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Effects of Pre-Treatments and Conservation Conditions on Seed Viability and Germination of Two Varieties of an Endangered Species *Anacyclus pyrethrum* (L.) Link (Asteraceae)

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Abstract: This research presents, for the first time, a study of seed germination for two varieties, '*Anacyclus pyrethrum* var. *pyrethrum* (L.) Link' and '*Anacyclus pyrethrum* var. *depressus* (Ball.) Maire', of an endemic and endangered medicinal species listed in the IUCN red list as *Anacyclus pyrethrum* (L.) Link. Our objective was to provide information for their protection as well as a sustainable development strategy, by understanding whether the seeds germinate easily or have very specific germination requirements, and whether the storage conditions provided for the seeds were sufficiently favorable for the development of the species. A study of seed germination of the two varieties was conducted on lots of 25 seeds for each variety/treatment. Five treatments were used to break seed dormancy, which were chemical scarification with sulphuric acid (H_2SO_4), hydrogen peroxide (H_2O_2) and potassium nitrate (KNO_3); hot water (80 °C); and cold scarification at 4 °C. The viability was examined for stored seeds (6 months, 1 year and 2 years) and for those that did not germinate after the germination test at harvest, using the tetrazolium test. The dormancy was broken via cold scarification (4 °C) and chemical scarification (H_2O_2, H_2SO_4, NP (KNO_3)). The results also showed that a long storage time in wet cold (4 °C) causes a loss of seed viability, whereas a long storage time at −17 °C causes seed dormancy, which can be broken by cold scarification and chemical scarification. It was concluded that the rarity of the species was not due to the reproductive system, but to the overexploitation of the species during the flowering season, which influenced the natural regeneration of the species.

Keywords: germination; thermal scarification; chemical scarification; seed viability; *Anacyclus pyrethrum* L.

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1. Introduction

Seed germination represents an important step in the life history of plants, and is the beginning of a new life cycle [1]. Several external signals affect seed germination, including humidity and nutrients, while light and temperature are important environmental signals in the upper soil layer that can determine the level and speed of seed germination [2–5]. During the germination process, humidity has a different but complementary effect [6], while temperature affects the breaking of dormancy [7].

Seed dormancy of a species can be defined as the inability to germinate when incubated under favorable conditions, such as adequate rain and temperature [8]. Dormancy-breaking

pre-treatments are necessary to obtain high germination percentages and rates [9], and they break dormancy through physiological, chemical and mechanical effects [10,11].

Several plant species, including those in the genus *Anacyclus*, exhibit seed dormancy frequently. Hard seed coatings, which prevent water and air from reaching the seed and preventing germination, are the primary cause of seed dormancy in *Anacyclus* [12,13]. There are two types of seed dormancy, innate and induced dormancy. While induced dormancy can be brought on by outside causes, such as environmental conditions, innate dormancy can be brought on by physiological and morphological characteristics that are present in every seed [13–17]. In some *Anacyclus* species, innate dormancy can be overcome with chemical scarification, which involves treating the seeds with chemicals, such as concentrated sulfuric acid or potassium nitrate, to break down the hard seed coat. In the *Anacyclus* species, temperature can also alter seed dormancy; for example, certain species need to undergo a period of cold stratification before their seeds can germinate [18,19].

Endemic and threatened species, such as *Anacyclus pyrethrum* (L.) Link, present challenges for their recovery and management in the face of overexploitation, habitat destruction, degradation and fragmentation. Their small populations are susceptible to inbreeding and genetic erosion [20,21]. Once the population size starts to decrease, some species may experience declines in seed production and germination [22,23], as well as disturbances in their reproductive system function, dispersal syndrome and longevity [24]. Studying the germination of these endemic and vulnerable species can broaden our understanding of their endangerment, and provide a knowledge base for their sustainable management.

Anacyclus pyrethrum (L.) Link (Asteraceae), is a threatened medicinal species endemic to Morocco, Algeria and Spain. It is a gynomonic species, with a mixed autogamy–allogamy reproductive regime and a high predominance of allogamy [25]. The species is known for its various therapeutic characteristics as an antioxidant [26–30], anti-inflammatory [31–33], sialagogue [34–36], aphrodisiac [37–41], immunostimulant [42,43], an antidepressant [44] and a muscle relaxant [45], as well as its antimicrobial [46–49], antibacterial [27,50], antifungal [51], insecticidal [52–54], local anesthetic [55], anticonvulsant [28,37,56,57], antidiabetic [49,58–60], androgenic and fertilizing [41,61] antiamnesic [28] and anticancer properties [62]. It is also used as a memory stimulant [63]. The species was identified in 1979, with two varieties, *Anacyclus pyrethrum* var. *pyrethrum* (L.) Link (*A.P* var. *pyrethrum*) and *Anacyclus pyrethrum* var. *depressus* (Ball.) Maire (*A.P* var. *depressus*) [64–66]. Recently, it has been placed in the 'vulnerable' category, according to the IUCN Red List and the Red List of Vascular Plants of Morocco.

To the best of our knowledge, no previous research has investigated the germination of the two varieties of *Anacyclus pyrethrum* (L.) Link. Thus, we studied, for the first time, the effect of different pre-treatments (chemical and thermal stratification) on seed dormancy break and germination, as well as the influence of ex situ storage conditions on the longevity of the seeds. The ultimate goal was to understand the implications of these data for the evaluation of future breeding and conservation strategies for the study species.

