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Determination of Some Biological Properties of The Seeds of The Johnson Grass (*Sorghum halepense* (L.) Pers.)

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ABSTRACT

Dormancy is an important factor affecting seed germination and the emergence of weeds. In this study, the intensity of dormancy in freshly collected seeds of *Sorghum halepense* (L.) Pers. was determined, and various methods to break this dormancy were evaluated. These methods included sulfuric acid application, sanding, soaking in distilled water, folding method application, and gibberellic acid application. Additionally, the study aimed to determine the optimal, maximum, and minimum germination temperatures and assess seed germination at different burial depths. According to the experimental results, the most effective method for breaking dormancy was the application of 62.5% sulfuric acid for 30 minutes. The ideal germination temperature was found to be 30°C, with a germination rate of 87%. The minimum temperature for germination was 15°C (6% germination), and the maximum temperature was 40°C (48% germination). In terms of sowing depth, the highest germination rate was observed at a depth of 3 cm, where 50% of seeds germinated. It was also noted that the germination rate gradually decreased with increasing burial depth. The results of these experiments provide valuable information on effective methods for breaking seed dormancy in *Sorghum halepense* and offer insights into the optimal conditions for seed germination.

Keywords: Johnson grass, Dormancy, Seed, Seedling, Depth

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Kanyaş (*Sorghum halepense* (L.) Pers.) Bitkisinin Tohumlarının Bazı Biyolojik Özelliklerinin Belirlenmesi

ÖZ

Dormansi, tohum çimlenmesini ve yabancı otların ortaya çıkmasını etkileyen önemli bir faktördür. Bu çalışmada, taze toplanan *Sorghum halepense* (L.) Pers. tohumlarının dormans yoğunluğu belirlenmiş ve bu dormansı kırmak için çeşitli yöntemlerin etkinliği değerlendirilmiştir. Bu yöntemler arasında sülfürik asit uygulaması, zımparalama, distile suya batırma, katlama yöntemi uygulaması ve gibberellik asit uygulaması yer almaktadır. Ayrıca, tohum biyolojisi ve çimlenme çalışmaları açısından optimal, maksimum ve minimum çimlenme sıcaklıkları ile farklı ekim derinliklerinde tohum çimlenmesi belirlenmiştir. Deneme sonuçlarına göre, dormansı kırmak için en etkili yöntem %62.5 sülfürik asit uygulaması ve 30 dakika bekletme olmuştur. İdeal çimlenme sıcaklığı 30°C olarak bulunmuş ve bu sıcaklıkta çimlenme oranı %87'ye ulaşmıştır. Minimum çimlenme sıcaklığı 15°C (çimlenme oranı %6) ve maksimum çimlenme sıcaklığı 40°C (çimlenme oranı %48) olarak belirlenmiştir. Derinlikle ilgili yapılan çalışmalarda ise en yüksek çimlenme oranı 3 cm derinlikte elde edilmiş ve bu derinlikte %50 çimlenme gözlemlenmiştir. Ayrıca, tohum çimlenme oranının derinlik arttıkça giderek azaldığı gözlemlenmiştir.

Anahtar Kelimeler: Kanyaş, Dormansi, Tohum, Çimlenme, Derinlik

1. Introduction

Weeds, the unwanted guests of agriculture, have been a persistent challenge throughout human history, causing significant harm to agricultural production. This enduring struggle against weeds, which dates back to the dawn of agriculture, underscores the urgency and seriousness of the issue. One of the most formidable traits of weeds is their prolific seed production and remarkable adaptability to their environment. Moreover, the seeds they produce often exhibit a high dormancy rate [1, 2], a state in which a seed temporarily halts or slows its growth and development activities [3, 4]. Dormancy is a natural survival mechanism that ensures seeds do not germinate under unfavorable conditions, which enables the weed to persist over time and in various environments [27]. This state of dormancy can be caused by various internal and external factors, including seed coat characteristics, environmental cues, and chemical inhibitors present within the seed. Dormant seeds remain viable in the soil for extended periods, leading to the challenge of managing weed populations, as seeds may remain in the soil seed bank until conditions are favorable for germination [28]. The persistence of seed dormancy in weeds makes controlling their spread particularly difficult, as traditional weed management methods often fail to address the full life cycle of the weed, especially when seeds remain dormant in the soil. As such, understanding and breaking seed dormancy is a critical aspect of integrated weed management strategies aimed at reducing the seed bank and limiting weed emergence [27].

