

Research Article

Impact of different storage ecospheres on germination and growth characteristics of Cork Oak (*Quercus suber* L) seeds

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Abstract

Quercus suber L. is one of the world's greatest forest trees, particularly in the Mediterranean Basin, because of its high environmental and social value year-round. However, these trees generally suffer from specific issues affecting their germination, growth, survival, and other biotic and abiotic factors influencing natural regeneration. For this reason, there is a need to discover solutions to the irregularity of cork oak tree seed production annually and its impact on the natural regeneration of oak forests. The present study focused on resolving high moisture content issues and preventing the loss of cork oak seeds due to moisture and fungal infection during storage. To improve seed viability and germination capacity after storage, the experiment aimed to study the effect of different storage media, including wood sawdust, cork sawdust, and standard treatment (control), at a temperature of +4°C. This work aimed to discover an alternative technology that provides cork oak seeds throughout the year, as well as to find a solution to the irregularity of oaks in annual seed production, to compensate for the loss of forests and the natural regeneration of forests whose trees have been killed, burnt, or deforested. The results obtained showed that the storage media, storage periods, and their interaction significantly influenced the seedling's germination rate, length, and diameter. The statistical analysis showed that the impact was significant at 5%. Storing seeds in cork sawdust resulted in a significantly higher germination percentage, length, and diameter of 64.41%, 26.18 cm, and 2.36 mm, respectively, compared to those stored in wood sawdust 48.77%, 24.19 cm, and 2.44 mm.

Keywords: Germination, growth, *Quercus suber* L, Seeds of Cork Oak, Storage of seeds, Seeds vitality

INTRODUCTION

The globe's forests includes many of the planet's most productive ecosystems. . These forests play an important role, preventing soil erosion, helping to regulate

the climate, and providing clean water for many products such as fuel, food, furniture, volatile oils, and plant-based medicines.. Moreover, forests are necessary for the lives and well-being of people worldwide, and forests are under enormous pressure and facing challeng-

es such as deforestation, increased carbon emissions, and shortages in water and nutrients. Despite the knowledge of the threats to the survival of endemic species in forests, many people do not realize the dangerous situation in which some trees exist, as studies indicate many types of trees are threatened with extinction. Unfortunately, highly economically important tree species are at risk of extinction due to the unsustainable use of forests.

In this study, the focus was on the cork oak tree, *Quercus suber* L., as one of the most important forest trees in the world because of its environmental and economic benefits provided throughout the year, as these trees face problems in their natural regrowth to meet the challenges faced by forests in general. Also, oak trees face unique problems that affect them in terms of germination, growth, and survival. The most important of these problems is the annual irregularity of trees in the production of seeds, in addition to other biotic and abiotic causes affecting natural regrowth; this was confirmed by Perez-Ramos *et al.* (2014).

In addition, Martin Vicente *et al.* (1998) studied the impact of environmental factors on cork oak seed production for a period of eight years (1991–1998). The results showed that the number of seeds produced fluctuated between 11.6 and 285.8 g/m² based on dry weight. Seed productivity fluctuated from year to year in all regions under study around the average by 68% to 176%. In addition, Lopez-Carrasco *et al.* (2007) confirmed significant differences in seed yield between years and between the quantities of uninfected (healthy) seeds, which reached 62.8%, while the percentage of seeds infected with fungi was 22.5% (Gonzalez-Rodriguez and Villar, 2012) proved that the herbivores affected annual cork oak seed production. When animals feed on fallen seeds, it affects the growth of oak forests. The results showed that the percentage of seed disappearance was generally about 85%. These results indicate the importance of large-sized herbivores in the disappearance of solitary seeds.

