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Research Article

The Effect of Sowing Times on the Flower and Seed Yields of Different Varieties of Calendula (*Calendula officinalis* L.) Flowers and Seeds under Çukurova Conditions

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ABSTRACT

This study was carried out to determine the effects of sowing times (March 15, April 1, and April 15) on the flower and seed yields of different varieties (Pot Marigold and Orange King) of Calendula (*Calendula officinalis*) during 2020-2021 growing seasons under Çukurova conditions. The experiment was set up randomized complete block design with a split-plot arrangement and 3 replications. According to the results of the study, the highest dry flower yield (360.63 kg/ha) was obtained from the Pot Marigold variety sown on 15th March 2021, the highest fresh flower yield (1308.67 kg/da) and flower essential oil (0.05 %) were obtained from Orange King variety, sown at 15th March 2020 and 15 March 2021, respectively. The highest seed yield of 35.6 kg/da and the highest seed fixed oil 15.39 % were obtained from balloon smooth-shaped seeds. The main component in flower essential oil was α-cadinol and in seed oil, it was identified as calendic acid (α+β). Both varieties exhibit similar trends in terms of essential oils for use in commercial medicinal and food industries. In contrast, the Pot Marigold variety stands out in terms of dry flower yield, seed yield, and seed oil.

Keywords: Calendula, sowing time, cultivar, flower, seed

Çukurova Koşullarında Farklı Ekim Zamanlarının Aynısafa (*Calendula officinalis* L.) Çeşitlerinde Çiçek ve Tohum Verimi ile Kalitesine Etkisi

ÖZ

Bu çalışma, Çukurova koşullarında 2020-2021 büyüme sezonlarında Pot Marigold ve Orange King olmak üzere farklı çeşitlerin ve ekim zamanlarının (15 Mart, 1 Nisan ve 15 Nisan) Aynısafa (*Calendula officinalis*) bitkisinin çiçek ve tohum verimi ile kalitesi üzerindeki etkilerini belirlemek amacıyla yürütülmüştür. Deneme, 3 tekrarlamalı tesadüf bloklarında bölünmüş parseller deneme desenine göre kurulmuştur. Araştırma sonuçlarına göre, en yüksek kuru çiçek verimi (360.63 kg/ha, 15 Mart 2021) Pot Marigold çeşidinden elde edilmiş, en yüksek taze çiçek verimi (1308.67 kg/da, 15 Mart 2020), çiçek esansiyel yağı (%0.05, 15 Mart 2021) Orange King çeşidinden elde edilmiştir. Düzgün balon şekilli olanlardan en yüksek tohum verimi (35.6 kg/da) ve en yüksek tohum sabit yağı (%15.39) elde edilmiştir. Çiçek esansiyel yağdaki ana bileşen α-cadinol iken tohum yağında calendic asit (α+β) olarak belirlenmiştir. Hem Pot Marigold hem de Orange King çeşitleri Çukurova koşullarında başarılı bir şekilde yetiştirilebilir. Her iki çeşit de ticari tıbbi ve gıda endüstrisinde kullanım için esansiyel yağlar açısından benzer eğilimler gösterirken, Pot Marigold çeşidi kuru çiçek verimi, tohum verimi ve tohum yağı açısından öne çıkmıştır.

Anahtar Kelimeler: Aynısafa, ekim zamanları, çeşit, çiçek, tohum

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Introduction

Calendula officinalis L., commonly known as Marigold, belongs to the Asteraceae family and has been used as a medicinal plant from ancient times to the present day (Çolak, 2018). Its origin is Central and Southern Europe, Western Asia, and the USA (Ashwlayan et al., 2018). It has been used for medicinal purposes since the 12th century.

