Effect of Pre-germination treatments on Seed Germination of *Helianthemum lippii* (L.)Dum.Cours.

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Abstract
Helianthemum lippii (L.) Dum. Cours. is a perennial shrubby plant 10-45 cm tall that belongs to the family Cistaceae. The effects of pretreatments on germination of *Helianthemum lippii* provide information regarding germination requirements of this species, which could be used for conservation studies. Five different pretreatment were applied to enhance seed germination. Scarification followed by germination at 6 different constant temperatures (10, 15, 20, 25, 30 and 35°C) with continuous light or dark, Scarification followed by GA_3 soaking (100, 250, 500, 750 and 1000 ppm), Heat and moist stratification (only intact seeds), KNO_3 and H_3BO_3 soaking (only intact seeds). The results obtained from this study indicate that germination in *H.lippii* was promoted by scarification. Scarification of seeds resulted in high germination while increasing germination temperature (35°C) decreased the germination of scarified seeds. Exposure to light and dark had no effect on germination. Heat stratification, cold stratification, KNO_3 and H_3BO_3 treatments were ineffective in increasing germination of *H.lippii* seeds. Scarification yielded maximum germination without soaking in GA_3. It increased the germination of *Helianthemum lippii* seeds from 1 to 99%. Increase in GA_3 concentration decreased the germination of this species. Scarification succeeded in breaking dormancy of *H.lippii* seeds suggesting that this species exhibits seed coat dormancy and in nature it may happen due to the abrasion of seed coat by sand particles or other biotic and abiotic factors.
Keywords: Germination, scarification, dormancy, conservation, biotic, abiotic factors.

Introduction

The vegetation of Kuwait is under severe pressure due to multiple interacting factors such as overgrazing, uprooting of woody shrubs, increased recreation, gravel quarrying, environmental factors and natural processes. Additionally, the Gulf War increased the constraints and pressures on the desert ecosystem (Omar et al. 2000). *Helianthemum lippii* (L.) Dum. Cours. is distributed in small patches in northern central and southern part of Kuwait. *Helianthemum lippii* is confined to the extreme desert, such as the southern Negev and the Arava Valley, where mean annual rainfall is less than 70 mm and the vegetation is restricted to wadis. The plant is between 10 and 45 cm high with white stems (Fig. 1). Seeds are very tiny and brown in color (Fig. 2). Seeds of *Helianthemum lippii* mature during April and May and are dispersed during summer by wind. Seeds of different plant species have been found to require different conditions for germination. These conditions enable them to germinate in the right period of the right season, and in the right place, and to survive in extremely hot deserts with completely unpredictable dates and distribution of rainfall (Gutterman, 1993). Omar et al. (2000) stated those desert truffles such as *Tirmania* and *Terfezia* are associated with the roots of *Helianthemum lippii*. In Saudi Arabia, *Helianthemum lippii* and *Helianthemum ledifolium* are the main host plant associates for desert truffles (Bokhary 1987). In the past, Al Dubdibah the southwest part of Kuwait was full of the desert truffle (*Tirmania* and *Terfezia*) locally known as Fuq'a or Kamaa. They are believed to be associated with *Helianthemum lippii*, which was common in the area. However, Fuq'a cannot be seen nowadays due to land degradation problems in the area (Omar et al. 2000). *Helianthemum lippii* has high potential for use in conservation practices.

Figure 1. *Helianthemum lippii*

Figure 2. *Helianthemum lippii* seeds

Propagation from seeds is the most common method in many range plants. However many range plants have dormancy and various methods are used to overcome dormancy. Baskin and Baskin (1998) stated that dormancy is widespread among desert shrubs, with only a few species having non-dormant seeds. Scarification enhanced germination of many range species. Thanos et al. (1992) stated that seed coat hardness and impermeability to water might be the most important causes of the dormancy present in several species of Cistaceae. The germination studies in native desert plants are the preliminary step in its conservation and also for revegetation program. This study was therefore designed to determine the effects of seed treatments on germination of *H. lippii,* seeds. The results of this research will help in the establishment of a protocol for seed multiplication of *Helianthemum lippii* to enhance the services of the desert ecosystems in Kuwait.