Studies have also shown the effect of climatic factors, such as moisture stress in the summer and temperatures in the spring, on the annual production of oak seeds. This was explained by Pons and Pausas (2012), who found that the fluctuation of seed production in dry areas such as the Mediterranean climate is due to the degree of water availability and its impact on the fluctuation of seed production. It was pointed out (Perez-Ramos *et al.*, 2015) in a study to determine the reasons for the difference in the number of seeds produced for two types of oak trees (*Q. suber*) evergreen, (*Q. canariensis*) deciduous. The researchers found that deciduous oaks had fewer seeds and were more in the number of seeds produced. For these reasons, the present study aimed to find an alternative to the annual fluctuation of cork oaks (*Quercus suber*). This translates into

the use and planting of these trees to obtain the necessary forest regeneration throughout the year and compensate for the fluctuation in annual tree production for seeds

MATERIALS AND METHODS

To preserve cork oak seeds for as long as possible while preserving their moisture content, non-infection with fungi, ability to germinate, and good growth characteristics after storage operations, and the ability of cork seeds to retain their properties after storage, an experiment was carried out in the lab and nursery of the Forest Research Center located in Rabat, Kingdom of Morocco, in 2020. Two storage media were used (cork sawdust and wood sawdust); these seed storage methods are carried out at a temperature of +4 °C to solve the main problem, such as the annual irregularity in seed production of cork oak trees. This is to take advantage of the physical properties during storage of both the sawdust and the cork sawdust so that the seeds are stored in them throughout the storage period. Additionally, the studied parameters were storing seeds at a temperature of +4°C in a cork shavings storage medium and storing seeds at a temperature of +4°C in a wood shavings storage medium. The process of collecting the seeds required for the storage process was from the forest of Maamoura, purifying the seeds and treating them against fungi in the soil using a fungicide (Previcor) at a rate of 10 ml per 7 liters of water. In this experiment, the storage process was carried out in 07 liter airtight plastic containers; these containers are (24 cm in diameter and 21 cm in height) small in size and are used to store seeds and are airtight during storage, where each container contained 105 seeds, which were stored in a medium different from the other (cork sawdust – wood sawdust), carried out to find out the impact of the study factors (temperature-storage medium) on the studied traits, which are the percentage of germination, the height of plants, the diameter of the stems of plants and was repeated in three replications.

The experiment was carried out in a randomized complete block design in three replicates (RCBD). Therefore, the cultivation process started 30 days after the start of storage. The cultivation process was repeated every 30 days, meaning the second cultivation was after 60 days from the beginning of storage. The third cultivation was 90 days after the beginning of storage. Thus, the cultivation operations continued until the last planting date, 300 days from the beginning of storage, where the cultivation for each treatment was (storage +4°C cork shavings) and (storage +4°C wood shavings) per month.

Hence, each treatment was planted in plastic experimental units (37cm * 49cm) and divided from the inside

into 35 alveoli; the unit size was 500cc by one alveoli. One seed was planted in each alveolus, and the treatment was carried out in three replicates. That is for each treatment, 105 seeds were planted for both storage mediums (cork shavings, wood shavings). The planting medium was prepared: the soil (natural with fertilizers). It is way for the plant to receive material and nutritional sustenance as the root system of the plant develops. As for the local soil that was used, it was compost from the Maamoura forest consisting of materials rich in humus and soil rich in organic matter mixed with commercial fertilizers. By mixing the soil with peat moss as a growth medium (organic fertilizer). It is a fertilizer consisting of a fermented mixture of organic residues and mineral salts. Once the medium of germination and growth is mixed, the experimental units are filled and moved to compact, then filled completely, then moved for the last time, and excess soil is removed. The units are placed on platforms with a height of 30 cm from the ground's surface.

The plastic experimental units used in agriculture for seeds were solid plastic units in the form of a truncated pyramid. These units were perforated at the base and fixed from the inside with grooves, or "root guides." All agricultural operations were carried out, starting with planting seeds, irrigation, removing weeds, and using some treatments (post-planting tasks such as weeding, insect, pest and fungus control), when necessary, against fungal or insect diseases.

The results were taken monthly until 31/3/2021 by taking measurements of the studied traits and calculating their rates by taking the arithmetic mean of them and obtaining the rate of the percentage of germination per treatment, the average length of plants per treatment, the average diameter of the stem of plants per treatment. The obtained results were compared as averages of the factors under study and compared with the average results of the treatment (control) (treatment of cultivation without seed storage). After that, these results were converted into graphs to illustrate the differences between the germination and growth rates of *Quercus suber* L. seeds.

Statistical analysis

The results were subjected to statistical analysis using SAS version 9.1 (2002) to determine the least significant difference (LSD) for the study factors and the significance of their impact on the studied traits with a value of 0.05 to find a clear vision and answer the study questions.

