

A geometric morphometric study of the geographic populations of Asian citrus psyllid, *Diaphorina citri* (Hem.: Liviidae), in Iran and Pakistan

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Abstract

Asian citrus psyllid, *Diaphorina citri* Kuwayama, is an important pest of citrus that transmits the bacterial pathogens responsible for citrus greening disease, also called Huanglongbing. The aim of the current study was to investigate the shape variations of the forewing among the Iranian geographic populations of *D. citri*, as well as one population from Pakistan. The adult psyllids used in this study were collected on Lime, *Citrus aurantifolia* (Christm.) Swingle, from orchards in Southern Iran in 2011. The forewings of 12 *D. citri* populations (30 randomly selected adult females from each population) were examined using geometric morphometric analysis. Multivariate and univariate analysis of variance indicated significant variation of wing shape and size among the examined populations. Based on forewing shape, Sistan-Baluchestan and Pakistan populations were different from other populations, including Hormozgan (Minab, Rudan, Fareghan, Hajiabad), Kerman (Manujan, Kahnuij, Jiroft-Anbarabad, Jiroft-Blook, Orzoie), and Fars (Darab). Considering the following reasons, geographic variation was developed by the interaction of genetic mechanisms and environmental processes: (1) there was a significant difference in the shape and squared Mahalanobis distance between some populations, (2) the correlation between geographic distances matrix and Mahalanobis distances matrix was significant, and (3) the wings grew allometrically, and geographic populations had separate allometric direction but parallel to each others. It is suggested that wing morphology could be beneficial for the preliminary assessment of population structure and pest control methods of *D. citri* in Iran.

Key words: geometric morphometrics, Iran, Pakistan, Liviidae, *Diaphorina citri*

چکیده

بررسی ریخت‌سنجی هندسی جمعیت‌های جغرافیایی پسیل آسیایی مرکبات، *Diaphorina citri* (Hem.: Liviidae)، در ایران و پاکستان محمد رضا لشکری، احد صحراگرد، شهاب منظری، فریبا مظفریان و رضا حسینی
پسیل آسیایی مرکبات، *Diaphorina citri* Kuwayama، آفت مهم مرکبات است که عامل بیماری باکتریایی میوه‌سبز مرکبات، که Huanglongbing نیز نامیده می‌شود، را منتقل می‌کند. هدف از مطالعه‌ی حاضر، بررسی تفاوت‌های شکل بال جلو در میان جمعیت‌های جغرافیایی پسیل آسیایی مرکبات در ایران و همچنین یک جمعیت از پاکستان بود. در این مطالعه، حشرات بالغ پسیل از باغ‌های درخت لیمو، *Citrus aurantifolia* (Christm.) Swingle، از جنوب ایران در سال ۱۳۸۹ جمع‌آوری شدند. بال جلویی دوازده جمعیت پسیل آسیایی مرکبات (۳۰ پسیل بالغ ماده از هر جمعیت که به‌طور تصادفی انتخاب شده بودند) به‌وسیله‌ی آنالیز ریخت‌سنجی هندسی بررسی شد. آنالیز واریانس چندمتغیره و یک‌متغیره، نشان‌دهنده‌ی اختلاف معنی‌دار در شکل و اندازه‌ی بال جمعیت‌های مورد بررسی بود. بر پایه‌ی شکل بال جلویی، جمعیت‌های سیستان و بلوچستان و پاکستان از سایر جمعیت‌ها، شامل هرمزگان (میناب، رودان، فارغان، حاجی آباد)، کرمان (منوجان، کهنوج، جیرفت-عنبرآباد، جیرفت-بلوک، ارزوئیه) و فارس (داراب)، متفاوت بودند. با توجه به دلایل زیر، تنوع جغرافیایی با تقابل مکانیسم‌های ژنتیکی و فرآیندهای محیطی توسعه یافت: (۱) تفاوت معنی‌داری در شکل و مربع فواصل ماهالانوبیس (Mahalanobis) بین برخی از جمعیت‌ها وجود داشت، (۲) همبستگی بین ماتریس فاصله‌ی ماهالانوبیس و ماتریس فاصله‌ی جغرافیایی معنی‌دار بود و (۳) بال‌ها به‌صورت آلومتریک (allometric) رشد یافته و جمعیت‌های جغرافیایی، دارای جهت آلومتریک مجزا ولی موازی نسبت به هم بودند. می‌توان اظهار داشت که شکل‌شناسی بال شاید بتواند در ارزیابی‌های مقدماتی ساختار جمعیت و روش‌های کنترل پسیل آسیایی مرکبات در ایران مفید واقع شود.

واژگان کلیدی: ریخت‌سنجی هندسی، ایران، پاکستان، Liviidae، *Diaphorina citri*

Introduction

Asian citrus psyllid, *Diaphorina citri* Kuwayama, may be the most serious pest of citrus in the world if any of the pathogens that cause citrus greening accompany it (Halbert & Manjunath, 2004). This species has been reported from the Arabian Peninsula (Saudi Arabia and Yemen), Afghanistan, Pakistan, India, Japan, Taiwan, Hong Kong, China, Philippine

Islands, Indonesia, New Guinea, USA, Bahamas, Cayman Islands, Argentina, Jamaica, Guadeloupe, Dominican Republic, Puerto Rico, Cuba, Honduras, Mexico, Brazil, Paraguay, Uruguay, Venezuela and the Indian Ocean Islands of Mauritius and Réunion (Halbert & Manjunath, 2004; Boykin *et al.*, 2012). The first report of *D. citri* from Iran goes back to 2000 in the Southern part of Iran near the border of Pakistan

(Bové *et al.*, 2000). Afterward, Faghihi *et al.* (2009) found the citrus greening disease pathogens in *D. citri* and its host plants. The species is now established throughout the southern provinces of Iran, such as Sistan-Baluchestan, Hormozgan, Kerman and Fars, which are citrus growing regions (Salehi *et al.*, 2012). The citrus greening disease is also widely distributed in citrus growing regions of Sistan-Baluchestan and Hormozgan provinces (Salehi *et al.*, 2012).

