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# Farklı Sıcaklık ve Sürelerde Bekletilen Kasımpatı Polenlerinde Polen Kalitesinin Belirlenmesi

Hilal Beyza DURSUN[1], Soner KAZAZ[1], Tuğba KILIÇ[2*]

**Öne Çıkanlar:**
- Polen tanelerinin canlılığını koruma süresi bilinmelidir
- Kasımpatı polenleri için Monnier kültür ortamı uygun bir çimlenme ortamıdır
- Kasımpatı polenleri +4ºC'de bekletilerek 4 güne kadar kullanılabilir

**Anahtar Kelimeler:**
- Krizantem
- Canlı polen oranı
- TTC yöntemi
- Monnier
- Asılı damla

**ÖZET:**

Polen kalitesi, tohum oluşumu üzerindeki etkilerinden dolayı ıslah programlarında en önemli faktörlerden biridir. Etkili bir ıslah programı için ıslahçılar, polenin canlılığını, çimlenme oranını ve polenlerin canlılığını koruma süresini mutlaka bilmelidir. Bu çalışma, farklı sıcaklık ve sürelerde bekletilen 'Chic' ve 'Barolo' sprey kasımpatı çeşitlerinin polen canlılığı ve çimlenme oranlarını belirlemek amacıyla yapılmıştır. Her iki çeşide ait polenler, tam otomasyonlu bir ıslah serasında yetiştirilen bitkilerden elde edilmiştir. Polenler, +24°C'de ve +4°C'de 7 gün boyunca bekletilmiştir. 0. gün dahil olmak üzere 8 gün boyunca günlük olarak TTC yöntemi ile polen canlılıkları, modifiye $ME_{3\text{-}m}$ ortamı ile asılı damla yönteminde ise çimlenme oranları belirlenmiştir. Elde edilen bulgulara göre, 'Chic' çeşidinin 'Barolo' çeşidinden daha fazla canlı polene sahip olduğu saptanmıştır. Canlı polen ve çimlenme oranları, bekletme sürelerinin artmasıyla sürekli olarak azalma eğilimi göstermiştir. 7. günde çimlenme oranı 'Barolo' ve 'Chic' çeşitlerinde sırasıyla %93.44 ve %71.64 oranında azalmıştır. Ancak +4°C'de tutulan polenin canlılığını koruma kapasitesi her iki çeşitte de daha iyi bulunmuştur. Çalışmada, polenlerin öncelikle taze olarak uygulanması, gerektiğinde +4°C'de saklanarak kullanılması, +24°C'de bekletilen polenlerin ise 2 güne kadar kullanılması gerektiği sonucuna varılmıştır. +4°C'de bu süre çeşide göre 4 güne kadar uzayabilir.

# Pollen Quality of Some Spray Chrysanthemum Varieties at Different Holding Time and Temperature

**Highlights:**
- The period of preservation of the viability of pollen should be known
- Monnier culture medium is a suitable germination medium for chrysanthemum pollen
- Chrysanthemum pollen can be used for up to 4 days by keeping it at +4°C

**Keywords:**
- Mums
- Viable pollen rate
- TTC methods
- Monnier
- Hanging drop

**ABSTRACT:**

Pollen quality is one of the most important factors in breeding programs because of its effects on seed formation. For an effective breeding program, breeders must know pollen viability, germination rate, and the duration of maintaining pollen viability. This study was carried out to determine pollen viability and germination rates of 'Chic' and 'Barolo' spray chrysanthemum varieties kept at different temperatures and times. Pollen from both varieties was obtained from plants grown in a fully automated greenhouse. Pollens were stored at +24°C and +4°C for 7 days. Pollen viability was determined by the TTC method and germination rates were determined by the hanging drop method with modified $ME_{3\text{-}m}$ medium, daily for 8 days, including day 0. The 'Chic' varieties had more viable pollen than the 'Barolo' variety. Viable pollen and germination rates tended to decrease continuously with time. On the $7^{th}$ day, the germination rate decreased by 93.44% and 71.64% in the 'Barolo' and 'Chic' varieties, respectively. However, the capacity to maintain the viability of pollen kept at +4°C was found to be better in both varieties. In the study, it was concluded that the pollen should be applied freshly but stored at +4°C when necessary. The pollen kept at +24°C can be used for up to 2 days. This period can be extended up to 4 days at +4°C, depending on the variety.