RESULTS AND DISCUSSION

Previous studies showed that there are factors affecting seed production (annual production pattern), such as a decrease in the dry period in the summer and a de-

crease in frost in the spring, which leads to a decrease in the production of seed-bearing trees (every two years). In the same context, LingLi *et al.* (2013) indicated that the resulting seed yield was affected by the weevil infection rate and that the percentage of healthy, dead and infected seeds in 2008 was 20.09%, 55.90%, 20.01%, respectively. In 2009, they were 50.16%, 30.28%, and 19.56%, respectively, and Perez-Ramos *et al.* (2014) indicated several other reasons affecting the yearly variation in seed yield. They evaluated the number of acorns produced by two species of oak trees (*Q. suber* and *Q. canariensis*). The findings also showed that seed production can be affected primarily by soil fertility, while trees planted in moist, fertile soil produced more seeds than trees planted in specific fertility zones, in addition to their genetic composition and the environmental conditions surrounding them. The present research has also shown that the quality of trees, whether deciduous or perennial, influences the number of seeds produced annually. In this regard, numerous research studies have examined various methods of preserving cork oak seed at various temperatures in order to obtain a high germination rate, good morphological character, and vitality for as long as possible to make up for changes in the cork oak trees' yearly seed production, but the seeds can also face other problems during storage, such as the low moisture content inside the seeds when storing them. According to Zulueta and Montoto (1992), the germination rate reduced considerably when *Q. suber* seeds had 40% of their moisture gone. In addition, oak seeds cannot be stored easily for long periods because of their high water content. While Santos and Bernardino (1995) demonstrated that seeds with a moisture content of more than 50% and less than 40% have a low life. According to Belletti *et al.* (2001), studies showed that the seeds' moisture content should be greater than 40%, which supported this interpretation. Consequently, the germination rate and percentage of seeds that were held at zero degrees Celsius and had a 42% moisture content were comparable to those of fresh seeds. This is in line with a study by Merouani *et al.* (2001), in which they stored cork oak seeds at zero degrees Celsius for six months after drying them. Their results showed a link between seed vitality and water content, with germination rates falling significantly when seed water content was below 30%. Monteleone *et al.* (2002) also studied the impact of seed storage on temperatures (-5, -2, 0, 3 °C). Their results show that the seeds were stored at different humidity and temperature levels for three to twelve months.

The results of Monteleone *et al.* (2002) indicated that after 9 months, the percentage of germination and germination of seeds with a moisture content of 42% and stored at zero centigrade was similar to that of non-stored seeds. The insect infestation of seeds during

Table 1. Means deviations square on the impact of seed storage periods and storage medium on germination percentage, length and diameter of oak seedlings

Source of variations (S.O.V.)	Degrees of freedom (d.f.)	Length (cm)	Diameter (mm)	Germination %
Replication (Rep)	2	6.670	0.001	130.368
Media	1	113.620**	0.364**	6974.189**
Periods	18	285.039**	0.893**	2426.062**
Media* Period	18	66.855**	0.161**	305.233**
Error	54	9.101	0.045	82.452

** : highly significant differences with a probability ($p < 0.01$)

harvesting or storage also affected germination quality, seed viability, and seedling growth. It was shown by Branco *et al.* (2002) in a study that insect infestations impact *Q. suber* oak seeds immediately after harvest or during storage at zero degrees Celsius. The fungal infections during the storage period also affected the vitality of the seeds and their germination rate due to the high humidity during storage. Merouni *et al.* (2005) confirmed this when *Q. suber* oak seeds were stored at 0°C and 85% relative humidity, which led to an increase in the rate of infection with fungi, which led to a significant decrease in the percentage of germination. It reached 42%, and 18% of the seeds showed fungal growth. They showed the sensitivity of oak seeds during storage at low temperatures (very less than zero Celsius). This was confirmed by Xin *et al.* (2010), and the present study carried out different storage operations for cork oak seeds to provide the planting requirements for the entire season. The present study determined the best treatment in a storage medium for oak seeds to maintain their viability for the longest possible storage period and achieved optimal seed germination and growth rates where two media were used for storage (cork sawdust and wood sawdust) at a temperature of +4 degrees Celsius to take advantage of the physical properties during storage for each of the sawdust medium, which has physical properties which are that it is not resistant to rotting, is not resistant to biological attacks, has a high ability to absorb moisture and water vapor, is relatively low in thermal conductivity, and is not good at sound insulation as stated earlier (Ahmed

Ali 5.8.2021. Wood defects <https://e3arabia.com>).

