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YIELD AND THE CHEMICAL COMPOSITION OF FLOWER HEADS OF POT MARIGOLD (*Calendula officinalis* L. cv. Orange King) DEPENDING ON NITROGEN FERTILIZATION

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Abstract. Marigold belongs to the oldest medicinal plants, which had been used in the ancient times. At present it supplies its material of a great significance for pharmaceutical and cosmetic industry. The study was conducted in the period 2007–2009 on loess soil with the granulometric composition of silt loam. The aim of the experiment was to determine the effect of different nitrogen doses (0, 40, 80, 120, 160 kg $N \cdot ha^{-1}$) on some plant morphological features as well as on yield and quality of pot marigold inflorescences. Flowering of pot marigold was shortest in the control treatment (30 days) and longest (40 days) in the plot where nitrogen fertilization had been applied at the highest rate (160 kg $N \cdot ha^{-1}$). Nitrogen fertilization had a significant impact on the number of flower heads per plant, but no differences were found in inflorescence diameter under investigated factor. Raw material yield was found to increase significantly after the application of 80 kg $N \cdot ha^{-1}$, compared to the control treatment. Further increase in the amount of nitrogen (120, 160 kg $N \cdot ha^{-1}$) did not result in growth of yield. Nitrogen fertilization modified essential oil content in flowers heads (this content increased along with increasing nitrogen rates), but it did not affect the percentage of flavonoid compounds.

Key words: N dose, inflorescences yield, essential oil, flavonoids

INTRODUCTION

Pot marigold (*Calendula officinalis* L.) is an annual ornamental plant whose cultivars with dark-orange full flowers are also grown for herbal material used in the pharmaceutical and cosmetic industries [Taczanowska and Hołderna-Kędzia 1998].

Numerous studies [Golcz et al. 2006; Dzida and Jarosz 2006a; Biesiada and Kuś 2010] have shown that nitrogen fertilization plays a large role in determining yields of herbal plants. In the Polish literature there are few papers on pot marigold fertilization.

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Rumińska [1983] thinks that marigold does not need intensive mineral fertilization, whereas Dedio and Kozłowski [1998] found that high nitrogen rates reduced the percentage content of oleanosides. Pharmacopoeial raw material obtained from marigold includes its flower heads – *Calendulae anthodium,* or ligulate flowers – *Calendulae flos.* Their usefulness is dependent on their chemical composition. In the available literature there are few studies on the effect of nitrogen fertilization on the content of biologically active components in marigold. Biesiada et al. [2006] did not observe a clear correlation between nitrogen fertilization and the chemical composition of marigold flower heads. But they noted a slight decrease in polyphenol content at higher nitrogen rates.

The aim of the present study was to determine the effect of different doses of nitrogen fertilization on some plant morphological features as well as on yield and quality of pot marigold inflorescences.

MATERIALS AND METHODS

A three-year-long field experiment (2007–2009) was conducted on loess soil with the granulometric composition of silt loam, characterized by a neutral pH (pH 1 M KCl – 6.7) as well as an average content of humus (1.61%) and nutrients (51.6 P; 168.3 K; 50.1 Mg in mg·kg^{-1} of soil). The experiment was set up in a randomized block design in 5 m^2 plots, in quadruplicate. Seeds of pot marigold cv. 'Orange King', characterized by large, full orange-coloured flowers, were used for the investigations.

In the experiment there were used 4 nitrogen fertilization levels: 40, 80, 120, 160 kg N·ha^{-1}. Plots without nitrogen fertilization were the control treatments. The same phosphorus (single superphosphate) and potassium (potassium salt) fertilization was used in the experiment at the following amounts: P-21.8 and K – 62.3 kg·ha^{-1}. Phosphorus and potassium fertilizers as well as a half of the nitrogen dose (in the form of ammonium nitrate) were incorporated during soil preparation before seeding. The remaining portion of nitrogen fertilizer was applied after emergence, during the first weeding and soil loosening.

Seeds (dressed with the fungicide Dithane M-45) were sown in the third decade of April using a garden seed drill at a row spacing of 40 cm (8 kg·ha^{-1}). After emergence, thinning was done, leaving about 60 plants per m^2 for future growth. The start and end dates of flowering were recorded as well as the flowering duration was determined for all treatments.

Flower heads were harvested gradually as the plants bloomed, at 5-day intervals (from the first half of July until the second decade of August). After each harvest, the diameter of flower heads and the number of ligulate flowers were determined (n = 10), and subsequently the overall average was calculated for flower heads from all harvests. During the study, the number of flower heads per plant was determined based on 20 randomly selected plants from each treatment. After the end of harvest, the total yields of dry flower heads and ligulate flowers (t·ha^{-1}) were determined. The results were analysed statistically using the analysis of variance and Tukey's test.

