The Libyan Journal of Science (An International Journal): Volume 24, 2021

The Effect of Mechanical Scarification on Seed Germination of *Helianthemum lippii* (L.) Dum. Cours.

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Abstract

Improving germination of *Helianthemum lippii* is important for conservation efforts regarding the regeneration of the desert flora and for truffle cultivation. As with other members of Cistaceae, the species is characterized by hard-coated seeds that causes long seed dormancy. In this experiment, the effect of mechanical scarification of seeds using sandpaper, shaker or household mixer on seed germination of *Helianthemum lippii* was investigated. The results showed that manual rubbing of seeds of *H. lippii* between two pieces of sandpaper grid size 400μm 4, 8, 16, or 32 times enhanced germination rates from 19% for the control to 92-99%. Using a Shaker for 5, 10 or 15 minutes improved the germination rates upto 74-79%. Significant differences between the control and the treated seeds appeared very early and lasted throughout the experiment. The number of rubbing times or the time of exposure of the seeds to shaker had no significant effect. Using a household mixer for 1, 2, or 4 minutes increased the germination rates to 41, 99 and 90% respectively. However, stretching the time of exposure to 6 minutes gave nil germination. Mechanical scarification using sandpaper, mixer or shaker was found very effective for seed germination in *H. lippii*, but the first method was especially practical and easy to apply.

Key words: Germination; Scarification; *Helianthemum lippii*; Cistaceae.

المستخلص

زيادة معدلات إنبات نبات .L *Helianthemum lippii* يكتسب أهمية خاصة في إنجاح عمليات الحماية وإعادة تعمير الغطاء النباتي الصحراوي, بالإضافة إلى أهمية إكثاره بحثيا كعائل للكماء باعتبار علاقتهما التكافلية. كما هو الحال مع باقي الأنواع التابعة لعائلة Cistaceae يتميز هذا النوع بصلابة القشرة التي تسبب سكون البذور.

في هذه التجربة تم دراسة تأثير خدش بذور نبات *H. lippii* على معدلات إنباتها وذلك باستعمال ثلاث أليات هي ورق الصقل (Sandpaper) , هزاز(Shaker) والخلاط المنزلي (Household mixer). أظهرت النتائج أن فرك البذور بين ورقتي صقل حجم 400μm يدويا 4, 8, 16 أو 32 مرة أدى إلى تحسين نسبة الإنبات إلى 92-99% مقارنة بنسبة 19% في الشاهد. استعمال الهزاز لمدة 5, 10 أو 15 دقيقة أيضا رفع نسبة الإنبات إلى 74-79%. ظهرت فروق معنوية بين البذور المعاملة والشاهد منذ بداية التجربة واستمرت إلى نهايتها. عدد مرات فرك البذور أو زمن تعرضها للهز لم يكن لهما تأثير معنوي. استعمال الخلاط المنزلي لمدة 1, 2 أو 4 دقائق زاد نسبة الإنبات إلى 41, 99 و90% على التوالي, لكن تمديد استعماله لمدة 6 دقائق ادى الى عدم الإنبات نهائيا. استعمال اليات الخدش الثلاث كان فعال جدا في زيادة معدلات انبات بذور *H. lippii* ولكن طريقة الفرك بواسطة ورق الصقل تمتاز بكونها عملية وسهلة التطبيق.

Accepted for Publication: 2/5/2021

Introduction

Helianthemum lippii (L.) Dum. Cours. is a perennial plant adapted to the semi-arid climate dominatant in many vulnerable ecosystems world wide including North Africa and Mediterranean regions (Escudero et al., 2007). Zaman et al., (2019) reported that "*H. lippii* is confined to extreme desert climate where the mean annual rainfall is less than 70 mm". Besides the advantages of this species adaptation to these extreme conditions, the literature referred to the economical, ecological, medicinal and pastoral importance of *H. lippii* and to its pivotal role in the struggle against desertification and the stabilization of vulnerable sites (Hamza et al., 2013).

Seed germination is a critical step in the life cycle of Cistaceae members as their seed coats are impermeable to water and gas exchange (Thanos et al., 1992). Fenner (1985) and Thompson (1992) considered seed hardness as advantageous for maintaining a persistent soil seed bank, a characteristic that is highly appropriate for the viability of plant populations, especially in regions with unpredictable climate such as precipitation. Cerabolini et al., (2003) and Probert et al., (2009) added that seed dormancy of the Cistaceae family, allows them to be stored under laboratory conditions for long periods (more than five years, in some cases more than twenty years) without any loss of viability.

