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Genetic Diversity Determination for Peganum Harmala Species using Shannon's, Simposn, Eveness, Density and Richness Parameters In Jordan

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Abstract
The diversity indices are very essential components to estimate the plant species diversity at ecosystems. *Peganum harmala* is an essential medicinal plant with high value of phytochemicals as well it has fertility potential in sheep. It is considered as an indicator for climate change particularly at the arid regions. This research was carried out to investigate the plant species diversity of desert area at four sites representing different habitats within Ma'an governorate southern part of Jordan via comparison biodiversity indices. Quadrat-transect method was randomly used to determine the density, richness, dominance and evenness indices under selected sites. Results of plant species list showed 23 taxa recorded. *Peganum harmala* registered the high density value (0.667) during the year 2013 at Adruh site, while high frequency value (0.800) was found at Ma'an site. The year 2013 showed high plant diversity compared to 2014. During 2013 and 2014 Wadimusa recorded the high Shannon's index 1.998 and 1.488, respectively compared to the other sites. Two medicinal plants were recorded during this study namely *Achillea fragrantissima* and *Artemisia herba alba* used as antidiabetic disease in the folk medicine. Studying the desert biodiversity is a crucial for evaluate the health of rangeland ecosystems.
Keywords: Diversity, Biodiversity, Ecosystem, Harmal, Medicinal plant,

Introduction:
Harmal or hamel (*Peganum harmala* L.), is a poison plant grown naturally at the wild fields, belongs to the Zygophyllaceae family. It is considered as a medicinal plant that all parts used as anti-inflammatory, sudorific, sedative and narcotic (Al-Esawi and Takruri, 1989). Al-Haboby and Bounejmate, (1999) investigated the relationship between harmel smoking and sheep production and they found that the smoked rams had higher sexual activity than the non-smoked rams this related to the highly influenced chemical composition on follicular growth and ovulations. According to Al-Esawi, (1998), *Peganum harmala* L. described as a perennial herb, 30-50cm long, with fleshy stems, growing from basal root stocks, with evil smelling. Leaves irregularly divided, with stipules, fleshy, hairless. Flowers white, 2-3cm in diameter. Fruits capsule, 1cm in diameter. Habitat: marginal and desert lands; Irbid, Salt, Karak, Tafila, Jordan valley, dead sea, Wadiaraba, Zarka, Mafraq and Eastern desert. Flowering during March-May. Many studied have been investigated the pharmacological and biological activities of *Peganum harmala* L. such as anti microbial and antifungal (Al-Izzy, 2010), antimicrobial efficacy of alkaloid, harmaline alone and in combination with chlorhexidine digluconate against clinical isolates of *Staphylococcus aureus* (Xing *et al*., 2012), antitumor and cytotoxic effects (Zaker *et al*., 2007). Asgarpanah and Ramezanloo, (2012) stated that *Peganum harmala* has been used in traditional medicines for the relief of pain and antiseptic agent, also anti viral, antidiabetic, antitumor, insecticidal and antinociceptive. The methanolic extract of *Peganum harmala* observed highly effective as metformin in reducing the blood glucose level of normoglycemic and streptozotocin-induced diabetic rats (Singh *et al*., 2008). The compound peganine of *Peganum harmala* had antileishmanial activity (Rahimi-Moghaddam *et al*., 2011) and this species exhibited a significant narcotic analgesic activity (Twaij and El-Jalil, 2009). In Masjed-Daghi, Julfa, NW Iran, Farjandi *et al*., (2011) studied that the ability of *Peganum harmala* L. used as an indicator for hperaccumulator of biogeochemicals.

Biodiversity as a variation at all levels is very important to natural seize area delineation and handling. Biodiversity was investigated using several diversity indices such as richness and evenness (Davari *et al*., 2011; Zhang and Zhang, 2007). Banerjee and Srivastava, (2010) define the species richness as the number of species present in a given area or in a given sample without necessitate any particular regard for the number of individuals observed for individual species.