Directly after harvest, flower heads were dried in a drying room at a temperature of 35°C. Then, after drying, there was determined essential oil content in the dry inflorescences by Deryng's method and flavonoid content (expressed in hyperoside and quercetin equivalents) using the method described in Farmakopea Polska VIII [2008]. The results presented in this paper are three-year averages.

RESULTS AND DISCUSSION

Depending on the nitrogen rate, flowering of pot marigold lasted from 30 to 40 days. The plants in the control treatment started flowering the latest and the duration of this stage was the shortest (30 days). As the amount of nitrogen increased, the flowering duration lengthened, since it lasted 40 days in the plots where 160 kg N·ha^{-1} had been applied. The lowest number of harvests was recorded in the control treatment (6), whereas inflorescences were harvested eight times in the plots where the highest nitrogen dose had been applied. The number of flowers was initially small, gradually increasing until the fourth and fifth harvests. During the next harvests, the number of inflorescences decreased and they came primarily from the third-tier shoots. As reported by Rumińska [1983], inflorescences borne on the main stem and on the first-tier branches, which produce the largest heads, bloom first. At the end of anthesis, flower heads are becoming increasingly less shapely and have a smaller number of ligulate flowers; hence their pharmaceutical value is lower. In our experiment, the head diameter also decreased on successive branches, but no difference was found in inflorescence size under the effect of different nitrogen doses (tab. 2).

Table 1. The effect of nitrogen dose on the number of flower heads, number·plant^{-1}
Tabela 1. Wpływ dawki azotu na liczbę koszyczków kwiatowych, sztuka·roślina^{-1}

Dose of nitrogen Dawka azotu kg N·ha^{-1}	Total number of inflorescences Ogólna liczba kwiatostanów	Including inflorescences on branches W tym kwiatostanów z odgałęzień		
		first rank I rzędu	second rank II rzędu	third rank III rzędu
Control – Kontrola	41.6	8.8	26.0	5.8
40	56.0	9.5	34.8	10.7
80	67.5	11.0	43.3	12.2
120	68.2	11.7	43.2	12.3
160	69.0	11.9	43.3	12.8
Mean – Średnio	60.5	10.6	38.1	10.8
$LSD_{0.05}$ – $NIR_{0,05}$	20.41	n.s.	14.21	4.17

n.s. – difference not significant – różnica nieistotna

The plants in the control treatment produced the least amount of inflorescences (in total 41.6 – tab. 1), while nitrogen fertilization stimulated the formation of branches, thus affecting the increase in the number of inflorescences; however, the differences between the higher nitrogen rates (80–160 kg·ha^{-1}) were small and statistically insignificant. A comparison of the distribution of inflorescences on particular branches shows that nitrogen fertilization primarily impacted the formation of second- and third-tier shoots, whereas in the case of the first-tier shoots the difference between particular treatments was not statistically proven.

Table 2. The effect of nitrogen dose on the inflorescence diameter (mm) and number of ligulate flowers per head
Tabela 2. Wpływ dawki azotu na średnicę kwiatostanów (mm) oraz liczbę kwiatów języczkowych w koszyczku

Dose of nitrogen Dawka azotu kg N·ha^{-1}	Inflorescence diameter on branches Średnica kwiatostanów na odgałęzieniach			Inflorescence diameter irrespective of the branches Średnica kwiatostanu niezależnie od odgałęzienia	Number of ligulate flowers in the inflorescence irrespective of the branches Liczba kwiatów języczkowych w koszyczku niezależnie od odgałęzień
	first rank I rzędu	second rank II rzędu	third rank III rzędu		
Control – Kontrola	88.2	82.6	71.8	80.9	149.3
40	89.7	84.1	73.1	82.3	151.5
80	88.8	83.5	72.8	81.7	172.9
120	88.5	83.7	71.4	81.2	176.3
160	88.4	83.1	72.7	81.4	178.1
Mean – Średnio	88.7	83.4	72.4	81.5	165.6
$LSD_{0.05}$ – $NIR_{0,05}$	n.s.	n.s.	n.s.	n.s.	22.83

n.s. – difference not significant – różnica nieistotna

Rumińska [1983] reports that there can be up to 50 inflorescences on a single marigold plant. On the other hand, in the experiments of Martin and Deo [2000], carried out in New Zealand, marigold produced about 20 flower heads, whereas in the study of Khalid et al. [2006] in India this number ranged from 70 to 140 pieces·plant^{-1}. In our experiment, the average number of flower heads was 60.1 per plant. Large variations in this value result from the differences in climatic conditions, marigold cultivars, and experimental factors.

