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The comparison of allergenicity of *Platanus oriantalis* and *Olea europea*

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Abstract

In recent years the expansion of Tehran suburbs has increased the pollution of the city. Not only the amount of this pollution has not been stable, but it also has been on the rise. On the one hand, the necessity of constructing urban gardens and diversity of the species, and on the other hand, the removal of some existing species due to incompatibility has brought about addition of new species to flora of Tehran every now and then. *Olea europea* is suggested for cultivation in Tehran. The study indicates that skin sensitivity of *Olea europea* is less than *Platanus oriantalis*. However, Eosinophil fluctuations and Immunoglobulin E in guinea pigs' blood are almost the same as *Platanus oriantalis*. Olive cultivation is only suggested for clean parts of the city.

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Introduction

In recent years, suburban expansion of Tehran and nearby towns, an influx of large number of immigrants from every corner of the country, increase of vehicles and various polluting units, have turned the pollution of the city into a deep and fundamental challenge. The amount of the pollution has not only been unstable, but it has been on the rise year by year. On the one hand, the necessity of constructing urban gardens and diversity of the species, and on the other hand, the removal of some existing species due to incompatibility has brought about addition of new species to flora of Tehran every now and then. Introducing each new species entails consequences. Allergenicity of 75% plant species is caused by their pollens. In fact, some species have grown hundreds of years ago in Tehran, and in the meantime the skins of people have been relatively resistant to them. According to statistics, 10 to 15 percent of every community suffers from allergy. Announcement made by the ministry of health indicates that 20% of Tehran population is allergic. In other words, there are about 2 million allergic individuals in Tehran. Introducing new species without examining their allergic effects brings about serious problems to this huge community; and the number of newly affected individuals within this community may also increase (Moraghebi *et al.,* 2012).

By examining the influence of air pollutants emitted from stable and mobile resources, impacts of these pollutants on plants, and comparing the research results in Iran and other countries, it can be concluded that air pollution in Tehran has lead to some undesirable effects such as growth halt, decrease in absorption of necessary nutrients, contamination of water and soil, augmentation of tension on trees, and their gradual removal due to accumulation of toxicants in plant tissues (Mohammadi and Arman, 2010).

The study shows that more than 70% of trees along highways of Tehran are half-dead due to accumulation of layers of harmful particles on their branches and foliages. Moreover, due to severe air pollution plant species compatible with Tehran climate have been transformed from wide to needle-leafed ones gradually; and in fact, former buttonwood trees of Tehran turned into half-dead pine trees. This transformation is one of the other causes of increase in air pollution; because, air refinement capability of needle-leafed trees is much less than wide-leafed ones (Moraghebi *et al.,* 2012). In Finland, the effect of industrial pollution on the growth curve of *Empetrum nigrum* L. was investigated. Three zones were selected and observed based on their distances from the sources of pollution. Within these zones, species of the same age had the same linear growth. Although ages of the trees in non-polluted zones were one third of the ones in the polluted areas, their branches and leaves have grown three fold (Vitali, 2008).

Research conducted by Fernando *et al.,* (1999) , Moreno-Grau *et al.,* (2006), Feo- Brito *et al.,* (2011) show that *Olea Europea* is an allergic species.The studies erformed on *Platanus oriantalis* species indicate that this is an allergic species (Fernandez-Gonzalez *et al.,* 2010, Asero *et al.,* 2012, Alcazar *et al.,* 2011, Enrique *et al.,* 2004). But it is necessary to determine which species is less allergenic than others in order to cultivate it.The purpose of this investigation is to study whether Olea can increase the danger of allergenicity or not before it is cultivated copiously in Tehran.

Materials and methods

Plant selection

The data collection methods are as follow:

The survey of polluted and clean areas, selecting samples, taking samples- pollens are collected from the blossoms on the branches- separating anthers-cleaning anthers under the loupe and pollen collection. Then, pollens are kept in special containers with a vented lid made up of Organza textile. The containers were placed in three regions in the west and three regions in the center of Tehran (as polluted and clean areas) and they were examined after 7 to 10 days of exposure.

