

## ECOLOGICAL ASSESSMENT AND PHENOTYPIC AND FITNESS VARIATION OF SINAI'S REMNANT POPULATIONS OF *MORINGA PEREGRINA*

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**Abstract.** *Moringa peregrina* which is one of the most valuable and economically important medicinal species in the Egyptian desert has become one of the most endangered trees due to unmanaged grazing and over-collection. The present study aims to provide ecological assessment of the species and to investigate whether progeny from the remnant fragmented populations show reduced fitness. Sixteen sites containing a total of 197 trees were sampled upon survey of Wadis in South Sinai, where vegetation parameters and associated species were recorded. Variation in edaphic factors, phenotypic traits, germination, and early life-history fitness were assessed and analyzed. The results indicated that *M. peregrina* has narrow distribution and grows on cliffs and at the base of hills (300 - 800 m a.s.l.) with very rugged topography. Almost all the reproductive trees grow on south facing slopes and crevices of metamorphic rocks. The study clearly showed that the studied populations have very low early stage fitness estimated as an index of maternally affected life-history characters. The study suggested that the deteriorated environmental circumstances have affected negatively the fitness of maternal plants in small populations and the performance of their offspring. It concluded that direct protection is urgently needed to stop further deterioration of the populations and to improve their number and size.

**Keywords:** *Sinai, Moringa peregrina, conservation, fitness, medicinal plants.*

### Introduction

Egypt, due to explosive growth of its population, needs to conserve, reallocate, and sustainably use its natural biological resources. A special concern should be directed to underutilized, rare and endangered but economic species. In the last thirty years, the wild vegetation of South Sinai has been endangered which resulted in disappearance of palatable species, rarity of trees, and change in vegetation composition (Moustafa *et al.*, 2001). Abd El-Wahab *et al.* (2004) assessed the status, distribution, the causes of loss, and the specific threats facing conservation and sustainable use of medicinal plants in St. Catherine Protectorate. The study recommended that detailed and specific reproductive ecology, life-history characteristics, and population genetic diversity and structure studies are essentials to construct sound conservation and sustainable utilization plan for the threatened medicinal species.

Due to the destruction, deterioration and fragmentation of their habitats, many species (including *Moringa peregrina*) have recently been forced into small and isolated populations. *Moringa peregrina* which belongs to the monogeneric family Moringaceae, with just thirteen/fourteen species throughout the dry tropics of the world (Somali *et al.*, 1984), is one of the most economically important medicinal plant species in Egyptian desert. *Moringa* family consists of about ten Xerophytic species (FAO, 1988; Al Kahtani and Abou-Arab, 1993), distributed from tropical Africa to the East

Indies. Although it is a rare species, *M. peregrina* has a wide geographic range, growing from the Dead Sea area sporadically along the Red Sea to northern Somalia and around the Arabian Peninsula to the mouth of the Arabian (Persian) Gulf. In Egypt, it is recorded from the eastern desert, Red sea coast, Elba massive, and the Sinai Mountains (Täckholm, 1974; Boulos, 1999). It is recorded in South Sinai in small limited areas at W. Feiran, W. Me'ar, and W. Zaghra and locally called "Yassar". It grows in crevices and rocky slopes of mountains. *Capparis cartilaginea*, *Reseda decursiva*, and *Hamada elegans* are the main associated species. *M. peregrina* is adapted to a wide range of soil types but it does best in well-drained loam to clayeyloam (Abd El Wahab, 1995). It does not withstand prolonged water logging. It is observed to prefer a neutral to slightly acidic soil reaction, but it has recently been introduced with temperature ranges from 26 to 40 °C and annual rainfall at least at least 500 mm.

*Moringa peregrina* could soon become one of the world's most valuable plants, at least in humanitarian terms. Its seeds have different economic and medicinal importance due to its unique composition of oil (42-54%), fatty acids (14.7 % saturated, and 84.7 unsaturated), proteins (23%), fiber (3.6 %), and carbohydrates (15.3 %) in its kernel that contains 1.8% moisture, and 2.5% ash, (Somali *et al.*, 1984). They are the source of oil used by Egyptians since the old and middle kingdoms (3000-2000 BC) and currently are used in folk medicine "Al-Yassar" (Migahid, 1978; Abd El-Wahab *et al.*, 2004). The extracted oil is composed of 14.7 % saturated and 84.7 % unsaturated fatty acids including Palmitic (9.3 %), Palmitoleic (2.4 %), Stearic (3.5 %), Oleic (78.0 %), Linoleic (0.6 %), Araachidic (1.8 %), and Behenic (2.6 %) (Somali *et al.*, 1984). Refined oil has a yellowish color, a sweet taste, is non-sticking, and is odorless. As a result, it is highly valued for preparing cosmetics, cooking, and lubricating purposes. It does not turn rancid, is excellent on salads, can be used for soap making, and burns without smoke (Price, 2000). It has a reputation as watch oil (Verma *et al.*, 1976). The seedcake left after oil extraction can be used as soil fertilizer or in the treatment of contaminated water (the usual case in most local Bedouin communities). It could be used (as well as suspensions of ground seed) as primary coagulants to remove a high proportion of the suspended bacteria (Von Maydell, 1986).

According to Duke (1983), *Moringa* leaves can be described as "phytoactive". They are a source of vitamin A and, when raw, vitamin C. They are a good source of B vitamins and among the best plant sources of minerals (Price, 2000). The calcium content is very high for a plant while phosphorous is low. Iron content is high. It is an excellent source of protein and a very low source of fat and carbohydrates. The leaves are incomparable as a source of the sulfur-containing amino acids methionine and cystine, which are often in short supply in the Bedouin diet. The nutritional value is even higher for its pods. Both leaves and pods supply several amino acids including arginine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, and valine (e.g. Makkar and Becker, 1996).

