Türler ve Habitatlar

(2024) 5(1): 7–20
e-ISSN: 2717-770X
https://dergipark.org.tr/tr/pub/turvehab

## Research Article

https://doi.org/10.53803/turvehab.1425906

# Reproductive Ecology and Viability Assessment of *Jurinea cadmea* Subspecies Distributed in Western Anatolia: Implications for Conservation and Biodiversity

**Aida Tuğ*, Hasan Yıldırım**

Department of Biology, Faculty of Science, Ege University, TR-35040, İzmir, Türkiye
*Correspondence: Aida Tuğ, *aida.mujagic@gmail.com*

| Received: 26.01.2024 | Accepted: 29.02.2024 | Online: 02.06.2024 |
|---|---|---|

**Abstract**

In this study, the reproductive capacity of endemic *Jurinea cadmea* subspecies, *J. cadmea* subsp. *cadmea*, and *J. cadmea* subsp. *nifensis* was investigated, focusing on pollen, stigma, seed viability, and seed germination. Plant materials were collected from natural populations in Western Anatolia (Nif Mountain, Bozdağ, Babadağ and Muğla Oyuklu Mountain) between 2017 and 2020. The tests employed in this study included the MTT (2,5-diphenyl tetrazolium bromide-thiazolyl blue) test for pollen viability, the Peroxide test for stigma viability, the TTC (2,3,5-triphenyl tetrazolium chloride) test for seed viability, and seed germination tests under different conditions, with and without pre-treatment (wet-cold stratification). The study revealed high viability of mature pollen, stigma, and seeds, indicating successful reproductive capacity. Seed germination experiments showed that most populations exhibited high germination rates in light conditions. After 30 days of stratification, seeds showed very low germination in light and dark conditions, but after 60 days at 4°C, Nif Mountain, Bozdağ, and Muğla Mountain (short) populations exhibited germination rates above 80%. However, Babadağ population showed low seed germination in light and dark conditions. This study has significantly contributed to our understanding of the reproductive biology of both subspecies.

**Keywords**: *Jurinea cadmea* subspecies, endemic, Türkiye, reproductive capacity

# Batı Anadolu'da Yayılış Gösteren *Jurinea cadmea* Alt Türlerinin Üreme Ekolojisi ve Canlılık Değerlendirmesi: Koruma ve Biyoçeşitlilik Üzerine Çıkarımlar

**Özet**

Bu çalışmada, endemik *Jurinea cadmea* alt türlerinin, *J. cadmea* subsp. *cadmea* ve *J. cadmea* subsp. *nifensis*'in, polen, stigma, tohum canlılığı ve tohum çimlenmesine odaklanarak üreme kapasitesi araştırıldı. Bitki materyali, 2017-2020 yılları arasında Batı Anadolu'daki doğal popülasyonlardan (Nif Dağı, Bozdağ, Babadağ ve Muğla Oyuklu Dağı) toplandı. Bu çalışmada kullanılan testler arasında polen canlılığı için MTT (2,5-difenil tetrazolyum bromür-tiyazolil mavi) testi, stigma canlılığı için peroksit testi, tohum canlılığı için TTC (2,3,5-trifenil tetrazolyum klorür) testi ve farklı koşullarda tohum çimlenmesi için ön işlem (stratifikasyon) ile ve ön işlem olmadan yapılan testler bulunmaktadır. Çalışma, olgun polen, stigma ve tohumların yüksek canlılığını ortaya koyarak başarılı üreme kapasitesini gösterdi. Tohum çimlenme deneyleri, çoğu populasyonun ışık koşullarında yüksek çimlenme oranları sergilediğini gösterdi. Tohumlar, 30 gün stratifikasyon sonrasında ışık ve karanlık koşullarda çok düşük çimlenme gösterirken, 60 gün boyunca 4°C'de tutulduktan sonra Nif, Bozdağ ve Muğla (kısa) populasyonları %80'in üzerinde çimlenme oranları sergiledi. Ancak, Babadağ populasyonu hem

Suggested Citation:
Tuğ, A. & Yıldırım, H. (2024). Reproductive Ecology and Viability Assessment of Jurinea cadmea Subspecies Distributed in Western Anatolia: Implications for Conservation and Biodiversity. *Türler ve Habitatlar* 5(1): 7–20.

ışıkta hem de karanlıkta düşük tohum çimlenmesi gösterdi. Bu çalışma, her iki alt türün üreme biyolojisinin anlaşılmasına önemli bir katkı sağlamıştır.
**Anahtar kelimeler**: *Jurinea cadmea* alt türleri, endemik, Türkiye, üreme kapasitesi

## INTRODUCTION

The Asteraceae family stands out as one of the largest flowering plant families, boasting approximately 1,620 genera and over 23,600 species, making it a prominent member of the plant kingdom (Stevens 2001). In Türkiye, the Asteraceae family ranks as the second-largest family, comprising 134 genera and a total of 1,209 species, with 447 being endemic, contributing to a remarkable 37% endemism (Özhatay & Kültür 2006; Doğan et al. 2011). As part of the Asteraceae family, the *Jurinea* Cass. genus plays a vital role in ecological systems and offers insights into plant adaptation across diverse regions. With an extensive distribution spanning Central Asia, Iran, Türkiye, and the Eastern Mediterranean, this genus encompasses around 180 species (Szukala et al. 2019). *Jurinea*'s endemism in Türkiye is noteworthy, constituting approximately 42.1% of the genus (Aksoy et al. 2018). Eight species are endemic to Türkiye, particularly in the Mediterranean and Irano-Turanian phytogeographic regions, representing 18 species in total (Danin & Davis 1975; Doğan et al. 2007; 2009; 2010). The prevalence and endemism of *Jurinea* species in Türkiye highlight the genus's significance in contributing to the country's biodiversity. Numerous endemics within the *Jurinea* genus are threatened due to anthropogenic factors like deforestation, destruction of steppe vegetation, and the conversion of semi-desert areas for agricultural and industrial purposes.

*Jurinea cadmea* Boiss. is an endemic plant species, meaning it is native and restricted to a specific geographic region. Distinctive morphological features, such as the absence or rarity of a stem and the presence of scabrous pappus, set *Jurinea cadmea* apart from other species within the *Jurinea* genus (Danin & Davis 1975; Doğan et al. 2010). *J. cadmea*, a species with two subspecies, which are *J. cadmea* subsp. *cadmea* and *J. cadmea* subsp. *nifensis* Yıldırım & Şenol, holds ecological significance in Türkiye and Greece. While *J. cadmea* subsp. *cadmea* is distributed across Türkiye and Greece, *J. cadmea* subsp. *nifensis* is confined to a small range on Nif Mountain in Izmir, Türkiye, making it a local endemic taxon. According to Doğan (2012), *J. cadmea* subsp. *nifensis* was not accepted as a valid taxon and was considered synonymous under *J. cadmea*. However, as a result of a comprehensive revision study carried out by us on *J. cadmea*, which has not yet been published, *J. cadmea* subsp. *nifensis* distinctly separated from *J. cadmea* subsp *cadmea* both morphologically and molecularly. Thus, *J. cadmea* subsp. *nifensis* is treated as a valid taxon in this article. According to the Türkiye Plants Red Data Book, Ekim et al. (2000) suggested a potentially vulnerable (VU) categorization for *J. cadmea*. The impact of human activities has led to the critical endangerment (CR) classification for *J. cadmea* subsp. *nifensis* (Yıldırım & Şenol 2010) and the vulnerable (VU) classification for *J. cadmea* subsp. *cadmea* on the IUCN Red List. The critical endangerment of *J. cadmea* subsp. *nifensis* and the vulnerability of *J. cadmea* subsp. *cadmea* underlines the need for immediate action and a unified approach to protecting these valuable plant species. The unique morphological features and limited geographical ranges of *J. cadmea* subspecies highlight the urgency of conservation efforts.

