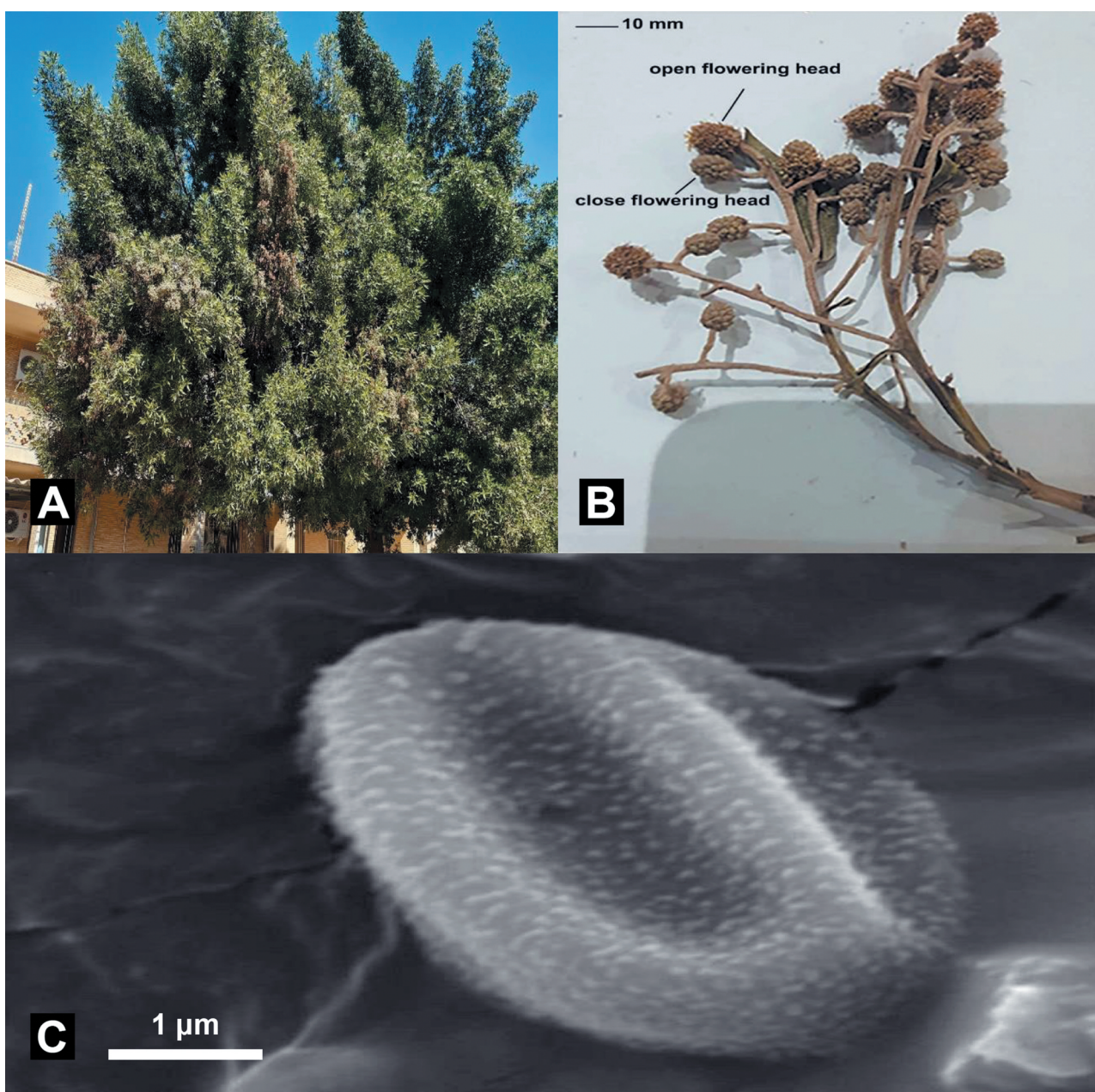


Figure 1. **A.** The *Conocarpus erectus* tree. **B.** Inflorescence of *Conocarpus erectus* plant, opened and closed flowering heads (Namjoyan et al. 2020). **C.** Scanning electron microscopy photograph of *Conocarpus erectus* pollen (Rad et al. 2019).

been widely planted in southern regions of Iran, due to their low water need and adaptation to the warm environment. Therefore, this study aimed to investigate the skin prick test (SPT) reactivity to the pollen of *Conocarpus erectus* and its association with some indoor and outdoor aeroallergens in respiratory allergic patients.

