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BEATA KRÓL

Influence of nitrogen fertilization on the seed yield and the content and quality of fat in pot marigold (*Calendula officinalis* L.) cultivars

Wpływ nawożenia azotem na plon nasion oraz zawartość i jakość tłuszczu wybranych odmian nagietka lekarskiego (*Calendula officinalis* L.)

Summary. Pot marigold seeds contain oil with a specific composition (presence of calendic acid); hence, they can be used as an alternative oil raw material. In 2011-2013, a field experiment was conducted to determine the effect of different nitrogen doses $(0, 30, 60, 90, 120, 150 \text{ kg N} \cdot \text{ha}^{-1})$ on the seed yield, oil content, and fatty acid composition in four pot marigold cultivars. Nitrogen fertilization exerted a positive effect on the number of flower heads and seed yield. However, the response of marigold cultivars to nitrogen doses applied varied, i.e. the yield of the 'Tokaj' and 'Radio' cultivars was significantly increased up to the dose of 60 kg N·ha⁻¹, whereas such an increase was noted in the 'Orange King' and 'Persimmon Beauty' cultivars at the dose of 90 kg $N \cdot ha^{-1}$. Nitrogen fertilization modified the content of fat: the low nitrogen doses (30 kg $N \cdot ha^{-1}$) did not influence the fat content in the seeds, whereas the increasing doses resulted in its gradual decline. The cultivars were characterised by significant differences in plant morphology, seed yield, and fat quality. During the three-year period, the highest seed and fat yields were determined for the 'Orange King' cultivar (1857.3 and 399.3 kg ha⁻¹, respectively) and the highest proportion of calendic acid in the oil (54.86%) was detected in the 'Persimmon Beauty' cultivar. The yield and quality of marigold oil depended on both nitrogen fertilization and the weather conditions prevailing during the growing seasons. The increase in the nitrogen doses was accompanied by an increase in the content of linoleic acid and a decrease in the level of oleic acid. The cooler and wetter growing season in 2011 turned out to promote the highest fat and calendic acid accumulation.

Key words: agronomic nitrogen efficiency, fat yield, fatty acid composition, calendic acid

INTRODUCTION

The pot marigold has long been cultivated as an ornamental and medicinal plant. Formulations derived from ray florets or inflorescences are used in treatment of many diseases [Muley et al. 2009]. Given the high fat content in marigold seeds (approx. 20%) with a specific fatty acid composition (presence of calendic acid), the plant has been in the focus of interest as an alternative oil-bearing crop [Zanetti et al. 2013]. The chemical properties of marigold seed oil facilitate its application in chemical industry for production of polymers and high quality paints [Biermann et al. 2010], and its antitumour activity can be used for pharmaceutical purposes [Li et al. 2013].

Numerous reports have indicated that nitrogen fertilization exerts an effect on the size and quality of the yield of pot marigold inflorescences [Mili and Sable 2003, Bie-siada et al. 2006, Szwejkowska and Bielski 2012]. However, there are only few publications on the nitrogen fertilization of pot marigold as an oil-bearing material and the information on this issue is unequivocal. Ahmad et al. [2017] found that the pot marigold requires intensive nitrogen fertilization (200 kg N·ha⁻¹), whereas Froment et al. [2003] recommended substantially lower amounts of nitrogen (50–100 N·ha⁻¹). Additionally, the suitability of the oil-bearing material is determined by the chemical composition of seeds, but the publication cited disregards this issue [Froment et al. 2003, Ahmad et al. 2017]. Nitrogen demand should be considered in relation to cultivars, as they may differ in this respect [Wielebski and Wójtowicz 1998, Kotecki et al. 2001].

The aim of the study was to determine the effect of differentiated nitrogen fertilization on elements of the yield structure and seed production and on the content and quality of fat in four pot marigold cultivars.

MATERIAL AND METHODS

The field study was carried out on an experimental farm of the University of Life Sciences in Lublin in 2011–2013 (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 6.7–7.2 and an organic matter content of 1.4–1.7%. The content of available P, K and Mg was estimated at 6.57–7.78, 12.7–13.1 and 4.86–5.59 mg·100g⁻¹ respectively.

The experiment was set up in a split-plot design with four replications (plot size of $5m^2$). The experiment involved the following factors: I – cultivars: 'Tokaj', 'Orange King', 'Persimmon Beauty', and 'Radio' (characterised by high seed yields and high content and quality of oil (Król and Paszko 2017)) and II – nitrogen doses: 0, 30, 60, 90, 120, and 150 kg N·ha⁻¹. Nitrogen (in the form of 34% ammonium nitrate) was applied twice: half before sowing the seeds and rest after plant thinning. The same level of phosphorus (single superphosphate) and potassium (potassium salt) fertilization at a dose of 31 kg P·ha⁻¹ and 70 kg K·ha⁻¹, respectively, was applied in all objects in autumn.

