Frequency of allergic sensitivity to local herbs pollen among medical students in East of Iran by use of skin prick test

Analiza uczuleń na pyłki lokalnych roślin wśród studentów medycyny we Wschodnim Iranie z użyciem testów nakłucia naskórka

Sajad Chamani[1,2], Sara Mahmoudzadeh[1,2], Hadis Rezapour[1,2], Mohammad Fereidouni[2,3]

[1] Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran
[2] Department of Immunology, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran
[3] Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

Summary

Background: Allergic disorders are among the most common health problems, have a high impact on patients' quality of life and constitute a significant economic burden. Pollen grains are the most significant trigger of allergic symptoms. Knowing pollen allergy potency of common plants in each area is essential for preventing allergic symptoms.

Objective: This study aims to evaluate allergic sensitivity to local plant pollen among medical students in East of Iran using skin prick test

Methods: Types of allergenic pollen grains were determined based on geoclimatic factors. Ten different pollen grains from common regional plants, including *Eucalyptus tree*, Helianthus trees, Rosa damascene flowers, Jasminum sp flowers, Chinaberry trees (Melia azedarach), pomegranate trees, black olive trees, pistachio trees, Palm trees, and Crambe cordifolia flowers, as well as seven standard commercial extracts including grass mixture, tree mixture, Fraxinus, Amaranthus retroflexus, Cypress, Betula, and Prosopis pollens were collected. After purification, the aqueous extract was prepared from each pollen. Skin prick test with ten domestic extracts and some commercial extracts was performed on the participants. The ethics committee of Birjand University of Medical Sciences approved the study and all participants signed consent form.

Results: One hundred and twelve volunteer medical students (mean age: 22.29 range: 19 to 34 years, M/F ratio:1.1) enrolled in this study. The overall frequency of sensitivity to any allergenic extracts was reported at 80.59%. In domestic pollen, the highest skin sensitivity rate was for Eucalyptus pollen, Rosa damascene flowers pollen, and Jasminum sp pollen (59.3%, 53.1%, and 50.0%, respectively). In the case of commercial extract, Amaranthus retroflexus and the tree mixture pollens were the most common (66.6% and 45.7%, respectively).

Conclusion: This study showed high allergic potency of some common regional plants, including Rose flower, Eucalyptus tree, and Jasminum sp flower.

Keywords: *Pollen allergy, Allergic rhinitis, Pollen, Prick test, Prevalence, Aeroallergen, Sensitization*

Streszczenie

Wprowadzenie: Choroby alergiczne stanowią jeden z najbardziej powszechnych problemów zdrowotnych, wpływają na jakość życia pacjenta i stanowią poważne obciążenie ekonomiczne. Ziarna pyłków roślin są najistotniejszym czynnikiem wywołującym objawy alergii. Znajomość siły alergizacji pyłków roślin występujących na danym obszarze ma zasadnicze znaczenie dla zapobiegania objawom alergii.

Cel pracy: Celem badania była ocena występowania uczuleń na pyłki lokalnych roślin wśród studentów medycyny we wschodnim Iranie za pomocą testów nakłucia naskórka (prick test).

Materiał i metody: Typy uczulających pyłków dobrane zostały w oparciu o analizę danych geoklimatycznych. Do badań użyto dziesięć ziaren pyłków roślin typowych dla regionu, w tym drzewa Eucalyptus, drzewa Helianthus, kwiatów Rosa damascene i Jasminum sp, drzewa Chinaberry (Melia azedarach), drzewa granatu, czarnych oliwek, drzewa pistacjowego, palm i kwiatów Crambe cordifolia, a także siedem standardowych, komercyjne dostępnych zestawów ekstraktów, w tym mieszanek traw, drzew i pyłków Fraxinus, Amaranthus retroflexus, Cypress, Betula i Prosopis. Po oczyszczeniu preparatów, przygotowywano wodne roztwory ekstraktów pyłków. Badania przeprowadzano u ochotników metodą nakłucia naskórka (prick test) ze wszystkimi dziesięcioma ekstraktami lokalnych roślin oraz kilkoma ekstraktami komercyjnymi. Badanie zostało zatwierdzone przez komisję ds. etyki Uniwersytetu Medycznego Birjand, a wszyscy uczestnicy podpisali formularz zgody.

