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Research Paper

AUTECOLOGY FOR TWO THREATENED SPECIES *Teucrium polium* AND *Verbascum sinaiticum* GROWING IN SOUTH SINAI FOR CONSERVATION APPROACH

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Abstract

The present study focused on the conservation of the endemic medicinal plant species *Teucrium polium* and *Verbascum sinaiticum* through studying of different ecological aspects; seed germination behavior against different treatments, seed storage behavior, moisture isotherm and ecological field conditions and distributions. Three hundred and twenty six plant species were recorded in St. Catherine Protectorate in recent surveys. Among these326 species, 115 species (35.6%) are considered as medicinal species the specific threats facing conservation and sustainable use of these globally significant medicinal species.. the results showed that seed germination experiments were done for the studied species and revealed that the highest germination percentage of *Teucrium polium* seeds (18%) resulted when seeds were soaked in calcium carbonate (1%)for 30 minute and then germinated in incubator at 15/20°C. *Verbascum sinaiticum* seeds germinated with a percentage of 54% after soaking seeds in GA_3 for 24h (500 ppm)and germinating at room temperature.After had been soaked in dilute tetrazolium salt solution for 24 hours, 30% of the *Verbascum sinaiticum* seeds were revealed to be viable. Meanwhile, 34% of the *Teucrium polium* seed were revealed to be viable after had been soaked in tetrazolium diluted solution for 48 hours. Data of vegetation and environmental variables were subjected to multivariate analyses using TWINSPAN for classification and CCA for ordination. Four main assemblages were recognized as a result of classification:1-*Teucrium polium* 2- *Chiliadenus montanus* 3- *Hypericum sinaicum* 4- *Origanum syriacum – Mentha longifolia*.

INTRODUCTION

Medicinal plants have been used by mankind for millennia; their use is as old as humanity itself. The range of species used and their scope for healing is vast. Cures as yet undiscovered may exist in plants as yet un-described. Currently, it is estimated that the number of higher plant species used worldwide for medicinal purposes is more than 50,000 (Schippmann *et al*., 2002). This equates to approximately 20% of the world's vascular flora and constitutes the biggest spectrum of biodiversity used by people for a specific purpose (Hamilton *et al*., 2006).

The genus *Teucrium*, which belongs to the family Labiatae, includes 300 species widespread all around the world. (Coste,1990 and Bonnier, 1990) about 100 species cosmopolitan, but especially Mediterranean region one of them is *Teucrium polium* L. or Jaadah as it is known in Egypt(Boulos,2002). It is mainly Mediterranean and west Irano-Turanian and can be found in countries such as Iraq, Saudi Arabia and Egypt (Feinbrun-Dothan, 1978).The essential oil of *Teucrium polium* possesses an antiphytoviral activity (Bezić *et al*., 2011).*Teucrium polium* can cause significant reduction in blood glucose concentration that could be due to enhancement of peripheral metabolism of glucose rather than an increase in insulin release (Gharaibeh *et al.*, 1988). The methanolic extract of *Teucrium polium* has been studied for its antioxidant activity. This plant species is a source of polyphenols and flavonoids, confirm their antioxidants activities and underline their potential either as natural preservatives or in pharmaceutical (Belmekki and Bendimerad, 2012).

On the other hand the family Scrophulariaceae is a cosmopolitan family with 300 genera and about 5400-5500 species. *Verbascum* is a genus having about 360 species in Europe and Asia. *Verbascum sinaiticum* is a biennial plant, 60 to 150 cm tall (Abebe and Ayehu, 1993).It is represented in Egypt by seven Species; *Verbascum sinuatum* L., *Verbascum sinaiticum* Benth, *Verbascum schimperianum* Boiss,*Verbascum letouneuxii* Asch, *Verbascum eremobium* Murb and *Verbascum fruticulosum* Post (Boulos, 2002).*Verbascum sinaiticum* is used in the treatment of abdominal dropsy (root), anthrax (root and leaves), diarrhea (root) and superficial fungal infections (flower and roots) (Asres*et al.,* 1986 and Hedberg and Edwards1989). The root is also used in the treatment of mental illness, amnesia, tape warm, syphilis, gonorrhoea, relapsing fever, rheumatic pain and elephanthiasis, while the leaf is used in the treatment of wound, measles and infections with *Tinea decalvans* (Omino andKokwaro, 1993). Investigation of the leaves of *Verbascum sinaiticum* has afforded two flavonols, hydrocarpin and the novel sinaiticum, as well as two flavones, chrysoeriol and luteolin. All compounds exhibited dose dependent cytotoxicity against leukaemia cells (Goll*et al*., 1983).

As those are two medicinal species subjecting to a number of threats in saint Catherine which include: over grazing, over-collection, over-cutting for fuel, feral donkeys, tourism activities, collection for scientific research, aridity and irregularity of rain fall, in addition to climate change and drought cycles. Some of the threats affecting rare and endemic plants in the St. Catherine area and Sinai deserts in general are specific to populations of medicinal plants, but the majority affects the functional communities and ecosystems in which these populations ultimately exist and interact with other species and the abiotic environment (Abd El-Wahab *et al*., 2004).

These threats can be classified in two categories: the first includes the natural threats; drought, floods, and natural enemies (rodents and insects). *Ex situ* conservation through long-term storage of seeds is, in principle, possible for a significant proportion of higher plants. Where feasible, long-term seed storage serves as a safe and relatively inexpensive method of plant genetic resources conservation. Although seeds are a crucial part of the life history of plants living in desert ecosystems, information such as moisture content, germination behavior, desiccation sensitivity, and storage behavior (orthodox, recalcitrant, or intermediate) and responses to environmental perturbations are not available for most species.

Therefore our objectives of this study is; studying the distributional behavior of the endemic medicinal plants species *Teucrium polium* and*Verbascum sinuatum*and figuring out the seed characteristics, germination, storage behavior, moisture content and moisture isotherms in order to recommend appropriate *ex situ* conservation measurements.

STUDY AREA

The study was carried out in St. Catherine protectorate located between 33°50'to 34°00'east, and 28°29'to 28°34' north (Figure 1). The area is approximately 25 Km^2 and is characterized by high and rugged mountains that range between 1500 and 2641 m a.s.l. The temperature varies from summer to winter in a great extent with a high temperature of 37°C in July and a low temperature of -2.7°C in January.

