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THE EFFECT OF DIFFERENT NITROGEN FERTILIZATION RATES ON YIELD AND QUALITY OF MARIGOLD (*Calendula officinalis* L. 'TOKAJ') RAW MATERIAL

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Abstract

Pot marigold (*Calendula officinalis* L.) is an annual ornamental plant which is also grown for herbal raw material (flower heads) used in the pharmaceutical and cosmetic industries. A field experiment was carried out in the years 2006-2008 in the Experimental Farm of the University of Life Sciences in Lublin. The study was conducted on loess soil with the granulometric composition of silt loam. The aim of the experiment was to determine the effect of different nitrogen rates (0, 40, 80, 120, 160 kg N×ha^{-1}) on some morphological features of flower heads as well as on yield and quality of pot marigold raw material. Flowering of pot marigold was shortest in the control treatment (32 days) and longest (43 days) in the plot where nitrogen fertilization had been applied at the highest rate (160 kg N×ha^{-1}). Nitrogen fertilization had a significant influence on the number of flower heads per plant, but no significant difference was found in diameter as well as in ligulate flowers and tubular flowers in the flower head. It was found to increase significantly raw material yield after the application of 80 kg N×ha^{-1}, compared to the control treatment. Yield of flower heads did not differ markedly for fertilization rates from 80 to 160 kg N×ha^{-1}. Nitrogen fertilization modified slightly essential oil content (this content increased with increasing nitrogen rates), but at the same time it decreased the percentage of flavonoid compounds.

Key words: pot marigold, nitrogen, yield, essential oil, flavonoid

INTRODUCTION

Marigold belongs to the oldest medicinal plants which had been used from the ancient times. At present, its raw material is of great significance for the pharmaceutical and cosmetic industries. Herbal raw material comprises whole flower heads, *Calendulae anthodium,* or ligulate flowers, *Calendulae flos,* of orange and yellow colour (Farmakopea Polska (Polish Pharmacopoeia), 2008). Their usability as pharmaceutical material depends on their chemical composition. Numerous studies (Golcz et al. 2006; Dzida and Jarosz, 2006; Biesiada and Kuś, 2010) have shown that nitrogen fertilization results in a significant increase in quantity and quality of herbal plant yields. In the references, there are few studies available on fertilization of marigolds and its impact on the content of active biological substances in the raw material. According to Rumińska et al. (1991), the marigold does not require intensive mineral fertilization, and high doses of nitrogen result in the decrease of yield of flower heads.

In their study, Biesiada et al. (2006) did not observe any clear correlation between nitrogen fertilization and chemical composition of marigold flower heads. However, a slight decrease in polyphenol content was noted under the effect of high nitrogen fertilization.

The aim of the experiment was to determine the effect of different nitrogen rates on some morphological features of flower heads as well as on yield and quality of pot marigold raw material.

MATERIALS AND METHODS

A thee-year field experiment (2006-2008) was conducted on loess soil with the granulometric composition of silt loam, characterized by a neutral pH (pH 1M KCl – 6.5) as well as an average content of nutrients (50.1 P; 124.8 K; 49.7 Mg in mg×kg^{-1} of soil). The humus content was 1.52%. The experiment was set up in a randomized block design in 5m^2 plots, in quadruplicate. Pot marigold cv. Tokaj was used, characterized by yellow-coloured flowers and anemone structure of flower heads.

In the experiment, there were 4 nitrogen fertilization levels: 40, 80, 120, 160 kg N ha^{-1}. Plots without nitrogen fertilization were the control treatments. The same phosphorus (in the form of 46% granulated triple superphosphate) and potassium (in the form of 50% 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 34% 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 beginning and end dates of flowering were recorded and flowering duration was determined for all treatments.

Flower heads were harvested gradually as the plants bloomed, at 4-day intervals (from the first half of July until the second decade of August). After each harvest, the diameter of flower heads (n=10) and the number of ligulate and tubular flowers per flower head were determined 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, air dry mass of a single flower head of marigold as well as the total yield of flower heads (g×m^{-2}) were determined. The study results were statistically verified, determining the significance of differences using Tukey's test, with a 5% risk error.

