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Effects of seed treatments on the germination of Golkoy palm (*Phoenix theophrasti* Greuter subsp. *golkoyana* Boydak)

Gölköy hurması (*Phoenix theophrasti* Greuter subsp. *golkoyana* Boydak) tohumlarına uygulanan bazı işlemlerin çimlenme özelliklerine etkileri

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Abstract

Datça palm *(Phoenix theophrasti* Greuter) is the only palm taxa in the European continent. Its subspecies, Golkoy palm (*Phoenix theophrasti* Greuter subsp. *golkoyana* Boydak) was recorded only in Turkey. This subspecies, which spreads in sensitive ecosystems, needs to be carefully protected. Although there are three natural populations of Datça palm in Turkey, there is only one population of Gölköy palm. This population is under threat due to factors such as tourism, pollution, urbanization, use of groundwater and forest fires. In this respect, the continuity of the species should be ensured by taking *in-situ* and *ex-situ* protection measures immediately. One of the ex-situ conservation measures involves the propagation of the species from seed. Cold-wet stratification is applied to remove dormancy in Gölköy palm seeds. In this study, GA_3, ultrasound and vacuum applications at different times were applied to remove dormancy in seeds and shorten germination time. At the end of the study that lasted for 10 weeks, it was determined that GA_3, vacuum and ultrasonic applications increased the germination rate of the seeds and shortened the germination period. While the highest germination rate was reached in the seeds treated with GA_3, (91.4%), the germination rate was 88.3% and 88.6%, respectively, in 60 and 120 min ultrasound applications. The germination rate of the seeds in which vacuum was applied for 60 and 120 minutes was found to be 29.9% and 48%, respectively. The lowest germination was 6.3% in the control group seeds.

Keywords: Golkoy palm, GA_3, ultrasound and vacuum treatments, seed germination

Öz

Datça hurması (*Phoenix theophrasti* Greuter) Avrupa kıtasının tek palmiye türüdür. Bu türün alt türü olan Gölköy hurmasının (*Phoenix theophrasti* Greuter subsp. *golkoyana* Boydak) sadece Türkiye'de kaydı bulunmaktadır. Hassas ekosistemlerde yayılış gösteren bu alt türün özenle korunması gerekir. Türkiye'de Datça hurmasının üç doğal populasyonu olmasına rağmen Gölköy hurmasının tek populasyonu bulunmaktadır. Bu popülasyon turizm, kirlenme, şehirleşme, yeraltı sularının kullanımı ve orman yangınları gibi faktörlerden dolayı varlığı tehdit altındadır. Bu bakımdan *in-situ* ve *ex-situ* koruma önlemlerinin acilen alınarak türün devamlılığı sağlanmalıdır. Ex-situ koruma önlemlerinden biri türün tohumdan çoğaltılmasını kapsamaktadır. Gölköy hurması tohumlarında dormansiyi gidermek için soğuk ıslak katlama uygulanmaktadır. Bu çalışmada tohumlardaki dormansiyi gidermek ve çimlenme süresini kısaltmak için GA_3, ve farklı sürelerde ultrason ve vakum uygulamaları yapılmıştır. 10 hafta süren çalışma sonunda GA_3, vakum ve ultrasonik uygulamaların tohumların çimlenme oranını artırdığı ve çimlenme süresini kısalttığı belirlenmiştir. GA_3 ile muamele edilen tohumlarda %91,4 oranı ile en yüksek çimlenme oranına ulaşılırken, 60 ve 120 dk ultrason uygulamalarında çimlenme ise sırasıyla %88,3 and %88,6 oranında gerçekleşmiştir. 60 ve 120 dk vakum uygulanan tohumlarda çimlenme oranı ise sırasıyla %29,9 ve %48 olarak bulunmuştur. En düşük çimlenme ise control grubu tohumlarda %6,3 olmuştur.

Anahtar Kelimeler: Gölköy palmiyesi, GA_3, vakum ve ultrason uygulama, tohum çimlenmesi

1. Introduction

Industrialization and urbanization threaten the future of sensitive forest ecosystems. This effect is much greater on rare and endemic species that have low ecological tolerance. The changes that occur increase the vulnerability of the ecosystems of such species, while they come to the point of extinction. These species can be protected through *ex-situ* or *in-situ* methods. The purpose of e*x-situ* protection is to generated saplings from the species and grow them in areas that are suitable for their ecology. For such products, the biological characteristics of the species especially their reproduction methods should be known well and the production barriers, if any, should be eliminated.

