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Evaluation of Germination under Different Storage Conditions of Four Endemic Plant Species from Ethiopia: Implications for Ex Situ Conservation in Seed Banks

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Abstract: The conservation of endemic plant species is a major concern, as the species are with restricted distribution range. Since in situ conservation alone will not guarantee their maintenance, ex situ conservation measures must be undertaken to support the conservation of these species. Investigation of the impact of the storage environment of seeds gives baseline information. Therefore, this study was conducted to investigate the effect of different storage conditions (room temperature, 4 °C and −10 °C) and different storage periods over one year. Four Ethiopian endemic plant species, namely *Euryops pinifolius*, *Kniphofia foliosa*, *Lobelia rhynchopetalum*, and *Solanecio gigas*, were considered. Multivariate analyses revealed a significant ($p < 0.05$) effect of storage condition and period on seed germination. The storage of seeds at room temperature before drying may not be recommended for short-term storage of the studied species, except *K. foliosa*. After drying of seeds, *E. pinifolius*, *K. foliosa*, and *L. rhynchopetalum* seeds showed high germination percentage (above 80%) after storage even at sub-zero temperatures for one year. The germination percentage of *S. gigas* stored at room temperature before drying for thirteen months was 60% and that stored at 4 °C was 6%, which indicates that the seeds may be categorized under intermediate storage behavior. The three species can be grouped in the orthodox seed storage category. Research on desiccation screening should be undertaken to predict an optimal combination of storage moisture content and suitable storage temperature to determine storage category with certainty.

Keywords: endemic species; seed germination; storage duration; storage behavior

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1. Introduction

A changing global climate threatens species and ecosystems, causing shifts in species distributions [1]. However, often barriers will provide no opportunity for distributional shifts. Species that are confined to mountaintops may not have sufficient suitable habitat left to escape climate change and, hence, may face high risks of extinction [2]. Mountains constitute centers of endemism for biodiversity, sheltering endangered species and ecosystems. However, they are amongst the most fragile environments in the world [3]. According to Chala [4], true mountaintop species stand to face local extermination with upslope range shifts.

Species extinction is an immediate concern because of its negative implications for human survival. Biodiversity is the multiplicity of life, including variation among genes, species, and functional traits in an ecosystem, and has a profound effect on the functioning of ecosystems [5]. Loss of biodiversity affects both the stability and function of the ecosystem and the resistance, i.e., the 'shock-absorbing' capacity of an ecosystem and resilience, which is the ability of an ecosystem to 'bounce back' after it has been severely disturbed [6,7].

Different studies have stressed the urgent need of developing conservation strategies. The in situ approach of conservation is at an ecosystem level and natural habitats, and it includes the maintenance and recovery of viable populations of species in their natural surroundings. The preservation of species in situ offers all the advantages of allowing natural selection to act, which cannot be recreated in ex situ conservation [8]. However, ex situ conservation methods should be applied as a backup system to avoid possible loss of genetic diversity [7].

In ex situ conservation, the genetic resources are conserved in identified genebanks. These genebanks can be storage of seeds (≈5 to −20 °C), in vitro storage of plantlets (≈4 to 25 °C), cryostorage of propagules using liquid nitrogen (≈−150 to −196 °C), or in the form of field genebank [9]. Successful ex situ conservation of wild plant species by seed banking is generally more complicated due to the inherent variability in seed longevity typical in wild species. The longevity of seeds varies from species to species even if they are provided with identical storage conditions [10]. This is because seed storage may influence seed viability and reduce seed vigor depending on the period and conditions of storage [11].

Plant life has diversified enormously since the evolution of seeds [12]. Once mature, seeds can persist in the soil seed bank, in the plant canopy (as in the case of serotiny), or in ex situ storage [13]. Seeds increase the likelihood of successful establishment by reaching more places and persisting over greater timescales [14]. The plant species distribution is related to the ability of seeds to germinate and establish plants in natural communities [15].

Seeds are classified as orthodox (desiccation-tolerant) and recalcitrant (desiccation-intolerant) according to their storage properties [16]. A large proportion of plant species produce seeds that undergo a period of desiccation before being shed from the tree [17] and also can be dried to sufficiently low levels of moisture content that permits them to be stored at low temperatures. These seeds are termed orthodox seeds [7]. The mature seeds of orthodox seeds survive desiccation to low moisture content, at least 2–6% depending on the species, and are generally easy to store if basic processing and storage facilities are available [18]. On the other hand, many species of tropical or subtropical origin have recalcitrant seeds, which are sensitive to drying and chilling and cannot be stored in conventional genebanks [19]. There is also an intermediate category between the orthodox and recalcitrant seed groups. Intermediate storage behavior implies that the seeds are shed at relatively high water concentrations, but will withstand considerable dehydration, although not to the extent tolerated by orthodox seeds [20,21]. Intermediate seeds can withstand partial dehydration, but they cannot be stored under conventional genebank conditions because they are cold-sensitive.

