American-Eurasian J. Agric. & Environ. Sci., 15 (12): 2512-2520, 2015
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2015.15.12.12691

Seed Bank Approach for Conservation of Two Threatened Endemic Medicinal Plant Species; *Hypericum sinaicum* Hocsht. & Steud ex Boiss. and *Plantago sinaica* (Barneoud) Decne.

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Abstract: The present study aimed to illustrate soil seed bank and its relationship to above ground vegetation in order to contribute in designing a sound long term conservation plan for the two threatened endemic, medicinal species; *Hypericum sinaicum* and *Plantago sinaica*. Ninety-two soil samples taken from thirty- six stands, each sample was taken from a 25 x 25 cm^2 quadrate and three cm depth, samples were labeled, air driedand stored in laboratory conditions until sowing. The total number of species was forty, including four grasses (Gramineae); *Schismus barbatus*, *Lophochloa cristata*, *Polypogon monspeliensis* and *Panicum coloratum*, showed emergence of eight endemic species: *Veronica kaiseri*, *Hypericum sinaicum*, *Nepeta septemcrenata*, *Plantago sinaica*, *Origanum syriacum*, *Phlomis aurea*, *Galium sinaicum* and *Primula boveana* among thethirty-one identified species. Some species were found in most of the studied localities as *Alkanna orientalis* and *Pulicaria crispa* in the contrary *Galium setaceum*, Phlomis aurea and *Chenopodium* sp. were found only in Garagnia stands. Based on the floristic composition (seed density), the stands were classified and separated by TWINSPAN to four main assemblages or communities; assemblage I: *Alkanna orientalis*, assemblage II: *Arenaria deflexa*, assemblage III: *Schismus barbatus* and assemblage IV: unknown sp. no. 3, which were separated at the second level of classification where the main indicator species were *Hypericum sinaicum* and *Mentha longifolia*. As a general conclusion, the present study clarified that the behavior of endemic species seeds in the soil seed bank can give us an indication of its status and can be a useful tool to restore rare species from the soil samples.

Key words: Ecology · Endangered · Sinai · *Jasonia montana* · Plantin · St john's wort

INTRODUCTION

Seeds are a crucial part of the life history of plants living in desert ecosystems. For annual species, which constitute 40% or more of the desert flora, they may only exist as seeds for long periods especially during drought years and the seed of most desert plant species is the only means of dispersal [1], that why the soil seed bank is considered the life cycle origin for the annual species, being fundamentally the cause of its persistence; in perennials, besides the seed bank, there is a bank of vegetative propagules like tubers, rhizomes and stolons [2].

The earlier studies of soil seed banks started in [3] with Darwin, when he observed the emergency of seedlings, using soil samples from the bottom of a lake. Many seed bank studies have been carried out in Egypt; Alaily *et al.* [4] carried out a seed bank study in the south-western desert of Egypt, while the seed bank study on soils of the most prominent communities in Wadi Feiran was carried out by Ramadan [5] and Batanouny *et al.* [6] in order to estimate the potential viable seed flora of such a desert area. The potential of both soils and different kinds of domestic and wild animal dung as reservoir for seeds in Sinai desert was studied by Ramadan and Shabana [7]. Although the seed bank is an important element in desert ecosystems, little is documented on the diversity of the soil seed bank and its relations to the above-ground vegetation in arid regions [8-10].

South Sinai, an arid to extremely arid region, is characterized by an ecological uniqueness, due to its diversity in landforms, geologic structures and climate that resulted in a diversity in vegetation types, which is characterized mainly by the sparseness and dominance of