Turkey is one of the most prominent countries in the world as regards its species diversity and endemism. Guner et al. (2012) stated that Turkey has 11466 natural and 3649 endemic species. Datca palm (*Phoenix theophrasti* Greuter), distributed only along the Mediterranean coast and the only natural palm species in Muğla, Turkey, and its subspecies, Golkoy palm (*Phoenix theophrasti* Greuter subsp. *golkoyana* Boydak), are among the rare and endangered species. Although *P. theophrasti* Greuter subsp. *golkoyana* Boydak is distributed in Turkey, Crete and Greece, ssp *golkoyana* has a limited distribution only in Turkey (Boydak, 2019).

P. theophrasti is one of the few tree species tertiary relict endemic to the eastern Mediterranean, and it is one of the two palm taxa native to continental Europe. Crete is its another distribution area, which constitutes the only natural distribution of palm in Europe, except Turkey. *P. theophrasti* Greuter was first found by (Boydak, 1983; Boydak and Yaka, 1983; Boydak, 1985; Surhone et al., 2011; Vardareli et al., 2019) in Datca Eksere valley and Hurmalıbük Karasüleyman and Karaali river. Another distribution area is 50-250 m. in Finike bay on the Kumluca-Karaöz coast between Karaöz port and Papaz port in Antalya. between altitudes Boydak (1986, 1987; Kavgaci, 2014).

A subspecies of Datca palm which is *P. theophrasti* subsp. *golkoyana* Boydak (Boydak and Barrow, 1995; Barrow, 1998; Esener, 1999; Boydak, 1986) was recorded in Bodrum Golkoy-Golturkbuku-Bodrum, Mugla. It is distributed in an area of 3.9 hectares mixed with *Pinus brutia* Ten. in marshland near Golkoy (Bodrum, Mugla). It is important to protect this species, whose number has decreased considerably, to ensure the continuity of ecosystems. (Yazici, 2007; Senol et al., 2016). For this reason, the region has been declared a natural protected area (Boydak, 1994; Boydak, 2019).

The distribution of Golkoy palm differs from that of Datca palm while it was found to be a subspecies of Datca palm (Boydak and Barrow, 1995; Boydak, 2019). There are certain differences between the two species of palm. Datca palm has several trunks from the bottom and can grow as high as 17 meters (Boydak, 1994). Golkoy palm, however, does not grow high, while it can only grow as high as 8 meters. Moreover, there are differences between the two as regards peduncle length (Boydak, 2019). The Datça palm, whose fruits ripen in September and October, is edible but has no commercial value. The seeds of the Datça palm are larger and sweeter than the Gölköy palm (Boydak, 1986; Boydak, 1994, Senol et al., 2016; Boydak, 2019). This subspecies is different from *P. theophrasti* at molecular level (Vardareli et al., 2019).

Datca and Golkoy palms are distributed only in a local area; therefore, they are exposed to various threats as regards their survival. Such threats include road construction, pressure from people and tourism activities, change of flow in groundwater due to climate change, red palm weevils leading to drying of palm trees and forest fires (Boydak, 1995; Dembilio et al., 2011; Hazir and Buyukozturk, 2013; Kontodimas et al., 2006). Orucu (2019) highlighted in his study that habitat loss of *P. theophrasti* Gr. due to climate change will be probably severe in Turkey, leading to narrowing of its distribution areas. Studies show that gene flow is under critical levels in Datca and Golkoy populations, which requires their protection through *in-situ* or *ex-situ* methods (Barclay, 1974; Vardareli et al., 2012). Hazir and Buyukozturk (2013) advised that, *P. theophrasti* and *P. theophrasti* ssp. *golkoyana* is of considerable scientific importance to Turkey's flora because they are the only representations of native palms. These endemic species should be conserved in terms of biodiversity against possible threats such as physical development on private property, tourism, cultivation and also against pests.

Golkoy palm (*P. theophrasti* subsp. *golkoyana* Boydak) is distributed in a small area and only in Turkey for now (Boydak and Barrow, 1995; Boydak, 2019). Since this species is distributed in sensitive ecosystems, it is exposed to constant environmental pressures. For this reason, Vardareli et al, (2019) recommend urgently to be placed on the IUCN red list for its conservation.