Therefore, the appropriate conservation methods for any taxa can only be decided after determining its seed storage behavior [18]. Moreover, the use of seeds for conservation and propagation needs prior knowledge of how soon the seeds would lose their viability. Seeds age during storage and eventually lose their ability to germinate [22]. Ageing is a process of deteriorating events that take place within the seed, which limits their viability and ultimately leads to the death of the seed. Causes of ageing may be grouped into internal (genetic, structural, and physiological) and external (mainly storage conditions: temperature and humidity) [23]. During storage, seeds are exposed to oxidative damage that affects germinability caused by reactive oxygen species (ROS) [24]. Viability testing through germination can be a rapid way of identifying problems with seed storage conditions [25]. Hence, investigation of the survival of seeds following storage in different environments has been practiced to determine seed storage behavior.

Viability tests through germination remained the most fundamental step to understanding the adaptive strategies of plants in an area. Moreover, viability tests can be used to determine effective conservation methods of specific species and can be a rapid way of identifying problems with the seed storage conditions [12,25]. Germination begins with the uptake of water by the dry seed imbibition and is completed when a part of the embryo, usually the radicle, extends to penetrate the structures that surround it (seed coat) [26]. As germination proceeds, presumably metabolism of the seed becomes engaged in those

processes vital for the emergence of the radicle [27]. Determination of how soon the seeds would lose their viability is mandatory to use seeds for propagation.

Assessment of the existing conservation methods of all plant species to establish detailed and appropriate management plans is a prerequisite. However, setting priority is very essential for the efficient allocation of scarce resources and time [28]. A list of priority species and/or habitats for conservation is developed based on the institutional remit and expertise, political priorities, and botanical information such as Red Data Lists [29,30]. Ethiopia is endowed with rich endemic species of higher plants, as there are 647 endemic species from 6027 total plant species recorded in the country [31]. Endemism is particularly high in the high mountains [32]. According to Vivero [33], montane and Afroalpine plants in Ethiopia suffer from a set of threats. Moreover, upward shifts in response to recent climate changes in mountain peaks have been reported as the major cause driving a species to face a very high risk of extinction following climate warming [4], hence the required immediate implementation of conservation measures.

Endemic species have long been targets for conservation efforts because they are not found anywhere else in the world, and if lost from their native habitat, they will be lost forever. In Ethiopia, studies have shown that there are endemic plant species which are threatened due to direct exploitation and climatic conditions. For example, *Lobelia rhynchopetalum* faces high risk of extinction following climate warming [4], and *Echinops kebericho* has been registered under the National Red List as vulnerable [34]. There is, thus, an urgent need to develop and implement conservation strategies, such as ex situ conservation through the collection and preservation of seeds of endemic species in seed banks to complement in situ conservation.

Undoubtedly, there is scarce information available on how long seeds can be stored and remain viable under a given set of conditions for Ethiopian endemic species. Therefore, the main objective of this study was to assess the impact of storage duration and storage environment on the germination of seeds for four endemic plant species of Ethiopia. Botanical description, geographical distribution, family, conservation status, and uses of the species are presented in Table 1.

2. Material and Methods

2.1. Study Site

Seed collection was conducted in the Choke Mountain Range. The Choke Mountain Range is located in East Gojjam Zone in the Amhara National Regional State, Northwestern Ethiopia, approximately 332 km northwest of Addis Ababa. The mountain range extends from 10°34′ E to 10°46′ N and 37°47′ to 38°01′ E, and the highest peak is located at 10°42′ N and 37°50′ E. Choke Mountain is the water tower of the region, serving as headwater of the Upper Blue Nile basin where a total of 59 rivers and many springs are identified to originate from this mountain range [35]. The area is endowed with beautiful views (Figure 1).

The mountain range is found within six climatic zones, where the peak of Choke Mountain is characterized as Wurch, which is above 3200 m altitude with a cold and moist climate and less than 11.5 °C average annual temperature [36]. The seeds of the three endemic species *Euryops pinifolius*, *Kniphofia foliosa*, and *Lobelia rhynchopetalum* were collected from the peak of Choke Mountain, while one species, namely *Solanecio gigas*, was collected from a forest patch in the mountain range located 45 km from Debremarkos (Table 1 and Figure 2).

2.2. Study Species Description and Seed Collection

A reconnaissance survey was conducted in December 2018 across the mountain range to obtain an impression of the site conditions (Figure 1) and asses the plant phenological stage for further seed collection plans. The two studied species, *E. pinifolius* (Figure 3) and *K. foliosa* (Figure 4) flower in November. In the case of *L. rhynchopetalum* (Figure 5), flowering individuals were observed throughout the year, as several trips were made during the study. Also, *S. gigas* (Figure 6), flowers in November.

Figure 1. Different views of Choke Mountain. Source: photo by Sinework Dagnachew.