Knowing species richness patterns are crucial to provide insights to environmental planners, nature reserve designers, ecologists and botanists (Sang, 2009). In Taif, Saudia Arabia, Ferrang , (2012) used Shannon's index and species richness to study the floristic composition in Wadi-Al Argy region. In Iran, changes of vegetation structure and biomass were investigated by (Zarekia *et al*., 2013) and changes in vegetation properties under short and long term protection in North Africa (Abdallah and Chaieb, 2014). In Southern Ethiopia, relative density, diversity and richness of woody plant species in disturbed secondary forest and river course by

(Gebretsadik and Telila, 2013). The indices of Simpson, Shannon's –Weiner and Birllouin were used to estimate the floral diversity on four locations at Sinia, in Egypt (Khafagi *et al* ., 2013). Rad *et al*., (2009) also used the Species richness, Shannon and Simpson indices to quantify diversity of the different communities. Variability in tree species, composition, richness, density and diversity across the northern forest-Savanna ecotone of Ghana was determined by (Attua and Pabi, 2013). In India, mainly at Estate-Pantnagar, Simpson's index of biodiversity was (0.28) representing presence of very less floral diversity; species evenness (0.36) signifying that only few species are dominant in the monitoring area and Shannon-Wiener index (0.506) signifying presence of less biological diversity (Banerjee and Srivastava, 2010). Frequency is necessary for spatial patterns and the evenness important for spatial distribution of plant species communities elsewhere. Frequency is a measure of the uniformity of the distributing of a species; thus a low frequency indicates that a species is either irregularly distributed or rare in particular stand or forest (Shameem and Kangroo, 2011). The results showed negative impact of overgrazing on vegetation and palatability in a traditional grazing ecosystem which is alarming to promote a monitoring program (Hassani *et al*., 2008). At the level of medicinal plants many studies conducted for assessment the diversity such as, diversity and regeneration aspects of medicinal plants at Western Ghats (Rao *et al*., 2014); diversity of medicinal plants among different forest-use type of Pakistani Himalaya (Adnan and Hölscher, 2012), in Cameroon, species composition, diversity and distribution at Takamanda rainforest estimated (Ndah *et al*., 2013). In Mexico, plant management and biodiversity conservation (Larios *et al*., 2013). In order to remain the medicinal plants in their natural ecosystems for times come, it is inevitable to adopt suitable conservation practices and create awareness among the people (Roa *et al*., 2014). However, to my knowledge there is no studies were dealt with biodiversity analysis or habitat variation in the Ma'an region particularly for *Peganum harmala* L. therefore this research carried out to study the diversity status of *Peganum harmala* L. and plant species diversity using diversity indices such as richness, density, frequency and Shannon's index parameters.

Materials and methods

Study area

Ma'an governorate considered as a desert city located at the southern part of Jordan. The climate of the area varied from cold at the winter and sunny most of the year days. The west side of this city such as Wadimusa and Alshuobak were occupied at higher elevations and colder than down town and its borders (Table 1). Three transects established with 50m length per transect. A total of 15 quadrats with an area (0.5mx0.5m) randomly done per each region. These quadrats were sampled during the survey in end of March to April of years 2013 and 2014. The number of individuals and occurrence of each species in the quadrats was then used to calculate its diversity indices also density and frequency, respectively. The collected data directly inserted in the excel sheet for analysis.

Data analysis

Species richness was estimated as the number of the species found in the quadrate. To quantify the diversity of the plant species, the Shannon index (*H'*) as a measure of species abundance and richness applied (Rad et al., 2009). The data for each site were analyzed separately. Excel program 2007 was used in the organization and presentation of data statistically. Density calculated according to Ambasht, (1982); frequency measured based on Rajan, (2001). Qualitative and quantitative diversity indices such as Dominance, Simpson, Menhinick, Marglef, Equitability, Fisher's alpha and Berger-Paker were analyzed using the PAST software program ver. 2.18c (Hammer *et al*., 2001).

Results

Species richness and diversity indices among studied areas illustrated at Table 1. During year 2013, high species richness (9) was recorded by Wadimusa site while Alshoubak subdistrict showed the lowest value of plant species diversity (4). High number of plant species was found at Wadimusa (36) compare to Alshoubak (23). Diversity index (Shannon's-Wiener, Simpson, Berger-Parker, Fisher's-alpha, Margalef), richness (Menhinick), evenness (Simpson), Dominance (Simpson) are given at (Table 1). The diversity value was ranged from 1.998 to 0.652 within the study locations. The year 2013 showed high plant diversity compared to 2014. During 2013 and 2014 Wadimusa recorded the high Shannon's index 1.998 and 1.488, respectively compared to the other sites (Table 1). High Simpson values were recorded during 2013, 0.684, 0.759, 0.841 and 0.694 for Ma'an, Adruh, Wadimusa and Alshoubak sites, respectively (Table 1). Among studied areas high Simpson value 0.841 and 0.754 was registered for Wadimusa during 2013 and 2014. High value of Menhinick and Margalef were found in Wadimusa region, 1.500 and 2.232, respectively followed by Ma'an 1.46 and 1.914 (Table 1). Fisher's-alpha was highest in Wadimusa 3.852 followed by Ma'an 3.427 and Adruh (Table1). High equitability-J observed in Adruh, Alshoubak and Wadimusa with values 0.935, 0.912 and 0.909, respectively. Ma'an site showed the highest value of Berger-Paker (0.533). In general, the year 2014 showed highest diversity indices compared to the year 2013