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## INTRODUCTION

Chrysanthemum (*Chrysanthemum* spp.) is known as one of the most important plant species used both as a pot and garden plant as well as a cut flower. It is the second-most traded species of cut flower in the world after the rose, and millions of cut flowers are produced every year. In the year 2020, a total of 399 million, in the year 2021, 392 million, and in the year 2022, 369 million units of chrysanthemums were sold (AIPH, 2021, Royal FloraHolland, 2023). At the same time, chrysanthemum varieties with flowers of different shapes, types, and colors are developed every year to meet consumer demands and expectations. Many countries maintain their position in the market with the new varieties they have introduced to the sector. It is known that more than 30.000 varieties have been developed in chrysanthemums to date (Wang et al., 2019).

Crossbreeding and mutation breeding are the most widely used methods in the development of new chrysanthemum varieties (Ibitoye & Akin-Idowu, 2011). Mutation breeding enables new varieties to be obtained in a short time due to the high heterozygosity of chrysanthemums and this increase in the mutation rate (Miler & Kulus, 2018). However, in mutation breeding, mutations occur suddenly in an unpredictable way and cause only one change, making it very difficult to develop varieties with many desired characteristics. For this reason, crossbreeding is preferred primarily in the development of new chrysanthemum varieties with desired characteristics (Kharkwal et al., 2004). It has been reported that 90% of the varieties developed in chrysanthemums to date have been developed by crossbreeding. The most important advantage of crossbreeding in chrysanthemums is the high success rate in interbreeding due to self-incompatibility. As a matter of fact, the greater the genetic difference among the parents, the greater the success of crossbreeding, and by increasing the genetic variation, it is possible to develop new varieties with desired characteristics (Zhang et al., 2018).

In addition to the advantages of crossbreeding in chrysanthemums, the low number of seeds per capitulum is one of the most frequently encountered problems. Considering that a large number of seeds are set per fruit in breeding programs, successful pollination and fertilization are of great importance for the breeder. Successful pollination and fertilization are possible by first selecting the pollen parent with high pollen quality suitable for the seed parents with high fertility (Nadeem et al., 2013). It is known that knowing the fertility of the pollen parents is one of the most important factors in seed formation, and the breeder must know the pollen viability and germination rate and the duration of maintaining the viability of pollen of the species/varieties that can be used as pollen parents.

The literature stated that pollen should not be kept for a long time in chrysanthemum breeding programs because they lose their viability in a short time, and pollen viability and germination rate differ significantly between species and commercial varieties (Zhao et al., 2005; Zhao et al., 2008; Yang & Endo, 2005; Wang et al., 2018). Various methods (TTC, IKI, FDA, acetocarmine, safranin, lactophenol cotton blue, monnier culture, Brewbaker and Kwack's medium etc. in hanging drop or petri dishes method) have been used to determine pollen viability and germination rate of chrysanthemums, and different results have been obtained from them. While pollen viability and germination rate could not be obtained from some methods, very low pollen viability and germination were obtained from other methods (Jie et al., 1995). Zhao et al. (2006) reported that in some chrysanthemum species and varieties, chemical methods are not suitable for determining pollen viability rates, and the most appropriate method is biological testing. In addition, successful results were not obtained when many germination medias recipes previously reported in the preliminary studies conducted by us were used.

When both web pages and cultivar catalogs of chrysanthemum breeders around the world were examined, no information could be found regarding pollen viability or pollen germination rates of the

varieties they developed. In addition, there is very little research on pollen viability and germination rate in chrysanthemums. The studies found that when pollen viability and germination rates in chrysanthemums were stored for 1 day and 16 days under room temperatures, pollen viability and germination rates decreased rapidly with the prolongation of the storage period, and therefore pollen should not be kept for more than 3 days (Zhao et al., 2008). In the oral interviews with the chrysanthemum breeders in the Netherlands, we were informed that pollen from chrysanthemums should be used in crosses immediately and not kept for any time. As a result, there is no clear information about how many days the pollen can be kept successfully after being taken from the flowers in chrysanthemum breeding by hybridization. Considering that the flowers used as seed and pollen parents in the greenhouse do not bloom at the same time and the need for pollen to make all hybridizations cannot be met on the same day, it makes it necessary to store pollen for a certain period of time for future use.

This study aimed to determine the pollen viability and germination rates of some chrysanthemum varieties and contribute to breeding programs by revealing the capacity of the pollen to maintain its viability.