Research on *Jurinea cadmea* has been limited, primarily focusing on morphological, molecular, cytological, and palynological studies. This study aims to enhance our understanding of the life cycles of the two taxa within *J. cadmea*, providing clarity on their taxonomic statuses.

Understanding the biological potential of *J. cadmea* subpopulations is crucial for effective conservation strategies. The study of pollen, stigma, seed viability, and seed germination is considered the only way to preserve the genetic diversity of populations. This becomes especially important in rare and endemic species and for species at risk of extinction. Understanding the germination abilities of these taxa is emphasized as crucial for their conservation and the continuity of generations. Species like *J. cadmea* may exhibit specific germination strategies adapted to local conditions, allowing them to respond to environmental changes and ensure their survival. Knowledge of pollen and stigma viability contributes to understanding reproductive barriers within and between *J. cadmea* populations. This information is crucial for addressing challenges related to gene flow and maintaining genetic diversity. Environmental factors such as temperature, humidity, and pollinator's presence can impact pollen and stigma viability in *J. cadmea* species. Studies on how these factors affect reproductive success are vital for conservation and cultivation efforts. Knowledge of seed germination is a critical event that shapes the life cycle of plants like *J. cadmea*, influencing population dynamics, genetic continuity, and the overall health of ecosystems. Understanding the germination requirements of *J. species* is crucial for reintroducing them into their native habitats and restoring ecosystems.

The findings of this research are expected to enhance knowledge regarding the reproductive characteristics of *Jurinea cadmea*, including pollen, stigma, seed viability, and seed germination. The comprehensive insights gained will not only contribute to the scientific understanding of this species but will also serve as a foundational resource for informed conservation efforts, ensuring the preservation of biological diversity in the country.

## MATERIAL AND METHOD

### Material

Plant materials for both *Jurinea cadmea* subspecies were collected during the vegetation seasons of 2017-2020 from natural populations in the following regions of Western Anatolia: Izmir / Nif Mountain, Izmir / Bozdağ, Denizli / Babadağ, and Muğla / Oyuklu Mountain (Table 1, Figure 1). Sampling was conducted during the flowering period (May-July) and the fruiting period (June-October) to encompass the entire vegetation cycle of *J. cadmea*. Plant materials, including flowers, were collected during the flowering period to capture the reproductive stage, while achenes and other reproductive structures were collected during the fruiting period to represent the later stages of the vegetation cycle.

### Seed morphology of *Jurinea cadmea* subsp. *cadmea*

Achenes are light brown, tetragonal, longitudinally striped, and crowned, 3.06-6.10 × 1.06-2.66 mm. The pappus is scabrous, dirty white, 8.34-15.18 mm, generally with 2-5 hairs longer than the others. When mature, the pappus does not emerge from the achene. Flowering time is May-June (population in Muğla), July-October (population in Bozdağ), and August-October (population in Babadağ). Achene maturity is June (population in Muğla), August-October (population in Bozdağ), and September-October (population in Babadağ, Figure 2).

The research areas in Bozdağ, Babadağ, and Muğla Oyuklu Mountain are characterized by rocks of silica origin, such as schist and gneiss. Notably, the population of *Jurinea cadmea* in Muğla was first recorded in 2019, and the study related to this population represents the first research conducted on it. In the populations of *J. cadmea* in Babadağ and Muğla Oyuklu Mountain, two forms have been identified: a short-stemmed form - s (with capitula on short peduncles up to 6.55 cm) and a long-stemmed form - l (with capitula on long peduncles up to 29 cm) (Figure 3). This variation in forms suggests a possible transitional form between *J. cadmea* and *J. mollis* species.

**Table 1**. The locations of studied populations of *J. cadmea* subspecies in Türkiye.

| ***Location*** | ***Subspecies*** | ***Coordinates*** | ***Altitude (m)*** |
|---|---|---|---|
| İzmir / Nif Mountain | *Jurinea cadmea* subsp. *nifensis* | 38;23;22.7280 N - 27;21;24.437999 E<br>38;23;3.32399 N - 27;21;18.929999E | 1457<br>1441 |
| İzmir / Bozdağ | *Jurinea cadmea* subsp. *cadmea* | 36;31;46.64999 N - 29;11;2.172000 E<br>38,19;20.07899 N - 28;6;14.491999 E<br>38;19;26.95199 N - 28;6;0.0660000 E<br>38;19;10.16399 N - 28;6;41.831999 E | 1924<br>2160<br>2125<br>2054 |
| Denizli / Babadağ | *Jurinea cadmea* subsp. *cadmea* | 37;42;9.761999 N - 28;59;27.77400 E<br>37;41;44.60999 N - 28;59;25.04399 E<br>37;41;36.00000 N - 28;59;29.04600 E<br>37;42;8.945999 N - 28;59;31.54200 E | 2039<br>1895<br>1831<br>2012 |
| Muğla / Oyuklu Mountain | *Jurinea cadmea* subsp. *cadmea* | 37;16;14.95800 N - 28;26;36.37200 E<br>37;16;16.64999 N - 28;26;30.72599 E<br>37;16;15.26399 N - 28;26;30.31799 E | 1601<br>1636<br>1624 |

