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Seed Ecology and Environmental Conditions of Hypericum sinaicum, Growing in South Sinai, Egypt

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ABSTRACT

There are increasing threats facing the rare plants including the endemic populations. Also the potential development of the earth's vast desert areas for agriculture and other human-needs demands an awareness of the ecological characteristics and requirements of desert vegetation. Saint Catherine area has a unique location and environment. The vegetation and wild life in Saint Catherine area is subjected to great disturbance through the unmanaged human activities. In the present study we had used seed ecology in order to contribute in designing a sound long term conservation plan for the threatened endemic studied medicinal species; *Hypericum sinaicum*, at two levels; (a) soil seed bank and its relationship to above ground vegetation and (b) the germination response at different conditions and pretreatments on wetted substrate. *Hypericum sinaicum* grows in Sinai on mountainous sheltered moist crevices and in Hijaz in the extreme north-west of Saudi Arabia and in Edom in Jordan. The results revealed that seven endemic species were identified in soil seed bank; *Veronica khaiseri*, *Hypericum sinaicum*, *Nepeta septemcrenata*, *Plantago sinaica*, *Origanum syriacum*, *Phlomis aurea*, and *Primula boveana*. Germination treatments on *Hypericum sinaicum* seeds showed that calcium carbonate ($CaCO_3$) and hot water of 50°C treatment was found to be most effective to improve seed germination depending on doses, while other treatments were efficient to a lesser degree. As a general conclusion, the present study clarified that the behaviour of endemic species along environmental gradients varies greatly, as well as in its strategies in struggling for existence.

Key words: Endemic, *Hypericum sinaicum, Plantago sinaica,* Saint Catherine, South Sinai.

INTRODUCTION

There are about three-hundred and seventy species of genus *Hypericum* found in temperate and tropical mountainous regions of the old world, some naturalized in North America (Boulos, 1999). The main center of the diversity of *Hypericum* could be in the Palaearctic area, where more than 45 % of the described species are native. A second center is located in the Neotropic with 30 % of the species. Compared to these numbers, the Indo-Malayan, Nearctic and Afrotropic regions harbor much less diversity, with 10 %, 8.5 % and 6.4 % of the known species, respectively (Nuerk and Blattner, 2010). Hypericum extract and Hypericin inhibits dopamine-beta-hydroxylase in vitro (Obry, 1996).

Hypericin also potentiated neurotransmitter and serotonin receptors (Curle *et al*, 1996). Hypericin has produced a potent antitumor activity in vitro against several tumor cells. However, it did not show any toxic effect on normal cells at much higher concentrations. Based on additional experiments it was concluded that Hypericin directly inhibits epidermal growth factor EGF-receptor and protein tyrosine kinase (PTK) activity (Kil *et al.*, 1996). In most countries, *Hypericum* products are marketed as dietary supplements, and therefore not subjected to stringent drug regulations. In the European community, however, *Hypericum* products are available both as food supplements and as drugs (Linde, 2009). *Hypericum perforatum* was among the top ten best-selling herbal dietary supplements sold in the USA in 2008.

In Egypt, *Hypericum sinaicum* grows in Sinai on mountainous sheltered moist crevices and in Hijaz in the extreme north-west of Saudi Arabia and in Edom in Jordan (Boulos, 1999). *Hypericum sinaicum* has a highly medicinal importance value. Extraction from aerial parts give substances like Hypericin, protohypericin, pseudo-hypericin, protopseudo-hypericin, and hyperforin which showed effect to inhibit the growth of retroviruses including HIV, the AIDS virus in animals beside the treatment of depression (Rezanka and Sigler, 2007). Sinaicin one is an adamantanyl derivative with the isoprenyl oxygenated side chain as a plant metabolite which was isolated from the Egyptian plant *Hypericum sinaicum* (Rezanka and Sigler, 2007).

MATERIALS AND METHODS

Study Area

The study was carried out in Saint Katherine Protectorate which is located between 33°30ˈ and 34°30ˈ E and 27°50ˈ and 28°50ˈ N and covers about 4350 km^2 with elevation ranges from 396 to 2642 m. 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 studied locations included: 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, Gebal Abu-Giffa, Wadi Gibal and Wadi Tobug (Figure 1).

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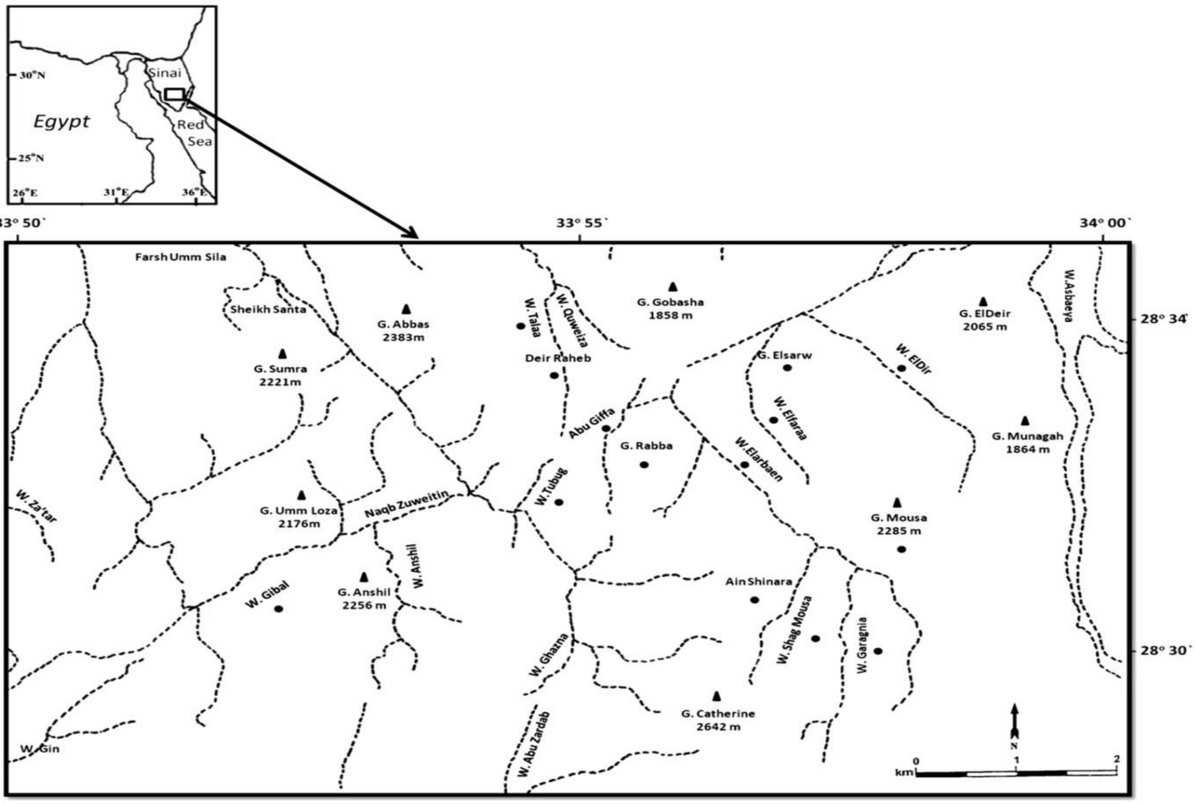