In nature, hard-coated seed plants utilize different mechanisms to erode seed coat and to break the dormancy of their seeds (Baskin and Baskin, 2014). Among these mechanisms are thermal shock produced during fires, alternating moisture (by dew, mist or rain) and drought and/or major soil temperature changes (Tebar et al., 1997). Also, fungal attack and rainfall wash (Robles and Castro, 2002). Another mechanism is the natural scarification is abrasion of seed coat by soil particles or by herbivores ingestion. Robles and Castro (2002) found that incubation of *H. apenninum* seeds in sheep rumen enhanced their germination from 12% to 32%. Gardener *et al*., (1993) suggested that the proteolytic, amylolytic and lipolytic enzymes that the ingested seeds are exposed to may soften their seed coat and thereby increase their germination rate, a mechanism which has been reported for some Cistaceae species (Malo and Suarez, 1996). Trabelsi et al. (2017) investigated the role of camel's alimentary canal in the regeneration of the desert flora by germinating the seeds dispersed by camel's faeces. They found that out of 712 seedlings emerged from 48 faecal samples examined, 570 seedlings belonged to Cistaceae including 316 seedlings that were *H. lippii*.

Abrasion of hard-coated seeds between two pieces of sandpaper, for example, softens the seed coat and increases water permeability and gas exchange. This kind of mechanical scarification was found to break dormancy and increase the rate of seed germination dramatically in many species of the genus *Helianthemum* like: *H. almeriense, H. appeninum, H. cinereum, H. hirtum* and *H. squamatum* (Perez-Garcia and Gonzalez-Benito, 2006). Also *H. kahiricum* (Hamza and Neffati, 2015), *H. salicifolium* (Yeşilyurt *et al*., 2017) and *H. lippii* (Zaman *et al*., 2019). Because of seed dormancy, germination of *H. lippii* is very low without treatment. Investigating germination requirements of this species could be used for conservation practices as well as to understand and develop their symbiotic relationship with truffle (Jamaila and Banihashemi, 2012; Bradai et al., 2014).

Materials and methods

Plant materials: Ripe fruits (capsules) containing mature seeds were handpicked from wild *H. lippii* population located in the Faculty of Agriculture Farm, University of Tripoli, Libya during June 2018. They were then stored in a closed glass container under lab conditions for a few months. Seeds were cleaned by removing capsule remains. The seeds were then treated as follows:

1- Control: Intact seeds receiving no treatment (1 lot).
2- Scarification using sandpaper: Intact seeds were manually scarified by gentle rubbing between two pieces of sandpaper grid size of 400 µm for 4, 8, 16 or 32 times (4 lots).
3- Scarification using household mixer: Intact seeds were placed in the container of a regular household mixer (Atlas) model MJ-2071 volume 1.5 liter. The container was then half-filled with distilled water. The mixer was then run on medium speed for 1, 2, 4 or 6 minutes. After that, the water was filtered with filter paper to separate the seeds (4 lots).
4- Scarification using shaker: Intact seeds were placed in a 1000 ml beaker containing 5 pieces of gravel size no. 4. The beaker was then half-filled with distilled water and shaked using a shaker (Flac) at the speed of 140 R.P.M. for 5, 10 or 15 minutes. The water was then filtered with filter paper to separate the seeds (3 lots).

Each of the last 12 treatments (lots) was represented by four replicates (Petri dishes) with 25 seeds each. Germination experiments were conducted in 9cm diameter disposable Petri dishes lined with Whatman filter paper moistened with distilled water. All Petri dishes were then placed in the dark (Zaman et al., 2019) in an incubator at a constant temperature of 20±0.5°C, (Thanos *et al.*, 1992). The Petri dishes were moistened regularly as needed. Seeds showing radicle emergence were recorded daily and removed from the Petri dishes. The final germination was recorded after 20 days from the start of the experiement.

Differences in germination rates among treatments were subjected to one-way analysis of variance (ANOVA) and Tukey honestly significant differences post-hoc test.

Results

Fig.1 shows that the mechanical scarification using sandpaper increased the germination rate in *Helianthemum lippii* significantly ($P<0.01$) regardless of the number of rubbing times. Germination rates increased from 19% for the control to 92, 97, 97 and 99% for seeds rubbed 04, 08, 16 and 32 times respectively. Since the first day, significant differences in germination rates appeared between the control and the seeds scarified by sandpaper for 16 or 32 times. After that and throughout the experiment, the differences between the control and any lot of the scarified seeds were significant ($P<0.01$). The differences among the scarified seeds were not significant.