2. Materials and Methods

2.1. Study Area

The plant material (cypselae, hereafter seeds) was collected in July (25 July 2018) from two populations, one for the variety *A.P* var. *pyrethrum* (P4), and the other for the variety *A.P* var. *depressus* (P3). For seed harvesting, 10 individuals of each population from each variety were sampled for collection. The latitude, longitude and altitude of the areas where the samples were collected are shown in Table 1. Timahdite was the only station where the two varieties coexisted. It should be noted that the *A.P* var. *pyrethrum* variety was rare.

Table 1. Contact details for the harvested sites.

Harvested Stations	Population No.	Latitude	Longitude	Altitude	Varieties
Timahdite (Tassemagt el maadane)	P3 P4	33.14311626° N	5.15923206° W	1948 m	*A.P* var. *depressus* *A.P* var. *pyrethrum*

The germination study was carried out in the Laboratory of Biotechnology, Environment, Agro-Food and Health, at the Faculty of Sciences, Dhar al Mahraz Fez.

2.2. Germination Study

2.2.1. Seed Cleaning

Each capitulum was manually opened up to separate the seeds. Two types of seeds were extracted, the (GL) peripheral seeds (seeds resulting from the ligulated flowers) and the (GT) central seeds (seeds resulting from the tubulated flowers). The seeds were mixed with impurities (bracts, dust, etc.), thus they were cleaned by sieving. Using a 3 mm mesh, the large impurities were retained. Then, with a 1 mm mesh, the dust and the small impurities were eliminated. After sieving, the last impurities were removed manually with a spatula.

2.2.2. Determination of Fresh Weight (FW), Dry Weight (DW) and Moisture Content of Seeds

The weights of the fresh and oven-dried samples (at 80 °C for 3 days) were determined using a precision balance. The water content of the seeds was determined with the following formula [67,68]:

$$\mathrm{TE}(\%) = \frac{\mathrm{PMF} - \mathrm{PMS}}{\mathrm{PMF}} \times 100$$

PMF: weight of fresh matter; PMS: weight of dry matter.

2.2.3. Seed Germination Assay

The different lots of seeds were subjected to pre-germination according to different treatments (chemical and thermal stratification), which aimed to eliminate seed dormancy, consequently facilitating exchanges between the embryo and the external environment and, in particular, the absorption of water [69,70]. The chemical stratification and heat treatment participated to break the seeds' physiological, morphological and physical dormancy [71–73]. These methods are similar the effects of fires and strong periods of heat and cold, which occur in the natural environment [69].

The lots were of 25 seeds for each variety/treatment, with 3 replications. Five treatments were used in our study: chemical scarification with concentrated sulphuric acid (H_2SO_4) by soaking for 1 min, then rinsing thoroughly with running water to remove all traces of acid [74]; chemical scarification with hydrogen peroxide (H_2O_2), in which the seeds were soaked under agitation in warm water (40 °C) for 1h, then in diluted hydrogen peroxide 50% (v/v) for 1h, then in pure hydrogen peroxide for 20 min [75]; chemical scarification with a 0.2% potassium nitrate (KNO_3) solution that was prepared by dissolving 2 g of KNO_3 in 1 litre of water, used to moisten the germination substrate at the beginning of the test [71]; thermal treatment with hot water (80 °C) to remove the waxy cuticle from the seeds [76]; cold scarification, where the seeds were placed on sand in closed polyethylene bags and stored at 4 °C for 15 days [71].

When the pre-germination treatments were completed, the seeds were sterilized in an alcohol solution, with the aim eliminating microorganisms that could be potentially harmful to germination. The seeds were then placed in petri dishes on germination substrate (coal soil and sand, filter paper), and the seeds were watered with distilled water to keep the substrate moist. Germination tests were carried out for a minimum of 21 days, and the seeds were examined every two days to check their germination. A seed was considered to have germinated as soon as the radicle emerged from the radicle. The temperature

and light conditions consisted of alternating day and night for 12 h each; the temperature ranged between 15 °C and 25 °C.

2.2.4. Calculation of Germination Parameters

To express seed germination, we used the following parameters:

- Germination kinetics

To better understand the physiological significance of the germination behavior of the seeds of the two varieties studied, the numbers of germinated seeds were counted daily until the 21st day of the experiment [77].

- Germination %(TG) (% germination) [78]

$$\mathrm{TG} = \frac{\mathrm{NGg}}{\mathrm{NTG}} \times 100$$

NGg: number of seeds germinated during the test; NTG: total number of seeds sown at the beginning of the test.

- Germination capacity (GPC) [79]

$$\mathrm{GPC} = \frac{\mathrm{ni}}{\mathrm{N}}$$

n_i: the cumulative number of germinated seeds at each observation i; N: the total number of seeds germinated.

- Germination speed [78]

The germination speed is an expression of the germination energy responsible for the depletion of seed reserves. It can be expressed by the median germination time (T50) (the time after which 50% of the germinated seeds were reached) [80]; the germination velocity coefficient (CV) [81–83] corresponds to the reciprocal of the average germination time, and makes it possible to evaluate the uniformity of germination [84]. These measures allowed us to take into consideration the germination behavior of all the seeds for each treatment.

$$\mathrm{T50} = \mathrm{T1} + \left(0.5 - \frac{\mathrm{G1}}{(\mathrm{G2} - \mathrm{G1})}\right) \times (\mathrm{T2} - \mathrm{T1})$$

G1: cumulative percentage of sprouted seeds with a value closest to 50% by lower value; G2: cumulative percentage of sprouted seeds with a value closest to 50% per higher value; T1: the day corresponding to the germination of the seeds whose value was closest to 50% by lower value; T2: the day corresponding to the germination of the seeds whose value was closest to 50% by higher value.

$$\mathrm{Cv} = \frac{\Sigma \mathrm{Ni}}{\Sigma\ \mathrm{NiTi}} \times 100$$

N_i: the number of newly germinated seeds at time Ti; N_{i+1}: the number of seeds germinated between time Ti and Ti + 1.