According to the World Health Organization (WHO), there are more than 20,000 plant species used for medicinal purposes worldwide (Kendir ve Güvenç, 2010). The flowers have been used in Mediterranean and Middle Eastern cuisines, in soups and stews in Germany, and the golden ray petals have been used to add color to butter and cheese (Reider et al., 2001). Calendula contains two types of pigments that can be used as yellow and orange natural dyes in the food industry, making it increasingly important as an acceptable natural colorant by food producers (Ul-Islam and Kumar, 2014). In Arab, Indian, and Ancient Greek cultures, it has been used as a medicinal herb in cosmetics, food, and fabric dyeing (Bown, 2002; Lubbe & Verpoorte, 2011). In modern times, the desire of people to consume nutritious foods has led to a new trend towards healthier diets. Consumers demand foods that are high in nutritional value, have a long shelf life, are free from chemical additives, and are environmentally friendly. *Calendula officinalis*, which holds potential in terms of edible flower coatings, is being used as both a herbal tea and a food dye, highlighting its compatibility with the environment (Lekshmi et al.,2023; Tomou et al., 2023). Among the fixed oils traditionally used for medicinal purposes worldwide, St. John's Wort, black seed, pomegranate, and Marigold oils are prominent. Marigold exhibits various biological effects such as antibacterial, antifungal, antiviral, anthelmintic, cytotoxic, antioxidant, analgesic, anti-inflammatory, liver protective, heart protective, burn healing, and more (Şahingil, 2019; Alsaraf et al., 2019). Today, *C. officinalis* is approved for food use in the USA and appears on the Food and Drug Administration's list of GRAS (Generally Recognized as Safe) substances (Hamzawy ve ark., 2013). Marigold flower is also successfully used in the treatment of diaper dermatitis in infants (Panahi et al., 2012), as a topical treatment for second-degree or higher acute dermatitis induced by radiotherapy in breast cancer patients (Pommier et al., 2004), in reducing lesions in homogeneous leukoplakia disease (Singh and Bagewadi, 2017), in reducing oral and pharyngeal mucositis in patients undergoing radiotherapy for head and neck cancer (Babaee et al., 2013), in accelerating the healing of ulcers (Buzzi et al., 2016), and in the treatment of vaginal candidiasis with vaginal lotion in patients (Saffari et al., 2016).

Marigold flower extract containing compounds like vitexin, rutin, quercetin, luteolin, quercitrin, myricetin, apigenin, and kaempferol can be used as a skin protectant due to its soothing sunburn effects and delaying skin aging. High doses of Marigold applied in animal experiments have been reported to have a sedative effect. In mice, sleep time induced by hexobarbital could be prolonged with Marigold (Silva et al., 2021). Marigold seeds also contain a significant amount of oil (5-22%) (Özgül and Yücel, 2005; Walisiewicz-Niedbalska et al., 2009). Linoleic acid in Marigold seeds is synthesized into conjugated calendic acid through desaturation (Crombie and Holloway, 1985; Reed et al., 2002). The increasing interest in plants producing conjugated fatty acids stems from the findings of recent research on their biological effects. Conjugated linolenic acids have significant lipid-lowering, anti-obesity (Chardigny et al., 2003; Miranda et al., 2014) and anti-carcinogenic effects, exhibiting apoptotic activity against various tumor cells such as the U-937 human leukemia cell line and colon cancer cells (Caco-2) (Hennessy et al., 2011; Suzuki et al., 2001; Yasui et al., 2006). In vitro studies have shown that α and β calendic acids increase cancer cell death in monocytic leukemia, colon cancer, and choriocarcinoma (a type of cancer that occurs in the womb during pregnancy) patients (Suzuki et al., 2001; Yasui et al., 2006; Li et al., 2013).

Therefore, in our study, the sowing time and cultivar characteristics of Calendula officinalis, a cool climate plant that has regained commercial importance in recent years due to scientific studies contributing to its medicinal and food industry use, have been examined in the Çukurova region, where intensive global warming has begun to be observed, and the highest yield and quality features have been determined.