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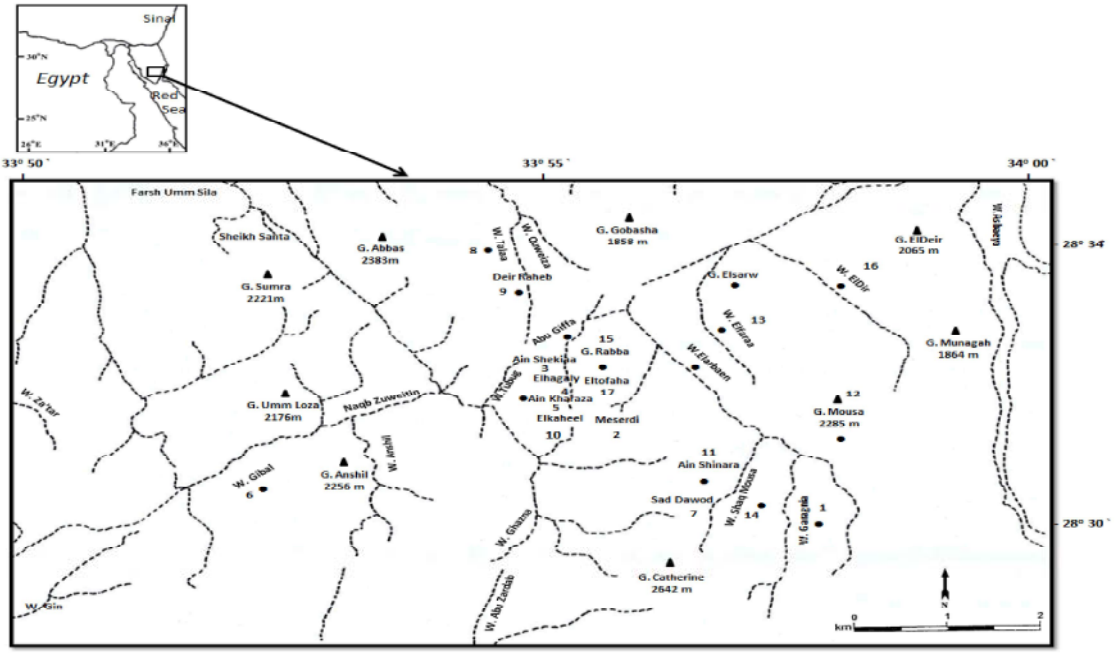

Fig. 1: Location map of the study area (Saint Kathereine Protectorate) in the southern part of Sinai. Mountain tops (Gebel = G) are represented by (▲), Wadis or valleys (W) and main location of the study represented by (•).

shrubs and sub-shrubs and the paucity of trees and a variation in soil properties [11]. Three-hundred and twenty three species were identified in Saint Katherine Protectorate. The mountainous area of South Sinai harbors 26 endemic species [12]. Of the species in Saint Katherine's Protectorate, 10 are extremely endangered, 53 are endangered and 37 species are vulnerable. Nearly half of the endemic species found in Saint Catherine's are vulnerable, rare, endangered, or extremely endangered [12] are subjected to great disturbance due to the severe impact of the human activities. The continuous over-grazing, over-collection (cutting and uprooting for fuel and medicinal uses), tourism and urbanization resulted in disappearance of pastoral plants, paucity of trees and shrubs as well as disappearance of many rare and endemic species

The present study aims to contribute in designing a sound long term conservation plan for the studied threatened medicinal species by using of soil seed bank in different populations to determine species composition and seed densities in different seasons during the study period as a tool in understanding the seed behavior of each studied species.

Study Area: The study was carried out in Saint Katherine Protectorate which is located between 33°30' and 34°30' E and 27°50'and 28°50'N and covers about 4350 km^2 with elevation ranges from 396 to 2642 m. The region is characterized by outcrops of smooth-faced granite uplifted to form several mountain peaks. Its diversity in landforms, geologic structures and climate led to the differentiation of a number of microhabitats, each of them has its peculiar environmental conditions and resulted in a relatively rich and unique flora [11]. Saint Catherine is the coolest area in Sinai and Egypt as a whole due to its high elevation. The lowest minimum temperature was recorded in January and February (-3°C and -6°C), while the highest maximum temperature was in June and August (42°C and 43°C, respectively). The flora of South Sinai comprises five-hundred and twenty species [13]. Three hundred and twenty-three species were identified in Saint Katherine Protectorate. The mountainous area of South Sinai harbors twenty-six endemic species [12]. In many of these plants, Bedouins find sources of nutrition, medicine and pasture. In the last ten years unmanaged human activities have threatened endemic and rare species resulting in disappearance of pastoral plant communities and have caused an increased dominance of unpalatable plant species.