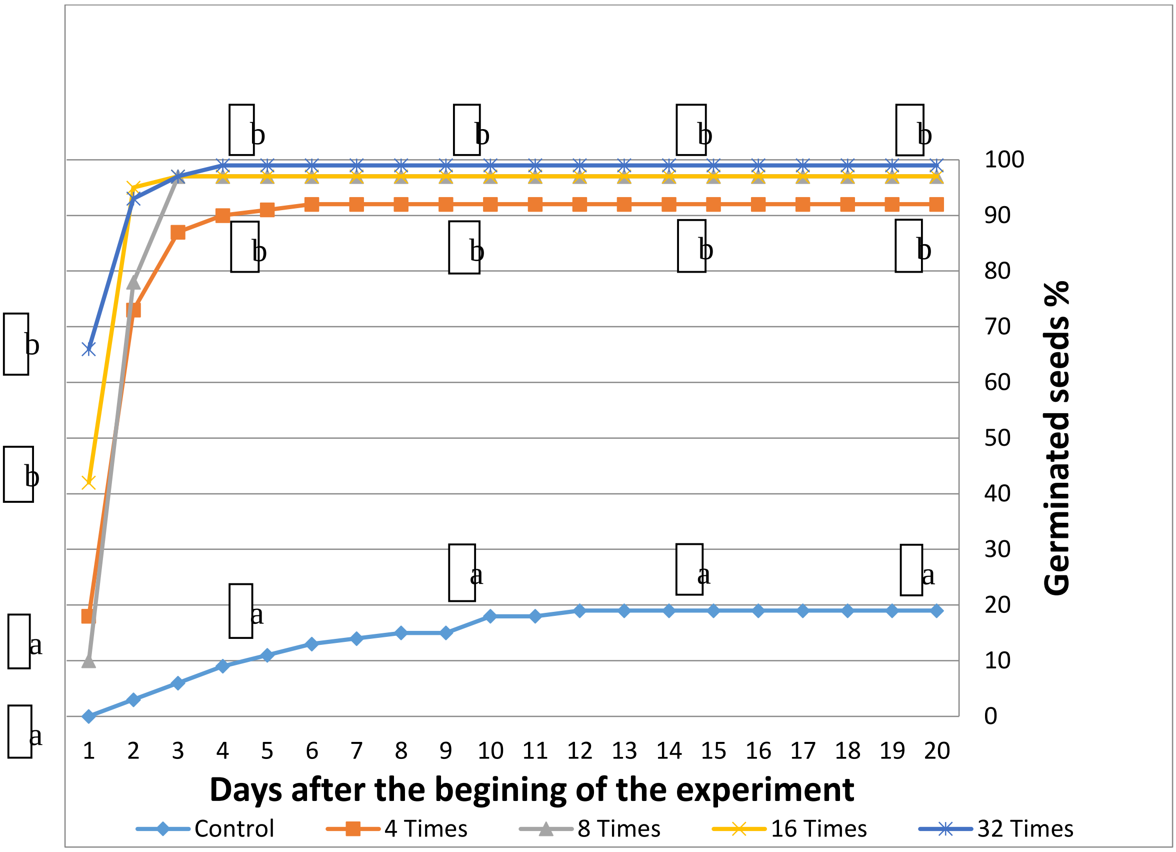

Fig. 1. The effect of mechanical scarification using sandpaper on seed germination of *Helianthemum lippii.*
*For any particular day, figures denoted by different letters are significantly different at (P<0.01).

Fig.1 reveals that mechanical scarification using sandpaper also reduced the time to reach the maximum germination rate, Which was reached on day 12 in the control compared to days 6, 3, 3 and 3 in seeds rubbed 04, 08, 16 and 32 times respectively. Mechanical scarification using the shaker increased the germination rate of *H. lippii* significantly (P<0.01) regardless of the time of exposure. Germination rates increased from 19% for the control to 74, 79 and 75% for seeds scarified by shaker for 05, 10 or 15 minutes respectively. Shaker scarification also shortened the time needed to reach the maximum rate of germination which was shorter in the seeds shaken for 15, 10 and 5 days respectively. During the first five days after treatment, the rate of germination was higher in the lots of seeds that were shaken 15, 10 and 05 minutes respectively even though the differences between treatments were not significant (Fig. 2).