Sorghum halepense (Pers.) L., commonly known as Johnson grass, is a member of the Poaceae family and is categorized as a perennial weed. Biologically, it is a C4 plant, meaning it uses a specialized photosynthetic pathway that enables it to thrive in hot and dry environments, giving it a competitive advantage over many other plant species in such conditions. This adaptation not only enhances its survival but also contributes to its ability to invade diverse ecosystems. As a highly invasive weed, *Sorghum halepense* has shown a remarkable ability to spread rapidly, particularly in agricultural and non-agricultural areas across Asia, Africa, America, and Europe. The weed is reported associated with 30 crop plants in 53 countries [29-30]. Its wide distribution is attributed to its high seed production and adaptability to different environmental conditions, making it difficult to control through conventional methods.

Beyond its invasive nature, *S. halepense* is notorious for its high competitiveness with cultivated plants. It competes for essential resources such as water, nutrients, and sunlight, which negatively affects crop growth and agricultural yields. As a result, it is considered one of the most problematic weeds in crop production, particularly in the cultivation of soybeans. For instance infestation by weed during all growing season causes 59-88% reduction in soybean yield [31].

Moreover, *S. halepense* is commonly found in meadows, pastures, and disturbed lands, where it often outcompetes native vegetation. This widespread presence in grazing areas poses a threat to livestock, as it can reduce the availability of more nutritious forage and, in some cases, can even be harmful to grazing animals. The grass contains compounds such as cyanogenetic glycosides that can be toxic to livestock when consumed in large quantities, leading to potential health risks [32].

The invasive nature of *S. halepense* and its detrimental effects on both agricultural systems and grazing lands highlight the importance of effective management strategies to control its spread and reduce its impact on ecosystems and agricultural productivity.

Sorghum halepense is a plant that has the ability to reproduce via its seeds and rhizomes. Problematic in several plants, weed can enter a period called dormancy in its seeds, where it can overcome unsuitable conditions [9]. In this way, *S. halepense* is ensured to survive successfully in different conditions. Due to the biology of the plant, it becomes extremely difficult to control. Cultural methods remain ineffective during control procedures due to these biological traits. As a result of the failure of various herbicides used against *S. halepense*, the plant has spread and continues to spread over a wide area in the ecosystem [10, 11].

2. Material and Method

1. Material

The main material used in the study consists of the mature seeds of the Johnson grass (*S. halepense*) plant, which were collected from the cultivated areas in the Ereğli district of Konya in September 2018. After the seeds collected from the field were cleaned, they were stored at +4°C until the experiments were established.

2. Method

2.1. Dormancy Breaking Studies

Various methods have been used to break the intense dormancy found in Johnson grass seeds. For this purpose, dormancy-breaking studies started within a month from the seeds collected in 2018. Petri dishes with a diameter of 9 cm were used during the experiment, and two layers of Whatman filter papers were placed in each petri dish. Then, 20 seeds subjected to various treatments were placed in Petri dishes, 6 ml of pure water was added, and they were surrounded with parafilm. Daily observations were made, and pure water was added to the petri dishes when necessary. The trials were monitored daily for a month and concluded after 30 days. The experiments were set up according to the randomized parcel design with 4 replications and 2 repetitions, and since there was no statistical difference between the replicates, the results were combined [12, 13].

In order to statistically determine the effects of dormancy-breaking studies on Johnson grass seeds, they were grouped by the Duncan test, a widely used statistical test for comparing the means of different treatments, at a significance level of $p \leq 0.05$ using the SPSS (Statistical Package for Social Sciences 19.0) software package.

Dormancy-breaking methods used in the study are as follows;

a. Soaking in Distilled Water (H₂O): The collected weed seeds were kept in distilled water for 24, 48 and 72 hours.

b. Sulfuric Acid (H₂SO₄) Application: The seeds used in the experiment were kept in 96% purity sulfuric acid for 15, 30, 45, 60, 75, 90 seconds and 2, 3, 5, 10, 20 and 30 minutes. Expired seeds were quickly washed under running water to remove the sulfuric acid. Then, the seeds were washed with pure water and placed in petri dishes after drying.

c. Mechanical Sanding: The seeds were sanded using water sandpaper until the top of the seed coat was worn away. After the etching process, the seeds were arranged in petri dishes in an orderly manner.

d. **Folding Method Application:** The Sorghum seeds used in the experiment were wrapped in moistened drying papers and kept at +4°C and (-20°C) temperatures for 7, 15, 30, 45, and 60 days. After waiting, the extracted seeds were placed in petri dishes and monitored.

e. **Gibberellic Acid (GA₃) Application:** GA₃ concentrations prepared at different concentrations (500, 750, 1000, 1500 and 2000 ppm) were added to the blotting papers in Petri dishes, and the germination of the seeds to be used in the experiment was observed there [12-15].