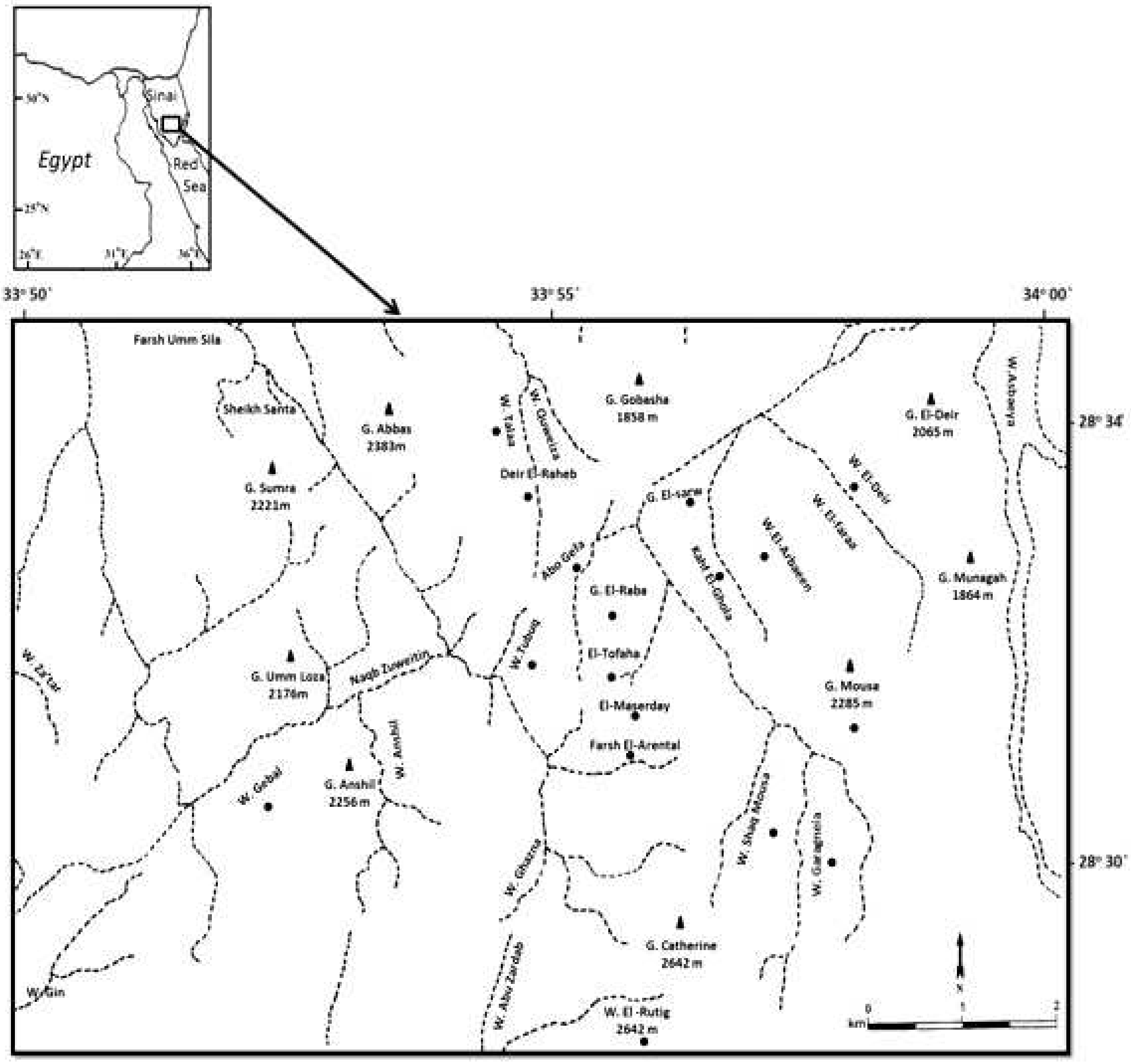


Figure1: Location map of the study area (Saint Catherine) in the southern part of Sinai. Mountain tops (Gebel = G) are represented by (▲). Dashed lines indicate drainages into wadis (W) or valleys. Sampling sites are represented by (●).

MATERIAL AND METHODS

Seeds of *Teucrium polium* and *Verbascum sinaiticum* were collected as bulked seeds from study area in February 2013 from Gabel Mousa, Wadi El-Arbaeen and Kahf El-Ghola.One hundred seventy-seven stands (5x5m) have been sampled in various major locations in the study area: Gabel El-sarw (27 stands), Kahf El-Ghola area (33 stands), Abo Gefa (13 stands), Wadi El-Tofaha (33 stands), Gabel El-Raba (10 stands), Wadi El-Rutig (6 stands), Wadi Gebal (11 stands), Gebal Catherine (17 stands), Wadi Talaa (2 stands), Farsh El-Arentla (10 stands), Gabel Mousa (3 stands), Wadi Garagneia (2 stands) and Wadi El-Maserday (10 stands).

To estimate the seed viability, a quick 24-hour viability test was applied. The isolated seed embryos were soaked in water for 24 hours and then in a diluted (1%) solution of 2, 3, 5-triphenyltetrazolium chloride for 24 hours, according to (Peters, 2000), but for*Teucrium polium*was soaked for 48 hours, because24 hours was not enough to change the color of the seed embryo.with tetrazolium solution,upon the action of dehydrogenases, the viable embryos gain a pale pink colour (Peters, 2005).

To study the effect of different treatments on the germination behavior of the studied species, Different pretreatments were used including soaking in hot water (80°C) for 5 and 15 minutes, wet chilling at 5°C for different periods (2 weeks, 4 weeks and 8 weeks), soaking in 0.1 and 1% citric acid for 96 hours,in 10, 50, 100, 200, and 500 ppm gibberellic acid (for 24 hours), and in 1, 2, and 3% Calcium Carbonate ($CaCO_3$) for 30 minutes and then the seeds were washed thoroughly under tap water to remove all the saltresidual at were kept at three different temperature degrees; at 25°C ±2 in dark incubator and at 25°C ± 2 in light for 12 hours fluctuated with 15°C ± 2 in dark for 12 hours in fluctuating growth chamber

Moisture content was determined by weighing empty crucibles with lids and clearly labels with sample numbers.Three replicates were used for each sample.After adding the seeds, the crucibles were reweighed to estimate the fresh weight of the seeds, using approximately 0.2 gram of seeds in each crucible. Then the crucibles were placed in the oven for 4 hours at 130°C, and then the samples were let to cool in desiccators for 30 minute. Then, the crucibles were reweighed again to estimate the dry weight.

Moisture isotherms can be constructed by allowing seeds to reach equilibrium in environments with known RH maintained by saturated salt solutions at a given temperature. Saturated salt solutions are prepared by adding the indicated salt to warm (about 40°C) distilled water with stirring until no more salt dissolves. Additional salt is added to ensure an excess of the saturating salt. Table 1 provides approximate compositions for saturated solutions. The saturated solution is then cooled to ambient temperature and allowed to set for at least 24 hour before use.After that the prepared saturated salt solutions are placed in labeled glass jar at least 20% of the total volume. Seeds were weighed (approximately 0.2 gm) and packed in tulle fabric and then placed in the jar above thesolutions. The jars then were sealed and allow enough time

Table 1: Approximate composition of saturated salt solutions.

Salt	**Humidity at 25 °C**	**Salt (g)**	**Water (ml)**
Lithium Chloride (Li Cl)	11.3	50	52
Magnesium Chloride(Mg Cl)	32.8	50	17
Potassium Carbonate (K_2CO_3)	43.2	50	22
Sodium Chloride (Na Cl)	75.3	50	15
Potassium Chloride(K Cl)	84.3	50	30
Sodium Hydroxide (Na OH)	11.1	50	35

Determining the equilibrium seed moisture content at each RH was carried out by oven-drying as described in the previous section. Put seeds in salt for a certain period of time 6 weeks after it germinated in Petri dishes to compare the percentage of germination moisture.
Following Robertson *et al.* (1999) a number of soil samples were collected from each stand as a mixture of 0-25 cm depth for chemical and physical analysis, organic matter, Moisture content,Soil (pH, EC, TDS) and water holding capacity were measured. Particle size analysis was determined by dry sieving for the coarse sand and by pipette method for fine sand, silt, and clay (Richards, 1954). Inorganic carbonate in soil was analyzed using a volumetric calcimeter method (Loeppert and Suarez, 1996).

RESULTS

SEED VIABILITY

After had been soaked in dilute tetrazolium salt solution for 24 hours, 30% of the *Verbascum sinaiticum* seeds were revealed to be viable. Meanwhile, 34% of the *Teucrium polium* seed were revealed to be viable after had been soaked in tetrazolium diluted solution for 48 hours.

SEED GERMINATION

Teucrium polium

The highest germination percentage (18%) in the *Teucrium polium* resulted from soaking seeds in calcium carbonate (1%) for 30 minutes before germination in room temperature. The lowest germination ratio (2%) was obtained after pre-treating seeds with calcium carbonate (3%) for 30 minutes. Germinating seeds without any pre-treatment either in fluctuating temperature (15/20°C) or in room temperature resulted in very far germination percentages (9% and 13%, respectively).Mann-Whitney statistical analysis determinednonsignificant difference (P =0.14) in germination rate of the seeds betweengerminating in 15/20°Cand room temperature (Figure 2).