Immediately after forecrop (white mustard) harvest, postharvest cultivation treatments and ploughing were carried out. Seeds were sown using a garden seed drill on April 15–22 in each study year at 25-cm row spacing and a seeding rate of 8 kg·ha⁻¹. After emergence, the seedlings were thinned and approximately 60 plants per 1 m² were left. Later during the growing season, the soil in the interrows was scarified and weeded. The plants were harvested at maturity of 60–70% seeds (in the first half of August). Due to uneven seed maturation, the plants were desiccated 5–9 days before the harvest, as recommended by Froment et al. [2003]. The harvested plants were dried in a foil tunnel,

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the seeds were threshed, cleaned, and dried to approximately 10% humidity, and the yield and thousand-seed weight were determined. Prior to desiccation, the number of flower heads was determined in 20 plants randomly collected from each object and the plant vegetative mass (stems and leaves), was evaluated after drying.

Seeds collected from each object were ground separately in a stainless-steel grinder, and fat was extracted from a 5-g sample with n-hexane for 8 h using a Soxhlet apparatus. Fatty acid methyl esters were extracted with the BF₃-CH₃OH method [AOCS 1997]. Chromatographic analyses were carried out with the use of a Varian GC 3800 device (Walnut 123 Creek, CA USA) equipped with an autosampler and a flame-ionising detector (FID). Separation of the fatty acid methyl esters was performed in a UltiMetalTM UCP-WAX 52CB capillary column (\emptyset 25 µm, length 60 m) with a polyethylene glycol stationary phase. Helium with a flow rate of 1.4 cm³·min⁻¹ was used as a carrier gas. The initial temperature of the column was 120°C, and the maximum temperature was 210°C. The analysis lasted for 127 min. The injector and detector temperatures were set at 160°C. The peaks of the fatty acid methyl esters were identified using Supelco 37 Component FAME Mix and LGC standards.

Agronomic nitrogen efficiency (AE) was calculated according to the following equation:

$$AE = \frac{S_F - S_C}{N}$$

where: S_F is the seed yield of the fertiliser treatment; S_C – the seed yield of the control treatment (kg·ha⁻¹); N – the fertiliser rate (kg N·ha⁻¹) [Dordas 2010].

The theoretical fat yield calculated per 1 ha area is a quotient of the seed yield and fat content in seeds.

The results were analysed statistically with analysis of variance for the orthogonal factorial experiments in the split-plot design. The significance of the differences between the values of the parameters was tested with Tukey's confidence semi-intervals at a significance level $\alpha = 0.05$.

Year/ Rok	Month/ Miesiąc						
I cal/ KOK	IV	V	V VI VII VIII		VIII	IV – VIII	
Air temperature/ Temperatura (°C)						Mean Średnia	
2011	10.8	14.3	18.0	18.1	19.0	16.0	
2012	9.5	15.8	18.9	21.5	19.5	17.0	
2013	8.1	15.5	18.5	19.2	19.1	16.1	
1950-2010	7.4	13.0	16.3	18.0	17.2	14.4	
	Total Suma						
2011	29.9	42.2	67.8	179.0	85.3	404.2	
2012	34.0	56.3	52.8	52.3	37.6	233.0	
2013	51.1	101.6	70.2	86.1	47.8	356.8	
1950–2010	39.0	60.7	65.9	82.0	70.7	318.3	

Table 1. Weather conditions during the marigold growing season in 2011–2013Tabela 1. Warunki pogodowe w okresie wegetacji nagietka lekarskiego w latach 2011–2013

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The weather conditions prevailing in 2011–2013 varied and exerted a significant effect on the course of pot marigold growth (tab. 1). In 2011, the highest rainfall level was recorded but its distribution during the growing season was not beneficial. Moisture deficiencies noted in April and May delayed plant emergence and growth; in turn, the high precipitation levels recorded in July and August (2-fold higher than the multiyear rates) impeded seed maturation and harvesting. The growing season of 2012 was characterised by low rainfall rates and high temperatures, which shortened the plant vegetation period and had an adverse effect on pot marigold yields. The most favourable weather conditions (evenly distributed precipitation and moderate temperatures) promoting the growth and development of pot marigold plants were recorded in 2013.

RESULTS AND DISCUSSION

The length of the growing season of the pot marigold depended mostly on the weather conditions during the study years. The longest (on average 127 days) and the shortest (107 days) vegetation periods were noted in 2011 and 2012, respectively. Thedifferences between the cultivars ranged from 5 day in the dry and warm season of 2012 to 13 days in the rainy season of 2011. Each year, the longest vegetation period was noted for the 'Radio' cultivar (on average 121 days), whereas the 'Persimmon Beauty' cultivar was characterised by the shortest vegetation time (114 days). The length of the flowering period is important in cultivation of the pot marigold as an oil-bearing plant. A long flowering period is desirable in ornamental species, but in cultivation focused on seed raw material it results in uneven maturation and problems with determination of the optimal harvesting time. The longest flowering period (22–36 days) was recorded in the 'Radio' cultivar, while 'Orange King' was characterised by the shortest flowering (18–26 days) (data not presented in the tables).

The different dosage of nitrogen fertilization only slightly modified the rate of the consecutive developmental stages in the pot marigold, with the strongest effect on the course of flowering and seed maturation. Plants that did not receive nitrogen fertilization began flowering at the latest term and the length of this phase was the shortest. With the increasing nitrogen dose, the flowering phase increased, which was in agreement with the reports by Chauhan and Kumar [2007] and Król [2011]. In our investigations conducted in plots fertilized with the high nitrogen doses (120–150 kg N·ha⁻¹), the process of seed maturation was extended by 2–10 days (data not present in the tables). In contrast, the experiments carried out by Johnson and Gesch [2013] did not reveal an effect of nitrogen fertilization on the marigold flowering and maturation rate.