Wyniki: W badaniu wzięło udział 112 studentów medycyny (średni wiek: 22,29, zakres: 19–34 lata, stosunek M / K: 1,1). Ogólna częstość występowania uczulenia na pyłki co najmniej jednego z ekstraktów wynosiła 80,59%. W przypadku pyłków roślin lokalnych największe odczyny skórne stwierdzono dla pyłków drzewa Eucalyptus, kwiatów Rosa damascene i Jasminum sp (odpowiednio 59,3%, 53,1% i 50,0%). W odniesieniu do ekstraktów dostępnych komercyjnie najczęściej stwierdzano uczulenia na Amaranthus retroflexus i mieszaninę pyłków drzew (odpowiednio 66,6% i 45,7%).

Wnioski: Wyniki badań wskazują na wysoką siłę alergizującą niektórych pospolitych roślin regionalnych, w tym kwiatu róży, drzewa eukaliptusa i kwiatu jaśminu.

Słowa kluczowe: *Alergia na pyłki, Alergiczny nieżyt nosa, Pyłki, Test punktowy, Częstość występowania, Aeroalergeny, Uczulenie*

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Adres do korespondencji / Address for correspondence
Mohammad Fereidouni
Cellular and molecular research center, Birjand University of medical sciences, Birjand, Iran.
Email address: dr.m.fereidouni@gmail.com
Telephone number: 09353886124

Introduction:

Allergy is an overreaction of the immune system to environmental substances that are not potentially harmful to the body. According to the World Health Organization estimates, 20% of people suffer from one type of allergic disorders [1].The prevalence of allergies in Iran is estimated to be 36.3% in some reports [2]. The prevalence of allergic rhinitis changes from country to country and region to region, and based on previous research, childhood asthma and allergies vary from 20 to 45% of the total country's population [3,4]. In recent years, various allergic disorders such as anaphylaxis, hay fever, atopic dermatitis, and asthma have increased [5]. This increase should be seen worldwide as a public health issue. During an allergic reaction, the human body produces a significant amount of immunoglobulin E in response to an allergen. Re-exposure to this antigen stimulates mast cells and releases inflammatory mediators such as histamine, which causes allergic symptoms. Allergen is an antigen that causes an allergic reaction. Various types of allergens include food allergens, pollen grains, house dust mites (HDMs), insect bites, latex, animal hair, and some medicine [6].

Allergic rhinitis is commonly known as „hay fever", characterized mostly by rhinorrhea, sneezing, itching, and nasal congestion [7]. It is the most common allergic disorder, and 400 million people worldwide suffer from allergic rhinitis [1].

Variety in climatic conditions and vegetation of each region leads to certain plants' growth in that region [8]. Iran has a hot, dry climate described by long, dry summers and short, cool winters. These special climatic conditions have led to the growth of a wide range of plants. The eastern part of Iran includes several provinces with arid to semi-arid climate, low rainfall, and hot summers. Despite the geo-climatic situation, many different plant species, including trees, grass, weeds and flowers, grow in the area, especially in the cities and towns where the municipality does the artificial watering. These region's special climatic conditions led to many plants' growth, which we selected ten plants in this study. These plants have been selected due to their abundant growth in Iran. In addition to their enormous use in the region.

Considering the high prevalence of allergic rhinitis and the importance of pollen grains in triggering symptoms and regarding the lack of information about the allergenicity of some common pollen in the east of Iran, this study aimed to determine the frequency of skin sensitivity to ten pollen extracts, to control and prevent allergies to these plants

Methods:

Study population and design:

This cross-sectional study was conducted on 112 randomly selected atopic or non-atopic medical students of Birjand University of Medical Science (BUMS), Birjand, Iran. The Ethics committee of BUMS approved the study (Code IR.BUMS.REC.1398.281), and a consent form was received from all participants. A questionnaire (based on ISAAC [9]) collected demographic data and information about the history of any allergies and drug use. Participants who had a severe health problem or using medication that interact with the Skin Prick Test (SPT) excluded from the study. The diagnosis of atopic dermatitis was confirmed using Hanifin and Rajka criteria. The parameters, consisting of four major and 23 minor factors, include a collection of clinical symptoms, aggravating or environmental conditions, irregular results from invasive testing, ophthalmic findings, and personal or family history of atopic diseases [10].