Material and Method

Two *Calendula officinalis* varieties, Pot Marigold and Orange King, were used in the study. The flowers of the Pot Marigold and Orange King varieties differ in that the hermaphrodite flowers in the center of the receptacle and the ligulate (with stigma) flowers surrounding the receptacle are doubled. Orange King has a smaller flower pad with multiple, multiple ligulate, and fewer hermaphroditic flowers than the Pot Marigold varieties. Calendula has heteromorphic seeds (Figure 1). Five different seed shapes (Balloon-like without spines, insect leg, winged, curved, and ring-like) were observed in both Calendula cultivars under the Çukurova conditions.

The field experiment was conducted using a split-plot design over randomized completed blocks with three replications at the experimental area of the Department of Field Crops at Cukurova University in Adana, Turkiye (37^{o}00'55.00"N, 35^{o}21'2690" E) during the 2019-2020 and 2020-21 growing seasons. The main plot was allocated by the sowing dates (March 15, April 1, and April 15) while the subplot was occupied by a variety of *Calendula oficinalis* L. (Pot Marigold and Orange King)(Figure 1). In the experiment, the subplots are 2x3.5 m² in size, with a sowing interval of 25x50 cm, consisting of 7 rows (a total of 49 plants), while the main plots are 4x7 m² in size, consisting of 14 rows. Flower and seed harvest numbers varied according to sowing times, varieties, and years. Although the flowers were harvested with an interval of 4 days, the seeds were harvested with an interval of 8 days. While the number of flower harvests varied between 13-16 in the Pot Marigold variety and 12-16 in the Orange King variety, depending on sowing times in 2020, it varied similarly between 11-18 in both varieties in 2021 Seed harvest numbers were similar in both varieties in 2020 (3-9 times) and 2021 (4-9 times). The climate is usually typical of the Mediterranean, with hot, dry summers and moderate, rainy winters.

Clevenger-type apparatus was used for the isolation of essential oils by hydro-distillation method. Dried flowers (50 g each) were added to a beaker containing distilled water (500 ml). The distillation process was performed for 4 h and essential oil samples were stored in analyses.

5 g ground seed sample was extracted by Automatic Soxhlet Extractor using petroleum ether at the Department of Field Crops, Faculty of Agriculture, Çukurova University. Seed oil samples are stored in the refrigerator for GC-MS/MS.

The analyses conducted at the Central Research Laboratory in Çukurova University, performed by an Agilent GC-MS/MS (7890B GC -7010B MS) with an autosampler (Gerstel, Germany) equipped with a flame ionization detector and a capillary Agilent J& amp; amp; W DB-WAX column (60mx0.25 µm x0.25µm) The oven temperature was held at 50 o C for 1 min, raised to 200 ° C at a rate of 25°C/min held for 10 min and then to 230 ºC at a rate of 3 ° C/min held this temperature for 18 min. The injector (250 ºC) and detector temperatures (300 ºC) were set. The sample size was 1µl and the flow rate of carrier gas was 1mL/min. The split used was 1:40. Fatty acids were identified by comparing the retention times of FAME with the standard 37-component FAME mixture (Figure 2).

Figure 1. Overview and flower of cultivars and seeds. 1: A. Orange King flowers, B. Pot Marigold flowers. 2: Experiment, 3: Seed types.