According to the report, the central region is polluted during most part of the year, and the western region has more clean air during most days of the year. If the allergenicity of polluted area is different from clean area, we can say that the allergenicity of the polluted area has intensified in relation to the clean area.

Skin allergenicity Analysis

Pollen extract, in phosphate buffer with different densities of 5 and 10 percent, was injected to three-month old and 300-gram Hartli guinea pigs. The control test was administered using salty phosphate buffer.

Also, sham (pure control) samples which received no injection were used for determining the effect of phosphate buffer. The tests administered for examining allergic symptoms will be applied in two ways: hypodermic around abdomen and eyes. Wheal and flare assessment was implemented according to millimeter; and it will be used as a criterion in evaluating and comparing the skin allergenicity intensity of pollen extract (Chelbian *et al.,* 2009). In the eye test, one to two drops of pollen extract are to be instilled to one of the guinea pig's eyes and the other eye will be considered as a control.

The test was repeated five times. Injection amount was 75 micro liters per test. The injection intervals were once in 7 days, which is good for eliminating allergic factors in the guinea pigs' bodies. Overall, there were 4 injections (Ghaemi *et al.,* 2010). Sampling was done 12 hours after the last injection.

Blood extensive Analysis

Blood extensives were used after staining using giesma method for counting blood cells and possible morphological changes. IgE assessment of blood serum using total IgE, and all serologic tests were carried out comparatively among sensitized animals and control sample. Full automatic Elisa instrument (Awareness stat fax 2100) was used for analysis. At first, according to the protocol of producing kit company - Glory Science Co - the standard curve was prepared by standard solution 64, 32,16, 8, 4, 2 micro gram in milliliter, and then the samples were evaluated and determined based on the standard curve (Fig. 1, 2, and 3).

Fig. 1. The pack containing standard immunoglobin kit of guinea pig and its standards.

Fig. 2. Full Automatic Elisa (Awareness stat fax 2100).

Fig. 3. Shaving the hair for injection.

Data Analysis

Results were obtained using SPSS software and Variance Analysis method (ANOVA analysis) and in case of observing any difference, average comparison will be implemented through Duncan method.

Results

Skin sensitivity measurement (Wheal and flare)

In this method, at first, part of the abdominal skin of guinea pigs was shaved and antisepticised. After 24 hours injections were administered in shaved parts. The highest inflation was observed one hour after the injection. It was carefully measured and recorded with millimeter precision. The data was used for statistical comparison (table 1).

Table 1. first data of Wheal and flare skin sensitivity.

sample	Repeat one	Repeat two	Repeat three	Repeat four	Repeat five	Average
clean Platanus 5%	19	21	13	15	17	17
clean Platanus10%	26	30	31	25	28	28
Contaminated Platanus 5%	25	35	22	28	26	27
Contaminated Platanus10%	27	27	35	24	28	28.2
clean Olea5%	20	18	17	1725	18	18
clean Olea10%	18	17	20	25	20	20
Contaminated Olea 5%	20	17	16	25	20	19.5
Contaminated Olea10%	17	18	25	20	19	19.8
Phosphate buffer	15	10	14	15	16	14

Phosphate Buffer

Phosphate buffer was used for solving pollens. Phosphate buffer was first injected to control guinea pigs, and then the skin sensitivity was measured. The average length of skin blister was measured 14 mm, and the height between 1 to 2 mm (table 1). Buffer and tree species were compared. Statistically, there was no meaningful difference between *Platanus oriantalis* and buffer but there was a meaningful difference at 5% between buffer and *Olea europea* extract.

Platanus oriantalis: with regard to table 2, comparing guinea pigs' skin sensitivity, the difference between treated clean *Platanus oriantalis* the 5% and the 10% was meaningful at 1%. The difference became meaningful at 1% between treated clean *Platanus oriantalis* 5% and contaminated ones 5%. Also, there was no meaningful difference between treated contaminated *Platanus oriantalis* 5% and contaminated ones 10%, clean *Platanus oriantalis* 10% and contaminated ones (table 2 and Fig. 4).