2.2. Seed Depth Studies:

Following dormancy studies, 30 minutes of sulfuric acid application was determined as the ideal dormancy-breaking method used at this study stage. Sorghum seeds exposed to sulfuric acid for 30 minutes were planted in plastic containers (12 cm in diameter) at a depth of 3, 5, 7, 9, 12, and 15 cm, with 5 seeds. The experiment, which was performed on a clay loam structured and oven-sterilised soil, was set up with 4 replications and 2 repetitions. The trial was monitored for 30 days and checks and counts were taken daily. The experiments were set up in a controlled incubator at 24°C [12-15].

2.3. Seed Germination Temperature Studies:

In line with the data obtained at the end of the dormancy-breaking studies, the seeds were kept at 5, 10, 15, 20, 25, 30, 35, 40 and 45°C for 30 days as a result of the study established to determine the ideal germination temperatures of the Johnson grass seeds whose dormancy was broken with sulfuric acid. The experiments were set up in a controlled incubator for 21 days.

3. Results and Discussion

1. Dormancy Breaking Experiments:

Table 1. gives the results obtained from the dormancy studies. As a result of dormancy-breaking studies, germination data and statistics show that sulfuric acid application (62.5% for 30 minutes) is the most effective method. Research results in various literature reveal that applying sulfuric acid as an abrasive is extremely effective in various plants, especially with thick seed coats. Studies conducted by various researchers have shown that there is an intense dormancy in Sorghum plants, which is one of the biggest problems in agricultural areas [12, 16, 17].

Table 1. Results of dormancy breaking studies

Applications	Germination rate (%)
Control	0k
Sulphuric acid Application (H₂SO₃)	
15 sec	11h
30 sec	12h
45 sec	14g
60 sec	14g
75 sec	15fg
90 sec	14g
2 min	16f
3 min	15fg
5 min	18e
10 min	30d
15 min	54c
20 min	57b
30 min	62,a
Sanding	5j
Folding method	
4°C 7 day	0k

4°C 15 day	Ok
4°C 30 day	Ok
4°C 45 day	Ok
4°C 60 day	
(-20)°C 7 day	Ok
(-20)°C 15 day	Ok
(-20)°C 30 day	Ok
(-20)°C 45 day	Ok
(-20)°C 60 day	Ok
Soaking in distilled water	
24 hour	Ok
48 hour	Ok
72 hour	Ok
Gibberellik acid (GA₃) application	
500 ppm	Ok
750 ppm	Ok
1000 ppm	Ok
1500 ppm	Ok
2000 ppm	Ok

*P<0.05 significance value

Huang and Hsiao [18] conducted a study aiming to create various deformations in the thick weed seed coat with H₂SO₄. According to the results obtained, it has been reported that water and air can enter through the wounds opened in the seed coat; thus, the dormancy of weed seeds is broken, and seeds show the ability to germinate. Within this study we have conducted, it is seen that the application of sulfuric acid gives consistent results in breaking the seed dormancy of Sorghum. In contrast, there is no germination in the mechanical sanding method. Again, Monaghan [19] and Warwick and Black [20] confirm our study by reporting that removing the seed coat or applying sulfuric acid to break seed dormancy can yield successful results.

In another study, Yazlık and Üremiş [17] achieved a germination rate of 64.80% due to a 75-second sulfuric acid application. Their study showed a similar result due to the 30-minute application of sulfuric acid. When our study is compared with this, unlike our study, Yazlık and Üremiş kept the seeds at room temperature for 6 months after harvest. Our study revealed that setting the experiment immediately after harvest may have prevented a time-dependent dormancy break.

No results were obtained in this study due to applications such as folding, gibberellic acid applications, and keeping in distilled water without sanding the seed coat. A similar result was obtained in the study conducted by Yazlık and Üremiş [17] due to the application of gibberellic acid and potassium nitrate, which is parallel to our study. Huang and Hsiao [18] also reported in their study that the application of gibberellic acid was ineffective.

