

Preliminary study of the pre-germinative treatments of *Juniperus oxycedrus* L. and *Pistacia lentiscus* L. in the Saida region (Western Algeria)

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Abstract. *Juniperus oxycedrus* L. and *Pistacia lentiscus* L. are two taxa with a rigid or rigid enough structure caused by a solid pericarp resulting in a strong inhibition of the germination. The objective of this work was to test the effectiveness of certain pre-germinative treatments on the germination rate of the *Juniperus oxycedrus* and *Pistacia lentiscus* seeds with the aim of restoring perturbed ecosystems. In this context 180 seeds from each of the 2 examined species were tested in the laboratory (the number of repetitions is 5, to reach the degree of freedom 'ddl'). The seeds were divided into 4 groups of 20 seeds of each species and were treated as follows: 1) Immersion in the concentrated (95%) sulphuric acid (H₂SO₄) for 10, 30 and 60 minutes; 2) Mechanical scarification with a scalpel; 3) Soaking in hot water (at 100°C) for 30 seconds, 60 seconds and 2 minutes; 4) Cold stratification at 5°C for 24 hours. There is also a control group of 20 seeds representing each of the two species, which remained neutral. In the case of the *Juniperus oxycedrus* seeds the results showed that the germination rate improves after the mechanical scarification with a scalpel (92%), followed by the cold stratification with water (86%) and a treatment with a concentrated sulphuric acid for 60 minutes (82%). The seeds were treated with H₂SO₄ for 10 and 30 minutes, respectively, soaked in hot water; the control lot did not germinate. The germination rate for the *Pistacia lentiscus* seeds is 88% after the mechanical scarification and 84% after the cold water stratification. On the other hand, the treatment with H₂SO₄ or after soaking in hot water does not allow for the germination of these seeds. The same result is observed on the control group (0% for 30 days).

Key words: dormancy, *Juniperus oxycedrus*, *Pistacia lentiscus*, pre-germinative treatments, Saida

1. Introduction

The Doui Thabet State Forest occupies the western region of the Saida Mountains. These Mountains are a part of the biogeographical subsector of the Oranese

Tellien Atlas. Several scientific studies on the functioning of the ecosystems in this region showed a regression of the vegetations as a result of the anthropozoogenic action and the climate aridification (Benabdeli 1996; Nasrallah 2014; Kefifa 2015; Aouadj *et al.* 2020a,

2020b). This regressive dynamic requires the restoration and rehabilitation of the degraded ecosystems. The plant communities of the Saida Mountains are characterized by the variable physiognomic aspects (forests, pre-forests, lawns). These communities develop on poor soils and suffer from hard climatic conditions (Zouidi *et al.* 2019; Aouadj *et al.* 2020c; 2020d). These conditions affect the biological potential of plants and the natural vegetation cover depends on these parameters (soil and climate). The anthropogenic impact is added to this dual climatic-edaphic action. Pedoclimatic synergy coupled with the anthropozoogen stress conditioned the richness of plant structures and their appearances (Hasnaoui 2008; Hasnaoui *et al.* 2014; Medjati *et al.* 2014; Hasnaoui & Bouazza 2015; Zouidi *et al.* 2018; Aouadj 2021; Kehal *et al.* 2022).

The *Juniperus* (Badri 2003; Ferradous *et al.* 2013) and *Pistacia* (Benmahiou 2009) occupy an important place in the North African landscape, mainly because of their hardiness and dynamism. They are the pioneer species that are not ecologically demanding and present from the seaside to the Atlas peaks. Their hardiness allows them to withstand intense human aggressions which they are subjected to. In fact, in many areas they represent the only tree or shrub element that can be exploited for wood, foliage or even for industrial or medicinal purposes (Nasrallah *et al.* 2021). According to Doghbage *et al.* (2020) these two species are among the threatened species in Algeria due to the scarcity of viable seeds, the limit of natural seed germination, overgrazing, cutting offences, fires and above all, the lack of effective management programs for their rehabilitation, which increases the risk of their regression (Allam *et al.* 2019; Aouadj *et al.* 2020e, 2020f). However, the concerned authorities are now more conscious than ever about the risk of the extinction of these important species. Consequently, a law was passed in 2012 by the Algerian Ministry of Agriculture and Forestry with the aim of allowing its regeneration, rehabilitation and conservation to protect the *Juniperus*.