The study area includes: Wadi El-Arbaen and its surrounding mountains namely Gebel El-Rabba and Gebel El-Sarw, Gebal Mousa and Garagnia, Wadi Tofaha and its surrounding mountains namely Gebel Tofaha and Gebel El-Talaa, Meserdi ridge and Gebal Abu-Giffa, Wadi Gibal, Wadi Tobug (Fig. 1).

MATERIALS AND METHODS

Soil Sampling: The soil sampling was carried out during the winter seasons of 2013 and 2014 after seed shedding of most plant species of the vegetation in the study area. Ninety-two soil samples were taken from thirty-six stands. Each sample was taken from a 25 x 25 cm^2 quadrate and three cm depth samples were labeled, air dried and stored in laboratory conditions until sowing, then samples were sieved through two mm-mesh sieve to separate and eliminate large gravel particles to guarantee not to produce any microhabitat effect in sowing which may give a false variation among samples. The above sieve's mesh was chosen to be sure that it is large enough not to eliminate any seed [14].

Sowing of Soil Samples (Seedling Emergence): Generally, in this method of determining the numbers of seeds in a sample, the soil is placed directly into a shallow container or spread in a thin layer on suitable medium, kept moist and the seedlings that emerge are identified and recorded. In this study, the seed bank experiment was carried out in the laboratory during the spring periods of 2013 and 2014. Before soil sowing, the bottoms of circular plastic plates (≈21 cm diameter) were filled with three cm depth seed-free sand. This substrate allows only the viable seeds of the investigated soil sample to germinate and stimulate a quick development of roots searching for nutrients. An amount of one-hundred and seventy cm^3 from each soil sample was sown in each plate and was done in three replicas. This amount was spread in a half cm thick layer over the sandy substrate. It was irrigated every other day and sometimes every day. The germinated seedlings were marked by color-headed pins whenever a new seedling is noticed and were coded. Seedlings were left to form foliage leaves and grow in order to be identified completely.

RESULTS

Soil samples showed high species richness where the total number of species was forty, including four grasses (Gramineae); *Schismus barbatus, Lophochloa cristata, Polypogon monspeliensis* and *Panicum coloratum*. In seedling and young stages, these species look very similar and could not be distinguished and many individuals died in young stages, so these species were treated collectively under common name " grasses" until some of them where indentified. Nine species could not be identified because the seedlings died in a too young stage. Biological crust (algae, mosses) grew on soil samples of ten stands. In natural habitats, each of these stands either has biological crust (at least one component), or it is located near another stand that has biological crust in its natural vegetation.

The results of seed bank test (Table 1) showed emergence of eight endemic species: *Veronica kaiseri, Hypericum sinaicum*, *Nepeta septemcrenata, Plantago sinaica, Origanum syriacum, Phlomis aurea*, *Galium sinaicum* and *Primula boveana* among the thirty-one identified species. Some species were found in most of the studied localities as *Alkanna orientalis* and *Pulicaria crispa* in the contrary *Galium setaceum*, *Phlomis aurea* and *Chenopodium* sp. were found only in Garagnia stands.

The emergent seedlings from soil seed bank samples showed the highest density in Ain Shekiaa site (15052 seedling/m^2), followed by El-Raheb field site (12879 seedling/m^2). The lowest density (96 seedling/m^2) was found at Ain-Shenara site. Meanwhile, the end of Talaa site had no seedling emergence at all. The richness is highly variable between locations with the highest (29 species) recorded in Garagnia followed by Meserdi (24 species), Shag-Mousa and Ain-Shekaia (11 species) for each and W. Gibal (10 species). G.El-Rabba, El-Tofaha, Wadi El-Deir site and El-Raheb field showed the lowest species richness in collected soil seed bank samples (2,3, 4 and 4, respectively) (Table 2 & Fig. 2).