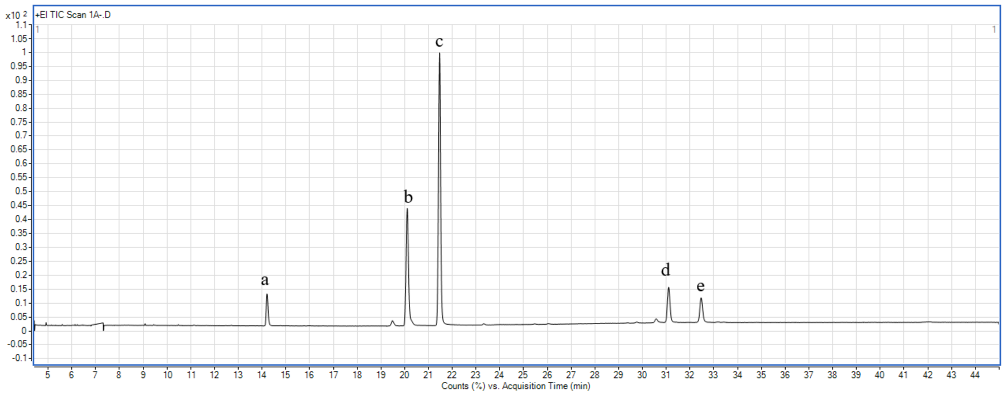

Figure 2. Representative GC-MS chromatogram obtained in the seed oil composition analysis (March 15, 2020, Pot Marigold) (a: Palmitic acid, b: Oleic acid, c: Calendic acid (α+β), d: Eicosapentaenoic acid, e: Behenic acid).

Results and Discussion

Fresh flower and dry flower yields were varied according to years, cultivar, and sowing date (Table 1 and 2). While the highest fresh flower yield was obtained from the Orange King cultivar (999.26 kg/da), In contrast to the Pot Marigold, which has a larger receptacle and fewer petals, the Orange King has denser and more double-layered petals. The petals contain less dry matter compared to the receptaculum, therefore, the highest dry weight is obtained from Pot Marigold (202.47 kg/da), which has a larger receptacle. The highest fresh flower yield (1308.67 kg/da) was obtained from the Orange King cultivar, on March 15, 2020. Differently from this, the highest dry flower yield (360.63 kg/da) was obtained from the Pot Marigold cultivar, on 15 March 2021. Increased temperatures in late sowing times caused the flowers to shrink in both cultivars. After the flowers were collected from the first harvest, small but numerous flowers appeared at the ends of the branches, which increased exponentially following each harvest. The shrinkage in flowers was caused by the high temperatures in July and August and the physiological aging of the plant. Thus, fresh and dried flower yields decreased in late sowing times. Our values are similar to the fresh flower yield values (500-1400 kg/da) obtained by Martin and Deo (2000) for the Pot Marigold variety; It is considerably higher than the fresh flower yield (84.90-166.10 kg/da) values obtained from the study conducted by Król (2012). For the Orange King variety, our values are higher than Crnobarac et al. (2008) (334.4-503.1 kg/da) and Balijagic et al. (2018) (290.8- 417.3 kg/da). Dry flower yields were higher than other reported results, with both

Orange King 166.10 kg/da (Krol, 2012.) and Pot Marigold 56.68 kg/da (Çalışkan and Kurt, 2018). The values of essential oil content in flowers do not show significant differences across varieties, sowing times, and years; however, they have been determined as 0.034% in the Pot Marigold variety and 0.042% in the Orange King variety (Figure 3). Our findings are similar to those of Ayran et al. (2023) (0.02-0.08%) and lower than those of Barut et al. (2024) (0.17%). The main components of the essential oil obtained from the flowers were identified as α-cadinol, δ-cadinene, γ-gurjunene, endo-borneol, and tau-cadinol (Table 3). The amount of α-cadinol and δ-cadinene detected in the flower essential oil was higher in 2021, which was warmer compared to 2020. In the Orange King variety, there were more fluctuations in the levels of cadinol and cadinene compared to the Pot Marigold variety. This change, as indicated by El-Hawary et al., was due to factors such as environmental conditions, variety, and harvest time. (El-Hawary et al., 2018). The variability in the content of compounds such as gamma-gurjunene and alpha-terpineol may depend on climatic conditions, and they were not detected in samples from 2021. The essential oil of *Calendula officinalis* flowers contains bioactive compounds (terpinen-4-ol, α-cadinol, muurolene) that exhibit antioxidant activity. With this property, the essential oil can be considered a natural antioxidant and antibacterial alternative to synthetic bactericides, allowing its use in the food industry. Additionally, its ability to completely inhibit the growth of food-borne fungal pathogens provides opportunities for use in the food industry (Hsu et al., 2021). It is claimed that gurjunene, one of the main components, has anti-cancer properties (Tomko et al., 2020).