Also, the cork sawdust has physical properties, which are that it is resistant to rotting, resistant to absorbing water, moisture, and water vapor, non-flammable, resistant to attacks of insects, rodents, and fungi, and one of the best sound and thermal insulators (Housing, insulation and heating. <https://ar.Econologue.com>.cork insulation. January 24, 2008 // May 26, 2016, Christophe) to find solutions to the difficulties faced by the cork oak seeds during storage, which are the high moisture content of the storage medium and the inability of the seeds to retain the moisture content of the cork oak seeds and their infection with fungi during storage to solve the problem of the basic study.

Therefore, the annual irregularity in the production of seeds for cork oak trees and finding appropriate answers to the questions of the study. Does storing cork oak seeds in a specific medium maintain the moisture of the storage medium and prevent the moisture content of the seeds from decreasing or rising during storage? What is the appropriate medium for storage that affects the best results on the germination and growth characteristics of cork oak seeds?

In this present work, oak seeds were stored in wood sawdust or cork sawdust at +4°C. The length and diameter of the seedling and the germination percentage were measured a month after the beginning of planting, and this was repeated monthly for ten months. The results recorded in Table 1 indicated that each of the storage mediums and storage periods and the interaction between them had a significant impact ($P < 0.01$) on

Table 2. Impact of periods and medium of storage on the overall average of germination rate, length and diameter of *Q. suber* seedling after 300 days of storing in the nursery of CRF (forestry research center).

Measured parameters	Length (cm)	Diameter (mm)	Germination rate (%)
Cork sawdust	26.18a	2.44a	64.41a
Wood sawdust	24.19b	2.36b	48.77b
L.S.D. _{0.05} media	1.12	0.07	3.38
L.S.D. _{0.05} period	3.47	0.24	10.44
L.S.D. _{0.05} media*period	4.92	0.34	14.82

The means in a row followed by the different letter are significantly different at the 5% threshold

the germination percentage and the length and diameter of the seedling.

On the other hand, Table 2 shows the average impact of storage averages and storage period and the interaction between them on the percentage of germination. Therefore, it is clear from the data that the percentage of germination achieved from storing seeds in cork sawdust (64.41%) was significantly higher than that stored in sawdust (48.77%). This may be due to the ability of sawdust to absorb and retain moisture from the seeds and the surrounding environment, which negatively affects the germination of oak seeds, unlike cork sawdust. These results were in line with those of (Ahmed Ali 5.8.2021. Wood defects <https://e3arabia.com>) in the physical properties of wood and its ability to absorb moisture and water vapor. As explained by Belletti *et al.* (2001), oak seeds do not tolerate drought during storage.

On the other hand, the present results indicated that the rate of germination gradually decreased with increasing storage period (Fig. 1). Also, the germination rate was the highest in the control (comparison) condition, which recorded (78.09%) even after 240 days for seeds that were stored for 60 days (79.04%). After this period, the germination percentage began to decrease significantly, as studies showed a regression relationship between the moisture level and each germination percentage and root length by Li *et al.* (2013).

As for the impact of the interaction between storage media and storage periods on the germination rate, the results in Fig. 1 indicated that the seeds that were stored in cork sawdust for 30 days and tested after 120 and 240 days of storage showed the highest germination rate (92.38%, 91.43%) respectively. These percentages were significantly equal to those estimated in the comparison treatment and storage for 30 days in either type of sawdust and tested after 330 days, as well as those stored in cork sawdust for 60 days and tested after 120 and 240 days (79.04%, 81.90%), respectively.