Water uptake and oxygen availability are the essentials of seed germination (Ashraf and Foolad. 2005). Seed coat impermeability is usually caused by the presence of one or more layers of palisade cells in the testa. These palisade layers are composed of sclereid cells with thick lignified secondary cell walls. There is a two-fold effect of the intact coat: primarily, it causes the retention of inhibitor, and secondarily, it can act as a barrier to oxygen, preventing the entry of sufficient oxygen from the surrounding air to support the oxidation of the inhibitor (Bewley et al., 2013).

Sound waves transported through a medium via the mechanism of particle interaction are characterized as mechanical waves (Pierce. 1989). It can be transmitted through gases, liquids or solids. As waves propagate, they transport energy (Chowdhury et al., 2014). When sound waves are applied to seeds, they cause some morphological and physiological changes. Different metabolic activities including enzyme activation and hormonal changes occur during seed germination, and sound is known to directly affect biological systems including those involved in seed germination (Braam and Davis. 1990; Chowdhury et al., 2014). In many studies, it is reported that ultrasonic applications increase the germination of seeds (Nazari and Eteghadipour. 2017).

Ultrasounds are mechanical waves of a frequency higher than 20000 Hz. Therefore, ultrasonic waves should alter the seeds' characteristics. Proper sound waves can reduce cell membrane penetrability (Chowdhury et al., 2014) and more water and oxygen are available. In water, ultrasonic waves lead to cavitation (Piyasena et al., 2003; Yaldagard et al., 2008), Sound stimulation increased the cell wall and membrane fluidity, which facilitated cell division and growth (Keli et al., 1999; Zhao et al., 2003). Ultrasonic waves have vastly been applied as an efficient technique for breaking seed dormancy and improving the germination characteristics (Nazari and Eteghadipour, 2017). Cavitation is a phenomenon by which micro-bubbles are created in the water. The cavitation created by the ultrasound causes a mechanical pressure on the seeds (Yaldagard et al., 2008; Hu et al., 2007). This mechanical pressure then leads to the cell wall fluidity (Yaldagard et al., 2008), and the creation of micro-pores and micro-cracks on the cell wall (Jaime et al., 2014). The creation of micro-pores and micro-cracks caused by sonication means that the seeds are more permeable to water and oxygen entry (Miano et al., 2016a; Luo, 2016). Researchers have indicated that exposure of the seeds to the ultrasound enhances hydration (Toma et al., 2001; Jambrak et al., 2007; Yaldagard et al., 2008; Miano et al., 2016b). It seems that increase in hydration of the seeds treated with ultrasonic waves increases in enzymatic activities especially alpha-amylase (Yaldagard et al., 2008; Sharififar et al., 2015; Miano et al., 2016a). Consequently, the starch hydrolysis is enhanced. Ultrasound can be used to accelerate the germination process, (Miano et al., 2016a).

The breaking of physiological dormancy and the induction of germination are regulated via hormone signalling pathways and mainly through the GA-(gibberellin) and ABA-(abscisic acid) biosynthetic and catabolic pathways. While ABA and GA are the primary inhibitory and promotive hormones in regulating seed dormancy and germination. There are more than 130 different structures of GA molecules in plants (Bewley and Black, 1994; Bewley et al., 2013). Gibberellins are a class of tetracyclic diterpene carboxylic acids, functioning as plant hormones (phytohormones) and influencing a range of developmental processes including dormancy, germination, root and shoot elongation, and flowering (Vehn and Sauer, 2017).

GA_s plays a key role in dormancy release and promotion of germination (Baskin and Baskin, 2004; Kucerna et al., 2005; McDonald and Kwong, 2005; Cetinbas and Koyuncu, 2006; Bewley et al., 2013; Vehn and Sauer, 2017). Gibberellic acid (GA_3) is widely used to break the dormancy of seeds of various plant species. Dormant seeds, which require stratification, dry storage after-ripening and light as a germination stimulator, are often treated with GA_3 to overcome their dormancy (Gupta, 2003). A lot of studies demonstrate that seeds treated with GA_3 have increased germination (Acikgoz and Kara, 2019). GA_3 dosage trials revealed that 100 mg/l GA_3 had a better result for seed germination (Madakadze et al., 2000; Nasri et al., 2013; Baskin and Baskin, 2014; Ge et al., 2018; Ayranci and Oner, 2019).