Materials and Methods

Environmental Conditions of Kuwait

Kuwait is a small arid country extending between latitudes 28° 33'and 30° 05' N and longitudes 46° 33' and 48° 30' E in the northeastern part of the Arabian Peninsula. It has a surface area of 17,818 km^2 covering the mainland and a number of offshore islands. Summer is hot and the temperature rises up to 51°C with very low humidity. Winter is cool and the average temperature stays around 13°C with maximum 23°C. The rainfall is minimal, averaging about 115 mm y^{-1} (fluctuates between 25 and 250 mm), but evaporation is very high, ranging from 3.1 to 21.6 mm d^{-1}. Rainfall occurs anytime between mid October and late April. Soil of Kuwait is mostly sandy in texture, has a high infiltration rate and is calcareous in nature (Omar et al. 2000). Underground water resources are limited and brackish in nature with total dissolved solids (TDS) concentrations ranging from 3.0 to 10.0 g L^{-1}.

Seed Collection

Helianthemum lippii seeds were handpicked from natural rangeland population in Sulaibiya, (N 29° 8' 42.3"; E 47° 40' 59.2") southern part of Kuwait (Fig. 3) during April 2004. The fully dried seeds were collected, manually cleaned, and stored at room temperature (22±3°C and 30-40% RH). After 4 months under laboratory conditions the germination rates were determined.

Seed Treatments

Germination Conditions

Germination experiments were conducted in 9cm diameter disposable petri dishes lined with Whatmann filter paper. The filter paper was moistened with distilled water. Four replicates of 25 seeds were used for each treatment. Germination was recorded everyday and the seeds were considered germinated when the radicle protruded to the length of 2mm.

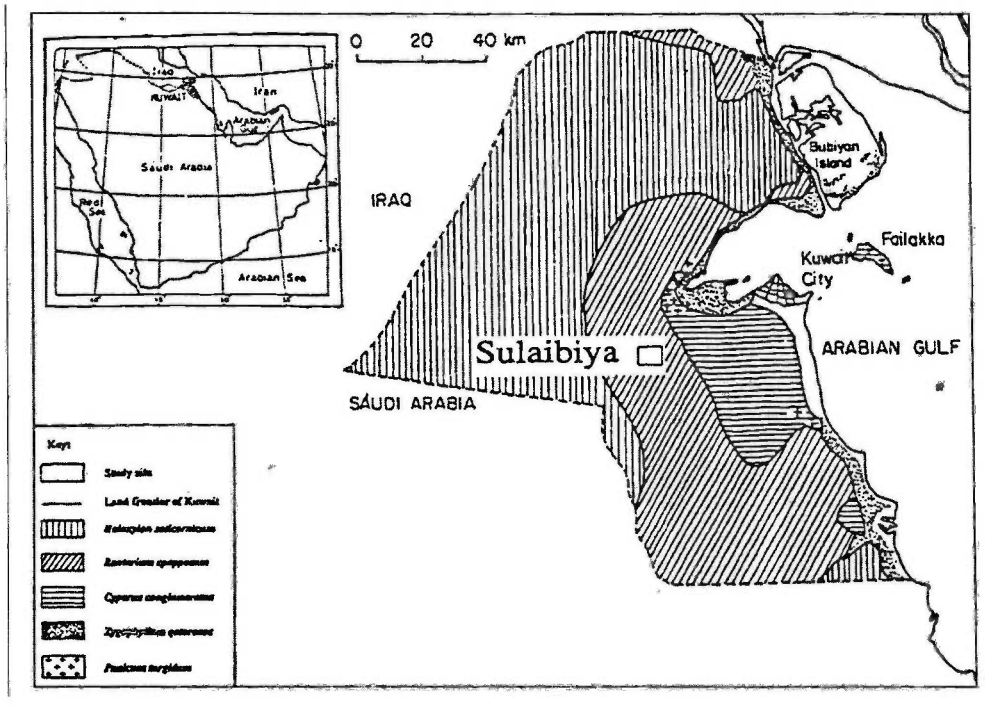

Figure 3. Location of Kuwait, the seed collection site and the major types of vegetation in Kuwait

Control – Intact seeds receiving no treatment
Scarification – Intact seeds were scarified using sandpaper.
Temperature and light – Intact seeds and scarified seeds were exposed to different combinations of temperature (10, 15, 20, 25, 30, and 35°C), light and dark. Darkness was obtained by wrapping the petri dishes with 2 layers of aluminium foil.
Heat and moisture stratification – Intact seeds were exposed to periods of time at 50°C and different moisture levels at (4°C).
KNO_3 – Intact seeds were soaked in different concentrations of potassium nitrate.
H_3BO_3 – Intact seeds were soaked in different concentrations of Boric acid.
GA_3 – Scarified seeds were soaked in different concentrations of Gibberellic acid.