(Table 1), except Shannon diversity indices was highest at 2014. Density and frequency among plant species were depicted at Table 2. *Peganum harmala* registered the high density value (0.667) during the year 2013 at Adruh site, but high frequency value (0.800) at Ma'an site. A total number of taxa was 23 recorded (Table 2), among these species *Echinops polyceras* Bioss*, Anabasis syrica IIjin*, *Eryngium bourgatii* and *Artemisia herba alba* showed density values as 0.133, 0.267, 0.267 and 0.200, respectively (Table 2). In Ma'an site, *Anabasis syrica IIjin* was the dominant species whereas *Artemisia herba alb* was the predominant at Alshoubak. Nearly, both species considered as an associated for *Peganum harmala*. *Hordeum spontaneum* recorded high frequency values 0.667 and 0.467 at Wadimusa and Adruh, respectively. Two medicinal plants were recorded during this study namely *Achillea fragrantissima* and *Artemisia herba alb* used in the traditional system of medicine. In 2014, *Peganum harmala* showed density value (0.267), 0.00, 0.467 and 0.533 at Ma'an, Adruh, Wadimusa and Alshoubak, respectively (Table 3), high frequency value 0.900 for *Peganum harmala* was found at Adruh. Both medicinal plants *Achillea fragrantissima, Artemisia herba alba* had high density values 0.33 and 0.267 at Wadimusa and Alshoubak, respectively.

Discussion

The study area differs from rest of the Jordan biomes in terms of vegetation cover and species composition. It is considered as a desert area characterized with low species diversity. Nearly, 80% of plant species are restricted to the valley bottoms and street banks particularly to *Peganum harmala* L. Joshi *et al*., (2006) reported that nearly 78-80% of plant species in Nubra, India are restricted to the valley bottom. Also, Hassani *et al*., (2008) pointed that the results showed the importance value of unpalatable species such as *Peganum harmala* was increased in critical areas around watering points. *Peganum harmala* L. is native plant species, with aromatic odor which related to high alkaloids concentration such as harmaline and harmine, is unpalatable plant either for human or livestock. Due to low availability of certain wild medicinal plants in the study area Ma'an, a travel far and wide outside will allow for find more species. However, in addition to the regions that identified by Al-Esawi, (1998) for *Peganum harmala* L. habitat, also we found this species at Ma'an governorate southern part of Jordan. So, this species is wide distributed over all Jordan areas and considered as desert plant species that has highly adaptability with harsh conditions. Wadimusa located at altitude (1485m) with a dominant *Ahillea fragrantissima* species while Alshouback studied region (1389m) was dominant with *Artemisia herba alba*. In Egypt, Khafagi *et al*. (2013) found that *Achillea fragrantissima* (Forssk.) represented the highest cover in Wadi Gebal with mean elevation (1823 m) and the *Aretmisia judaica* L. was the highest cover in Wadi Gharaba. These results confirm that each species has a specific site adaptability with highly positively correlated. On the contrary, a negative correlation between altitude and vegetation attributes, including species diversity, richness and community maturity was observed in the investigated area of Kashmir, Pakistan by (Shaheen *et al*., 2011). Plant species diversity decreased with east direction and increased by west directions such as Alshoubak and Wadimusa with high altitudes. This results confirmed what Gebretsadik, and Telila (2013) they found in their study, the species diversity and richness at eastern base of Abaro mountain is low. Zhang and Zhang, (2007) stated that elevation was the most important variable in terms of variations in species diversity. Species richness, evenness and diversity of different functional groups showed similar responding model to changes in elevation. i.e. the maximum diversity occurring at intermediate elevations. This study emphasis that the species richness increased with increased elevation this findings is in agreement with Sang, (2009) results who found that the plant species richness increased with altitude from the lowest to highest points (450 and 1,500 m a. s. l.). In spite of this study was implemented during consequence years (2013-2014) at the same time and spatial, but some of plant species weren't found in the next year, this could be related to their early flowering and grazed by animals as well variation and distribution in the amount of rainfall, for this reason any species will be appearing with seasonal changes should be monitored in the future research of this area. Variation in altitudinal and latitudinal variation which leading to the variation in climatic conditions and consequently, make changes in all ecosystem components (Khafagi *et al*., (2013). Zarekia *et al*., (2013) stated that the diversity and composition of plant communities may be strongly influenced by management practices in response to the livestock grazing. Davari *et al*., (2011) reported that Margalef and Menhinick indices had significant relationship with the rangeland conditions. Therefore, further studies should focus on the ecology and biodiversity patterns using high resolution satellite data with a separate set on environmental variables. The data obtained through this study with a high value for a medicinal plant or other species as baseline data for regular monitoring of target areas in the future. On the other hand, studies regarding molecular markers and phytochemical analysis is needed to explore the database of *Peganm harmala* which considered as key for breeders in the future. `

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Table 1: Coordinates and biodiversity indices for *Peganum harmala* species in four sites at Ma'an governorate at Jordan.