Pressure is usually applied to ensure the penetration of water or hormones into seeds. However, there are studies in which vacuum technique has been applied (Loveys and Jusaitis, 1994; Custódio et al., 2016).

There are two basic approaches to protect endangered species as *in-situ* and *ex-situ*. *Ex-situ* conservation is the preservation of a species outside its natural habitat. This protection method also includes the generative production of the species. It is important to protect the Gölköy palm, one of the endangered, rare and endemic species, by producing it from seed. Long-term stratification of seeds in traditional production also increases the risks of germination. Treatments applied to seeds with modern methods can eliminate dormancy and accelerate germination. For this purpose, the effects of ultrasound, vacuum, GA_3 and soaking applications on germination were investigated to break dormancy in seeds. The effects of these pretreatments on the germination percentage and speed of the seed were revealed.

2. Material and Methods

2.1. Material

The study material included seeds collected from Golkoy palm populations in 2018. The fleshy fruit seeds were collected and mesocarps were removed and then cleaned. They were kept in polyethylene bags at +4 ^{0}C until the study was conducted.

The materials used at the lab included Petri dishes with a diameter of 9 cm for germination, GA_3 hormone (Merck), ultrasonic device (Bandelin DL 510 H, Germany), vacuum device (Binder VD 23), precision balance (Radwag AS 220.R2), incubators (Liebherr-Lovibond TC 140 G), pure water device (Elga DV 35), flow cabinet (biosafety cabinet class II), alcohol, filter paper, stretch wrap and fungicide (Maxim XL 035 FS).

2.2. Methods

2.2.1. Sterilization

For seed sterilization, ethanol and sodium hypochlorite were used and all seeds were soaked in ethanol for one minute for surface sterilization. Then, they were treated with 10% sodium hypochlorite for 10 minutes and rinsed three times with pure water. Glass Petri dishes, plates where the seeds were placed, and filter papers were sterilized at 120 ^{0}C for 1 hour.

2.2.2. GA_3, ultrasonic treatment and vacuum application

The seeds were soaked only in 100 ppm GA_3 for 12 hrs. For "ultrasonic application", the seeds are placed in the plastic net and the ultrasound device (Bandelin Sonorex Dıgıplus, Typ DL 510 H, Berlin Germany). Seeds were subjected to "ultrasonic application" at 35 kHz for 60 and 120 mins. During ultrasonic application, the water temperature was kept at ± 30 ^{0}C by adding ice from time to time. For "vacuum application the seeds" were placed in the vacuum device (Binder, VD 23, Tuttlingen, Germany) in beakers filled with 200 cc of pure water. They were retained in the vacuum device at 35 ^{0}C and 100 millibar for 60 and 120 min to ensure that water penetrated the whole seed.

2.2.3. Seed germination test

Each treatment was done in triplicate and 50 seeds were used in each replication. Seeds were sown on 20 December 2019. After the seeds were subjected to treatments; they were sown in Petri dishes with a diameter of 9 cm that had one filter paper and 5 ml of water each and they were sprayed with 1 % fungicide.

All Petri dishes were wrapped in two folds with stretch film. The seeds were kept in the climate cabinets at a constant temperature of 20 ^{0}C and in dark conditions. Those seeds that developed 2 mm radicles were considered as germinated. After germination started, the seeds were counted weekly and the germinated seeds were transferred to another Petri dish. To prevent the infection of the seeds in Petri dishes, they were sprayed with a fungicide after every count and wrapped again.

2.2.4. Statistical analysis

The data of the study were analyzed with SPSS (22) statistical analysis software. The germination percentages in % found in counting were subjected to arc-sin conversion. One-way ANOVA analysis of variance was applied to the data and the differences were determined with Duncan multiple comparison test.

3. Results and Discussion

The study was carried out for 75 days, as the germination of the seeds of the control group started after the 8th week. Germination checks were weekly to determine the germination percentages and statistical analyses were conducted to determine the differences between the treatments. The statistical analysis revealed statistically significant differences between the treatments applied to Golkoy palm seeds as regards their effects on germination (Table 1).

Duncan multiple comparison test was applied to determine the differences between the treatments. The test results showed that the control seeds had the lowest germination percentage (6.3 %). Those seeds treated with GA_3 and ultrasound had the highest germination percentage (91.4 %). The seeds had better germination properties when treated with ultrasound compared to the control seeds whereas no difference was found between the vacuum applications for one and two hours (Table 2).