- Mean Daily Germination (MDG) [85]

$$\mathrm{MDG} = \frac{\mathrm{PGF}}{\mathrm{NGF}}$$

P_{GF}: final germination percentage; N_{GF}: number of days to final germination.

- Germination index (GI) [17]

$$\mathrm{IG} = \mathrm{N1} + \left((\mathrm{N2} - \mathrm{N1}) \times \frac{1}{2}\right) + \left((\mathrm{N3} - \mathrm{N2}) \times \frac{1}{3}\right) + \ldots + \left((\mathrm{Nn} - \mathrm{N}(\mathrm{n}-1) \times \frac{1}{\mathrm{n}}\right)$$

N_1: number of seeds germinated on the first day; N_2: number of seeds germinated on the second day; N_n: number of seeds germinated on day n; $N_{(n-1)}$: number of seeds germinated on day (n − 1).

- Germination reduction percentage (RG%) [17]

$$RG\% = \frac{G_S}{G_T} \times 100$$

G_S: number of unsprouted seeds; G_T: number of control seeds.

2.2.5. Viability Assay for Preserved Seeds

A viability assay for preserved seeds was a test to determine whether the seeds were still capable of germinating and growing into healthy plants.

The seeds were stored at three different temperatures: ambient temperature where the temperature for seed storage was between 10 to 15 °C and the relative humidity was between 20% to 35%; at 4 °C (with wet sand); and at −17 °C.

The seeds from preserved lots (6 months, 1 year and 2 years) and those that did not germinate after the germination test at harvest were tested for viability using the germination test and the tetrazolium test [86]. First, we proceeded to scarify the seeds, avoiding damage to them, by soaking them in tap water for 24 h, which allowed for complete hydration of the tissues, hence facilitating extraction of the seed coats. The denuded seeds, rinsed with distilled water, were completely immersed in the 0.5% tetrazolium chloride solution at an average temperature of 40 °C for 24 h. After staining, the seeds were rinsed several times with distilled water to remove excess colorant. Then, the seeds were classified into two categories according to their staining mode; fully stained seeds were viable, while uncolored seeds were not.

3. Results

3.1. Seed Water Content

The seed moisture content was determined for each variety, using the difference between the fresh sample weight (FW) and the dry sample weight (DSW). The results obtained are described in Table 2.

Table 2. Average water content for each seed type (GL: peripheral seeds; GT: central seeds) of *A.P* var. *pyrethrum* and *A.P* var. *depressus*.

Samples		PMF (g)	PMS(g)	TE (%)
A.P var. *depressus*	GL	0.056	0.047	16.07
	GT	0.061	0.052	14.75
A.P var. *pyrethrum*	GL	0.122	0.099	18.85
	GT	0.147	0.124	15.64

The data in Table 2 show that seeds from ligulate flowers (GL) contained a higher amount of water than seeds from tubulate flowers (GT) for the two varieties studied. The water content (WC) of the seeds of the two varieties, *A.P* var. *pyrethrum* and *A.P* var. *depressus*, was on the order of 14% to 18% in relation to the fresh material. The water content of the seeds indicated to us their degree of maturity.

3.2. Germination Type

The seeds of the two varieties studied showed an epigeal germination pattern (Figure 1), since the cotyledons emerged into the open air.

Anacyclus pyrethrum var. *depressus* (Ball.) Maire   *Anacyclus pyrethrum* var. *pyrethrum* L.

Figure 1. Seeds of the two varieties of *Anacyclus pyrethrum* (L.) Link considered as germinated.

3.3. Germination Assays

The first germination parameter considered was the final germination percentage. We undertook a comparative germination test of the *A.P* var. *pyrethrum* and *A.P* var. *depressus* seeds collected in Timahdite (Tassemagt al maadane) on 25 July 2018. The germination tests were conducted on seeds from ligulate flowers GL (peripheral seeds), and on seeds from tubulate flowers GT (central seeds) of the two varieties studied, on different substrates (filter paper and soil) without any treatment. Regular monitoring of germination made it possible to establish cumulative germination curves for these four seed lots (GLAPP: peripheral seeds of *A.P* var. *pyrethrum*; GTAPP: central seeds of *A.P* var. *pyrethrum*; GLAPD: peripheral seeds of *A.P* var. *depressus*; GTAPD: central seeds of *A.P* var. *depressus*). The results of these observations are shown in Figure 2.

From these results, the seeds were able to germinate as early as the 2nd day of germination on filter paper; meanwhile, for the seeds germinated on soil, the seeds were able to germinate as early as the 3rd day. In the four seed lots, it appeared that germination was relatively well grouped in an interval of 13 days for seeds germinated on filter paper and 12 days for those germinated on soil. Although the test was continued until day 21, no germination was obtained beyond day 16.

The germination percentage determined for the four lots, GLAPP, GTAPP, GLAPD and GTAPD, germinated on filter paper, were 25%, 31%, 26% and 32%, respectively; and 20%, 28%, 20% and 26%, respectively, for soil as the germination substrate. The low germination obtained may have been related to seed dormancy.