Mojtaba et al. [21] reported in their study that the application of sulfuric acid at a concentration of 95-98% promoted germination by breaking dormancy at the highest rate. This study, conducted in Iran, also has similar results to our study.

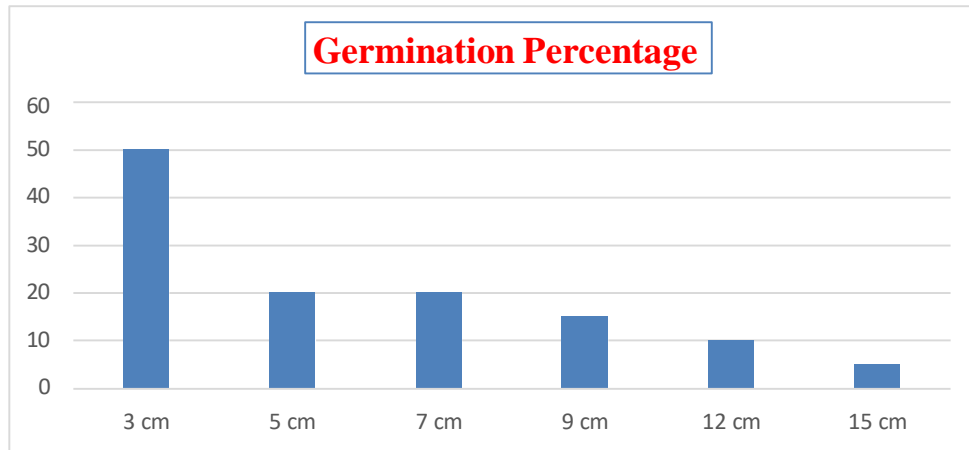
2. Seed depth studies

Five seeds were planted at depths of 3, 5, 7, 9, 12 and 15 cm. Seeds were checked daily and monitored for 30 days. At the end of 30 days, the total number of plants on the soil surface was counted, and germination percentages were calculated (Table 2). As a result of the experiment, it was determined that the best depth was 3 cm (50%), followed by 5 and 7 cm (20%), and this rate decreased as the depth increased. At a depth of 15 cm, this rate was recorded as 5%. Warwick and Black [20] reported in their study that the most suitable depth for germination of Johnson grass seeds is 7 cm, but seed germination can also occur at a depth of 15 cm. It is stated that this may be due to the type of soil used, light and temperature conditions, the age of the seeds used in the experiments and other environmental factors. Yazlık and Üremiş [17] report that the ideal germination depth is 10 cm (25%). In a similar study that was

carried out in our country, Kadioğlu [14] investigated the germination rates of 7 weed species in cotton fields at different temperatures and different depths. In the study, *S. halepense* seeds were planted at depths of 2, 5, 10, 15, 20 and 25 cm. The seeds were left to germinate for 18 months. As a result of the experiment, it was found that *S. halepense* seeds germinated best at a depth of 2 cm, with maximum germination at a depth of 25 cm. In their study, Podrug et al. [22] conducted a depth experiment with 10-year-old seeds and, in line with their results, stated that the most effective depth was in the range of 1-3 cm and that as the depth increased, the seed germination power decreased. Again, in various studies, it is mentioned that soils at a depth of 0-4 cm are ideal depths for emergence germination for *S. halepense* [22-25].

Several factors can influence seed germination during soil depth studies, including the number of seeds used, their proximity to each other, soil structure, temperature, and seed age. Our study used seeds harvested within 3 months and clay loam field soil as the soil type. These factors were chosen to represent typical conditions in agricultural settings and to provide a realistic context for the study.

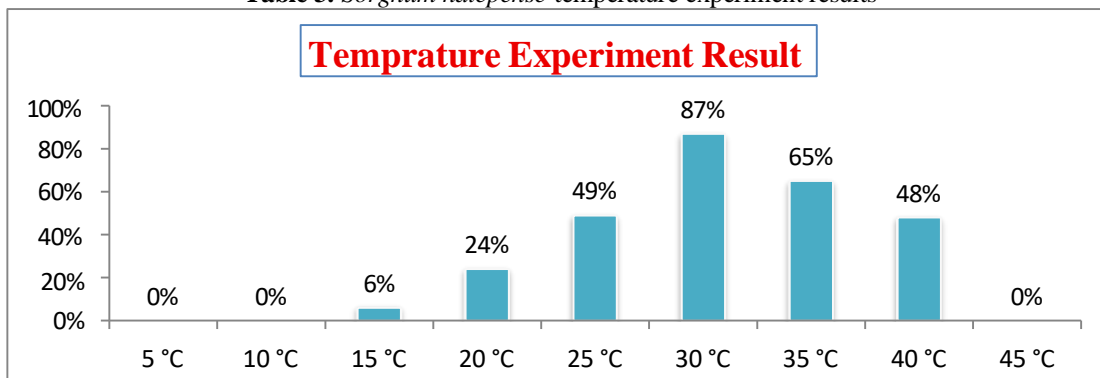
Table 2. Seed depth study of *Sorghum halepense*