Based on the floristic composition (seed density), the stands could be classified and separated by TWINSPAN to four main assemblages or communities (Figure 3) which were separated at the second level of classification where the main indicator species were *Hypericum sinaicum, Mentha longifolia* and unknown sp.

Assemblage I: ***Alkanna orientalis***
Assemblage II: ***Arenaria deflexa***
Assemblage III: ***Schismus barbatus***
Assemblage IV: **unknown sp. no. 3**

The soil seed bank samples in *Alkanna orientalis* assemblage was dominated by *A. orientalis* with high frequency (88%) and two associated species *Mentha longifolia* with frequency 76% and *Hypericum sinaicum* with frequency 60%. This assemblage comprised of twenty-five stands that were found in the main locations of study area (W. El-Deir, Meserdi, El-Tofaha, Garagnia, W. El-Talaa, Sad-dawood, Ain-Shekiaa, W. Gibal and El-Hagaly). Most of these stands located at elevation ranges from 1600 to 1920 ma.s.l., with highest soil

Table 1: Summary of species list emergent from soil seed bank and their distribution in the studied localities.

	Species	Distribution
1.	*Alkannaorientalis*	1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11
2.	*Arenariadeflexa*	1, 2, 4, 6, 11, 2, 13 and14
3.	*Ballotaundulata*	1 and 2
4.	*Cotoneaster orbicularis*	1 and 3
5.	*Chenopodium* sp.	1
6.	*Dipotaxisacris*	1, 4 and 5
7.	*Ficus pseudo-sycomorus*	1, 3, 4, 6, 7, 12 and 14
8.	*Funaria* sp.	1 and 2
9.	*Galiumsetaceum*	1
10.	*Galiumsinaicum*	2, 13 and 14
11.	*Hypericumsinaicum*	1, 2, 3, 4, 6, 15 and 16
12.	*Iflogaspicata*	1 and 11
13.	*Lophochloacristata*	1, 2 and 14
14.	*Menthalongifolia*	1, 2, 3, 6, 7, 8, 9 and 16
15.	*Nepetaseptemcrenata*	1, 2 and 14
16.	*Origanumsyriacum*	1, 2 and 8
17.	*Panicumcoloratum*	1, 2 and 3
18.	*Phlomisaurea*	1
19.	*Plantagosinaica*	2, 10, 13 and 15
20.	*Polypogonmonspeliensis*	1, 2 and 13
21.	*Primulaboveana*	1 and 12
22.	*Pulicariacrispa*	2, 3, 8, 10, 11, 12, 1 4 and 17
23.	*Schismusbarbatus*	1 and 14
24.	*Scrophularia* sp.	4 and 5
25.	*Sisymbriumerysimoides*	1 and 14
26.	*Stachysaegyptiaca*	1, 6, 12, 13 and 14
27.	*Tanacetumsinaicum*	1 and 2
28.	*Teucriumpolium*	1, 2, 6, 12 and 14
29.	*Trigonellastellata*	3 and14
30.	*Verbascumsinaiticum*	1, 2, 12, 13 and14
31.	*Veronica kaiseri*	1, 12 and 14

Distribution locations:1; W. Garagnia, 2;Meserdi, 3; AinShekiaa, 4; Elhagaly,5; AinKharaza, 6; W.Gibal, 7;Sad Dawod, 8;W.Talaa, 9;DeirRaheb, 10;Elkaheel11;Ainshinara, 12; Gebel Mousa, 13;ElFaraa, 14; ShaqMousa, 15;G.Rabba, 16; W.Eldeir, 17; El-Tofaha.