The analysed pot marigold cultivars were characterised by significant differences in morphological traits. The 'Radio' cultivar plants produced the greatest amounts of vegetative mass, 'Orange King' had the greatest number of flower heads, and 'Tokaj' exhibited the highest thousand-seed weight (tab. 2). Significant morphological and developmental differentiation depending on the genotype was reported by other authors as well [Cromack and Smith 1998, Gesch 2013]. The morphological traits of the pot marigold exhibited changes induced by the humidity and thermal conditions. The substantial water shortages noted in 2012 had a negative effect on biomass formation and plant development (number of inflorescences and seed formation). The traits had the lowest values of all the 3 years of the study.

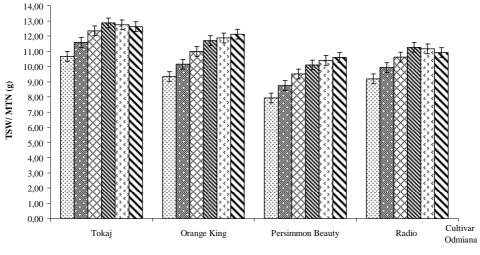
Table 2. Number of flower heads per plant, air-dry mass of the vegetative parts of the plants, and thousand-seed weight of the pot marigold, depending on the factors of the experiment Tabela 2. Liczba koszyczków kwiatowych na roślinie, powietrznie sucha masa części wegetatywnych roślin oraz masa tysiąca nasion nagietka lekarskiego w zależności od czynników doświadczenia

Factor Czynnik	Flower heads Koszyczki kwiatowe	Dry mass of the vegetative parts Sucha masa części wegetatywnych (g)	Thousand-seed weight MTN (g)					
Cultivar/ Odmiana								
Tokaj	aj 37.7 ^c 24.8 ^b 12.							
Orange King	46.8 ^a	20.8 °	11.02 ^b					
Persimmon B.	43.1 ^b	20.6 ^c	9.54 ^d					
Radio	38.6 ^c	28.4 ^a	10.51 ^c					
	Dose of nitrogen/ Dawka azotu (kg N·ha ⁻¹)							
0	30.1 ^d	15.2 ^f	9.28 ^d					
30	35.8 °	19.1 ^e	10.09 ^c					
60	41.2 ^b	22.8 ^d	10.86 ^b					
90	45.9 ^a	25.8 °	11.48 ^a					
120	47.0 ^a	28.4 ^b	11.55 ^a					
150	48.7 ^a	30.6 ^a	11.56 ^a					
Years/ Lata								
2011	43.6 ^b	23.6 ^b	11.12 ^a					
2012	33.9 °	19.9 °	9.78 ^b					
2013	47.1 ^a	27.5 ^a	11.51 ^a					

Values denoted in columns with different letters (a–f) differ significantly (p = 0.05)

Wartości liczbowe oznaczone w kolumnach różnymi literami (a–f) różnią się istotnie (p = 0.05)

The nitrogen fertilization exerted a significant effect on the growth and morphological characteristics of the plants. It has the greatest effect on the production of vegetative mass (stems and leaves), whose average value from the 3 years was two-fold higher in the variants treated with the highest dose of 150 kg N·ha⁻¹ than in the control object (tab. 2). Noteworthy, all the nitrogen doses used in the experiment resulted in a significant increase in the vegetative mass. The nitrogen fertilization stimulated branching, thereby increasing the number of inflorescences; the differences between the nitrogen doses (90–150 kg·ha⁻¹) were small and statistically insignificant. The favourable effect of nitrogen in promoting plant growth might be attributed to the more efficient metabolite transport and synthesis of protein, which increased photosynthesis and cell multiplication, resulting in increased vegetative growth. These results are in good agreement with those reported by Chauhan and Kumar [2007]. A positive effect of the nitrogen addition on the thousand-seed weight (TSW) was found (tab. 2) with correlations between the cultivars and nitrogen doses. In the case of the 'Tokaj' and 'Radio' cultivars, reduction of TSW was noted in the variants with the fertilization doses of 120–150 kg N·ha⁻¹, which was probably associated with shedding of the largest seeds (fig. 1). This phenomenon was especially pronounced in 2011, which was characterised by high precipitation rates.



🖸 ON 🖾 30N 🖾 60N 🔯 90N 🖸 120N 🗳 150N

Fig. 1. Thousand-seed weight (TSW) of the pot marigold (interactions between the cultivars and nitrogen fertilization); error bars – Least Significant Difference (p = 0.05)
Rys. 1. Masa tysiąca nasion (MTN) nagietka lekarskiego (współdziałanie odmian i nawożenia azotowego); słupki błędów – najmniejsza istotna różnica (p = 0.05)

The mean 3-year marigold yields obtained in the present investigations ranged from 1191.7 kg·ha⁻¹ ('Radio') to 2028.3 kg·ha⁻¹ ('Orange King') (fig. 2). Similar yields were reported by Breemhaar and Bouman [1995], Cromack and Smith [1998], Forcella et al. [2012] and Gesch [2013]. In turn, the yields reported by Wilen et al. [2004] were substantially lower and did not exceed 800 kg·ha⁻¹. As explained by the authors, the low marigold yields may have been associated with seed shedding during mechanical harvest. As shown by Zanetti et al. [2013], marigold seed losses during harvest can reach 50–60%.