## MATERIALS AND METHODS

### Plant Material

The study was carried out in the cytology laboratory of Ankara University, Faculty of Agriculture, and Department of Horticulture between September and December 2020. Pollens of commercial spray varieties 'Chic' and 'Barolo', belonging to *Chrysanthemum morifolium* L., were used as plant material. Pollens were obtained from plants grown in soil from cuttings in the greenhouse of the Ankara University Faculty of Agriculture, Department of Horticulture (39°57′40.2′′N 32°51′51.7′′E). The cuttings were purchased from Royal Van Zanten companies. In May, planting was done in 8 rows at 12.5 x 12.5 cm intervals on the beds prepared in one meter width and 20 m length. 600 ppm IBA was applied before planting. When cuttings became seedlings about 35 cm tall four weeks after planting, the blackout application was started, and then the application was terminated after seven weeks. When the buds reached the size of a pea, the apical buds were removed. The temperature and humidity of the pollen taken from the greenhouse between April and October varied between 20ºC and 30ºC and 65% and 70%, respectively. The EC of the nutrient solution given to the plants was 1.5-1.7 mS $cm^{-1}$ at the beginning of the development period of the plants and between 1.7-2.0 mS $cm^{-1}$ during the flowering period, and the pH of the nutrient solution is 6.5.

### Method

Pollen of the 'Chic' and 'Barolo' varieties was collected in glass petri dishes with the help of a sable brush when the pollen matured on the anthers during the period when tubular flowers opened 4-5 rows from outside to inside. Pollen viability and germination rates were determined immediately in some of the pollen (control, day 0, fresh pollen), and the remaining pollen was divided into two parts, half of which were kept in a climate cabinet at +24°C with 60±5% humidity and dark conditions, and the other half were kept in a refrigerator at +4°C for different periods of time. In both the climate cabinet and the refrigerator, the pollen was placed in glass petri dishes, covered with lids, and wrapped with aluminum foil.

The pollen was kept at both +24°C and +4°C for 1, 2, 3, 4, 5, 6, and 7 days, and the viability and germination rates were determined for each day of holding time. The viability and germination rates of the pollen kept at +24°C were determined immediately after the pollen was removed from the climate cabinet. While the viability and germination rates of the pollen kept in the refrigerator at +4°C were

determined after they were removed from the refrigerator and kept in the climate cabinet for one night (12 hours) at +24°C and 60±5% humidity conditions.

In the study, the TTC (2,3,5 Triphenyl Tetrazolium Chloride) staining test was used to determine pollen viability rates. One drop of the prepared TTC solution was placed on the slide, and pollen was sown on each drop with a brush. Then, the sown drops were covered with coverslips and kept in the dark for 2 hours for staining. In pollen counting, pollen grains stained dark red, red, or dark pink were considered "viable", those stained light red, or pink were considered "semi-viable", and those stained yellow or unstained and abnormally shaped pollen were considered "non-viable" (Figure 1). Theoretically, 50% of semi-viable pollen grains were considered viable, and this value was added to the absolute amount of viable pollen (Eti, 1990; Eti, 1991). The rate of viable pollen was expressed as %.

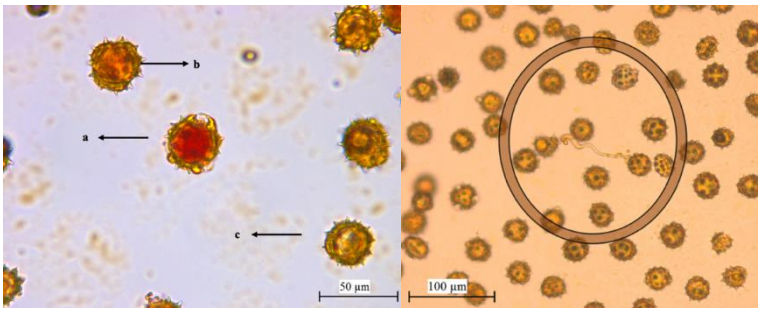


**Figure 1.** Viable (a), semi-viable (b), and non-viable (c) pollen grains (left); pollen considered germinated (right)

The hanging drop method was used to determine pollen germination rates. Firstly, liquid culture medium was prepared, and modified Monnier culture medium ($ME_{3\text{-}m}$) was used as liquid culture medium (Table 1). The hanging drop preparations were placed in petri dishes containing moist filter paper and kept in a climate cabinet with 60±5% humidity at +24°C for 24 hours for germination. Pollen counts were made under a microscope, and pollen forming a grass tube 1.5 times larger than its own diameter was considered "germinated" (Figure 1) (Sun et al., 2010; Chen et al., 2009). Pollen germination rates were expressed as % and calculated as the ratio of the number of germinated pollen grains to the total number of pollen grains.