A population is a group of conspecific individuals occupying a defined area that both ecological and reproductive interactions occur more frequently within the group than with the members of other populations (Futuyma, 1986). The differentiation of populations involves both genetic and environmental systems (Kim & McPheron, 1993). Populations of a species can be genetically differentiated by one of the four following events: (1) colonization of a new habitat or host, (2) colonization of a new territory or region, (3) landscape changes (bottleneck effect), and (4) genetic changes by stochastic events, such as gene flow, genetic drift and mutation or natural selection (Kim & McPheron, 1993). Interactions between each of these factors will depend on the species considered and environmental conditions. Important components of these interactions are the geographical scale, the weather, the extent of the treated areas relative to gene flow, and the cost of resistance for resistant genotypes in the absence of insecticides (Kazachova & Ekbohm, 2008). Another important component is the level of stress that insects must face in stressful environments such as agricultural ecosystems, which is much greater than in natural ecosystem. In stressed environments, insect pests must continually adapt to severe environmental conditions to survive (Kim & McPheron, 1993). Among the pests, invasive species are rapid evolutionary events (Sakai *et al.*, 2001). The term 'invasive species' is usually used to refer to introduced species that have a negative impact on the biodiversity, human economic activities, and health (Ross & Shoemaker, 2008). Introduced species that rapidly colonize in broad areas would lead to a higher occurrence of short-term stochastic or

deterministic evolutionary changes, which it might result in speciation. Another important factor in modulating these genetic effects is related to the mode of reproduction of the introduced organism (Lynch, 1984; Simon *et al.*, 1999). The most enduring concerns about invasive species could be considered as: the origin of the colonies, transportation of colonies from their native range to the new area they invade (Ross & Shoemaker, 2008), and also the possible difference of genetic diversity and biology of invasive species in their native vs. introduced areas (Sakai *et al.*, 2001).

In geometric morphometrics, a set of landmarks are incorporated to generate comprehensive information about the shape of specimens (Bookstein, 1996). Insect wings, as an old factor that has been used in many previous geometric morphometric analyses (Rohlf & Slice, 1990), are very beneficial in this regard, because they are suitable to be analyzed in two dimensions with biological realism. Generally, wing morphometric analyses can provide good insights into various categories, such as identifying populations within a species, as shown by the analysis of geographic variation in populations of *Drosophila lummei* Hackman (Haas & Tolley, 1998), *D. serrata* Malloch (Hoffman & Shirrifs, 2002), *Scythris obscurella* (Scopoli) (Roggero & Dentreves, 2005), *Calopteryx splendens* (Harris) (Sadeghi *et al.*, 2009). In Iran, several geometric morphometric studies have recently been carried out on some major lepidopteran insect pests, such as the study of geographic variations among the populations of *Chilo suppressalis* (Walker) (Zahiri, 2003), *Cydia pomonella* L. (Alipanah *et al.*, 2004; Khaghaninia *et al.*, 2008), *Ectomyelois ceratoniae* (Zeller) (Mozaffarian *et al.*, 2005, 2007b), and *Helicoverpa armigera* (Hübner) (Khiaban *et al.*, 2010a), as well as the variations among host-plant associated populations as in *E. ceratoniae* (Mozaffarian *et al.*, 2007a), and *H. armigera* (Khiaban *et al.*, 2010b).

Diaphorina citri causes severe damages to citrus trees in Iran. Despite its economic importance, the intraspecific variation of this species has not been

studied in different geographic areas in Iran. Therefore, in this study, the geometric morphometric method was employed to determine the morphometric variations among 12 populations of *D. citri*, 11 from Iran and one from Pakistan.

Material and methods

Psyllid samples

The adult psyllids, used in this study, were collected on Lime, *Citrus aurantifolia* (Christm.) Swingle, from orchards in the Southern parts of Iran (table 1, figs. 8-9), in 2011. An additional sample was provided by Dr. Shahid Nadeem Chohan (Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan).

Geometric morphometric analysis

A total of 360 specimens including 30 adult females from each population were randomly selected as sample size. The right forewing of each specimen was slide-mounted using Canada balsam as the mounting medium. Photographs were taken from the wings using a digital camera (Dino digital microscope) coupled to a stereo microscope with 30X magnification. Eleven homologous landmarks, type I, were digitized and subjected to geometric morphometric analysis (fig. 1). The raw x and y coordinates of the biological homologous landmarks were aligned and superimposed using the least-squares method based on the generalized Procrustes analysis (GPA) in a non-Euclidean Kendall's shape space and transferred to a linear Euclidean tangent space, allowing for the removal of variations which were not due to shape (e.g. rotation, movement) (Rohlf, 1999; Slice, 2001). Digitalization was performed using tpsDig software (Rohlf, 2004) and aligned individuals were compared using TpsRelw program (Rohlf, 2010), performing a thin-plate spline interpolation function to project the data onto a Euclidean plane.

The matrix of partial warp scores (the weight matrix, W) was used as a "data matrix" (18 variables) to perform various multivariate statistical analysis to

compare shape (Rohlf *et al.*, 1996). Size was eliminated by dividing the coordinates by centroid size. Maximal shape variation was shown along the two first relative warp axes to illustrate ordination of the shapes' consensus by a relative warp ordination plot using tpsRelw. In this analysis, alpha was set equal to zero to weight all partial warps equally as recommended by Rohlf for exploratory studies such as taxonomy (Rohlf, 1993).

The TpsSmall software, V.1.02 (Rohlf, 1997), was used to assess the correlation between Procrustes and the Kendall tangent space distances to ensure that the amount of shape variation in a data set was adequately represented after projection in the tangent space to allow subsequent statistical analysis. High correlation of Procrustes and the Kendall shape spaces ($R^2 = 1.000$) allowed further statistical testing using the projected data set (Rohlf, 1998b).

To determine whether geographically separated populations from different area differ in wing shape, a one-way multivariate analysis of variance (MANOVA) was performed with the squared Mahalanobis distance calculated on the landmark coordinates data set using SAS statistical program, V. 9.1 (SAS Institute, 2003). In this analysis, the Wilks' lambda was used and when the MANOVA showed significant overall differences between groups, the analysis proceeded by pair-wise comparisons (post-hoc) through pair-wise Hotelling's tests. Canonical variate analysis (CVA) was conducted on the landmark coordinates data set using NTSYSpc software (Rohlf, 1998a). Relationships among the populations were summarized by UPGMA clustering method using NTSYSpc program. Isolation by distance was assessed as the correlation between geographic and morphometric distance matrices by the Mantel test with 5000 permutations using the NTSYSpc program. Minimum geographic distances among populations were measured using the GIS software.