Mechanical scarification using a regular household mixer had a significant effect (P<0.05) on seed germination of *H. lippii* (Fig. 3). There was an interaction between the effect and time of exposure to the mixer scarification.

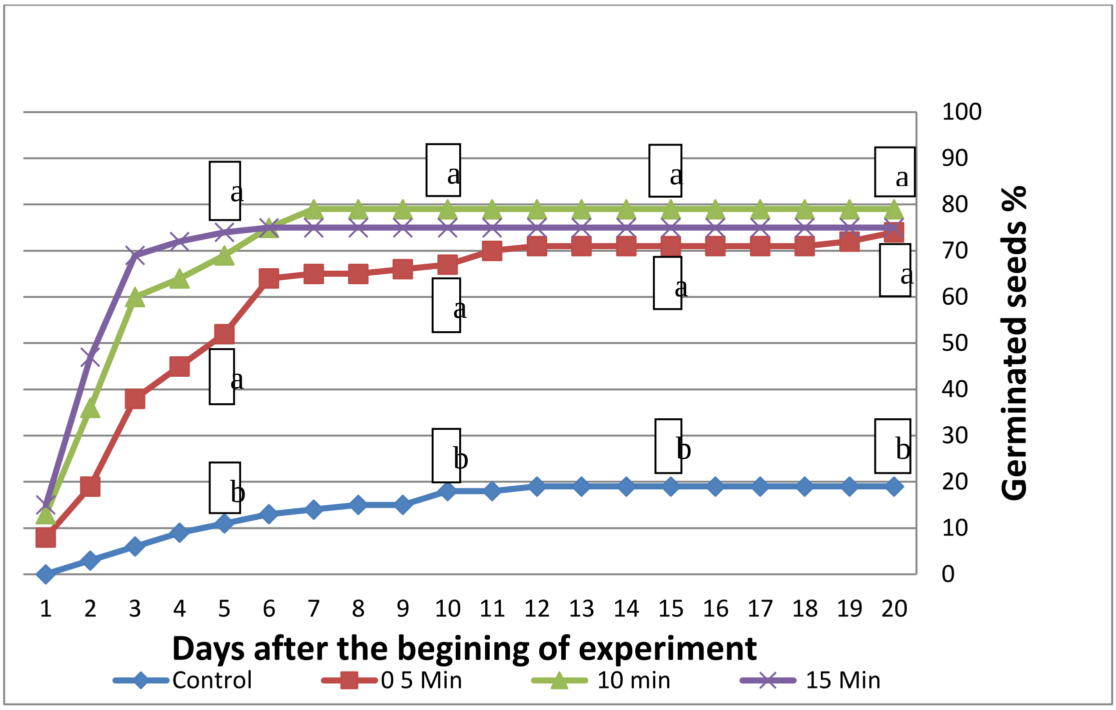

Fig. 2. The effect of mechanical scarification using shaker on seed germination of *Helianthemum lippii.*

* For any particular day, figures denoted by different letters are significantly different at $p<0.01$.

The rate of germination in the control seeds was 19%, whereas the germination rate for seeds treated with a household mixer for one minute was 41%, two minutes was 99%, four minutes was 90% while, six minutes produced nil germination. Fig. 3 also shows that mechanical scarification using a regular household mixer also reduced the time needed for the seeds to reach the maximum rate of germination which was 7, 6, and 5 days for seeds scarified with mixer for 1, 2 and 4 minutes.