The nitrogen fertilization applied in this experiment caused a significant increase in the seed yield, with plant response to the nitrogen doses dependent on the cultivar (fig. 2). In the 'Tokaj' and 'Radio' cultivars, the seed yield increased significantly up to the dose of 60 kg N·ha⁻¹, while the higher dose of 90 N·ha⁻¹ resulted in only a slight increase in the yield. In turn, in the variant with high doses (120–150 kg N·ha⁻¹), reduced yields were recorded (except for 2012). In the case of the 'Orange King' and 'Persimmon Beauty' cultivars, a significant increase in the seed yield was noted in the variants with

the dose of 90 kg $N \cdot ha^{-1}$ as well. The increase in the fertilization doses to 120 and 150 kg $N \cdot ha^{-1}$ resulted in further enhancement of the yield, but the differences were small and statistically insignificant. The different responses of the analysed pot marigold cultivars may be associated with the specific genetically determined nutrient requirements of the species.

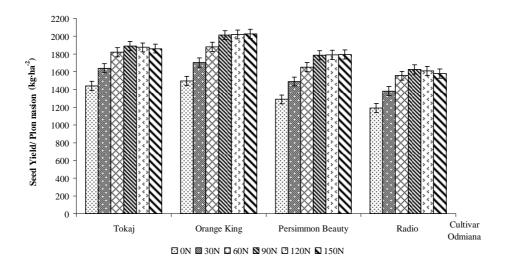


Fig. 2. Pot marigold seed yield depending on nitrogen fertilization (mean for 2011–2013); error bars – Least Significant Difference (p = 0.05) Rys. 2. Plon nasion odmian nagietka w zależności od nawożenia azotem (średnie z lat 2011–2013); słupki błędów – najmniejsza istotna różnica (p = 0.05)

Based on the 3-year results, it can be concluded that, depending on the cultivar, the best production effects of nitrogen fertilization of the pot marigold cultivated for seeds can be obtained at the doses of 60–90 kg N·ha⁻¹. The further increase in the nitrogen doses caused yield decline (tab. 3). Similar nitrogen fertilization was recommended by Froment et al. [2003], whereas Johnson and Gesch [2013] and Samoon and Kirad [2013] claim that a higher dose should be applied (100–150 kg N·ha⁻¹) and Ahmad et al. [2017] obtained the highest seed weight at a dose of 200 kg N·ha⁻¹. Such large discrepancies in the data may be related to the specific agroclimatic conditions in which the investigations were conducted and to differences between analysed cultivars.

The yield of the pot marigold largely depended on the weather conditions prevailing during each growing season (tab. 3). All cultivars achieved the highest yields in 2013, when the moderate precipitation was distributed evenly over time, and the lowest yields were reported in the dry and warm season of 2012. The response to the weather conditions varied between the cultivars. 'Persimmon Beauty' exhibited the highest yielding stability (coefficient of variation (CV) – 11.7%), whereas the greatest fluctuations of the yields were noted in the 'Radio' cultivar (CV – 21.5%) (data not presented in the tables).

The agronomic efficiency of fertilization is a measure of its effectiveness expressed in yield increments per unit (1 kg) of the fertiliser component. In all the cultivars, the value of this parameter was the highest at the lowest fertilization level (30 kg N·ha⁻¹) and declined with the increasing nitrogen doses (fig. 3). The 'Orange King' cultivar was characterised by the highest nitrogen efficiency and 'Radio' exhibited the lowest values. The nitrogen productivity measured as a ratio of the yield increase to the nitrogen dose increase differed between the cultivars (fig. 4). In the ranges of 0–30 and 30–60 kg N, the nitrogen efficiency values were similar in all the cultivars; at high doses, i.e. 90–120 and 120–150 kg N·ha⁻¹, negative values were noted in the 'Radio' and 'Tokaj' cultivars.

Table 3. Seed yield and the content and yield of crude fat, depending on the factors of the experiment

Tabela 3. Plon nasion, zawartość oraz plon tłuszczu surowego w zależności od czynników doświadczenia

Factor Czynnik	Seed yield Plon nasion (kg·ha ⁻¹)	Fat content Zawartość tłuszczu (%)	Fat yield Plon tłuszczu (kg·ha ⁻¹)					
Cultivar/ Odmiana								
Tokaj	1754.1 ^b	21.0 ^{ab}	368.9 ^b					
Orange King	1857.3 ^a	21.5 ^a	399.3 ^a					
Persimmon B.	1633.3 °	19.5 ^b	317.8 °					
Radio	1490.4 ^d	18.2 °	271.2 ^d					
	Dose of nitrog	en∕ Dawka azotu (kg N·ha¹)					
0	1354.5 ^d	20.8 ^a	283.8 ^d					
30	1554.0 ^c	20.7 ^{ab}	324.9 ^c					
60	1727.0 ^a	20.4 ^{ab}	354.5 ^{ab}					
90	1827.7 ^b	19.9 ^{bc}	367.2 ^a					
120	1823.6 ^{ab}	19.5 ^{cd}	358.5 ^{ab}					
150	1815.8 ^{ab}	18.9 ^d	346.9 ^b					
Years/ Lata								
2011	1773.1 ^b	20.7 ^a	368.8 ^a					
2012	1405.2 °	19.1 ^b	269.1 ^b					
2013	1873.0 ^a	20.3 ^a	380.0 ^a					