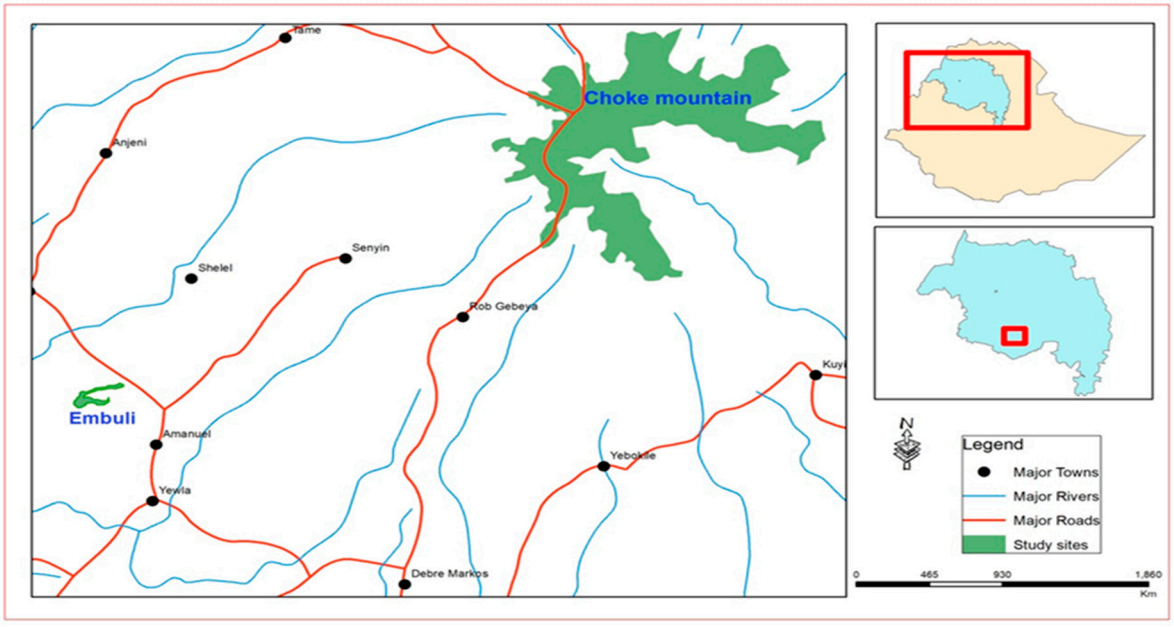

Figure 2. Map of seed collection sites in the Choke mountain range.

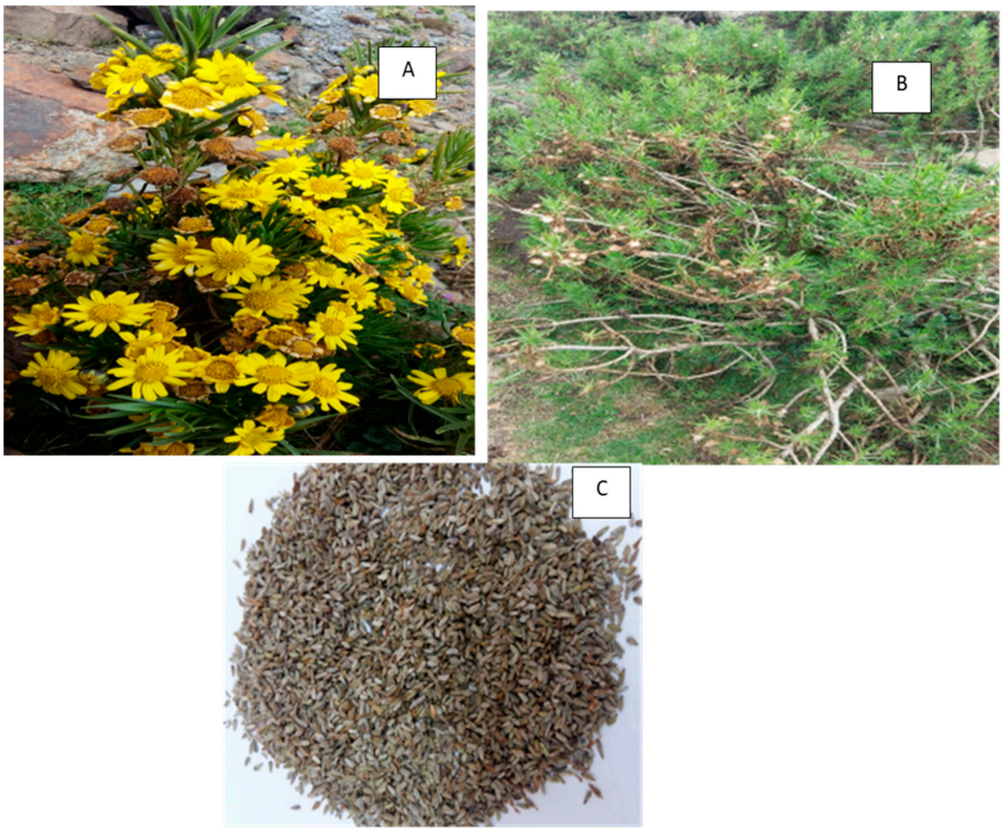

Figure 3. *Euryops pinifolius*: (**A**) flowering stage (November 2018), (**B**) fruit dispersing stage (December 2018), and (**C**) seeds. Source: photo by Sinework Dagnachew.

Figure 4. *Kniphofia foliosa*: (**A**) flowering stage (March 2018), (**B**) fruit dispersing stage (March 2019), and (**C**) seeds. Source: photo by Sinework Dagnachew.