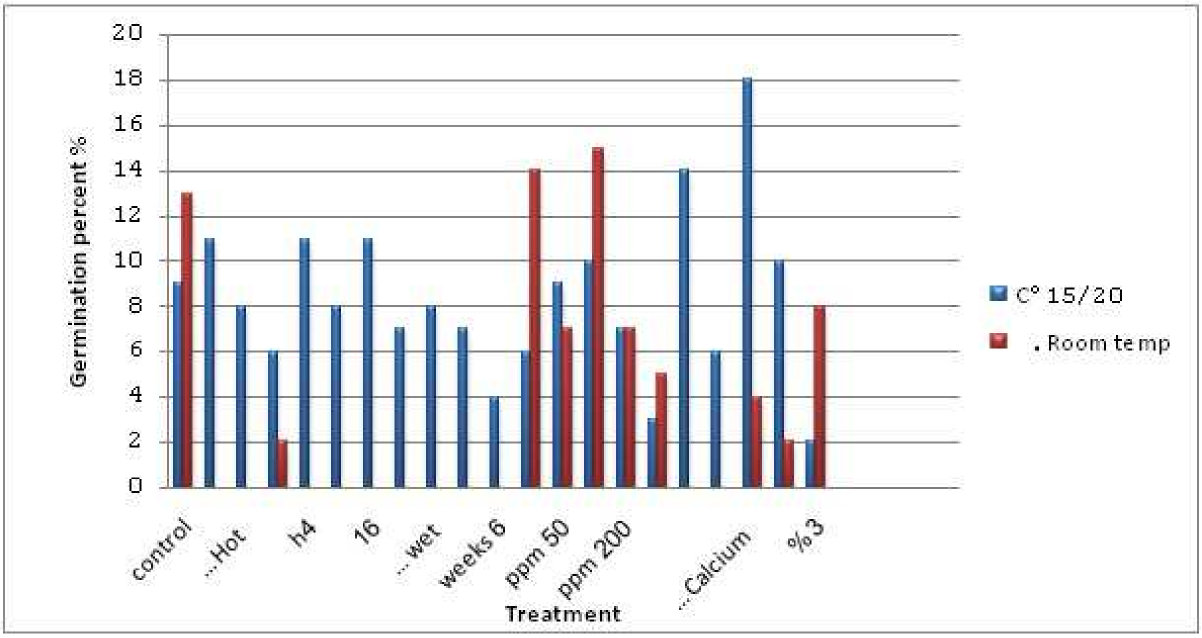


Figure 2: Chart shows the effect of different treatments on seed germination of *Teucrium polium*.

Verbascum sinaiticum

The highest germination percentage (54%) in the *Verbascum sinaiticum*showed from soaking seeds in Gibberellic acid (500 ppm) for 24 hours and germination in 15/20°C (photo 1, figure 3).The lowest germination ratio (zero %) was obtained after treatment seeds with 1%citric acid for 96 hours.The highest percentage when germinating seeds at room temperature was only45% that was obtained when the seeds were soaked in Gibberellic acid (50 ppm) for 24 hours.Mann-Whitney test resulted in non-significant difference (P =0.9) between germination rate of the seeds indifferent applied temperatures (room and 15/20° C).Generally, for all pre-treatments,germination began within 7 days of sowing.

Photo 1: Germination of *Verbascum sinaiticum* on moistened cotton in Petri-dish after incubation in15±2°C.

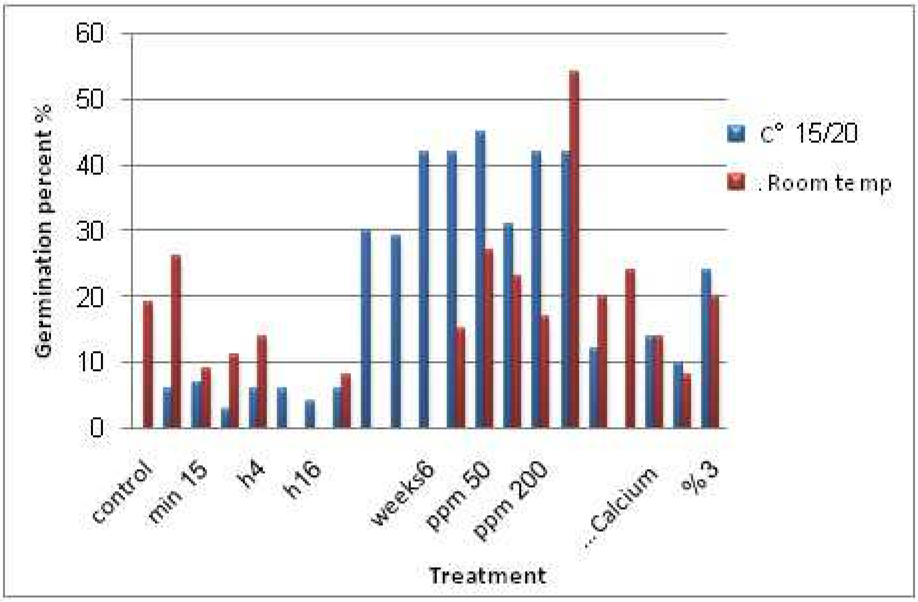


Figure 3: Germination percent of *Verbascum sinaiticum*with different pretreatments.

SEED MOISTURECONTENT AND MOISTURE ISOTHERM

Moisture content

The moisture content of each sample was determined in a drying oven at 130°C for 4 hours (AOAC, 1990).The results showed that seeds moisture contentfor *Teucrium polium* and *Verbascum sinaiticum* are 6.6% and 5.5%, respectively.

Moisture isotherm

Themoisture content of *Teucrium polium*, and *Verbascum sinaiticum* were equilibrated by incubation in six different saturated salt solutions (LiCl, NaCl, Mg Cl, K_2CO_3, Na OH, and KCl) at 25ºC(Figure 4 and 5).The targeted moisture degrees were ranging from 7.5 to 85% according to the used salt (Greenspan, 1976).Equilibrium moisture content was reached after 6 weeks in different salts.The highest rate of germination after incubating the seeds with different salts for 6 weeks at 25 ° C for *Teucrium polium* it was13% in NaCl and for*Verbascum sinaiticum* it was 37% in KCl. As evidenced by the Pearson correlation coefficient, thelinear correlation between germination and the moisture content in the seeds of *Teucrium polium* (P = 0.4) and *Verbascum sinaiticum* (P= 0.58) was non-significant.

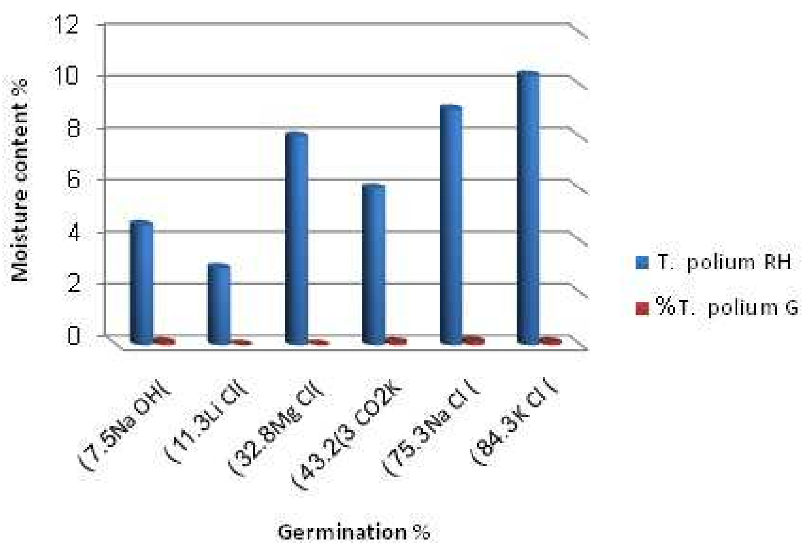


Figure 4: Seedmoisture content and germination after incubation with different salts for 6 weeks in *Teucrium polium*.