Discussion

Cistaceae is characterized by hard-coated seeds. This is regarded as advantageous for plants living in inconsistent climatic zones. It also secures the germination of seeds in the right circumstances. Baskin and Baskin (2014), stated that dormancy is widespread among desert shrubs, with only a few species having non-dormant seeds. Evolutionary, these plants utilize variable mechanisms to erode their seed coats and overcome seed dormancy. Mechanical scarification is a way to mimic some of these natural processes. In this experiment 3 ways of seed mechanical scarification were investigated; manual sandpaper, shaker and house hold mixer. They all enhanced the germination of *H. lippii* significantly. Also, shortened the time needed for the treated seeds to reach their maximum rate of germination. The results of this experiment show that the maximum seed germination of the control was 19%. Mechanical scarification improved the germination percentage in scarified seeds significantly. The percentages were 92-99% using sandpaper, 41-99% using mixer and 74-79% using shaker. The differences between the control and the scarified seeds appeared very early and were significant throughout the experiment. Very similar results are found in the literature which support the strong positive impact (reaching values approaching 100%) of manual sandpaper scarification on seed germination of *Helianthemum lippii* (Zaman et al., 2019) as well as other species in the same genus such as; *H. squamatum* (Escuedero et al. 1997), *H. apenninum* (Robles and Castro, 2002) and *H. kahiricum* (Hamza and Neffati, 2015). Time of exposure of the seeds to treatments was a limiting factor when mixer was used as a tool for seed scarification. The germination rates achieved using the mixer for 1, 2 and 4 minutes were 41, 99 and 90%. However, subjecting the seeds to 6 minutes of mixing damaged the seeds and killed their embryos resulting in nil germination. Another disadvantage of using a mixer or shaker as tools for scarification of seeds is that the seeds are very small and must be submerged in water which makes their separation from the water time consuming as compared to sandpaper method.

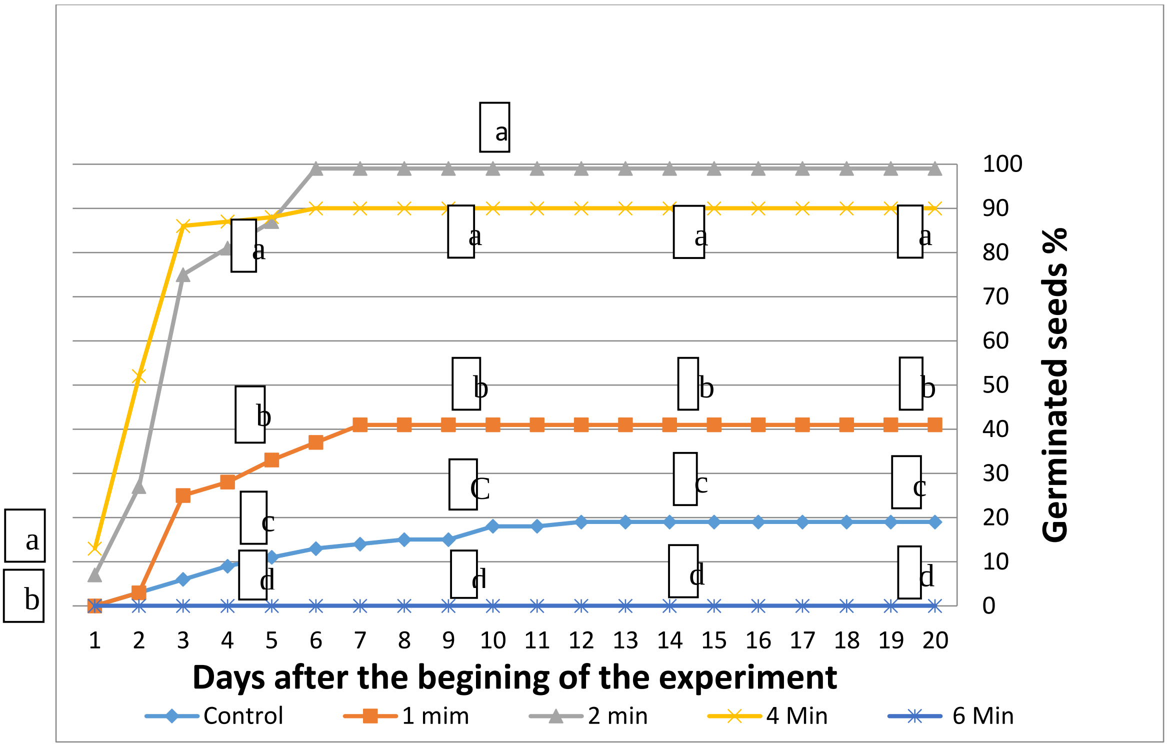

Fig. 3. The effect of mechanical scarification using regular house hold mixer on seed germination of *Helianthemum lippii*.

* For any particular day, figures denoted by different letters are significantly different at $p<0.05$.

Conclusion

Helianthemum lippii is characterized by having hard coated seeds impermeable to water and gas exchange which causes long seed dormancy. The seeds have a potentially high rate of germination (≥90%) if the coats are eroded by some mechanism. This experiment supports the findings of others that mechanical scarification is very effective, particularly the use of manual sandpaper scarification. It is extremely effective and practical.

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