Figure 5. *Lobelia rhynchopetalum*: (**A**) vegetative, (**B**) fruiting stage (March 2018), (**C**) capsule, and (**D**) seeds. Source: Photo by Sinework Dagnachew.

Figure 6. *Solanecio gigas*: (**A**) flowering stage (December 2018), (**B**) dispersal stage (January 2019), and (**C**) seeds. Source: photo by Sinework Dagnachew.

Mature seeds were hand-collected before they had fallen to the ground to avoid the risk of contamination and to obtain healthy seeds. Seeds of each species were collected randomly from more than 20 individuals to maintain the heterogeneity of the population of the

samples. During transportation, the samples were placed in cotton bags and kept ventilated in ambient conditions to avoid suffocation and decay. The samples were transported to the forest seed laboratory at the Ethiopian Biodiversity Institute, Addis Ababa. Seeds were cleaned and air-dried under shade (around 20 °C) immediately upon arrival until their temperature reached equilibrium with the surrounding air. Herbarium specimens of the studied species were collected, pressed, and identified in the National Herbarium of Addis Ababa University, Ethiopia. Plant species nomenclature follows that used in the published volumes of the *Flora of Ethiopia and Eritrea* [37–39]. The date of seed collections, altitude, geographical coordinates of seed collection sites, and final moisture content during storage for germination trials are presented in Table 1.

2.3. Seed Storage

In the laboratory, cleaned seeds of four species were randomly divided into two groups each. Seeds in the first group were categorized as fresh and the seeds in the second group were placed in a drying room to lower their moisture contents. The first group of seeds representing fresh seeds was further divided into two, where one subgroup was stored at room temperature (15 to 21 °C) and the other in a cold room adjusted to 4 °C. The subgroups were kept in paper bags closed with stapled pins after double folding. The seeds were stored for 3, 6, 9, and 13 months of storage for the subsequent germination test. For *L. rynchopetalum*, seeds were stored only at room temperature due to scarcity of seeds.

The second group of seeds was placed in a drying room set at 15 °C and 15% relative humidity for three weeks. The moisture content of seeds was determined gravimetrically with 5 g of three replications using the low-constant-temperature oven method (103 ± 2 °C for 17 ± 1 h) following the method recommended by Rao et al. [40]. After drying, seeds were tested for initial germination. The remaining seeds were stored at two different temperatures set at 4 °C and −10 °C with sealed aluminum foil bags. The seeds were stored for 3, 6, 9, and 12 months of storage for the subsequent germination test.

Table 1. List of endemic plants studied with their families, description, geographical distribution with altitudinal ranges in Ethiopia, month, altitude, geographical coordinates of seed collection sites, uses, and conservation status.

S No	Species	Local Name	Family	Altitudinal Range and Brief Botanical Description	Geographical Distribution throughout the Country	Place, Month, Year, and Altitude of Collection (Geographical Coordinates)	Seed Moisture Content (%) during Storage	Local Uses	Conservation Status *
1	*E. pinifolius* A. Rich.	Gimiy	Asteraceae	Alt 3200–3700 m.Shrub or shrublet, 30–100 cm high. Branches leafy in upper parts, leafless below with prominent leaf scars.	GJ, WU, and SU	Choke; January–February 2019; 3920 m (10°41′47.8″ N × 37°50′14.5″ E)	2.8	Used to treat stomachache. (*kurba*)	VU
2	*K. foliosa* Hochst.	Ashengdiy	Asphodelaceae	Alt 2500–4000 m.Robust plants forming dense clumps, with thick erect rhizomes, sometimes with a stem up to 40 cm high.	TU, GD GJ, WU, SU, AR, BA, and HA	Choke; March 2019; 3757 m (10°44′01.2″ N × 37°47′05.5″ E)	2.8	Used for the treatment of abdominal cramps and for wound healing, inhibit the growth of malaria parasite.	unknown

Table 1. *Cont.*

S No	Species	Local Name	Family	Altitudinal Range and Brief Botanical Description	Geographical Distribution throughout the Country	Place, Month, Year, and Altitude of Collection (Geographical Coordinates)	Seed Moisture Content (%) during Storage	Local Uses	Conservation Status *
3	*L. rhynchopetalum* Hemsl.	Gibira	Lobeliaceae	Alt 3000–4350 m.Plant up to 7 m high when in flower; stem erect, more or less woody towards the base.	GD, GJ, SU, AR, BA, and HA	Choke; March 2019; 3906 m (10°39′47.8″ N × 037°69′34.2″ E)	3.5	The hollow woody stems serve as long trumpets for shepherd boys, used to treat evil eye.	High riskof extinction
4	*S. gigas* (Vatke) C. Jeffrey	Boz	Asteraceae	Alt 1750–3350 m.Shrub or small tree, 1.75–6 m high. Stems and branches leafless below, but with a leafy crown near apex.	GD, GJ, WU, SU, AR, SD, IL, KF, BA, and HA	Embuli; January 2019; 2302 m(10°29′19.7″ N × 037°31′17.5″ E)	2.9	Used to treat dysentery and bloating, juice of leaves used as internal medicine for cattle and hedge plant around small home gardens.	LC

AR = Arsi, BA = Bale, GD = Gonder, GJ = Gojjam, HA = Harerge, IL = Illubabor, KF = Keffa, SD = Sidamo, SU = Shewa Upland, TU = Tigray Upland, WU = Welo Upland. * LC = least concern; VU = vulnerable. Sources: [4,41–44].