Pollen grains collection and extraction:

Polliniferous materials from *Eucalyptus* tree (blue gum), *Helianthus* trees (sunflower), *Rosa damascene* flowers (Damask rose), *Jasminum sp* flowers (Persian Jasmine), *Chinaberry* trees (*Melia azedarach*), *Punica granatum* trees (pomegranate,) *black olive* trees, *Pistacia vera* trees (pistachio), *Phoenix dactylifera* trees (Palm) and *Crambe cordifolia* flowers (Flowering Sea Kale) were collected by vacuuming during the pollination period from different plants in different parts of the region, from April 2019 to October 2019. Collected pollen was dried in a clean container at 37°C and then passing serially through 500, 300, 150, 100 microns and smaller meshes until the purity of >95% was achieved by microscopy examination. The image of each plant and its corresponding pollen grains micrograph was shown in Figure 1.

Pollens extraction was performed according to the protocol used in previous studies [11]. In brief, 200 mg of each pollen grains, defatted two times in 10 milliliters of acetone at 4 ° C overnight. After drying, the residual pollen content was powdered in a mortar and mixed with PBS buffer ten times at 4°C overnight while shaking. The next day, the solution was centrifuged at 10,000 g at 4ºC for 30 min. The supernatant was removed, and its protein concentration was measured by the Bradford method. The extract was then sterilized by passed through a 0.22 um syringe filter (SORFA, China). And mix with Glycerin in a 1:1 ratio in a clean vial and stored at 4ºC. To confirm the sterility of prepared extracts, 50 microliters of each extract, was added to an LB agar plate and incubated for 48 h at 37°C. The process of extracting the pollen and filtration is done again.

Gel electrophoresis of pollens extracts:

SDS-PAGE electrophoresis on 12.5% polyacrylamide gel was performed to confirm all allergenic components' presence. Briefly, six microliters of the extract were mixed with 24 microliters of sample buffer (4x) and then heated to 95 ° C for 2 minutes. Then, 20 microliters of each extract were loaded into the gel. Multicolored protein marker (Bio-Rad, Hercules, CA, USA), is used to estimate molecular weight. Coomassie Brilliant Blue staining was used to visualize protein bands. Figure 2 shows the electrophoresis profile of ten extracts.

Skin Prick Testing:

To evaluate the safety of prepared extracts, first, all ten extracts were tested on the skin of six volunteers, including the research team, and the immediate and late reactions during the first 24 hours, and the following week were recorded to see if there are any side effects. After ensuring the safety of the extracts. In addition to ten native plants, to evaluate the cross-reactivity and compare the allergenic potency of these plants, the sensitivity to 7 common allergenic plants including grass mixture (*Agrostis gigantea* pollen, *Dactylis glomerata* pollen, *Festuca pratensis* pollen, *Lolium perenne* pollen, *Phleum pratense* pollen, *Poa*