Material and methods

Sampling

In the present case series, and multicentre study conducted between Bushehr University of Medical Sciences (BPUMS) and Ahvaz Jundishapur University of Medical Sciences (AJUMS) in Iran, 104 patients participated with respiratory allergies such as AR, allergic rhinoconjunctivitis, chronic rhinosinusitis with nasal polyp (CRSwNP), chronic rhinosinusitis without nasal polyp (CRSsNP), and

asthma. Demographic data of the patients were registered by using a standard questionnaire at the allergy clinic centre, Bushehr, Iran (Figures 2, 3). SPTs were performed for all patients. In addition, paraclinic tests such as pulmonary function test (PFT), fractional exhaled nitric oxide (FeNO), and laboratory tests such as total serum immunoglobulin E (IgE), eosinophil count, and vitamin D₃ were carried out. This study was approved by the Ethics Committee of the BPUMS, Bushehr, Iran (approval code: IR.BPUMS.REC.1396.374).

Skin prick test (SPT)

SPT was performed for all patients by using regional common allergen extracts (Greer, USA) and *Conocarpus erectus* pollen extract (prepared by AJUMS, Ahvaz, Iran). Allergens were selected for the study based on the type of plants grown in the area and other allergens which were identified and introduced by the authori-

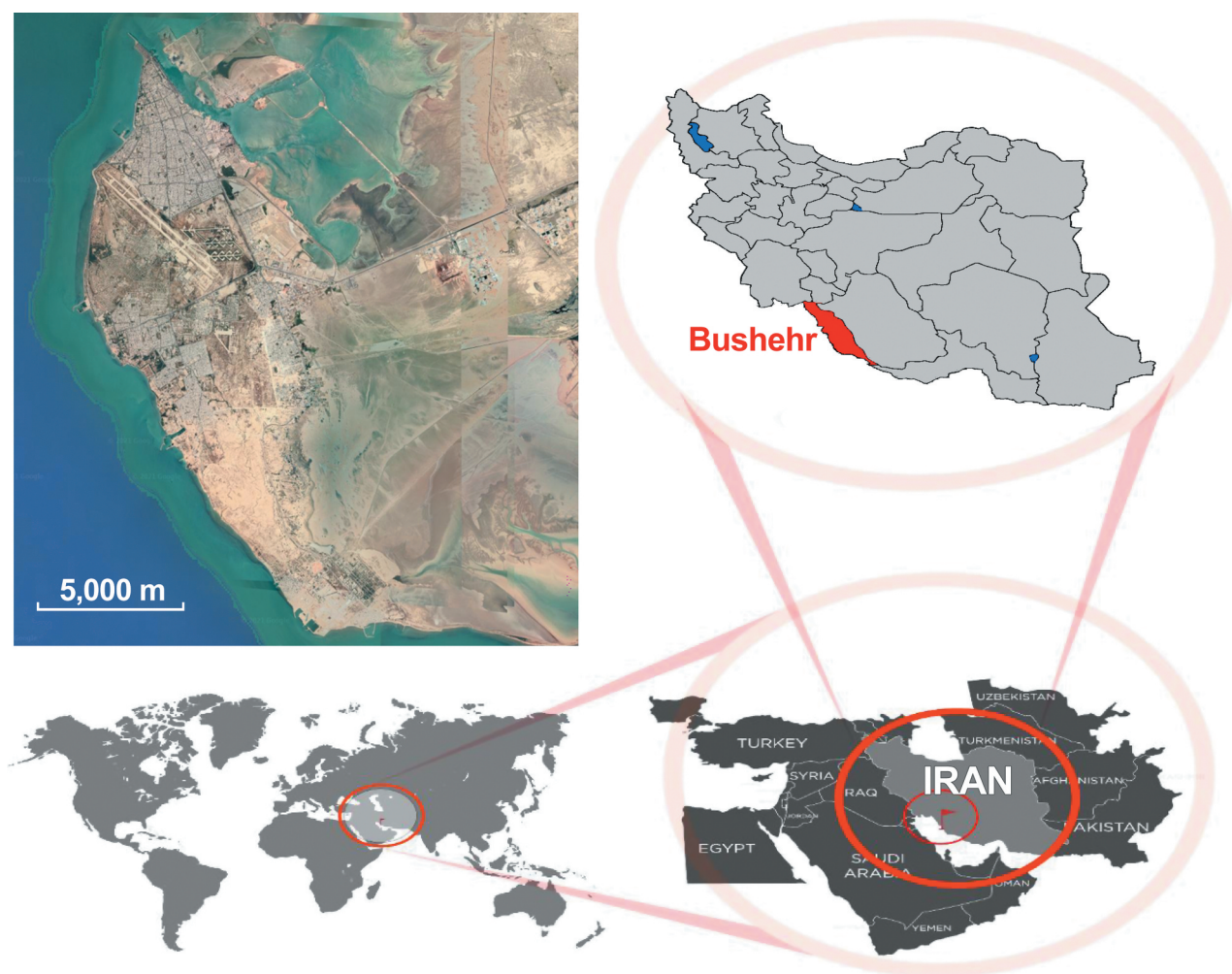


Figure 2. Geographic location of the study area.

Food Allergens

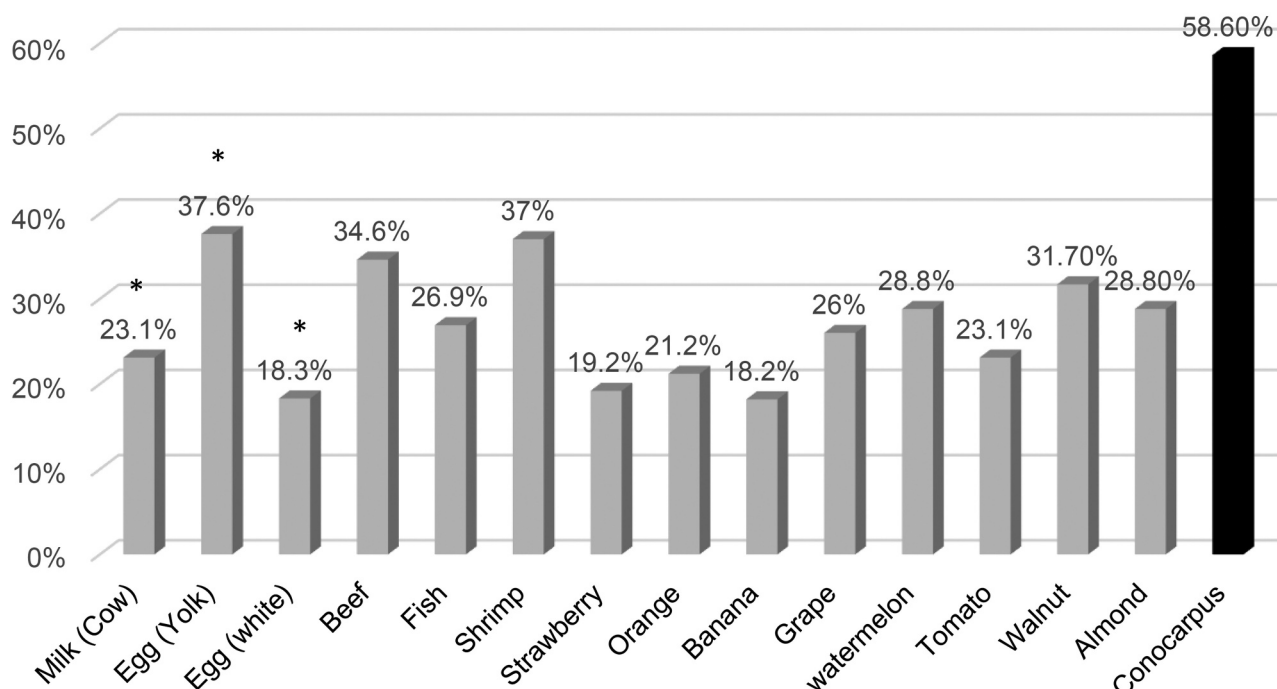


Figure 3. The frequency of skin prick reactivity of food allergen in the patients and association with *Conocarpus*. (* $P < 0.05$).