2.4. Germination Tests

Four replicates of 25 seeds per treatment were allowed to germinate before the storage of seeds and after each storage interval to determine the germination response of seeds of different ages. Germination tests were conducted at room temperature maintained at 15 to 21 °C with 12 h day and 12 h night. The seeds were periodically tested for their germination percentage at the three-month interval. In all the germination tests, seeds were placed in petri dishes, on filter paper, which was kept moist with distilled water. Seeds were considered to have germinated when the radicle penetrated the seed coat. Germinating seeds were counted every week and discarded. Tested seeds were incubated for up to 60 days in which all experiments continued until no seed germinated for two weeks. The number of germinated seeds was converted to germination percentages.

2.5. Statistical Analyses

The percentage data from the different germination tests were first arcsine transformed before being subjected to two-way ANOVA to meet the requirement of normality [45]. The effects of storage temperatures and durations on the germination of seeds were evaluated separately using a generalized linear model (GZLM) for multiple comparisons between treatments. The adjusted p values of $p < 0.05$ using the Bonferroni–Holm method were considered significant. Tukey's Honestly Significant Difference (HST) Test was used to compare significant differences between treatment means.

3. Results

3.1. Germination Test before Drying

3.1.1. *Euryops pinifolius*

Storage temperatures and durations had major effects on the germination of seeds, and the two-way ANOVA revealed that the percentage of germination was significantly different ($F_{(4,30)} = 7.509$, $p = 0.0001$). In the first three months of storage, there was no significant reduction in the germination percentage of seeds stored both at room temperature and 4 °C (Figure 7a). After six months, seeds stored at room temperature showed a significant reduction in germination percentage. Initially, seeds of *E. pinifolius* exhibited a germination of 91%. The seeds showed no dormancy and, hence, germinated easily at harvest. Seeds

stored at room temperature resulted in a reduction in germination percentage to 59% after thirteen months of storage. However, seeds stored at 4 °C exhibited no reduction in germination percentage (89%) compared with seeds stored at room temperature (Figure 7a).