Statistical analysis

The data analysis was conducted by using R 2.2.1 statistical program. The standard error of mean and the level of significance were obtained by analysis of variance (ANOVA). All reported values were used to determine if the difference were significant among treatments.

Results

Effect of Scarification, Light and Temperature

In *Helianthemum lippii* light did not influence seed germination and did not present significant interactions with any of the other variance factors, temperature or pre germinative treatments (Fig. 4). Temperature significantly affected seed germination ($P<0.001$). Increase in temperature (35°C) decreased the germination of control and mechanically scarified seeds. At 35°C, irrespective of light conditions seed germination of intact seeds reached its lowest values (>3%). Scarification significantly promoted germination ($P<0.001$) and the seeds germinated faster within 24 hours and reached the maximum germination. The final germination percentage of the control seeds (without any pretreatment) ranged from 1-13%. In intact seeds no significant difference in germination was found among the light and dark treatment. However the incubation temperatures significantly affected the final germination of scarified and intact seeds.

Effect of Heat and Cold Stratification treatment

Heat and cold stratification treatments had no significant effect on germination (Fig. 5). When comparing the germination percentages of control with 75 days heat stratified seeds there was no significant difference ($P = 0.49$). Germination of 10, 20, 30, 40, 60, 100 and 160 days heat stratified seeds were significantly lower than the control (10 days ($P = 0.78$), 20 days ($P = 0.58$), 30 days ($P = 0.77$), 40 days ($P = 0.12$), 60 days ($P = 0.57$), 100 days ($P = 0.56$) and 160 day ($P = 0.79$). Similarly cold stratification at 5°C for 10, 20, 30, 50, 75, 100, 135, 150 days did not improve seed germination percentages ($P = 0.53$, $P = 0.17$, $P = 0.72$, $P = 1$, $P = 0.26$, $P = 0.16$, $P = 0.73$ and $P = 0.69$).

Effect of H_3BO_3 and KNO_3 treatment

After 24 hours soaking, percentages of seed germination at different concentration of H_3BO_3 were summarized (Fig. 6). When comparing the germination of the control (without any treatment) with germination of 0.02, 0.04, 0.06, 0.08, 0.1 and 0.2% boric acid treatment there were no significant differences (0.02 ($P=1$), 0.04 ($P=0.52$), 0.06 ($P=0.74$), 0.08 ($P=0.72$). In potassium nitrate treatment (Fig. 4) average germination percentage of 10% in 0.2% KNO_3 ($P<0.05$) was higher than the 0.1, 0.3, 0.4 and 0.5 % KNO_3 ($P = 1$). (Fig. 7)

Effect of Scarification and GA_3

The germination experiment in *H.lippii* revealed that the manual scarification was the most effective treatment to promote germination ($P<0.001$) and 90 - 100% of germination was achieved after 1 day. While in the same time the corresponding percentages for all other GA_3 concentration immersed seeds ranged between 51% and 93%. Increasing GA_3 concentration significantly reduced ($P<0.01$) the germination percentage (Fig. 8).