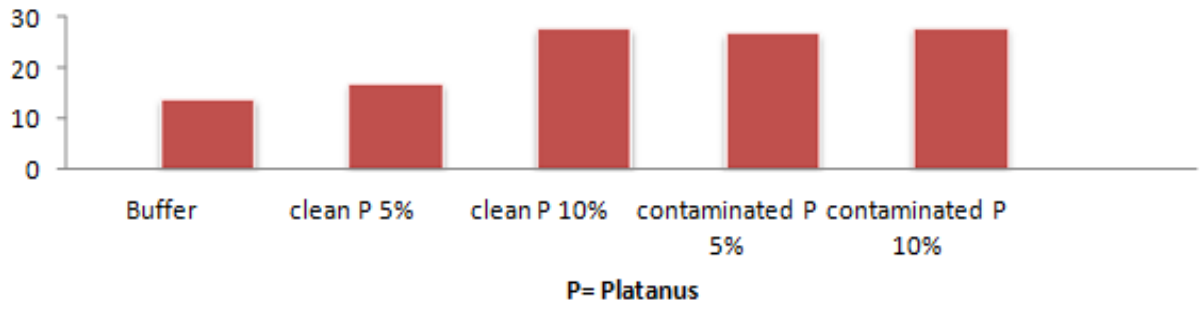

Fig. 4. Comparison of skin blister resulting from skin sensitivity of different treated *Platanus oriantalis* with phosphate buffer.

Table 2. Statistical comparison of *Platanus oriantalis* and *Olea europea* from the perspective of skin sensitivity with *Olea europea*- t-test.

sign	test	species	sign	test	species	sign	test	Species
ns	Clean 5%	Platanus	ns	Clean 5% and 10%	Olea	**	Clean 5% and 10%	Platanus
*	Contaminated 5%	Platanus	ns	Clean 5% and Contaminated5%	Olea	**	Clean 5% and Contaminated 5%	Platanus
**	Clean 10%	Platanus	ns	Contaminated 5% and Contaminated 10%	Olea	ns	Contaminated 5% and Contaminated 10%	Platanus
**	Contaminated 10%	Platanus	ns	Clean 10% and Contaminated 10%	Olea	ns	Clean 10% and Contaminated 10%	Platanus

ns statistically meaningless *difference at 5% is meaningful **difference at 1% is meaningful

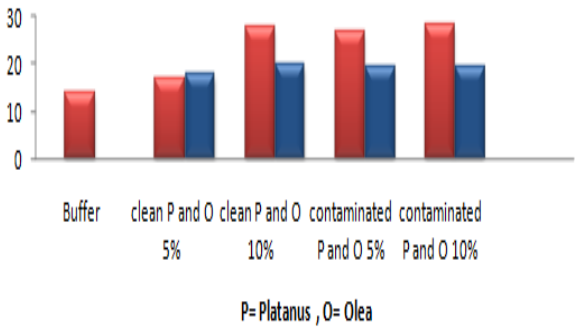

Fig. 5. The comparison of skin blister resulting from skin sensitivity of different treated *Olea europea* with *Platanus oriantalis* and phosphate buffer. Red column = *Platanus oriantalis*.

Olea europea: with regard to table 2, guinea pigs' skin comparison Wheal and flare, there was no difference between treated *Olea europea*. According to table 2 and Fig. 5, it is indicated that allergenicity of olive trees were clearly less than *Platanus oriantalis* especially at 10% density of clean and contaminated one.