Table 1. Fresh flower yield of *Calendula officinalis* (kg/da)

Cultivar	Year	Sowing Dates				Year Average	
		March 15	April 1	April 15	Year x Cultivar		
Pot Marigold	2020	937.73 bcd	971.47 bcd	762.76 de	890.65	941.39	913.39
	2021	992.03 bc	874.63 cd	594.53 e	820.40		
SDXC		**964.88**	**923.05**	**678.65**	**855.52 B**		
Orange King	2020	1308.67 a	1099.40 ab	568.33 e	992.13		
	2021	1079.63 bc	941.87 bcd	997.67 bc	1006.39		
SDXC		**1194.15**	**1020.63**	**783.00**	**999.26 A**		
SD		1079.52 A	971.84 A	730.83 B			
CV (%): 12.69	**LSD $_{YxSDxC}$: 209.3 LSD $_{SD}$:197.8 LSDc: 85.45**						

SD: Sowing Date, C: Cultivar, Y: Year.

Table 2. Dry flower yield of *Calendula officinalis* (kg/da)

Cultivar	Year	Sowing Dates				Year average	
		March 15	April 1	April 15	Year x Cultivar		
Pot Marigold	2020	207.90 bcd	159.60 cde	107.77 of	158.42 B	154.56	208.78
	2021	360.63 a	239.03 b	139.90 e	246.52 A		
SDXC		**284.27**	**199.32**	**123.83**	**202.47 A**		
Orange King	2020	223.07 b	156.30 de	72.73 f	150.70 B		
	2021	218.90 bc	150.80 de	143.43 e	171.04 B		
SDXC		**220.98**	**153.55**	**108.08**	**160.87 B**		
SD		252.63 A	176.43 B	115.96 C			

CV (%): 19.13	**LSD $_{YxSDxC}$: 61.82 LSD$_{YxC}$: 35.69 LSD $_{SD}$: 58.01 LSDc: 25.24**

SD: Sowing Date, C: Cultivar, Y: Year.

Figure 3. Essential oil content of *Calendula officinalis* flowers (%)

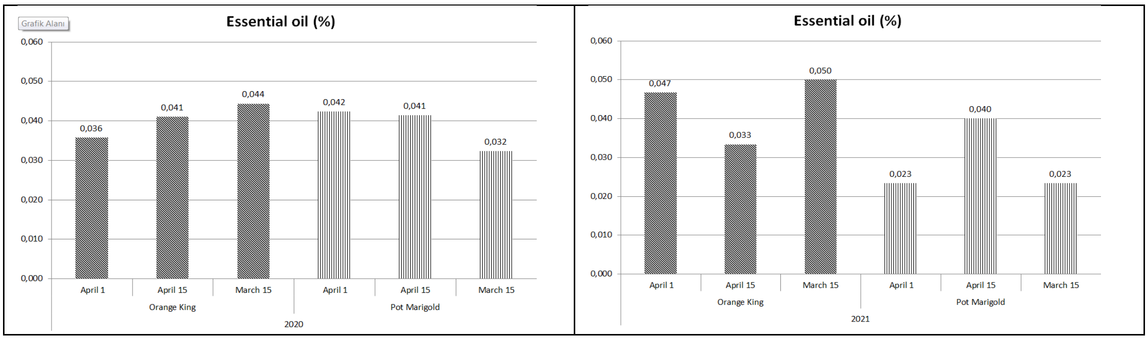

Table 3. Essential oil components of *Calendula officinalis* flowers (%)