The values on the same line followed by the same letters are not significantly different at $P<0.05$.

Radicles were found to be formed in the opposite side of the groove direction of the seeds, which was the micropyle side (Figure 1).

The seeds were retained in germination cabinets for 75 days and checked weekly. The first germination was observed 4 weeks after sowing. The seeds that were germinated first were the ones treated with ultrasound and GA_3 hormone. Maximum

Table 1. One way analysis of variance on Golkoy palm
Tablo 1. Gölköy hurması tohumlarının tek yönlü varyans analizi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11028.351	6	1838.059	34.088	0.000
Within Groups	754.888	14	53.921		
Total	11783.240	20			

Table 2. Duncan multiple comparison test on the treatments
Tablo 2. Uygulanan işlemlerin Duncan çoklu karşılaştırma testi

Treatments	Petri dish (N)	Germination percentage (%) and standart devision
T1-Soaking in ethanol for 1 mins+soaking in pure water for 12 hrs at +5 ^{0}C (Control-760 mm/Hg)	3	6.9000±11.95115 a
T2-reatment with 100 ppm GA_3 for 12 hrs and soaking in pure water at +5 ^{0}C	3	73.8533±6.86079 b
T3-Soaking in pure water for 12 hrs at +5 ^{0}C	3	59.7167±12.46840 c
T4-Soaking in pure water for 12 hrs at +5 ^{0}C + ultrasonic treatment for 1 hr	3	70.1233±2.60450 cd
T5-Soaking in pure water for 12 hrs at +5 ^{0}C + ultrasonic treatment for 2 hrs	3	70.6000±4.45689 cd
T6-Soaking in pure water for 12 hrs at +5 ^{0}C + vacuum application for 1 hr (100 mm/Hg)	3	32.7733±1.97356 b
T7-Soaking in pure water for 12 hrs at +5 ^{0}C + vacuum application for 2 hrs (100 mm/Hg)	3	43.8533±1.24114 b

Figure 1. Germination of Golkoy palm seeds
Şekil 1. Çimlenen Gölköy hurması tohumları

germination was observed by the end of week 7. Overall, germination speed and percentage were observed to decrease after week 7. The control seeds had a very slow germination speed and they could start germinating after week 8, which indicated that there was a time difference of 4 weeks in germination compared to the other treatments. The germination percentage was very low in the control group (Figure 2).

This study revealed that the highest germination percentage was found in the group of seeds treated with GA_3 (91.4 %), while the lowest was found in the control group (6.3 %). Germination started 4 weeks before the one in the control group and the germination percentage was 85.1% higher than that of the control group. The results of the study are consistent with the other studies showing that GA_3 increases seed germination. There are not many studies on the effects of GA_3 application on the germination of palm trees. For this reason, studies in other species were examined.

In these studies, it was determined that GA3 increased seed germination and was effective in cracking the seed coat (Follett et al., 2005; Dilip et al.