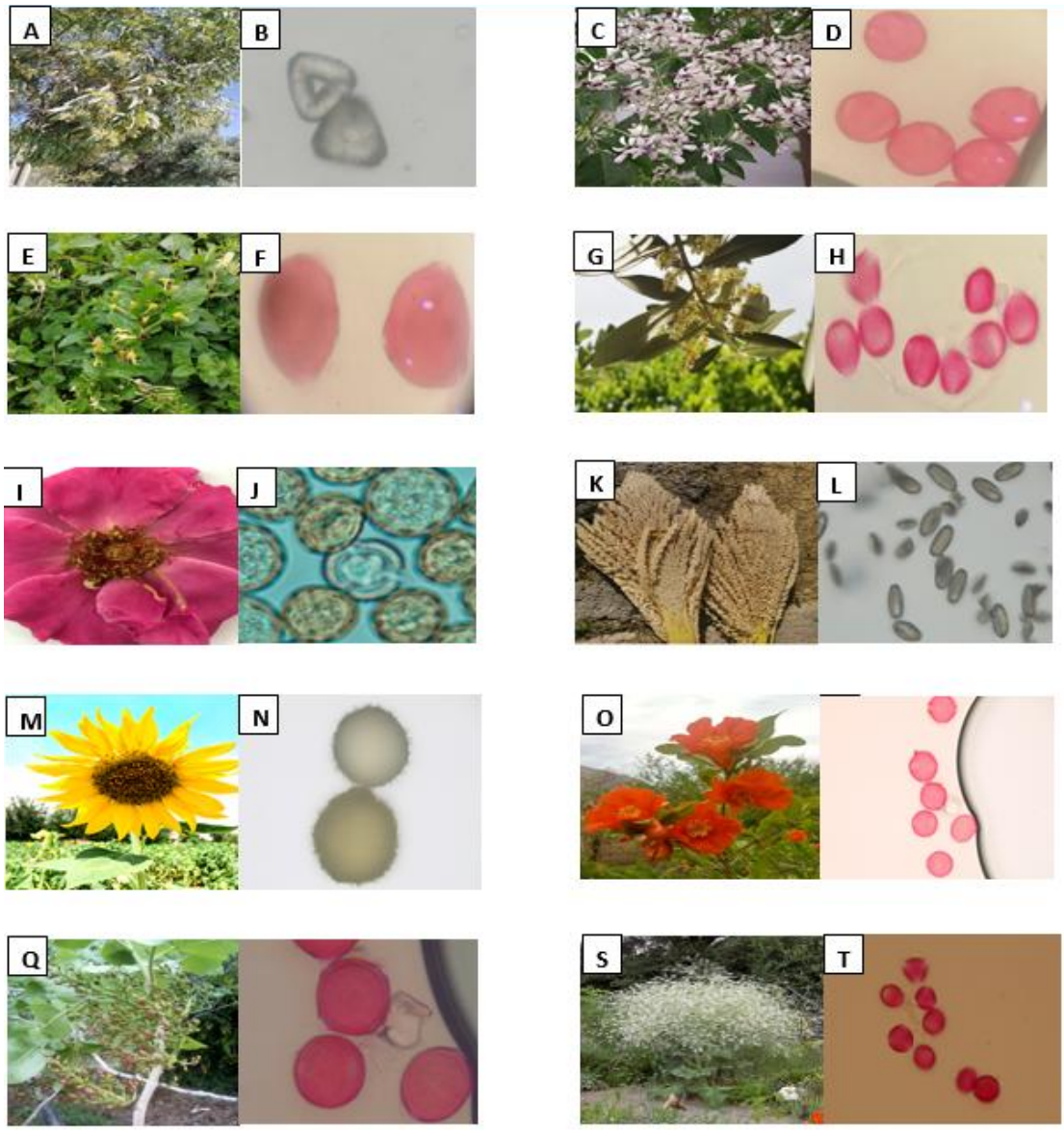


Figure 1: The flower image of each plant and its pollen grains' microscopic appearance are shown. Pollen grains images are photographed at 1000× zoom, using a light microscope. A) *Eucalyptus* tree's flower, B) Pollen grains of *Eucalyptus* tree's flower, C) *Chinaberry* tree's flower, D) Pollen grains of *Chinaberry* tree's flower, E) *Jasminum sp* flower, F) Pollen grains of *Jasminum sp* flower, G) Black olive tree's flower, H) Pollen grains of Black olive tree's flower, I) *Rosa damascene* flower, J) Pollen grains of *Rosa damascene* flower, K) *Palm* tree's flower, L) Pollen grains of *palm* tree's flower, M) *Helianthus* tree's flower, N) Pollen grains of *Helianthus* tree's flower, O) *Pomegranate* tree's flower, P) Pollen grains of *Pomegranate* tree's flower, Q) *Pistachio* tree's flower, R) Pollen grains of *Pistachio* tree's flower, S) *Crambe Cordifolia* flower, T) Pollen grains of *Crambe cordifolia* flower

pratensis pollen, and *Anthoxanthum odoratum* pollen), tree mixture (Betula pendula, olive tree, poplar, peanut, oat, black alder, and white ash), *Fraxinus*, *Amaranthus retroflexus, Cypress, Betula,* and *Prosopis* pollens (Hollister-Stier, USA) were performed for all participants by an expert allergist, according to European guidelines [12]. Next, the mean diameter of wheal reaction in every patient was measured and compared with negative (50% glycerine solution in PBS. pH 7.4) and positive (Histamine dihydrochloride, 10mg/ml) controls. Patients with a wheal diameter more than 3 mm greater in diameter than the negative control, surrounded by erythema, were designated as a positive SPT. The size of the wheal is an indication of the severity of allergic reaction

Statistical Analysis:

The data were analyzed by SPSS version 21 software (Chicago, USA). A chi-squared test was used for comparison of frequencies. A P-value of less than 0.05 was considered significant.

Results:

Demographic data

In total one hundred and twelve students enrolled in this study and nine were excluded. Finally, 103 cases remained in the study (mean age: 22.29 range: 19 to 34 years, M/F ratio:1.1). Based on the questionnaire form, the prevalence of current allergic rhinitis among the participants was 44.6 %.