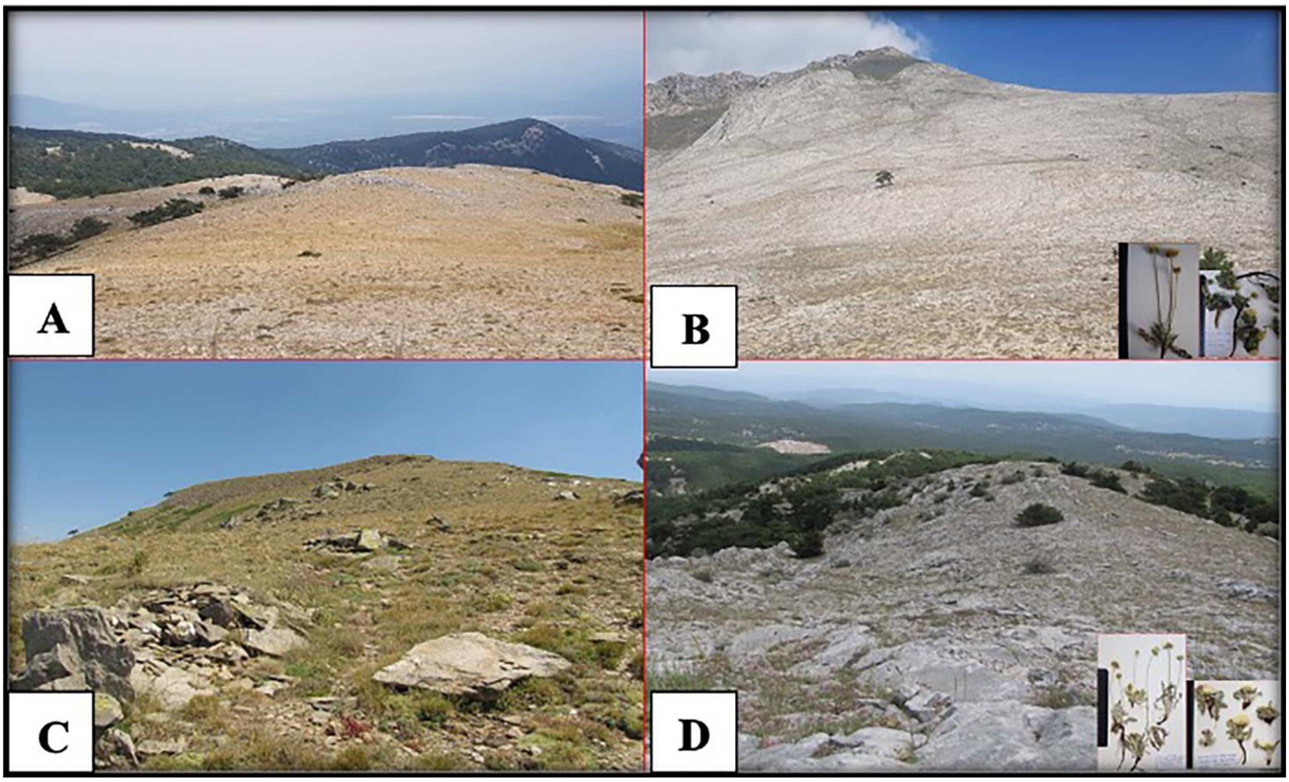


**Figure 1**. Studied populations of *Jurinea cadmea* subspecies (photos by Aida Tuğ). A) İzmir / Nif Mountain – *J. cadmea* subsp. *nifensis*; B) Denizli / Babadağ – *J. cadmea* subsp. *cadmea*; C) İzmir / Bozdağ – *J. cadmea* subsp. *cadmea*; D) Muğla / Oyuklu Mountain – *J. cadmea* subsp. *cadmea.*