Directly after harvest, flower heads were dried in a drying room at a temperature of 35^{o}C. Then, essential oil content was determined by steam distillation (method 2.8.12, Polish Pharmacopoeia VIII). Flavonoid content (expressed as hyperoside and quercetin equivalents) was determined by the use of a spectrophotometer (method 2.2.25, Polish Pharmacopoeia VIII).

RESULTS AND DISCUSSION

Depending on the nitrogen rate, flowering of pot marigold lasted from 32 to 43 days. The plants in the control treatment started flowering latest and the duration of this stage was shortest (32 days). As the amount of nitrogen increased, the flowering duration lengthened, since it lasted 43 days in the plots where 160 kg×N a^{-1} had been applied. The lowest number of harvests (7) was recorded in the control treatment, whereas flower heads were harvested ten times in the plots where the highest nitrogen dose had been applied. The number of flowers was initially small, gradually increasing until the fifth and sixth harvest. During the next harvests, the number of flower heads decreased and they came primarily from the third-tier branches.

Analysing the impact of different nitrogen doses on the number of flower heads, it was noted that the plants in the control treatment produced the smallest number of flower heads (Table 1). Nitrogen fertilization stimulated the formation of branches, thus affecting the increase in the number of flower heads. The differences between the higher nitrogen rates (80-160 kg×ha^{-1}) were small and statistically insignificant. A comparison of the distribution of flower heads on particular branches shows that nitrogen fertilization primarily impacted the formation of second- tier branches, whereas in the case of the first- and third- tier branches the difference between particular treatments was not statistically proved. There were no significant differences between the size of flower heads, their mass and the number of flowers (ligulate and tubular) in the flower head as a result of different nitrogen doses (Table 2). In the experiment of G a n j a l i et al. (2010), the nitrogen fertilization also caused an increase in the number of flower heads per; however, it did not differentiate the diameter of the flower heads.

R u m i ń s k a (1991) maintains that there can be up to 40-50 flower heads on a single marigold plant. Similar results were obtained in our experiment. On the other hand, in the experiments of M a r t i n and D e o (2000), carried out in New Zealand, marigold produced about 20 flower heads, whereas in the study of K h a l i d and Z a g h l o u l (2006) this number ranged from 70 to 140 pieces·plant^{-1}. Such large variations in this value result from the differences in climatic conditions and experimental factors.

Information on the yield of marigold flower heads varies and depends mainly on the place where the experiment is conducted and the type of the marigold cultivar. In Polish studies (B i e s i a d a et al. 2006, D e d i o et al. 1986, H o j d e n et al. 1990), the yield of air dry flower heads ranged from 100 to 280 g×m$^{-2.}$ Similar results were obtained in India (G a n t a i t and C h a t t o p a d h y a y , 2004) and in Chile (B e r t i et al. 2003). At the same time, in the experiments carried out in New Zealand (M a r t i n and D e o , 2000) and in Italy (P i c c a g l i a et al. 1997) the yields of marigold were significantly lower and did not exceed 50 g×m^{-2}. In our experiment, the yield of cv. Tokaj was lower than yields reported in the domestic literature (Table 3).

D e d i o et al. (1986) shows that nitrogen fertilization levels of 30-50 kg N×ha^{-1} are sufficient under good soil conditions. In the experiments of M i l i and S a b l e (2003) as well as of R a h m a n i et al. (2009), conducted under different climatic and soil conditions, the highest yield of flower heads was obtained at a nitrogen rate of 100 kg N.

In our own experiment, flower head yield increased by 11.5%, after the application of 40 kg N×ha^{-1} compared to the control treatment (this difference, however, was statistically insignificant). An increase in nitrogen fertilization (80 kg N×ha^{-1}) caused a significant increase in the yield (by 36.2% compared to the control treatment). Further increase of nitrogen doses (120-160 kg×ha^{-1}) practically had no effect on the yields of dry flower heads (Table 3). As nitrogen fertilization did not influence the size and mass of a single flower head, the differences in the yield of flower heads between the compared treatments resulted from the umber of flower heads on the plant. It is characteristic that the yield of vegetative matter (stems and leaves) increased systematically (and significantly) together with an increase in nitrogen doses (Table 3).