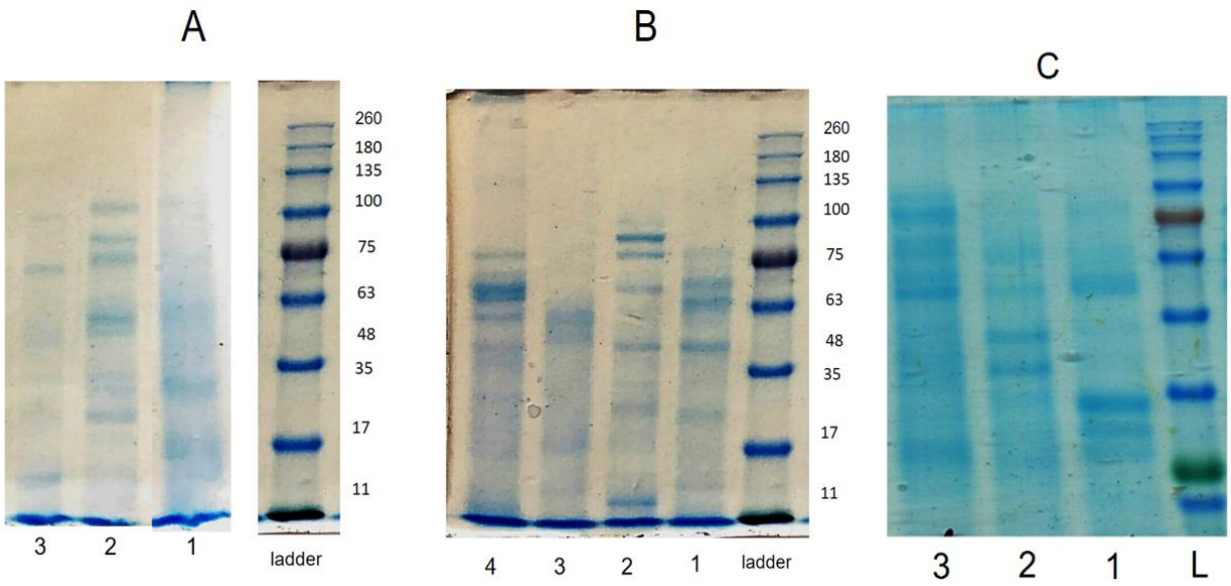


Figure 2. protein profile of pollen grains. A) Lane 1: *rosa damascene* flower pollen extract, lane2: *Helianthus* tree pollen extract, lane3: *Pomegranate* tree pollen extract B) Lane1: *Persian jamine* flowers pollen extract, lane2*: pistachio* tree pollen extract, lane3: *Palm* tree pollen extract, lane4: *crambe cordifolia* flowers pollen extract C) Lane1: *Eucalyptus* tree pollen extract, lane 2: Chinaberry tree pollen extract, lane 3: *black olive* trees pollen extract

Table I shows the prevalence of different allergy-related symptoms among participants who remained in the study based on the questionnaire.

Protein profile of pollen grains:

The prepared extracts' protein concentration measured by the Bradford method was between 800 μg/mL for black olive tree pollen to 3700 μg/mL for Rosa damascene pollen. Analysis of SDS-PAGE showed that almost all the prepared extracts have major and minor allergenic bands, although a different bands' intensity (Figure 2).

Skin Prick Testing:

In total,80.59% of cases had a positive skin test to at least one extract and 67% had a positive skin test to one of the ten domestic extracts. Among domestic extracts, the highest and lowest rate of skin sensitivity in the atopic patient were related to the pollen extracts of Rosa damascene flowers (59.37%), Eucalyptus tree (53.12%), and Jasminum sp flowers (50%). The mixture of grasses and Amaranthus retroflexus had the highest rate of sensitivity among the atopic group. There was no significant difference in each pollen extract's sensitivity in the SPT between men and women except in Jasminum sp, Betula, and Cypress pollen extract. None of the participants reported any moderate or severe side effects during a week after testing. Table II shows the prevalence of skin reactivity to extracts. Also, the mean wheal size of each pollen extract is shown in Table III. 63.4 % of patients were poly-sensitized (positive skin reaction to more than three allergens) and about 18.75 % of them had positive SPT to more than ten different allergens. The highest rate was between Amaranthus, grass mixture, Tree mixture, Rosa damascene, and Eucalyptus in co-sensitization. Table IV shows cross-reactivity in allergic reactions between extracts of each pollen