Nevertheless, it should be noted that despite the measures taken by the managers to preserve the ecosystem of the study area, the natural germination of the species in question seems to be a real problem for the ecological restoration. This is mainly due to the formation of seeds with a solid to semi-solid protective pericarp, preventing access to the basic elements necessary for the resumption of active life (e.g., oxygen, water and light). In fact, it is estimated that only 5% of the *Juniperus oxycedrus* seeds germinate naturally. As a result, the natural regeneration of these two species and the self-repair of our ecosystem cannot take place in the long term. This would therefore imply the need for a germinative pre-treatment to increase the germination rate.

J. oxycedrus seeds are characterized by very hard pericarp (teguments) (Badri 2003) while *P. lentiscus* seeds have moderately hard pericarp (Côme 1970; Bewley & Black 1994), inducing an inhibition of natural germination. This inhibition phenomenon of the seed germination may be related to internal factors, such as: species, variety, growing conditions, genetics (seed size and weight) (Chaussat & Chapon 1981) and seed age (Barton 1936) or external factors, such as temperature, oxygen, light and water (Chaussat & Chapon 1981).

The nature of the soil also appears to be a limiting factor that can influence the germination quality, as it is responsible for the behaviour of each species according to its composition (Chaussat & Chapon 1981). All these factors allow or do not allow the successful germination of the seeds of any plant species.

Thus, the persistence of the seed coat will prevent the absorption of water and the entry of oxygen, which causes the phenomenon of dormancy. The dormancy of a seed in its state of maturation is: “the inability to germinate when all environmental conditions should apparently allow germination”. While Rohde & Bhalerao (2007) define it as the inability to initiate the meristematic growth under favourable conditions. Whatever the definition, it appears that seed dormancy may be due either to the structures surrounding the embryo (Bewley & Black 1994) and/or to the phenolic compounds produced in the fruit (Isfendiyaroglu & Ozeker 2001; Baskin & Baskin 2004). In order to overcome this integumentary dormancy and increase the germination rate, the seeds should be subjected to specific pre-treatments to obtain a more satisfactory germination.

The germination of *J. oxycedrus* and *P. lentiscus* have not been studied extensively in Algeria and definitely not yet in the Saida region, despite their ecological and economical importance, hence the pertinence of this current study.

The objective of this work was to test the effectiveness of certain pre-germinative treatments (chemical, mechanical, thermal) in order to increase the speed and rate of the seed germination of those two species.

2. Material and methods

2.1. Origin and the choice of seeds

The used seeds in this study were collected in the Doui Thabet State Forest (located in the western Region of the Saida Mountains) in October 2020 from different feet of *J. oxycedrus* and *P. lentiscus*. During the picking, we took into consideration the sanitary aspect of the plant. On the other hand, the selected seeds were used in the fresh state, were morphologically ripe, were not

Table 1. Germination pre-treatment protocol (GENMEDOC 2006)

Essences	Pre-germinative treatment
	Description of the experimental protocol
	1 – Disinfection: The disinfection of all of the seeds in bleach for 10 minutes and rinsing with distilled water 3 to 4 times
<i>Juniperus oxycedrus</i>	2 – Separation of the seeds into different lots of 20 seeds per group: a – One group for a natural germination (as a control group without any pre-treatment) b – Three groups for a chemical treatment according to the time of exposure c – One group for a mechanical scarification d – Three groups for a hot water pre-treatment (100°C) according to the time of exposure e – One group for a cold treatment at 5°C for 30 days
and	
<i>Pistacia lentiscus</i>	3 – Pre-germinative treatments lots in detail: a – Immersion of the seeds in H ₂ SO ₄ (95%). Each batch is soaked in H ₂ SO ₄ for 10 minutes, 30 minutes and 60 minutes b – Mechanical scarification with a scalpel c – Soaking in hot water at 100°C of the three groups (for 30 seconds, 60 seconds and 2 minutes) d – Cold treatment at 5°C for 30 days

attacked by parasites. Those which were tested by tempering in water in order to retain only the viable ones. The floating seeds were not taken into account because we decided they were physiologically unripe.