3.4. Influences of Pre-Treatment on Seed Germination Quality

To lift possible dormancy, the seeds were subjected to different pre-treatments. The results of the germination parameters of the different seed lots of the two varieties, *A.P* var. *pyrethrum* and *A.P* var. *depressus*, according to the different pre-treatments, are recorded in Tables 3–6. The germination kinetics curves of the different seed lots are shown in Figure 3.

From the results of seed germination for variety *A.P* var. *pyrethrum* (Tables 3 and 4 and Figure 3), it was observed that after soaking the seeds (GT: central seeds and GL: peripheral seeds) in oxygenated water, the germination percentage did not exceed 48%. Meanwhile, a treatment by cold scarification at 4 °C, potassium nitrate and sulphuric acid, made it possible to increase the germination to 88%, 80% and 60%, respectively, for the central seeds on filter paper, and 68%, 64% and 32%, respectively, on the soil. For the peripheral seeds, the germination results were 84%, 64% and 55%, respectively, on filter paper (Table 3), and 76%, 53% and 36%, respectively, on the soil (Table 4). It should be noted that boiling water practically canceled germination of the seeds. According to the germination results of *A.P* var. *depressus* (Tables 5 and 6 and Figure 3), the most efficient

pre-treatments were cold scarification (4 °C) and potassium nitrate and hydrogen peroxide, which increased the germinations to 70%, 58% and 50%, respectively, for the central seeds on filter paper, and 64%, 43% and 36%, respectively, for germinations on soil; meanwhile, for the peripheral seeds, the germination results were 66%, 44% and 19%, respectively, on filter paper (Table 5), and 52%, 23% and 10%, respectively, on soil (Table 6). However, the acid treatment was not efficient; the germination capacity decreased significantly after the sulphuric acid treatment, and even more strongly after the boiling water treatment, where there was no germination; it appeared that these two pre-treatments killed the seeds. In addition, all of the pre-treatments, except the boiling water treatment, had a positive effect on the germination of the seeds of the two varieties studied, and consequently on the germination behavior explained by the different parameters calculated (germination speed (T50, CV), germination capacity (GPC), germination percentage (TG), germination index (GI) and mean daily germination (MDG)).

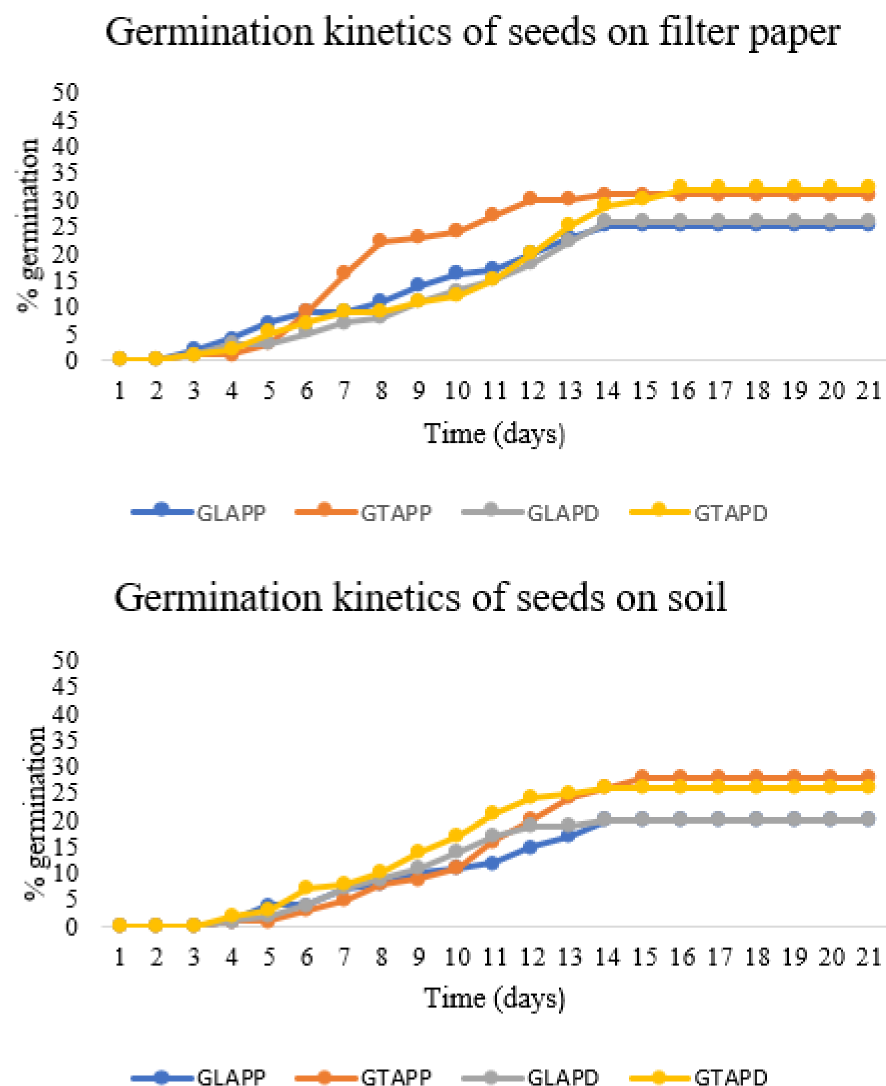

Figure 2. Germination kinetics of the two studied varieties on soil and filter paper (GLAPP: peripheral seeds of *A.P* var. *pyrethrum*; GTAPP: central seeds of *A.P* var. *pyrethrum*; GLAPD: peripheral seeds of *A.P* var. *depressus*; GTAPD: central seeds of *A.P* var. *depressus*).