ties from the Agricultural Research Centre, Bushehr, Iran. Aeroallergens such as trees that commonly release pollen (i.e. *Phoenix dactylifera*, *Morus rubra* and Orange), grasses (i.e. *Elymus repens*, and *Phleum pratense*), weeds (i.e. *Chenopodium album*, *Salsola kali* and *Rumex acetosa*), house dust mites (HDMs) (i.e. *Dermatophagoides farina* and *Dermatophagoides pteronyssinus*), moulds (i.e. *Aspergillus fumigatus* and *Alternaria alternata*), cat and dog, and cockroach extracts were tested. The SPT was performed according to the international guidelines (Dreborg 1989) as a single-test on two fore-arms with lancets and standardised allergens. A wheal size ≥ 3 mm was measured as a positive reaction. Histamine hydrochloride (10 mg/ml) and glycerine saline were used as positive and negative controls, respectively. All subjects who were analysed had a positive reaction to histamine and none of them had any reaction to the negative control.

Preparation of *Conocarpus* pollen extract

Conocarpus erectus flowers were collected during pollination season (November–December) from trees in the urban green space of Ahvaz, the capital city of Khuzestan. Dried materials were passed through a sieve with mesh size 20 μ m and for more purification

and removal of dust from 10 μ m under vacuum condition (Bradford 1976; Assarehzadegan et al. 2009). The pollen grains were collected on a 10 μ m sieve. Preparation of pollen extract was carried out according to Assarehzadegan et al. (2009), with a slight modification. In brief, the pollen (3 g) was defatted with 15 ml of petroleum ether, dried in an incubator at 37 °C and mixed with 15 ml phosphate-buffered saline (PBS, 0.01 M; pH 7.4) by continuous stirring for 18 h at 4 °C. Then, the solution was centrifuged with 10 000 g, at 4 °C for 20 min. The supernatant was separated and centrifuged again with the same condition for 5 min. Then, the extract was dialysed against PBS 150 mM pH 7.4 overnight and freeze-dried. The protein content of the extract was measured by Bradford's method.

Statistical analysis

The frequency of SPT reactivity to food and aeroallergens in patients with respiratory allergies was calculated. The severity of SPT reactivity and its association with *Conocarpus* was compared by age group, gender (male or female), food and aeroallergens by using Chi-squared tests. The P values of < 0.05 were considered to be statistically significant,

and the statistical analyses were performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA).

Results

In this case series study, 104 patients (52.9% men and 47.1% women) with respiratory allergic diseases were enrolled. The mean age of the participants was 34 ± 14.6 years. The age of most of the patients was in the range 13–45 years, and 13.5% of them were smokers (Table I).

Reactivity to *Conocarpus erectus* pollen in SPT was positive in 61 patients (58.6%); 32 (30.8%) and 29 (27.8%) of them were mild and moderate to severe reactive SPT, respectively. Moreover, the highest frequency of positive reactivity to the aeroallergens was with HDM (78.8%), *Salsola kali* (71.2%), and cockroach (71.2%), while egg yolk (37.6%), shrimp (37%), and beef (34.6%), were the most reactive of the food allergens (Figures 3, 4, Table II).

There was no significant associations ($P > 0.05$) between moderate to severe reactivity to *Conocarpus erectus* pollen and age groups, sex, first complaint, and final diagnosis of the patients. Moreover, we observed a significant association between moderate

Table I. Demographic data of the patients.

Variable	N (%)
Men	55 (52.9)
Women	49 (47.1)
Age group (year)	
≤12	9 (8.7)
13–45	75 (72.1)
46–60	17 (16.3)
>60	3 (2.9)
Smoking status	
Smokers	14 (13.5)
Non-smokers	90 (86.5)
Allergic diseases	
AR	89 (85.6)
ARC	8 (7.7)
CRSwNP	26 (25)
CRSsNP	70 (67.3)
AS	25 (24)

AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; CRSwNP, chronic rhinosinusitis with nasal polyp; CRSsNP, chronic rhinosinusitis without nasal polyp; AS, asthma.

to severe reactivity to some foods, aeroallergens and *Conocarpus erectus* (Figure 4, Table II).