Table I. Prevalence of allergy symptoms in participants according to the questionnaire form

Mean age	19.13 (19-34)
Female	51.45 % (53)
Male	48.54 % (50)
Active rhinoconjunctivitis	44.66 % (46)
Family history of allergy	52.42 % (54)
Wheezing	20.38 % (21)
Dyspnea	26.21 % (27)
Dry cough	16.50 % (17)
Itchy skin lesions	10.67 % (11)
Eczema	21.35 % (22)
Itching and tearing eyes	78.64 % (81)

Discussion:

Pollen grains are the most important triggers of allergic symptoms worldwide, but the pattern of sensitivity varies in different regions. This study was conducted to evaluate the allergenicity of ten standard regional pollen extracts by SPT in the East of Iran.

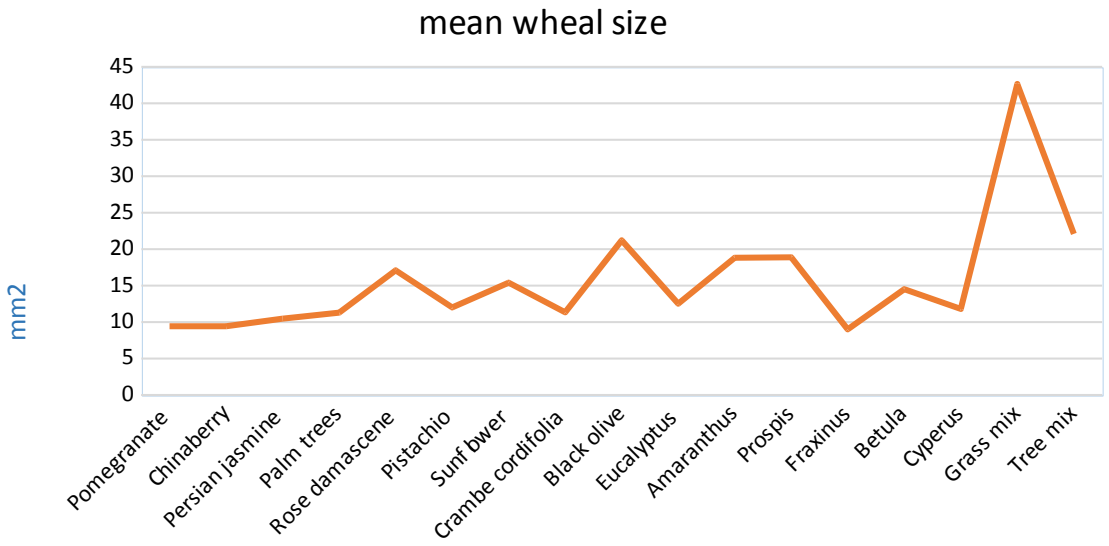
Our study showed the allergenicity of ten pollen plants in the region. Identifying allergenic plants can help control and prevent allergic rhinitis. *Rosa damascena* and *Jasminum sp* were among the most allergenic pollen in our study. These plants grow more in areas such as Iran, with cold and semi-arid climates in winter and temperate and dry in summer. The plants used in our study grow to a large extent in the region and they are widely used for economic, agricultural and decorative purposes, and therefore the sensitivity to these plants can be high. Insect pollination is very common among plants. Flowers that are pollinated

Table II . Frequency of positive skin prick test for individual pollen extract among participants