Figure (1): Location map of the study area (Saint Kathereine Protectorate) in the southern part of Sinai. Mountain tops (Gebel = G) are represented by (▲), Wadis or valleys (W) and main location of the study represented by (•).

Recording of environmental parameters

In each stand, the following parameters were measured; altitude (in meters above sea level), slope degree, exposure degree and landform type. Land form type was determined according to Moustafa and Klopatek (1995) as; gorge, slope, Wadi, ridge, plain and outcrop of smooth-faced rock and terraces. Nature of soil surface was described using the following scale; fine fraction (< 2mm), gravel (2-75 mm), cobbles (75-250 mm), stones (250-600 mm), and boulders (> 600 mm) (Hausenbuiller, 1985).

Germination behavior

Seed collection of study species was carried out in the winter seasons of 2013 and 2014. Seeds were stored in the laboratory conditions till germination tests. In the period from May to October 2013 preliminary germination experiments were carried out in laboratory conditions to determine the germination behaviour and dormancy (if present) for each species. The seeds were pre-treated by soaking in different concentrations of gibberellic acid (GA), sulphoric acid (H_2SO_4), calcium carbonate ($CaCO_3$), citric acid (CA), and hot water. Then seeds were sown on moistened cotton layer in Petri dishes. The used concentrations were as follows: 50, 100 and 200 mg/L GA; 0.5, 1 and 1.5% H_2SO_4; 1, 2 and 3 % $CaCO_3$; and 0.1 and 1 % citric acid solutions and hot water pre-treatment (40, 50 and 60° C) according to Mendoza-Urbina *et al*., (2012), all was kept at 25/15 °C and 16/8 light/dark incubator.

Biomass

Thirty seven individuals for *Hypericum sinaicum* were selected for biomass assessment. These samples were collected from certain sites at Saint Catherine area; El-Tofaha, Gebel El-Rabba, Ain-Shiekiaa, Mid of Wadi El-Shag, Ain-kharaza, Wadi Gibal, El-Hagaly and W. Talaa. Size of representative samples of each plant species was measured in terms of volume through measuring their diameters and height. Dry weight of the shoot system at 105°C was determined. The relationship between volume and weight of different plant species was assessed by simple regression analysis (Barbour *et al*., 1987).

Soil Seed bank

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 labelled, 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 micro habitat 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 (Zaghloul, 1997).

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 colour-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.

Multivariate and statistical analysis of data

Classification of the phytosociological data set (eighty nine sites and sixty one species) was carried out using TWINSPAN (Two-Way Indicator Species Analysis) technique in PC-ORD computer program (McCune and Mefford, 1999), version 4 for Windows; a program for multivariate analysis of ecological data. The statistical analysis of data was carried out by using Minitab 15 and Systat programs.

RESULTS

Classification of stands

The TWINSPAN classification of eighty-nine stands and sixty-one species resulted in four main vegetation groups (Figures 2). These groups were separated at the second level of classification where the main indicator species are *Jasonia montana*, *Tanacetum sinaicum* and *Origanum sinaicum.*

The four assemblages separated by TWINSPAN can be explained as follows:
Assemblage I: *Jasonia montana*
Assemblage II: *Plantago sinaica*
Assemblage III: *Hypericum sinaicum*
Assemblage IV:*Hypericum sinaicum - Adiantum capillus-veneris*

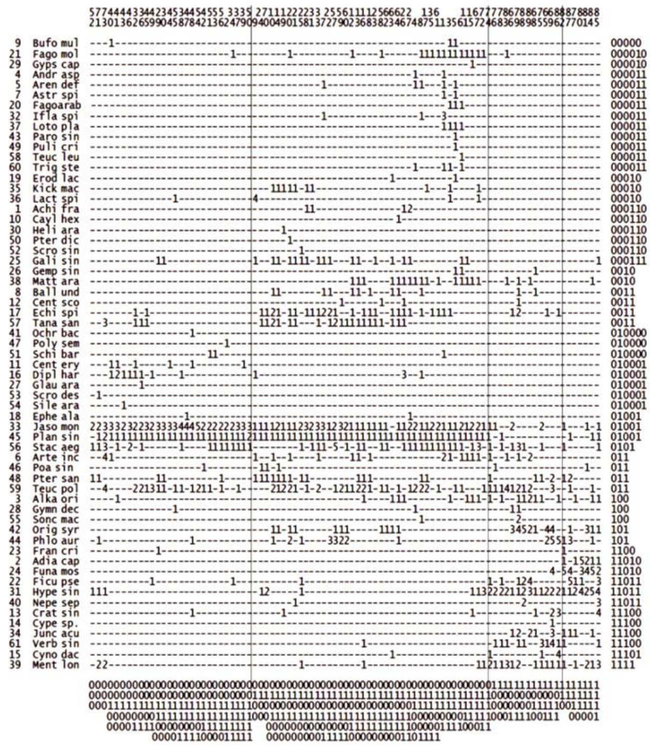

Figure (2): Two-way species–figure print out of TWINSPAN results showing two dendrograms: species groups on the right hand side and site clusters on the bottom of the figure, for the classification of 89 stands based on the cover percentage of 61 plant species.