Values denoted in columns with different letters (a–d) differ significantly (p = 0.05)

Wartości liczbowe oznaczone w kolumnach różnymi literami (a–d) różnią się istotnie (p = 0.05)

The cultivars and nitrogen fertilization were factors that predominantly determined the content of crude fat in the seeds. The mean content of fat in the analysed cultivars ranged from 18.2% ('Radio') to 21.5% ('Orange King') (tab. 3). Varied levels of fat in pot marigold cultivars were reported by other authors as well [Cromack and Smith 1998, Dulf et al. 2013, Król et al. 2016]. The increasing nitrogen doses led to reduction of the fat content in the seeds of all the cultivars (the mean difference between the control object and the highest dose was 1.9 percentage points) (tab. 3). The greatest reduction of the fat content was noted in the 'Radio' and 'Tokaj' cultivars in 2011, which may have been associated with shedding of the most mature seeds (data not presented in the tables).

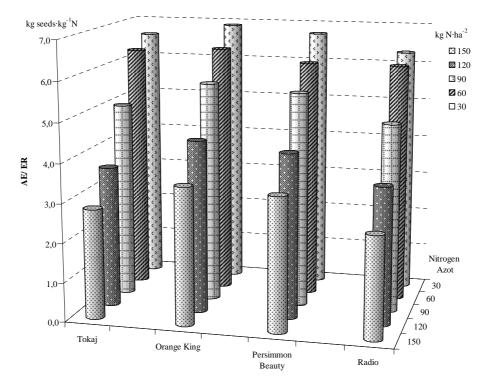


Fig. 3. Agronomic nitrogen efficiency (AE) – mean for 2011–2013 Rys. 3. Efektywność rolnicza azotu (ER) – średnie z lat 2011–2013

The available literature on the pot marigold does not provide data of the analysed issue. Investigations of other oil-bearing plants [Rathke et al. 2006, Dordas 2010, Wielebski 2011, Jiang et al. 2014] showed that nitrogen fertilization exerted a negative effect on the seed fat content in a majority of cases.

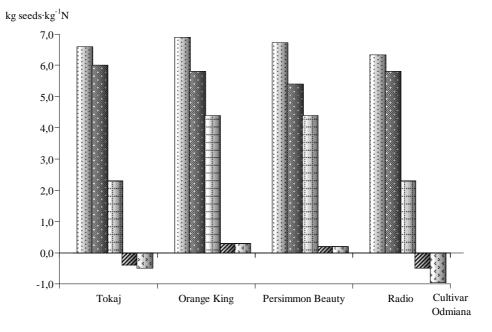
The content of crude fat was also dependent on the weather conditions prevailing in the study years. The high precipitation recorded in 2011 and 2013 accompanied by moderate temperatures promoted accumulation of fat. Substantially lower fat levels were detected in the dry a hot season of 2012 (tab. 3).

The profitability of oil-bearing plant cultivation is determined by the fat yield [Zanetti et al. 2013], which is a function of the seed yield and the percentage level of lipids contained therein. In the present experiment, the greatest mean 3-year fat yields were obtained in the $60-120 \text{ kg N}\cdot\text{ha}^{-1}$ dose variant (the individual values did not differ statistically). As expected, the lowest fat yield was recorded in the non-fertilised variant (tab. 3). The highest yield of all the cultivars compared was noted for 'Orange King', which produced on average a 47% higher fat yield than that exhibited by 'Radio', i.e. a cultivar with the lowest yield. The differences in the fat yield were also induced by the weather conditions prevailing during the vegetation period: a substantially higher fat yield was noted in all the cultivars in 2013 and 2011 than in 2012 (tab. 3).

B. KRÓL

The proportion of fatty acids in the pot marigold oil depended primarily on the weather conditions in the study years and the genotype, but only slightly on the nitrogen fertilization (tab. 4). The analysis of the major fatty acids revealed significant differences between the cultivars in the case of palmitic, stearic, oleic, and linoleic acids as well as calendic acid, which is classified as a linolenic acid isomer (CLNA). The highest levels of calendic acid were accumulated by the 'Persimmon Beauty' cultivar, which exhibited the lowest content of oleic and linoleic acids. An inverse relationship was noted in the case of the 'Radio' cultivar, since both these acids are precursors of calendic acid [Cahoon et al. 2001, Król et al. 2016].

Differences in the proportions of the individual acid groups were noted over the study years as well (tab. 4). A more favourable fatty acid composition (highest content of CLNA) was noted for the seeds of all the cultivars in 2011. Therefore, it seems that the high air humidity that and low mean temperatures during seed formation and maturation had a positive effect on the fat composition, which was shown in other studies as well [Król and Paszko 2017].