In addition, the mean levels of serum total IgE, eosinophilia, vitamin D₃, FeNO, forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and peak expiratory flow (PEF) were 165 ± 20.2 IU/ML, 3.2%, 30.5 IU, 13 ppb, 63%,

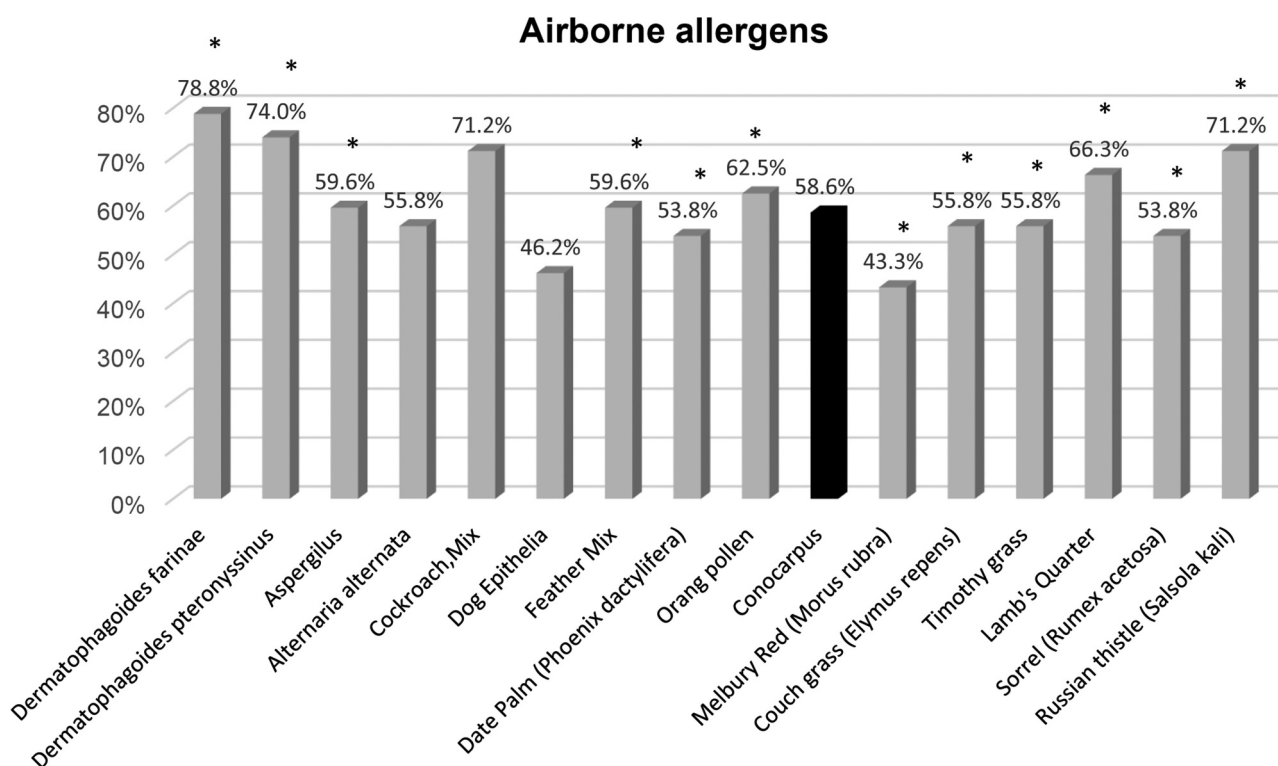


Figure 4. The frequency of skin prick reactivity of aeroallergens in the patients and association with *Conocarpus*. (* $P < 0.05$).

Table II. The frequency of the severity of skin prick test reactivity to food and aeroallergens in the patients.