Table 2: Soil seed bank results showing the seed density (seedlings/m^2) and species richness at sampled locations of the study area of Saint Catherine

Locations	No. of Sites	Seed density (seedling/m^2)	Species richness
Gargnia	04	5868.0	29
Meserdi	12	3198.0	24
Ainshekiaa	02	15052	11
El hagaly	02	4422.0	9.0
Ainkharaza	01	147.00	5.0
WadiGibal	01	7600.0	10
Sad Dawod	01	8360.0	6.0
W. Talaa	02	3386.0	7.0
El Raheb field	01	12879	4.0
End of Talaa	01	0.000	0.0
Wadi El Deir	01	256.00	4.0
G. El Rabba	01	768.00	2.0
Tofaha	01	752.00	3.0
Elkaheel	01	1008.0	7.0
Ainshenara	01	96.000	6.0
Gebel Mousa1	01	141.00	7.0
Gebel Mousa2	01	1505.0	8.0
El faraa	04	408.00	6.0
Shaq Mousa	12	640.00	11

Fig. 2: Seed bank results show; (a) density of *Mentha longifolia* seedlings emergence at Meserdi site, (b) the dense emergence of seedlings at Garagnia site, (c) the experiment of soil seed bank during the study at the laboratory, (d) the emergence of seedlings of different species at different study sites, (e) the emergence of Graminae species at vegetative stage (*Schismus barbatus*), (f) seedlings of Ficus pseudo-sycomorus at Ain-Shekiaa site

bicarbonate concentration (11.8 meq/L), a range of electric conductivity from 400 to 4000 µS, highest percentage of organic matter 13.7 %, highest percentage of moisture content 5.9% and a wide range of pH 6.9 - 8.14. The second assemblage *Arenaria deflexa* was characterized by high frequency 100%, while the prominent species was *Verbascum sinaiticum* with frequency 75%. This assemblage was represented by four stands that were in Ain-Shenara, G. Mousa, Shag-Mousa and W. El-faraa, which is characterized by high elevations that reached 1971 m and with high electric conductivity which reached 4000 µ.s. and highest chloride, calcium and magnesium concentrations 89.73, 53.2 and 31.6 meq/L respectively.

The third assemblage *Schismus barbatus* was dominated by *S. barbatus* with frequency 100 % and is characterized by 100% presence of unknown no.3. This assemblage was represented by three stands located at Ain-Shekiaa, El-Hagaly and El-Kehal, which was found at high elevations reaching 1852 m and the nature of the soil surface of these stands consisted mainly of boulders and high percentage of gravel in the soil texture 51.4%. Unknown no. 3 was dominating the fourth assemblage with frequency 100%, associated *Pulicaria crispa* and *Plantago sinaica* with frequency 66.7% for each. Three stands were represented in this assemblage in G. El-Rabba, El-Tofaha and Meserdi, which is characterized by elevation range of 1600 to 1650 m and high gravel percentage in soil texture 56.1%.