**Table 1.** Nutrients and concentrations used in modified $ME_{3\text{-}m}$ medium

| Macro Element | Amount (mg/L) | Micro Elements | Amount (mg/L) |
|---|---|---|---|
| $MgSO_4.7H_2O$ | 370 | $Na_2EDTA$ | 7.45 |
| $KNO_3$ | 950 | $CuSO_4.5H_2O$ | 0.025 |
| $KH_2PO_4$ | 85 | $CoCl_2 \cdot 6H_2O$ | 0.025 |
| $CaCl_2 \cdot 2H_2O$ | 880 | $FeSO_4.7H_2O$ | 5.55 |
| $NH_4NO_3$ | 412.5 | KCl | 175 |
| **Micro Elements** | | **Vitamins** | |
| $MnSO_4.H_2O$ | 16.80 | B1 | 1.0 |
| $ZnSO_4.H_2O$ | 10.50 | B6 | 1.0 |
| $H_3BO_3$ | 50 | **Others** | |
| KI | 0.83 | PEG4000 (g/L)* | 200 |
| $Na_2MoO_4.2H_2O$ | 0.25 | - | - |

*(Zhao et al., 2005; Zhao et al., 2008)

## Experimental design and statistical evaluation

The studies to determine pollen viability and germination rates were established according to a factorial, completely randomized design with three replicates. In pollen viability tests, 8 slides for each variety and a total of 16 areas, 2 on each slide, were counted. In germination rate tests, counting was performed by scanning the whole area on 16 slides. At least 350 pollen counts were made in each count. The data obtained were subjected to an analysis of variance after applying an angle transformation. The difference between the means was evaluated by the 'Duncan Multiple Comparison Test'. The IBM SPSS 20 statistical package program was used for both variance and Duncan test analysis. The mean standard

errors of variables were calculated. Means were given at the tables, and standard errors were given at the figures as error bars.

## RESULTS AND DISCUSSION

As a result of the analysis of variance applied to the data obtained, the effects of 'variety', 'holding temperature', and 'holding time' factors on the viable pollen and germination rate were statistically significant. At the same time, the effects of 'variety x holding time' and 'holding temperature x holding time' interactions on viable pollen rate and 'holding temperature x holding time' interactions on pollen germination rate were found to be statistically significant.

### Pollen Viability Rate

The effects of different holding times and temperatures on the pollen viability rates of 'Barolo' and 'Chic' are given in Table 2. According to Table 2, the highest viable pollen rate was recorded at 'Chic' on day 0 with 19.24%. The viable pollen rate of 'Barolo' was also higher on day 0 (8.82%) than on other days. Regardless of the holding times and holding temperatures, 'Chic' showed a higher viable pollen rate than 'Barolo'.

For both 'Chic' and 'Barolo', viable pollen rates decreased as the holding times increased, and the lowest viable pollen rates were found on day 7 with 4.35% and 2.43% values, respectively, regardless of the holding temperatures. After day 0, the highest viable pollen rate was recorded on day 1. Considering the holding temperatures, both varieties were kept at +4°C for both day 1 ('Chic': 14.39%; 'Barolo': 8.27%) and day 7 ('Chic': 10.14%; 'Barolo': 6.22%), and the viability rates obtained from pollen kept at +4°C were higher than those obtained from pollen kept at +24°C for both day 1 ('Chic': 14.39%; 'Barolo': 8.27%) and day 7 ('Chic': 3.33%; 'Barolo': 1.85%) (Figure 2).

In addition to the viability rates of the pollen, the potential to maintain their viability also differed between varieties and holding temperatures. On the 7$^{th}$ day, regardless of the holding temperatures, the rate of viable pollen decreased by 72.45% and 77.39% in 'Barolo' and 'Chic', respectively, compared to the fresh pollen. As of the 5$^{th}$ day, the loss of viability rate in 'Barolo' was over 50%, while the same rate of decrease was realized in 'Chic' as of the 3$^{rd}$ day. In the 'Barolo', the loss of viability in pollen kept at +4°C reached above 50% on the 7$^{th}$ day, while the loss of viability in pollen kept at +24°C reached above 50% on the 5$^{th}$ day. In the 'Chic', the loss of viability was faster than in the 'Barolo', and the loss of viability in pollen kept at +4°C reached above 50% on the 4$^{th}$ day, and the loss of viability in pollen kept at +24°C reached above 50% on the 2$^{nd}$ day.