Table 3. Averages of the main germination parameters of *A.P* var. *pyrethrum* seeds (GL: peripheral seeds; GT: central seeds) on filter paper, according to the different pre-treatments (EB: boiling water, H_2O_2 oxygenated water, NP: potassium nitrate; SF 4 °C: cold scarification 4 °C; H_2SO_4: sulphuric acid).

Germination Characteristic	Control		EB		H_2O_2		NP (KNO_3)		SF 4 °C		H_2SO_4	
	GT	GL	GT	GL	GT	GL	GT	GL	GT	GL	GT	GL
TGF (%)	31 ± 0.011	25 ± 0.020	0	0	48 ± 0.011	32 ± 0.02	80 ± 0.025	64 ± 0.005	88 ± 0.020	84 ± 0.005	60 ± 0.011	56 ± 0.015
T_{50} (j)	4.1 ± 0.26	6.83 ± 0.005	0	0	4.12 ± 0.003	4.87 ± 0.020	5 ± 0.1	5.11 ± 0.179	4.215 ± 0.003	3.83 ± 0.004	5.5 ± 0.011	3.6 ± 0.040
No. of germination days total	14 ± 0	14.66 ± 1.15	0	0	12 ± 1	12 ± 0	12 ± 1	13.33 ± 0.577	13 ± 1	14 ± 0	14 ± 1	11.33 ± 0.57
MDG	2.21 ± 0.002	1.78 ± 0.003	0	0	4 ± 0.066	2.66 ± 0.004	6.66 ± 0.006	4.92 ± 0.006	6.76 ± 0.002	6.04 ± 0.052	4.28 ± 0.002	5.09 ± 0.006
CV	7 ± 0.004	6.95 ± 0.003	0	0	7.16 ± 0.004	7.20 ± 0.001	7.42 ± 0.005	7.06 ± 0.003	7.05 ± 0.003	7.18 ± 0.008	7.04 ± 0.004	7.45 ± 0.005
IG	6.55 ± 0.007	6.36 ± 0.003	0	0	10.89 ± 0.020	7.63 ± 0.002	26.96 ± 0.007	15.89 ± 0.011	19.72 ± 0.003	24.48 ± 0.001	15.48 ± 0.003	20.14 ± 0.002

Table 4. Averages of the main germination parameters of *A.P* var. *pyrethrum* seeds (GL: peripheral seeds; GT: central seeds) in soil, according to the different pre-treatments (EB: boiling water, H_2O_2 oxygenated water, NP: potassium nitrate; SF 4 °C: cold scarification 4 °C; H_2SO_4: sulphuric acid).

Germination Characteristic	Control		EB		H_2O_2		NP (KNO_3)		SF 4 °C		H_2SO_4	
	GT	GL	GT	GL	GT	GL	GT	GL	GT	GL	GT	GL
TGF (%)	28 ± 0.015	22 ± 0.005	0	0	29 ± 0.025	20 ± 0.01	64 ± 0.015	53 ± 0.005	68 ± 0.015	76 ± 0.02	32 ± 0.015	36 ± 0.005
T_{50} (j)	8.3 ± 0.006	3.66 ± 0.015	0	0	6.07 ± 0.059	6.37 ± 0.002	5.75 ± 0.050	6.25 ± 0.004	4.5 ± 0.052	4.28 ± 0.020	4.27 ± 0.015	4 ± 0.167
No. of germination days total	15.33 ± 0.577	14 ± 1	0	0	13 ± 1	13.33 ± 0.577	12.33 ± 0.577	13.33 ± 1.527	10.66 ± 0.577	13 ± 0	13.66 ± 1.15	14.66 ± 1.154
MDG	1.86 ± 0.009	1.57 ± 0.018	0	0	2.23 ± 0.004	1.53 ± 0.002	5.33 ± 0.029	4.07 ± 0.002	6.8 ± 0.022	5.84 ± 0.005	2.46 ± 0.009	2.57 ± 0.002
CV	6.46 ± 0.002	6.66 ± 0.002	0	0	6.86 ± 0.003	7.01 ± 0.003	6.95 ± 0.001	6.95 ± 0.001	7.45 ± 0.002	7.13 ± 0.014	7.30 ± 0.006	5.25 ± 0.001
IG	4.53 ± 0.002	7.05 ± 0.007	0	0	6.06 ± 0.003	6.75 ± 0.003	12.14 ± 0.004	13.44 ± 0.001	21.47 ± 0.010	26.41 ± 0.005	8.78 ± 0.006	5.67 ± 0.004

Table 5. Averages of the main germination parameters of *A.P* var. *depressus* seeds (GL: peripheral seeds; GT: central seeds) on filter paper, according to the different pre-treatments (EB: boiling water, H_2O_2 oxygenated water, NP: potassium nitrate; SF 4 °C: cold scarification 4 °C; H_2SO_4: sulphuric acid).