Discussion

The effects of pretreatments on seed germination are given in Fig. 4, 5, 6, 7, and 8 respectively. In *Helianthemum lippii* seeds the highest germination was obtained in manual scarification. Similar results were reported in *H. almeriense, H. appeninum, H. cinereum, H. hirtum* and *H. squamatum* (Perez-Garcia and Gonzalez-Benito, 2006). Light and dark had no significant effect on germination percentage. However the incubation temperatures significantly affected the germination percentage of scarified and unscarified seeds. Seeds of many shrubs germinate equally well in light and darkness and those of some species germinate to higher percentage in darkness than in light (Baskin and Baskin 1998). The complete ineffectiveness of light on Cistaceae seed germination could be explained (Thanos and Georghiou 1988) by either a very low Pfr threshold level for phytochrome action (satisfied even under Far-Red light) or an inconspicuous level of phytochrome action (minimized by evolution). The lowest germination percentage of scarified seeds was obtained at the highest constant temperature (35°C). Agami and Gutterman (1987) observed similar result in *H. vesicarium* and *H.ventosum*. The germination of seeds in light and dark suggesting that they can germinate if they come slightly buried in the soil where water may be available.

In *Helianthemum lippii* dry heat and moist chilling had a significantly negative effect on the germination. However some pretreatments (H_3BO_3, KNO_3) slightly increased germination percentage. In *Helianthemum lippii* heat treatment was not effective in increasing germination which agreed with results for other *Helianthemum* species. (Perez and Gonzalez 2006). Drake et al. (1998) reported only 9% of germination after weeks of cold pretreatment in *Aster curtus*. Stratification treatments were not effective in improving seed germination and this means that no physiological dormancy was involved. The boric acid treatment applied to the *Helianthemum lippii* seeds was not harmful to the seeds but did not significantly enhance germination compared to the control. A study of seed germination and growth of three populations of *Atriplex polycarpa* under greenhouse conditions using water cultures showed it to be very tolerant to high concentrations of boron in the growth medium. Germination was not affected by boron (Chatterton, 1969). In *Helianthemum lippii* 0.2% KNO_3 slightly increase the germination (10%). The International Seed Testing Association (1996) recommends 0.2% potassium nitrate (KNO_3) as a chemical that may break physiological dormancy in some species, including grasses (Pons 1989). Potassium nitrate is believed to penetrate the embryo and stimulate metabolic activity (Bradbeer 1988).

The germination experiments revealed that the mechanical scarification was the most effective treatment to promote germination and 90-100% of germination was achieved within 24 hours in *Helianthemum lippii*. While in the same time the corresponding percentages for all other GA_3 concentrations immersed seeds ranged between 51% and 95%. GA_3 at higher concentration (1000ppm) highly inhibited germination. GA_3 in high concentrations could be turned toxic for permeable seeds. Scarification treatment significantly improved germination of *Helianthemum lippii* seeds over water control. In the absence of GA_3, scarification yielded higher germination than controls. In previous studies seed dormancy of *Helianthemum squamatum* has been broken by mechanical scarification (Escuedero et al. 1997). The highest germination percentages were obtained with manual scarification. Mechanical scarification resulted in dramatic increases in germination when completed in less than 2 days. On the other hand, heat treatment lowered the germination rate to levels similar to those of control seeds.

In conclusion *Helianthemum lippii* seeds have low germination rate due to hardness and impermeability of the seed coat. The results of the present study showed that scarification was the most effective method in breaking the dormancy of *Helianthemum lippii* seeds used in this study. This sandpaper scarification has the advantage of requiring less time, however, small amounts of seeds must be handled during scarification. An alternative scarification method should be explored for this species for most restoration and propagation needs.