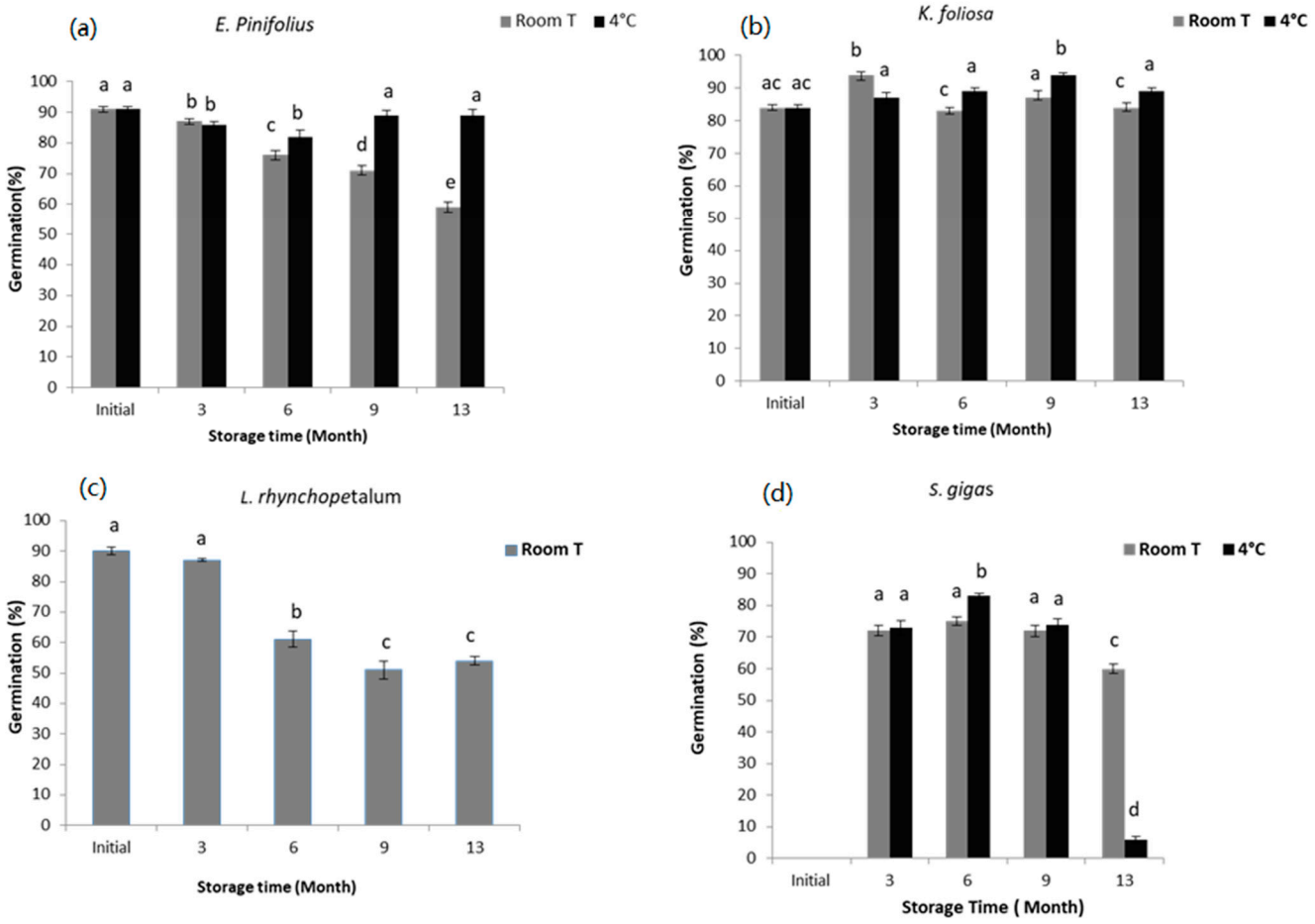

Figure 7. Effects of storage time and temperature on germination of seeds of (**a**) *E. pinifolius*, (**b**) *K. foliosa*, (**c**) *L. rhynchopetalum* and (**d**) *S.gags* before seed drying (vertical bars indicate the standard deviation). Error Bars marked with different letters indicate statistically significant difference ($p < 0.05$) according to Tukey test.

3.1.2. *Kniphofia foliosa*

A two-way ANOVA revealed that the germination percentages were not affected by either storage duration or storage temperature ($F_{(4,30)} = 1.698$, $p = 0.177$). However, a slightly higher reduction in germination percentage was observed after thirteen months of storage for seeds stored at room temperature (Figure 7b). In both storage conditions, seeds showed no reduction in percent germination after thirteen months of storage.

3.1.3. *Lobelia rhynchopetalum*

In this species, the germination test was undertaken for only one storage condition, which is room temperature. The percentage of germination was reduced from 90% initially to 54% after thirteen months of storage. However, the reduction in germination percentage was small in the first three months (Figure 7c). A significant fall in seed germination percentage was observed after three months, where a 61% germination was recorded after 6 months of storage. One-way ANOVA revealed a significant reduction in germination percentage with the increasing storage duration ($F_{(4,15)} = 23.603$, $p = 0.0001$). Progressive and significant reductions in the percentage of seed germination were observed with increasing duration of the storage.

3.1.4. *Solanecio gigas*

The initial germination of *S. gigas* was zero. After three months of storage, the germination percentages recorded were 72% and 73% for seeds stored at room temperature and 4 °C in the cold room, respectively. The percentage showed a slight increase up to 6 months in both storage conditions, where 75% germination was recorded at room temperature and 83% at 4 °C cold rooms (Figure 7d). However, a significant difference in germination percentage between the two temperature conditions was observed after nine months of storage. The percentage germination of seeds stored at 4 °C in a cold room showed an abrupt reduction. The lowest germination percent (6%) was recorded for seeds stored at 4 °C, while seeds stored at room temperature recorded 60% after thirteen months of storage (Figure 7d). The two-way ANOVA revealed a significant change in germination percentage ($F_{(4,30)} = 42.072$, $p = 0.0001$) during the storage period.

3.2. Germination Test after Drying

3.2.1. *Euryops pinifolius*

In the initial test before the storage of seeds, the germination percentage was 95%. Fortunately, in both storage conditions, 4 °C and −10 °C, seeds showed no reduction in percent germination after 12 months, which were recorded as 89% and 90%, respectively (Figure 8a). A two-way ANOVA also revealed that there was no statistically significant germination reduction ($F_{(4,30)} = 2.190$, $p = 0.094$) during the storage time.