	2020						**2021**					
	March 15		**April 1**		**April 15**		**March 15**		**April 1**		**April 15**	
	P.M.	**O.K.**	**P.M.**	**O.K.**	**P.M.**	**O.K.**	**P.M.**	**O.K.**	**P.M.**	**O.K.**	**P.M.**	**O.K.**
Terpinen-4-ol	1.34	0.68	1.73	1.38	1.10	1.23	1.28	1.16	1.40	0.78	1.42	1.45
Lavandulo/Humulene	-	0.38	0.23	0.12	0.27	0.20	0.44	0.49	0.44	0.45	0.37	0.25
γ-Muurolene	-	0.89	-	-	0.75	-	1.53	1.69	1.02	1.76	-	0.19
α-Terpineol	0.45	1.54	1.48	0.31	0.74	-	-	-	-	-	-	-
endo-Borneol	6.74	11.89	3.08	6.35	2.28	7.08	2.81	3.32	3.11	3.33	2.07	8.88
cis-muurola-3,5-diene	0.32	0.54	-	0.24	0.31	0.23	0.79	0.74	0.68	0.77	0.27	0.34
D-Carvone/ α-Muurolene	1.85	3.21	2.08	1.08	2.72	1.45	3.17	3.72	2.73	3.60	1.76	1.36
(+)-δ-Cadinene	18.01	28.46	19.18	11.43	20.04	14.89	23.09	5.90	20.49	23.51	20.73	17.62
Cubebol	2.57	5.44	2.40	3.02	2.66	2.20	-	2.38	2.42	2.28	2.78	3.22
Epicubebol/α-Copaene	2.03	1.23	1.87	2.23	2.12	1.60	1.60	1.88	1.90	1.65	2.01	2.38
Ledol	-	2.40	-	-	-	-	1.56	1.98	-	1.59	1.91	4.62
(-)-Globulol	3.35	-	1.80	5.63	1.70	4.30	1.56	1.98	2.04	1.59	1.91	4.62
γ-Gurjunene	10.06	6.74	4.45	17.17	3.87	13.34	-	-	-	-	-	-
Epicubenol	-	1.32	-	-	2.41	-	2.10	1.88	1.90	2.10	2.24	2.20
α-Cubebene	2.30	2.25	2.03	2.26	2.41	3.89	3.97	5.63	5.69	4.62	5.49	15.42
Naphthalene	3.81	-	3.20	4.21	-	-	-	-	0.28	0.48	-	0.35
(+)-Ledene	3.95	2.93	2.52	6.47	1.99	5.04	-	-	0.20	0.19	-	0.20
β-copaene/.tau.-Cadinol	3.26	2.09	4.25	2.54	5.64	3.18	5.25	6.62	4.93	4.98	3.76	2.01
δ-Cedrol	5.65	3.60	6.88	5.06	8.51	5.76	-	-	-	-	6.45	3.65
δ-Cadinol	1.28	0.79	1.44	1.21	2.05	1.27	1.66	2.24	1.62	7.38	1.39	0.81
α-Cadinol	24.57	14.00	29.69	23.31	29.71	24.85	28.39	35.61	26.33	26.26	32.49	17.48