**Table 2.** Viable pollen rates of chrysanthemum varieties kept at different temperatures and times

| Variety | Holding Temperature (ºC) | Holding Time (Days) | Viable Pollen Rate (%) |
|---|---|---|---|
| Barolo | +4 | 0 | 8.82 a |
| | | 1 | 8.27 ab A |
| | | 2 | 8.31 ab A |
| | | 3 | 7.62 b A |
| | | 4 | 7.26 b A |
| | | 5 | 5.31 c A |
| | | 6 | 4.82 c A |
| | | 7 | 3.01 d A |
| | | **Average** | **6.68 a B** |
| | +24 | 0 (Control) | 8.82 a |
| | | 1 | 6.22 b B |
| | | 2 | 6.28 b B |
| | | 3 | 5.31 c B |
| | | 4 | 4.89 c B |
| | | 5 | 3.73 d B |
| | | 6 | 2.67 e B |
| | | 7 | 1.85 f B |
| | | **Average** | **4.97 b B** |

**Table 2.** Viable pollen rates of chrysanthemum varieties kept at different temperatures and times (Continued)

| | | | |
|---|---|---|---|
| **Chic** | **+4** | 0 (Control) | 19.24 a |
| | | 1 | 14.39 b A |
| | | 2 | 11.69 cd A |
| | | 3 | 10.19 cd A |
| | | 4 | 8.82 de A |
| | | 5 | 9.30 de A |
| | | 6 | 7.39 ef A |
| | | 7 | 5.37 f A |
| | | **Average** | **10.80 a A** |
| | **+24** | 0 (Control) | 19.24 a |
| | | 1 | 10.54 b B |
| | | 2 | 8.11 c B |
| | | 3 | 7.74 c B |
| | | 4 | 6.53 cd B |
| | | 5 | 5.57 de B |
| | | 6 | 4.16 ef B |
| | | 7 | 3.33 f B |
| | | **Average** | **8.15 b A** |

*p is statistically significant at the probability level of ≤0.05. Lowercase letters represent the difference between days in the same variety, in the same column, at the same holding temperature. Capital letters, the difference between holding temperatures of the same variety and on the same days, in the same column. Bold lowercase letters indicate the difference between holding temperatures within the same variety, at the same holding time. Bold capital letters indicate the difference between the average holding temperatures of the varieties

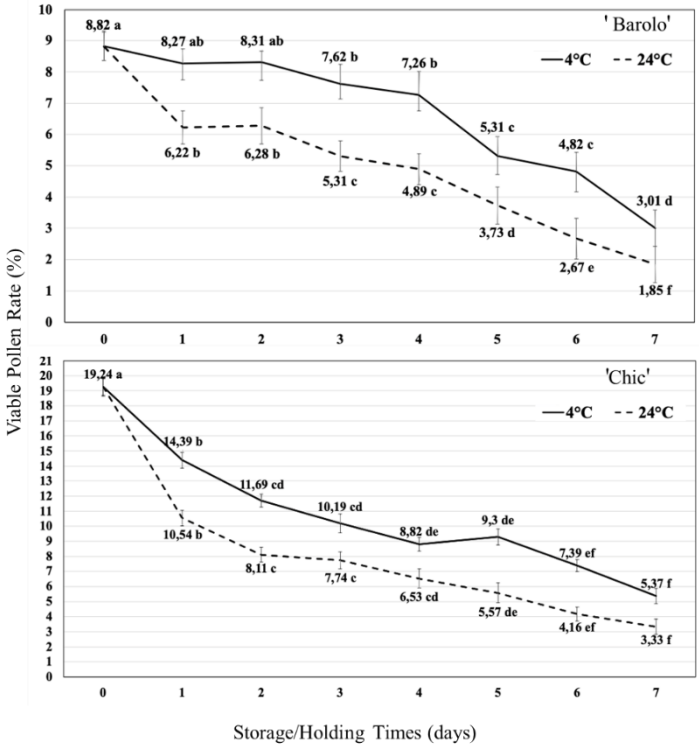


**Figure 2.** Viable pollen rates of chrysanthemum varieties at different holding times and temperatures (error bars show standard errors)

## Pollen Germination Rate

The effects of different holding times and temperatures on the pollen germination rates of 'Barolo' and 'Chic' are given in Figure 3 and Table 3. The highest pollen germination rate was determined on day 0 in 'Chic' with 7.58% and 'Barolo' with 5.18% (Figure 3). Similar to the viable pollen rates, 'Chic' showed a higher pollen germination rate than 'Barolo', regardless of holding times and temperatures (Table 3).