In contrast, oak seeds stored in any storage medium (cork sawdust - sawdust) for 210 days or more showed

the lowest percentages of germination of 50.47% - 12.38%, respectively. This may be due to Perez-Ramos and Maranon (2009)'s study, which showed that the germination rate was not affected for 30 days. Also, the germination rate is affected and decreases whenever the seeds lose part of their moisture during storage, which was confirmed by Zulueta and Montoto (1992). On the other hand, storing oak seeds in cork sawdust resulted in longer seedlings (26.18 cm) compared to those stored in sawdust (24.19 cm) (Table 2), with a confidence interval ± 1.99 cm. The seeds were also stored for 60 days and tested after 330 days or 240 days. In addition, the seeds stored for 120 days and tested after 300 days produced the longest seedlings (35.98, 34.48, 34.22 cm), respectively - where the results here showed the strength of the seedlings and the preservation of their qualities (Fig. 1). The results of the stored seeds for 120 days after testing them after 300 days of planting showed that they retained their qualities and achieved continuous growth and development during growth. This may be due to the nature of the storage medium (Housing, insulation and heating. <https://ar.Econologue.com>. cork insulation. January 24, 2008 may 26, 2016 Christophe) (Fig. 1)

As for the impact of interference on seedling length, the results of Fig. 2 showed that storing oak seeds in cork sawdust for 90 days and testing them after 330 days gave the longest seedlings (38.17 cm). It was statistically equal to that stored for 60 days in either sawdust type and tested after 240 or 330 days as well as seeds stored for 90 days in cork sawdust and tested after 240 days (34.27 cm) and stored in any of the storage medium for 120 days and tested after 300 days. Conversely, storing oak seeds in either a 300-day storage medium and testing after 330 days produced the shortest seedlings (13.22, 14.77 cm). The seedlings resulting from seed storage in sawdust for 210 days and tested after 330 days (11.58 cm) were equal. Here the results showed the impact of both the storage medium on the stored seeds and the ability to maintain their moisture during storage.

Thus, on its vitality as it showed the characteristic of

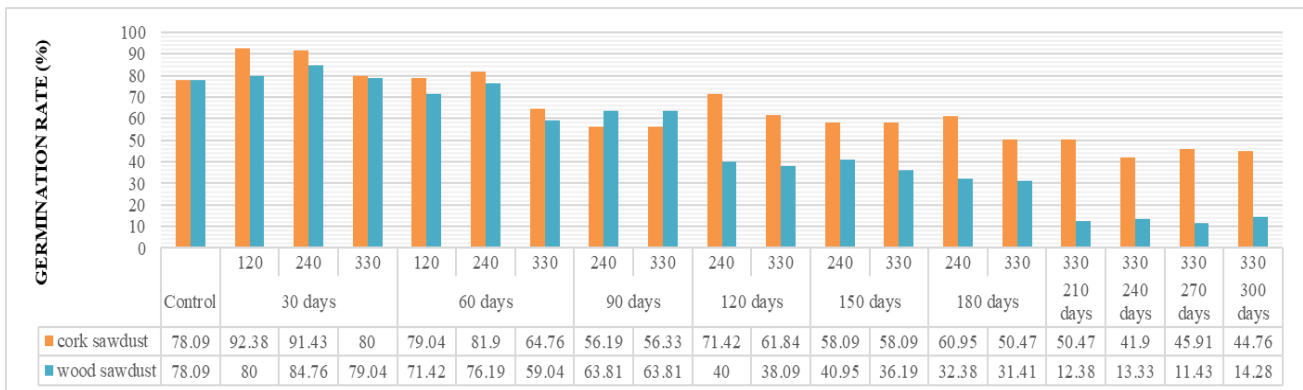


Fig. 1. Impact of storage periods on the germination rate for cork and wood sawdust