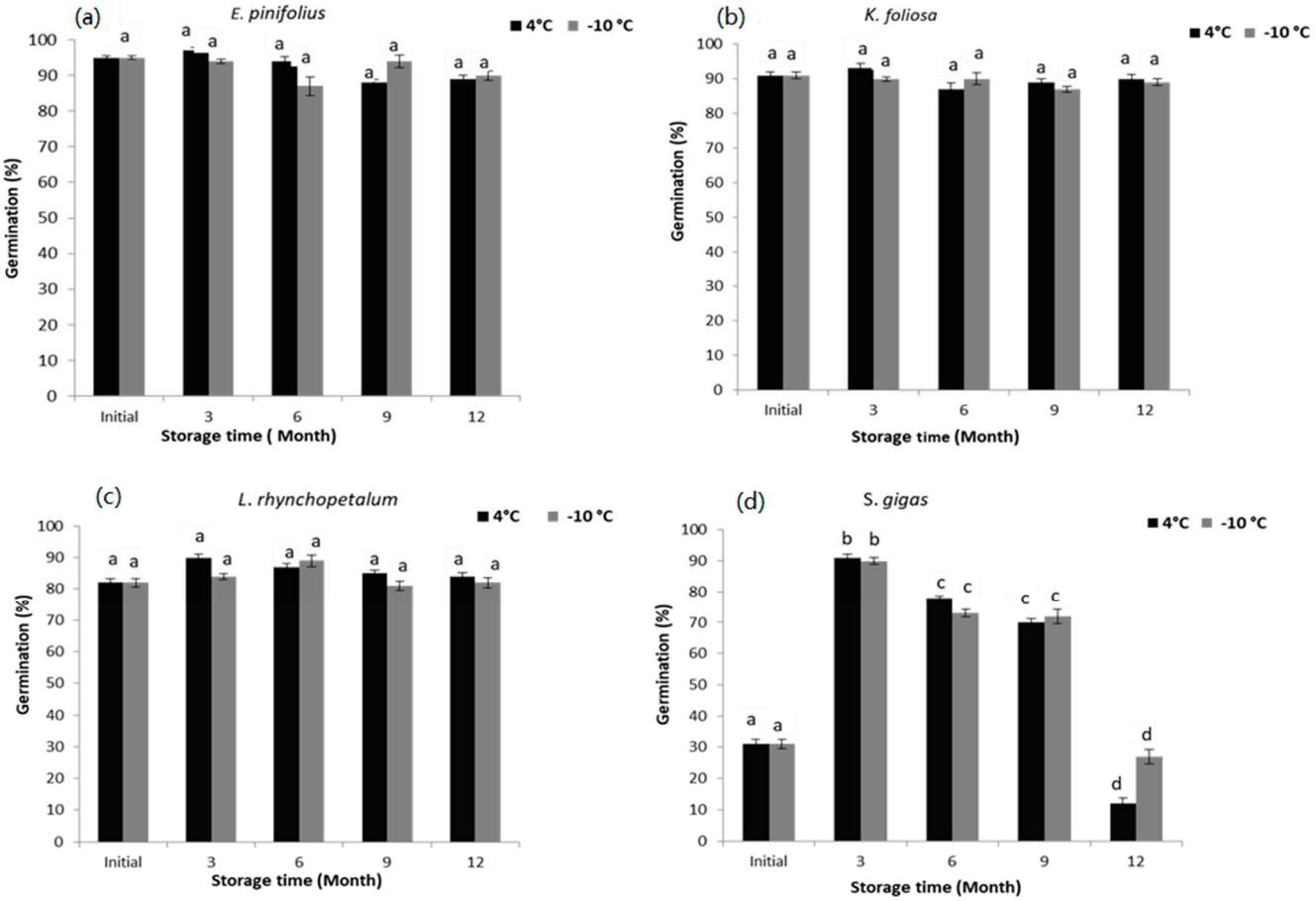

Figure 8. Effects of storage time and temperature on germination of seeds of (**a**) *E. pinifolius*, (**b**) *K. foliosa*, (**c**) *L.rhynchopetalum* and (**d**) *S.gags* after seed drying (vertical bars indicate the standard deviation). Error bars marked with different letters indicate statistically significant difference ($p < 0.05$) according to Tukey test.

3.2.2. *Kniphofia foliosa*

The seed germination percentage of *K. foliosa* was high in both storage environments throughout the storage time. When tested initially immediately after drying, the germination percentage was 91%. After twelve months of storage, the percentage of germination was recorded as 90% for seeds stored at 4 °C and as 89% for seeds stored at −10 °C (Figure 8b). A two-way ANOVA showed that the germination percentage was not significantly affected by the duration and the two storage temperatures ($F_{(4,30)} = 0.736$, $p = 0.575$).

3.2.3. *Lobelia rhynchopetalum*

In both storage conditions, seeds showed no significant reduction in the percentage of germination after twelve months of storage. The germination percentage showed a slight increase from an initial record at three months of storage time for seeds stored at both storage conditions (Figure 8c). A two-way ANOVA ($F_{(4,30)} = 0.847$, $p = 0.505$) revealed that the overall germination percentage did not show significant change during the storage time.

3.2.4. *Solanecio gigas*

After drying, the seed germination percentage was 31%. The germination percentage increased for seeds stored for three months in both storage conditions, recorded as 91% for seeds stored at 4 °C and 90% for seeds stored at −10 °C (Figure 8d). The percentage showed a slight decline after three months in both storage conditions. However, after nine months of storage, the percentage germination of seeds stored at 4 °C showed an abrupt reduction, with a noticeable difference in germination percentage between the two temperature storage conditions (Figure 8d). Hence, after twelve months of storage of seeds, a low seed germination percentage was recorded (12%) for seeds stored at 4 °C, while seeds stored at −10 °C showed 27%. In general, the results from the ANOVA revealed ($F_{(4,30)} = 3.442$, $p = 0.020$) that the *S. gigas* germination percentage showed significant change during the storage period.