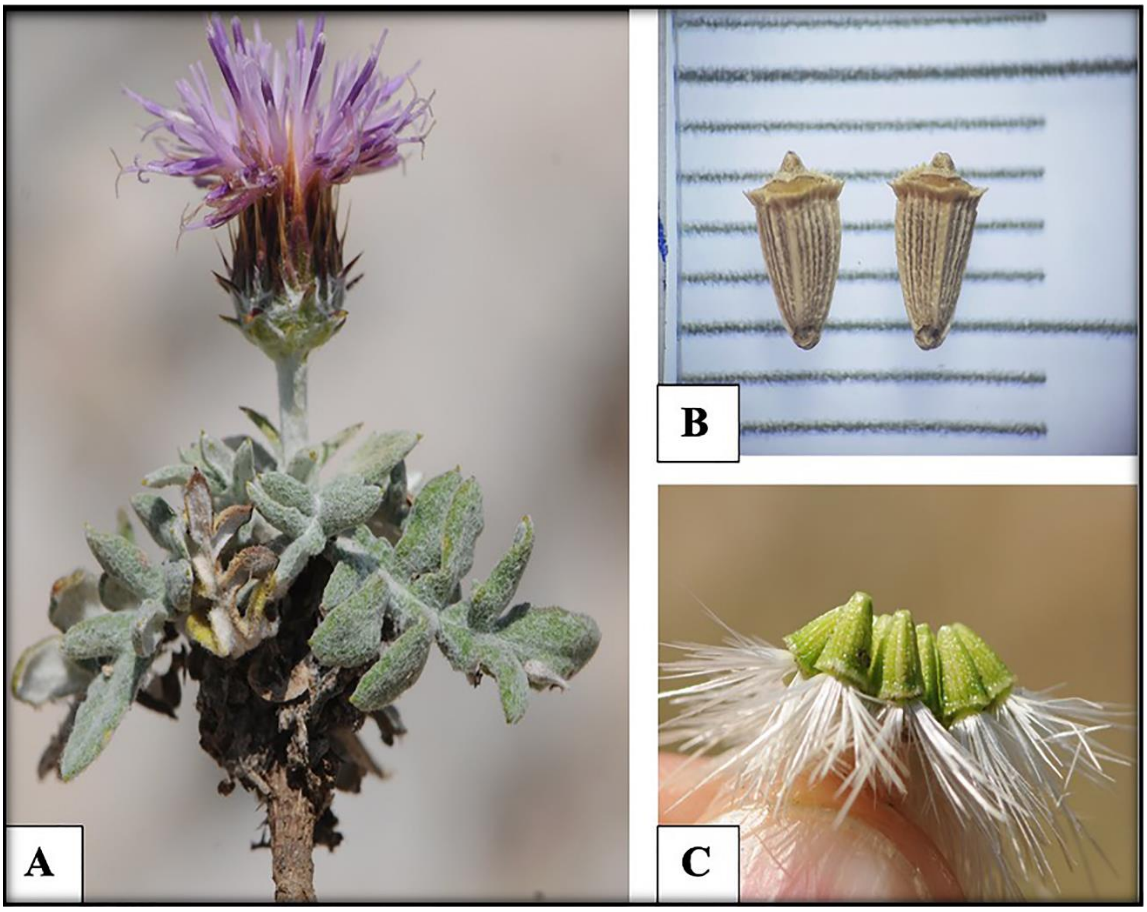


**Figure 2**. *Jurinea cadmea* subsp. *cadmea.* A) Plant's general appearance; B-C) Achenes.

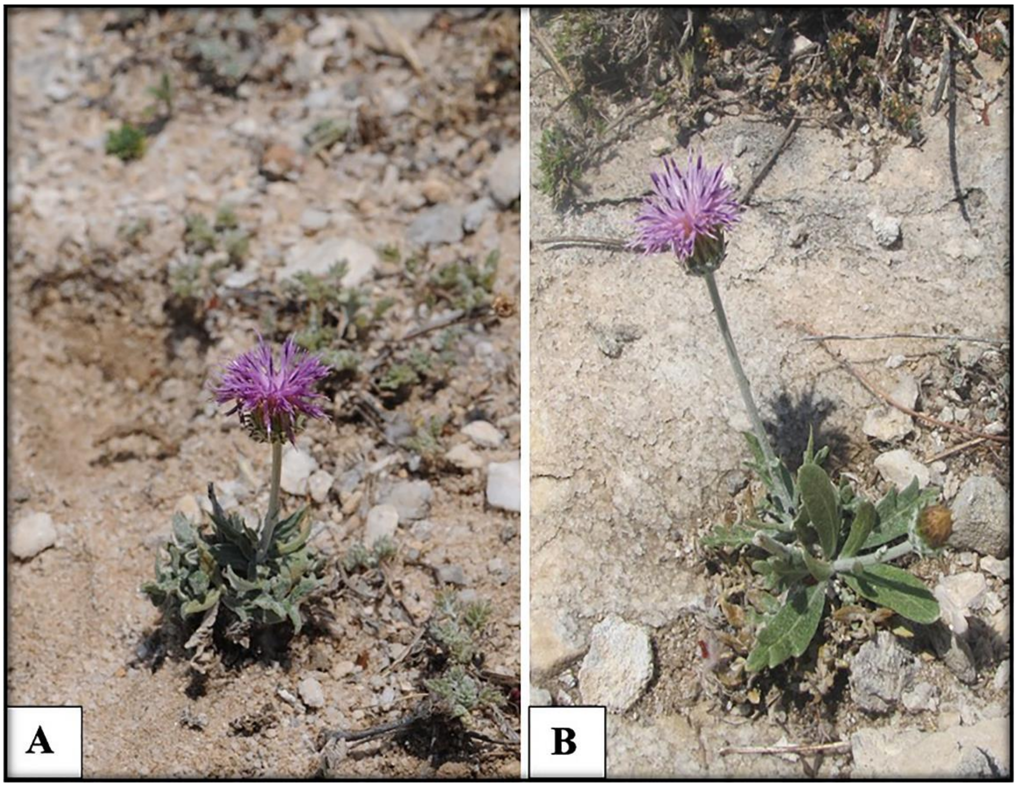


**Figure 3**. *Jurinea cadmea* subsp. *cadmea.* A) short form; B) long form.

***Seed morphology of Jurinea cadmea* subsp. *nifensis***

The achenes are tetrahedral, 4-5 × 1.5-2 mm, not glandular hairy, lightly lined, longitudinally striped light brown and transversely striped dark brown, naked. The pappus is 10-17 mm long, whitish, and scabrous. Mature pappus does not persist on the achene (Figure 4). This subspecies was described by Yıldırım and Şenol (2010) based on specimens from Nif Mountain in Izmir. It is distributed on calcareous soils at elevations between 1400-1500 m in the Nif Mountain within the boundaries of the Kemalpaşa district of Izmir. It has a restricted distribution in high elevations and open subalpine regions. Due to anthropogenic pressures, this subspecies is classified as critically endangered according to the IUCN Red List.

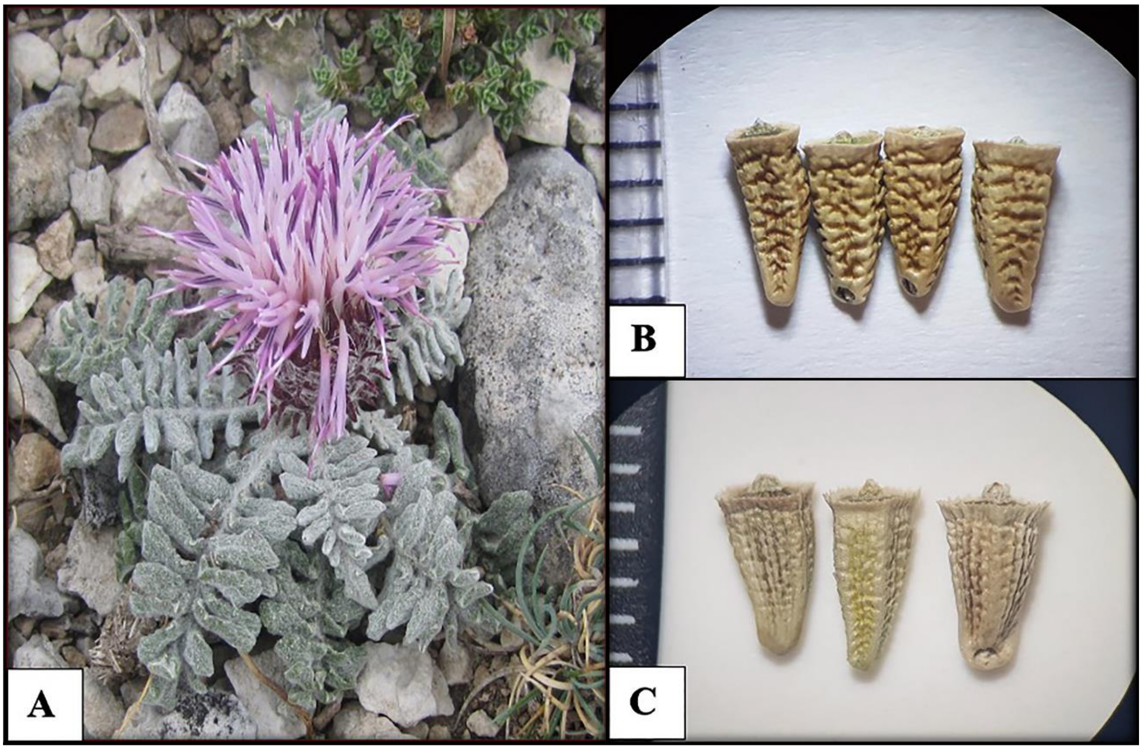


**Figure 4**. *Jurinea cadmea* subsp. *nifensis.* A) Plant's general appearance, B-C) Achenes.

## Methods

***Pollen viability analyses***

To assess the pollen viability of the target taxa, *Jurinea cadmea* subsp. *cadmea* and *J. cadmea* subsp. *nifensis,* we employed a 5% sucrose solution in the MTT (2,5-diphenyl tetrazolium bromide-thiazolyl blue) test (Rodriguez-Riano & Dafni 2000; Zeng-Yu et al. 2004; Dafni 2007). Given the occurrence of secondary pollen presentation in *J. cadmea*, fresh pollen grains were randomly collected both before the presentation (pre-anthesis) and during the anthesis stage (Figure 5). A total of 108 samples from fresh buds and flowers (36 plants) of both subspecies underwent the MTT test for pollen viability analysis. Using fine tweezers, pollen grains were transferred onto glass slides, and 1-2 drops of the MTT solution were added. Pollen grains, with a minimum of 300 grains per preparation field, were counted. The viability percentage for each phase was calculated by dividing the number of colored pollen grains by the total number of pollen grains in the field of view and multiplying by 100. In the MTT test, live pollen grains exhibiting a dark purple or black color change due to the presence of the dehydrogenase enzyme in the mitochondria were distinguished from non-viable or damaged pollen, which did not undergo any color change.