Food allergens	Mild reactivity N (%)	Moderate to severe reactivity N (%)	Aeroallergens	Mild reactivity N (%)	Moderate to severe reactivity N (%)
Milk, cow	16 (15.4)	8 (7.7)	<i>Dermatophagoides farina</i>	27 (26)	55 (52.9)
Egg (yolk)	27 (26)	12 (11.6)	<i>Dermatophagoides pteronyssinus</i>	32 (30.8)	45 (43.3)
Egg (white)	14 (13.5)	5 (4.8)	<i>Aspergillus</i>	29 (27.9)	33 (31.7)
Beef	23 (22.1)	13 (12.5)	<i>Alternaria alternata</i>	27 (26)	31 (29.8)
Fish	21 (20.2)	7 (6.7)	Cockroach	31 (29.8)	43 (41.4)
Shrimp	26 (25)	13 (12.5)	Dog epithelia	29 (27.9)	19 (18.3)
Strawberry	15 (14.4)	5 (4.8)	Feather	29 (27.9)	33 (31.7)
Orange	13 (12.5)	9 (8.7)	<i>Phoenix dactylifera</i>	28 (26.9)	28 (26.9)
Banana	12 (11.5)	7 (6.7)	<i>Citrus X sinensis</i>	33 (31.7)	32 (30)
Grape	21 (20.2)	6 (5.8)	<i>Conocarpus erectus</i>	32 (30.8)	29 (27)
Watermelon	26 (25)	4 (3.8)	<i>Morus rubra</i>	18 (17.3)	27 (26)
Tomato	19 (18.3)	5 (4.8)	<i>Elymus repens</i>	28 (26.9)	30 (28.8)
Walnut	23 (22.1)	10 (9.6)	<i>Phleum pratense</i>	31 (29.8)	27 (26)
Almond	20 (19.2)	10 (9.6)	<i>Chenopodium album</i>	27 (26)	33 (40.4)
			<i>Rumex acetosa</i>	30 (28.8)	26 (25)
			<i>Salsola kali</i>	36 (34.6)	38 (36.5)

72%, and 78%, respectively. There was no significant association between these factors and moderate to severe reactivity to *Conocarpus erectus* pollen ($P > 0.05$).

Discussion

The result of the present study for the first time in Iran showed that 58.6% of patients with respiratory allergies had positive SPT reactivity to *Conocarpus erectus* pollen, out of which 30.8% was mild, and 27.8% was moderate to severe. Our data showed that reactivity to *Conocarpus erectus* pollen was similar to other outdoor aeroallergens of trees in the region.

Over the last decade, *Conocarpus* trees have been extensively planted in the southern regions of Iran, especially in Khuzestan province (Namjoyan et al. 2020). Actually in Iran, *Conocarpus* trees are very common ornamental species in many cities including Ahvaz and Busheher (Namjoyan et al. 2020). During 2013 to 2015, there were reports of several respiratory attacks after the autumn rainfall in Khuzestan, especially in patients with history of seasonal allergies (Namjoyan et al. 2020). Accordingly, the presence of pollen from *Conocarpus* trees in the autumn season was considered to be one of the potential triggers of these attacks (Namjoyan et al. 2020). It has been suggested that airborne pollen grains, such as *Conocarpus erectus*, as well as fungal spores and high levels of air pollutants may trigger autumnal thunderstorm asthma attacks, and the frequency of thunderstorm asthma was reported to decrease in the Khuzestan province after pruning of the *Conocarpus erectus* trees (Rad et al. 2019).

In other countries, planted ornamental species may be an important source of pollen allergens. For example, in southern Spain, the Cupressaceae family has been cultivated in a large area for reforestation. A study conducted in this area showed that 30% of the allergic patients reacted positively to Cupressaceae pollen allergens by using SPT (Díaz de la Guardia et al. 2006). In another country Poznań (Poland), due to intensive planting of *Platanus* trees in the city, the pollen concentrations increased and exceeded the threshold above at which the first symptoms of allergy occur in people sensitised to *Platanus* pollen (Nowak et al. 2012). As the authors stated, the observed tendency to plant a lot of young *Platanus* trees in Poznań seems to be disturbing, as it can only worsen the allergological situation in the city (Nowak et al. 2012). Gassner et al. (2013) described another important example of the impact of ornamental trees on pollen allergy in the city of Buchs in Switzerland. The authors showed that after planting alien species of alder (*Alnus spaethii*), the prevalence of pollen allergy to alder pollen allergens markedly increased among schoolchildren (Gassner et al. 2013). Taken together, and based on the earlier-mentioned studies, it is clear that proper planning of urban green spaces is one of the most important issues in municipalities, and therefore the management of green urban spaces should be considered carefully, especially when green urban spaces are directly related to peoples' health, including those with respiratory chronic diseases (Cariñanos & Casares-Porcel 2011).