Coordinates								
E°	035 42.323"		035 35 .857"		035 30.632"		035 33.577"	
N°	30 13.599"		30 19.747"		30 19.836"		30 31.285"	
Elevation (m)	1137		1315		1485		1389	
Diversity indices	Ma'an		Adruh		Wadimusa		Alshoubak	
	2013	2014	2013	2014	2013	2014	2013	2014
Taxa_S	7	3	5	2	9	5	4	4
Individuals	23	8	29	14	36	21	22	15
Dominance_D	0.3157	0.375	0.241	0.541	0.159	0.247	0.306	0.404
Shannon_H	1.515	1.04	1.506	0.652	1.998	1.488	1.264	1.063
Simpson_1-D	0.684	0.625	0.759	0.459	0.841	0.753	0.694	0.596
Evenness_e^H/S	0.650	0.943	0.901	0.960	0.811	0.886	0.885	0.723
Menhinick	1.46	1.061	0.929	0.534	1.500	1.091	0.853	1.033
Margalef	1.914	0.962	1.188	0.379	2.232	1.314	0.971	1.108
Equitability_J	0.7785	0.964	0.935	0.940	0.909	0.925	0.912	0.767
Fisher_alpha	3.427	1.743	1.742	0.639	3.852	2.076	1.431	1.785
Berger-Parker	0.522	0.500	0.345	0.643	0.278	0.333	0.364	0.533

Table 2: Density and frequency of plant species diversity at four sites at Ma'an governorate during 2013.

Year 2013	Density	Frequency	Year 2013	Density	Frequency	Year 2013	Density	Frequency	Year 2103	Density	Frequency
Species/ Ma'an			Species/ Adruh			Species/Wadimusa			Species Alshoubak		
Achillea fragrantissima	0.133	0.133	*Anabasis syrica Iljin*	0.267	0.400	*Achillea fragrantissima*	0.200	0.267	*Artemisia herba alba*	0.200	0.2667
Anabasis syrica Iljin	0.133	0.133	*Centaurea halolepis Bioss*	0.133	0.200	*Achillea santolina*	0.667	0.400	*Centaurea sinaica*	0.133	0.133
Atriplex halimus L.	0.067	0.067	*Hordeum spontaneum*	0.133	0.467	*Artemisia herba alba*	0.267	0.267	*Avena sterilis*	0.067	0.533
Echinops polyceras Bioss.	0.200	0.200	*Peganum harmala*	0.667	0.667	*Carthamus tenuis*	0.133	0.133	*Peganum harmala*	0.533	0.533
Hordeum spontaneum	0.067	0.067	*Urtica pilulifera* L.	0.067	0.200	*Eryngium bourgatii*	0.667	0.067			
Malvella sherardiana (L.) Joaub & Spach	0.067	0.133				*Hordeum spontaneum*	0.067	0.667			
Peganum harmala	0.533	0.800				*Onopordon ambiguum*	0.133	0.133			
						Peganum harmala	0.333	0.333			
						Silybum marianum	0.133	0.133			

Table 3: Density and frequency of plant species diversity at four sites at Ma'an governorate during 2014.

Year 2014	Density	Frequency	Year 2014	Density	Frequency	Year 2014	Density	Frequency	Year 2014	Density	Frequency
Species/ Ma'an			Species/ Adruh			Species/ Wadimusa			Species/ Alshoubak		
Achillea fragrantissima	0.133	0.133	*Anabasis syrica Iljin*	0.333	0.333	*Achillea fragrantissima*	0.333	0.267	*Artemisia herba alba*	0.267	0.333
Anabasis syrica Iljin	0.133	0.133	*Peganum harmala*	0.600	0.933	*Achillea santolina*	0.067	0.133	*Centaurea sinaica*	0.067	0.067
Peganum harmala	0.267	0.267				*Artemisia herba alba*	0.333	0.400	*Avena sterilis*	0.067	0.067
						Hordeum spontaneum	0.133	0.133	*Peganum harmala*	0.533	0.533
						Peganum harmala	0.467	0.467			

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