*Moringa peregrina* is an extremely fast growing small tree. Perhaps the fastest-growing of all trees, it commonly reaches about three meters in height just 10 months after the seed is planted (Abd El-Wahab, 1995). Wildly, it is 5-15 m high, with grayish-green bark, long leaves, and bisexual yellowish white to pink, showy, fragrant flowers (Täckholm, 1974; Boulos, 1999). The flowers are good for beekeepers as they provide nectar. *M. peregrina* wood is very soft, it makes acceptable firewood which is a basic requirement in traditional Bedouin life in the Sinai.

Due to the over-stressing demand and use of *Moringa peregrina*, there are increasing threats facing its populations in wild. It is subjected to severe over-harvesting, overgrazing, over-cutting, and uprooting (for fuel and medicinal uses) as well as disturbance through unmanaged human activities. These activities have caused *M. peregrina* to become extremely rare in Egypt. The overall result has been the extirpation of whole populations and a reduction in size of the remaining populations. There has also undoubtedly been an erosion of genetic diversity. A loss of genetic variation may reduce the ability of local populations to adapt to changes in the environment and the potential for evolutionary change (Frankel and Soulé, 1981; Beardmore, 1983). The continued over-exploitation of natural populations threatens this species with local extinction unless these practices are stopped and natural populations are restored. In addition, since this species is a promising candidate for cultivation, the loss of genetic variation that accompanies the reduction of population sizes could jeopardize selection for increased productivity. Due to a shortage of native *M. peregrina*, Egyptian medicinal plant traders have to import pods of other *Moringa* species to cover the demand for *Moringa* products.

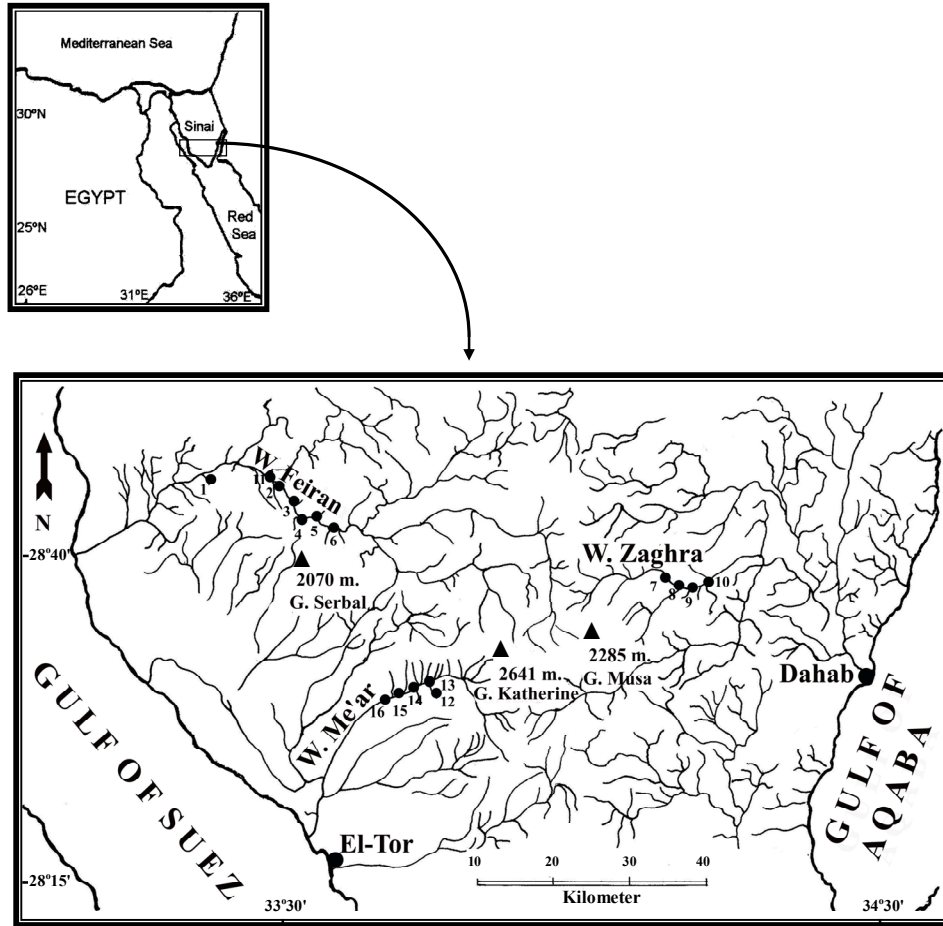
The present study aimed to provide ecological assessment of the species which will greatly assist the design of rehabilitation as a part of *in situ* and *ex situ* conservation program and to investigate whether progeny from the remnant and fragmented small populations of *Moringa peregrina* show reduced fitness.

## Materials and methods

### Study area

The study was carried out in St. Catherine Protectorate, which covers 4300 km<sup>2</sup> and lies in the middle of the triangular mass of mountains of the southern part of the Sinai Peninsula that is bounded to the east and the west by Aqaba and Suez Gulf, respectively. This triangular mass of mountains, with its apex at Ras Mohammed to the south, is 7500 Km<sup>2</sup> in surface area and formed of igneous and metamorphic rocks, chiefly granites (Said, 1990). This mass of mountains is intensively rugged and dissected by a complicated system of deep Wadis, some of which reach a considerable length (e.g. Wadi Feiran, and Wadi Zaghra) and some are shorter, narrow and steeper, and represent tributaries of the main Wadis (e.g. Wadi El-Arbae'en) (Said, 1990; Moustafa and Klopatek, 1995).