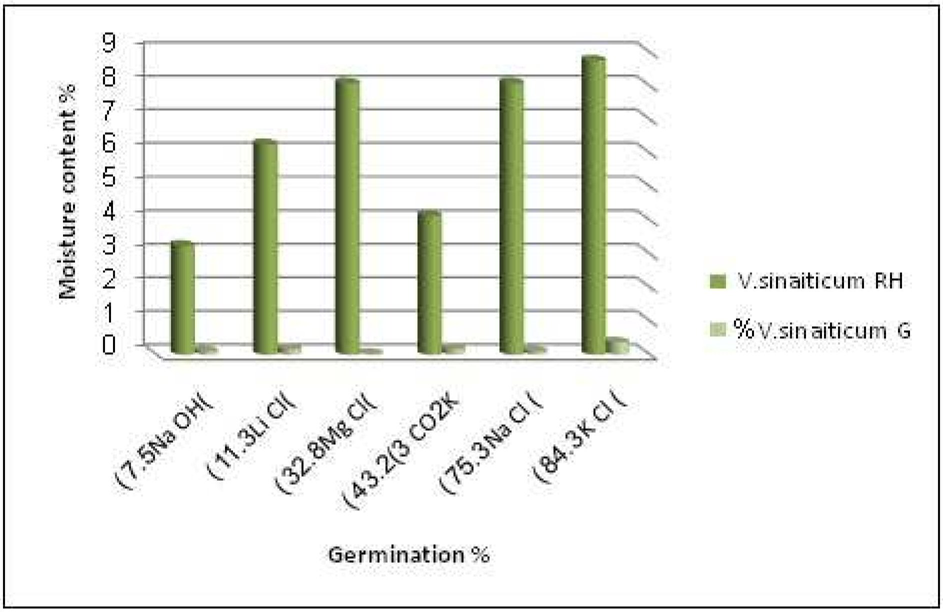


Figure 5: Seedmoisture content and germination after incubation with different salts for 6 weeks in *Verbascum sinaiticum*.

Quantitative vegetation analysis

Preliminary inspection of data

The study included twenty-two physical and chemical environmental factors as well as habitat features. These factors can be classified into three main groups. The first groupencompasseshabitat features which include landform type, exposure degree, elevation (m a.s.l.) and slope degree. The second group is thephysical characteristics of the soil and includes nature of soil surface, soil texture, soil moisture content, and organic matter content. The parameters of this group are represented as percentages of weight except nature of soil surface which is represented as a percentage of area. The third group which is the chemical characteristics of soil includes six parameters; acidity (pH), electric conductivity (EC), calcium, magnesium, bicarbonate and chloride, which arerepresented as a percentages.

Classification of stands

The TWINSPAN classification of one-hundred seventy-seven stands and seventy-seven species resulted in four main vegetation groups (Figure 6). These groups are separated at the second level of classification where the main dominantspecies are *Teucrium polium, Chiliadenus montanus, Hypericum sinaicum*and*Origanum syriacum-Mentha longifolia.*

The four vegetationgroupswerenamed according to the dominant species based on their presence percentages in each group:

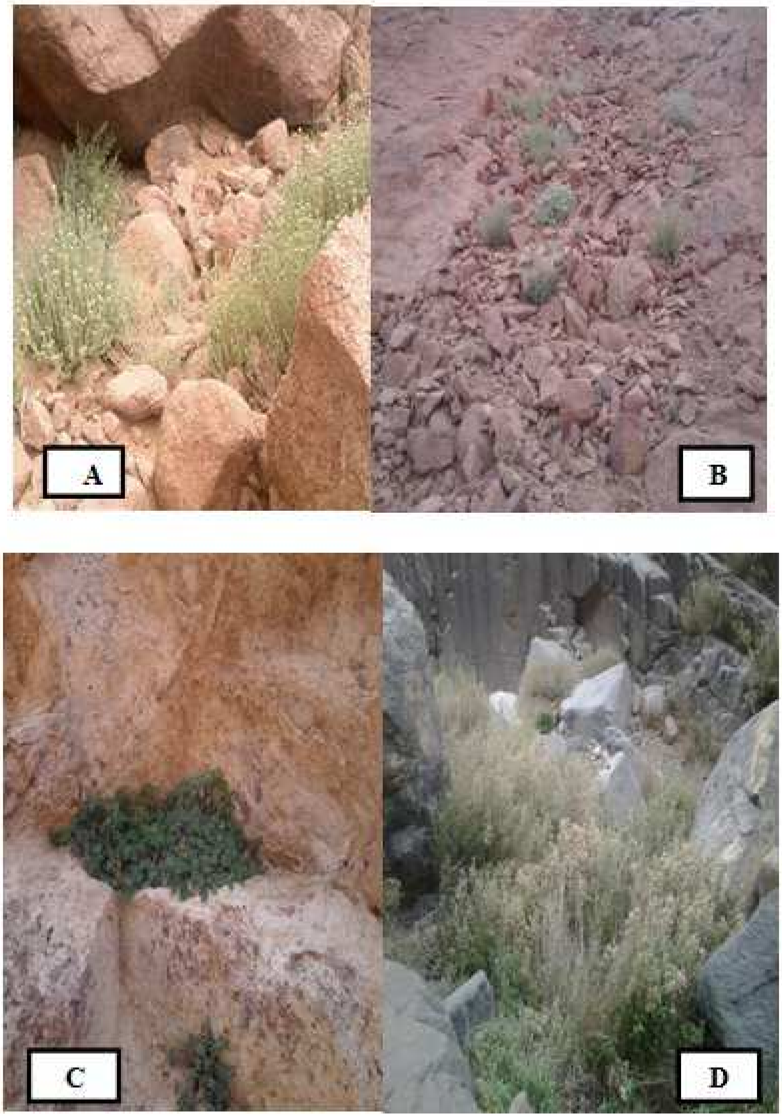
Assemblage I: ***Teucrium polium***
Assemblage II: ***Chiliadenus montanus***
Assemblage III: ***Hypericum sinaicum***
Assemblage IV: ***Origanum syriacum - Mentha longifolia***

The four assemblages can be described as follows:

Assemblage I: ***Teucrium polium***

Assemblage I is dominated by *Teucrium polium* (98.9%) withaverage abundance 1.43. The most important associated species include *Chiliadenus montanus* (89.0%) with average abundance 1.09, *Seriphidium herba-album* (83.9%) with average abundance 1.43, *Matthiola Arabica* (76.0%) with average abundance 1.43, *Stachys aegyptiaca* (67.5%) with average abundance 1.05, and *Galium sinaicum* (61.2%) with average abundance 1.01. This assemblage is found on ridges with fissures, steep slope, gorge and terraces habitats of G. El-Sarw, Kahf El-Ghola, W. El-Tofaha, Abo Gefa, W. El-Rutig, W. Gebel and G. El-Rabah (photo 2). This assemblage is characterized by average altitude of 1746 m a.s.l. with exposure degrees ranging from 15° to 340° (North) and the average slope degree is 21.75° with high organic matter content (18.53%), gravel percentage (48.98%), pH (8.16%) and cobbles (11.73). Most sites where this assemblage was recorded have a high boulders (54.42%) covering the surface area, while the other parameters such as coarse sand, medium sand, fine sand, silt and clay, EC, Ca, Mg, HCO_3, Cl, are moderate in their range.

Figure 6: The TWINSPAN four main vegetation groups resulted fromtheclassification of 177 stands based on the cover of77 plant species.



Photos 2: the plant species of the vegetation assemblages of the TWINSPAN. (A)*Teucrium polium* (I) at wadi El-Tofaha, (B) *Chiliadenus montanus* (II)at wadi El-Rutig,(C) *Hypericum sinaicum* (III)at Al-Maserday and (D) *Origanum syriacum* - *Mentha longifolia* (IV)at wadi Garagneia.

Table 2: TWINSPAN output of vegetation classification showing stand number at the top and differential clusters at bottom. Species names arranged at left hand-side and species clusters on right hand-side.