For both varieties, germination rates tended to decrease continuously as holding times increased. The highest germination rate was obtained on day 1 after day 0. The lowest pollen germination rate was 2.58% for the 'Chic' and 0.53% for the 'Barolo' on day 7, regardless of the holding temperatures (Figure 3). When temperatures were analyzed regardless of cultivars, the germination rate of pollen kept at +4°C

on day 1 (5.17%) was higher than that of pollen kept at +24°C for 1 day (4.81%). Except for the 3rd and 7th days, the germination rates obtained from pollen kept at +4°C were higher than those obtained from pollen kept at +24°C (Figure 3, Table 3). Within the varieties, there was no difference between the holding temperatures up to day 3 for the 'Barolo' and up to day 2 for the 'Chic'.

**Table 3.** Pollen germination rates of chrysanthemum varieties kept at different temperatures and times

| Variety | Holding Temperature (ºC) | Holding Time (Days) | Viable Pollen Rate (%) |
|---|---|---|---|
| Barolo | +4 | 0 | 5.18 a |
| | | 1 | 3.74 b A |
| | | 2 | 2.58 c A |
| | | 3 | 1.46 de A |
| | | 4 | 1.65 d A |
| | | 5 | 1.50 de A |
| | | 6 | 1.04 de A |
| | | 7 | 0.72 e A |
| | | **Average** | 2.23 a B |
| | +24 | 0 (Control) | 5.18 a |
| | | 1 | 3.37 b A |
| | | 2 | 2.10 c A |
| | | 3 | 1.38 d A |
| | | 4 | 0.99 de B |
| | | 5 | 0.67 e B |
| | | 6 | 0.51 e B |
| | | 7 | 0.34 e B |
| | | **Average** | 1.82 b B |
| Chic | +4 | 0 (Control) | 7.58 a |
| | | 1 | 6.61 b A |
| | | 2 | 6.16 b A |
| | | 3 | 5.22 c A |
| | | 4 | 5.05 c A |
| | | 5 | 4.27 d A |
| | | 6 | 3.70 d A |
| | | 7 | 3.01 e A |
| | | **Average** | 5.20 a A |
| | +24 | 0 (Control) | 7.58 a |
| | | 1 | 6.24 b A |
| | | 2 | 5.80 b A |
| | | 3 | 4.62 c B |
| | | 4 | 4.29 c B |
| | | 5 | 2.85 d B |
| | | 6 | 2.77 d B |
| | | 7 | 2.15 e B |
| | | **Average** | 4.54 b A |

*p is statistically significant at the probability level of ≤0.05. Lowercase letters represent the difference between days in the same variety, in the same column, at the same holding temperature. Capital letters, the difference between holding temperatures of the same variety and on the same days, in the same column. Bold lowercase letters indicate the difference between holding temperatures within the same variety, at the same holding time. Bold capital letters indicate the difference between the average holding temperatures of the varieties

When the potential of the pollen to maintain their viability was evaluated in terms of germination rates, pollen germination rates of 'Barolo' and 'Chic' on day 7 showed a decrease of 89.77% and 65.96%, respectively, compared to fresh pollen, regardless of the holding temperatures. As of day 2, the loss of germination rate in 'Barolo' was over 50% for both holding temperatures. In the 'Chic', the same percentage decrease was realized on day 6 (51.19%) for pollen kept at +4°C and on day 5 (62.40%) for pollen kept at +24°C. In 'Chic', the loss of viability was slower than in 'Barolo' in terms of pollen germination rate, contrary to the findings obtained in the viable pollen test.