Frequencies and average abundance of species composition for these assemblages showed that the first assemblage (I) has only one species with frequency 100% (*Jasonia montana*) with average abundance 2.60. In the second assemblage (II) the species with frequency 100% was (*Plantago sinaica*) with average abundance equals 1. In the third assemblage (III) also one species had frequency 100% presence (*Hypericum sinaicum*) with average abundance 1.75. Two species of frequency 100 % (*Hypericum sinaicum* and *Adiantum capillus-veneris*) and average abundance of about 2.40 and 1.5 respectively are in the fourth assemblage (IV).

Assemblage I: Jasonia montana

This assemblage is dominated by *Jasonia montana* with frequency 100 % and average abundance of about 2.6. The co-dominant species is *Plantago sinaica* (96.4%), with average abundance 1 and the prominent species are *Stachys aegyptiaca and Teucrium polium* each had 53.5% frequency. This assemblage is found in G. El-Sarw, Meserdi, Ain-Shekiaa, Ain-Kharaza and El-Hagaly, in different landforms; ridges, gorges and fissures walls with exposure degrees 40°-50° east to 340° south east, high elevations 1834.8 m. Soil of this assemblage characterized by the highest chloride (29.7 Meq/L) concentrations, relatively high electric conductivity of about 2315 µ.s. and high organic matter 23.87 %. (Photo1)

Photo (1): *Jasonia montana* found at assemblagess at study sites.

Assemblage II: Plantago sinaica

This assemblage is dominated by *Plantago sinaica* with frequency 100 % and average abundance of about 1. The co-dominant species is *Jasonia Montana* (97.6%), with average abundance 1.6. The associating species are *Stachys aegyptiaca* and *Echinops spinosus* with frequency 60 % for each. This assemblage is found in Meserdi, W. Gibal and Garagnia with different landforms; slope, gorge, terraces steep slope with spring, gentle slope and steepy ridges with exposure degrees 20° east to 340° south east. Species of this assemblage is found at stands with soil of alkaline type 8.78 pH, and high calcium and magnesium concentration of 34.8 and 33.7 (Meq/L) respectively, high EC 4000 µ.s. and high chloride concentration of 38.2 (Meq/L) (Photo 2).

Photo (2): *Plantago sinaica* growing with *Hypericum sinaicum* in a very special case which is unusual form.

Assemblage III: *Hypericum sinaicum*

This Assemblage is dominated by *Hypericum sinaicum* with frequency 100 % and average abundance of about 1.8. The associating species are *Verbascum sinuatum* with frequency 75% and *Mentha longifolia* 83%. This assemblage is found in Meserdi, W. Talaa, W. El-Dier and Garagnia with different landforms; slopes, slope with fissures, terraces with ponds and ridges with exposure degrees 30° north-east to 340° south east, high elevations 1920 m.

Species of this assemblage is found at stands of alkaline soil type of 8.35 pH, high EC 3945 (µ.s) and high magnesium and calcium concentration of 33.7 and 37.2 (Meq/L) respectively, with high gravel percentage 62.5% (Photo3).

Photo (3): Steep slope covered with *Hypericum sinaicum* on the walls of the water pond at Meserdi area.

Assemblage IV: Hypericum sinaicum – Adiantum capillus-veneris

This Assemblage is dominated by *Hypericum sinaicum* and *Adiantum capillus-veneris* with frequency 100 % and average abundance of about 2.4 and 1.5 respectively. The associating species are *Funaria* sp. and *Mentha longifolia* with frequency 85% and 71% respectively. This assemblage is found in Meserdi and W. Talaa with landforms between slopes and fissured slopes, exposure degrees 40° north-east to 150° north west, high elevations 1870 m. Species of this assemblage is found at stands of soil with high gravel percentage (62.5%), high EC 2867 (µ.s.) and alkaline soil of 8.09 pH .

Classification of species

There are sixty-one species recorded in the eighty-nine stands, including eight endemic species. These species belong to 23 taxonomic families. Compositae is the most represented family (12 species), followed by Labiatae (9 species), Caryophyllaceae (6 species) and Scrophulariaceae (4 species). The floristic structure in the studied stands in Saint Catherine area includes 25% annuals (15 species), 41% perennials (25 species), 16% frutescent (10 species) and 16% shrubs (10 species) and 2% biennials (1 species). The TWINSPAN output revealed that all species can be grouped into eight groups at the third level of classification.

First group comprised of twenty-two species and includes; *Kickxia macilenta, Pulicaria crispa, Achillea Fragrantis sima* and *Pituranthos triradiatus*. Second group comprised of six species; the most prominent of them are *Ballota undulata*, *Echinops spinosus* and *Matthiola arabica*. The third group contained twelve species, among which are, *Plantago sinaica*, *Stachys aegyptiaca*, *Schismus barbatus and Diplotaxis harra*, the fourth group was represented by four species only; *Artemisia inculta*, *Poa sinaica*, *Pterocephalus sanctus* and *Teucrium polium*. The fifth group was represented only by three species; *(Sonchus macrocarpus, Gymnocarpos decanderum* and *Alkanna orientalis)*. The sixth group was composed of two species; *Origanum syriacum* subsp. *sinaicum* and *Phlomis aurea*, both species are endemic. The seventh group comprised of six species which include; *Hypericum sinaicum*, *Adiantum capillus-veneris* and *Nepeta septemcrenata*. The eighth group also contains six species from which are; *Mentha longifolia*, *Juncus rigidus*, *Verbascum sinaiticum* and *Crateagus x sinaica*.

Species – environment relationship

In the ordination diagrams (Figure 3), twenty-two environmental factors (organic matter, moisture content, exposure, slope, elevation, land form type, pH, electric conductivity, silt and clay, gravels, total chloride, total carbonate, total magnesium, total calcium, fine, coarse and medium sand and fines (soil texture), cobles, gravel, stones and boulder (nature of soil surface) are represented as vectors (lines from centre) and sixty-one species as stars (*).