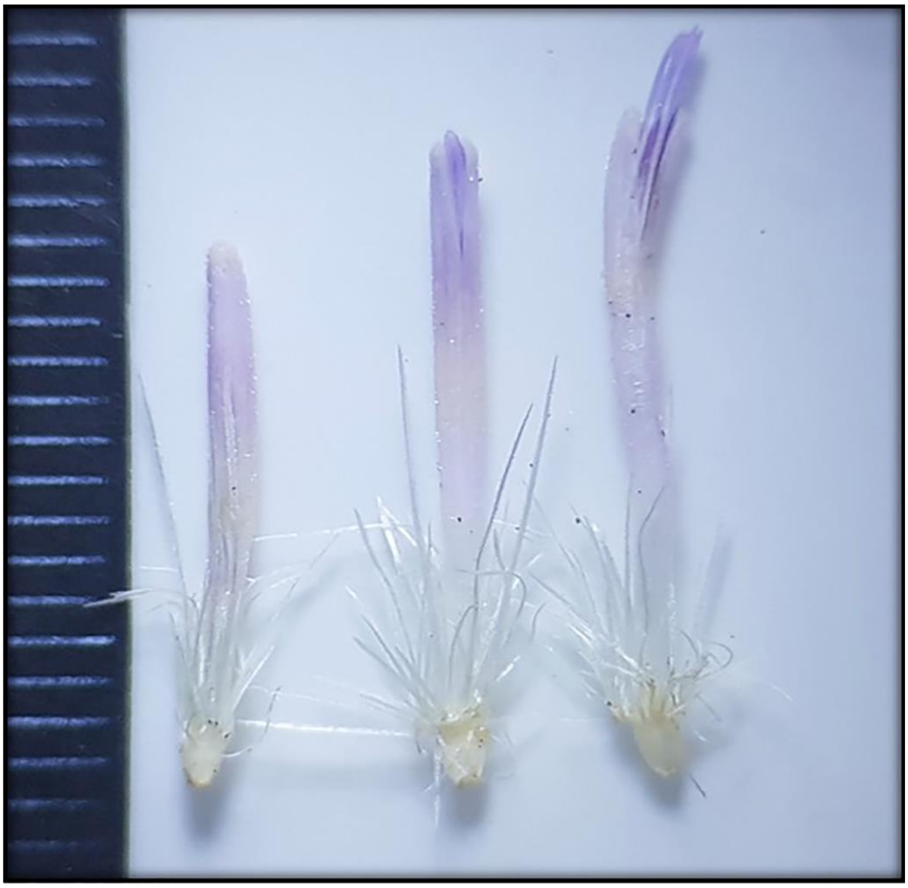

**Figure 5**. *Jurinea cadmea* flowers in pre-anthesis and anthesis phases.

***Stigma viability analyses***

We employed the Peroxide test to evaluate the stigma viability in the studied subspecies of *Jurinea cadmea* (Dafni & Firmage 2000). This test is designed to detect the presence of hydrogen peroxide in tissues. When hydrogen peroxide is present, the enzyme induces a color change to dark orange. The test kit includes a color scale indicating peroxide concentrations in the 10-5000 ppm range. Fresh flowers after secondary pollen presentation, just before fruiting, were utilized for analyzing stigma viability. A total of 108 stigmas, obtained from samples collected from 36 individuals, were included in this study. The procedure involved adding one or two drops of the Peroxide test solution to the isolated stigma on a glass slide. After waiting 1-4 minutes for the enzyme to activate, the color of the tissue was compared to the Peroxide test scale values to determine the viability of the stigma. Compared to the Peroxide test scale, the tissue's color change enabled the determination of stigma viability.

***Seed viability test***

A total of 180 mature achenes were collected from four populations of each of the two subspecies, each with two replicates. The potential seed viability was assessed using the TTC test. Seeds were soaked between moistened filter papers for 24 hours. Subsequently, they were soaked in a 1% TTC solution for one day. Viability levels were determined by observing color changes. The tetrazolium solution is initially colorless but turns red when it comes into contact with hydrogen produced by enzymes during the respiration process (reduction process). Live embryos turn red, while partially stained or unstained embryos are considered non-viable.

***Seed germination tests***

To determine seed germination, achenes were collected at full maturity, cleaned, and stored in a dark place at room temperature until the experiments were conducted. A total of 720 achenes were used for germination trials, encompassing all four populations. Before the experiment, achenes were immersed in a 5% sodium hypochlorite solution for 1 minute to prevent mold formation. For the stratification method, achenes were placed between two layers of filter paper in an 8 cm petri dish and moistened with distilled water to maintain moist and dark conditions. The dishes were then wrapped in aluminum foil and kept in the refrigerator at 4°C for 30 and 60 days (two groups consisting of 40 achenes each per working population). Two groups with 20 achenes each from each population were used for control. For the room temperature germination method, achenes were arranged between two layers of filter paper in an 8 cm petri dish and kept in both bright and dark environments at room temperature. Distilled water was added during the experiment to keep the filter paper moist. Every 3-4 days, germinated achenes were removed from the petri dish. Seeds were considered germinated if they were the same length or longer than the achene. These seed germination methods provide insights into the reproductive success and potential of *J. cadmea* subspecies, contributing valuable information for further research and conservation initiatives. The experiment was set up as follows: **Control group (room temperature)**: two repetitions with 20 seeds each were placed between moistened filter papers in a petri dish. a) Kept at room temperature in a bright environment for 30 days. b) Kept at room temperature in a dark environment for 30 days. **Stratification group (cold-wett treatment)**: two repetitions with 40 seeds each were placed between moistened filter papers in a petri dish for stratification. a) After 30 days at +4°C, transferred to 20°C and kept in both bright and dark environments for an additional 30 days. b) After 60 days at +4°C, transferred to 20°C and kept in a bright environment for an additional period. In both the control and stratification groups, the petri dishes were regularly checked to maintain the moisture of the filter papers. Every 3-4 days, germinated seeds were removed from the dishes for assessment. The experiment aimed to observe the germination responses of *J. cadmea* subspecies seeds under different conditions, including room temperature and stratification.

## RESULTS AND DISCUSSION

The pollen viability and stigma activity for *Jurinea cadmea* subsp. *nifensis* were observed to be approximately 74.18% before presentation in the male phase and 52.13% during presentation. The Bozdağ population showed an average pollen viability of 76.60% before presentation in the male phase and 53.13% during presentation. The Babadağ population (short form) exhibited a pre-presentation pollen viability of 89.39% and a pollen presentation viability of 79.39%. In the long-form Babadağ population, the pre-presentation pollen viability was 82.28%, and it was 65.23% during the presentation. In all studied populations, it was observed that all stigmas were active in the female phase (Figures 6 and 7). Pollen viability and stigma activity tests were not conducted for the Muğla Oyuklu Mountain populations in this study.

Based on the staining intensity with the tetrazolium solution, live seeds are stained red, while partially stained or unstained seeds are considered non-viable (Figure 8). Using the tetrazolium test, it was determined that the majority of seeds in all studied populations were viable (Figure 9).