Germination Characteristic	Control		EB		H_2O_2		NP (KNO_3)		SF 4 °C		H_2SO_4	
	GT	GL	GT	GL	GT	GL	GT	GL	GT	GL	GT	GL
TGF (%)	32 ± 0.01	26 ± 0.02	0	0	51 ± 0.04	20 ± 0.006	58 ± 0.004	44 ± 0.015	70 ± 0.007	66 ± 0.012	18 ± 0.01	15 ± 0.008
T_{50} (j)	8.53 ± 0.05	4.51 ± 0.04	0	0	3.48 ± 0.07	3.66 ± 0.002	5.51 ± 0.16	5.04 ± 0.051	4.91 ± 0.001	3.56 ± 0.05	7.07 ± 0.064	7.17 ± 0.017
No. of germination days total	16.33 ± 0.57	14 ± 1	0	0	13.33 ± 1.52	10 ± 1	13.33 ± 0.577	12.33 ± 1.52	14 ± 1	11 ± 2	11.66 ± 1.15	12.33 ± 1.52
MDG	2.03 ± 0.05	1.85 ± 0.002	0	0	3.84 ± 0.002	2.06 ± 0.1	4.46 ± 0.001	3.66 ± 0.005	5.04 ± 0.052	6.06 ± 0.055	1.63 ± 0.002	1.25 ± 0.004
CV	6.58 ± 0.004	6.66 ± 0.007	0	0	7.52 ± 0.004	7.59 ± 0.09	6.97 ± 0.004	7.14 ± 0.006	7.18 ± 0.001	7.55 ± 0.004	6.81 ± 0.003	6.66 ± 0.005
IG	5.65 ± 0,.005	4.96 ± 0.002	0	0	19.15 ± 0.001	7.45 ± 0.001	12.47 ± 0.003	12.28 ± 0.005	17.72 ± 0.002	27.07 ± 0.002	3.30 ± 0.005	2.24 ± 0.002

Table 6. Averages of the main germination parameters of *A.P* var. *depressus* seeds (GL: peripheral seeds; GT: central seeds) in soil, according to the different pre-treatments (EB: boiling water, H_2O_2 oxygenated water, NP: potassium nitrate; SF 4 °C: cold scarification 4 °C; H_2SO_4: sulphuric acid).

Germination Characteristic	Control		EB		H_2O_2		NP (KNO_3)		SF 4 °C		H_2SO_4	
	GT	GL	GT	GL	GT	GL	GT	GL	GT	GL	GT	GL
TGF (%)	28 ± 0.015	23 ± 0.01	0	0	36 ± 0.02	10 ± 0.015	43 ± 0.02	23 ± 0.005	64 ± 0.006	52 ± 0.015	16 ± 0.005	12 ± 0.006
T_{50} (j)	6.02 ± 0.06	5.82 ± 0.03	0	0	4.33 ± 0.011	6.75 ± 0.01	5.07 ± 0.064	3.50 ± 0.017	4.81 ± 0.004	6.84 ± 0.002	5.71 ± 0.010	6.5 ± 0.01
No. of germination days total	14.66 ± 1.15	14.33 ± 0.57	0	0	13.66 ± 0.15	11.33 ± 0.57	12.66 ± 1.15	12.33 ± 0.577	14.33 ± 0.577	11.33 ± 0.57	10.66 ± 0.57	11 ± 1
MDG	2.06 ± 0.11	1.78 ± 0.12	0	0	2.76 ± 0.005	0.91 ± 0.001	3.58 ± 0.002	1.91 ± 0.003	4.57 ± 0.01	4.72 ± 0.005	1.6 ± 0.004	1.09 ± 0.02
CV	6.74 ± 0.002	6.68 ± 0.02	0	0	7.06 ± 0.001	1.98 ± 0.005	6.98 ± 0.004	7.13 ± 0.002	7.07 ± 0.011	6.89 ± 0.06	7.01 ± 0.005	5.79 ± 0.012
IG	6.78 ± 0.003	5.05 ± 0.005	0	0	10.28 ± 0.002	2.38 ± 0.01	10.4 ± 0.007	12.14 ± 0.004	20.26 ± 0.004	13.91 ± 0.003	3.75 ± 0.001	2.31 ± 0.014