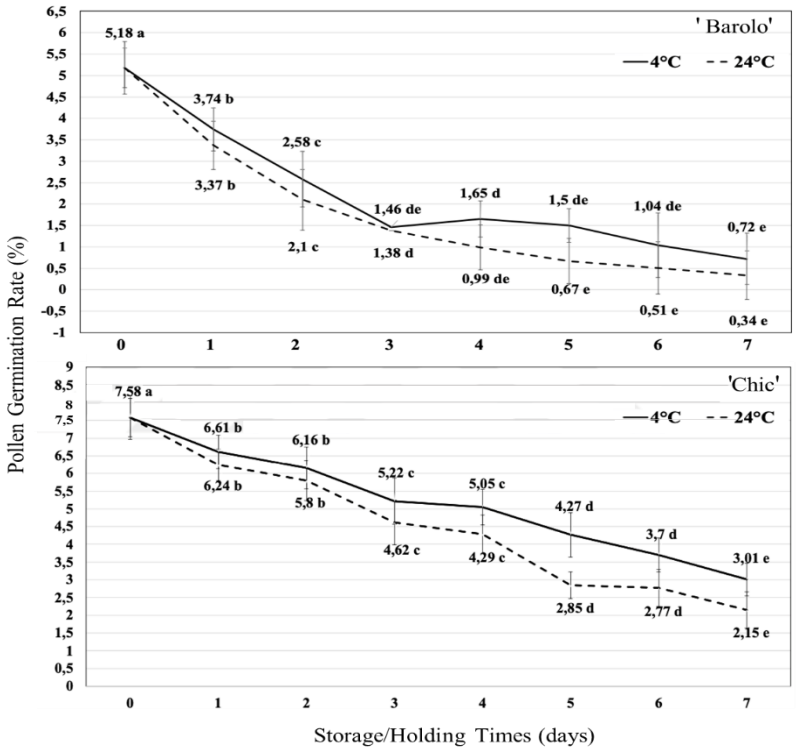


**Figure 3.** Pollen germination rates of chrysanthemum varieties at different holding times and temperatures (error bars show standard errors)

When the research on the determination of pollen viability in chrysanthemum species and varieties was examined, very few studies were found in which staining methods were used. Biological methods were generally used to determine the rate of viable pollen. However, Yang & Endo (2005) reported that viable pollen rates ranged between 83.50% and 96.30% in some chrysanthemum species and cultivars by the acetocarmine method. Kattera et al. (2013) reported that viable pollen rates of six different chrysanthemum genotypes varied between 70.0% and 87.0% in a 5% TTC solution. Zhao et al. (2006) reported that TTC, modified TTC, IKI, and FDA methods were not suitable for determining pollen viability rates in chrysanthemums. The viability rates obtained in this study differed from the studies mentioned above, and lower viable pollen rates (8.82%-19.24%) were obtained than the viability rates reported by Yando & Endo (2005) and Kattera et al. (2013). The variation in pollen viability rates among the studies may be due to the differences between the methods used and the species and cultivars. However, contrary to Zhao et al. (2006), the TTC test is considered a suitable method for determining viable pollen rates in chrysanthemums since it gives results close to germination rates. The suitability of the methods may vary depending on the differences in species and varieties' responses to the solution dose, active ingredient, and method of application. It has been reported by many researchers that the results obtained from viability tests performed on the same species or variety and under the same conditions vary and that the rate of viable pollen varies depending on the species and variety, the dyes used, and the dose of the active substance (Bolat & Güleryüz, 1994; Kalyoncu et al., 2013; Erbaş et al., 2015).

In the studies carried out to determine pollen viability in chrysanthemum species and varieties, biological tests were generally applied to determine germination rates. Yang & Endo (2005) found the germination rate of pollen in five different wild chrysanthemum species and three different chrysanthemum varieties to be between 69.40% and 76.40%. Zhao et al. (2005) reported that pollen germination rates varied between 46.0% and 54.0% in chrysanthemum species and between 23.0% and 25.0% in chrysanthemum varieties. Zhao et al. (2006) also reported that pollen germination rates varied

between 20.0% and 26.0% in some chrysanthemum species and varieties. Kattera et al. (2013) determined that pollen germination rates in six different chrysanthemum genotypes varied between 0% and 67.0% depending on the holding time. Miler & Wozny (2021) reported that pollen germination rates varied between 5.30% and 6.63% in four different chrysanthemum varieties at different storage temperatures and times. The germination rates obtained in this study are generally similar to the findings of Miler & Wozny (2021) and partially similar to the findings of Kattera et al. (2013). In the other studies mentioned, very low germination rates (5.18%, 7.58%) were obtained. It is thought that the difference between the lower and upper limits of pollen germination rates between the studies may be due to factors such as the species and/or variety used, the temperature, light, and humidity conditions of the greenhouse where the plant material is grown, the cultivation technique, the methods used to determine the germination rates of the pollen, the season and time of pollen collection, and the temperature and duration of pollen storage. As a matter of fact, it has been reported by many researchers that pollen germination rates differ depending on genotype, ploidy levels, climatic conditions, pH, sucrose content, etc. of the germination medium, nutritional status of the plant, pollen collection time (season, flowering period, flower development period), storage conditions, and duration (Günes et al., 2005; Mert & Soylu, 2006; Richer et al., 2007; Zlesak, 2007; Sulusoglu & Cavusoglu, 2014; Martins et al., 2017; Fragallah et al., 2019).