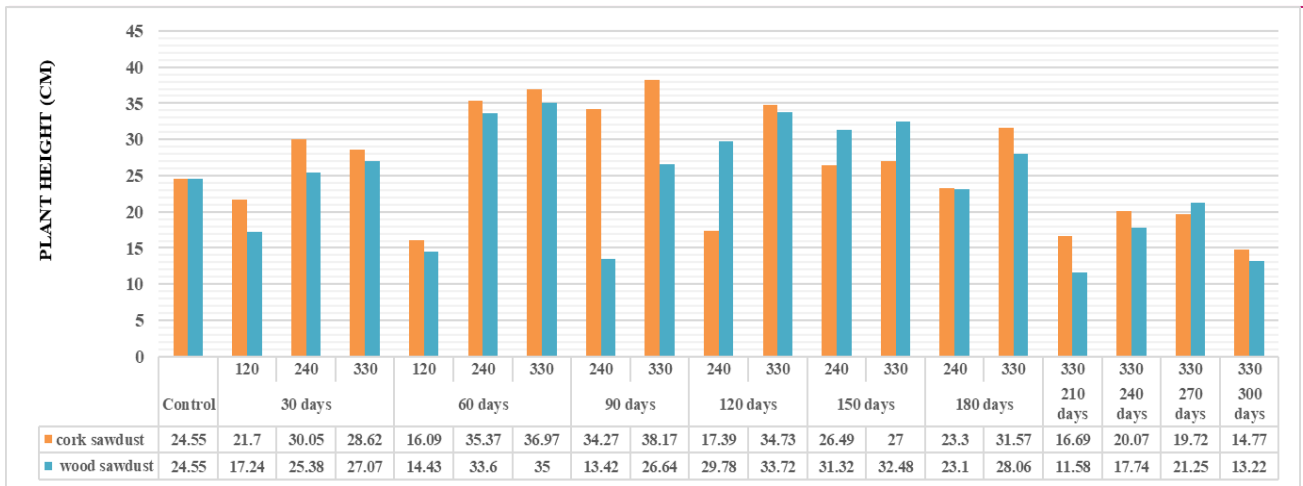


Fig. 2. Impact of storage periods on plant height (cm) for cork and wood sawdust

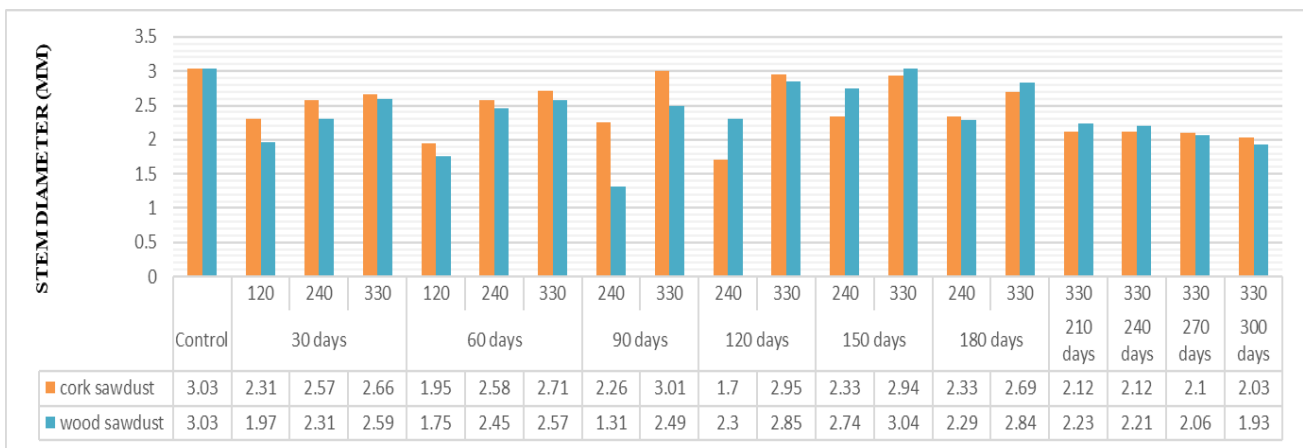


Fig. 3. Impact of storage periods on stem diameter (mm) for cork and wood sawdust

the seedling length of the seeds stored for 120 days for the medium of storage, and it was tested after 300 days, the length of the seedling was (34.27 cm) but with the increase in the storage period, the seedling length of the seeds stored for 300 days and tested after 330 produced the shortest seedlings. This may be due to the seeds being affected by the length of the storage period and the loss of part of their moisture during storage. This trait was affected by seeds stored in sawdust for 210 days and tested after 330 days (11.58 cm). This was confirmed by (Santos and Bernardino, 1995), which is the difficulty of storing *Q. suber* seeds for long periods due to their high moisture content; where it was found that the seeds with a moisture content of more than 50% and less than 40% had a low life.