⊡ 0–30 🖾 30–60 🖾 60–90 🖾 90–120 🖾 120–150

Fig. 4. Productivity of 1 kg of nitrogen (in kg of seeds) at doses increasing every 30 kg (mean for 2011–2013)
 Rys. 4. Produktywność 1 kg azotu (w kg nasion) w przedziale dawek co 30 kg (średnie z lat 2011–2013)

	Fatty acid/ Kwasy tłuszczowe (%)									
Factor				18:2	18:3n6	18:3n6	Σ	Σ	Σ	Σ
Czynnik	16:0	18:0	18:1 (n9)	(n6)	(8t,10t,12c)	(8t,10t,12t)	CLNA	SFA	MUFA	PUFA
Cultivar/ Odmiana									I	
Tokaj	3.88 ^b	2.56 ^b	6.72 ^b	33.76 ^b	45.55 ^b	4.44 ^c	49.99 ^b	7.81 ^b	7.43 ^b	84.77 ^{ab}
Orange King	4.01 ^b	2.63 ^b	5.52 °	34.96 ^b	43.36 ^c	4.71 ^b	48.07 ^b	9.12 ^a	6.99 ^c	83.89 ^b
Persimmon B.	3.66 ^c	2.05 °	4.73 ^d	32.00 ^c	51.90 ^a	2.96 ^d	54.86 ^a	6.76 ^c	5.53 ^d	87.71 ^a
Radio	5.27 ^a	3.07 ^a	7.10 ^a	36.93 ^a	39.15 ^d	5.11 ^a	44.26 ^c	10.00^{a}	7.99 ^a	82.04 ^b
Dose of nitrogen/ Dawka azotu (kg N·ha ⁻¹)										
0	4.07	2.50	6.69 ^a	33.25 ^b	45.71	4.17	49.88	8.18	7.89 ^a	84.03
30	4.13	2.54	6.42 ^{ab}	33.72 ^b	45.45	4.21	49.65	8.26	7.55 ^a	84.22
60	4.11	2.52	6.21 ^b	34.17 ^{ab}	45.13	4.30	49.43	8.32	7.17 ^b	84.51
90	4.27	2.58	5.81 ^c	34.60 ^a	44.93	4.37	49.30	8.53	6.63 ^c	84.83
120	4.31	2.64	5.53 ^d	35.12 ^a	44.58	4.39	48.97	8.66	6.38 ^d	84.96
150	4.32	2.67	5.45 ^d	35.63 ^a	44.16	4.40	48.55	8.65	6.28 ^d	85.06
Years/ Lata										
2011	3.95 ^b	2.10 °	5.50 °	33.58 ^b	47.50 ^a	4.02 ^b	51.52 ^a	7.43 ^c	6.60 ^b	85.99 ^a
2012	4.85 ^a	2.98 ^a	6.59 ^a	35.25 ^a	42.24 ^c	4.82 ^a	47.06 ^c	9.59 ^a	7.29 ^a	83.12 ^b
2013	3.81 ^b	2.65 ^b	5.97 ^b	34.42 ^{ab}	45.24 ^b	4.07 ^b	49.31 ^b	8.25 ^b	7.06 ^a	84.69 ^{ab}

Table 4. Fatty acid composition in the oil from pot marigold seeds, depending on the factors of the experiment Tabela 4. Skład kwasów tłuszczowych oleju z nasion nagietka lekarskiego w zależności od czynników doświadczenia

16:0 – palmitic acid/ palmitynowy; 18:0 – stearic acid/ stearynowy; 18:1n9c – oleic acid/ oleinowy; 18:2n6 – linoleic acid/ linolowy; 18:3n6 (8t,10t, 12c) – α-calendic acid/ α-nagietkowy; 18:3n6 (8t,10t,12t) – β-calendic acid/ β-nagietkowy; \sum CLNA – \sum α-calendic and β-calendic acid/ α-nagietkowy i β-nagietkowy; SFA – saturated fatty acids/ nasycone kwasy tłuszczowe; MUFA – monounsaturated fatty acids/ jednonienasycone kwasy tłuszczowe; PUFA – polyunsaturated fatty acids/ wielonienasycone kwasy tłuszczowe; Values denoted in columns with different letters (a–d) differ significantly (p = 0.05)/ Wartości liczbowe oznaczone w kolumnach różnymi literami (a–d) różnią się istotnie (p = 0.05) The effect of nitrogen fertilization on the proportion of fatty acids was limited to a significant increase in the content of linoleic acid and a decrease in the oleic acid level. The slight decrease in the CLNA content was statistically insignificant. Only in 2011, a significant decline in the CLNA content, compared to the control, was observed in the variants with the high nitrogen doses $(120-150 \text{ kg N}\cdot\text{ha}^{-1})$ in the 'Radio' and 'Tokaj' cultivars (data not presented in the tables). This may have been a consequence of more intense seed shedding; as demonstrated by Martin et al. [2005] as well as Król and Paszko [2017], calendic acid is accumulated during marigold seed maturation. Modification of the fatty acid composition induced by application of nitrogen fertilization was reported in investigations of other oil-bearing plants [Pisulewska et al. 1999, Urbaniak et al. 2008, Wielebski 2011, Jiang et al. 2014].