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               9347259869802568883112490447802340145679983578912661261303789235112404028701456358323771356790930154

37  Jas mont  1211111-11111111111121111121111111111211111211111212211112121111121121111111112223121211211111111111-1  000
20  Ech spin  11--2-1---------11-----1--1-1-1111-1-111-1------1------1-11--111-11-1-11-111----1111-1-11-2211--22-1-  001
30  Gemsini  -------------------1--------------11111------------11---------1--------------------------------------  001
69  Staaegy  111111--11--1-111--11-111111111--111111-111111-111111111-1---------11111111111121111---1-11-11---1-  001
74  Teupoli  1111111111111111111111111111111111321432212221112411111112212122111133121322113232231112111121121111  001
16  Croaegy  -------------------------1--------------------------------------------------------------------------  010000
19  Dip harr  11-11-11------1--------1-----1--1--------------------------------1-----1-----------111---------------  010000
51  Peg harm  ----------------------------------------------------------------------------------------------------  010000
56  Pol sina  ----------------------------------------------------------------------------------------------------  010000
66  Silleuc  -------1--------------------------------------------------------------------------------------------  010000
29  Gal sina  111-11---1-11--11-1---111--11-1-1-11-111--1-11-111--111--1111111-1111--11--1---111111121----11-11--1  010001
1  Ach frag  ------2------------------------------1--------------------------1------------------111--1---------3---  010010
31  Glaarab  ----------------------------------------------------------------------------------------------------  010010
34  Helarba  ----------------------------------------------------------------------------------------------------  010010
44  Mel indi  -----------------------------------------------------------------------------------1-------------  010010
55  Pol semi  ----------------------------------------------------------------------------------------------------  010010
70  Stiradd  ---------------------------------------------------------------------1----1-------------------------  010010
21  Ephalat  -------------------------------------------------1-----------------------------11----11---------1---  010011
39  Kicmaci  ----1------------------------------------------------------------------------2----12-111-1111111--1  010011
71  Tan sant  -1----1--1------1---------------1111-1------------------11-1-1111-1-1-111-1-11-111--1-211-111-11-11  010011
6  Atr spin  ------------------------------------------------------------1---------------------------------------  010100
36  Ifl spic  -----------------------------------------------------------1---------1------1-------1----------------  010100
59  Ptedich  ---------1----------------------1-------------------------------------------------1-11----121--1-1---  010100
61  Res sten  -----------------------------------------------------------1----------------------------------------  010100
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65  Scr dese  -----------------------------------------------------------------------1------------------------  010100
67  Silarab  -1---------------------------------------------------------------------------121-------------  010100
5  Are defl  1---1-1--11-11---111---1-----------1---1-1-----1--1-----11-1111-1-1-1-1---1-1--1-1--1--11-11---1--11  010101
33  Gyp capi  -------1----------------------------------------------1---------------------------1--------------  010101
49  Par rug  --------------------------------1----------------------111-1-------------------------1----------11  010101
60  Rum cypr  1-----------------------------------------------------1-----------------------------------------  010101
72  Teuleuc  ------------------------------------------1-11-----1---111112------------------1----------------1--  010101
4  And aspe  ----------------------------------------------111----------------1-----------11------------------  010110
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40  Lac spin  1-----1111--1---1----------------11----1----1-----------------------1---------------------11------11-  010110
63  Sch barb  --------------------------1-1-1----1--------111-------------1--------------------------------------  010110
75  Tri stel  ---------1-----1-11-1-1-11--------1-11--------1111111---------------------------111-1--1-------------  010110
77  Zil spin  ------1--11-1-1111--------------------------------------1-1-------------------------------1--111-  010110
9  Ast spin  --1-1------------1-1-11----11--11---1----1----------------1---1-1----1-------------1-1------1-------  010111
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27  Fun Moss  ---------------------------------------------------------------------------------------------------  101001
35  Hypsina  ---------------------------------------------------------------------------------------------------  101001
38  Jun acut  ---------------------------------------------------------------------------------------------------  101001
45  Men long  ---------------------------------------------------------------------------------------------------  101001
76  Versina  ---------------------------------------------------------------------------------------------------  101001
17  Crasina  ---------------------------------------------------------------------------------------------------  10101
28  Fra cris  ---------------------------------------------------------------------------------------------------  10101
46  Nep sept  ---------------------------------------------------------------------------------------------------  10101
68  Son macr  ---------------------------------------------------------------------------------------------------  10101
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52  Phlaure  ---------------------------------------1----2-------------------1----1----1--------11-------31----2---  11
53  Plasina  -------------------1--------------------------------1------------1----11-----------------------------  11
54  Poa sina  --1------------------------------------1-------------------------------------------------------------  11

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Table 2.cont.

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59  Ptedich  1-----------1------------------------------------------------------------------  010100
61  Res sten  -------------------------------------------------------------------------------  010100
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67  Silarab  ----------------------------------------------------1--------------------------  010100
5  Are defl  1----------------------------1-------------------------------------------------  010101
33  Gyp capi  -------------------------------------------------------------------------------  010101
49  Par rug  --1-----------------------------------------1----------------------------------  010101
60  Rum cypr  -------------------------------------------------------------------------------  010101
72  Teuleuc  1-1----------------------------------------------------------------------------  010101
4  And aspe  -----------------------------1-------------------------------------------------  010110
13  Cay hexa  ----------------------------------1--------------------------------------------  010110
25  Far aegy  ---1---------------------------------------------------------------------------  010110
40  Lac spin  111--1--------------------------------------1-----------------------------------  010110
63  Sch barb  ------------------------------------1------------------------------------------  010110
75  Tri stel  ----------------------------1----------------1---------------------------------  010110
77  Zil spin  -2--------1---------------------1----------------------------------------------  010110
9  Ast spin  -------------------------------------------------------------------------------  010111
11  Bufmult  --------------------------------11---------------------------------------------  010111
14  Cen eryn  -------------------------------------------------------------------------------  010111
22  Erolaci  -------------------------------------------------------------------------------  010111
23  Fag arab  -------------------------------------------------------------------------------  010111
41  Lot cret  -------------------------------------------------------------------------------  010111
42  Lot plat  ----------------------------------1--------------------------------------------  010111
50  Par sina  ---------------------------------1---------------------------------------------  010111
58  Pulcris  -------------------------------------------------------------------------------  010111
62  Sen flav  -------------------------------------------------------------------------------  010111
7  Art incu  -11----1--1-1--1-------------2-----------11-111--11------11---1-----------------  01100
24  Fag moll  --111----1--1------------1----1------------11---------------1-1-------------  01100
32  Gym deca  ----------------------------1---------------------------1--------------------  01100
43  Mat arab  ------1--1------------------1-1---------------1---------11----1-1----1-------  01100
10  Balundu  --1--1--1-1----1--1------------------------------------1-------------1-----  01101
15  Cen scop  ----------------1-1--11---1--------------------------1--------------------  01101
47  Ono ambi  -1--------------------------------------------------------------11-------  0111
73  Teupilo  ---------------------------------------------------------1---------------------  0111
3  Alkorie  1---------------------------------------------------11111-1---1---111-1---  100
12  Cap spin  ------------------------------------------------------------------------1----  101000
2  Adicapi  ---------------------------------------------------------------111----------1  101001
8  Art juda  ---------------------------------------------------------------------1-------  101001
18  Cyndact  -------------------------------------------------------1--1--------------1---  101001
26  Ficpseu  ---------------------------------------------------------11--1---111122--1---  101001
27  Fun Moss  -----------------------------------------------------------------------------3  101001
35  Hypsina  ----------------------------1-------------------------1-1111112-111111111111  101001
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 38  Jun acut  --------------------------------------------------------------11-11---1  101001
 45  Men long  --------------------------1----------------------11111111112-22--112111  101001
 76  Versina  -----1-----------------1--------------------------311-1-111-1---1--11--  101001
 17  Crasina  ----------1-------------------------------------------------1----111---  10101
 28  Fra cris  -----------------------------------------------1----------1------------  10101
 46  Nep sept  1-----------1-----------------------------------------------1---2------  10101
 68  Son macr  --------------------------------------------------1--1-----------------  10101
  57  Ptesanc  --------11111--11-----------------------------11--11-1----1--1---11----  1011
   48  Orisyri  12---1--1---11-1-----1111111---24----------------------1-----1311--211-412  11
   52  Phlaure  --2---------2--1-----3-22-----------1---------1--------------11-3-----112--  11
   53  Plasina  ------11111111111--111111-1-111----11---1----------111--1-1-11---11----------  11
   54  Poa sina  --------1--------------------------------------1---------1---------------1  11

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Assemblage II: ***Chiliadenus montanus***

This assemblage is dominated by *Chiliadenus montanus* (100%) with average abundance 2.22. The associating species include *Teucrium polium*(98.4%) with average abundance1.28, *Stachys aegyptiaca*(48.5%) with average abundance1.06,*Plantago sinaica*(47.5%) with average abundance 1,*Origanum syriacum*(45.2%) with average abundance 1.28, and*Tanacetum sinaicum* (34.5%) with average abundance1.05.