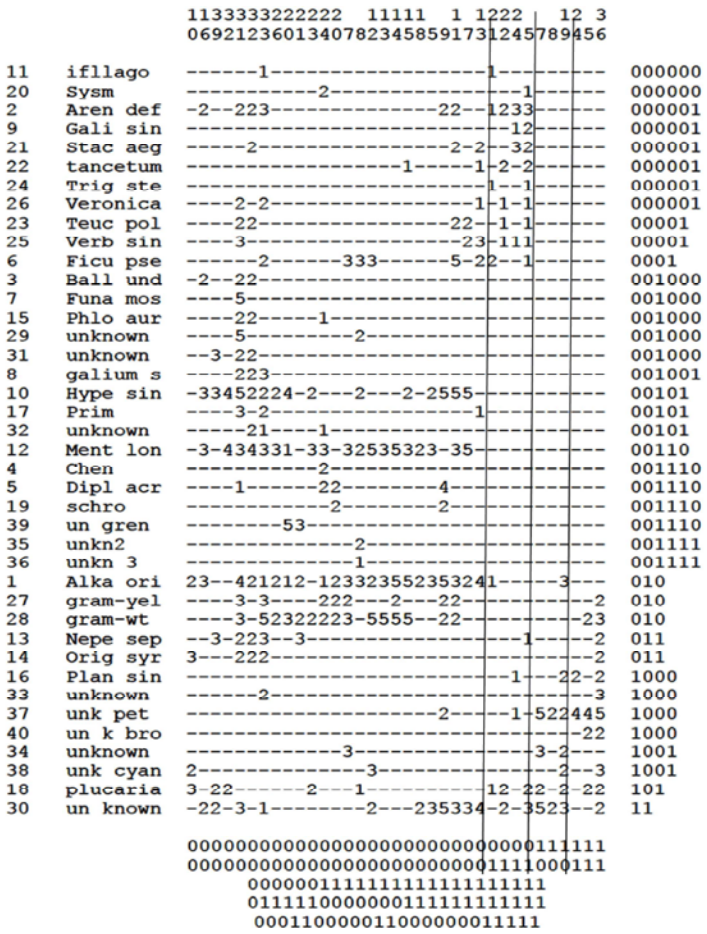

Fig. 3: Two-way species-figure print out of TWINSPAN results showing two dendrograms: species groups on the right hand side and site clusters on the bottom of the figure, for the thirty-five locations and forty plant species of the studied soil seed bank samples.

DISCUSSION

In context of climate change, plant genetic composition may change in response to the selection pressure and some plant communities or species associations may be lost as species move and adapt at different rates [15]. Therefore, soil seed banks are considered as essential constituents of plant communities [16], since they have a significant contribution to ecological processes. The ability of vegetation recovery after disturbance is believed to lie mainly in the buried seed populations [17, 18]. The replacement of individuals from the seed bank may have reflective effects on the composition and patterns of the vegetation within the community [19, 20]. Therefore, restoration and conservation of plant species diversity rely on understanding levels of diversity, spatial distribution and processes that influence these levels and the pathways by which plant species colonize sites. In arid ecosystems soil seed banks are characterize by high spatial and temporal variability [21] and are affected particularly by spatial patterns of vegetation [22].

Seed banks are a crucial component in desert ecosystems and other stressful habitats where favorable conditions for seed germination and seedling establishment are quite unpredictable both in space and time [23]. Abundance of germinable seeds did not always satisfactorily predict seedling emergence of species, although it did so at the community level. At the population level, the relationship between the numbers of germinable seeds and emerged seedlings largely depended on species identity [24]. In the present study, of the sixty-one species recorded in the standing above ground vegetation, only twenty-two of the identified species were present in the seed bank. Among the nine species recorded only in the seed bank and not found in

the standing vegetation, there were two endemic species; *Primula boveana* and *Veronica kaiseri*, two species are endangered; *Cotoneaster orbicularis* and *Panicum coloratum*, two species are very rare; *Sisymbrium erysimoides* and *Galium setaceum* and three common species; *Chenopodium* sp., *Dipotaxis acris* and *Panicum coloratum*.

The TWINSPAN analysis of soil seed bank samples results in four assemblages, the identified dominant species of the four assemblages in the seed bank samples were; *Alkanna orientalis*, *Arenaria deflexa* and *Schismus barbatus*. Those assemblages differed from that of the standing crop analysis, which confirmed the dissimilarity between them. Soil seed bank TWINSPAN analysis acts as a prediction tool for the next vegetation or in other words the upcoming communities out of the soil, as the standing crop is already established. This non-similarity was also found in the desert in south-west of Egypt [4] and in the seed bank of endemic species in Saint Catherine area [5, 14], while it was on the contrary to what Gomaa [25] found in soil seed bank in different habitats of the Eastern Desert.

In general, seed banks have been exploited in two contexts: to manage the composition and structure of existing vegetation and to restore or establish native vegetation. Zaghloul *et al*. [26] found that genetic differentiation among populations of *H. sinaicum* was significantly different between the standing crop and soil seed bank. Honnay *et al* [27] reported that the standing crop showed modest differentiation among populations, while the differentiation among soil seed bank was much lower and assumed that it was very likely the result of local selection acting either directly or indirectly as a filter on the alleles present in the seed bank.