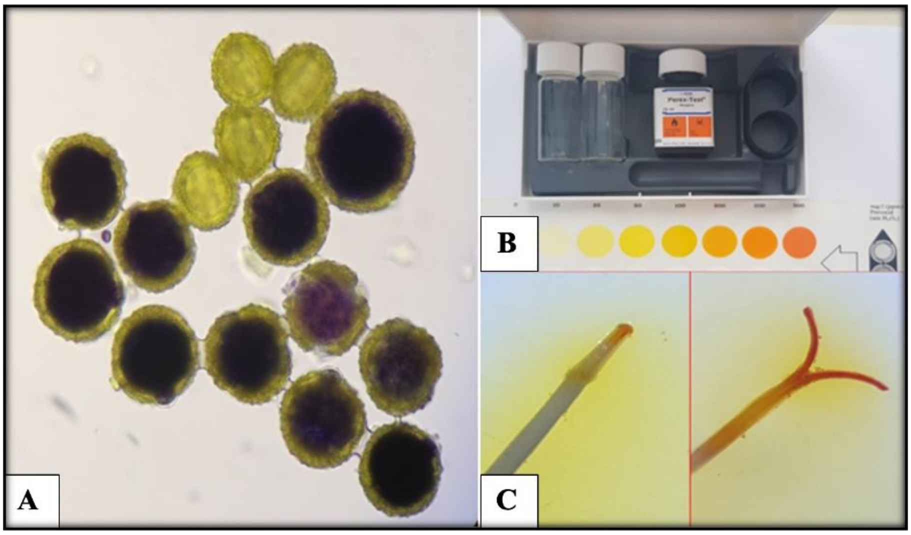


**Figure 6**. Pollen and stigma viability. A) Live (purple dark) and death (yellow) pollen grains; B) Peroxide test scale for determination of stigma viability; C) Viable stigmas.

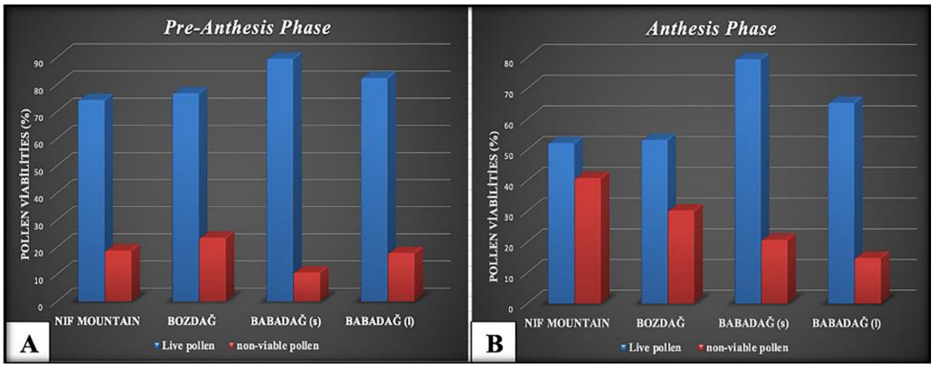


**Figure 7**. Pollen viability test. A) Pre-anthesis phase; B) Anthesis phase.


**Figure 8**. Non-viable seed (-); viable seed (+).

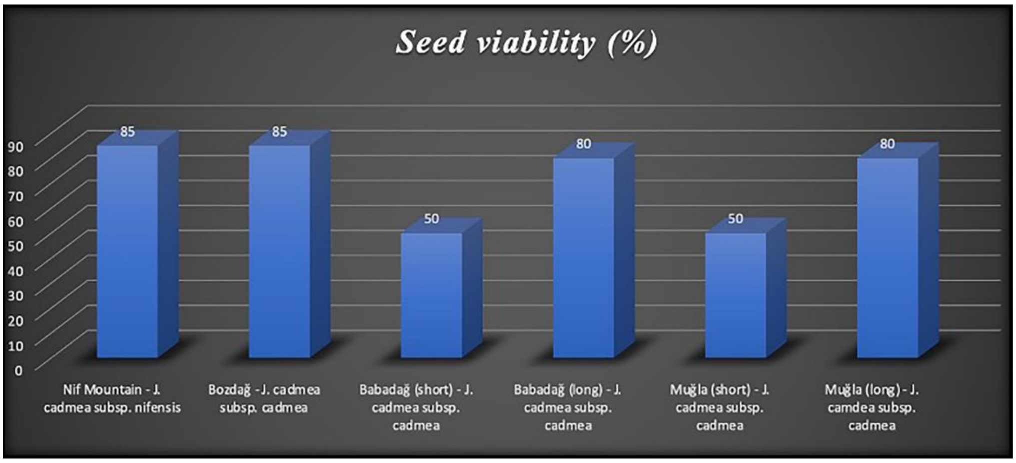


**Figure 9**. Seed viability in *Jurinea cadmea* subspecies showed by populations.

When seeds were examined for germination without pre-treatment (control) in a lighted area, most populations in the study exhibited high germination rates. An exception was observed in the Muğla Oyuklu Mountain short population and Babadağ short population, where the germination percentage was slightly lower than in other examined populations. The germination percentage was 25% in the Muğla Oyuklu Mountain short population and 65% in the Babadağ short population. For most populations studied, the minimum time for germination to start ranged between 5 to 7 days. Seeds from the Nif Mountain population showed 100% germination on the seventh day of the experiment. In the other studied populations, seeds reached maximum germination between 14 to 18 days after the initiation of the experiment (Figure 10).

When seeds were examined for germination in the dark without pre-treatment (control), seeds obtained from the Nif Mountain and Bozdağ populations showed germination rates exceeding 60%. In other studied populations, germination ranged between 10% and 40%. While the minimum germination time in the dark was 3 to 5 days for the Nif Mountain and Babadağ populations, it ranged from 10 to 12 days for seeds from the Bozdağ and Muğla Oyuklu Mountain populations. Seeds obtained from the Nif Mountain population reached maximum germination (85%) in the dark after 18 days of initiating the experiment. Seeds obtained from the Bozdağ and Babadağ populations reached maximum germination after 20 and 26 days, respectively, from the initiation of the experiment in the dark. Regardless of light conditions, seeds from the Muğla Oyuklu Mountain population showed low germination without pre-treatment (Figure 10).

To determine whether the stratification process influenced the germination of *Jurinea cadmea* subspecies seeds and to what extent seeds from the studied populations were subjected to stratification after pre-treatment (control). For this purpose, the seeds were kept in the refrigerator at 4°C for 30 and 60 days. After 30 days of stratification at 4°C, the *J. cadmea* subspecies seeds were divided into two groups and kept at room temperature in light and dark conditions. Germination of seeds was monitored and recorded daily, and the experiment was stopped after 30 days when no further changes in germination were observed. The seeds of the *J. cadmea* subspecies showed very low germination in light and dark conditions after 30 days of the stratification process (Figure 10). The seeds obtained from the Nif Mountain and Muğla Oyuklu Mountain populations did not germinate. Seeds from the Bozdağ population showed slightly better germination in the dark (15%) compared to the illuminated place (5%). On the other hand, seeds from the Babadağ population exhibited better germination in the illuminated place (25% for the short form and 45% for the long form) compared to the dark place (15% for the short form and 40% for the long form).

