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# Harvest date as a factor affecting crop yield, oil content and fatty acid composition of the seeds of calendula (*Calendula officinalis* L.) cultivars

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ABSTRACT

Recent years have witnessed a growing interest in calendula (*Calendula officinalis* L.), as an oil-bearing plant whose seeds contain unique polyunsaturated fatty acids that can be used in pharmaceutical, paint and coatings industries. The influence of harvest date on seed yield and oil biosynthesis in calendula seeds has not been widely researched. A three-year field experiment was carried out to examine the effect of harvest dates at 50%, 65% and 80% seed maturity on the yield and fatty acid composition of seven calendula cultivars. The highest seed (1096–1950 kg $ha^{-1}$) and oil (181–391 kg $ha^{-1}$) yield and the highest oil content (16.39–20.55%) were achieved when calendula plants were harvested with 65% of mature seeds, and yields were somewhat smaller with 80% of mature seeds. The oil from calendula seeds harvested at 80% seed maturity was characterized by the highest content of α-calendic acid (43.60%–54.39%) and the lowest content of the remaining fatty acids. The Partial Least Squares Regression analysis revealed that oil ($r^2$ = 95.8) and seed ($r^2$ = 98.1) yield depends on the morphological features of the examined cultivars, such as the number of flower heads, percentage of morphological seed types and climatic factors (mean temperature and total precipitation during the growing season). The results clearly indicate that seed and oil yield was highest in cultivars with the highest percentage of winged and hooked seeds and the highest number of flower heads.



## 1. Introduction

Calendula (*Calendula officinalis* L.), also known as pot marigold, is a plant native to southern Europe, which has been cultivated around the world for ornamental and medicinal purposes for many centuries. Calendula flower heads (*Calendulae anthodium*) are used as in the production of pharmaceuticals, cosmetics and foods (Khalid and Teixeira da Silva, 2012).

In recent years, calendula seeds have attracted scientific interest as a source of oil with unique properties. The oil content of calendula seeds ranges from 5% (Angelini et al., 1997) to 22% (Dulf et al., 2013; Gesch, 2013). Calendula seed oil is abundant in conjugated linolenic acids (CLNAs) such as α-calendic acid ((8*E*, 10*E*, 12*Z*)-octadeca-8, 10, 12-trienoic acid) and contains small amounts of β-calendic acid ((8*E*,10*E*,12*E*)-octadeca-8,10,12-trienoic acid) (Özgül-Yücel, 2005; Walisiewicz-Niedbalska et al., 2012).

Oils containing CLNAs are used in the chemical industry; for example, α-eleostearic acid as a drying oil in resins, varnishes, inks, paints and coatings (Biermann et al., 2010; Metzger, 2009). CLNAs demonstrate a wide spectrum of bioactive properties: they regulate lipid metabolism and exert antiadipogenic, antioxidant and anti-inflammatory effects. CLNAs also deliver anti-carcinogenic properties (Hennessy et al., 2011; Yuan et al., 2014; Hennessy et al., 2016). *In vitro* studies have demonstrated that exposure to α- and β-calendic acids can induce apoptosis in human monocytic leukemia cells, colon cancer cells and choriocarcinoma (Suzuki et al., 2001; Yasui et al., 2006; Li et al., 2013).

Harvesting is a critical operation, especially in plants with indeterminate inflorescences and shedding of mature seeds. The flowering stage lasts several weeks in calendula (Król, 2012). A long flowering period is desirable in ornamental cultivars, but in oil-bearing plants such as calendula, it causes uneven seed ripening and prevents the determination of the optimal harvest date (Froment et al., 2003). Seed and oil yield may be compromised when plants are not harvested on the optimal date. Delayed harvest contributes to seed loss (Gesch et al., 2005; Breemhaar and Bouman, 1995), while premature harvest of underdeveloped seeds leads to a considerable reduction in both seed yield and oil content due to poor seed filling and low oil deposition (Martin et al., 2005; Ghasemnezhad and Honermeier, 2008).

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Calendula seeds are represented by differently shaped achenes, including larval (annular achenes), winged (cymbiform achenes) and hooked (rostrate achenes) (Cromack and Smith, 1998; De Clavijo, 2005; Joly et al., 2013), which differ in size and weight (Kiełtyka-Dadasiewicz and Król, 2015). The uniformity of calendula seeds facilitates mechanical sowing and contributes to uniform plant emergence (Joly et al., 2013). One of aims of breeding works is to ensure that oil-bearing cultivars of calendula produce seeds whose shape is compatible with seeding equipment and facilitates seed cleaning. For example, cultivar 'Carola' produces primarily larval-shaped (annual) seeds (Eberle et al., 2014). However, according to Robbelen et al. (1994), a large share of larval-shaped seeds does not seem compatible with high yields and oil content.

The aim of this study was to determine the optimum harvest date of calendula seeds that guarantees maximum seed and oil yields. The second objective was to compare the chemical composition of oils obtained on each of the examined harvest dates. To the best of our knowledge, no such investigations have been carried out to date. The results of our experiment were processed statistically to develop a mathematical model describing the relationships between yield, specific features of the examined cultivars and climatic factors.