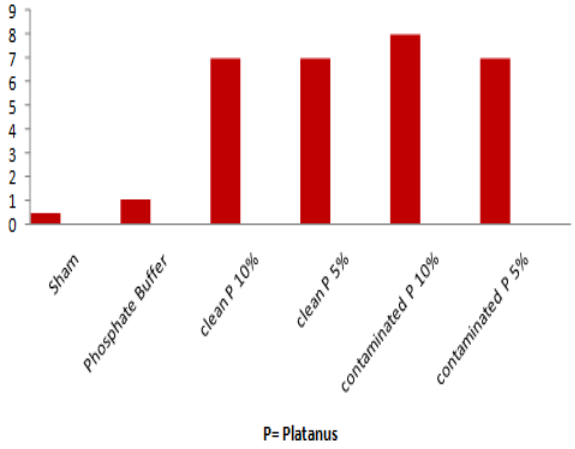

Fig. 6. The comparison of Eosinophils activity in different treated *Platanus oriantalis* with control and sham.

Cytological study

The measurement results of Eosinophils and Immunogolobin (IgE) are shown in table 3.

Table 3. Cytological raw data.

guinea pig number	Eosinophils %	Eosinophils average	IgE	IgE average	sample
1	13		7		
2	11		8.6		
3	3	7	7.6	7.5	clean Platanus10%
4	0		6.8		
5	7		5.5		
6	12		6.8		
7	11		7.3		
8	4	7	7.4	7.2	clean Platanus5%
9	1		7.2		
10	7		7.4		
11	15		7.5		
12	6		7.4		ContaminatedPlatanus10%
13	0	8	7.6	7.5	
14	11		7.4		
15	8		7.6		
16	1		8.2		
17	13		7.6		ContaminatedPlatanus5%
18	8	7	7	7.5	
19	8		7.6		
20	6		7.4		
21	10		7.7		
22	10		8.9		
23	0	5	7.7	7.8	Clean Olea 10%
24	2		7		
25	5		7.8		
26	9		7.5		
27	6		7.9		
28	12	9	7	7.4	clean Olea 5%
29	11		7.8		
30	9		7.2		

31	5		9.2		
32	12		9.2		
33	7	7	8.2	9	Contaminated Olea10%
34	6		9		
35	7		9.2		
36	0		9.6		
37	9		7.2		Contaminated Olea5%
38	4	5	8.1	8	
39	3		7.2		
40	5		8		
41	2		7.3		
42	1		8.4		
43	2	1	7.8	7.8	control
44	0		7.7		
45	0		7.9		
46	0		6.6		
47	0		7		
48	2	0.4	7.4	7	sham
49	0		7.1		
50	0		6.9		

Platanus oriantalis

Eosinophils data comparison in *Platanus oriantalis* is shown in Fig. 6. The figure indicates that *Platanus oriantalis* pollens had a meaningful difference of 5% compared to phosphate buffer samples and sham. In other words, pollens were the sensitivity factor regardless of density and collection area.

It is observed in *Platanus oriantalis* trees that although allergenicity in density and various areas was different, the difference is not meaningful in blood.

Olea europea

The comparison of Eosinophils data in *Olea europea* is shown in figure 4. The figure indicates that *Olea europea* pollens had a meaningful difference of 1% compared to sham samples and phosphate buffer. In other words, regardless of density and collection area, pollens were the sensitivity factor.

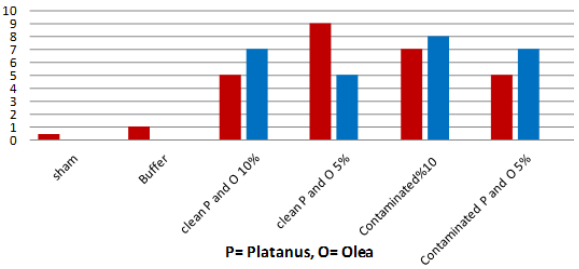

Fig. 7. comparison of Eosinophil activity in different treated *Olea europea* with *Platanus oriantalis,* phosphate buffer and sham. Red column = *Platanus oriantalis*

However, in 5% clean and contaminated *Olea europea*, 5% meaningful difference is measurable. It indicates that *Olea europea* trees cause allergenicity increase in contaminated areas. According to table 6 and Fig. 7, although allergy activity of *Olea europea* is less than *Platanus oriantalis* most of the time, this difference is meaningless statistically. It means that cultivating this tree does not lead to measurable allergy increase in blood. The comparison of immunoglobin activity in *Platanus oriantalis and Olea europea* is shown in table 5 and 6. Immunoglobin data comparison in *Platanus oriantalis* is shown in figure 5. Table 5 indicates that *Platanus oriantalis* pollens have a meaningful difference of 5% compared to sham samples.According to Fig. 8 and table 4, although the allergenicity of *Platanus oriantalis* pollens is different, this difference is not meaningful statistically.