Seed yields and seed oil rates were varied according to sowing date, cultivar, and years. Similarly Barut et al. (2023), differences were observed in the yield and seed oil rate of different seed shapes in parallel with the temperature increases during the harvest dates. The same five seed types (Balloon-like without spines, insect leg, winged, curved, and ring-like) were formed in both *Calendula officinalis* varieties under Çukurova conditions. Barut et al. (2022) reported that seed types vary according to environmental factors. While Soliman (2003) and Barut (2022) identified 6 seed types in different ecological conditions, Froment et al. (2003), Jolly et al. (2013) and De Clavijo (2005) identified 3 seed types. The highest seed yield was obtained from curved and ring seed shapes (respectively 22.93 and 20.53 kg/da at Pot Marigold) on March 15 (Figure 3.) according to the different sowing dates and seed shapes. Likewise our findings, Biçen (2020) obtained the highest seed yield from curved (40.75 kg/da) and ring-like (38.98 kg/da) seed types. The highest total seed yield was obtained from the Pot Marigold cultivar, March 15 sowing time (99.55 kg/da) according to cultivars and sowing times. The average total seed yield obtained in 2020 (59.55 kg/da) was higher than in 2021 (Figure 4). Different results were reported by other researchers, such as 63.42-179.72 kg/da (Barut et al.,2022), and 101.0-189.0 kg/da (Krol and Paszko, 2017). The seed oil rates obtained in both varieties in 2020 (5.7-15.4%) were higher than in 2021 (5.3-11.3%). The oil content of Orange King and Pot Marigold varieties depending on their seed types has changed over the years (Figure 4.). According to seed shapes, the highest oil rate was obtained in balloon-like without spines-type seeds (15.4%) in 2020 and ring-like type seeds (11.3%) in 2021 (Figure 5).

Different results were reported by other researchers, such as 13% -15% (Zarrinabadi et al., 2019), 2%-12% (Angelini et al., 1997), 12.86% (Barut and Tansi, 2024), 20% (Dulf et al., 2013). Calendula seed oil's main components have been identified as calendic acid, oleic acid, and eicosapentaenoic acid. The calendic acid in seed oil, which holds significance in both medical and food industries (Dulf et al., 2013; Chardingy et al., 2003; Hennessy et al., 2011; Suzuki et al., 2001; Yasui et al., 2006), is influenced by the sowing time, variety, and years. The highest calendic acid content (47.3 %) was obtained from the Pot Marigold variety on March 15, 2020 (Table 4).

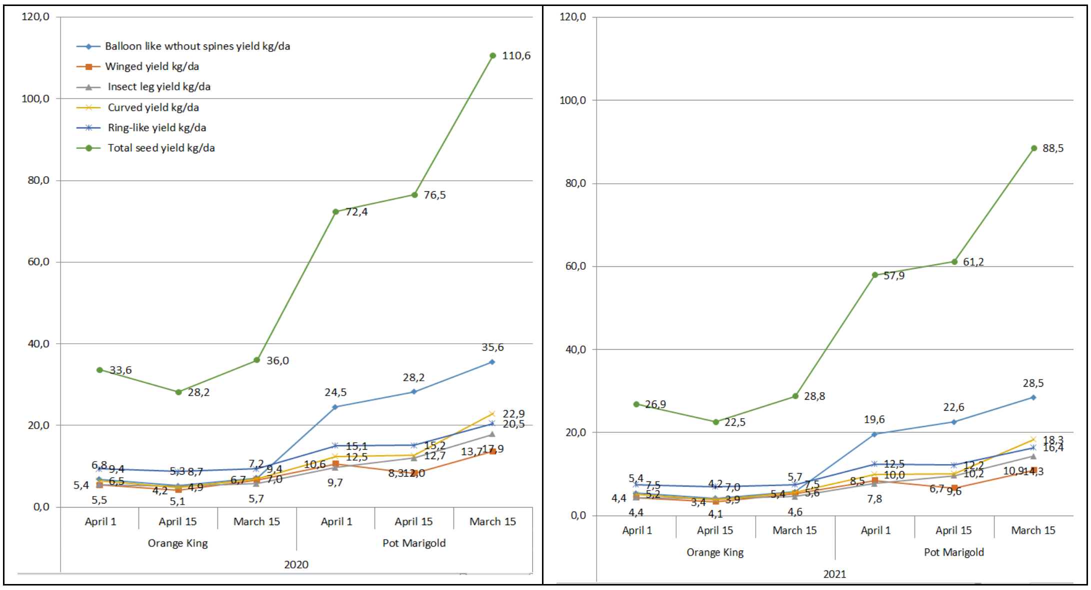

Figure 4. Total and different seed type yields of *Calendula officinalis* (kg/da)

