



Comparing of Cultivated Annual and Perennial *Calendula officinalis* L. Species

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ABSTRACT

The aim of this study was to determine the highest dry flower yield and essential oil content of yellow and orange flowered annual and perennial marigold plants. Annual and perennial *Calendula officinalis* L. plant seeds with yellow and orange flower obtained from Research and Application Center of Botanical Garden and Herbarium, Ege University were used as a study material. The experiment was established to the 1000 m² area of Ege University Odemis Vocational School field according to split-split plot parcel design with three replications on 28th of March 2016. Flowers were harvested when the plants in full blossom stage, every ten days. Annual yellow and orange flowers and perennial yellow and orange flowers were evaluated separately for each harvest. Totally seven harvest were done. All fresh flowers were weighed after then dried in a drying cabinet at 30 °C and essential oil contents were obtained by hydro distillation method. The highest dry flower yields for perennial plants were provided for yellow flowered plants; at 7.54 kg da⁻¹ and for orange flowered plants; 7.28 kg da⁻¹. The highest essential oil contents in perennial were determined for yellow flowered plants as 0.051%, and orange flowered plants; 0.020%. In general, it can be said that content of essential oil is higher in perennial plants than single years.

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Tek ve Çok Yıllık *Calendula officinalis* L. Türlerinin Kültür Şartlarında Karşılaştırılması

ÖZET

Bu çalışmada tek yıllık ve çok yıllık sarı ve turuncu çiçekli aynısafa bitkilerinde en yüksek drog çiçek verimi ve uçucu yağ oranını belirlemek amaçlanmıştır. Materyal olarak kullanılan tek ve çok yıllık sarı turuncu renkli *Calendula officinalis* L. tohumları Ege Üniversitesi Botanik bahçesinden temin edilmiştir. Deneme Ege Üniversitesi Ödemiş Meslek Yüksekokulu deneme tarlasında 1000 m² alanda üç tekerrürlü olarak bölünen bölünmüş parseller deneme desenine göre 28 Mart 2016 tarihinde kurulmuştur. Çiçekler tam çiçeklenme döneminde her 10 günde bir toplanmış ve her hasatta sarı ve turuncu çiçekler, tek yıllık ve çok yıllık olarak ayrı ayrı değerlendirilmiştir. Hasat edilen çiçekler tartıldıktan sonra 30 °C' de kurutma dolabında kurutulmuştur. Uçucu yağ içeriği su distilasyonu metoduna göre volümetrik olarak belirlenmiştir. En yüksek drog çiçek verimi çok yıllık *Calendula* türlerinden sağlanmış ve sarı renkliler için 7.54 kg da⁻¹ iken, turuncu renklilerde 7.28 kg da⁻¹ olmuştur. En yüksek uçucu yağ içeriği ise yine çok yıllıklardan sarı renk çiçeklilerde % 0.051, turuncu renk çiçeklilerden ise % 0.020 olarak ölçülmüştür. Genel olarak uçucu yağ içeriklerinin çok yıllık bitkilerde tek yıllıklara göre, arı renklilerin ise turuncu renkliler göre daha yüksek verim sağladığı belirlenmiştir.

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Uçucu yağ
Drog herba verimi

INTRODUCTION

Calendula officinalis L. is an annual or a perennial flowering plant in *Asteraceae* family. It is widely used as an ornamental plant due to its beautiful looking orange and yellow flowers. While annual plants are generally cultivated, perennial plants grow naturally from southern Europe to central Europe (Okoh et al., 2008) and in the Mediterranean. (Gazim et al., 2008; Khalid and Silva, 2012; Khodadadi et al., 2013). It is reported that the plant is considered as a medicinal plant in central and southern Europe, western Asia and the United States and has phytochemicals such as terpenoid, flavonoid, coumarin, quinon, karatonoid and essential oils (Muley et al., 2009). Plant flower colors can range from yellow to orange, depending on the region, and full bloom occurs in spring summer (Okoh et al., 2008). Pharmacological studies have confirmed that flowers have a large number of biological effects and pharmacological activity of the liver and antispasmodic protection (Mohammad and Kashani, 2012; Arora et al., 2013; Jan et al., 2017). Essential oils of the *C. officinalis* species are intensely found in the flowers of the plant. The essential oils obtained from these flowers have been reported to soothe the central nervous system and heal wounds (Milliauskas et al., 2004). The essential oils contained in flowers are also used in the pharmaceutical and cosmetics industry, except for its traditional use (Gazim et al., 2008; Bielski and Szejewska, 2013; Rezaei et al., 2018).