*Moringa peregrina* populations are recorded in the Wadi Feiran, Wadi Me'ar, and Wadi Zaghra basins where it grows in crevices and on rocky slopes of metamorphic mountains. Wadi Feiran represents one of the longest Wadis in Southern Sinai. It is bounded by igneous and metamorphic mountains with different varieties of dykes. Its main tributaries include Wadi El-Sheikh, W. Solaf, W. El-Akhdar, W. Nesrin, W. Tarr, W. Mekatab, and W. Alliat. The vegetation cover throughout the Wadi ranges between 5 and 10%. The Wadi basin supports vegetation consists of about 40 plant species and is dominated mainly by *Acacia tortilis* subsp. *raddiana*. The species that associate *A. tortilis* subsp. *raddiana* associations include *Achillea fragrantissima*, *Anabasis articulata*, *Artemisia judaica*, *Capparis sinaica*, *Echinops glaberrimus*, *Fagonia mollis*, *Fagonia arabica*, *Launaea spinosa*, *Lycium shawii*, *Mentha longifolia*, and *Retama raetam* (Zaghloul et al., 2008).



**Figure 1.** Location map of the studied *Moringa peregrina* populations in Southern Sinai

Wadi Me'ir is a rocky plain with more than 80% of the surface been covered with cobbles and stones, and about 20% with fine sand. The Wadi-bed is dissected with channels and deltas due to the water erosion. Generally, the total plant cover ranges between 1-5% all over the Wadi and 20-30% at some tributaries at the middle of the Wadi (Moustafa *et al.*, 1998). The following associations are recognized at the Wadi (Moustafa *et al.*, 1998): *Haloxylon salicornicum* - *Acacia tortilis* subsp. *raddiana*, *Haloxylon salicornicum* - *Zygophyllum coccineum*, *Acacia tortilis*, *Cleome droserifolia*. The associated species include *Achillea fragrantissima*, *Artemisia judaica*, *Capparis sinaica*, *Chrozophora plicata*, *Cleome droserifolia*, *Fagonia mollis*, *Iphiona scabra*, *Ochradenus baccatus*, *Panicum turgidum*, *Pergularia tomentosa*, *Zilla spinosa*, and *Zygophyllum coccineum*. *Moringa peregrina* is found as associated species or as co-dominant with *Hyoscyamus muticus* and/or *Fagonia mollis*. Wadi Me'ir is characterized by 50 plant species including one endemic and 26 medicinal species including 11 threatened species (Moustafa *et al.*, 1998).

Wadi Zaghra supports vegetation with cover percentage ranges between 1-5% in Wadi-bed, and 5-10% at foothill (Abd El-Wahab *et al.*, 2004). It supports the following main associations; *Haloxylon salicornicum*, *Solenostemma argel*, *Moringa peregrina*, *Artemisia judaica*, *Zygophyllum coccineum* – *Aerva javanica*, *Moringa peregrina* – *Acacia tortilis*, *Acacia tortilis*, and *Retama raetam*. The associated species are: *Acacia tortilis* at the foot hills, *Capparis spinosa* on cliffs and *Aerva javanica*, *Cleome*

*droserifolia*, *Ochradenus baccatus*, *Senna italica*, and *Crotalaria aegyptiaca* in the main Wadi (Abd El-Wahab *et al.*, 2004).

Medicinal plants in Wadi Zaghra include nine threaten species, only one of them is endemic, *Origanum syriacum*. These medicinal species include *Artemisia judaica*, *Capparis spinosa*, *Citrullus colocynthis*, *Cleome droserifolia*, *Cucumis prophetarum*, *Hyoscyamus muticus*, *Moringa peregrina*, *Pulicaria arabica*, *Senna italica*, *Solenostemma arghel*, and *Zygophyllum coccineum*. The following medicinal plant species are dominant in Wadi Zaghra: *Artemisia judaica*, *Fagonia mollis*, *Haloxylon salicornicum*, *Iphiona scabra*, *Moringa peregrina*, *Solenostemma arghel*, *Zilla spinosa*, and *Zygophyllum coccineum* (Abd El-Wahab *et al.*, 2004).

Threats affecting *Moringa peregrina* in the aforementioned Wadis include over-collection mainly of seeds for trade and of fuel wood for cooking and heating. Breaking down of the tree branches as a result of over-grazing effect is obviously shown around the area.

**Table 1.** Summary of the number of *Moringa pergerina* trees, range of cover, and height of *M. pergerina* at the sixteen sites selected at three different wadis in South Sinai