3. Seed Temperature Trials

Sorghum seeds (3 months old), whose dormancy was broken after being subjected to 30 minutes of sulfuric acid application, were subjected to germination at 5, 10, 15, 20, 25, 30, 35, 40 and 45°C for 30 days. As a result of the study, while no germination was observed at 5, 10 and 45°C, the highest germination rate of 87% was detected at 30°C. This temperature was followed by 35°C with a 65% germination rate, and a 49% germination rate was observed at 25°C. In their study, Yazlık and Üremiş [17] found that the lowest germination temperature of weed seeds is 15°C the highest temperature is 40°C, and the ideal germination temperature is 25-30°C results fit our study. In another study conducted in Türkiye, Kadioğlu [14] determined the optimum germination temperature of *S. halepense* as 25-35°C, the minimum germination temperature as 25°C, and the maximum germination temperature as 40°C. In their study, Mojab et al. [21] concluded that the ideal germination temperature of weed seeds with broken dormancy is 25-45°C. In their study, Krenchinski et al. [26] stated that the seeds of *S. halepense* can germinate at the highest temperature of 30°C. They also investigated the effect of light on germination temperature during their study.

Table 3. *Sorghum halepense* temperature experiment results



4. Conclusion

Johnsongrass (*Sorghum halepense*) is a major perennial weed that poses serious challenges in both agricultural and non-agricultural areas. This plant is particularly troublesome due to its resilience and ability to spread rapidly, causing damage to crops and ecosystems. Working with plants like Johnsongrass, which have strong dormancy mechanisms, is a particularly difficult task for researchers and farmers alike. Dormancy is a condition in which seeds or plants delay germination or growth under favorable conditions, making it a significant obstacle to managing their spread. Because of this dormancy and the plant's ability to regenerate from its rhizomes and seeds, Johnsongrass is particularly difficult to control. Therefore, understanding the biology of the plant and developing strategies to manage it is a critical aspect of agricultural research and weed management.

In this study, researchers investigated the conditions under which Johnsongrass seeds germinate. The results showed that Johnsongrass seeds can begin to germinate at a minimum temperature of 15°C. However, the ideal temperature for maximum germination was found to be 30°C. This temperature range is critical for developing control strategies that target the early stages of seedling development. The study also found that the optimal seed planting depth for best germination was 3 cm. Planting seeds at this depth promotes more consistent germination, which could have implications for both crop management practices and weed control efforts.

One of the most notable findings of the study was the discovery that Johnsongrass seeds have a particularly hard seed coat. This hard seed coat is a significant barrier to germination and is one of the main reasons why Johnsongrass is difficult to control. To overcome this barrier, the researchers treated the seeds with sulfuric acid for 30 minutes. This treatment effectively broke down the tough seed coat, allowing the seeds to germinate more easily. By breaking down the physical defenses of the seed coat, this approach significantly improved the germination process. Interestingly, other methods of breaking seed dormancy - such as mechanical treatments or the use of other chemicals - were found to be insufficient to break the dormancy of Johnsongrass seeds.

These findings highlight the importance of understanding the unique biology and dormancy mechanisms of Johnsongrass in order to develop more effective control methods. This study, along with other similar research, is critical to advancing our understanding of Johnsongrass biology. It provides important insights that could help inform future scientific studies aimed at better management and control of this invasive plant. By laying the groundwork for future research, these studies are helping to develop innovative strategies to control Johnsongrass.

5. Declaration

5.1. Competing Interests: The authors declare that they have no conflict of interest

5.2. Author Contributions: İK and BŞ designed the study and set up the experiments, BŞ and CY conducted the study, BŞ and İK analysed the data, and BŞ, İK and CY wrote the article.

5.3. Ethics Committee Approval: Ethics committee approval is not required.

5.4. Acknowledgements: A part of this study was represented as an abstract in the Bialic Congress.

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