For overall wing size assessment, the centroid size was used and tested for normality using the Shapiro-Wilk test implemented in SAS statistical program. Leven's test was employed to test the

homogeneity of the variance (Milliken & Johnson, 1984). An ANOVA procedure was conducted on the whole data set to test centroid size differences among populations in SAS statistical program.

The null hypothesis, i.e. the shape of the wing in *D. citri* changes isometrically with an increase in size, was tested by regressing each partial warp on size using the TpsRegr program (Rohlf, 2009). The size of a landmark configuration was measured by its centroid size (Slice *et al.*, 1996). Subsequently, the homogeneity of regression slopes among the groups was tested as described by Rohlf (2009).

Results

Wing shape variation

Generalized Procrustes analysis superimposition of 360 *D. citri* specimens showed the range of variation at each landmark after the translation, rotation, and scaling of data from individual specimens (fig. 2). The first two relative warps (RW1 and RW2) explained 18.93% and 15.36% of the shape variation among specimens, respectively (fig. 3).

The positive and negative extremes of wing shape variation along RW1 axis are shown in fig. 4, representing the directions of maximal shape variation obtained from the principal component analysis (PCA) of the partial-warp scores (W matrix). However, the

scatter plot of first two principal components (PC-I and PC-II) (fig. 3) showed an overlap among Iranian populations but revealed shape variation between the Pakistani and all Iranian populations. Through PC-I, the vectors on landmarks showed their inclination, specially landmarks 1 and 2, to the apical part of the wing, which led to increasing distance between landmarks 1 and 2 with landmarks 6 and 8 (figs. 3-4). Moreover, the veins R, M1 and M2 in the positive extreme were longer than those in the negative one, resulting in a larger size of apical cell and changing the geometric characters (figs. 4, B and 4, D). In contrast, at the negative extreme, the outline landmarks (1, 2, 3, 4, 5 and 8) were more close to each other and defined a roughly concave shape (figs. 4, C, 4, E).

One-way MANOVAs revealed a significant difference among the shape components of the populations (Wilks' lambda = 0.454, $P < 0.01$). Therefore, the null hypothesis of MANOVA test, which assumes the distances among the groups are similar, was rejected. Pairwise generalized squared distances among groups showed significant differences between Pakistan (P) and Sarbaz (S) populations comparing to other populations (table 2). Three dimensional ordinations using the mean canonical variate scores (CV1 and CV2) of the 12 populations (fig. 5) gave the same result.

Table 1. Collection sites, codes, their geographic coordination, and the number of examined specimens for *Diaphorina citri* populations.

Locality	Region	Code	N.	E.	Altitude	n
Iran, Kerman	Orzoiye	KO	28°27'37.56"	56°7'35.64"	1030	30
	Jiroft-Anbarabad	KJA	28°16'0.88"	57°36'7.26"	508	30
	Jiroft-Blook	KJB	28°12'3.77"	57°28'0.48"	653	30
	Kahnuj	KK	27°43'7.42"	57°35'10.64"	343	30
	Manujan	KM	27°28'35.19"	57°30'57.74"	308	30
Iran, Hormozgan	Rudan	HR	27°10'3.6"	57°9'40.70"	370.5	30
	Minab	HM	26°52'55.7"	57°28'58.48"	185	30
	Fareghan	HF	28°02'1.56"	56°14'32.2"	1100	30
	Hajiabad	HH	28°18'32.1"	55°54'42.7"	731	30
Iran, Fars	Darab	FD	28°46'52.92"	54°22'13.62"	1094	30
Iran, Sistan-Baluchestan	Sarbaz	S	26°3'26.73"	61°24'25.45"	300	30
Pakistan, Punjab	Multan	P	-	-	50-380	30

The correlation coefficient is used as a measure of goodness of fit for a cluster analysis in NTSYSpc software. The dendrogram plotted by UPGMA method

(fig. 6) in the latter program had a very good degree of fit ($r = 0.96577$). Based on the cluster analysis, the Pakistan (P) and Sarbaz (S) populations were clustered

distinctly from the others. The KO and HH populations were quite similar to each other. Also, HM and HR were clustered together, and the result was the same for HF and FD populations. The KM population could be considered as the sister-group of remaining populations

(fig. 6). Mantel test revealed a significant correlation between geographic distances matrix and Mahalanobis distances matrix at $\alpha = 0.05$ ($R = 0.743$, $p[\text{rand } z \geq \text{obs } z] = 0.014$)

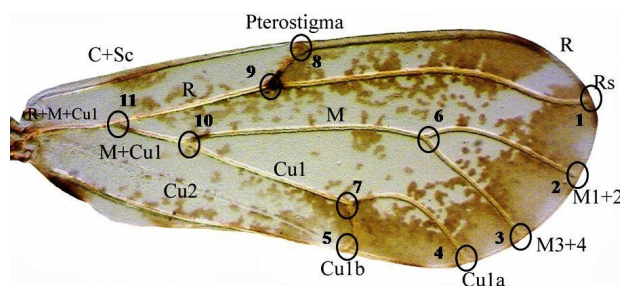


Fig. 1. Position of landmarks (circles) in the right forewing of *Diaphorina citri*. Wing vein terminology follows that of Hodkinson & White (1979).



Fig. 2. Superimposed landmarks obtained from the studied populations of *Diaphorina citri* using the generalized Procrustes analysis.

Wing size variation

The Shapiro-Wilk test revealed a normal distribution of all populations ($P > 0.05$) and the Levene's test showed the significant homogeneity of variances ($P < 0.05$) based on means. A one-way ANOVA indicated a significant difference in the centroid size ($F = 8.28$, $df = 11$, $P < 0.01$) among the populations. Pairwise comparisons of samples from the 12 populations, using HSD post-hoc test, indicated that Sarbaz (S) and Pakistan (P) populations had smaller wings size than the other ones (table 3). In contrast to wing shape analysis, the Manujan (KM), Pakistan (P) and Sarbaz (S) populations had no significant

difference among their wing size. Generally, based on the centroid size, two significantly different groups were obtained: (1) populations with small-sized wings including S, P and KM, and (2) those with large-sized wings, i.e. HF, HH, KJA, KJB, KK, KO, HM, HR, and FD (fig. 7).