The content of biologically active components is the evidence of the quality of herbal raw material; in the case of marigold, the content of essential oil and flavonoids (expressed as hyperoside and quercetin equivalents) was analysed. According to the studies of Dedio et al. (1986) as well as Taczanowska and Hołderna-Kędzia (1998), Polish marigold cultivars contain approximately 0.2% of essential oil in the flower head. In France (Chalchat et al. 1991), 0.3% of essential oil was obtained, while in other regions of the world (Gazim et al. 2008; Khalid and Zaghloul, 2006) from 0.1% to 0.2% of this ingredient was recorded. In our experiment, the average content of essential oil in the raw material was 0.34% (Table 4).

Nitrogen fertilization affects not only yield, but also chemical composition of herbal raw material. In the experiments with marjoram, Dzida and Jarosz (2006) found an increased concentration of essential oil in the herb under increasing nitrogen fertilization. The beneficial impact of nitrogen on essential oil has also been shown in the experiments with camomile (Nikolova et al. 1999), sweet basil (Golcz et al. 2006), and summer savoury (Alizadeh et al. 2010). However, in the case of lemon balm (Abbaszadeh et al. 2009) and thyme (Baranauskiene et al. 2003), different nitrogen fertilization levels did not have a significant effect on oil content in the herb.

In the available literature, there are few data on the impact of fertilization on essential oil content in marigold flower heads. In the experiment of Khalid and Zaghloul (2006), organic fertilization caused an increase in essential oil content. In our experiment, the least amount of oil was in the raw material from the control treatment (0.29%). With increased doses of nitrogen, there was an increase of its content (the difference between the control treatment and the highest dose of N was 0.1 percentage point).

The content of flavonoid compounds in marigold raw material is most frequently expressed as quercetin or hyperoside equivalents. In the experiment 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 approximately 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 (Farmakopea Polska 2008), the required content of active components in marigold raw material should not be less than 0.4% of flavonoids expressed as hyperoside equivalents. In our experiment, the hyperoside content (regardless of fertilization) was 0.4%. In case of the plants which received higher doses of nitrogen (120 and 160 N×ha^{-1} – Table 4), the content of these compounds was slightly lower than the one required by the Pharmacopoeia. Felgines et al. (2000) consider that nitrogen deficiency increases the content of flavonoids in plants. In the study of Biesiada et al. (2008) with lavender, a decrease in polyphenol content in raw material was noted under the effect of high nitrogen fertilization.

Table 1
The effect of different nitrogen rates on the number of flower heads per plant (in pieces) – mean for 2006-2008

Rate of nitrogen kg N ha^{-1}	Total number of flower heads	Including flower heads on branches		
		first tier	second tier	third tier
Control	25.7^{c}	7.0	13.2 ^{b}	4.5
40	37.5 ^{b}	9.5	19.5 ^{b}	7.5
80	54.1^{a}	12.0	29.6 ^{a}	11.5
120	54.7 ^{a}	12.3	29.8 ^{a}	11.6
160	56.2 ^{a}	12.5	30.9 ^{a}	11.8
Mean	45.6	10.7	24.6	9.4
$LSD_{0.05}$	8.04	n.s.	8.35	n.s.

n.s. – difference not significant

a, b, c values designated with the same letters within column do not differ significantly at $p \le 0.05$.