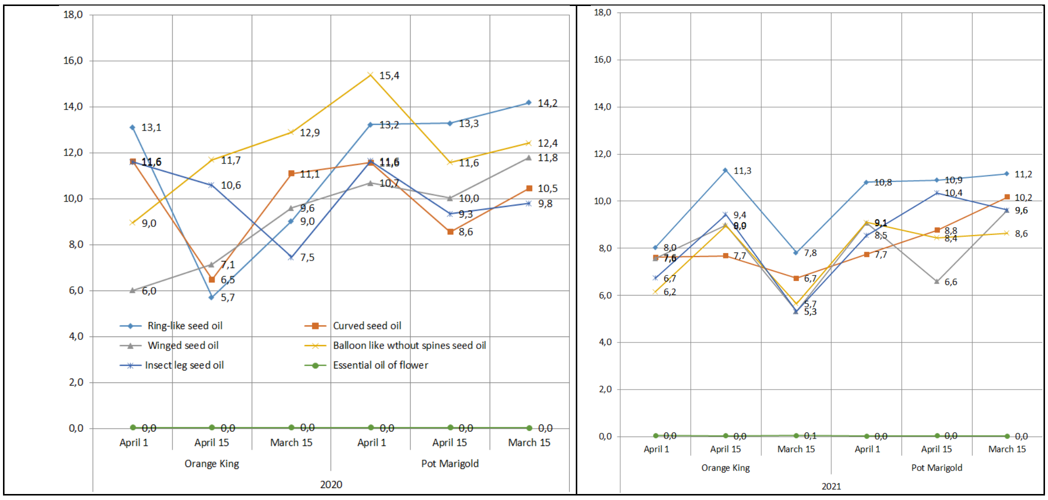

Figure 5. Different seed type oil rates of *Calendula officinalis* (%)

Table 4. Seed oil components of *Calendula officinalis* (%)

	2020						2021					
	March 15		**April 1**		**April 15**		**March 15**		**April 1**		**April 15**	
	P.M.	**O.K.**	**P.M.**	**O.K.**	**P.M.**	**O.K.**	**P.M.**	**O.K.**	**P.M.**	**O.K.**	**P.M.**	**O.K.**
Palmitic Acid	5.9	5.8	5.0	4.8	5.1	5.9	4.5	4.7	4.8	5.3	5.3	4.7
Stearic Acid	2.1	1.6	0.8	1.2	-	1.4	-	-	1.4	0.5	1.4	1.3
Oleic Acid	22.0	19.1	26.4	22.9	24.5	20.9	22.4	19.4	22.9	15.4	19.9	21.5
Calendic Acid (α+β)	47.3	39.9	44.6	43.5	46.3	44.9	44.4	41.5	43.4	35.6	45.4	41.6
Linolenic Acid	0.5	0.3	0.5	0.5	0.5	0.9	0.6	0.1	0.6	0.9	0.5	0.6
Eicosapentaenoic Acid	11.0	14.5	19.8	22.2	18.0	22.6	18.6	13.4	21.9	19.5	20.7	20.2
Behenic Acid	8.7	3.3	2.9	3.7	4.9	3.0	9.1	0.5	4.4	-	4.3	6.5

Conclusion

Early sowing is recommended under Çukurova conditions as it allows plants to benefit from the cool climate conditions for a longer period.

Flowers should be gradually harvested every 4 days throughout the flowering period to collect them before seed formation, while seeds should be gradually harvested every 8 days to prevent them from shedding before harvest.

Both Pot Marigold and Orange King varieties can be grown successfully under Çukurova conditions. Both varieties show similar trends in terms of essential oil for commercial medicinal and food purposes, and the Pot Marigold variety stands out in terms of dry flower yield, seed yield, and seed oil.

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