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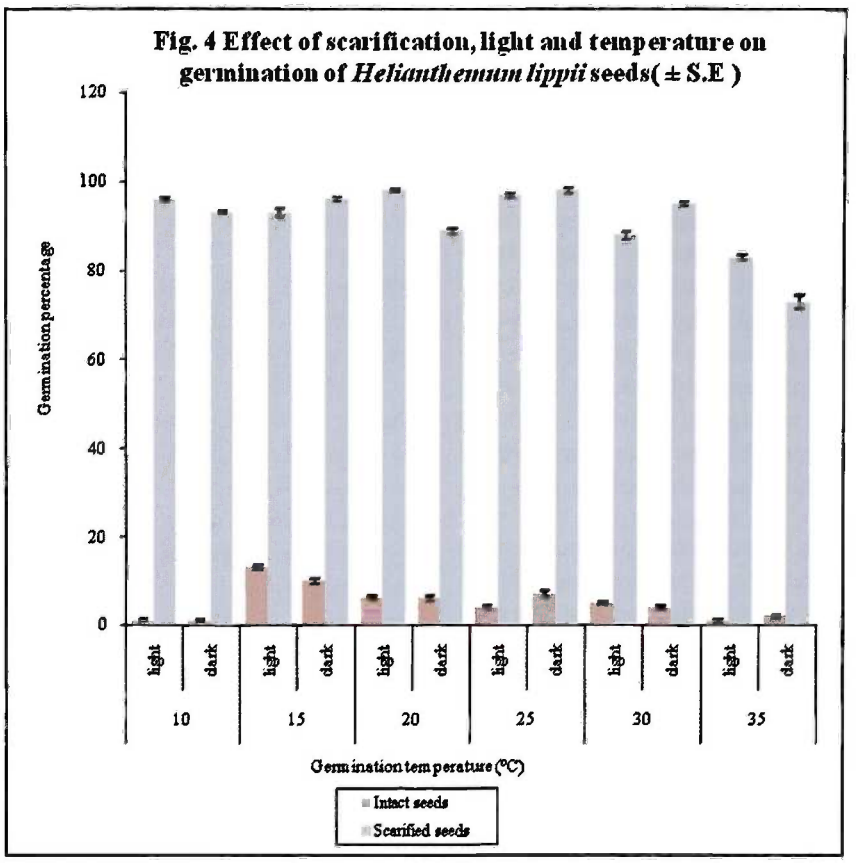
Fig. 4 Effect of scarification, light and temperature on germination of Helianthemum lippii seeds(± S.E)
Germination percentage
120
100
80
60
40
20
0
light
dark
10
15
20
25
30
35
Germination temperature (°C)
Intact seeds
Scarified seeds

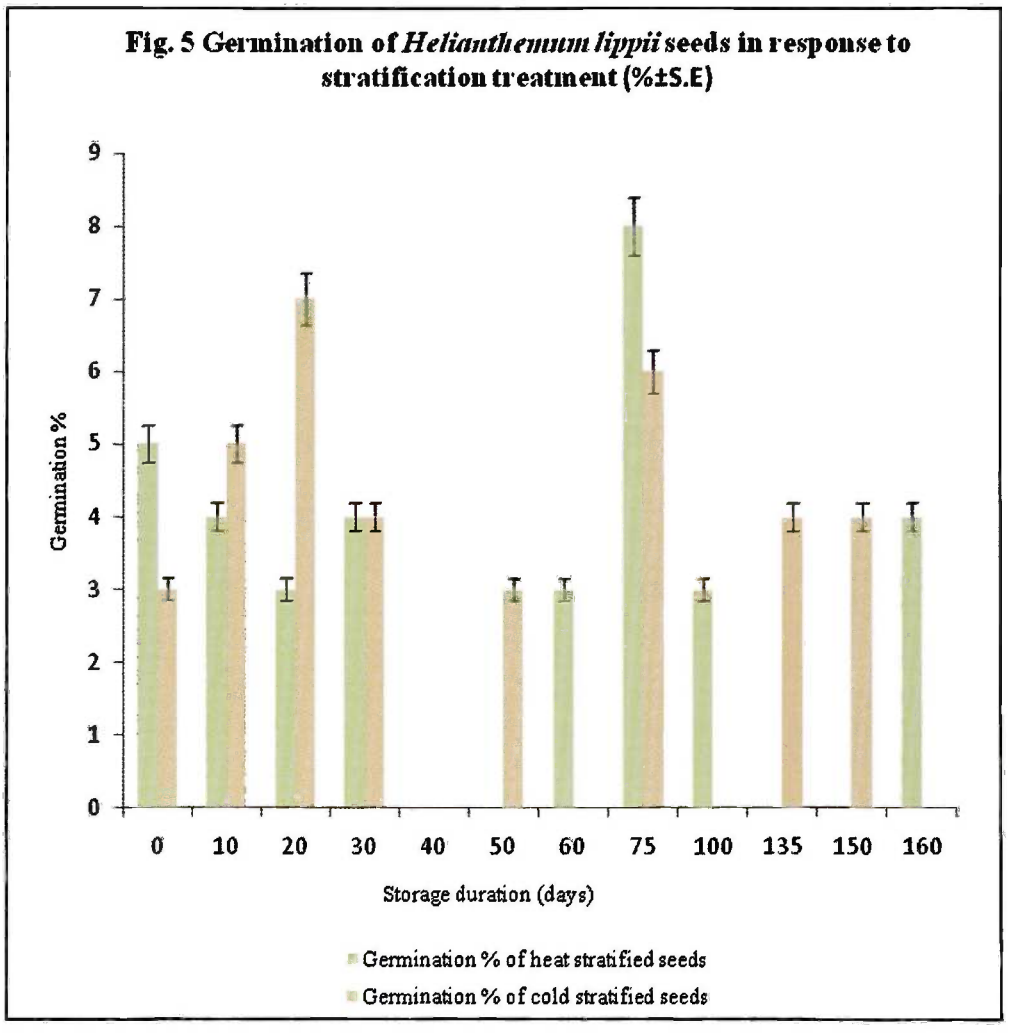
Fig. 5 Germination of Helianthemum lippii seeds in response to stratification treatment (%±S.E)
Germination %
9
8
7
6
5
4
3
2
1
0
0
10
20
30
40
50
60
75
100
135
150
160
Storage duration (days)
Germination % of heat stratified seeds
Germination % of cold stratified seeds