CONCLUSIONS

1. Nitrogen fertilization of the pot marigold has a positive effect on formation of flower heads as well as thousand-seed weight and, consequently, on the seed yield.

2. The best production effects of nitrogen fertilization of the pot marigold cultivated for seeds were obtained at the doses of 60 and 90 kg $N \cdot ha^{-1}$, depending on the cultivar. The further increase in the nitrogen doses is economically irrelevant.

3. The content and quality of marigold oil (assessed on the basis of the fatty acid composition) is a species-related trait only slightly modified by the nitrogen fertilization. The increased fertilization doses were accompanied by a decline in the fat content, in which the proportion of linoleic acid increased and that of oleic acid decreased. These features depended also on the weather conditions prevailing during the growing seasons. The cooler and wetter growing season was the most favourable for accumulation of fat and calendic acid (CLNA).

4. Among the cultivars compared in the experiment, 'Orange King' was characterised by the highest seed and fat yields; in terms of the oil quality, 'Persimmon Beauty' accumulated the highest levels of calendic acid.

REFERENCES

- Ahmad I., Jabeen N., Ziaf K., Dole J.M., Khan M.A. S., Bakhtawar M.A., 2017. Macronutrient application affects morphological, physiological, and seed yield attributes of *Calendula officinalis* L. Can. J. Plant Sci. April. DOI: 10.1139/CJPS-2016-0301.
- AOCS (American Oil Chemists Society), 1997. Preparation of methyl esters of fatty acids. 452 Official Method Ce 2–66. Champaign (IL): AOCS Press.
- Biermann U., Butte W., Holtgrefe R., Feder W., Metzger J.O., 2010. Esters of calendula oil and tung oil as reactive diluents for alkyd resins. Eur. J. Lipid. Sci. Tech. 112, 103–109.
- Biesiada A., Sokół-Łętowska A., Kucharska A., Wołoszczak E., 2006. Wpływ formy i dawki azotu na plonowanie i skład chemiczny koszyczków nagietka (*Calendula officinalis* L.) Folia Hort. 18, supl.1, 61–65.
- Breemhaar H.G., Bouman A., 1995. Harvesting and cleaning *Calendula officinalis*, a new arable oilseed crop for industrial application. Ind. Crop. Prod. 4(4), 255–260.

- Cahoon E.B., Ripp K.G., Hall S.E., Kinney A.J., 2001. Formation of conjugated Δ^8 , Δ^{10} -double bonds by Δ^{12} -oleic-acid desaturase-related enzymes Biosynthetic origin of calendic acid. J. Biol. Chem. 276, 2637–2643.
- Chauhan A., Kumar V., 2007. Effect of graded levels of nitrogen and VAM on growth and flowering in calendula (*Calendula officinalis* L.) J. Orn. Hort. 10(1), 61–63.
- Cromack H.T.H., Smith J.M., 1998. Calendula officinalis production potential and crop agronomy in southern England. Ind. Crop. Prod. 7, 223–229.
- Dordas C.A., 2010. Variation of physiological determinants of yield in linseed in response to nitrogen fertilization. Ind. Crop. Prod. 31(3), 455–465.
- Dulf F.V., Pamfil D., Baciu A.D., Pintea A., 2013. Fatty acid composition of lipids in pot marigold (*Calendula officinalis* L.) seed genotypes. Chem. Cent. J. 7–8.
- Forcella F., Papiernik S.K., Gesch R.W., 2012. Postemergence herbicides for calendula. Weed Technol. 26(3), 566–569.
- Froment M., Mastebroek D., van Gorp K., 2003. A growers manual for *Calendula officinalis* L. Plant Research International, Wageningen, 11.
- Gesch R.W., 2013. Growth and yield response of calendula (*Calendula officinalis*) to sowing date in the northern US. Ind. Crop. Prod. 45, 248–252.
- Jiang Y., Caldwell C.D., Falk K.C., 2014. Camelina seed quality in response to applied nitrogen, genotype and environment. Can. J. Plant Sci. 94(5), 971–980.
- Johnson J.M., Gesch R.W., 2013. Calendula and camelina response to nitrogen fertility. Ind. Crop. Prod. 43, 684–691.
- Kotecki A., Malarz W., Kozak M., 2001. Wpływ nawożenia azotem na rozwój i plonowanie pięciu odmian rzepaku jarego. Rośliny Oleiste – Oliseed Crops, 22(1), 69–80.
- Król B., 2011. Yield and the chemical composition of flower heads of pot marigold (*Calendula officinalis* L. cv. Orange King) depending on nitrogen fertilization. Acta Sci. Pol. Hortorum Cultus, 10(2), 235–243.
- Król B., Paszko T., Król A., 2016. Conjugated linolenic acid content in seeds of some pot marigold (*Calendula officinalis* L.) cultivars grown in Poland. Farmacia, 64(6), 881–886.
- Król B., Paszko T., 2017. Harvest date as a factor affecting crop yield, oil content and fatty acid composition of the seeds of calendula (*Calendula officinalis* L.) cultivars. Ind. Crop. Prod. 97, 242–251.
- Li Q., Wang H., Ye S.H., Xiao S., Xie Y.P., Liu X., Wang J.H., 2013. Induction of apoptosis and inhibition of invasion in choriocarcinoma JEG-3 cells by α-calendic acid and β-calendic acid. Prostag. Leukotr. Ess. 89, 367–376.
- Martin R.J., Porter N.G., Deo B., 2005. Initial studies on seed oil composition of calendula and lunaria. Agron. N. Z. 35, 129–137.
- Mili R., Sable A.S., 2003. Effect of planting density and nitrogen levels on growth and flower production of calendula (*Calendula officinalis* L.). Indian. J. Hortic. 60(4), 343–345.
- Muley B.P., Khadabadi S.S., Banarase N.B., 2009. Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae): a review. Trop. J. Pharm. Res. 8(5), 455–465.
- Pisulewska E., Lorenc-Kozik A., Borowiec F., 1999. Wpływ zróżnicowanego nawożenia azotem na plon, zawartość oraz skład kwasów tłuszczowych w nasionach dwóch odmian soi. Rośliny Oleiste – Oliseed Crops, 20(2), 511–520.
- Rathke G., Behrens T., Diepenbrock W., 2006. Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): a review. Agric. Ecosyst. Environ. 117, 80–108.
- Samoon S.A., Kirad K.S., 2013. Effect of nitrogen and phosphorus on seed yield parameters of calendula (*Calendula officinalis* L.) var. Touch of Red Mixture. Prog. Hortic. 45(1), 149– 151.