Location	Population Number	Number of trees	Cover (m <sup>2</sup> )**				Height (m)**			
			Min	Max	Mean	SD	Min	Max	Mean	SD
W. Feiran	1	4	1.26	11.78	6.74	2.87	0.4	5.0	2.95	1.09
	2	6	2.51	14.13	7.09	4.76	1.0	5.0	2.75	1.54
	3	40	1.88	8.64	5.28	2.22	0.8	4.5	2.84	1.17
	4	6	1.88	14.13	7.56	4.72	1.0	5.0	3.08	1.69
	5	8	1.88	26.69	13.68	8.65	1.0	18.0	8.31	5.9
	6	11	8.64	21.98	15.70	5.38	3.0	10.0	6.81	2.86
	11	1	14.92	14.92	14.92	-	6.0	6.0	6.0	-
Subtotal	7	76	1.26	26.69	8.11	5.61	0.4	18.00	3.83	2.56
W. Zaghra	7	8	26.69	39.25	29.20	5.62	13.0	21.0	17.0	3.81
	8	21	3.93	34.54	20.34	7.24	3.5	20.0	9.83	4.61
	9	12	4.71	31.40	17.40	7.88	4.0	15.0	9.96	3.76
	10	1	29.83	29.83	29.83	-	12.0	12.0	12.0	-
Subtotal	4	42	3.93	39.25	20.81	8.01	3.50	21.00	10.30	4.93
W. Me'ar	12	17	3.14	13.35	7.76	3.53	2.5	7.0	4.76	1.39
	13	27	1.18	11.78	6.44	2.97	1.0	8.5	3.57	1.98
	14	15	1.57	8.64	4.81	2.37	1.0	5.5	2.77	1.4
	15	15	1.57	14.13	6.9	3.86	1.0	8.0	4.4	2.47
	16	5	1.57	5.5	2.83	1.81	1.0	3.0	1.5	0.87
Subtotal	5	79	1.18	14.13	6.28	3.33	1.00	8.50	3.70	2.00
Total	16	197	1.18	39.25	9.86	7.69	0.40	21.00	5.09	4.05

Note: \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.02$ , NS = non significant variation

### Field survey and seed collection

Three Wadis known to support *M. peregrina* trees were surveyed and sixteen sites were sampled (Figure 1 and Table 1): six sites at W. Fieran (seventy-six trees), four at W. Zaghra (forty-two trees), and six at W. Me'ar (seventy-nine trees). Associating

species, human activities, and land use were recorded to assess the ecological status of the *M. peregrina* populations in selected Wadis. Identification and nomenclature of plant species were according to Täckholm (1974) and Boulos (1995; 1999; 2000; 2002; 2005).

Forty eight surface soil samples, 0-30 cm depth and 2kg each were collected from W. Feiran, W. Zaghra and W. Me'ar near the main trunk of trees at different sites (Table 2) to characterize the soil condition that support *M. peregrina* populations. Three soil samples were collected from each population for physical and chemical analysis. Physical parameters included particle size distribution and soil texture whereas chemical analyses included soil pH and electric conductivity (EC).

By using nets and carefully shaking the *M. peregrina* tree branches, seeds from *M. peregrina* trees growing at W. Feiran, W. Me'ar, and W. Zaghra were collected for germination and propagation experiments at the Suez Canal University (SCU, Ismailia, Egypt) botanical garden.

**Table 2.** List of associated species with *M. peregrina* populations at different sites

Family	Species
Amaranthaceae	<i>Aerva javanica</i> (Burm. f.) Juss. ex Schult.
Asclepiadaceae	<i>Solenostemma arghel</i> (Delile) Hayne
Capparaceae	<i>Capparis sinaica</i> Veill. <i>Capparis spinosa</i> L.
Chenopodiaceae	<i>Anabasis articulata</i> (Forssk.) Moq. <i>Haloxylon salicornicum</i> (Moq.) Bunge ex Boiss.
Cleomaceae	<i>Cleome droserifolia</i> (Forssk.) Delile
Compositae	<i>Artemisia judaica</i> L. <i>Iphiona scabra</i> DC. <i>Launaea spinosa</i> (Forssk.) Sch. Bip. ex Kuntze <i>Pulicaria arabica</i> (L.) Cass.
Cucurbitaceae	<i>Citrullus colocynthis</i> (L.) Schrad. <i>Cucumis prophetarum</i> L.
Euphorbiaceae	<i>Chrozophora oblongifolia</i> (Delile) Spreng.
Labiatae	<i>Otostegia fruticosa</i> (Forssk.) Penz. <i>Teucrium polium</i> L.
Leguminosae	<i>Acacia tortilis</i> (Forssk.) Hayne <i>Retama raetam</i> (Forssk.) Webb & Berthel. <i>Senna italica</i> Mill. <i>Zilla spinosa</i> (L.) Prantl
Resedaceae	<i>Ochradenus baccatus</i> Delile <i>Reseda</i> sp.
Solanaceae	<i>Hyocyamus muticus</i> L.
Zygophyllaceae	<i>Fagonia mollis</i> Delile <i>Peganum harmala</i> L.

### ***Variation in phenotypic traits, germination and early-life growth***

Vegetative parameters including crown cover, diameter, height, and average seed number/fruit and weight for each population was also measured (*Table 1*) to assess the variation in phenotypic traits between studied populations. Seeds of *M. peregrina* germinate well without any pre-treatment. So, a total of 2788 seeds from forty-seven trees representing different sampled populations were sown directly on Fafard mix no.3B (Canadian Sphagnum Peat, Vermiculite, Perlite, and Processed Pine Bark.) and placed in a greenhouse. Germination percentage and variation between sampled populations were assessed.

A total of 1302 *M. peregrina* seeds representing thirty-two trees grown at Wadi Fieran (165 seeds from five trees) and Wadi Zaghra (1137 seeds from twenty-seven trees) were planted in sand-Peat Moss 1:1 mixture and placed under open-air conditions in SCU botanical garden during the growing season (March and April). Germination percentage was assessed for every tree and the mean for every population and overall mean were calculated. Early-life growth parameters including height, number of branches, and vitality of 10 seedlings were measured every two weeks for six months. Variation in germination percentages and growth parameters between trees and populations were assessed.