This assemblageis high terraces with fissures and ridges with fissures steep slope with fissures. It is found in all sites except W. Garagneia(photos 2). In this group the altitude is 1813 m. with exposure degree ranging from 20º to 340º(North, North-Western, South-Eastern) and the slope degree is 45.18º.This assemblage is characterized by low organic matter mean percent (3.13%) while the other parameters landform, slope degree, exposure degree, elevation, gravel, coarse sand, medium sand, fine sand, silt and clay, pH, electrical conductivity, moisture content, calcium, magnesium, bicarbonate, chloride, and nature of soil surface are moderate in its range.

Assemblage III:***Hypericum sinaicum***

Assemblage III is dominated by*Hypericum sinaicum* (91.7%) with average abundance 1.05. The associating species include *Mentha longifolia* (81.5%)with average abundance 1.34,*Teucrium polium* (77.4%)with average abundance1,*Alkanna orientalis* (57.1%)with average abundance1,*Juncus acutus*(57.1%)with average abundance 1,*Ficus pseudo-sycomorus*(56.5%)withaverage abundance1.2,*Stachys aegyptiaca*(50.6%) with average abundance 1,*Plantago sinaica*(50%)with average abundance 1, and *Verbascum sinaiticum*(47.6%) with average abundance 1.12.

This assemblage is found in gorges at springs and steep slopes with fissuresof W.El-Tala'a, W. Gebel, G.Catherine, Al-Maserday, W. El-Tofaha and G.Mousa (photos 21). This assemblage is characterized by average altitude of 1762 m with exposure degree ranging from20º to 340º (North, North-Western, South-Eastern)and the slope degree is (30.23º).Most sites where this assemblage was recorded have a high percentage of boulders (68.09%) covering the surface area and the soil has a high medium sand percentage(39.00%), while the other parameters are moderate in its range.

Assemblage IV: ***Origanum syriacum - Mentha longifolia- Hypericum sinaicum***

This assemblage is co-dominated by *Origanum syriacum* - *Mentha longifolia*-*Hypericum sinaicum*(100%) with average abundance 2.33, 1and 1 respectively. The associating species include*Galium sinaicum*(33.3%)with average abundance3, *Phlomis aurea* (33.3%) with average abundance 2,and *Verbascum sinaiticum*, *Adiantum capillus-veneris*and *Poa sinaica*(33.3%) with average abundance1. This assemblage is found on slopes with fissures habitats of Al-Maserday and W. Garagneia. This assemblage has average altitude of 1876m a.s.l. with exposure degree ranging from 20º to 120º(North, North-Eastern) and average of slope degree of 13.33º.This assemblage is characterized by lowest (medium sand)(26.07%), fine sand (15.45%), moisture content (1.13%), cobbles (0), and stones percentage (2%).

Most sites where this assemblage was recorded have a low percentage ofboulders (32%) covering the surface area. This assemblage is characterized by high coarse sand (46.39%), silt and clay (12.08%), calcium (23.84%), magnesium (8.6%), bicarbonate (5.88%), chloride (16.88%), and gravel percentages (19.14) and high electricalconductivity (2640.33).

Classification of species

The TWINSPAN technique revealed that all species can be grouped into seven groups at the third level of classification. The first group comprises one species includes; *Chiliadenus montanus,* the second group comprises four species includes *Echinops spinosus*, *Gomphocarpus sinicus, Stachys aegyptiaca, Teucrium polium.* The third group comprises fourty–fourspecies including *Achillea fragrantissima,Andrachne aspera*, *Arenaria deflexa*, *Atraphaxis spinosa*, *Bufonia multiceps*, etc. The fourth group contains eight species includes; *Ballota undulate*, *Centaurea scoparia, Seriphidium herba-album, Fagonia mollis, Gymnocarpos decandum, Matthiola arabica, Onopordum ambiguum, Teucrium polium.* The fifth group comprises only one species includes *Alkanna orientalis.* The sixth group comprises fifteen species includes *Adiantum capillus-veneris,Artemisia judaica*, *Capparis spinosa, Crateagus x sinaica, Cynodon dactylon, Ficus pseudo sycomorus, Funaria sp, Farsetia aegyptia, Hypericum sinaicum, Juncus acutus, Mentha longifolia, Nepeta septemcrenata, Verbascum sinaiticum*, etc. The seventh group comprises four species.

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 is considered in the interpretation. The reason is that the eigenvalues of the CCA axis I is (0.352) and the CCA axis II (0.211) and the CCA AxisIII (0.187).

The one-hundred seventy-seven stands were classified by TWINSPAN technique at the second level into four community types (assemblages). The ordination diagram (Figure 7) shows the position of these assemblages and their interrelation with environmental factors. The first assemblages (*Teucrium polium*) is found in the center of the diagram and associated with sand and silt fractions of soil texture, elevation, exposure, slope, EC, calcium, HCO_3, Cl, and gravel. It occurs along axis1 and axis2 and so it occupies the higher left-hand corner in axis1-axis2 plane. The assemblages II, III and IV (*Chiliadenus montanus*, *Hypericum sinaicum* and *Origanum syriacum* - *Mentha longifolia*) occur along axis1 and axis2 and so it occupies the lower right-hand corner in axis1-axis2 plane of the diagram.

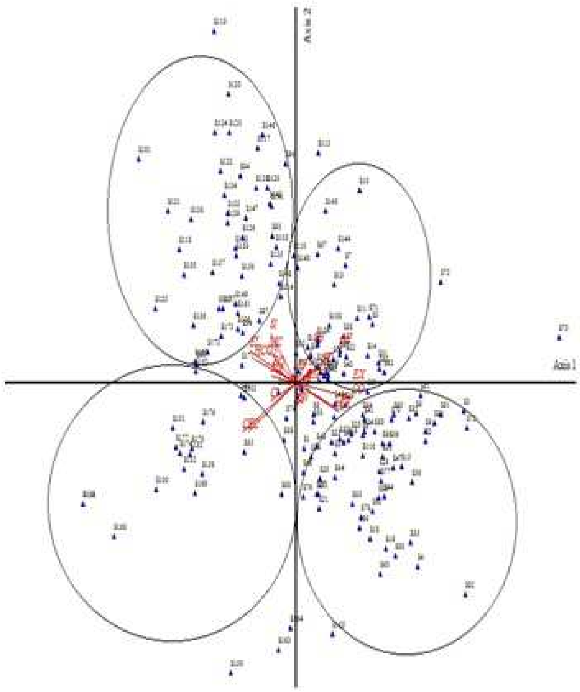

Figure 7: Output of Canonical Correspondence Analysis (CCA) diagram (axis1 – axis2 plane). Thestands are representedby (Δ).

(b) Species - environment relationship

In the ordination diagrams (Figure 8) twenty-two environmental factors landform type, exposure degree, elevation (m) and slope degree,nature of soil surface, soil texture, soil moisture content, organic matter content,acidity (pH), electric conductivity (EC), calcium, magnesium, bicarbonate and chloride.

In this graph species as *Heliotropium arbainense*, *Silene leucophylla*, *Stachys aegyptiaca* exhibit high correlation with calcium, electrical conductivity(EC), *Hypericum sinaicum*,*Origanum syriacum*, *Mentha longifolia, Phlomis aurea,* while are not correlated, whilespecies as *Zilla spinosa* are not correlated. Other species as *Teucrium polium* exhibit high correlation with areas with high exposure, silt and clay, pH, medium sand, coarse sand , gravels, while other as,*Gomphocarpus sinicus*, *Tanacetum sinaicum* are correlated. While other species as *Cotoneaster orbicularis* is not correlated.