Although there were differences among chrysanthemum varieties in terms of pollen viability and germination rates, the viable pollen and pollen germination rates obtained as a result of chemical and biological methods in the same variety also differed from each other. The rate of viable pollen obtained by the TTC method was higher than the germination rates obtained by the hanging drop method. Parfitt & Ganeshan (1989) determined that chemical methods were not similar to biological methods. Considering that both methods reveal pollen viability, a linear relationship between viable pollen and germination rate is expected (Martins et al., 2017). However, since immature pollen can also be stained by chemical methods, higher viable pollen rates can be obtained when compared to the results obtained by biological methods (Sensoy et al., 2003).

In the study, the highest viability and germination rates were obtained from fresh pollen of both 'Barolo' and 'Chic'. In both cultivars, the viability and germination rates of pollen kept at both +4°C and +24°C decreased significantly with increasing holding time. At the same time, it was determined that the ability to maintain their viability differed between the varieties. Many researchers (Zhao et al., 2008; Xu et al., 2012; Miler & Wozny, 2021) also reported that viable pollen rate and germination rate decreased with the prolongation of storage and/or holding time in chrysanthemum species and varieties. It is thought that the fact that pollen loses its viability over time or its ability to maintain its viability is related to its resistance to dehydration. In addition, it is thought that there is variation in the holding times due to the difference in dehydration resistance between species and varieties. Pacini & Dolferus (2019) reported that the resistance of pollen to dehydration may vary depending on plant species and varieties.

The viable pollen and pollen germination rates kept at +24ºC in 'Barolo' and 'Chic' were lower than pollen kept at +4ºC. Similarly, Miler & Wozny (2021) reported that pollen germination rates decreased with increasing storage temperature in chrysanthemum varieties; Güclü et al. (2021) in different blackberry varieties; Perveen & Sarwar (2011) in *Jasminum sambuc* L. and *Nycanthes arbortristis* L. species; Giovannini et al. (2015); and Macovei et al. (2016) in rose varieties. Similar to the results obtained in our study, Erbaş et al. (2015) reported that the viability rate of pollen kept at +4ºC was higher than that of pollen kept at +25ºC in oil rose (*R. damascena* Mill.). Temperature is one of the most important factors affecting pollen quality (Paupıe`re et al., 2017), and the variation between viable pollen

and pollen germination rates at different temperatures may be related to changes in the metabolic activities of pollen. Pollen kept at +24ºC may have strong respiratory and metabolic activity. Pollen kept at +4ºC may maintain pollen viability because it has lower respiratory and metabolic activities. Indeed, Almeida et al. (2011) reported that high temperatures accelerate metabolic activity, respiration, and dehydration in pollen.

## CONCLUSION

In this study carried out to determine the viability and germination rates of chrysanthemum pollen and to investigate the effects of different holding times and temperatures on viability and germination rates, the viability and germination rates of chrysanthemum pollen were quite low. The TTC method and $ME_{3\text{-}m}$ medium were found to be applicable tests for chrysanthemum pollen. The highest pollen viability and germination rates for both varieties were obtained from fresh pollen. Therefore, the pollen of parents should be used immediately after collection without waiting for the breeding of chrysanthemums by hybridization. However, considering the fact that the flowers used as parents in the breeding greenhouse do not bloom at the same time and that the pollen requirement for all crosses cannot be met on the same day, it becomes a necessity to keep the pollen for a certain period of time for future use. Therefore, the results obtained show that pollen should be kept at +4°C and used when necessary (within a short time). The chrysanthemum pollen kept at +24°C can be used for up to 2 days, while at +4°C this period can be extended up to 4 days, depending on the variety. The results obtained from our study would be appropriate to plan repetitively. In addition, comparing the results obtained from pollen holding temperatures and times with the crossing success in greenhouse conditions will allow more precise findings to be obtained.

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### Conflict of Interest

The article authors declare that there is no conflict of interest between them.

### Author's Contributions

Conceived and planned the experiments: SK. Performed the experiments: SK and HBD. Analyzed the data: TK. Wrote the paper: TK and HBD. Reviewed and edited the paper: SK.

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