Using MINITAB release 14 computer software, One Way ANOVA was used to test the variation between groups. As the ANOVA procedure depends on data being at least a reasonably close approximation to a normal distribution, the Anderson-Darling test was used to allow the possible conclusion that the departures from normality are detectable or not. Bartlett's or Levene's test was used to test for variance homogeneity. Bartlett's test was used when the data come from a normal distribution and Levene's test was used when the data come from continuous, but not necessarily normal, distributions. Kruskal-Wallis was used as an alternative to ANOVA with data sets that failed that test for normality and/or homogeneity of variance.

### ***Variation in early life-history fitness***

Survivorship of any plant in such harsh desert environment depends on its fitness to this environment. Since fitness is the probability of a plant to survive until the reproductive age and gives offspring, it is a net function of the total number of seeds that the mother plant produces, the viability of the produced seeds, the probability of germination and producing seedlings, and the probability of these seedlings to grow ahead of seedling (juvenile) stage and get established (adult). Abd El-Wahab (1995) revealed that the seeds of *M. peregrina* is almost hundred percent viable. As a result, the viability does not contribute to differentiate between populations in their fitness. The net number of seeds been produced by a tree could be, logically, calculated through estimating the average number of seeds each fruit has, multiplied by the total number of that fruits a tree produce. Unless been in fenced enclosures, practically in highly palatable seeds, the total number of fruits been produced by a tree could not be estimated. Meanwhile, the average number of seeds per fruit is still a good indicator for the overall seed production and that is what we adopted here.

Growth parameters like number of branches, height, and vitality reflects the healthiness of the tree and hence refers directly to establishment probability. As *M. peregrina* seeds are highly palatable, its weight (as it reflects its size and storage material content) represents a major factor in determining the risk of been eaten. The

higher weight of seeds the higher probability of been eaten, i.e. low establishment probability. Therefore, six measured life-history parameters (average number of seeds/fruit, seed weight, germination percentage, height, number of branches, and vitality) were subjected to principal component analysis to develop a smaller number of artificial variables (principal components) that will account for most of the variance in the estimated fitness in case there is some redundancy in those variables. As long as *Moringa peregrina* seeds have no innate dormancy, the germination rate may reflect the total fitness including female (production of ovules) and male fitness (fertilization of produced ovules).

The transformed data (divided by the maximum measured value) of average number of seeds/fruit and seed weight, germination percentage (in greenhouse and open-air), and growth parameters (height, number of branches, vitality) were used to construct a fitness index for the Early life-history as follows:

- Fitness = average number of seeds/fruit X seed weight X germination (greenhouse & open air) X height X number of branches X vitality
- Statistical analyses of fitness traits and fitness index were performed using MINITAB release 14 computer program. One-way ANOVA or Kruskal-Wallis was used to test for significant differences between populations. Kruskal-Wallis test was used when ANOVA could not be used due to a violation of its assumptions, normality and/or variance homogeneity.

## Results

### *Ecological status assessment*

Three Wadis in South Sinai known to support *M. peregrina* populations were surveyed. Sixteen sites containing a total of 197 *M. peregrina* trees were recorded (Table 1). The largest population (N = 40) was recorded in W. Fieran (site 3). Two isolated trees were recorded, one in site 10 in W. Zaghra and another in site 11 in W. Fieran. The most noticeable field observation was that almost all reproductive trees grow on south facing slopes and crevices of metamorphic rocks. At the same Wadi, trees growing on north facing slopes do not flower. This observation may indicate the importance of the light and temperature as reflected by slope exposure as a limiting factor for growth, flowering and fruiting by *M. peregrina*. Most *M. peregrina* populations grow on cliffs and at the base of hills with very rugged topography. The elevation of these hills ranges between 300 to 800 m above sea level. The narrow distribution of *M. peregrina* is due to over-grazing (the seeds are highly grazed by goats and sheep), soil moisture content and over-cutting.

Twenty five species were recorded as commonly associating species to *M. peregrina* in the surveyed sites. Associating species (Table 2) include *Acacia tortilis*, *Aerva javonica*, *Capparis spinosa*, *Ephedra alata*, *Hyoscyamus muticus*, *Peganum harmala*, and *Pergularia tomentosa*.

Soil physical analysis showed that surface soils of the different sites supporting *M. peregrina* populations are characterized by very narrow (non-significant, Table 3) variation in soil texture with more or less neutral to slightly alkaline sandy soil (pH = 6.5 – 7.9 and sand fraction = 62.7 – 96.8 %) and with low salinity level (EC = 0.4 – 15.4 mS/cm). Pearson linear correlation test showed highly significant positive correlation



between EC and Clay content ( $CC = 0.582$ ,  $P = 0.001$ ) and negative one between silt and sand fractions ( $CC = -0.989$ ,  $P = 0.000$ ).