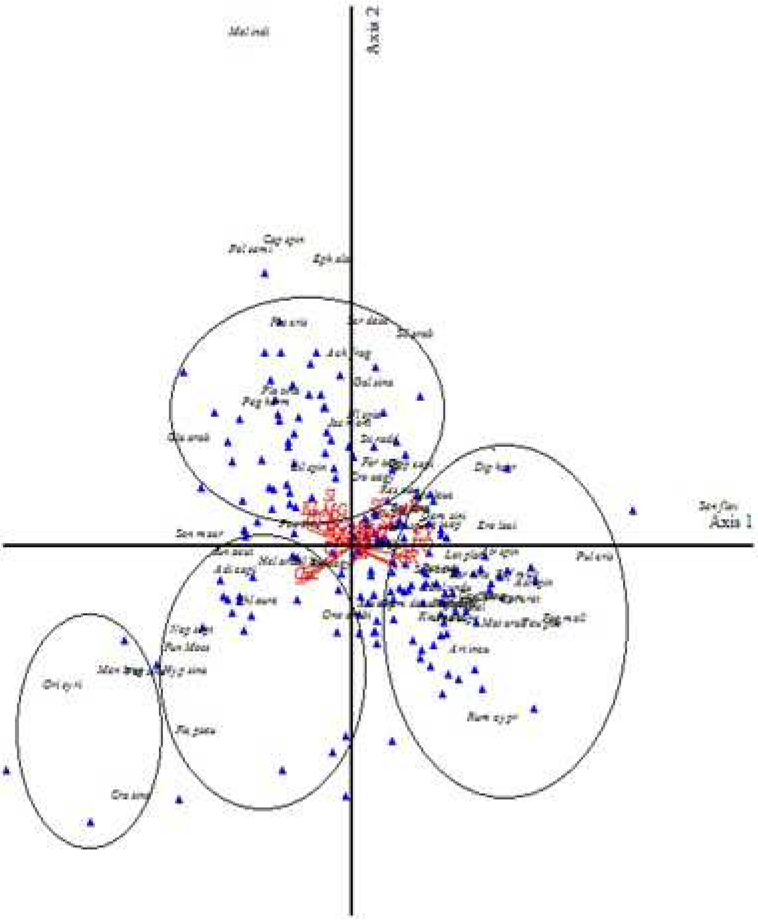

Figure 8: Ordination (CCA) diagram (axis1-axis2 plane) with plant species represented as (**Δ**) and environmental variables as centroid lines (Abbreviations are listed in table 4).

DISCUSSION

Sinai Peninsula is floristically one of the richest of all phytogeographical regions of Egypt. Contact with different floras, past climatic changes, and present local influence of habitat could favor plants from different floras and increase the number of species (Danin, 1978). According to Boulos (1995) species growing in Sinai belong to 121 families comprising 742 genera. Out of these, 63 species are endemic distributed in different phytogeographical regions of Egypt. More than 65% of these endemic species (41) occur in Sinai: 25 species in Sinai only and 16 species in Sinai and other regions of Egypt (Boulos, 1995, 1999, 2000, 2002, 2005). Most of the endemic species of Sinai (>70%) are recorded from the Southern mountainous area.

The flora of South Sinai has been subjected to threats causing declines in population number and size. So, only 326 species were recorded in St. Catherine Protectorate in recent surveys (Moustafa *et al*., 1998, 1999, 2001, Abd El-Wahab *et al*., 2004). Among these326 species, 115 species (35.6%) are considered as medicinal species (Abd El-Wahab *et al*., 2004). Medicinal plants refer to species having medicinal (including veterinary) aromatic, and culinary importance. Distributions, utilization in folk medicine and active constituents of medicinal plants in Sinai have attracted many ecologists, taxonomists and phytochemists (Hanafi and Abdel-Wahab, 2000; Abd El-Wahab *et al*., 2004).

A lot of disturbances due to human impact are recorded throughout the Saint Catherine, including over-grazing, over-collection, feral donkeys, over-cutting for fuel wood, land conversion (construction of new settlements, infrastructure, cultivation areas and digging new wells), other land use, tourism, and solid wastes (due to urbanization and tourism activities). Each site may have its specific order of threats but the most important threat. These dangerous problems lead to destruction of natural habitats and the disappearance of plant communities in which many threatened species are found.

The results of vegetation survey of the present study identified 77 plant species during the study period in the chosen stands. The identified species belong to 27 families. Compositae, Labiatae, Caryophyllaceae and Leguminosae are the families that are represented by the largest number of species, respectively. Zaghloul (1997) identified 132 species throughout the area of Saint Catherine mountainous area belonging to 31 family including Compositae, Labiatae and Gramineae.

Although the endemic species represent 3.2% of the total flora of Sinai (Danin, 1986) and 3.8% of the upper Sinai massif flora (Danin, 1986 and 1987), in the present study, from the identified 77 species, 7 species are endemic according to Täckholm (1974) and Boulos (1995,1999,2000, 2002 and 2005) with a percentage of 10%. On the other hand, Zaghloul(1997) identified 13 endemic species (12.2%).

The recorded seventy-seven species included thirty species vulnerable, sixteen species are endangered,sixteen species are common, fourteen species are rare, seven species are endemic, and five species are critically endangered.Within these threatened species there are thirteen medicinal species are vulnerable(*Achillea fragrantissima*, *Alkanna orientalis, Artemisia judaica, Chiliadenus montanus, Ephedra alata, Lactuca spinosa, Plantago sinaica, Pituranthos tortuosus, Scrophularia sinaica, Teucrium leucocladum, Teucrium pilosum, Trigonella stellata* and *Zilla spinosa*),ten medicinal species are endangered (*Andrachne aspera, Astragalus spinosus, Atraphaxis spinosa, Glaucium arabicum, Juncus acutus, Mentha longifolia, Melilotus indica, Nepeta septemcrenata, Teucrium polium* and *Verbascum sinaiticum*),eight medicinal species are rare (*Adiantum capillus-veneris*, *Centaurea scoparia*, *Ficus pseudo-sycomorus*, *Francoeuria crispa*, *Gomphocarpus sinaicus*, *Gypsophilla capillaris*subsp.*Antari*, *Polypogon semiverticillata, Pulicaria crispa*) and four medicinal species are critically endangered (*Bufonia multiceps*, *Hypericum sinaicum*, *Origanum syriacum* subsp. *sinaicum* and *Polygala sinaica*).

To understand the behavior of the study species along the environmental gradient, the collected vegetation data were subjected to multivariate analysis techniques, that classification by TWINSPAN computer program and ordination by CAA computer program. In general, distribution of these vegetation groups or plant communities were more related to altitude, slope degree, exposure degree, soil moisture, soil organic matter, sand, silt and clay fractions and gravel percentage rather than to soil reaction (pH and EC).

These findings are in agreement with many previous studies indicated that distribution of plant communities in arid ecosystems are controlled by edaphic conditions, physiographic features, and topographical irregularities, which all act through modifying the amount of available moisture (Kassas and Batanouny, 1984; Salama and Fayed 1989 and 1990; El-Ghareeb and Shabanna, 1990; Salama and El Naggar, 1991, Moustafa and Klopatek, 1995 and Salama *et al.,* 2005).

Teucrium polium is the main significant species characterizing vegetation assemblage I as while the important species are *Chiliadenus montanus*, *Seriphidiumherba-album*, *Matthiola arabica*, *Stachys aegyptiaca* and *Galium sinaicum*. This assemblage *Teucrium polium* is found at elevation (1746 m a.s.l) on ridges with fissures, steep slope, gorge and terraces habitats and North exposure, and characterized by having the highest percent of high organic matter content, gravels percentage, pH, and cobbles. This assemblage characterized a high percentage of boulders covering the surface area.

Chiliadenus montanus assemblage, associated with *Teucrium polium*, *Stachys aegyptiaca*, *Plantago sinaica, Origanum syraicum* and *Tanacetum sinaicum* are associated species, occupies habitats of different landforms with north, north-western, south-eastern aspects with altitude of 1762 m a.s.l. The prominent characters of this assemblage are the low organic matter.