Generally, most of the species which are either recorded only in the standing vegetation and are absent from seed bank or abundant in the vegetation but rare in the seed bank are shrubs and long-lived perennials, these life forms in hot deserts have minimal dependence in soil seed bank for regeneration and protection against climatic uncertainty [28]. Their strategy is to produce few seeds almost every year, most of which do not persist in the seed bank [29]. To the extent that the onset of good conditions is predictable (i.e., the warming of spring or the onset of a rainy season), cues such as temperature, photoperiod, moisture, or seed age may be used to trigger germination [30]. Philippi [30] also stated that desert annuals species, in addition to having mechanisms that allow seeds to germinate only under appropriate conditions, also must have some trait that allows them to persist in the face of environmental unpredictability and may have traits that specifically exploit it. Seed dormancy for more than one year is thought to be a bet-hedging adaptation to environmental uncertainty in desert annuals

The seed bank identified in this study revealed a high degree of spatial heterogeneity, or in other words, the seed distributions are distinctly patched (clumped). These highly clumped distributions of seeds in soil are common for desert seed banks. In this study eight endemic species were identified in soil seed bank; *Veronica khaiseri, Hypericum sinaicum*, *Nepeta septemcrenata, Plantago sinaica, Origanum syriacum, Phlomis aurea*, *Gallium sinaicum* and *Primula boveana*. In this study the main target of the soil seed bank was that of the two endemic species; *Plantago sinaica* and *Hypericum sinaicum*. The behavior of the seeds in the soil seed bank of the two species was completely different and also was different than that of their status in the standing vegetation.

Hypericum sinaicum soil seed bank samples reflected the standing vegetation in species diversity, as most of the associated species were found in most of the samples especially *Mentha longifolia*, which was so distinctive at the *Hypericum* stands in the study area. From the thirty-five soil seed bank samples of the study *H. sinaicum* was found in twenty samples. W. Gibal samples were the highest in seed density; it was about 7600 seedlings /m^2 from which *H. sinaicum* formed 5504 seedlings /m^2 (72%), this was the highest representation of the species among all the other samples, followed by Garagnia samples; 5868 seedlings /m^2 in which *H. sinaicum* represented 40%.The lowest seed density of *H. sinaicum* was at one of the Meserdi site samples it was only 24 seedlings /m^2. Seeds of *P. sinaica* in the seed bank was found in Meserdi, El-kehal, W.El-Faraa and G. El-Rabba and the total seed density in those four sites was 236 seedlings /m^2, reaching its highest value of 96 seedlings /m^2 at El-kehal and its lowest of 16 seedlings /m^2 at W. El-Faraa. It was found in fifteen samples out of the thirty-five studied soil samples.

The study of soil seed bank of the endemic species is a fundamental part of understanding the processes by which they (as a part of endemic in the study area) have become adapted to their harsh and uncertain environment and enable us to make an effective management for conserving such endemic species as a contribution of the overall wild conservation processes.

As a general conclusion, the present study clarified the behavior of endemic species along environmental gradients and seed behavior in soil seed bank, as well as in its strategies in struggling for existence. This variation could be explained and summarized through the studied endemic species as following:

Plantago sinaica seeds may be predated as it contain large amounts of polysaccharide which may act as means of attractant for rodents. Although this plant species has a high germination rate without no pretreatments, it is not found as an abundant species in the standing vegetation nor in the soil seed bank samples, This may be due to bet hedging strategy. While Hypericum sinaicum populations produce the dusty seeds which some are dispersed and others fall in crevices of bare rocks that have more available moisture content and organic matter, some of the seeds germinate in the next spring, whereas the other seeds that fall on soil pockets and become buried by the effect of rains on smooth-faced granite that accumulate fine-textured soils in pockets, these seeds accumulate soil seed bank as a strategy for restoration under circumstances of disturbance. Seed germination and soil seed bank represent two main opportunities for habitation of this species under disturbance.

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