The regression of partial warps on the centroid size was significant (Wiliks' Lambda = 0.894, $P < 0.05$). Comparing allometric slope and shape in constant size revealed the homogeneity of regression slopes (Wiliks' Lambda = 0.526, $P > 0.05$). In other words, geographic populations had separate allometric direction but parallel to each others.

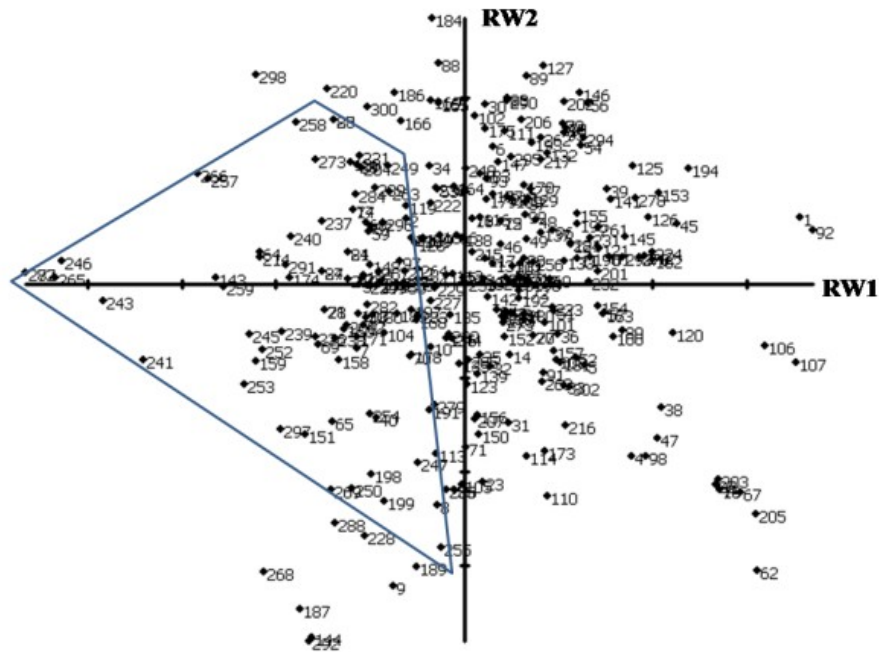


Fig. 3. Scatter plot of the first two principal components of the forewing of *Diaphorina citri*. The numbers (230-260) enclosed by a trapezoid show the Pakistan (P) population.

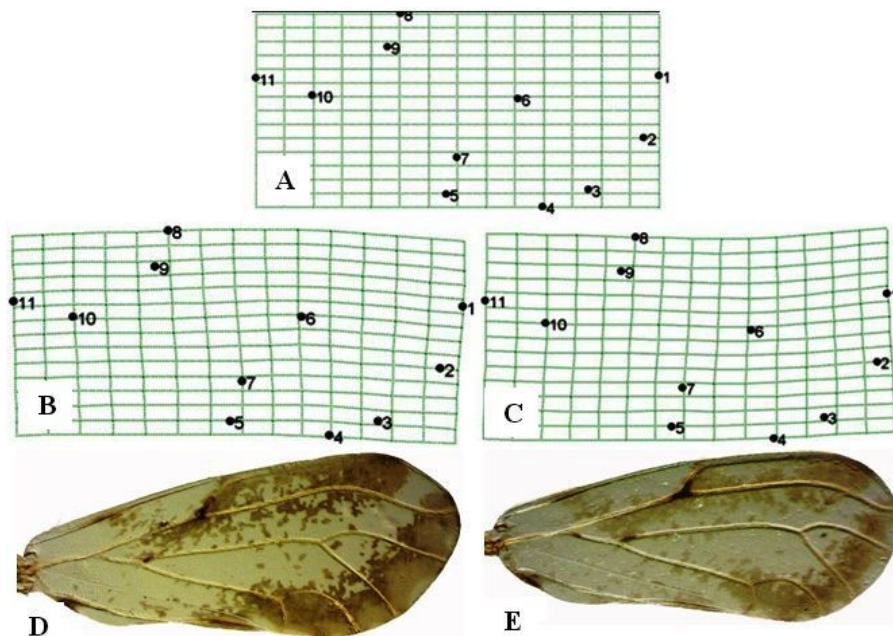


Fig. 4. Shape variation along the negative and positive extremes of RW1 in the wings of the Iranian populations of *Diaphorina citri*: A, the consensus configuration; B, positive deformation; C, negative deformation; D, wing deformation, positive extreme; E, wing deformation, negative extreme.

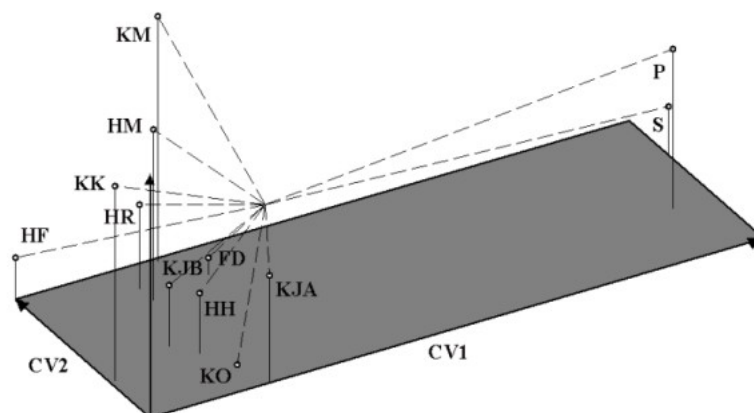


Fig. 5. Ordination of the group means along the first three canonical variate (CV) axes (the third axis not labelled) based on the partial warp scores matrix. Dotted lines show the edges of a minimum length spanning tree that joint neighbouring points based on the generalized distance matrix given in table 2. See table 1 for abbreviations.