In the third assemblage, *Hypericum sinaicum* is the dominant while *Mentha longifolia*, *Hypericum sinaicum*, *Teucrium polium*, *Alkanna orientalis*, *Juncus acutus*, *Ficus pseudo-sycomorus*, *Stachys aegyptiaca*, *Plantago sinaica* and *Verbascum sinaiticum* are the most prominent associated species. This assemblage is usually found in a very narrow scope of environmental factors with exposure north, north-western, south-eastern, high boulders and high medium sand percentage.

Origanum syriacum- *Mentha longifolia* assemblage includes *Hypericum sinaicum*, *Galium sinaicum*, *Phlomis aurea* and *Verbascum sinaiticum*, *Adiantum capillus-veneris* as associating species, is very restricted to main wadi Garagneia and Al-Maserday parts and the slope with fissured habitats. This assemblage is found on north, north-eastern exposure, the prominent characters of this assemblage are the low medium sand, fine sand, moisture content, cobbles and stones. This assemblage characterized a high percentage calcium, magnesium, bicarbonate and electrical conductivity. Moustafa (1990) recorded *Origanum syriacum* - *Plantago arabica* assemblage as a disjunct assemblage. Moustafa and Zaghloul (1993) recognized a cluster of *Tanacetum santolinoides - Origanum syriacum* assemblage, *Origanum syriacum* - *Fagonia mollis*, and *Mentha longifolia* - *Origanum syriacum* as disjunct assemblages.

According to Baskin and Baskin (2004), different germination behaviors were detected that can be attributed to the conditions under which each of the species grows. The release of dormancy and promotion of germination in unfavorable conditions is achieved by a variety of promontory treatments, including pre-chilling, light, GA3 which are usually effective in species of the family Labiatae (Ellis *et al*., 1985; Takano *et al*., 1990).

In present study, germination *Teucrium polium* resulted in maximum percentage of 18% in seeds soaked in calcium carbonate (1%) for 30 minutes before germination in room temperature. When seeds were pre-treated with GA3(100 ppm), it resulted in 15% germination in room temperature, while it was only 10%whenthe seeds germinated in the incubator at a constant temperatureof15 ± 2 ° Cat the same concentrations. Increasing GA3 concentration resulted in increased germination percentage. Nadjafi *et al*. (2006) showed when applying GA3, the germination of *Teucrium polium* highest germination percentage was obtained at 500 ppm GA3 (45%) with a positive response across all applied GA3 concentrations. Treatment with GA3 has also been proved effective for breaking dormancy in other *Teucrium* species growing in the Mediterranean region, such as *Teucrium polium* (Nadjafi *et al*., 2006) and the Iberian endemic *Teucrium oxylepis* ssp. *marianum* (Heranz *et al*., 2002).Meanwhile, our results showed that the maximum germination percentage (8%) in constant temperature of 15 ± 2° C for wet chilling at 5°C was obtained after two weeks chilling. Nadjafi *et al*. (2006) showed that washing and chilling (5°C) for a period of 14 days was most effective in breaking dormancy in *Teucrium polium*. Washing and chilling are standard procedures which have been used to enhance the germination of dormant seeds (ISTA, 1999).

In *Teucrium kyreniae* all seeds are non-dormant, while in *Teucrium divaricatum* ssp. *canescens*, even in the favorable temperature, germination was low (> 25%), but it was enhanced by treatments with nitrates, Gibberellic acid, and light(Ferriol *et al*., 2006). Such differences are not uncommon and they can occur even between closely related species that grow in different conditions. Five *Teucrium* species that are geographically restricted to central and south Spain have differences in germination behavior (Ferriol *et al*., 2006). In *Teucrium marum*, the most favorable temperature for germination was 15-20°C, while GA3 and light were also effective in further promoting seed germination (Benvenuti *et al*., 2006).

Comparing with other relative taxa, seed germination in the dark for the taxa *Micromeria cypria*, *Salvia veneris,* and *Teucrium divaricatum* ssp *canescens*, was found to be favored at relatively low temperatures (10 - 20°C), while in *Nepeta troodi* and *Salvia willeana* is achieved at even lower temperatures (5 - 10°C and 5°C, respectively). Germination in low temperature is a common trait in plants from the Mediterranean region (Thompson, 1970; Ellis *et al*., 1985; Perez-Garcia *et al.,*2003; Skordilis and Thanos, 1995; Thanos and Scordilis, 1995; Doussi and Thanos, 2002) and it has been documented specifically for several Mediterranean labiates (Kadis *et al*., 1994; Kadis, 1995; Thanos and Scordilis, 1995; Thanos *et al*., 1995; Perez-Garcia *et al.,* 2003; Kadis and Georghiou , 2010). This trait is related to the necessity for germination after the beginning of the rainy season, thus allowing seedlings to avoid arid conditions during summer. In all the above taxa, germination rate is relatively low. This characteristic can be also related to the survival strategy of these plants, since low germination rate assures that germination and subsequent seedling establishment do not take place during the frequently encountered dry spells in the early phase of the rainy period.

The results of germination tests in present study showed that the highest germination percentage of*Verbascumsinaiticum*without any treatments obtained at room temperature was 19%, while there was no germination when seeds were germinated in incubator at constant temperature of 15 ± 2°C. Treating seeds in hot water (80°C) for 5 minutes resulted in 26% germination in room temperature, while it was only 6% when seeds were germination in incubator at constant temperature of 15 ± 2°C.Soaking seeds in GA_3, the maximumgermination percentage(54%) was obtained at room temperature at concentrationsof500 ppmGA3.Germination rate was45%whenthe seeds weregerminated in the incubator at a constant temperatureof15 ± 2 °Cat concentrationsof50 ppmGA3. Increasing GA3concentration increased germination rate. Wright (1992) obtained 78% germination in *Verbascumthapsus* in room temperature.

Conservation of plant genetic resources comprises two types of approach; *in situ* and *ex situ* conservation. *In situ* conservation has been defined as the conservation of whole ecosystems and natural habitats where wild or cultivated species are maintained and may continue to evolve. *Ex situ* conservation maintains germplasm outside its original habitats, in the form of whole plants in botanical gardens and field genebanks, seeds as in seed genebanks, or certain other parts of the plant such as roots, dormant buds, pollen, explants as in vitro genebanks, or possibly as DNA. Knowledge of the seed storage behavior of a target species is required in order to determine whether or not seed storage is suitable as a method of genetic conservation, and how to handle seeds during collection and germplasm exchange (Hong *et al.*, 1996).

The results of seed moisture content and the response germination rates of seeds after incubation in different moisture present leveled a clue that the seeds of the studied species may be classified as orthodox. Orthodox seeds can be dried, without damage, to low levels of moisture content and, over a wide range of environments, their longevity increases with decrease in seed storage moisture content and temperature in a quantifiable and predictable way (Roberts, 1973). The latter is defined by the improved seed viability equation (Ellis and Roberts, 1980). In essence, in order for seed storage behavior to be defined as orthodox two conditions must be satisfied:

First; mature seeds survive desiccation to low moisture contents, at least to 2-6% depending on the species. Above this value (but within the air-dry range) there is a negative logarithmic relation between seed moisture content and longevity (Ellis and Roberts 1980; Ellis *et al*., 1988; Ellis *et al*., 1991).

Second; with regard to the effect of temperature on longevity, there is a negative relation between temperature (at least between –20 and 90°C) and seed longevity at constant moisture content (Roberts, 1973). The precise form of this relation is a negative semi-logarithmic relation modified by a quadratic term such that the relative benefit to longevity of a reduction in temperature declines the cooler the temperature (Ellis and Roberts 1980; Ellis *et al*., 1985; Ellis, 1988).

Storage environment is obviously very important in extending the life of seeds. The general objective is to reduce the metabolism of the seeds as much as possible without damaging them and to prevent attack by microorganisms. The ideal metabolic rate in storage will conserve as much of the stored food reserves in the seeds as possible, yet operate at a level that maintains the integrity of the embryos. Seed moisture is the most important factor in maintaining viability during storage; it is the primary control of all activities. Metabolic rates can be minimized by keeping seeds in a dry state. For true orthodox and sub-orthodox seeds, optimum moisture contents for storage are 5 to 10% (Hong *et al.*, 1996).

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