The chemical compounds found in medicinal and aromatic plants are affected by the region where the plant is cultivated, climate conditions, vegetation

period, genetic factors as well as cultivation conditions and geographical regions (Miliauskas et al., 2004). Genetic and environmental factors have been reported to have important effects on essential oil contents in *C. officinalis* flowers (Okoh et al., 2008). In studies on essential oil in different regions and conditions, it was found that essential oil contents in *Calendula* flowers varied between 0.04% and 0.97% (Okoh et al., 2007; Gazim et al., 2008; Alves Paim et al., 2010; Berimavandi et al., 2011; Khalid and Silva, 2012; Ourabia et al., 2019). The aforementioned studies were carried out in different regions, soil and climate conditions. Therefore, there are significant differences in both yield and pharmacological properties. However, researchers do not specifically explain whether the plants in their research are annual or perennial, or the plant flower colors (yellow-orange). This research was carried out to determine the yield and essential oil contents depending on the flower colors of annual and perennial plants.

MATERIAL and METHOD

Annual and perennial *Calendula officinalis* L. seeds with yellow and orange flower obtained from Research and Application Center of Botanical Garden and Herbarium were used as materials. The experiment was established in the fields of Ege University Ödemiş Vocational School. Ege University soil analysis laboratories ran soil analyses of the experiment area and the results are in Table 1. The properties of the soil are as follows; texture; clay-loam, neutral, salinity; non-saline, poor in terms of organic matter. Climate data for the experiment period is given in Table 2.

Table 1. Physical and Chemical Properties of the Soil of the Experiment Area

Çizelge 1. Deneme alanına ait toprağın fiziksel ve kimyasal özellikleri

| Properties | Depth (0-30 cm) | Depth (30-60 cm) |
|---------------------------|---------------------|-------------------|
| Organic Matter (%) | 1.34 (low) | 0.78 (very low) |
| Total Nitrogen (%) | 0.081 (low) | 0.047 (very low) |
| Phosphorus (ppm) | 24 (medium) | 28.8 (medium) |
| Potassium (ppm) | 210 (medium) | 300 (medium) |
| pH | 7.9 (slightly high) | 7.7 (good) |
| Satucontentn (%) | 60.9 (clay loam) | 62.9 (clay loam) |
| E.conductivity(mmohms/cm) | 0.08 (non-saline) | 0.17 (non-saline) |

C. officinalis were planted in seed plots with a seedbed compost of digested farmyard manure, sand and soil at 1:1:1 content on 10th of February 2016. Necessary maintenance such as irrigation, weed management and ventilation was monitored daily. These seedlings were then planted to the experiment area of 1000 m² according to randomized design in split-split plot parcel with three replications and 70x40 cm row spacing on 28th of March. Annual and perennial plants were distributed to the main parcels and flower colors as sub-parcels. Each block was 3 x 67.2 m, each main parcel (perennial and annual) was 3 x 33.6 m and sub-

parcels size were 3 x 16.8 m (24 rows orange or yellow color). The distance between blocks was set to 3 meters. A drip irrigation system was established, and the plants were irrigated in accordance with the plant irrigation requirement.

Flower Harvest

As of 25th April 2016, when the plants started to bloom in full, the receptacles were harvested every 10 days. A total of 7 harvests were made until 26th June 2016. Annual yellow and orange flowers and perennial yellow and orange flowers were evaluated separately

for each harvest. Fresh flowers were weighed immediately after the harvest and taken to be drying cabinet at 30 °C. Dried flowers were weighed and yields per acre were determined.

Essential Oil Content

In order to determine the essential oil content in the

flowers, 100 g of the dried flowers were weighed and boiled in a 2-liter glass flask for 3 hours in the Clevenger type device according to the hydro distillation method (Wichtl, 1971). Results obtained after volumetric measurement were converted to a percentage (% w w⁻¹).