Fig. 6 Effect of Boric acid on *Helianthemum lippii* seed germination (%±S.E)

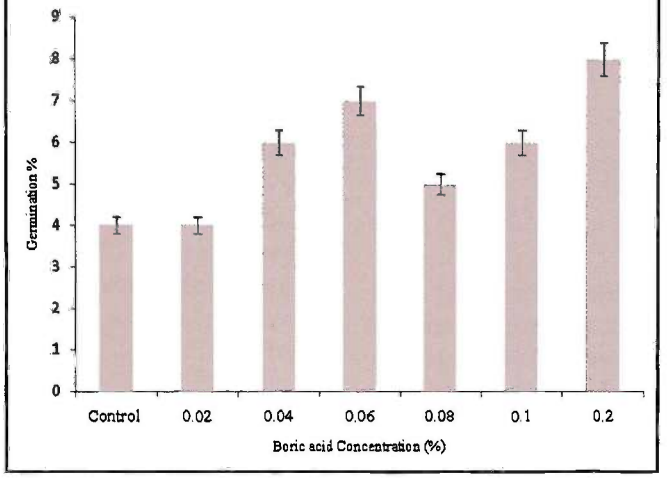

Fig. 7 Effect of Potassium nitrate on *Helianthemum lippii* seed germination (%±S.E)

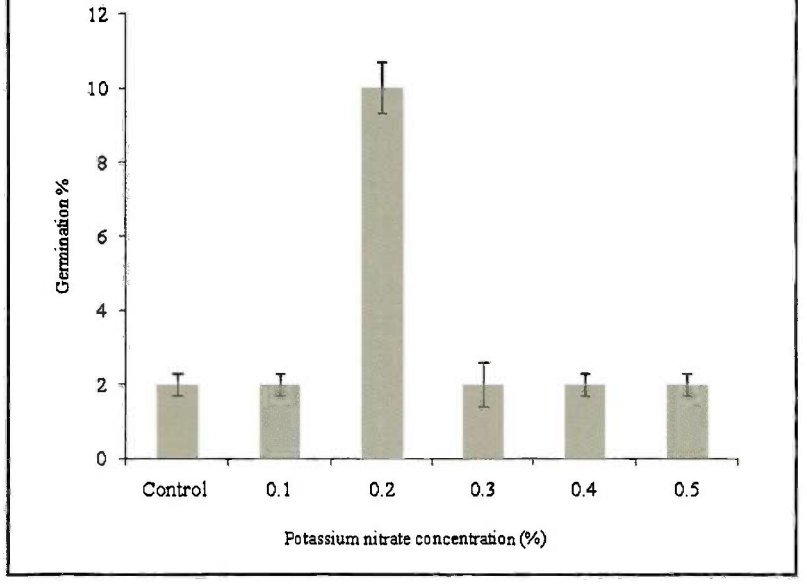

Fig. 8 Effect of scarification and GA_3 on germination of Helianthemum lippii seeds (%±S.E)