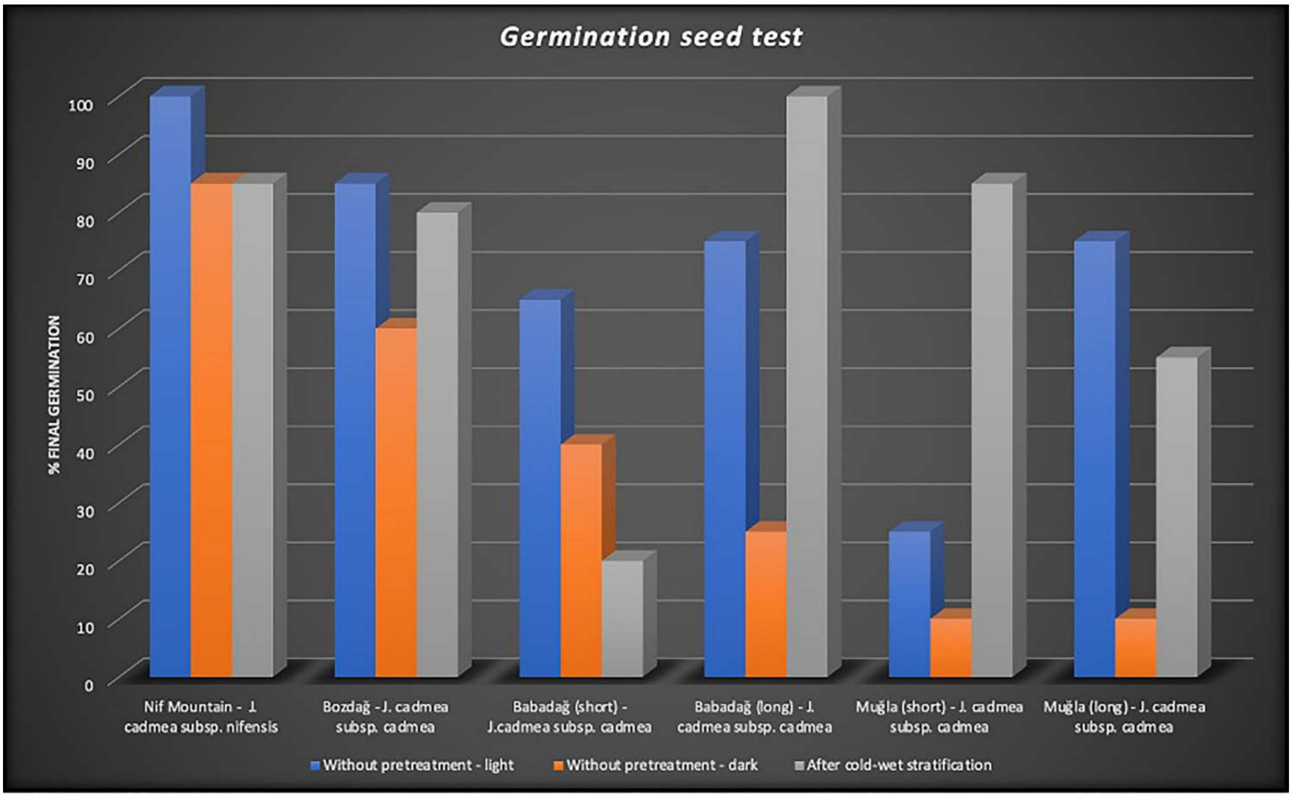


**Figure 10**. Germination seed test by populations and by treatments.

After 60 days of seed stratification at 4ºC, the germination percentage of seeds in most studied populations was above 60%. Specifically, the germination percentage for the Nif Mountain population was 85%, Bozdağ was 80%, Babadağ (short form) was 15%, Babadağ (long form) was 55%, Muğla Oyuklu Mountain (short form) was 85%, and Muğla Oyuklu Mountain (long form) was 55% (Figure 10).

Later, the seeds were left in a lighted place at room temperature, and changes in germination were monitored. The experiment was terminated because there was no change in germination after the third day. The germinated seeds were recounted, and the percentages were determined. While the germination percentages of seeds from the Nif Mountain, Bozdağ, and Muğla Oyuklu Mountain populations remained unchanged, the germination percentage of seeds from the Babadağ population increased after three days. For the short individuals, the germination percentage became 20% (an increase of 15% from before), and for the long individuals, it became 100% (a rise of 55% from before).

This study examined the reproductive capacity of two subspecies of *Jurinea cadmea* (pollen, stigma, seed viability, and seed germination) to determine some fundamental features of their reproductive biology. Seed production is closely related to pollen and stigma viability (Dafni & Firmage 2000; Wilcock & Neiland 2002; Hu 2005), and it is known to facilitate the successful realization of reproductive capacity, an essential prerequisite for maintaining the population size (Wang et al. 2012). The study explored seed germination by subjecting the seeds of the studied subspecies to a cold-wet folding pretreatment, aiming to assess the impact of the stratification process on germination. High viability for both pollen and stigma (above 70% for individuals from Nif Mountain and Bozdağ populations, and above 80% for individuals from Babadağ population), and high seed viability (above 85% for individuals from Nif Mountain and Bozdağ populations, above 80% for individuals from Babadağ-short, above 50% for individuals from Babadağ-long, above 80% for individuals from Muğla Oyuklu Mountain -short, above 50% for individuals from Muğla Oyuklu Mountain-long) indicate that the reproductive capacity was successfully achieved in both *J. cadmea* subspecies. Most seeds from the studied *J. cadmea* subspecies showed high germination without pretreatment (above 65% in most populations). Maximum germination percentage (100%) was observed in the first seven days for seeds obtained from Nif Mountain population (*J. cadmea* subsp. *nifensis*), while for the other *J. cadmea* subsp. *cadmea* populations, maximum germination was observed 14 to 18 days after the experiment was set up. The faster and maximum germination of *J. cadmea* subsp. *nifensis* seeds may be attributed to the absence of glandular hairs on the seed surface and potentially thinner seed coat. These reasons should be approached with caution until additional analyses are conducted on the germination of seeds of the *J. cadmea* subspecies. Muğla Oyuklu Mountain population seeds (25%) showed lower germination without pretreatment compared to the other studied populations, and the lower germination in these populations is thought to be due to the short waiting period (7 months) after seed collection. Seeds of other populations were collected and left to wait for more than 12 months. According to Urbanska and Schütz (1986) and Körner (1999), alpine plants may not germinate in the current season and enter a dormancy process to avoid low winter temperatures and be ready to germinate early in spring. The dormancy process terminates the embryo’s development, removing physical constraints in the seed coat through alternate freezing and thawing. After 30 and 60 days of stratification pretreatment, seed germination significantly decreased in all studied populations, indicating a negative impact of the stratification process on the germination of *J. cadmea* subspecies. Our results partly overlap with the findings of Giménez-Benavides et al. (2005), who studied the germination of 20 Mediterranean species from higher altitudes. In most studied subspecies, high seed germination was found without any pretreatment. Larger seeds did not germinate after the stratification process in this study, while *Biscutella laevigata* L. subsp. *gredensis* (Guinea) Malag. and *Senecio pyrenaicus* L. subsp. *carpetanus* (Willk.) Rivas Mart. lost their viability during the stratification process.