Table 2. Climatic Data of 2016 (Anonymous, 2020)

Çizelge 2. 2016 yılına ait iklim verileri

| | Monthly Average Temperature (C°) | Monthly Average Relative Humidity (%) | Monthly Total Precipitation (mm=kg+m ²) |
|-----------|----------------------------------|---------------------------------------|---|
| January | 6.8 | 72.6 | 154.6 |
| February | 11.9 | 72.4 | 38.6 |
| March | 11.7 | 71.2 | 123.0 |
| April | 17.7 | 60.8 | 6.2 |
| May | 19.1 | 62.4 | 77.0 |
| June | 26.7 | 49.8 | 1.4 |
| July | 28.4 | 48.5 | 0.0 |
| August | 28.3 | 53.3 | 6.6 |
| September | 23.3 | 55.9 | 30.7 |
| October | 17.6 | 65.3 | 0.2 |
| November | 11.8 | 69.2 | 85.1 |
| December | 4.7 | 68.9 | 3.0 |



Figure1. Orange and yellow flowered *Calendula officinalis* L. plants
Şekil 1. Turuncu ve sarı renk çiçekli *Calendula officinalis* L. bitkileri



Figure 2. Essential oil analysis of orange flowered *Calendula officinalis* L. plants
Şekil 2. Turuncu renk çiçekli *Calendula officinalis* L. bitkilerinde uçucu yağ analiz

Statistical Analyses

The measured data were determined according to analyses of variance using MSTAT-C software program. Differences significant treatment means were separated using least significant difference (LSD) at $\leq P$ 0.01.

RESULTS AND DISCUSSION

Fresh Flower Yield

Results of fresh flower yields of *Calendula officinalis* annual and perennial plants with different flower colors are given in Table 3. When perennial plants are considered, it is determined that yellow flowered plants have higher fresh flower yield than orange flowered plants. It has been determined that there is a 1% statistically significant difference between annual and perennial plants, different flower colors and harvest periods. The highest fresh flower yields were obtained at the fourth harvest, they were 51.96 for yellow flowered plants and 36.88 kg da⁻¹ in orange flowered plants, the lowest fresh flower yields were obtained at the first harvest and they were 3.08 kg da⁻¹

and 2.40 kg da⁻¹ for yellow flowered plants with and orange flowered plants respectively. Considering perennial plants, fresh flower yields from yellow flowered plants were higher than orange flowered plants during all harvest periods.

In annual plants, although first harvests were similarly the lowest fresh flower yields, they were higher than the perennial plants (3.34- 3.12 kg da⁻¹, respectively). The highest fresh flower yields were obtained in both yellow flowered and orange flowered plants at the fifth harvest, 10.80 kg da⁻¹ and 11.02 kg da⁻¹, respectively. Except for the first harvest period, orange flowered plants had better results in terms of fresh flower yield than yellow flowered plants. Fresh flower yields from perennial in both flower colors were higher than annual plants. It is observed that in perennial and annual plants with yellow and orange flowers, the yield of fresh flowers increases from the first harvest to the fourth and fifth harvests, however, it then decreases significantly in the subsequent harvests (Table 3).

Table 3. Fresh Flower Yields of Perennial and Annual *Calendula officinalis* L. Plants with Yellow and Orange Flowers at Different Harvest Periods (kg da⁻¹)

Çizelge 3. Tek yıllık ve çok yıllık *Calendula officinalis* L. bitkilerinde farklı biçim dönemlerindeki taze çiçek verimleri (kg da⁻¹)