Four pre-germinative treatments were applied: 1) sulfuric acid H₂SO₄ concentrated at 95%; 2) mechanical scarification using a scalpel; 3) hot water (at 100°C) and 4) cold stratification (at 5°C).

2.2 Seeds treatment

The different steps of the treatment are reported in Table 1 and they are related to the seeds of the two species.

2.3 Germination and the statistical analysis

The seed germination of the different lots was very well monitored. We took into consideration the percentage of the seeds that really germinated (Côme 1970), the latency T1 and T50 (the time necessary for 50% of the seeds to germinate). The T50 gives information on the germination speed. All of the obtained data were statistically analyzed using version 12.0 of the Statistica software. The difference between the growth parameters of the two species were evaluated by a variance analysis (ANOVA) of one factor.

In this context we were interested in the density of accumulation of the seedling growth each month. The

obtained results were compared to the threshold value indicated in Tables 2 and 4 according to the number of degrees of freedom (DFD): If $p > 0.05$: threshold difference is insignificant between averages; If $p \leq 0.05$: threshold difference is significant between averages; P: plus-value.

3. Results

3.1. Effect of the pre-germination treatments on the *Juniperus oxycedrus* seeds

The statistical analysis of the germination of the *J. oxycedrus* seeds, under the effect of various pre-germinative treatments was very significant from the 17th day (Table 2). Three out of five treatments (H₂SO₄ for 60 minutes, cold (5°C) and a mechanical one) had a very significant influence. The mechanical pre-treatment was the most effective one (92%). The pre-treatment by cold and H₂SO₄ for 60 minutes had a fairly high germination rate (86% and 82% respectively), while the hot water and the use of the Sulfuric acid (H₂SO₄) for 10 and 20 minutes, respectively, was null for the control group (Table 3; Fig 1).

It is important to consider that the results presented in this study should always be interpreted as valid only for the test lot and not for the species in general.

Table 2. Statistical results of the germination treatment of *Juniperus oxycedrus*

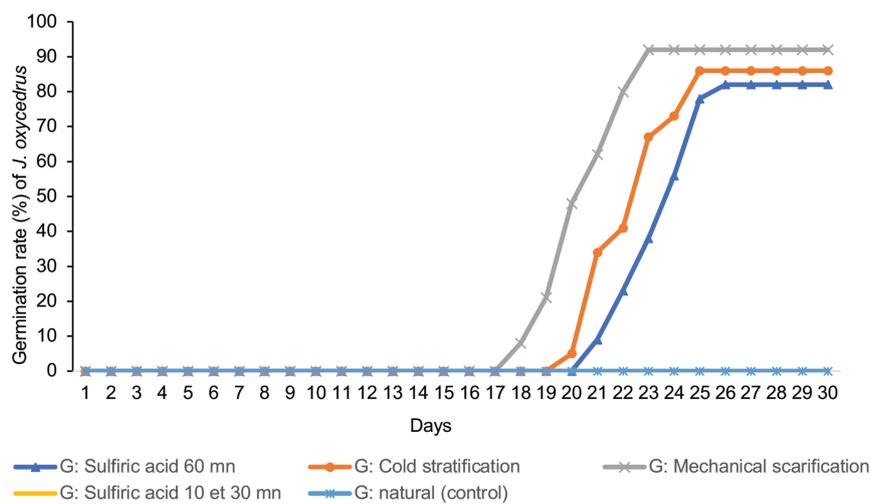
DFD	SS	AS	F	P value and meaning
5	5002457	833743	245.18	0.0000***

Explanations: DFD – degree of freedom, SS – Sum of squares, AS – Average squares, Fobs – F. observed, P – added value, meaning

Table 3. Germination rate of *Juniperus oxycedrus*

Pre-treatment	Pre-treatment duration	Germination rate	T1	T50
Control	/	0%	/	/
Mechanical Scarification	/	92%	17 days	19 days
Sulphuric acid (H ₂ SO ₄)	10 mn	0%	/	/
	30 mn	0%	/	/
	1 h	82%	20 days	23 days
Hot water	30 s	0%	/	/
	60 s	0%	/	/
	2 mn	0%	/	/
Cold water	30 days	86%	17 days	22 days

Explanations: T1 – Latency time (in days), T50 – time required to obtain 50% of the germination capacity (in days)