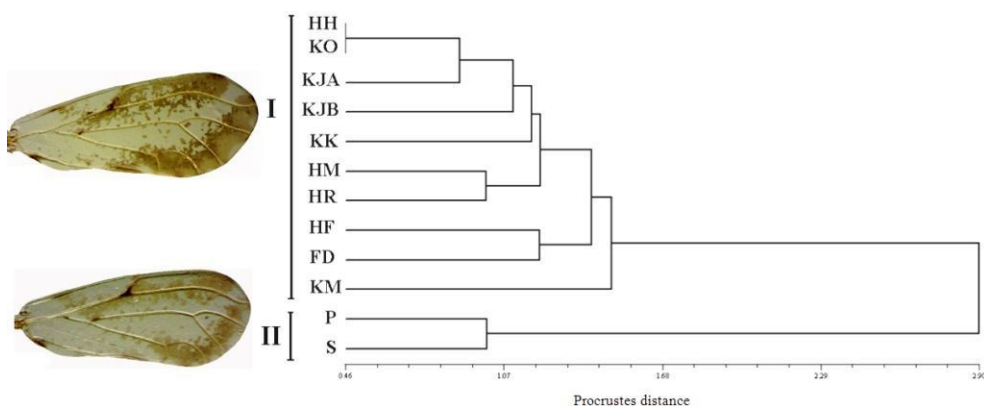


Fig. 6. Dendrogram plotted by UPGMA method based on clustering of the generalized distance matrix of *Diaphorina citri* populations. See table 1 for abbreviations.

Table 2. Generalized distance Matrix (Mahalanobis distances) among 12 populations of *Diaphorina citri*. P values based on Hotelling’s pairwise comparisons. See table 1 for abbreviations.

Pop.	FD	HF	HH	HM	HR	KJA	KJB	KK	KM	KO	P	S
FD	0											
HF	1.193	0										
HH	0.557	0.537	0									
HM	0.283	0.819	0.193	0								
HR	1.221	0.043	0.509	0.775	0							
KJA	0.410	0.810	0.139	0.093	0.815	0						
KJB	0.798	0.924	0.273	0.512	0.801	0.656	0					
KK	0.805	1.718	0.722	0.955	1.762	0.609	1.145	0				
KM	0.511	1.346	0.802	0.414	1.257	0.414	1.334	0.870	0			
KO	0.610	0.685	0.163	0.433	0.784	0.368	0.443	0.913	1.366	0		
P	4.16**	5.00**	5.66**	4.26**	4.74**	5.02**	5.69**	6.56**	3.05**	6.76**	0	
S	3.38**	4.15**	4.81**	3.68**	3.80**	4.53**	4.61**	5.95**	2.83**	5.75**	0.53**	0

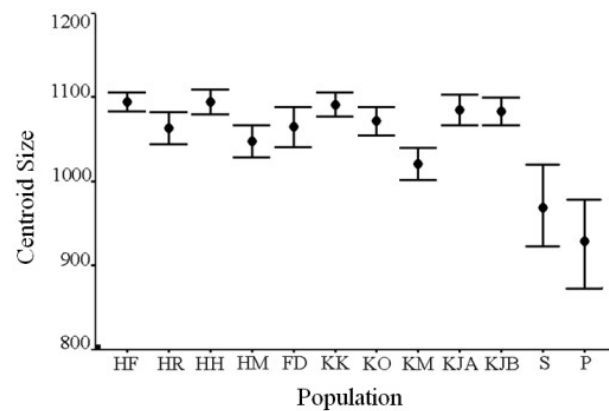
** Significant differences ($\alpha = 0.01$).

Pop. = Population.

Discussion

The results of the current study suggest that the examined geographic populations of Asian citrus psyllid can be categorized into two major groups. According to these results, there was a significant difference in the shape and squared Mahalanobis distance of Sarbaz (S) and Pakistan (P) populations compared to the other ones. Removal of size as a differentiation criterion is believed to reveal patterns of variation to be less influenced by environmental factors

Fig 7. The mean centroid size of 12 populations of *Diaphorina citri*. Black dots indicate mean values and whiskers show standard deviations. See table 1 for abbreviations.



According to the thin-plate spline deformation grids of wing shape variation, it seems that the apical half of the wing played a more important role than the basal part in population differentiation (fig. 4), which was in agreement with the results of Sadeghi *et al.* (2009) on significant differences among geographic populations of *C. splendens*. Based on the position of landmarks in deformation grids, two main types of wing deformation were distinguishable in the *D. citri* populations (fig. 4).

Distance can influence the gene flow among populations, even in species that are able to move long distances. This is known as “isolation by distance” (Wright, 1943; Hartl & Clark, 1989; Britten *et al.*, 1995). In the current study, it can be suggested that either Sarbaz (S) or Pakistan (P) populations had the least gene flow with the other populations because of some geographical barriers, such as Makran mountains

(Dujardin *et al.*, 1999) and, hence, much related to evolutionary differences (Hutcheson *et al.*, 1995; Dujardin *et al.*, 1999). As shown in fig. 5, the two populations, P and S, were obviously distinct from the others along the first three canonical variate axes based on partial warp scores matrix. However, the other populations were not quite distinguishable from each other. This was also confirmed in the cluster analysis of the examined populations (fig. 6).

and Espakeh desert (26°52' N to 27°4' N and 59°49' E to 60°31' E) in Sistan-Baluchestan province (fig. 8). There was also a significant correlation between form differences and geographic distances, suggesting that the amount of morphological differences could be predicted by the level of geographic isolation, without needing to infer them from climatic or ecological differences (Dujardin *et al.*, 1999). This result was consistent with that of Dujardin *et al.* (1999) and Mozaffarian *et al.* (2007b), who found a significant correlation between form differences and geographic distances in geographical populations of *Lutzomyia quinquefer* (Dyar) and *E. ceratoniae*, respectively.

Analysis of allometric relationships showed a significant shape difference among populations, even after removing the allometric component. This result was in agreement with that of Dujardin *et al.* (1999), Mozaffarian *et al.* (2007b) and Khiaban *et al.* (2010a),

i.e. founding a significant allometric growth in geographical populations of *L. quinquefer*, *E. ceratoniae* and *H. armigera*, respectively. These findings may indicate the genetic basis of the variation rather than environmental factors (Dujardin *et al.*, 1999).