## 2. Materials and methods

### 2.1. Field experiment

A field experiment was conducted in 2011–2013 on the Experimental Farm of the University of Life Sciences in Lublin (Eastern Poland – 51°13′ 21.9″ N, 22° 37′ 55.85″ E). The farm is situated in an area dominated by Luvisol soil group with a texture of silt loam. The Ap horizon had a pH (KCl) of 6.6–7.1 and an organic matter content of 1.5–1.8%. The content of available K, P and Mg was estimated at 129–135, 55–63 and 44–54 mg kg$^{-1}$, respectively. The study comprised seven different ornamental cultivars (cv.) of calendula: 'Tokaj', 'Santana', 'Radio', 'Persimmon Beauty', 'Orange King', 'Promyk', and 'Szlem'. The experiment had a randomized block design with four replications. Each experimental plot was 5 m long and 2 m wide. On 16 April 2011, 22 April 2012 and 17 April 2013, 8 kg seeds ha$^{-1}$ were sown in rows with 25 cm spacing. Emerged seedlings were thinned to 60 plants per m$^2$. The NPK rate was 80–31–60 kg ha$^{-1}$, respectively.

The experimental treatments were harvest times: 50%, 65% and 80% mature (brown) seed head. One of the three adjacent 10 m$^2$ plots was harvested on each harvest date. Calendula develops indeterminate inflorescences, therefore, the plants were desiccated chemically according to the recommendations of Froment et al. (2003). Before desiccation, plant height, the number of flower heads, and biomass (after drying for 3 days at 50 °C) were determined in 20 randomly selected plants per replicate. Plants from each plot were harvested 5–9 days after desiccation (refer to the Supplementary material, Table A1). Plants were harvested manually with pruning shears and placed in canvas bags. The bags were stored in a hoop tunnel until dry. Next, the seeds were mechanically threshed with a threshing machine. The seeds were cleaned and weighed to determine seed yield which was adjusted to 10% moisture content. In each cultivar, morphological seed types were determined according to the method proposed by De Clavijo (2005).

### 2.2. Determination of oil content and fatty acid composition

The seeds of every analyzed cultivar were ground separately in a stainless-steel grinder, and fat was extracted from 5 g samples over a period of 8 h with n-hexane in the Soxhlet apparatus. Then, n-hexane was evaporated at 40 °C from the extract in a rotary evaporator, and the resulting oil was stored in a freezer at a temperature of −18 °C. Methyl-esterification of the obtained fatty acids was carried out according to the $BF_3$-$CH_3OH$ method (AOCS, 1997).

Chromatographic analyses were performed in the Varian GC 3800 system (Walnut Creek, CA USA) equipped with an auto injector and a flame-ionizing detector (FID). Cis-trans fatty acid methyl esters (FAMEs) were separated on the UltiMetal™ capillary column CP-WAX 52CB (id 0.25 μm, length 60 m) with a polyethylene glycol stationary phase. The carrier gas was helium with a flow rate of 1.4 mL min$^{-1}$. Initial column temperature was 120 °C, temperature was increased in increments of 2 °C min$^{-1}$, and maximum temperature was 210 °C. The duration of the analysis was 127 min. Injector and detector temperatures were set to 160 °C. FAME peaks were identified based on the Supelco 37 Component FAME Mix and LGC standards.

### 2.3. Statistical analysis

The statistical analysis was carried out in the Statgraphics Centurion XVI program (Shapiro-Wilk and Bartlett's tests; Pearson's, Spearman's and partial correlations, multifactor ANOVA and Principal Component Analysis (PCA)). A Partial Least Squares Regression (PLSR) analysis was performed in the Tanagra 1.4.50 program to handle multicollinearity between variables (Rakotomalala, 2005). The number of latent vectors was selected based on the critical values of Wold's $Q^2$ (Wold, 1978), where the latent vector was considered to be significant when the minimal $Q^2$ value exceeded 0.097. The variable selection process was similar to the automatic procedure in stepwise regression analysis based on the values of standardized coefficients, Variable Importance in the Projection (VIP) values, and changes in $r^2$ (for details, see Chong and Jun, 2005, and Paszko, 2014).

## 3. Results and discussion

### 3.1. Climatic conditions, growth and development of plants

The highest total precipitation was noted in 2011, and rainfall was unevenly distributed throughout the growing season (Table 1). Dry spells in April and May delayed plant emergence, and heavy rainfall in July and August extended the period of flowering and seed maturation (refer to the Supplementary material, Table A1). The growing season of 2012 was characterized by low precipitation rates and high temperatures, which inhibited plant growth and development (Table 2 and Supplementary material, Table A1). The most favorable conditions for the growth of calendula plants were noted in 2013 which was characterized by uniform distribution of precipitation and moderate temperatures (Table 1).

The investigated calendula cultivars had varied morphological traits. Plants of cv. 'Tokaj' had the tallest shoots, cv. 'Orange King' produced the highest number of flower heads, and cv. 'Radio'

**Table 1**
Air temperature and precipitation during the growing season of calendula in the field experiment (Lublin, Poland).

| Year | Month | | | | |
|---|---|---|---|---|---|
| | April | May | June | July | August |
| Average air temperature (°C) | | | | | |
| 2011 | 10.8 | 14.3 | 18.0 | 18.1 | 19.0 |
| 2012 | 9.5 | 15.8 | 18.9 | 21.5 | 19.5 |
| 2013 | 8 .1 | 15.5 | 18.5 | 19.2 | 19.1 |
| Monthly precipitation (mm) | | | | | |
| 2011 | 29.9 | 42.2 | 67.8 | 179.0 | 85.3 |
| 2012 | 34.0 | 56.3 | 52.8 | 52.3 | 37.6 |
| 2013 | 51.1 | 101.6 | 70.2 | 86.1 | 47.8 |