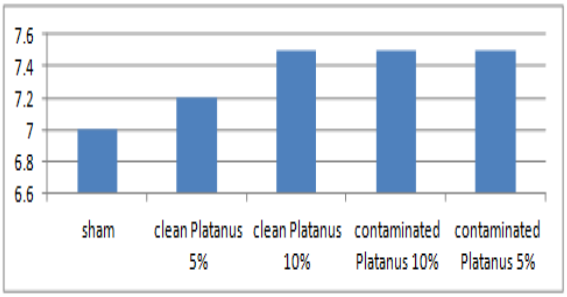

Fig. 8. comparison of immunoglobin activity in different treated *Platanus oriantalis* and sham ones.

Table 4. statistical comparison of Eosinophil data average based on t-test.

sign	test	species	sign	test	species	sign	test	species
ns	Clean 5% and 10%	Olea	ns	Clean 5% and 10%	Platanus	*	Sham- Platanus	
*	Clean5% and Contaminated5%	Olea	ns	Clean5% and Contaminated 5%	Platanus	**	Sham-Oleae	
ns	Contaminated 10% and Contaminated 10%	Olea	ns	Contaminated 5% and Contaminated 10%	Platanus	*	Buffer-Platanus	
ns	Clean 10% and Contaminated 10%	Olea	ns	Clean 10% and Contaminated 10%	Platanus	**	Buffer-Olea	

Olea europea

Immunoglobin data comparison in *Oleaeur opea* is shown in Fig. 9. Table 5 indicates that *Olea europea* pollens have no meaningful difference compared to sham samples.

According to Fig. 9 and table 5, allergenicity of *Olea europea* pollens in 10% contaminated areas is 1% meaningful, and in other cases is meaningless. In other words, cultivating *Olea europea* trees in contaminated areas leads to immunoglobin changes.

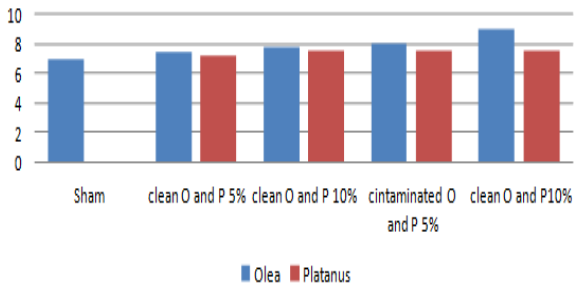

Fig. 9. comparison of immunoglobin activity in different treated *Platanus oriantalis, Olea europea* and sham ones. Red column = *Platanus oriantalis*.

Table 5. statistical comparison of IgE data average based on t-test.

sign	test	species	sign	test	species	sign	test	species
ns	Clean 5% and 10%	Olea	ns	Clean 5% and 10%	Platanus	*	Sham-Platanus oriantalis	
ns	Clean 5% and Contaminated 5%	Olea	ns	Clean 5% and Contaminated 5%	Platanus	ns	Sham-Olea europea	
ns	Contaminated 10% and Contaminated 10%	Olea	ns	Contaminated 10% and Contaminated 10%	Platanus	*	Buffer-Platanus	
**	Clean 10% and Contaminated 10%	Olea	ns	Clean 10% and Contaminated 10%	Platanus	ns	Buffer-Olea	

Statistical Comparison of Platanus oriantalis and Olea europea

According to the aforementioned table 6, it is shown that *Olea europea* causes less skin sensitivity than *Platanus oriantalis* in susceptible individuals; however, immunoglobin is increased in contaminated areas. It is a sign of more stimulation to allergy in sensitive individuals. Therefore, only this species can be cultivated in clean areas of Tehran.