**Table 3.** Summary of soil analysis results at different sites

Wadi		pH <sup>NS</sup>	EC <sup>NS</sup> (mS/cm)	Clay <sup>NS</sup> (%)	Silt <sup>NS</sup> (%)	Sand <sup>NS</sup> (%)	Soil Texture
Feiran	Min	6.87	1.51	2.72	2.60	89.22	Sandy
	Max	7.72	3.71	3.86	7.68	93.54	
	Mean	7.22	2.43	3.27	5.07	91.66	
	SD	0.35	0.96	0.44	1.80	1.60	
Zaghra	Min	6.47	2.02	1.46	1.97	62.70	Sandy loamy to Sandy
	Max	7.83	15.35	5.58	33.94	95.36	
	Mean	7.17	6.00	3.76	6.66	89.59	
	SD	0.37	4.07	1.15	8.78	8.66	
Me'ar	Min	6.94	0.42	2.62	0.61	90.36	Sandy
	Max	7.93	11.63	5.00	6.87	96.78	
	Mean	7.33	3.56	3.41	2.93	93.67	
	SD	0.30	3.48	0.73	1.92	2.19	
Total	Min	6.47	0.42	1.46	0.61	62.70	Sandy loamy to Sandy
	Max	7.93	15.35	5.58	33.94	96.78	
	Mean	7.24	4.37	3.53	4.84	91.63	
	SD	0.33	3.67	0.89	5.93	5.94	

**Note:** \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.02$ , NS = non significant variation

Human activities that threaten *M. peregrina* populations in the studied three Wadis are the over-grazing, over-cutting, and over-collection. Over-cutting and breaking down *M. peregrina* fragile branches are one of the best preferred sources for cooking and heating for most of Bedouins living near *M. peregrina* populations and especially when there are no other substitutes (e.g. *Acacia tortilis*, *Anabasis articulata*, *Artemisia judaica*, and *Haloxylon salicornicum*) in very dry seasons and years like the recent ones.

Although *M. peregrina* is one of the most suffering medicinal plants from over-collection of ripened seeds for feeding livestock animals, local trade, and research, this collection has more profound effect on *M. peregrina* populations than over-grazing and over-cutting where most of the produced seeds are eaten by grazing shoats *in situ*. Of course the magnitude of the anthropogenic effect differs from population to another and from Wadi to another depending on the closeness and size of the human population size nearby the targeted *M. peregrina* population. It is higher on populations grow at W. Fieran (more than one thousand families) and W. Me'ar (two Bedouin settlements, seven-one families, Abd El-Wahab *et al.*, 2004) than that at W. Zaghra where only one Bedouin settlement (five families, Abd El-Wahab *et al.*, 2004) occurs. These human unmanaged activities add on the regional natural hazards including the unprecedented prevailing aridity conditions in recent years that made most of the medicinal plants in South Sinai are considered as either endangered or vulnerable species. Although some traders import *Moringa* seeds for abroad sources, the shortage supply of local *M. peregrina* seeds at different herb shops is a clear evidence of being threatened and a reason for over-collection for trade. The seeds have been sold by herbalists under the Arabic name of "Habba Ghalia" for 30-40 LE per Kg (MPCP, 2006). Its vernacular name may reflect its scarcity and/or importance. In herbal traditional medication, being

edible, its seeds are mixed with other herbs and used mainly as anti-malnutrition food (MPCP, 2006).

### ***Phenotypic variation***

#### ***Seed characters (weight and number)***

Although there was variation detected between populations growing at different Wadis in the average seed weight, it was not significant. Generally, seeds collected from populations at W. Feiran (mean = 0.74g, SD = 0.11) have higher weight than that collected from populations at W. Zaghra (mean = 0.58g, SD = 0.12) and W. Me'ar (mean = 0.58g, SD = 0.058), respectively (*Table 4*). It varied from 0.61 to 0.89g for the populations growing at W. Fieran, from 0.31 to 0.89g at W. Zaghra, and from 0.33 to 0.97g at W. Me'ar. The overall mean is 0.60g (SD = 0.14). Unlike the average seed weight, number of seeds per pod showed a highly significant variation ( $P = 0.000$ ) between populations and between individual. It ranged from two to eighteen in trees at W. Feiran and to twenty-three in trees at W. Zaghra.

#### ***Vegetative parameters***

Tree crown cover and height showed a highly significant variation ( $P = 0.000$ ) between populations and Wadis (*Table 1*). Trees at Wadi Zaghra showed the highest cover (mean = 20.81, SD = 8.01) and height values (mean = 10.30, SD = 4.93) followed by trees at Wadi Fieran (mean cover = 8.11, SD = 5.61, mean height = 3.83, SD = 2.56) and trees at Wadi Me'ar (mean cover = 6.28, SD = 3.33, mean height = 3.70, SD = 2.00). Pearson correlation coefficient showed a highly significant positive correlation between the trees crown cover and their height which is very logical, but did not show any significant correlation with the measured soil characters.

### ***Variation in growth and fitness traits***

#### ***Germination***

Germination percentage of *M. peregrina* seeds planted in greenhouse was significantly higher than 55.56 %. It had highly significant difference among trees ( $P = 0.000$ ) and ranged highly from 0% to 100% with an overall mean percentage of 89.7 % (SD = 17.42). Although this highly significant variation between trees together with the apparent variation in mean germination percentage in different Wadis (97.38%, 88.87%, and 80.00 % for seeds from W. Fieran, W. Zaghra, and W. Me'ar, respectively), this apparent variation between populations was not significant ( $P = 0.12$ ). Pearson correlation coefficient showed a positive highly significant correlation between *M. peregrina* seed weight and germination percentage in greenhouse ( $P = 0.001$ ).

Germination under the open-air condition in botanical garden at Ismailia did not show a significant variation between populations in Wadi Fieran and W. Zaghra. However, the mean germination percentage differed between the two wadis. While it was significantly higher than 70.7 % for populations at W. Zaghra, it was only higher than 42.2 % for populations at W. Fieran (*Table 4*). This open-air germination did not show significant correlation with germination in greenhouse condition (*Table 5*).