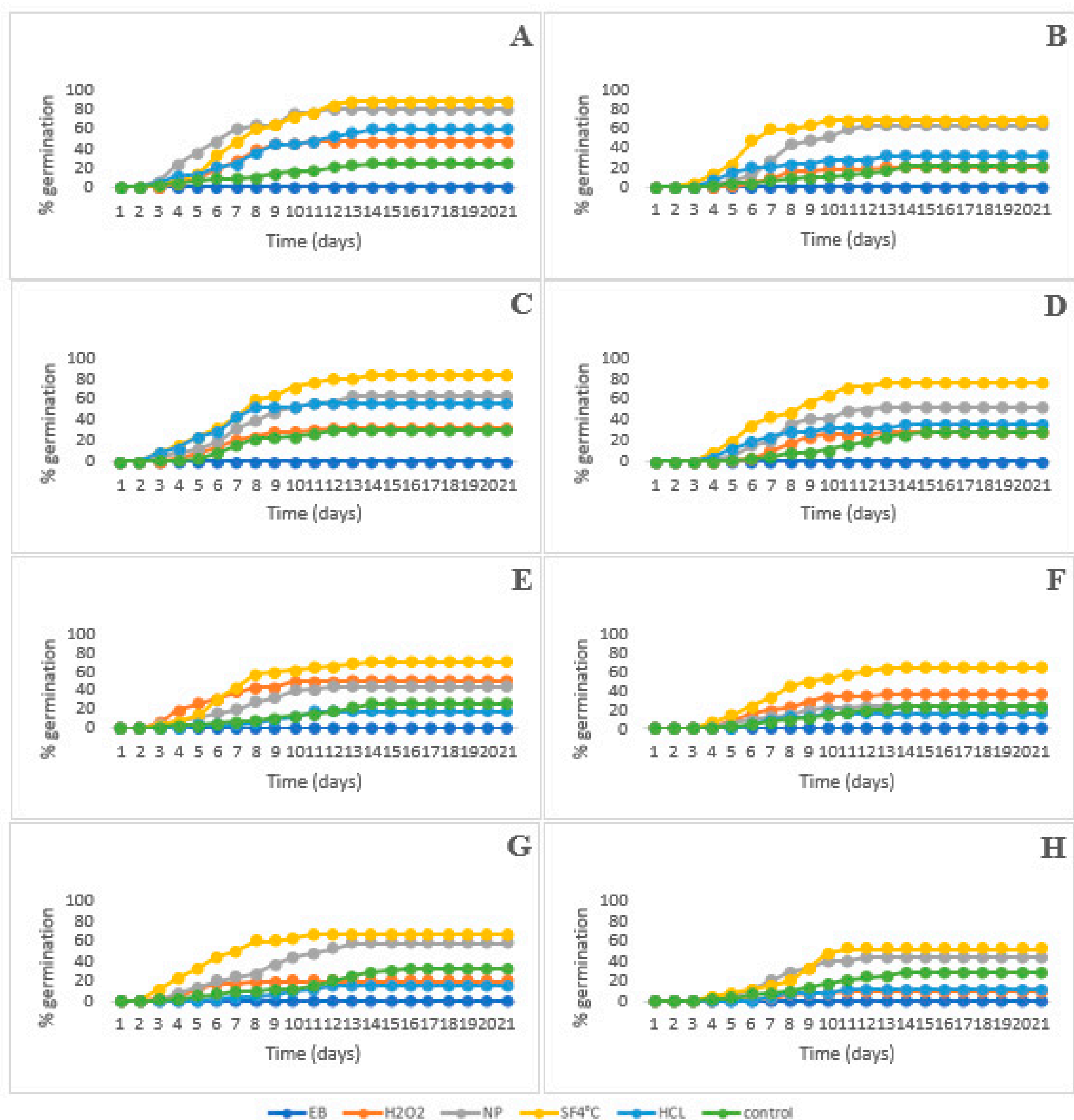

Figure 3. Germination kinetics of the two studied varieties germinated on soil and filter paper (FP), according to different pre-treatments (EB: boiling water, H_2O_2: hydrogen peroxide, NP(KNO_3): potassium nitrate; SF 4 °C: cold scarification 4 °C; H_2SO_4: sulphuric acid). (**A**) peripheral seeds of *A. P* var. *pyrethrum* germinated on filter paper; (**B**) peripheral seeds of *A.P* var. *pyrethrum* germinated on soil; (**C**) central seeds of *A. P* var. *pyrethrum* germinated on filter paper; (**D**) central seeds of *A.P* var. *pyrethrum* germinated on soil; (**E**) peripheral seeds of *A.P* var. *depressus* germinated on filter paper; (**F**) peripheral seeds of *A.P* var. *depressus* germinated on soil; (**G**) central seeds of *A.P* var. *depressus* germinated on filter paper; (**H**) central seeds of *A.P* var. *depressus* germinated on soil.

3.5. Seed Viability Assay

Viability Assay for Preserved Seeds

From the results presented in Table 7, it can be seen that seeds stored at ambient temperature and at −17 °C remained viable during the whole storage period. Meanwhile, the percentage of viability of seeds stored in the refrigerator at 4 °C decreased.

Table 7. Viability of different types of seed (GL: peripheral seeds; GT: central seeds) as a function of time and storage method.

The Period	*A.P* var. *pyrethrum*						*A.P* var. *depressus*					
	GL			GT			GL			GT		
	Ambient T°	4 °C	−17 °C	Ambient T°	4 °C	−17 °C	Ambient T°	4 °C	−17 °C	Ambient T°	4 °C	−17 °C
At harvest	96%			95%			95%			97%		
6 months	96%	94%	96%	95%	92%	95%	95%	93%	95%	97%	95%	97%
1 Year	90%	59%	93%	91%	63%	94%	93%	61%	95%	95%	67%	96%
2 Years	88%	19%	92%	90%	21%	94%	91%	20%	93%	92%	23%	93%

3.6. Influences of Storage Conditions on Seed Germination Qualities

From the results presented in Table 8, it can be seen that after 2 years of storage at 4 °C and −17 °C, the germination capacity (CG) of the seeds was null; thus, it was very likely that the reduction in germination capacity corresponded to a loss of viability or dormancy caused by the long cold storage time. However, the tetrazolium viability test (Table 7) revealed that the seeds stored at −17 °C were viable, whereas the seeds stored at 4 °C were not. Thus, we can say that a long storage time at 4 °C causes disturbances in the physiology of the seed, which causes its loss of viability.

Table 8. Germination characteristics of different types of seeds (GL: peripheral seeds; GT: central seeds) in relation to time and storage method.