Furthermore, our study showed that among aeroallergens, the most frequent reactivity was related to

HDM, *Salsola kali*, and cockroach, respectively. This study also showed that the most sensitivity to trees pollen was *Phoenix dactylifera* (61.5%) in the region; however, the tree is the most cultivated one in Bushehr. Additionally, our data showed that sensitisation to indoor allergens such as HDM and cockroach were more frequent in the patients than outdoor allergens. Moreover, a significant association was found between the moderate to severe SPT reactivity to some aeroallergens and *Conocarpus erectus*. Furthermore, we observed that a cross-reactivity exists between some indoor and outdoor aeroallergens, and *Conocarpus erectus* pollen. A study which was conducted on 163 allergic patients in Qazvin, Iran, showed that the highest reactivity was to the weed pollens such as *Salsola kali* (58.9%) and HDM (21.1%) (Mahram et al. 2013). In addition, the present study showed that the frequency of positive reactivity to weeds (*Salsola kali*) and grasses (i.e. *Phleum pratense*) was high, which is similar to our previous study in which the pollen from weeds, grasses, and trees as outdoor allergens were the most prevalent in patients with AR in the region (Farrokhi et al. 2015). In a study conducted in Khuzestan province in the year 2013 on 410 patients with allergic diseases, they observed that weeds such as *Salsola kali* (72.2%), and *Amaranthus retroflexus* (70.7%) were the most common aeroallergens and *Prosopis juliflora* was the most allergenic tree in the region (Shakurnia et al. 2013). Besides, another study that investigated about 35 common aeroallergens in five groups, reported that grasses, trees, and HDM were with the highest frequencies (Pazoki et al. 2018). In addition, Mahram et al. (2013) reported that sensitivity to weed pollen (*Salsola kali*), grasses, *Birch* tree pollen, and wheat was the most common in patients with AR (Mahram et al. 2013). In contrast to our results, the study which was performed on 451 patients in Kuwait, showed that indoor allergens including HDM play a lesser role in respiratory allergic diseases, and the most common reaction in patients with AR was related to pollen grains of *Salsola imbricate* (Al-Dowaisan et al. 2004). The controversies over the results obtained from the studies in different regions of Iran and the world may be related to the type of vegetation in each particulate region. Therefore, we suggest a study with a large sample size and uniformly designed that might be helpful in southern regions of Iran.

In addition, the results of this study revealed that egg yolk, shrimp, and beef were the most reactive food allergens. Also, an association was observed between some food allergens such as cow's milk, egg (yolk and white), and *Conocarpus erectus* pollen.

Another study carried out in China reported that the highest food reactivity in the general population was to egg (3.0–4.4%), and shrimp (0.17–0.42%) (Chen et al. 2012). This is in line with our results showing that milk, egg yolk, and shrimp were the most common food allergens. Also, in accordance with our results, a study in Tehran, Iran, reported that egg yolk is one of the most prevalent food allergens (Pazoki et al. 2018). Moreover, Chen et al. (2012) reported that egg, milk, shrimp, and fish were the most common food allergens in children (Chen et al. 2012).

Finally, the level of serum total IgE, eosinophilia, vitamin D₃, FeNO, and PFT were not associated with the moderate to severe reactivity to *Conocarpus erectus* pollen.

Conclusions

The results of this study showed that the frequency of reactivity to *Conocarpus erectus* pollen was high and similar as the pollen grains from other trees widely grown in the southern part of Iran. Regarding the fact that *Conocarpus erectus* is widely cultivated in the region, and urban green spaces cause interaction between the citizens and the environment, the lack of proper planning in the selection of ornamental species can be an important triggering factor of allergic diseases in urban populations. Furthermore, this study can be a prelude to larger projects in which, the assessment of the prevalence of sensitivity to pollen grains of cultivated plants in each region could be done, also to investigate the main allergen proteins of the pollen grains. Finally, further studies should be designed that lead to the production of extracts for allergen immunotherapy based on local plants in each region.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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