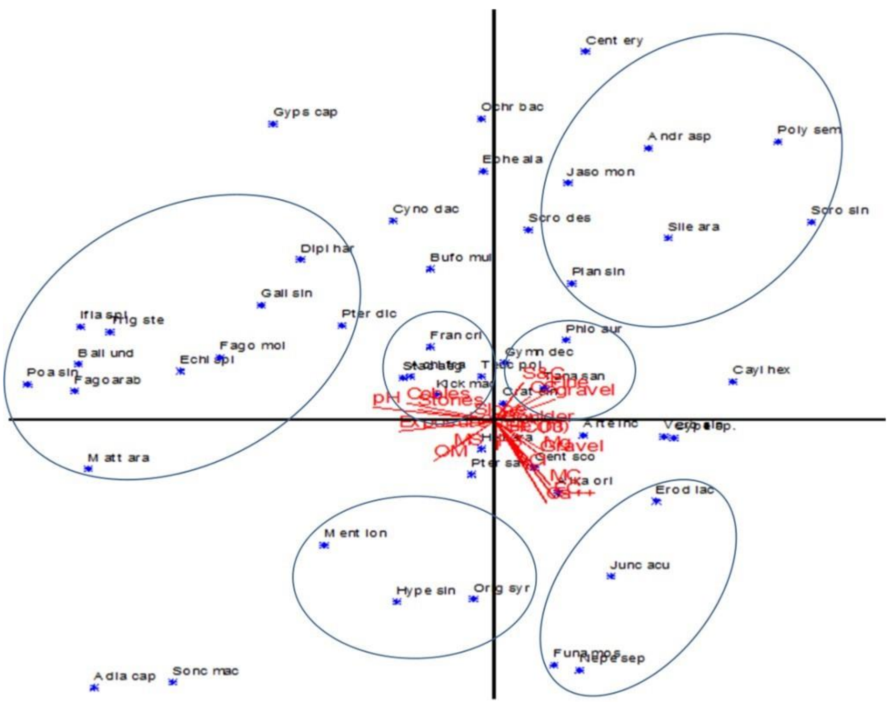

Figure (3): Ordination (CCA) diagram (X1-X2 plane) with plant species represented as (*) (abbreviations are listed in Table 16) and the centroid lines represents the environmental variables; OM; organic matter, MC; moisture content, exposure, slope, elevation, land form type, pH, EC; electric conductivity, silt and clay, gravels, Cl; total chloride, CO_3; total carbonate, Mg; total magnesium, Ca; total calcium, FS; fine sand , CS; coarse sand and MS; medium sand and fine sand (soil texture), cobles, gravel, stones and boulder (nature of soil surface).

In this graph species as *Hypericum sinaicum, Mentha longifolia, Juncus acutus, Origanum syriacum, Nepeta septemecrenata, Alkanna orientalis* and *Funaria* sp. exhibit positive correlation with high (organic matter, moisture content, electric conductivity, gravels, and calcium concentrations), while species as *Diplotaxis harra* and *Gallium sinaicum* are not correlated. Other species as *Plantago siniaca*, *Jasonia montana, Scrophularia desrti*, *Silene arabica* and *Polypogon semiverticillatus, Phlomis aurea, Crateagus x sinaica, Gymnocarpos decandrum and Tanacetum sinaicum* exhibit positive correlation with areas with silt and clay. Some species as *Francoeuria crispa, Gallium sinaicum, Fagonia mollis*, *Poa sinaica, Ballota undulata, Stachys aegyptiaca* and *Kikxia macelenta* are positively correlated with soil that has high pH and high cobles, and negatively correlated with organic matter and moisture content, while other species as *Verbascum sinaiticum and Matthiloa arabica* are located near the X1-axis and are not obviously correlated with any of the environmental variables.

Stands – environment relationship

CCA shows the species-environmental variables relationships by calculating axes that are products of the species composition and linear combinations of the environmental variables.

To explain these relationships CCA axes number I and II are considered in the interpretation. The reason is that the eigenvalues of the CCA axis I is 0.604 and the CCA axis II (0.418) is not much higher than Axis III (0.33). The eighty-nine stands were classified by TWINSPAN technique at the second level into four community types (assemblages). The ordination diagram (Figure 4) shows the position of these assemblages and their interrelation with environmental factors. The first assemblage (I) (*Jasonia montana*) occurs adjacent to axis 1 and so it occupies the lower left-hand corner in axis1-axis 2 plane of the diagram, it's obvious that it has a positive relation with exposure, medium sand and organic matter, while it is negatively affected with the soil pH, cobles and stones percentages. *Plantago sinaica* the second assemblage (II) is found on the two upper part of the diagram extending between the left and right corners of axis 1 and axis 2. This assemblage is most negatively affected by soil organic matter, total calcium, electric conductivity, moisture content and gravels percentage. The third assemblage III (*Hypericum sinaicum*) occurs at the lower right corner of the diagram between the two axes, it is most positively affected by soil organic matter, total calcium, total carbonate, electric conductivity and moisture content. The fourth assemblage (IV) (*Hypericum sinaicum - Adiantum capillius-venersis*) is found in the upper write-hand side across axis 1 and axis 2. This assemblage is positively affected mostly by soil gravel, fine and silt and clay percentages, it resembles the third assemblage to a great extent.