Aeroallergens family	Latin name	Common name	Atopic group(%) (n)[1]	All patients(%)	Male	Female	P value (Males vs. Females)
Myrtaceae	Eucalyptus trees	Blue gum	52.17 (24)	26.21 (27)	13.59 (14)	12.62 (13)	0.94
Rosaceae	Rosa damascene flowers	Damask rose	58.69 (27)	29.12 (30)	16.50 (17)	12.62 (13)	0.62
Plantae *	-	Tree mixture	45.65 (21)	32.03 (33)	17.47 (18)	14.56 (15)	0.36
Oleaceae	Jasminum sp flowers	Persian Jasmine	50 (23)	22.33 (23)	14.56 (15)	7.76 (8)	0.15
Amaranthaceae *	Amaranthus retroflexus	red-root amaranth	65.21 (30)	47.57 (49)	25.24 (26)	22.33 (23)	0.66
Meliaceae	Chinaberry trees	Melia azedarach	21.73 (10)	10.67 (11)	5.82 (6)	4.85 (5)	0.60
Oleaceae*	Fraxinus	Ash	15.21 (7)	8.73 (9)	4.85 (5)	3.88 (4)	0.68
Lythraceae	Punica granatum	pomegranate trees	15.21 (7)	6.79 (7)	3.88 (4)	2.91 (3)	0.60
Fabaceae*	Prosopis velutina	velvet mesquite	26.08 (12)	16.50 (17)	8.73 (9)	7.76 (8)	0.84
Arecaceae	Phoenix dactylifera trees	palm trees	28.26 (13)	13.59 (14)	8.73 (9)	4.85 (5)	0.62
Betulaceae*	Birch	Betula	13.04 (6)	7.76 (8)	5.82 (6)	1.94 (2)	0.28
Oleaceae	Olea europaea	black olive trees	13.04 (6)	6.79 (7)	3.88 (4)	2.91 (3)	0.58
Cupressaceae*	Cupressus	cypress	10.86 (5)	8.73 (9)	2.91 (3)	5.82 (6)	0.20
Anacardiaceae	Pistacia vera	pistachio trees	21.73 (10)	10.67 (11)	3.88 (4)	6.79 (7)	0.44
Poaceae*	*	grass mixture	71.73 (33)	63.10 (65)	33.01 (34)	30.09 (31)	0.96
Brassicaceae	crambe cordifolia	Sea Kale flowers	19.56 (9)	8.73 (9)	5.82 (6)	2.91 (3)	0.34
Asteraceae	Sunflower	Helianthus flower	19.56 (9)	8.73 (9)	4.85 (5)	3.88 (4)	0.94

*: commercial pollen extracts, [1]: diagnosis of atopic dermatitis was presented using Hanifin and Rajka criteria, n; Number

Table II . Frequency of positive skin prick test for individual pollen extract among participants



by insects have developed special adaptations. These flowers attract insects with their color, smell and taste. Also, in some of the plants we study, such as *Rosa damascena* and *Jasminum sp* flowers, in most areas, pollination is mainly done by insects. But in eastern areas of Iran such as Birjand, due to desert climate and low humidity and strong winds, pollen from plants which in Europe are counted as insect-pollinated is probably also transferred by the air. So far, no similar study has been performed on the pollen allergy of these two plants. More than half of the atopic individuals in our study (53.12%) were sensitive to the Eucalyptus pollen, which was near related to the study conducted in Kuwait [13] and higher than the study conducted in Australia and Turkey due to different vegetation and climatic conditions [14, 15].

Around one-third of atopic people were allergic to one type of *olive* pollens (*Chinaberry* and *black olive* trees), consistent with studies conducted in Zanjan City [16], Saudi Arabia [17] and, western Turkey [15] However, reports from Pakistan [18] and, Spain [19] showed a lower prevalence. *Palm* trees are abundant in the middle east region and also grows in the central desert of Iran. The frequency of allergy to *palm* pollen was not high, which was similar to a study conducted in Spain[20], but lower from reports from Saudi Arabia [17] and Kuwait [13]. possibly because of the higher number of palm trees in these countries. The sensitivity to *pistachio* pollen was similar to the studies conducted in Palestine [21]. One-fifth of our atopic cases were sensitive to *Helianthus* pollen, which was similar to studies in India [22], Turkey [23], and Spain [24].

Melia azedarach, commonly known as the *chinaberry* tree, is another plant found in abundance in Iran. In our study, 6.85% of all skin test participants were sensitive to *chinaberry* tree pollen extract, similar to a study in India [25].

Also, 15.62% and 18.75% of atopic individuals were allergic to *pomegranate* tree and *Crambe Cordifolia* flowers pollen extracts. A similar study has not been performed on the sensitivity of these two plants.

There were no statistical correlations between gender and susceptibility to inhaled allergens. This is similar to the research done by Oskouei in Mashhad [26] and Assarehzadegan in Ahvaz [27]. Different grasses and weeds grow abundantly in the east of Iran as they are adapted well to the geo-climate situation, and their pollen grains spread quickly because of the low humidity and wind. In commercial extracts, the highest sensitivity was for tree and grass mix extract, similar to studies in Turkey [28], southern [27,29], and northeastern Iran regions [26].

In our study, more than half of the patients had a positive SPT to *Amaranthus retroflexus* pollen, consistent with the studies conducted by Fereidouni [30], Assarehzadegan [27], and Tehrani [31] in Mashhad.

In domestic pollen, the mean wheal size was largest for *Black olive, Rosa damascene* flowers, and *Helianthus,* which shows their potential in allergenicity.