Table 6. statistical comparison of Platanus oriantalis from allergic perspective with other species.

IgE	Eosinophils	Wheal and flare	Experiment	species
Non-significant	Non-significant	Non-significant	Clean5%	Platanus-Olea
Non-significant	Non-significant	significant	Contaminated 5%	Platanus-Olea
Non-significant	Non-significant	significant	Clean10%	Platanus-Olea
significant	Non-significant	significant	Contaminated 10%	Platanus-Olea

Discussion

With regard to their life spans, trees have been present as a permanent factor in urban plant flora, and they cannot be replaced immediately. Thus, their proper selection is of utmost importance. According to the research, *Olea europea* cultivation has been prioritized expansively in Tehran. Hence, this species was selected for the study.

Because of the extended length of pollination in consecutive days, allergenicity was investigated chronically. Guinea pigs were exposed to pollen extract around 4 times in a month. It, in turn, verifies the research works of Ghaemi *et al.,* 2010, Amjad *et al.,* 2006, and Fernandez-Gonzalez *et al.,* 2010 in which allergenicity is investigated during a couple of weeks.

For investigating allergenicity, Wheal and flare reaction was studied as a skin sensitivity. It verifies the research works of Rezanezhad and Majd, 2005, Chalbian *et al.,* 2009. In addition to that, statistical numbers of this research are in agreement with other research studies.

Immunoglobin as a prevalent measurement indicator in blood was examined. It verifies the research works of Fernandez-Gonzalez *et al.,* 2010, Asero *et al.,* 2004, and Enrique *et al.,* 2010. The resulting numbers are within the number range of research study of Chalbian *et al.,* 2009.

Platanus oriantalis Trees

It is shown in table 4 and 5 that *Platanus oriantalis* trees have allergenicity. It verifies the research works of Asero *et al.,* 2012, Fernandez-Gonzalez *et al.,* 2010, Alcazar *et al.,* 2011, and Enrique *et al.,* 2004 which explain that *Platanus oriantalis* trees have medium allergenicity. It is also noticed that there is no meaningful difference between *Platanus oriantalis* trees in clean and contaminated areas. It is verifiable according to pollen form; because pollen particles of these plants have a colpus which makes it less susceptible to accumulation of pollution. It is a verification on the research work of Ghaemi *et al.,* 2010 which states that pores on pollen particle cause pollution accumulation and allergenicity intensification.

Olea europea Trees

It is shown in table 2, 4, and 5 that *Olea europea* has allergenicity; however, this species causes less skin sensitivity compared to *Platanus oriantalis*. In polluted areas of Tehran, it can cause more allergic reactions than *Platanus oriantalis*. Fernando *et al.,* 1999, Moreno-Grau *et al.,* 2006, and Feo-Brito *et al.,* 2011, introduced *Olea europea* as an allergic species in their research studies. Generally, it can be said that there is possibility of cultivating *Olea europea* in clean areas of Tehran; however, if the assumption is set on pollution increase in coming years, cultivating this species must be avoided.

Environmental summary

The problem of pollution increase and gradual removal of *Platanus oriantalis* in Tehran has currently become more complicated than what has been thought before. *Olea europea* is rather a short-stemmed tree, and there are some controversies over plausibility of cultivating this tree in Tehran or some of its suburbs; but these species in respect to environment: air filtration power, carbon dioxide absorption, oxygen production, temperature decrease, and humidity increase are never comparable to tall *Platanus oriantalis*. According to Kyoto treaty, developing countries like Iran are committed to eliminating temperature focus resulted from big cities, and diminishing carbon dioxide emitted from fossil fuels. However, replacing *Platanus oriantalis* with short trees has led to urban temperature increase. Thus, it has caused the dissatisfaction among the people during hot summer days, and exacerbation of inversion phenomenon during cold seasons. It is also against Iran's commitments to international communities.

According to what have been discussed, it is better to do more research on preserving *Platanus oriantalis* in Tehran.

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