Genetic forces (e.g. mutation and genetic drift) interact with natural selection favouring adaptation to local conditions, as well as leading to a genetic differentiation of local populations, whereas the force of gene flow opposes such differentiation. Moreover, a significant genetic bottleneck likely occurs when an expanding population colonizes a new locality (Kim & McPheron, 1993). A gene flow would have been occurred in the studied *D. citri* populations, especially Pakistan (P) and Sarbaz (S). It seems that transferring of agricultural materials, such as seedlings, may have also lead to the occurrence of gene flow among pest populations. This is expected considering the short geographic distance between citrus orchards in different locations in south of Iran, e.g. between Hajiabad and Orzoiye, Jiroft-Anbarabad and Jiroft-Blook, and Minab and Rudan.

Sarbaz (S) and Pakistan (P) populations had a smaller wing size compared with other ones, suggesting a temperature-dependent pattern and dependency on food quality. The observed size variation may be due to climatic elements such as temperature, as the studied geographic localities had different climates (fig. 9) and

altitudes (fig. 8). Additionally, the humidity and winter type of these localities were different. The HF, KO, FD and HH populations were collected from regions having a semi-arid climate, with cool winter and very warm summers (SA-C-VW); whereas, S, HM, HR, KJA, KJB, KM and KK populations belonged to arid climate regions, with moderate winter and very warm summer (A-M-VW). A number of studies reported that changes in the wing size in response to temperature led to changes in cell size and, to a lesser extent, in cell numbers (Alpatov, 1930; Robertson, 1959; Masry & Robertson, 1979; Partridge *et al.*, 1994). De Moed *et al.* (1997) showed that the wing length of *Drosophila melanogaster* Meigen, had a strong linear reduction with increasing temperature under optimal food conditions. However, under food-limited conditions, the response of the wing length levelled off at low temperature. Moreover, they argued that the wing size was highly sensitive to a reduction of food level.

It seems that the geographic variations observed in the studied populations were developed by the interaction of genetic mechanisms and environmental processes. According to Kim & McPheron (1993), these variations are expressed in various biological characteristics, including morphology, physiology, behaviour, life history traits and gene frequency. Such evolutionary processes would lead to the manifestation of different taxonomic status of local populations such as biotype and ecotype (Kim & McPheron, 1993). For

Table 3. Results of HSD (post-hoc) test on the wing centroid size of *Diaphorina citri* populations. See table 1 for abbreviations.

Loc.	HF	HH	KJA	KJB	KK	KO	HM	HR	FD	KM	S	P
HF	0											
HH	1.16	0										
KJA	8.97	7.81	0									
KJB	13.00	11.84	4.03	0								
KK	14.70	13.54	5.73	1.70	0							
KO	18.73	17.57	9.77	5.73	4.03	0						
FD	33.05	31.89	24.08	20.05	18.35	14.31	0					
HR	40.26	39.10	31.30	27.26	25.56	21.53	7.22	0				
HM	51.10	49.94	42.13	38.10	36.40	32.37	18.05	10.84	0			
KM	82.9**	81.7**	73.9**	69.90**	68.20*	64.17*	49.85	42.64	31.80*	0		
S	127.6**	126.5**	118.7**	114.67**	112.9**	108.9**	94.62*	87.40*	76.57*	44.77	0	
P	167.0**	165.8**	158.0**	154.0**	152.3**	148.2**	133.9**	126.7**	115.9**	84.1*	39.3	0

* Significant differences ($\alpha = 0.05$).

** Significant differences ($\alpha = 0.01$).

Loc. = Locality.

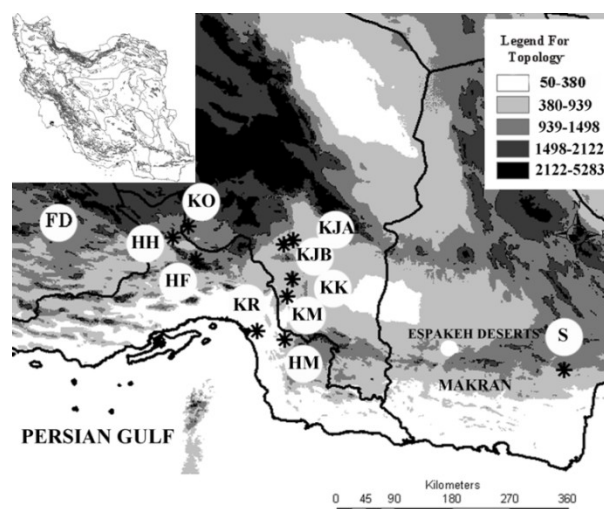


Fig. 8. Collection sites based on topology, where *Diaphorina citri* populations were collected. See table 1 for abbreviations and other geographical information.

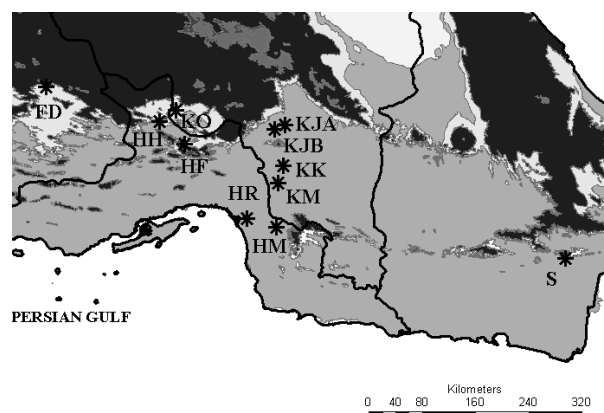


Fig. 9. Collection sites based on climate layers, where *Diaphorina citri* populations were collected. The HF, KO, FD and HH collection sites are located in semi arid climate, whereas S, HM, HR, KJA, KJB, KM and KK are located in arid climate regions. See table 1 for abbreviations.

example, *Ostrinia nubilalis* (Hübner) differs among populations by morphology (Kim *et al.*, 1967). Also, the three biotypes (B, C, E) of *Schizaphis graminum* (Rondani), show distinct morphometric differences (Inayatullah *et al.*, 1987).