| | Perennial | | | Annual | | |
|-------------------------|----------------------------|-------------------------------------|------------|--|--|------------|
| | Yellow | Orange | Mean | Yellow | Orange | Mean |
| 1 th Harvest | 3.08±0.61 ₁ | 2.40±0.42 ₁ | 2.74±0.36 | 3.34±0.74 ₁ | 3.12±0.34 ₁ | 3.24±0.37 |
| 2 nd Harvest | 15.22±2.24 _{efg} | 6.28±0.17 _{gh₁} | 10.74±2.24 | 5.80±0.23 _{gh₁} | 9.34±1.97 _{gh₁} | 7.57±1.18 |
| 3 rd Harvest | 27.26±2.26 _d | 20.18±0.41 _{de} | 23.72±1.89 | 7.22±0.72 _{gh₁} | 7.24±0.75 _{gh₁} | 7.23±0.47 |
| 4 th Harvest | 51.96±1.98 _a | 36.88±1.45 _{bc} | 44.42±3.54 | 7.62±0.72 _{gh₁} | 10.46±1.05 _{fgh₁} | 9.04±0.86 |
| 5 th Harvest | 45.44±2.81 _{ab} | 26.64±2.00 _d | 36.04±4.48 | 10.08±0.52 _{efgh₁} | 11.02±1.36 _{efgh₁} | 10.55±0.68 |
| 6 th Harvest | 28.38±1.49 _{cd} | 19.68±0.53 _{def} | 24.02±2.07 | 6.86±0.33 _{gh₁} | 8.60±1.89 _{gh₁} | 7.73±0.94 |
| 7 th Harvest | 13.02±1.02 _{efgh} | 8.12±1.66 _{gh₁} | 10.56±1.26 | 5.34±0.48 _{h₁} | 5.36±0.66 _{h₁} | 5.35±0.37 |
| Mean* | 26.33±3.00 | 17.16±2.57 | | 6.60±0.47 | 7.87±0.71 | |
| LSD (%1) | 9.48 | | | | | |

*: Means were given as ± standard error.

Caliskan and Kurt (2018) report that the fresh flower yield keeps increasing up to a certain period, and then there is a significant decrease in flower yields, and the yield varies between 0.77 and 37.7 kg da⁻¹ depending on the harvest period. These abovementioned threshold values are quite higher than the results of this study. There are also studies on planting density, planting time (Berimavandi et al., 2011) and fertilization (Bielski et al., 2013; Khodadadi et al., 2013) which significantly affect fresh flower yield and the number of flowers per plant.

Dry flower Yield

Dry flower yields obtained from yellow and orange flowered, annual and perennial *Calendula officinalis* L. plants from different harvest periods are given in Table 4. It has been determined that there is a 1%

statistically significant difference between annual and perennial plants, different flower colors and harvest periods. Similar to the fresh flower yields, the highest dry flower yield values for perennial plants were acquired at the fourth harvest for yellow flowered plants; at 7.54 kg da⁻¹, and at the fifth harvest for orange flowered plants; 7.28 kg da⁻¹. Dry flower yields from the 4th and 5th harvest periods for orange flowered plants were statistically in the same statistical group. While the lowest dry flower yield was 0.86 kg da⁻¹ for perennial yellow flowered plants, this number was 0.42 kg da⁻¹ for perennial orange flowered plants.

The highest yields of dry flowers in annual plants were obtained at the fourth harvest from yellow flowered plants; 1.74 kg da⁻¹, and at the fifth harvest from orange flowered plants; 3.12 kg da⁻¹. The lowest dry

flower yields were 0.60 kg da⁻¹ and 0.50 kg da⁻¹ for yellow flowered and orange flowered plants, respectively. Dry flower yields of the third, fourth, fifth and sixth harvests of yellow flowered plants were statistically in the same statistical group. Except for

the first harvests, dry flower yields from annual orange flowered plants were higher in all harvests than yellow flowered plants. However, considering both yellow and orange flower plants, dry flower yields from perennial plants were higher than annual plants.

Table 4. Dry flower Yields of Perennial and Annual *Calendula officinalis* L. Plants with Yellow and Orange Flowers at Different Harvest Periods (kg da⁻¹)

Çizelge 4. Tek yıllık ve çok yıllık *Calendula officinalis* L. bitkilerinde farklı biçim dönemlerindeki kuru çiçek verimleri (kg da⁻¹)