This study aimed to understand better the life cycles of the two subspecies of *Jurinea cadmea*, namely *J. cadmea subsp. cadmea* and *J. cadmea* subsp. *nifensis*, by examining reproductive biology features such as pollen, stigma, seed viability, and seed germination. Both subspecies exhibited high viability rates and successful reproductive capacity, indicating the importance of maintaining population size and capacity. This study has contributed significantly to understanding the reproductive biology of both subspecies, laying a foundation for future research endeavors that delve deeper into the intricacies of the *J. cadmea* subspecies' variability and taxonomic status. Successful seed germination ensures the continuity of the species. It allows for the transmission of genetic information from one generation to the next, facilitating the persistence of *J. cadmea* in its natural habitat. Understanding this interconnectedness provides insights into the plant's reproductive strategies, environment adaptation, and potential challenges it may face in maintaining viable populations. Conservation efforts should consider these aspects to promote the overall reproductive success and sustainability of *J. cadmea* in its native habitats.

However, further research is needed to understand these two subspecies, intra-population and inter-population relationships, further research is needed, encompassing genetic, effects of phytohormones, climatic, phytosociological, taxonomic, pedological, and other variability factors. Such studies could provide more detailed insights into the variability and taxonomic status of the *Jurinea cadmea* subspecies.

**ACKNOWLEDGEMENTS**

We express our gratitude to the Scientific Research Projects (Bilimsel Araştırma Projeleri - BAP) for their support of our research (Project Number: 18-FEN-018).

**AUTHOR CONTRIBUTION STATEMENT**

In this study; the authors made the study idea and design, data collection, analysis and interpretation of the results, and drafting of the article.

**REFERENCES**

Aksoy, N., Ataşlar, E. & Keskin, B.C. (2018). Morphological and molecular data reveal a new species of *Jurinea* (*Asteraceae)* from Western Black Sea Region, Turkey. Phytologia Balcanica 24(3): 351-359.

Dafni, A. & Firmage, D. (2000). Pollen viability and longevity: practical, ecological, and evolutionary implications. Plant Syst Evol 222(1-4): p.113-132. DOI: https://doi.org/10.1007/BF00984098.

Dafni, A. (2007). Manual of Pollination Ecology: Field Methods, (Text book) Laboratory of Pollination Ecology. Institute of Evolution University of Haifa.

Danin, A. & Davis, P.H. (1975). [*Jurinea* Cass.] In: Davis P.H. (Ed.). *Flora of Turkey and the East Aegean Islands*. Vol. 5. Edinnurgh University Press, Edinburgh.

Doğan, B., Duran, A. & Hakkı, E.E. (2007). Phylogenetic relationship analysis of *Jurinea* Cass. (Asteraceae) species from Turkey as revealed by Inter-Simple Sequence Repeat Amplification. *Ann Bot Fennici* 44(5): 353-358.

Doğan, B., Duran, A. & Hakkı, E.E. (2009). Numerical analyses of wild *Jurinea* spp. (Asteraceae) in Turkey. *Bangladesh J Bot* 38(1): 47-53. DOI: https://doi.org/10.3329/bjb.v38i1.5118.

Doğan, B., Hakki, E.E. & Duran, A. (2010). A phylogenetic analysis of *Jurinea* (Compositae) species from Turkey based on ITS sequence data. *African J Biotechnol* 9(12): 1741-1745. DOI: https://doi.org/10.5897/AJB10.1525.

Doğan, B., Duran, A., Martin, E. & Hakki, E.E. (2011). Karyotype analyses of the species of the genus *Jurinea* Cass. (Compositae) in Turkey. Afr J Biotechnol 10(5): 722-729. DOI: https://doi.org/10.5897/AJB10.907

Doğan, B. (2012). [*Jurinea* Cass.] In: Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M.T. (Eds.) *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, pp. 176–177.

Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z. & Adıgüzel, N. (2000). *Türkiye Bitkileri Kırmızı Kitabı*. Yüzüncü Yıl Üniversitesi ve Türkiye Tabiatını Koruma Derneği, Ankara.

Giménez-Benavides, L., Escudero, A. & Péez-García, F. (2005). Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability. *Ecol Res* 20(4): 433-444. DOI: https://doi.org/10.1007/s11284-005-0059-4.

Hu, S.Y. (2005). *Reproductive Biology of Angiosperms*. High Education Press, Beijing, China.

Körner, C. (1999). *Alpine Plant Life*. DOI: https://doi.org/10.1007/978-3-642-98018-3.

Özhatay, N. & Kültür, Ş. (2006). Check-list of additional taxa to the supplement Flora of Turkey III. *Turk J Bot* 30(4): 281-316.

Rodriguez-Riano, T. & Dafni, A. (2000). A new procedure to asses pollen viability. *Sex Plant Reprod* 12: 241-244. DOI: https://doi.org/10.1007/s004970050008.

Stevens, P.F. (2001). *Angiosperm Phylogeny Website*. Version 12, July 2012 [and More or Less Continuously Updated Since]. http://www.mobot.org/MOBOT/research/APweb/ [02.01. 2023].

Szukala, A., Korotkova, N., Gruenstaeudl, M., Sennikov, A. N., Lazkov, G. A., Litvinskaya, S. A., Gabrielian, E., Borsch & T. von Raab-Straube, E. (2019). Phylogeny of the eurasian genus *Jurinea* (Asteraceae: Cardueae): Support for a monophyletic genus concept and a first hypothesis on overall species relationships. *Taxon* 68(1): 112-131. DOI: https://doi.org/10.1002/tax.12027.

Urbanska, K.M. & Schütz, M. (1986). Reproduction by seed in alpine plants and revegetation research above timberline. *Botanica Helvetica* 96(1): 43-60.

Wang, Y.L., Guan, Z.Y., Chen, F.D., Fang, W.M. & Teng, N.J. (2012). Pollen viability, pistil receptivity, and embryo development in hybridization of *Nelumbo nucifera* Gaertn. *The Scientific World Journal*, Article ID 678706. DOI: https://doi.org/10.1100/2012/678706.

Wilcock, C. & Neiland, R. (2002). Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science* 7(6): 270-277.

Yıldırım, H. & Şenol, G. S. (2010). A new taxon for *Jurinea* from West Turkey. *OT Sistematik Botanik Dergisi* 17(1): 1-8.

Zeng-Yu, W., Yaxin, G., Scott, M. & Spangenberg, G. (2004). Viability and longevity of pollen from transgenic and non-transgenic tall fescue (*Festuca arundinacea*) (Poaceae) plants. *Am J Bot* 91(4): 523-530. DOI: https://doi.org/10.3732/ajb.91.4.523.