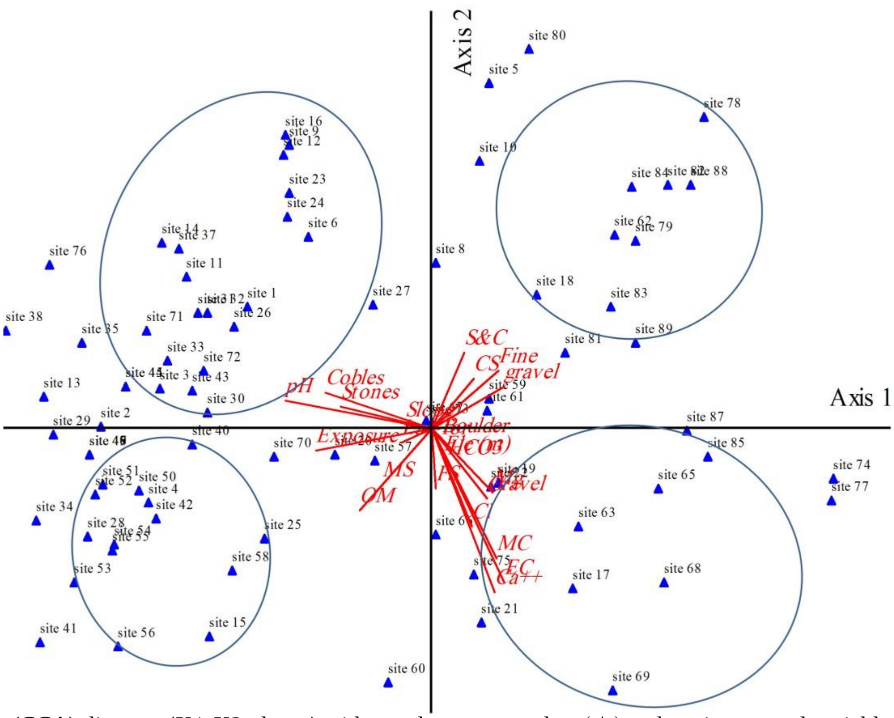

Figure (4): Ordination (CCA) diagram (X1-X2 planes) with stands represented as (▲) and environmental variables as centroid lines. The environmental variables are as follows: OM; organic matter, MC; moisture content, exposure, slope degree, elevation, land form type, pH, EC; electric conductivity, silt and clay, gravels, Cl; total chloride, CO_3; total carbonate, Mg; total magnesium, Ca; total calcium, FS; fine sand, CS; coarse sand and MS; medium sand, and fine sand (soil texture), cobles, gravel, stones and boulder (nature of soil surface).

Germination behavior

The seeds of *Hypericum sinaicum* are brown colour, cylindrically-shaped with longitudinal mesh wrinkling. Their average dimensions are: 1.2 mm length and 0.6 mm width. The average weight of 1000 seeds was 19 mg.

The Germination responses of seeds to the pre-soaking treatments are shown in table (1) and figure (5). In general, seed germination was low in most treatments and light was found to be an important factor affecting germination. Calcium carbonate ($CaCO_3$) treatment (Photos 4 & 5) and hot water (50°C) treatments was found to be most effective to improve seed germination depending on doses, while other treatments were efficient to a lesser degree. The test of variances, One way ANOVA, showed that hot water is the most significant treatment, $P \leq 0.036$, while all other treatments were non-significant (Table 1).

Highest mean germination percentages (94.66% and 89.33%) were induced by $CaCO_3$ (2%) and (3%) respectively. It was noted that hot water treatments of relatively high degree of temperature suppressed germination. That the germination percentage was 70.66% and 84% at 40°C and 50°C, respectively, while at 60 °C the germination percentage was declined to 2.66%. All the other treatments did not show any enhancement in germination but suppressed it as all results came in lower percentages than the control (82.66%). Immersing seeds in sulphoric acid (H_2SO_4) of 0.5, 1% and 1.5% concentrations showed germination percentages of 50.66%, 57.33% and 41.33%, respectively, while soaking with gibberellic acid (GA_3) 50 mg, 100 mg and 200 mg concentrations showed 76%, 72% and 74.66%, respectively, and that of citric acid was 81.33% at concentration of 0.1% and 77.33 % at concentration of 1% (Figure 5).

Photo (4): *H. sinaicum* seeds pre-soaked in hot water (40 ˚C) for 30 minutes with germination percentage of 100% at temperature of 15/20 ˚C.

Photo (5): *H. sinaicum* seeds pre-soaked in (2%) calcium carbonate ($CaCO_3$) solution for 30 minutes, with germination percentage of 100 % at temperature of 15/20 ˚C.

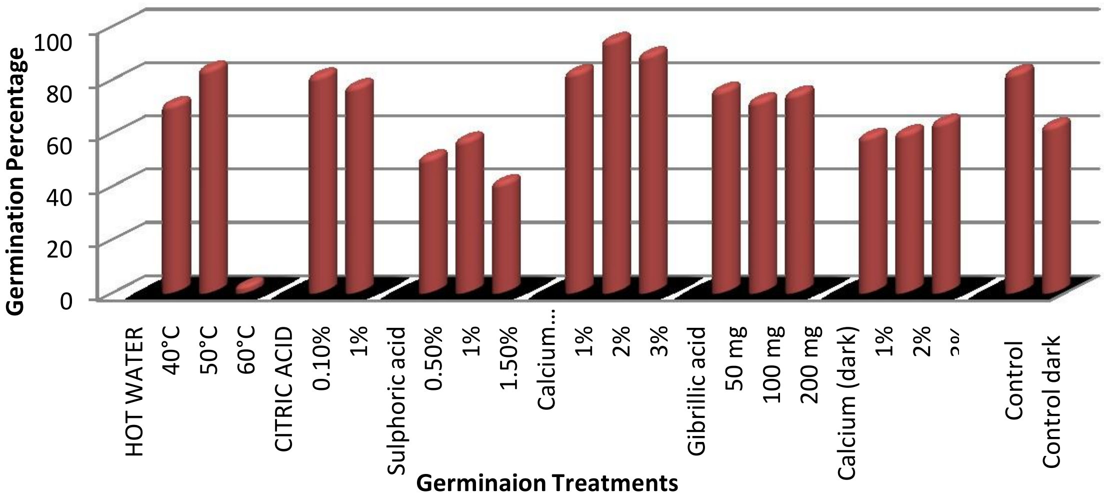

Figure (5): Germination rate of *Hypericum sinaicum*, using different pre-soaking treatments; hot water, citric acid, sulphoric acid, calcium carbonate, gibberellic acid, and calcium carbonate (dark).

Biomass assessment

Biomass of *Hypericum sinaicum* as dry weight per meter square was measured at certain sites of Saint Catherine. The relationship between volume of the medicinal plant and its dry weight was linear and showed high and significant correlation (Figure 6). The information of dry biomass per meter square gave an indication about the abundance of medicinal plants in different sites (Table 2). It was found that both species have low biomass in most sites, even in the sites supposed to have high biomass.