In considering the diameter of the seedling, the results showed in Table 2 that the oak seeds stored in cork sawdust were more in diameter (2.44 mm) than those stored in sawdust (2.36 mm). Also the diameter of the seedling differed from one storage period to another, where the comparison treatment recorded the most seedlings in diameter (3.03 mm) statistically equal with those resulting from seed storage for 120 days and tested after 300 days (2.90 mm), stored for 150 days

and tested after 330 days (2.99 mm). Because of the impact of the interaction between the two factors of the study on the diameter of the oak seedling, the results showed that most seedlings' diameter resulted from the control treatment and the seeds stored in either type of sawdust for 120 days and tested after 300 days or stored for 180 days and tested after 330 days as well as stored in cork sawdust, diameter for 60, 90, and 150 days and tested after 330 days was 2.71, 3.01, 2.94 mm, respectively, (Fig. 3).

This may be due to the nature of the storage medium and its characteristics that worked to preserve the stored seeds and their safety from infection from fungi and to maintain their moisture during storage (Housing, insulation and heating. <https://ar.Econologue.com>.cork insulation. January 24, 2008 may 26, 2016 Christophe), but when testing two storage mediums (wood sawdust and cork sawdust), the results showed that storage in the cork sawdust improved the storage conditions that kept reducing the moisture of the storage medium, maintaining the moisture of the stored seeds, preventing the growth of fungi and not infecting the stored seeds with fungi. The seeds stored in cork sawdust for 270 and 300 days achieved a germination rate of 45.91

and 44.76%, respectively. It is the best germination rate compared to sawdust for the same storage period, which was 270 and 300 days, where the germination rate was 11.43, 14.28%, respectively (Fig.1). This was due to the specificity of both mediums in terms of their physical properties, and these results were consistent with what was confirmed by Merouni *et al.*, (2005).

As Fig. 2 shows that the height in seedlings after 270 days and 300 storage days of the seeds stored in cork sawdust were 19.72 cm and 14.77 cm, respectively, while in sawdust after 270 days and 300 storage days, they were 21.25 cm and 13.22 cm, respectively, whereas, the length of the seedling achieved 80.32 % in the treatment 270 days of storage in the middle of cork shavings compared with the standard treatment (without storage). The seedling diameter in the same treatment after 270 days and 300 storage days was in the seeds stored in cork sawdust; it was 2.10 mm and 2.03 mm, respectively. As for sawdust after 270 days, 300 storage days were 2.06 mm and 1.93 mm, respectively (Fig.3). The seedling diameter maintained a percentage of 69.30% in the treatment 270 days of storage in the middle of the cork sawdust compared to the control cultivation (the standard treatment), where this treatment achieved 270 days of storage at a temperature of +4°C in the cork sawdust storage medium, ideal proportions and results for the storage treatment of seeds for nine months while maintaining an appropriate amount of their vitality and morphological characteristics. These results were consistent with what was confirmed by (Santos and Bernardino, 1995; Monteleone *et al.*, 2002), who worked on *Quercus suber* trees.

Their results showed that seeds stored for 9 months, or 270 days, were able to achieve a germination rate of 45.91% for seeds stored in cork conservation medium, while the germination rate of seeds stored in sawdust was 11.43% after the same storage period. And also that they found that cork sawdust helped maintain the moisture content of *Q. suber* seeds, while sawdust could not maintain the moisture content of *Q. suber* seeds and had a low germination rate. The novelty in our current work is to use two storage media that are different in terms of physical properties and to test the ability of both media to maintain seed moisture content, prevent an increase in moisture content of the storage medium, and prevent fungal infections during storage, which affect the vitality of stored seeds. The results showed that storage in a cork sawdust-based medium was ideal for storing *Q. suber* seeds.

Conclusion

To sum up, the results of the present study showed that the best storage medium treatment for cork oak seeds was the storage treatment in cork sawdust media, whereby the storage treatment after 270 days achieved

a germination rate of 45.91%. Also, the seedling length trait achieved 80.32% in the 270 days of a storage treatment in cork sawdust medium compared with the standard treatment (without storage). As for the seedling diameter maintained a percentage of 69.30% compared to the control cultivation (standard treatment), where this treatment achieved 270 storage days at a temperature of +4°C in the cork sawdust storage medium. These results were ideal for the storage treatment of seeds in this medium for nine months while maintaining an appropriate amount of their germination rate and morphological characteristics where the study addressed high humidity in storage mediums, loss of cork oak seeds due to moisture content, and fungi infection, focusing on the irregularity of *Quercus suber* L., tree production and its impact on oak forest renewal.

Conflict of interest

The authors declare that they have no conflict of interest.

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