**Fig 1.** Kinetics of the germination of *Juniperus oxycedrus***Table 4.** Statistical results of the germination treatment of *Pistacia lentiscus*

DFD	SS	AS	F	P value and meaning
5	5444692	5444692	381128.4	0.0000***

Explanations: DFD – degree of freedom, SC – Sum of squares, AS – Average squares, Fobs – F. observed, P – added value, meaning

Table 5. Germination rate of *Pistacia lentiscus*

Pre-treatment	Pre-treatment duration	Germination rate	T1	T50
Control	/	0%	/	/
Mechanical Scarification	/	88%	10 days	15 days
Sulphuric acid (H ₂ SO ₄)	10 mn	0%	/	/
	30 mn	0%	/	/
	1 h	0%	/	/
Hot water	30 s	0%	/	/
	60 s	0%	/	/
Cold water	2 mn	0%	/	/
	30 days	84%	11 days	14 days

Explanations: T1 – Latency time (in days), T50 – time required to obtain 50% of the germination capacity (in days)

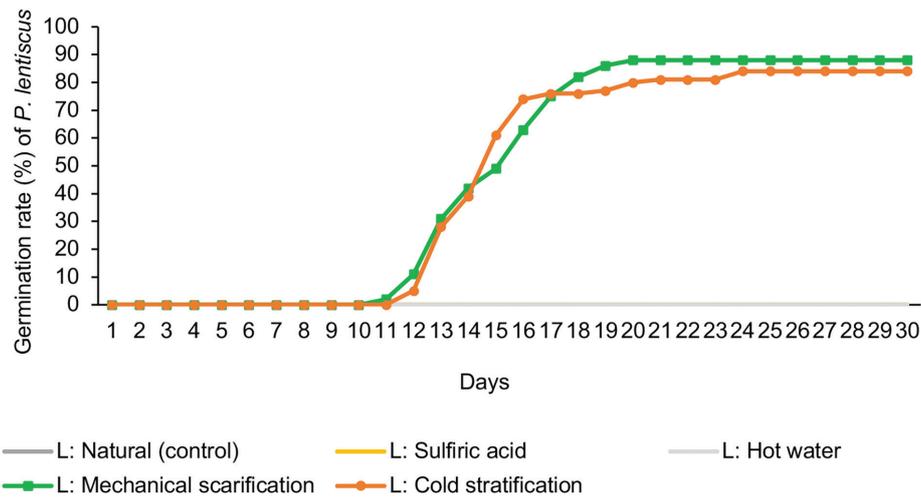


Fig 2. Kinetics of the germination of the *Pistacia lentiscus* seeds

3.2. Effect of the pre-germination treatments on the *Pistacia lentiscus* seeds

The statistical analysis of the germination of the *P. lentiscus* seeds was very significant from the 10th day (Table 5). We discovered that out of all of the experiments the two that have yielded a positive result were cold and scarification. The mechanical pre-treatment was found to be the treatment that produces a very significant germination (88%). As for the cold pre-treatment the result was also important, reaching 84% (Table 5; Fig. 2). It should be noted that other pre-treatments did not yield positive results.

4. Discussion

Seed germination is a key developmental process in the life cycle of plants. Our study indicated that hardening improves the germination performance of the *P. lentiscus* and *J. oxycedrus* seeds, which was also confirmed by the previous studies on several other species. Several authors have explained this rapid and synchronized germination to be caused by the activation of the pre-germination processes which induce the quantitative and qualitative biochemical modifications at the seed level (Varier *et al.* 2010; Maroufi *et al.* 2011), such as the repair of the membranes (Jowkar *et al.* 2012), strong synthesis and activation of the enzymes involved in the degradation and mobilization of the reserves (Varier *et al.* 2010; Wattanakulpakin *et al.* 2012), as well as the activation of the endo- β -mannase, which is the enzyme responsible for the synthesis of ethylene (the hormone which allows the degradation of the albumen to break the dormancy) (Varier *et al.* 2010). De Castro *et al.* (2000), showed that priming enhances and synchronizes the DNA replication in all of the embryo cells allowing

the cell cycle advancement from phase G1 to G2. This pre-activation cell cycle is one of the mechanisms by which priming induces a better sprouting performance. This mechanism is regulated by the activation of the cell cycle proteins, such as β -tubulin, cyclins and cyclin-dependent protein kinases. The improved germination in the hardened seeds may largely depend on the increased antioxidant enzyme activities (Ahmed *et al.* 2012). On the other hand, the beneficial effects of priming on the growth have been explained by Varier *et al.* (2010) by an acceleration of the nuclear replication in the roots and leaves.