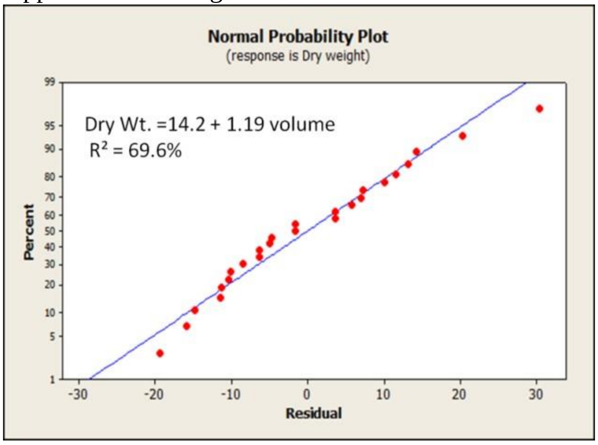

Figure (6): The regression equation for *H. sinaicum* biomass versus volume of the selected samples in the study area of Saint Catherine.

Table (2): Total biomass as gram per meter square of *H. sinaicum* at different sites of Saint Catherine area.

Sites	**Dry Wt.** gm/m^2
El-Tofahaa	-
G. El-Raba	-
Ain-Shekiaa	02.21
Mid of W. El Shag	00.11
Ain-kharaza	06.32
W. Gibal	17.40
El-Hagaly	101.70
El-Kehel	07.81
El-Raheb field	34.33

From table 2 we found that the highest biomass of *H. sinaicum* was at El-Hagaly (101.70 gm/m^2), which is north facing site characterized by high altitude (1850 m), alkaline soil (pH 8.34)and high total dissolves salts (1162 ppm) and of soil moisture content percentage (7.1%). While the lowest biomass of *H. sinaicum* (0.11 gm/m^2), which is east facing characterized by soil moisture content (1.733) and low total dissolved salts (256 ppm).

Table 1 Germination rate with mean values and standard deviation of *Hypericum sinaicum*, using different presoaking treatments; sulphoric acid, calcium carbonate, gibberellic acid, hot water and citric acid. Data is represented as mean of 3-replica ± SD, one way ANOVA; F-ratio and P value.

Treatments		Hot water (°C)			Citric acid (%)		Sulphoric acid (%)			Calcium carbonate (%)			Calcium carbonate (dark) (%)			Gibrillic acid (mg)			Control	Control dark
		40	50	60	0.1	1.0	0.5	1.0	1.5	1	2	3	1	2	3	50	100	200		
Mean Germination (%)		70.7	84	2.7	81.3	77.3	50.7	57.3	41.3	82.7	94.7	89.3	58.7	60	64	76	72	74.7	82.7	62.6
SD		50.8	13.8	4.6	6.1	12.2	10.6	2.3	20	9.2	9.2	10	18.4	31.7	27.2	26	4.6	22	4.6	50.96
One way ANOVA	F-ratio	6.13			0.26		0.94			1.49			0.08			0.06				
	P value	0.036			0.639		0.442			0.298			0.925			0.944				

Soil Seed Bank

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 3) 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. Mean while, 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 4).

Table (3): Summary of species list emergent from soil seed bank in the studied sites in Saint Catherine Mountain and their distribution in the studied localities.

Species	Distribution
1. ***Alkanna orientalis***	1,2,3,4,5,6,7,8,9,10,11
2. ***Arenaria deflexa***	1,2,4,6,11,2,13,14
3. ***Ballota undulata***	1,2
4. ***Cotoneaster orbicularis***	1, 3
5. ***Chenopodium* sp.**	1
6. ***Dipotaxis acris***	1,4,5
7. ***Ficus pseudo-sycomorus***	1,3,4,6,7,12,14
8. ***Funaria* sp.**	1,2
9. ***Galium setaceum***	1
10. ***Galium sinaicum***	2,13,14
11. ***Hypericum sinaicum***	1,2,3,4,6,15,16
12. ***Ifloga spicata***	1,11
13. ***Lophochloa cristata***	1,2,14
14. ***Mentha longifolia***	1,2,3,6,7,8,9,16
15. ***Nepeta septemcrenata***	1,2,14
16. ***Origanum syriacum***	1,2,8
17. ***Panicum coloratum***	1,2,3
18. ***Phlomis aurea***	1
19. ***Plantago sinaica***	2,10,13,15
20. ***Polypogon monspeliensis***	1,2,13
21. ***Primula boveana***	1,12
22. ***Pulicaria crispa***	2,3,8,10,11,12,14,17
23. ***Schismus barbatus***	1,14
24. ***Scrophularia* sp.**	4,5
25. ***Sisymbrium erysimoides***	1,14
26. ***Stachys aegyptiaca***	1,6,12,13,14
27. ***Tanacetum sinaicum***	1,2
28. ***Teucrium polium***	1,2,6,12,14
29. ***Trigonella stellata***	3,14
30. ***Verbascum sinaiticum***	1,2,12,13,14
31. ***Veronica kaiseri***	1,12,14

Distribution locations:1; Garagnia, 2;Meserdi, 3; Ain Shekiaa, 4; Elhagaly,5; Ain Kharaza, 6; W.Gibal, 7;Sad Dawod, 8; W.Talaa, 9; Raheb field, 10; Elkaheel 11;Ain shinara, 12; Gebel Mousa, 13; ElFaraa, 14; Shag Mousa, 15; G.Rabba, 16; W.Eldeir, 17; Tofaha.

Table (4): 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
Ain shekiaa	02	15052	11
El hagaly	02	4422.0	9.0
Ain kharaza	01	147.00	5.0
Wadi Gebal	01	7600.0	10
Dawood dam	01	8360.0	6.0
W. ElTalaa	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
Elkehal	01	1008.0	7.0
Ain shenara	01	96.000	6.0
Gebel Mousa	01	141.00	7.0
Gebel Mousa	01	1505.0	8.0
El faraa	04	408.00	6.0
Shaq Mousa	12	640.00	11

Based on the floristic composition (seed density), the stands could be classified and separated by TWINSPAN to four main assemblages or communities 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, 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 m a.s.l., with highest soil bicarbonate concentration 11.8 (Meq/L), a range of electric conductivity from 400 to 4000 µ.s., 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%.