Native research [Dedio et al. 1986; Biesiada et al. 2006] shows that nitrogen fertilization rates of 30–50 kg N·ha^{-1} are sufficient under good soil conditions. In the experiments of Biesiada et al. [2006], increased nitrogen doses above 50 kg N·ha^{-1} resulted in a significant growth only in herb yield. In the experiments of Mili and Sable [2003] conducted in different climatic and soil conditions, the highest inflorescence yield was obtained at a nitrogen rate of 100 kg N, whereas in the study of Gantait and Chattopadhyay [2004] an increase in fertilization from 100 to 200 kg N·ha^{-1} resulted in an increase in flower head yield by more than 90%. Hoffmann and Komosa [1974] report that the yield of flower heads and herb significantly increases along with increasing NPK rates.

In our own experiment, dry inflorescences yield was found to increase significantly, compared to the control treatment, after the application of 80 kg N·ha^{-1} (by 55% in the case of flower heads and by 74% for ligulate flowers – tab. 3). However, the increase in the yield of ligulate flowers was noted to be higher, which is probably attributable to the fact that nitrogen fertilization also contributed to an increase in the number of ligulate flowers per head (tab. 2). A further increase in nitrogen fertilization (120–160 kg·ha^{-1}) practically had no effect on the yields of dry flower heads and ligulate flowers. It is characteristic that the yield of vegetative matter (stems and leaves) increased systematically (and significantly) together with an increase in nitrogen rates (tab. 3).

Table 3. The effect of nitrogen dose on the dry weight of vegetative parts, yield of dry inflorescences and ligulate flowers, t·ha^{-1}

Tabela 3. Wpływ dawki azotu na suchą masę części wegetatywnych, plon suchych kwiatostanów i kwiatów języczkowych, t·ha^{-1}

Dawka azotu Dose of nitrogen kg N·ha^{-1}	The dry weight of vegetative parts Sucha masa części wegetatywnych	Yield of dry inflorescences Plon kwiatostanów	Yield of dry ligulate flowers Plon kwiatów |ęzyczkowych
Control – Kontrola	2.292	0.974	0.712
40	2.382	1.296	0.981
80	2.556	1.514	1.239
120	2.754	1.532	1.251
160	2.970	1.519	1.249
Mean – Średnio	2.590	1.367	1.086
$LSD_{0.05}$ – $NIR_{0,05}$	0.145	0.423	0.363

Marigold inflorescences yields varies and is dependent on the place of study and the cultivar. In the experiments conducted in Poland [Hojden et al. 1990; Biesiada et al. 2006; Dedio et al. 1986], yields of dry flower heads ranged from 1.0 to 2.88 t·ha^{-1}. Similar results were obtained in India [Gantait and Chattopadhyay 2004] and in Chile [Berti et al. 2003], whereas in the studies carried out in New Zealand [Martin and Deo 2000], in Italy [Piccaglia et al. 1997], marigold yields were much lower and did not exceed 0.5 t·ha^{-1}. In our experiment, average flower head yield was 1.37 t·ha^{-1}.

The content of biologically active components is evidence of the quality of herbal raw material; in the case of marigold, the content of essential oil and flavonoids (expressed in hyperoside and quercetin equivalents) was analysed. In the present study, the average content of essential oil in flower heads was 0.37 ml·100 g^{-1} and nitrogen fertilization modified its content. The least amount of oil was determined in the raw material from the control treatment (0.31 ml·100 g^{-1} and, along with an increase in nitrogen doses, its content increased (the difference between the control treatment and the highest N rate was 0.11 ml·100 g^{-1} – tab. 4).

Taczanowska and Hołderna-Kędzia [1998] as well as Dedio et al. [1986] report that marigold inflorescences contain ca. 0.2% of essential oil. Chalchat et al. [1991] obtained 0.3% of oil, while in marigold grown in other regions of the world [Gazim et al. 2008; Okoh et al. 2007; Okoh et al. 2008] only 0.1% of this component was found. In the literature there are few data on the effect of nitrogen fertilization on essential oil content in pot marigold inflorescences. Nevertheless, research on other herbal plants demonstrates that nitrogen fertilization affects not only yield levels, but also has an effect on the chemical composition of herbal raw material. In the experiments with marjoram, Dzida and Jarosz [2006a] as well as Kozłowski and Nowak [1983] found an increased concentration of essential oil in the herb under increasing nitrogen fertilization. The beneficial impact of nitrogen on the component in question has also been shown in the experiments with camomile [Nikolova et al. 1999], lemon balm [Kordana et al. 1997], sweet basil [Golcz et al. 2006; Golcz and Markiewicz 2002; Markiewicz et al. 2002], and peppermint [Mairapetyan et al. 1999]. However, in the case of summer savoury, different nitrogen fertilization did not have a significant effect on oil content in the herb [Dzida and Jarosz 2006b].