4. Discussion

4.1. Germination before Drying

The present study indicated that, after various storage durations, germination percentages of seeds varied considerably among species between the storage environments. The seed germination percentage of *E. pinifolius* stored at both 4 °C and room temperature showed a reduction with increasing storage duration. However, comparing the two storage environments, seeds stored at room temperature showed a relatively significant reduction in germination percentage than seeds stored at 4 °C. Likewise, *L. rhynchopetalum* seeds stored at room temperature showed a significant reduction in germination percentage with increasing duration of the storage time. This finding is supported by the justification that the relatively high storage temperature might have accelerated seed deterioration by the action of microflora and/or heating effect that eventually killed the seeds [46]. Other authors also reported the rapid loss of seed viability for seeds stored at room temperature. Hence, the storage of seeds at room temperature is an inappropriate condition for storing seeds for a longer period [47,48].

In our present study, *K. foliosa* seeds showed no reduction in the percentage of germination after thirteen months of storage at 4°C and room temperature. In addition, seeds stored at 4 °C showed a slight increment in germination percentage with an increase in duration. Increasing the percentage of germination after cold storage may be due to the loss of dormancy during storage. This finding is supported by results reported by Wang et al. [49], who reported an increase in seed germination with increasing storage duration of the seed of some subalpine species due to a dormancy break during cold, dry storage.

Different authors confirmed that storage temperature/condition significantly affects the seed germination of different plant species during storage [48]. This difference in germination percentages between species may reflect significant differences in the storability of

individual species [50]. Although controlled laboratory conditions differ from those found under field conditions [51], it is of practical interest to determine how storage conditions affect germination for ecological restoration and germplasm conservation [52]. This result can help for suggesting the suitability of species for short-term storage for most studied species. Hence, for *K. foliosa*, both room temperature and 4 °C can be effective storage environments for short-term storage.

Findings showed that room temperature affects viability [53]. The reduction in germination percentage for seeds stored at room temperature was also observed for *S. gigas*. In the case of *S. gigas* seeds, the initial germination was 'zero', while after three months of storage, the percentage of germination was improved. The failure of fresh seeds to germinate may be because of morphological dormancy, which needs after-ripening to increase the maturation of the embryo. According to Fenner [54], in morphological dormancy, the seed is immature when shed and a period of maturity is required before germination can take place. It has also been reported that for some species development of the embryo continues after the seed has been dispersed from the mother plant [55]. The seeds stored at room temperature sustain a relatively high percentage of germination, while an abrupt reduction in germination was observed for seeds stored at 4 °C. According to the current findings, the seeds of *S. gigas* showed intermediate storage characteristics. Intermediate seeds die rapidly when the temperature is lowered [18]. The result revealed that seed storage at 4 °C is not applicable for short-term storage of *S. gigas.*

4.2. Germination after Drying

For numerous reasons, the immediate use of fresh seeds in restoration practices may not be feasible. Thus, storage of seeds for several years may be necessary. One of the main factors that greatly affect the storage of seeds includes the initial moisture content of seeds when stored. Reducing moisture content and storing seeds under specific temperatures and moisture conditions can provide valuable information to predict the storability of seeds for later uses. The present study indicated that lowering the moisture content of seeds in a drying room can retain seed viability for a comparatively longer period. It has been shown that for seeds dried in the drying room, storage duration did not show much variation in percent germination in most studied species stored both at 4 °C and −10 °C. The three studied species namely *E. pinifolius*, *K. foliosa*, and *L. rhynchopetalum* showed no reduction in germination percentage during storage duration at 4 °C and −10 °C for twelve months. Comparing the two storage conditions, the germination percentage did not show any variation in both temperature regimes. Various authors have indicated that storage at low temperatures and seed moisture content generally maintains seed viability [18,56].

Responses to desiccation and storage at different temperatures can be used to assess the storage behavior of species [18]. From the current findings, the seeds of *E. pinifolius*, *K. foliosa*, and *L. rhynchopetalum* can be categorized as orthodox since the seeds tolerated considerable desiccation at sub-zero temperatures. On the other hand, *S. gigas* showed much reduction in germination percentage in one year of storage time, indicating lower levels of desiccation tolerance. The results revealed that the seeds may be categorized in the intermediate storage category.

5. Conclusions and Recommendations

Endemic plant species are more vulnerable to anthropogenic threats and natural changes and, therefore, should be given the highest conservation research priority. In this regard, the investigation of longevity and desiccation sensitivity of seeds gives baseline information for ex situ conservation of long- and short-term storage. The present study indicated that, after one year of storage, germination percentages varied considerably among species between the storage environments. The results showed that storage of seeds at room temperature before drying may not be recommended for short-term storage of the studied species, except *K. foliosa*. On the other hand, after drying, seeds of *E. pinifolius*, *K. foliosa*, and *L. rhynchopetalum* showed higher germination percentages after

storage even at sub-zero temperatures for one year. From the current findings, one can conclude that the three species can be grouped in the orthodox seed storage category. In the case of *S. gigas*, it is highly recommended to repeat the test to confirm the reason why the initial germination resulted in 'zero'. According to the current findings, the seeds of *S. gigas* showed intermediate storage characteristics. A desiccation screening test should be followed with a viability test at different temperature regimes and different moisture levels with different storage durations to determine the storage category with certainty.

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