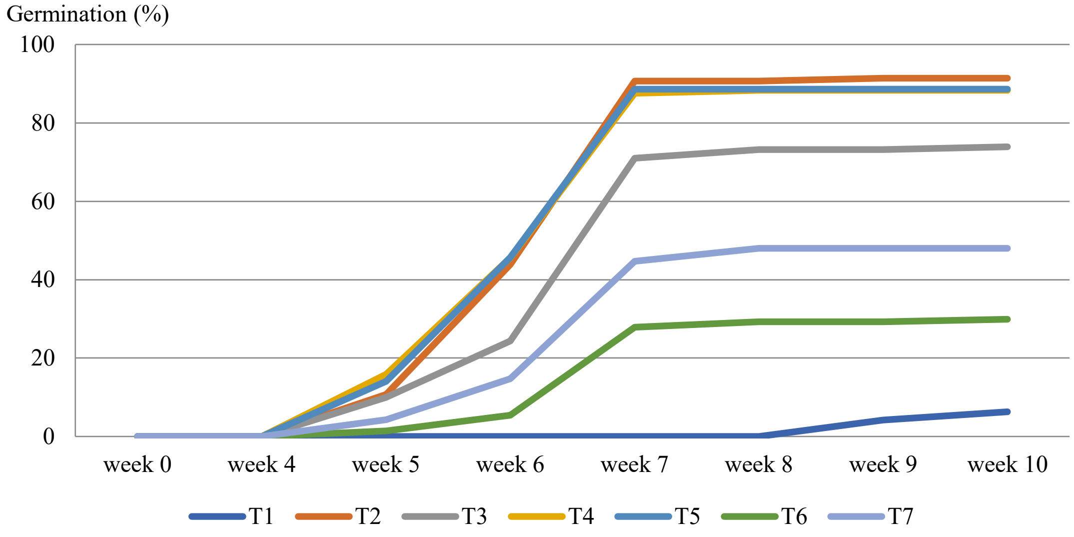

Figure 2. Percentage and speed of germination in different seed treatments
Şekil 2. Farklı işlemlere göre tohumların çimlenme oranları ve hızları

(2017). It was reported that GA_3 application increased seed germination in Taurus cedar (*Cedrus libani* A. Rich.), improved germination and seedling properties in *Eriobotrya japonica*, and increased seed germination in *Citrus aurantifolia* and different kiwi fruit varieties (Al-Hawezy, 2013; Okatan, 2017; Dilip). et al., 2017; Bishwas et al., 2018; Ayrancı and Öner 2019).

In this study, seeds treated with ultrasonic waves for 60 and 120 minutes had a germination percentage of 88.3 and 88.6, respectively. Compared to the germination percentage of the control group (6.3 %), it was found that ultrasound treatment both accelerated seed germination and increased germination percentage. The seeds treated with ultrasound started germinating 4 weeks before the control seeds.

The results of our study as regards the ultrasound treatment are consistent with the literature. Although there are no studies on the effects of ultrasound treatment on palm seeds, many studies on seeds of other species have shown that ultrasound treatment accelerates germination (Yaldagard et al., 2008; Wang et al., 2012; Luo, 2016; Nazari and Eteghadipour, 2018; Ameta et al., 2018; Wong et al., 2019). Ultrasound treatment accelerates and increases germination by enlarging the holes on the seed coat and facilitating water absorption. All these increase alpha-amylase activity causing faster germination (Yaldagard et al., 2008; Luo, 2016). In chickpea and mung bean, 60-minute ultrasound treatment increased water absorption by 2600% and 6350%, respectively, compared to the control group (Wong et al., 2019). Shekarı et al., (2015) reported that ultrasonic treatment of sesame seeds (*Sesamun indicum* L.) increased germination. López and Vicient (2017) also demonstrated that ultrasonic treatment did not have any effect on new seeds of *Arabidopsis thaliana*, while it increased germination further in older seeds. A study conducted by Kikuchi et al., (2006) demonstrated that water absorption in seeds was mostly through a hilum. Rişca and Fărtăış (2009) also obtained the best germination rate from the application over 40 seconds in their ultrasound study on *Picea abies*. In the study conducted by Poşta et al., (2020), it was determined that the seeds of *Liquidambar styraciflua L.* responded positively to ultrasound and the germination rate increased in the seeds. Machikowa et al., (2013) in their study on sunflower seeds, germination percentages were 44-48% lower than the others. Control when treated with ultrasonic at 80-100%. long-term higher intensity ultrasonic treatments possibly injury to the embryo. Treatments T6 and T7 (vacuum applications) increased both germination speed and percentages compared to the control group. As for the effect of duration of vacuum treatments on germination, seeds treated with vacuum for 60 minutes (29.9 %) had a lower germination percentage compared to those treated with vacuum for 120 minutes (48 %). Extended vacuum time increased germination by 20%. Custódio et al., (2016) reported that vacuum treated pine seeds increased the absorption of tetrazolium but it did not have an effect on the seeds of *Dactylorhiza fuchsii*.

In treatment T3, germination percentage was 73.9 %. The seeds treated width sodium hypochlorite had a higher germination percentage and speed compared to the control seeds.

4. Conclusion

In our study, there was no statistical difference in germination of seeds treated with GA_3 and seeds treated with ultrasound. GA_3 and ultrasound treatments accelerated germination by 4 weeks compared to the control group and increased the germination percentages. Since the germination process is accelerated by ultrasound application, losses due to environmental risks will be prevented. Given the cost and environmental impacts of GA_3, "ultrasound" can be used as a cheaper and more environmentally friendly option for cheaper and faster seed germination. Early-onset of seed germination will ensure that seeds will be less affected by negative biotic and abiotic factors and guarantee germination.

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