Table 4. Content (ml·100 g^{-1}) of essential oil and flavonoids (% – expressed in quercetin and hyperoside equivalents) in marigold inflorescences depending on nitrogen dose
Tabela 4. Zawartość (ml·100 g^{-1}) olejku eterycznego i flawonoidów (% – w przeliczeniu na hiperozyd i kwercetynę) w kwiatostanach nagietka w zależności od dawki azotu

Dose of nitrogen Dawka azotu kg N·ha^{-1}	Essential oil Olejek eteryczny	Flavonoids expressed in Flawonoidy w przeliczeniu na	
		Hyperosid Hiperozyd	Quercetin Kwercetyna
Control – Kontrola	0.31	0.53	0.37
40	0.36	0.50	0.35
80	0.39	0.46	0.32
120	0.39	0.46	0. 32
160	0.42	0.44	0.31
Mean – Średnio	0.37	0.48	0.33

Information on the content of flavonoid compounds in marigold dry flower heads is most frequently expressed in quercetin or hyperoside equivalents. In the experiments of Ociszyńska et al. [1977], the percentage of flavonoids (expressed in quercetin) was in a range of 0.2–0.8%, Hojden et al. [1990] found approx. 0.4%, while Kurkin and Sharova [2007] report values from 0.3% up to 0.7%. Such variation in the data may result from the fact that the respective studies were conducted with different cultivars and in varying climatic conditions. According to the Polish Pharmacopoeia [2008], the required content of active components in marigold raw material should not be less than 0.4% of flavonoids expressed in hyperoside equivalent. In the our experiment, the hyperoside content ranged from 0.44% to 0.53%, depending on nitrogen fertilization dose, and these results met the requirements of the Pharmacopoeia. Flower heads from the control treatment were characterized by the highest content of flavonoids, and a slight decreasing trend in their percentage was noted along with increasing nitrogen rates (tab. 4). In the study of Biesiada et al. [2008], a decrease in polyphenol content in lavender raw material was noted under the effect of high nitrogen fertilization levels.

CONCLUSIONS

1. Flowering period of pot marigold plants was shortest in the control treatment (30 days) and longest (40 days) in the plots where nitrogen fertilization had been applied at the highest dose (160 kg $N \cdot ha^{-1}$).

2. Nitrogen fertilization had a positive effect on the number of flower heads per plant (in particular on the second- and third-tier branches), but no differences were found in inflorescence diameter under the effect of the factor in question.

3. There was found a significant increase in yield (flower heads and ligulate flowers) after the application of nitrogen at 80 kg $N \cdot ha^{-1}$, compared to the control treatment. A further increase of nitrogen doses (120 and 160 kg $N \cdot ha^{-1}$) did not result in a significant growth in raw material yield.

4. Nitrogen fertilization modified essential oil content in pot marigold flower heads (this content increased along an increase in nitrogen doses), but it did not affect the percentage content of flavonoids.

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PLONOWANIE ORAZ SKŁAD CHEMICZNY KOSZYCZKÓW NAGIETKA LEKARSKIEGO (*Calendula officinalis* L. cv. Orange King) W ZALEŻNOŚCI OD NAWOŻENIA AZOTEM

Streszczenie. Nagietek lekarski należy do najstarszych roślin leczniczych wykorzystywanych już w starożytności. Obecnie dostarcza surowca zielarskiego o dużym znaczeniu dla przemysłu farmaceutycznego i kosmetycznego. Badania przeprowadzono w latach 2007–2009 na glebie lessowej o składzie granulometrycznym pyłu ilastego. Celem eksperymentu było określenie wpływu zróżnicowanych dawek azotu (0, 40, 80, 120, 160 kg $N \cdot ha^{-1}$) na wybrane cechy morfologiczne roślin oraz na plonowanie i jakość surowca nagietka lekarskiego. Kwitnienie nagietka najkrócej trwało w obiekcie kontrolnym (30 dni), zaś najdłużej (40 dni) tam, gdzie zastosowano azot w największej dawce (160 kg $N \cdot ha^{-1}$). Nawożenie azotem w istotny sposób wpłynęło na liczbę wytworzonych przez roślinę koszyczków kwiatowych, nie stwierdzono natomiast różnic w średnicy kwiatostanów pod wpływem badanego czynnika. Stwierdzono istotny wzrost plonów surowca po zastosowaniu 80 kg $N \cdot ha^{-1}$ w porównaniu z obiektem kontrolnym. Na dalsze zwiększanie ilości azotu (120, 160 kg $N \cdot ha^{-1}$) rośliny nagietka nie reagowały istotną zwyżką plonu. Nawożenie azotem modyfikowało zawartość olejku eterycznego w koszyczkach (wraz ze zwiększaniem dawek azotu jego zawartość wzrastała) nie wpłynęło natomiast na procentowy udział związków flawonoidowych.

Słowa kluczowe: *Calendula officinalis*, azot, plon kwiatostanów, olejek eteryczny, flawonoidy

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