| | Perennial | | | Annual | | |
|-------------------------|---------------|----------------|-----------|--------------|---------------|-----------|
| | Yellow | Orange | Mean | Yellow | Orange | Mean |
| 1 th Harvest | 0.86±0.17gh | 0.42±0.09h | 0.64±0.13 | 0.60±0.14gh | 0.50±0.06h | 0.56±0.07 |
| 2 nd Harvest | 2.40±0.39efgh | 1.50±0.27fgh | 1.94±0.29 | 1.06±0.09gh | 1.22±0.10fgh | 1.14±0.17 |
| 3 rd Harvest | 4.90±0.49bcde | 3.62±0.13cdef | 4.26±0.37 | 1.24±0.08fgh | 1.24±0.14fgh | 1.24±0.07 |
| 4 th Harvest | 7.54±0.14a | 7.16±0.27ab | 7.34±0.16 | 1.74±0.16fgh | 2.10±0.13fgh | 1.92±0.12 |
| 5 th Harvest | 5.72±0.99abc | 7.28±0.41ab | 6.50±0.59 | 1.14±0.09fgh | 3.12±0.35defg | 2.13±0.47 |
| 6 th Harvest | 4.84±0.44bcde | 6.04±1.82abc | 5.44±0.88 | 1.12±0.14fgh | 2.44±0.26efgh | 1.78±0.32 |
| 7 th Harvest | 5.28±0.61abcd | 2.90±0.30defgh | 4.08±0.61 | 1.04±0.11gh | 1.88±0.08fgh | 1.46±0.20 |
| Mean* | 4.50±0.488 | 4.13±0.61 | | 1.14±0.079 | 1.78±0.19 | |
| LSD (% 1) | 2.56 | | | | | |

*: Means were given as ± standard error.

In a study related to different flower harvest times of the *C. officinalis*, it is reported that dry flower yields vary between 0.14- 6.56 kg da⁻¹ depending on the harvest periods (Caliskan & Kurt, 2018). These values of dry flower yield are similar to this study. In another study investigating the effects of planting density and planting time on dry flower yield, dry flower yields vary between 75.00- 149.60 g m⁻² (Berimavandi et al., 2011). These values are quite higher than this study. Probably this is due to the cultural applications (planting density, fertilization, etc.) and the location of the experiment. As a matter of fact, Gazim et al. (2008) emphasize that large differences between the findings in *Calendula* cultivation may be due to climate conditions.

Essential Oil Contents

Essential oil contents obtained from dry flowers are

Table 5. Essential Oil Yields of Perennial and Annual *Calendula officinalis* L. Plants with Yellow and Orange Flowers at Different Harvest Periods (%)

Çizelge 5. Tek yıllık ve çok yıllık *Calendula officinalis* L. bitkilerinde farklı biçim dönemlerindeki uçucu yağ içerikleri (%)

| | Perennial | | | Annual | | |
|-------------------------|-----------|--------|-------|--------|--------|-------|
| | Yellow | Orange | Mean | Yellow | Orange | Mean |
| 1 th Harvest | tr* | tr* | tr* | tr* | tr* | tr* |
| 2 nd Harvest | tr* | tr* | tr* | tr* | tr* | tr* |
| 3 rd Harvest | tr* | tr* | tr* | tr* | tr* | tr* |
| 4 th Harvest | tr* | tr* | tr* | tr* | tr* | tr* |
| 5 th Harvest | tr* | tr* | tr* | tr* | tr* | tr* |
| 6 th Harvest | 0.027 | 0.015 | 0.021 | 0.025 | 0.005 | 0.015 |
| 7 th Harvest | 0.051 | 0.020 | 0.036 | 0.035 | 0.005 | 0,020 |
| Mean | 0,039 | 0,018 | | 0.030 | 0.005 | |

*trace: <0.005 %

In different parts of the world, very different results are reported about the essential oil rates in *Calendula*

flower. Ourabia et al. (2019) found that the contents of essential oil in dry flowers varied between 0.044 and

0.045%, while Okoh et al. (2007) emphasized that this content increased up to 0.97% depending on the harvest period. In many studies, the contents of essential oil in dry flowers have been reported among these values (Naguib et al., 2005; Gazim et al., 2008; Alves Paim et al. 2010 Berimavandi et al., 2011; Khalid et al., 2012). In terms of essential oil contents, the final harvest values for perennial yellow flowered plants were similar to previously reported minimum values, while other findings were lower in this study.

CONCLUSION

In this study of annual and perennial *C. officinalis* species with yellow and orange flowered plants, it was found that perennial plants were superior to annual plants and yellow flowered plants were superior to orange flowered plants in terms of fresh and dry flower yields and essential oil contents. It should be taken into consideration that genetic structure, cultural applications and locations have important effects on flower yields and essential oil contents.

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Researchers Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

Article authors declare that there are no conflicts of interest among them.

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