**Table 4.** Growth and fitness traits

Wadi (Location)	Pop	Avg. seeds/pod**				Avg. seed weight <sup>NS</sup>				Germination in <sup>NS</sup> Greenhouse				Avg. Germ. % in botanical garden <sup>NS</sup>				Avg. height (cm) **				Avg. # branches <sup>NS</sup>			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
W. Fieran	2	3	13	7.11	3.98	0.63	0.63	0.63	-	80.00	100.0	95.00	8.08	98.00	98.00	98.00	-	6.8	13.0	9.11	1.78	4	9	6.4	1.26
	5	2	18	5.17	4.30	0.78	0.89	0.83	0.06	85.71	100.0	98.26	4.29	54.00	100.0	79.00	23.30	5.0	12.5	6.71	1.86	4	9	6.0	1.23
	6	Bulked				0.71	0.71	0.71	-	96.00	96.00	96.00	-	-	-	-	-	-	-	-	-	-	-	-	-
	11	Bulked				0.61	0.61	0.61	-	100.0	100.0	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Subtotal		2	18	5.72	4.24	0.61	0.89	0.74	0.11	80.00	100.0	97.38	5.52	54.40	100.0	83.80	21.20	5.0	13.0	7.39	2.12	4	9	6.11	1.23
W. Zaghra	7	3	23	11.12	4.78	0.44	0.76	0.56	0.10	40.00	100.0	91.47	12.90	70.00	100.0	88.80	12.21	5.0	12.0	7.59	1.16	4	9	5.76	0.92
	8	2	19	9.92	3.90	0.31	0.89	0.58	0.14	0.00	100.0	87.59	20.26	86.00	100.0	95.85	4.45	4.5	9.5	7.14	1.1	3	8	5.94	1.03
	9	2	20	9.32	4.09	0.46	0.77	0.60	0.10	0.00	100.0	88.63	16.68	50.00	100.0	88.22	12.21	5.0	9.0	7.10	0.87	4	8	5.67	0.87
Subtotal		2	23	9.99	4.22	0.31	0.89	0.58	0.12	0.00	100.0	88.87	17.47	50.00	100.0	92.00	10.85	4.5	12.0	7.21	1.06	3	9	5.77	0.96
Me'ar	12	Bulked				0.43	0.68	0.56	0.18	96.00	100.0	98.00	2.83	-	-	-	-	-	-	-	-	-	-	-	-
	13	Bulked				0.33	0.97	0.67	0.32	0.00	100.0	66.7	57.7	-	-	-	-	-	-	-	-	-	-	-	-
	14	Bulked				0.43	0.49	0.46	0.04	70.6	95.8	83.2	17.8	-	-	-	-	-	-	-	-	-	-	-	-
Subtotal						0.33	0.97	0.58	0.22	0.00	100.0	80.00	37.00	-	-	-	-	-	-	-	-	-	-	-	-
Total		2	23	9.48	4.44	0.31	0.97	0.60	0.14	0.00	100.0	89.70	17.42	50.00	100.0	90.94	12.45	4.5	13.0	7.23	1.22	3	9	5.81	1.00

**Note:** \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.02$ , NS = non significant variation.

### Height, branching, and vitality

The height of *M. peregrina* juveniles grown in botanical garden of SCU at Ismailia under uncontrolled condition (open-air) during growing season (March – October) showed a highly significant variation between trees ( $P = 0.000$ ) and between populations ( $P = 0.000$ ). Although height was positively correlated with number of branches (C.C. = 0.967,  $P = 0.000$ ), the latter had variation between trees but not populations ( $P = 0.000$  and 0.201, respectively). Meanwhile, Pearson test did not detect significant correlation between the height and either germination in greenhouse or in botanical garden (C.C. = 0.063, 0.086, respectively).

Although Kruskal-Wallis test revealed a highly significant variation in measured vitality between trees and populations ( $P = 0.000$  for both), Pearson coefficient did not recognize any significant correlation between vitality and other measured parameters (Table 5).

**Table 5.** Pearson's product-moment correlation coefficients among the measured life-history characters

Variable	Germination % in greenhouse	Germination % (open air)	Height	Branch no.	Seed number	Seed weight
Germination % (open air)	-0.062 0.741					
Height	0.063 0.737	0.086 0.645				
Branch no.	0.080 0.667	0.034 0.857	<b>0.967</b> <b>0.000</b>			
Seed number	0.212 0.252	0.107 0.566	-0.065 0.727	-0.117 0.532		
Seed weight	<b>0.572</b> <b>0.001</b>	-0.075 0.687	-0.021 0.909	0.011 0.953	-0.331 0.069	
Vitality	0.173 0.353	-0.094 0.615	-0.119 0.523	-0.189 0.308	0.327 0.073	-0.070 0.710

### Early life-history fitness

Correlation coefficients among the measured life-history characters are presented in Table 5. Except for two sets of characters, one is related to seeds (germination in greenhouse and weight) and the other is related to seedlings (height and number of branches), all other intercorrelations were not significant (Table 5). PCA reduced the total set of partly intercorrelated variables to four uncorrelated principal components. The component loadings per original variable and the proportion of total variance explained by the four components are given in Table 6. Together, the four principal components explain 87.1 % of the total variation. The first main fitness component appears to be correlated with a combination of seedlings height and number of branches. Germination rate in greenhouse and seed weight have the highest loadings for the second fitness component while number of seeds per pod and produced seedling vitality have the highest loadings for the third fitness component. The last component is correlated with germination rate under open-air conditions.

Generally, the early life fitness index for *Moringa peregrina* populations was very low (mean = 0.06, SD = 0.03). Kruskal-Wallis test showed that the variation between

populations was not significant ( $H = 5.94$ ,  $P$  (adjusted for ties) = 0.203). While both are significantly higher than zero, populations at W. Zaghra showed very little higher mean fitness value (0.06, SD = 0.03) than those at W. Fieran (0.04, SD = 0.02).