DISCUSSION

Saint Catherine area is characterized by a high diversity of plant species. One of our main objectives in this study was to study the vegetation analysis in main locations dominated by *H. sinaicum*. The recorded species in the 89 stands are 61 species. These species belong to 23 taxonomic families. Compositae is the most represented family, followed by Labiatae, Caryophyllaceae and Scrophulariaceae. The floral structure in studied stands in Saint Catherine area includes 25% annuals, 41% perennials, 16% frutescent and 16% shrubs and 2% biennials. From the 61 identified plant species in present study, eight species are endemic according to Täckholm (1874) and Boulos (2002)-

(Bufonia multiceps, Hypericum sinaicum, Kickxia macilenta, Nepeta septemcrenata, Origanum syriacum, Galium sinaicum, Phlomis aurea and *Plantago sinaica*). This supports the results of previous studies on the area that Saint Catherine represents a centre of endemism (Zohary, 1973; Shmida, 1984; and Moustafa, 1990).

It has long been established that patterns in vegetation are correlated with gradients in environmental parameters (e.g. Whittaker, 1967; Smith and Huston, 1989). Multivariate analysis including classification and ordination can provide more detailed and comprehensive information on the patterns in vegetation and the response of plant species to the underlying gradients (Gauch, 1982; TerBraak, 1995). In Saint Catherine area, the patterns in vegetation are mainly influenced by the gradients in terrain variables such as altitude and slope. Vegetation in mountainous regions responds to small-scale variation in terrain like slope which affect microclimatic conditions such as temperature and soil moisture (Moustafa, 2000, 2002 a & b) which in turn affect plant species distribution. Altitude is an important terrain variable, since it affects atmospheric pressure, moisture and temperature, which in turn influence the growth and development of plants and the patterns in vegetation distribution (Hedberg, 1964).

In this study the vegetation survey was followed by applying multivariate analysis techniques, that classification by TWINSPAN computer program and ordination by CCA computer program. The main results of the vegetation analysis identified four main assemblages as follows: assemblage I: *Jasonia montana*, assemblage II: *Plantago sinaica*, assemblage III: *Hypericum sinaicum* and assemblage IV: *Hypericum sinaicum -Adiantumcapillus-veneris*.

The results of CCA analysis and TWINSPAN revealed that the first assemblage (*Jasonia montana*) which has *Stachys aegyptiaca*, *Teucrium polium* and *Plantago sinaica* as associated species had a positive relation with exposure, while it was negatively affected with the soil pH. A related assemblage was previously recorded by Moustafa (1990) on his study on species distribution on Sinai mountains, with *Jasonia montana* and *Stachys aegyptiaca* as the dominant species, those two species were also recorded by Salman (2004) as dominant species in two assemblages (*Alkanna orientalis- Jasonia montana* and *Alkanna orientalis-Stachys aegyptiaca*). The second assemblage *(Plantago sinaica*) is the first time to be recorded in Saint Catherine area. A related community type assemblage (*Origanum syriacum- Plantago sinaica*) was recorder as disjunct assemblage by Moustafa (1990). This assemblage has *Echinops spinosus*, *Jasonia montana* and *Stachys aegyptiaca* as associated species is most negatively affected by soil organic matter, soil calcium concentration and moisture content but exhibit positive correlation with areas with silt and clay. The negative correlation with the organic matter may be due to grazing, as those species are highly grazed (Guenther *et al.*, 2005) and as the organic matter increased it indicates that these localities are grazed which in turn means loss of vegetation of those species. Presence of *P. sinaica* in these assemblages reflecting the effect of environmental factors on its distribution put into consideration the causes of danger it is subjected to and give an idea into how to conserve and rehabilitation of this species.

The third and fourth assemblages are dominated by *Hypericum sinaicum* and *Adiantum capillus-veneris* and with associated species *Verbascum sinuatum* and *Mentha longifolia*. These two assemblages are also recorded for the first time in Saint Catherine area. A close study by Zaghloul (1997), he recorded *H. sinaicum, M. longifolia* and *A. capillus-veneris* as the most prominent associated species in the (biological crust- *Primula boveana*) assemblage. These assemblages exhibited positive correlation with high; organic matter, moisture content, gravels, fine, silt and clay percentages and soil calcium concentrations. Here we can find that the ultimate change in climatic conditions, which is leading to drought, is an important factor in the critically endangered status of *Hypericum sinaicum*, as it only grows in localities with high moisture content that’s why it only found at high elevations because of the low temperature. Due to this drought and destruction of the habitat of the fresh water springs it is subjected to extinction as it's becoming very rare and threatened.

In agreement with Shaltout and Ayyad (1990) and Abdel Wahab *et al.* (2004), the application of regression analysis using the volume of the plants is a good estimator for the biomass of the plants. The bio mass of medicinal plants varies greatly from site to site even in the same locality due to number of factors including water availability and degree of grazing. The biomass of threatened medicinal plants is relatively low, especially the endemic species as *Hypericum sinaicum* which indicates the high pressure of human impacts on those species. Abdel Wahab *et al.* (2004) recorded that *Hypericum sinaicum* had the lowest biomass during their study on the conservation of medicinal plants in Saint Catherine which reached 0.37gm/, which supports our data and revealed that *H. sinaicum* species is threatened and need to be protected.

A good understanding of natural regeneration in any plant community requires information on the presence and absence of persistent soil seed banks, quantity and quality of seed production, longevity of seeds in the soil, losses of seeds to predation and deterioration, triggers for germination of seeds in the soil and sources of re-growth after disturbances (Teketay, 2005). In the ongoing multi-prolonged efforts to halt species extinction and to promote the conservation, classification, evaluation and sustainable utilization of our rich plants heritage, this study was carried out in order to clarify and understand the ecological behaviour of seeds of endemic species in their natural habitats and its implications for conservation. The endemic species are endangered by the human impact through different ways of utilization. Therefore, the present study was directed to focus on the seed ecology of the threatened endemic species *Hypericum sinaicum*.