The Period		*A.P* var. *pyrethrum*						*A.P* var. *depressus*					
		GL			GT			GL			GT		
		Ambient T°	4 °C	−17 °C	Ambient T°	4 °C	−17 °C	Ambient T°	4 °C	−17 °C	Ambient T°	4 °C	−17 °C
6 Months	CG	27%	79%	68%	31%	76%	62%	28%	73%	62%	32%	69%	60%
	PR	73%	21%	33%	70%	24%	38%	72%	27%	39%	69%	31%	40%
1 Year	CG	26%	22%	19%	31%	19%	17%	26%	20%	16%	31%	17%	15%
	PR	74%	78%	81%	69%	81%	83%	74%	80%	84%	69%	83%	85%
2 Years	CG	26%	0%	0%	30%	0%	0%	27%	0%	0%	30%	0%	0%
	PR	74%	100%	100%	70%	100%	100%	73%	100%	100%	70%	100%	100%

4. Discussion

The water content of seeds can be an indicator of their degree of maturity. As seeds mature, they typically lose water content and become drier. The reason for this is because a seed's embryo begins to enter a state of dormancy, where it is metabolically inactive until conditions become favorable for germination. Knowing that the seeds were harvested at the same period, and that they were maintained under the same conservation conditions, the average water content of the seeds of the two varieties was on the order of 14% to 18%. This water content was normal for small seeds, and it was suitable to tolerate very low temperatures to ensure their conservation in cold conditions without a notable change in the germination capacity [17]. Therefore, they contained little water with slowed enzymatic reactions, which allowed them to be well preserved [87].

The determination of the germination of seeds was one of the important descriptors of seed quality. In addition, germination kinetics provide further information on the quality of the seeds tested in terms of clustering, or the time spread of germination. All of this information is essential for a successful plant management and conservation program. The low germination percentage obtained on the different substrates could be related to

seed dormancy that is linked to internal factors [87,88]. Indeed, the comparison between the lots of seeds showed that the germination percentages differed slightly between the seeds resulting from tubulated flowers (central seeds) and those resulting from ligulated flowers (peripheral seeds), as well as between the seeds germinated on soil and those germinated on filter paper. This difference was perhaps due to the water content of the seeds, the reproduction system of the species, and the environments from which they originated. In fact, according to a prior study, the morphological differences between peripheral and central seeds were not linked to a germination dimorphism [14,89–91]. Additionally, various authors have also found that the type of substrate, the aeration and the water content all have impacts on the success of the germination process [92–94].

The seed germination process was conditioned by different factors, such as temperature, humidity, light, osmotic stress, seed storage conditions and environmental exploitation [95]. Acquiring knowledge of the germinative properties of seeds allows for a better understanding of the colonization strategy of plant species. The study of the influence of the pre-treatments on seed germination of the two studied varieties highlights the existence of dormancy at the seed level. This dormancy was broken in our study with cold scarification (4 °C) and with chemical scarification (H_2O_2, H_2SO_4, NP (KNO_3)). The best treatment was cold scarification (4 °C), and these results agree with those of Come, who reported that wet cold treatment (4 °C) for some time breaks seed dormancy in most species [78]. In 1985, Weaver and Jordan tested dips in potassium nitrate and ammonium nitrate, and found variable results depending on the concentrations used and the species treated [17]. Scarification using heat shock (boiling water) was not efficient for all tested lots, while scarification via soaking in concentrated sulphuric acid was inefficient for seeds of the variety A.P var. depressus, as the acid caused damage to the seed embryos. According to Hilhorst, the breaking of dormancy was characterized by an increase in sensitivity to gibberellic acid, a substance that stimulates germination [96]. Previously, the germination process was characterized by germination kinetics [97].

Since 2005, the germination process has been defined and measured by several parameters, such as germination capacity, germination speed, daily average germination and germination index. Furthermore, according to the results obtained, it can be concluded that all pre-treatments (cold scarification (4 °C), H_2O_2, H_2SO_4, NP (KNO_3)) have a positive effect on the germination behavior explained by the different calculated parameters; however, the difference was in the days needed for total germination and the average daily germination. The results also showed that the center seeds germinated well compared to the peripheral seeds, and that the germination percentages on filter paper were always higher than those obtained on soil. These results are in agreement with those of Frasier, who showed that germination percentages on filter paper in the laboratory were generally always higher than those obtained on soil [98].

Under natural conditions, dormancy is said to be broken by various conditions, such as exposure to sunlight, snow action, alternating wet and dry periods, which cause hydration and desiccation of the seed coats; initiation of microbial action, which attacks the seed coats; and even trampling by livestock. The dormancy of the seeds is undoubtedly related to the mode of dissemination of the seeds, which sink into the substratum by themselves, and are subjected to winter cold and freeze/thaw cycles. Therefore, the seeds germinate mainly in the following spring, if the seed coats become permeable during the winter.

The results of the tetrazolium and germination tests, used to determine the viability of stored seeds, revealed that the seeds stored at −17 °C were viable, whereas the seeds stored at 4 °C were not. It was likely that the loss of seed viability was related to fluctuations in the water content of the seeds, due to the absorbance of moisture from the humid air; this led to an increase in the moisture content of the seeds, which eventually caused their deterioration. Alternately, the germination test results may have been due to the decrease in oxygen tension linked to the storage conditions, which would have negatively influenced the seeds' metabolic activity. An increase in the water content of the seeds influenced

their germination abilities during storage, as the moist seeds deteriorated more quickly, especially if stored under non-optimal conditions [87].

5. Conclusions

The germination study indicated that overharvesting of these plants' roots for their medicinal properties, rather than their inability to reproduce, was likely the primary cause of the species' decline. It is necessary to immediately and urgently reinforce the conservation of these plant varieties through programs of reintroduction, the installation of seed banks, and culturing the two varieties, primarily A.P var. pyrethrum, which is the most required and the rarest variety.

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