Table 2
The effect of different nitrogen rates on flower head diameter (in mm) and number of ligulate and tubular flowers per head – mean for 2006-2008

Rate of nitrogen kg N×ha^{-1}	flower head diameter	ligulate flowers	tubular flowers
Control	62.4	90.8	236.9
40	64.7	92.1	247.2
80	66.1	93.9	258.3
120	66.8	94.6	259.7
160	66.8	94.4	261.2
Mean	65.4	93.2	252.7
$LSD_{0.05}$	n.s.	n.s.	n.s.

n.s. – difference not significant

Table 3
The effect of different nitrogen rates on the weight of air-dry individual flower head (g), yield of air-dry flower heads and vegetative parts (g×m^{-2}) – mean for 2006-2008

Rate of nitrogen kg N×ha^{-1}	Weight of individual flower head	Yield of flower heads	Yield of vegetative parts
Control	0.332	67.6 ^{b}	280.2 ^{e}
40	0.351	75.4 ^{b}	299.4 ^{d}
80	0.357	92.1 ^{a}	309.7 ^{c}
120	0.351	93.9 ^{a}	321.1^{b}
160	0.353	95.7 ^{a}	334.4 ^{a}
Mean	0.349	84.9	308.9
$LSD_{0.05}$	n.s.	8.15	9.97

n.s. – difference not significant
a, b, c, d, e – values designated with the same letters within column do not differ significantly at $p \leq 0.05$.

Table 4
Content (%) of essential oil and flavonoids (expressed as quercetin and hyperoside equivalents) in marigold inflorescences depending on nitrogen rates – mean for 2006-2008

Rate of nitrogen kg N×ha^{-1}	Essential oil	Flavonoids expressed in	
		Hyperoside	Quercetin
Control	0.28	0.43	0.30
40	0.34	0.42	0.29
80	0.34	0.40	0.28
120	0.36	0.39	0.27
160	0.38	0.36	0.25
Mean	0.34	0.40	0.28

CONCLUSIONS

1. The period of flowering of pot marigold extended with the increase of nitrogen fertilization (from 32 days in the control treatment to 43 days in the plots where nitrogen fertilization had been applied at the highest rate).
2. Nitrogen fertilization had a significant impact on the number of flower heads per plant (especially on the second-rank branches).
3. A significant difference was found in their morphological features, such as diameter of the flower head as well as in numbers of ligulate flowers and tubular flowers in the flower head.
4. A significant increase in raw material yield was noted after the application of 80 kg N×ha^{-1} of nitrogen, compared to the control treatment. Yield of flower heads did not differ markedly for fertilization from 80 to 160 kg N×ha^{-1}.
5. The content of essential oils in flower heads increased with the increase of nitrogen rates, whereas the content of flavonoids slightly decreased.

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Wpływ zróżnicowanego nawożenia azotowego na plonowanie i jakość surowca nagietka lekarskiego (*Calendula officinalis* L.'Tokaj')

Streszczenie

Nagietek lekarski (*Calendula officinalis* L.) jest jednoroczną rośliną ozdobną, którą uprawia się również na surowiec zielarski dla przemysłu farmaceutycznego i kosmetycznego. Doświadczenie prowadzono w latach w latach 2006-2008 w Zakładzie Doświadczalnym UP w Lublinie. Badania prowadzono na glebie pochodzenia lessowego o składzie mechanicznym pyłu ilastego. Celem eksperymentu było określenie wpływu zróżnicowanych dawek azotu (0, 40, 80, 120, 160 kg $N \times ha^{-1}$) na wybrane cechy morfologiczne koszyczków kwiatowych oraz na plonowanie i jakość surowca nagietka lekarskiego. Kwitnienie roślin najkrócej trwało w obiekcie kontrolnym (32 dni), zaś najdłużej (43 dni) tam, gdzie zastosowano azot w największej dawce (160 kg $N \times ha^{-1}$). Nawożenie azotem wpłynęło istotnie na wzrost liczby wytworzonych przez roślinę koszyczków kwiatowych, nie miało natomiast znaczącego wpływu na ich średnicę oraz liczbę kwiatów języczkowych i rurkowych w koszyczku. Stwierdzono istotny wzrost plonów surowca po zastosowaniu 80 kg $N \times ha^{-1}$ w porównaniu z obiektem kontrolnym. Natomiast plon koszyczków nie różnił się istotnie dla nawożenia na poziomie od 80 do 160 kg$\times ha^{-1}$. Wzrastające nawożenie azotowe powodowało nieznaczne zwiększanie zawartości olejku eterycznego oraz obniżenie związków flawonoidowych.