**Table 6.** Principal component loadings of the measured life-history characters (fitness characters). Loadings given in boldface show the highest correlation between original values and principal component scores.

Variable	PC1	PC2	PC3	PC4
Germination %	-0.056	<b>-0.661</b>	0.323	-0.153
Germination % (open air)	-0.066	0.205	0.111	<b>-0.913</b>
Height	<b>-0.654</b>	0.078	0.235	0.089
Branch no.	<b>-0.668</b>	0.051	0.178	0.115
Seed number	0.205	0.106	<b>0.686</b>	-0.114
Seed weight	-0.1	<b>-0.698</b>	-0.216	-0.17
Vitality	0.258	-0.114	<b>0.529</b>	0.284
Eigen value	2.067	0.605	1.421	1.006
Proportion	0.295	0.229	0.203	0.144
Cumulative	0.295	0.524	0.727	<b>0.871</b>

## Discussion

Climate change and its consequences present one of the most important threats to biodiversity and the functions of ecosystems. The stress on biodiversity is far beyond the levels imposed by the natural global climatic changes occurring in the recent evolutionary past. It includes temperature increases, shifts of climate zones, melting of snow and ice, sea level rise, droughts, floods, and other extreme weather events (Omann *et al.*, 2009). During the past century, annual mean temperature has increased by 0.75°C and precipitation has shown marked variation throughout the Mediterranean basin (Osborne *et al.*, 2000). This change affected the wild vegetation of South Sinai in general and resulted in rarity of trees and change in vegetation composition (Moustafa *et al.*, 2001). Unmanaged anthropogenic practices and the natural sparseness of vegetation in desert ecosystems magnify the effect of climate change and results in more fragile and sensitive ecosystem to human impacts (Batanouny, 1983).

Habitat fragmentation is considered to be one of the major threats to biological diversity (Eriksson and Ehrlén, 2001; Oostermeijer, 2003). Human activities have had a strong impact on landscape structures, often associated with a decline in habitat size, an increase in habitat isolation, altered habitat conditions and loss of plant-animal interactions (e.g. Fischer and Matthies, 1998; Kearns *et al.*, 1998). Various studies have shown that plants in fragmented populations may be smaller, have lower reproductive output and reduced seed germinability compared to non-fragmented populations.

As a multi-use economical important medicinal species, *Moringa peregrina* has experienced unmanaged anthropogenic practices (especially the over-collection of ripened seeds) beside the harsh habitat conditions resulting in a sharp decline in number and size of its populations in Sinai (Abd El-Wahab, 1995; abd El-Wahab, 2004). This reduction in the number of populations and individuals has had a negative impact on the wildlife of the region since *M. peregrina* together with *Acacia* trees and various shrub species provide an essential food source for desert wildlife. Furthermore, the decline of

*M. peregrina* populations has resulted in the loss of a primary natural resource for the local Bedouin communities and their domestic animals.

The data presented in this paper clearly show that remnant populations of the rare medicinal species *Moringa peregrina* have very low early stage fitness estimated as an index of maternally affected life-history characters, represented by seed number and weight, germination rate under greenhouse and open-air conditions, and seedlings characters including height, number of branches, and vitality. All of the studied populations could be considered small ones, meanwhile Zaghloul *et al.* (in preparation) revealed that these populations have reduced genetic diversity. This may suggest that the deteriorated environmental circumstances, which the maternal plants in small populations encounter, affect the performance of their offspring negatively. Several previous studies (e.g. Schaal, 1984; Roach and Wulff, 1987) showed that environmental stress on maternal plants is carried over to the progeny, especially in the first stages of their development. Also, several previous works (e.g. Ouborg *et al.*, 1991; Oostermeijer *et al.*, 1994) demonstrated a positive relationship between population size and variation in fitness components for rare perennial plant species. Similar results were obtained in *Acacia tortilis* subspecies *raddiana* populations growing in the same habitats (Zaghloul, 2008).

Zaghloul *et al.* (in preparation) demonstrated that *Moringa peregrina* populations experience real selfing (16%) and open-pollinated seeds are a product of both selfing and outcrossing events. The potential for maternal plants in small populations to invest in offspring may also be reduced by inbreeding depression, or because they are, just by chance, a poorly performing sample of survivors from the previous larger populations, a phenomenon known as demographic stochasticity (Menges, 1991). However, it is not likely for the long-lived *Moringa peregrina* (unpublished data) that there has been a history of inbreeding. So, it is most likely that the very low maternally affected fitness is due to environmental stress (including threats imposed by contemporary prevailing drought and anthropogenic practices) on the remaining populations.

The results of current study showed a highly significant variation ( $P = 0.000$ ) between populations in number of seeds per pod, crown cover, and height of trees (Table 1). This variation could be attributed to very low observed heterozygosity ( $H_o = 0.04$ , Zaghloul *et al.*, in preparation) that is recorded in these populations that the reduced heterozygosity does not only lower individual fitness, but may also increase the amount of variation among progeny since 'developmental homeostasis' is disturbed in homozygotes (Mitton and Grant, 1984; Mitton, 1989).

In conclusion, current threats and the prevailing harsh habitat conditions are the main causes for the need of the species conservation and development of a plan for its sustainable management in the Sinai. Direct protection is urgently needed to stop further deterioration of *M. peregrina* populations and to improve their ability to maintain or improve population numbers.

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