Differences between the studied populations suggest that wing characteristics are valuable in the preliminary assessment of population structure and pest management strategies of *D. citri* in Iran. Definitely, for complete and precise conclusion, some molecular studies on these populations are required.

References

- Alipanah, H., Rezapanah, M. R. & Sari, A. S. (2004) Study of the morphometric variations of codling moth (*Cydia pomonella* L.) in some parts of Iran. *Proceedings of the 16th Iranian Plant Protection Congress, Vol. I, Pests*, p. 99.

Acknowledgements

We are grateful to Prof. J. Rohlf (State University of New York, USA) for valuable technical advice. We are also grateful to Dr. Shahid Nadeem Chohan (COMSATS Institute of Information Technology, Islamabad, Pakistan), Miss Farzaneh Parsi (Iranian Research Institute of Plant Protection, Tehran, Iran), Mr. Behnam Moetamedinia (Agricultural Research Center of Baluchestan, Iran), and Mr. Mahdi Naseri (Agricultural Research Center of Kerman, Iran) for providing insect samples.

- Alpatov, W. W.** (1930) Phenotypic variation in body and cell size of *Drosophila melanogaster*. *Biological Bulletin* 58, 85-103.
- Bookstein, F. L.** (1996) Combining the tools of geometric morphometrics. pp.131-150 in Marcus, L. F., Corti, M., Loy, A., Naylor, G. J. P. & Slice, D. E. (Eds) *Advances in morphometrics*. 585 pp. Plenum Press, New York.
- Bové, J. M., Danet, J. L., Bananej, K., Hassanzadeh, N., Taghizadeh, M., Salehi, M. & Garnier, M.** (2000) Witches broom disease of lime (WBDL) in Iran. *Proceedings of 14th Conference of International Organization of Citrus Virologists*, p. 207.
- Boykin, L. M., De Barro, P., Hall, D. G., Hunter, W. B., McKenzie, C. L., Powell, C. A. & Shatters, R. G.** (2012) Overview of worldwide diversity of *Diaphorina citri* Kuwayama mitochondrial cytochrome oxidase 1 haplotypes: two old world lineages and a new world invasion. *Bulletin of Entomological Research* 17, 1-10.
- Britten, H. B., Brussard, P. F., Murphy, D. D. & Ehrlich, P. R.** (1995) A test for isolation-by-distance in Central Rocky Mountain and Great Basin populations of Edith's checkerspot butterfly (*Euphydryas editha*). *Journal of Heredity* 86(3), 204-210.
- De Moed, G. H., De Jong, G. & Scharloo, W.** (1997) Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis. *Genetics Research* 70, 35-43.
- Dujardin, J. P., Pont, F. L. & Martines, E.** (1999) Quantitative morphological evidence for incipient species within *Lutzomyia quinquefer* (Diptera: Psychodidae). *Memórias do Instituto Oswaldo Cruz, Rio de Janeiro* 94(6), 829-836.
- Faghihi, M. M., Salehi, M., Bagheri, A. & Izadpanah, K.** (2009) First report of citrus huanglongbing disease on orange in Iran. *Plant Pathology* 58(4), 793-793.
- Futuyma, D. J.** (1986) *Evolutionary biology*. 2nd ed. 600 pp. Sinauer Associates, Sunderland, Massachusetts.
- Haas, H. L. & Tolley, K. A.** (1998) Geographic variation of wing morphology in three Eurasian populations of the fruit fly, *Drosophila lummei*. *Journal of Zoology (London)* 245, 197-203.
- Halbert, S. E. & Manjunath, K. L.** (2004) Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. *Florida Entomologist* 87(3), 330-353.
- Hartl, D. L. & Clark, A. G.** (1989) *Principles of population genetics*. 2nd ed. 682 pp. Sinauer Associates, Sunderland, Massachusetts.
- Hodkinson, I. D. & White, I. M.** (1979). *Homoptera: Psylloidea. Handbook for the identification of British insects*. Vol. 2, Part 5(a), 98 pp. Royal Entomological Society of London.
- Hoffman, A. A. & Shirrifs, J.** (2002) Geographic variation for wing shape in *Drosophila serrata*. *Evolution* 56, 1068-1073.
- Hutcheson, H. J., Oliver, J. H., Houck, M. A. & Strauss, R. E.** (1995) Multivariate morphometric discrimination of nymphal and adult forms of the blacklegged tick (Acari: Ixodidae), a principal vector of the agent of lyme disease in eastern North America. *Journal of Medical Entomology* 32, 827-842.
- Inayatullah, C., Fargo, W. S. & Webster, J. A.** (1987) Use of multivariate models in differentiating greenbug (Homoptera: Aphididae) biotypes and morphs. *Environmental Entomology* 16, 839-846.
- Kazachova, N. & Ekbom, B.** (2008) Genetic diversity in European pollen beetle, *Meligethes aeneus* (Coleoptera: Nitidulidae), populations assessed using AFLP. *European Journal of Entomology* 105(5), 807-814.
- Khaghaninia, S., Mohamadi, S. A., Sarafrazi, A., Hadade Iraninejad, K., Ebrahimi, E., Alavikia, S. & Zahiri, R.** (2008) Geometric morphometric approach on the sexual dimorphism of *Cydia pomonella* (Lep.: Tortricidae) in the North West of Iran. *Journal of Entomological Society of Iran* 28(1), 51-62.