In terrestrial vegetation, seeds and seedlings are implicated in various ecological phenomena. In the life history of higher plants, the seedling stage is the most vulnerable and is usually accompanied by extremely high mortality, while the seed stage is uniquely resistant to various environmental stresses. Since the process of germination links these two stages showing such greatly differing risk levels, any physiological mechanism confining germination only to circumstances associated with a high probability of sound seedling establishment would have a great adaptive value (Zaghloul, 1997, Moustafa *et al.,* 1999). These processes are economically important, as to determine uniformity, standing plant density, and the efficient use of the nutrients and water resources available to the crop and ultimately affect the yield and quality of the crop (Bench-Arnold, 2004; Gan *et al*., 1996). Seed germination is affected by a wide range of environmental factors, such as temperature, salt, water, oxygen concentration, and pH (Romo and Haferkamp, 1987; Balbaki *et al*., 1999; Karan *et al*., 1985; Swarn *et al*., 1999; Lu *et al*., 2006; Saeidi, 2008; Esmaeili *et al*., 2009; Mendoza-Urbina *et al.*, 2012).Clear understanding of the germination response of seeds to environmental factors and agronomic aspects are useful in screening crop tolerance to stress, identifying geographical areas where a crop can germinate and establish successfully and developing management models for the prediction of timing of crop development processes. Therefore, the germination behaviour of the studied species was tested.

For *Hypericum sinaicum*, highest germination (94.66%) was induced by $CaCO_3$ of (2%) concentration, this treatment was thought be done after our field notice, that *Hypericum* was found in places characterized by alkaline soil type. To some extent similar results were achieved by Mendoza-Urbina *et al.*, (2012), they found that scarifying with calcium hypochlorite appeared to be the best technique to break dormancy in *H. silenoides* seeds; 100% germination. Hot water treatments have been reported to enhance germination of hard coated seeds by elevating water and oxygen (O_2) permeability of the testa (Teketay, 1998; Aydin and Uzun, 2001). In our study it was noted that hot water treatments of relatively high degree of temperature significantly suppress germination comparing with control, where the germination percent was 84% at 50°C ($P \leq 0.036$), while at 60°C the germination percent was 2.66% and these results came similar to that of Cirak (2007).

He found that hot water treatment induced germination of *H. perforatum*, *H. origanifolium* and *H. pruniatum* in the lowest level, while it was not effective at all in germination of *H. orientale*, the same results were achieved by Camas and Caliskan (2011) on the Turkish species (*Hypericum leptophyllum*).

Mendoza-Urbina *et al.*, (2012) found that scarification of *Hypericum silenoides* seeds with hot water at 40°C and 50°C showed 91% and 97% germination after 20 days of planting, respectively, while the seeds treated with hot water of 60°C did not germinate at all. They assumed that immersion of dry seeds in hot water at temperatures up to 50ºC led to seed coat rupture allowing water to permeate faster through the seed tissues causing physiological changes and the subsequent germination process. Mendoza-Urbina *et al.*, (2012) also suggested that the negative effect of high degrees of hot water on the germination of *H. silenoides* seeds was probably due to the combination of both high temperature and time, which may cause damage to the embryo tissue as observed in other *Hypericum* species.

Our results showed that all the other treatments did not show any enhancement in germination, however it suppressed germination, as all came less than the control (82.66%), and this opposes the previous work done on other *Hypericum* species, as Camas and Caliskan (2011) found that gibberellic acid (GA_3) and sulphoric acid (H_2SO_4) increased germination, they assumed that this induction indicates the presence of physiological dormancy related to partially dormant embryo in case of GA_3 and presence of physical dormancy, related to hard seed coat and overcame by acid scarification in case of H_2SO_4. Ai-Rong (2007) reported that GA_3 promotes germination when combined with a scarification treatment. Cirak (2007) reported that seeds treated with 150 mg/l GA3+ 0.5% H_2SO_4 increased the germination of *Hypericum orientale* (50%), *H. origanifolium* (30%) and *H. pruinatum* (55%). Mendoza-Urbina *et al.* (2012) found that the mixture of 150 mg/l GA_3+ 0.5% H_2SO_4 increased the germination to 96%.

Hypericum sinaicum showed a strong dormancy which could be broken by fluctuating temperature, and pre-soaking in calcium carbonate. Our results support the study achieved by Nedkov (2007) that continuous soaking, heating and stratification considerably reduced germination percentage. This strong dormancy explains why seedlings of *H. sinaicum* are not seen in the field, as it undergo bet hedging in order to preserve the community against extinction.

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 (Rajjou and Debeaujon, 2008). Therefore, soil seed banks are considered as essential constituents of plant communities (Harper and Benton, 1966), 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 (Uhl *et al.*, 1981, 1982; Marks and Mohler, 1985; Lawton and Putz, 1988; Kalamees and Zobel, 2002). The replacement of individuals from the seed bank may have reflective effects on the composition and patterns of the vegetation within the community (Egler, 1954; Harper, 1983; Cheke *et al*., 1979; Fenner, 1985).

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 (Thompson, 1987; Rundel and Gibson, 1996), and are affected particularly by spatial patterns of vegetation (Guo *et al.*, 1998).

Seed banks are a crucial component in desert ecosystems and other stressful habitats where favourable conditions for seed germination and seedling establishment are quite unpredictable both in space and time (Kemp, 1989; Nathan and Muller-Landau, 2003; Meyer and Pendleton, 2005; Koontz and Simpson, 2010).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 (Kemp, 1989; Al-Faraj *et al*., 1997; Zaghloul, 2008). The present study aimed to study the behavior of endemic species in soil seed bank and its relationship to above ground vegetation. 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 (Rebollo *et al*., 2001).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 (Alaily *et al.*, 1987) and in the seed bank of endemic species in Saint Catherine area (Ramadan 1998; Zaghloul, 1997), while it was on the contrary to what Gomaa (2012) 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*. (2013) found that genetic differentiation among populations of *H. sinaicum* was significantly different between the standing crop and soil seed bank. Honnay *et al* (2008) 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 (Hegazy *et al*., 2009). Their strategy is to produce few seeds almost every year, most of which do not persist in the seed bank (Boyd and Burn, 1983). 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 (Philippi, 1993). Philippi (1993) 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 maintarget 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.

H. 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.

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