In our study, the presence of protein bands was confirmed by SDS-PAGE electrophoresis. The difference in the protein concentration of the extracts, the ingredients of each pollen grains, the Coomassie Brilliant Blue color's concentration, and the gel staining duration, causes the difference in the bands' intensity.

Conclusion:

The allergenicity of many types of pollen grains is unknown, and to our best knowledge, this is the first report about the allergenicity of ten common pollen in a relatively large number of patients. This study showed that some common pollen such as *Eucalyptus*, *Rosa damascene*, and *Jasminum sp* can cause allergic sensitivity in a large portion of atopic people and should be considered as a potent source of allergens. Knowing the allergenicity of regional plants' pollen is necessary and will improve allergic disorders' prevention and management. Further studies need to identify the exact allergenic component, which caused sensitivity for individual extracts.

Table IV. Cross-reactivity in allergic reactions between extracts of each pollen

Helianthus	9.70%	7.76%	3.96%	7.76%	8.16%	0.9%	3.125%
crambe cordifolia	2.91%	4.85%	3.92%	3.88%	4.08%	0.9%	2.08%
grass mix	16%	17.82%	28.88%	12.87%	42.22%	4.85%	9.37%
pistachio	8.73%	5.82%	2.97%	4.85%	5.88%	1.94%	1.04%
black olive	2.91%	3.88%	1.01%	3.88%	2.94%	0.9%	1.04%
palm	7.69%	7.76%	4.90%	6.79%	6.86%	3.88%	3.12%
Prosopis	3.88%	2.94%	9.37%	2.94%	13.54%	1.02%	6.25%
pomegranate	3.88%	5.82%	2.94%	4.85%	4.90%	1.94%	2.04%
Fraxinus	2.94%	2.94%	7.36%	2.94%	8.16%	2.04%	*
Chinaberry	5.82%	5.82%	2.91%	2.91%	4.85%	*	2.04%
Amaranthus retroflexus	14.70%	17.64%	24.49%	12.74%	*	4.85%	8.16%
Jasminum sp	14.56%	17.47%	10.89%	*	12.74%	2.91%	2.94%
Tree mixture	13/00%	13/00%	*	10.89%	24.49%	2.91%	7.36%
Rosa damascene	16.5%	*	13/00%	17.47%	17.64%	5.82%	2.94%
Eucalyptus	*	16.5%	13/00%	14.56%	14.70%	5.82%	2.94%
*	Eucalyptus	Rosa damascene	Tree mixture	Jasminum sp	Amaranthus retroflexus	Chinaberry	Fraxinus

Helianthus	3.88%	4.08%	4.85%	2.91%	6.80%	8.88%	2.91%	*
crambe cordifolia	2.91%	1.02%	2.91%	1.94%	1.94%	3.33%	*	2.91%
grass mix	5.20%	14.44%	7.77%	3.33%	7.77%	*	3.33%	8.88%
pistachio	1.94%	2.08%	1.94%	0.97%	*	7.77%	1.94%	6.80%
black olive	1.04%	1.04%	1.94%	*	0.97%	3.90%	1.94%	2.91%
palm	3.12%	3.12%	*	1.94%	1.94%	7.77%	2.91%	4.85%
Prosopis	1.04%	*	3.12%	1.04%	2.08%	14.44%	1.02%	4.08%
pomegranate	*	0/25%	3.12%	1.04%	1.94%	5.20%	2.91%	3.88%
Fraxinus	2.04%	6.25%	3.12%	1.04%	1.04%	9.37%	2.08%	3.125%
Chinaberry	1.94%	1.02%	3.88%	0.9%	1.94%	4.85%	0.9%	0.9%
Amaranthus retroflexus	4.90%	13.54%	6.86%	2.94%	5.88%	42.22%	4.08%	8.16%
Jasminum sp	4.85%	2.94%	6.79%	3.88%	4.85%	12.87%	3.88%	7.76%
Tree mixture	2.94%	9.37%	4.90%	1.01%	2.97%	28.88%	3.92%	8.16%
Rosa damascene	5.82%	2.94%	7.76%	3.88%	5.82%	17.82%	4.85%	7.76%
Eucalyptus	3.88%	3.88%	7.69%	2.91%	8.73%	16/00%	2.91%	7.76%
	pomegranate	Prosopis	palm	black olive	pistachio	grass mix	crambe cordifolia	Helianthus

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