- Szwejkowska B., Bielski S., 2012. Effect of nitrogen and magnesium fertilization on the development and yields of pot marigold (*Calendula officinalis* L.). Acta Sci. Pol. Hortorum Cultus, 11(2), 41–148.
- Urbaniak S.D., Caldwell C.D., Zheljazkov V.D., Lada R., Luan L., 2008. The effect of cultivar and applied nitrogen on the performance of *Camelina sativa* L. in the Maritime Provinces of Canada. Can. J. Plant Sci. 88(1), 111–119.
- Wielebski F., Wójtowicz M., 1998. Reakcja odmian rzepaku ozimego na wzrastające dawki azotu na glebach żytnich w Zielęcinie. Rośliny Oleiste – Oilseed Crops, 19(2), 507–514
- Wielebski F., 2011. Wpływ nawożenia siarką w warunkach stosowania zróżnicowanych dawek azotu na skład chemiczny nasion różnych typów odmian rzepaku ozimego. Rośliny Oleiste – Oilseed Crops, 32(1), 79–95.
- Wilen R.W., Barl B., Slinkard A.E., Bandara M.S., 2004. Feasibility of cultivation calendula as a dual purpose industrial oilseed and medicinal crop. Acta Horticulturae 629, 199–206.
- Zanetti F., Monti A., Berti M.T., 2013. Challenges and opportunities for new industrial oilseed crops in EU-27. A review. Ind. Crop. Prod. 50, 580–595.

Streszczenie. Nasiona nagietka lekarskiego zawierają tłuszcz o specyficznym składzie (obecność kwasu nagietkowego) i moga być wykorzystywane jako alternatywny surowiec olejarski. W latach 2011–2013 przeprowadzono doświadczenie polowe, którego celem było określenie wpływu zróżnicowanych dawek azotu (0, 30, 60, 90, 120, 150 kg N·ha⁻¹) na plon nasion, zawartość oleju i skład kwasów tłuszczowych czterech odmian nagietka lekarskiego. Nawożenie azotem dodatnio wpłynęło na liczbę wytwarzanych przez roślinę koszyczków kwiatowych oraz na plon nasion, przy czym poszczególne odmiany nagietka niejednakowo reagowały na zastosowane w doświadczeniu dawki azotu: Plony odmian 'Tokaj' i 'Radio' istotnie wzrastały do dawki 60 kg N·ha⁻¹, natomiast u odmian 'Orange King' i 'Persimmon Beauty' taki wzrost odnotowano także w dawce 90 kg N·ha⁻¹. Nawożenie azotem modyfikowało zawartość tłuszczu, przy czym niskie dawki azotu (30-60 kg N·ha⁻¹) nie miały wpływu na zawartość tłuszczu w nasionach, natomiast zwiększenie dawek powodowało systematyczny spadek jego zawartości. Badane odmiany charakteryzowały się istotnym zróżnicowaniem cech morfologicznych oraz plonem nasion i jakością tłuszczu. W trzyletnim okresie największą wydajnością nasion i tłuszczu wyróżniała się odmiana 'Orange King'(odpowiednio 1857,3 and 399,3 kg·ha⁻¹), najwyższym zaś udziałem kwasu nagietkowego w oleju (54,86%) - odmiana 'Persimmon Beauty'. Wydajność i jakość oleju nagietka zależała zarówno od nawożenia azotowego, jak i warunków pogodowych w okresie wegetacji. Wraz ze zwiększaniem dawek azotu zwiększał się udział kwasu linolowego, a zmniejszał kwasu oleinowego. Najbardziej sprzyjający gromadzeniu tłuszczu i kwasu nagietkowego okazał się chłodniejszy i wilgotniejszy sezon wegetacyjny w 2011 r.

Słowa kluczowe: efektywność rolnicza azotu, wydajność tłuszczu, skład kwasów tłuszczowych, kwas nagietkowy

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