- Khiaban, N. G. M. Z., Haddad Irani-Nejad, K., Hejazi, M. S., Mohammadi, S. A. & Sokhandan, N.** (2010a) A geometric morphometric study on geographical Iranian populations of the pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Entomological Society of Iran* 29(2), 13-24.
- Khiaban, N. G. M. Z., Haddad Irani Nejad, K., Hejazi, M. S., Mohammadi, S. A. & Sokhandan, N.** (2010b) A geometric morphometric study on the host populations of the pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in some parts of Iran. *Munis Entomology and Zoology* 5(1), 140-147.
- Kim, K. C., Chiang, H. C. & Brown, B. W.** (1967) Morphometric differences among four biotypes of *Ostrinia nubilalis*. *Annals of the Entomological Society of America* 60,796-801.
- Kim, K. C. & McPherson, B. A.** (1993) *Evolution of insect pests*. 479 pp. John Wiley and Sons, Inc.
- Lynch, M.** (1984) Destabilizing hybridization, general purpose genotypes and geographic parthenogenesis. *Quarterly Review of Biology* 59, 257-290.
- Masry, A. M. & Robertson, F. W.** (1979) Cell size and number in the *Drosophila* wing. III. The influence of temperature differences during development. *Egyptian Journal of Genetics and Cytology* 8, 71-79.
- Milliken, G. A. & Johnson, D. E.** (1984) *Analysis of messy data: designed experiments*. Vol. 1, 473 pp. Van Nostrand Rienhold Company, New York.
- Mozaffarian, F., Sarafrazi, A. & Nouri Ganbalani, G.** (2007a) Host-plant associated population variation in the Carob moth, *Ectomyelois ceratoniae*, in Iran: A geometric morphometric analysis suggests a nutritional basis. *Journal of Insect Science* 7, 1-11.
- Mozaffarian, F., Sarafrazi, A., Noori Ghanbalani, G. & Ahmadian, H.** (2005) Study on morphological variations of geographic populations of *Ectomyelois ceratoniae* (Zeller, 1839) on figs in Iran using geometric morphometric approach. *Journal of Agricultural Sciences (Islamic Azad University)* 11(1), 27-41. [In Persian with English summary].
- Mozaffarian, F., Sarafrazi, A., Nouri Ganbalani, G. & Ariana, A.** (2007b) Morphological variation among Iranian populations of the carob moth, *Ectomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae). *Zoology in the Middle East* 41, 81-91.
- Partridge, L., Barrie, B., Fowler, K. & French, V.** (1994) Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* 48, 1269-1276.
- Robertson, F. W.** (1959) Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in *Drosophila*. *Genetics* 44, 869-896.
- Roggero, A. & Dentreves, P. P.** (2005) Geometric morphometric analysis of wing variation between two populations of the *Scythris obscurella* species group: geographic or interspecific differences? (Lepidoptera: Scythrididae). *SHILAP Revista de Lepidopterologia* 33, 101-112.
- Rohlf, F. J.** (1993) Relative warp analysis and an example of its application to mosquito wings. pp. 131-159 in Marcus, L. F., Bello, E. & Garcia-Valdecasas, A. (Eds) *Contributions to morphometrics*. 257 pp. Monografias del Museo Nacional de Ciencias Naturales (CSIC), Madrid.
- Rohlf, F. J.** (1997) tpsSmall, version 1.02, Software. Available from: <http://life.bio.sunysb.edu/morph> (accessed October 2012).
- Rohlf, F. J.** (1998a) *NTSYSpc, version 2.02g*. Exter Software, Applied Biosystematics Inc.
- Rohlf, F. J.** (1998b) On applications of geometric morphometrics to studies of ontogeny and phylogeny. *Systematic Biology* 47, 147-158.
- Rohlf, F. J.** (1999) Shape statistics: Procrustes superimpositions and tangent spaces. *Journal of Classification* 6, 197-223.

- Rohlf, F. J.** (2004) tpsDig, version 1.4, Software. Available from: <http://life.bio.sunysb.edu/morph> (accessed October 2012).
- Rohlf, F. J.** (2009) tpsRegr, version 1.37, Software. Available from: <http://life.bio.sunysb.edu/morph> (accessed October 2012).
- Rohlf, F. J.** (2010) tpsRelw, version 1.46, Software. Available from: <http://life.bio.sunysb.edu/morph> (accessed October 2012).
- Rohlf, F. J., Loy, A. & Corti, M.** (1996) Morphometric analysis of Old World Talpinae (Mammalia, Insectivora) using partial-warp scores. *Systematic Biology* 45, 344-362.
- Rohlf, F. J. & Slice, D. E.** (1990) Extensions of the Procrustes method for the optimal superimpositions landmarks. *Systematic Zoology* 39, 40-59.
- Ross, K. G. & Shoemaker, D. D.** (2008) Estimation of the number of founders of an invasive pest insect population: the fire ant *Solenopsis invicta* in the United States. *Proceedings of the Royal Society of London, B* 275, 2231-2240.
- Sadeghi, S., Adriaens, D. & Dumont, H. J.** (2009) Geometric morphometric analysis of wing shape variation in ten European populations of *Calopteryx splendens* (Harris, 1782) (Zygoptera: Odonata). *Odonatologica* 38(4), 343-360.
- Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., O'Neil, P., Parker, I. M., Thompson, J. N. & Weller, S. G.** (2001) The population biology of invasive species. *Annual Review of Ecology and Systematic* 32, 305-332.
- Salehi, M., Faghihi, M. M., Khanchezar, A., Bagheree, A. & Izadpanah, K.** (2012) Distribution of citrus Huanglongbing disease and its vector in southern Iran. *Iranian Journal of Plant Pathology* 48(2), 195-208.
- SAS Institute** (2003) *SAS software, Version 9.1*. SAS Inc., Cary NC.
- Simon, J., Baumann, S., Sunnucks, P., Hebert, P., Pierre, J. & Le Gallic, J.** (1999) Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. *Molecular Ecology* 8, 531-545.
- Slice, D. E.** (2001) Landmark coordinates aligned by Procrustes analysis do not lie in Kendall's shape space. *Systematic Biology* 50, 141-149.
- Slice, D. E., Bookstein, F. L., Marcus, L. F. & Rohlf, F. J.** (1996) Glossary for geometric morphometrics. pp. 531-551 in Marcus, L. F., Corti, M., Loy, A., Naylor, G. J. P. & Slice, D. E. (Eds) *Advances in morphometrics*. 585 pp. Plenum Press, New York.
- Wright, S.** (1943) Isolation by distance. *Genetics* 28, 114-138.
- Zahiri, R.** (2003) A geometric morphometric investigation on geographic populations of rice stem borer, *Chilo suppressalis* W. (Lep.: Pyralidae) in northern parts of Iran. M. Sc. Thesis. Guilan University, Iran.

Received: 2 November 2012

Accepted: 6 March 2013