It is known that the plant is highly dependent on the water supplied to it and the slightest deficit in the water balance leads to a reduction in the growth and can lead to wilting. This can be observed through our own results; stopping the watering caused the yellowing and strong wilting of the plants from the untreated seeds. At the end of this study, we could conclude that the pre-treatment of the *P. lentiscus* and *J. oxycedrus* seeds improved the germination performance.

Several pre-treatments have been tested and the germination rates vary depending on the type of the pre-treatment used, the nature of the seeds and their provenance (Mezghenni *et al.* 2014).

The obtained results of the previous studies suggest that the teguments are a significant obstacle to the germination of the *J. oxycedrus* and *P. lentiscus* seeds. The effect of the removal of the seminal envelopes on the rate and the kinetics of the germination (Coe & Coe 1987; Medjati *et al.* 2014) would be beneficial. The germination difficulties should be investigated in the structure of the tougher teguments (El Wahidi *et al.* 2013).

The results of our present study confirm that the seeds of *J. oxycedrus* and *P. lentiscus* have problems

with the dormancy and that the pre-germinative treatments contribute to the lifting of it. These pre-treatments are a simple and effective way to improve the germinative rate of the concerned species (El Wahidi *et al.* 2013; Morsli *et al.* 2015).

The germination of the *J. oxycedrus* seeds is significantly influenced by the used pre-treatments (P: 0,000). The mechanical treatment, H₂SO₄ for 60 minutes and the cold scarification (5°C) provides much better results than other pre-treatments. The highest obtained germination rates are 92% with H₂SO₄ for 60 minutes, 86% by the cold treatment (5°C) and 82% with the mechanical treatment. The results of our work corroborate with the other research on the seed germination of *Olea*, *Argan*, and *Abies* (Derridj *et al.* 2000). These different pre-treatments seem to facilitate the release of the dormancy phenomena and allow the satisfactory germination rates.

As with the seeds of *J. oxycedrus*, the germination of *P. lentiscus* is significantly influenced by the used pre-treatments (P: 0,0000). The mechanical and thermal treatments produce much better results than the other pre-treatments. After only 11 days they gave the highest germination rates (88% and 84% respectively). In this context it should be noted that the work on the pioneer species such as *Pistacia atlantica* Desf., *Olea laperrini* Batt. & Trab., *Argania spinosa* (L.) Skeels, *Balanites aegyptiaca* (L.) Delile, *Cupressus atlantica* Gaussen, *Juniperus phoenicea* L. etc. confirm the resistance of seed teguments which requires artificial pre-treatment to obtain a better rate of germination (Derridj *et al.* 2000; El Wahidi *et al.* 2013). Two recent studies have reported very interesting results in terms of the germination rates (80%) of the *Pistachio* seeds with the pre-treatment with the Gibberellic acid (GA3) (Poon Syverson 2019).

5. Conclusion and perspectives

The results presented in this current study have highlighted the undeniable effect of pre-treatments (scarification, chemical and thermal treatment) on the germination of *J. oxycedrus* and *P. lentiscus* seeds. The three pre-treatments, that are effective for *J. oxycedrus*; mechanical, thermal (cold one) and a chemical treatment (H₂SO₄ concentrated at 95% for 60 minutes), yielded remarkable rates of the germination (92%, 86% and 82% respectively). While for *P. Lentiscus* only two out of four treatments are effective: The mechanical scarification and the thermal treatment (88% and 84% respectively). It should also be remembered that the scarification gives the best result in both species. These results are very encouraging and can be exploited by the decision makers as part of the ecological restoration of the degraded ecosystems in the study area.

In perspective it would be possible to test new techniques such as the seed pulping technique (using high-water pressure for example) in order to increase the germinative capacity of the species with the germination difficulties. Also the micro-propagation; a technique to regenerate an entire plant from the cells or plant tissues in a nutrient environment (the in vitro culture) can also be tested.

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