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ORIGINAL PAPER

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EFFECT OF NITROGEN FERTILIZATION AND PLANT DENSITY ON SEED YIELD AND FAT CONTENT AND QUALITY OF POT MARIGOLD (*Calendula officinalis* L.) UNDER CLIMATIC CONDITIONS OF BELARUS

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ABSTRACT

This paper presents the results of a study on the effect of different N fertilization and seeding rates on yield and some quality parameters (of seeds) of pot marigold. A field experiment was carried out during 2014–2016 under soil and climatic conditions of Belarus (Priluki Research Station near Minsk). The experiment included the following factors: N fertilization rate 40, 60, 80, 100 kg ha^{-1}; plant density per 1 m^2 (30, 50, 70, 90). The experiment was set up as a randomized block design in five replicates, with a single plot area of 8.0 m^2. The soil in Priluki was characterized by medium nutrient availability (at the level: P = 120–127, K = 164–174, Mg = 59–66 mg kg^{-1} soil). Regardless of the experimental factors, weather conditions had a significant impact on pot marigold productivity. The year 2016, characterized by an even distribution of rainfall during the growing season and moderate air temperatures, proved to be most beneficial for pot marigold productivity and quality. Hydrological conditions in the other years (2014 and 2015) there was a slight drought. The year 2014 should be considered to be cold, whereas the second year of the study (2015) was moderately warm. Best production effects (seed yield, fat yield) were obtained at rates of 60–80 kg N ha^{-1}. This rate of nitrogen fertilization also modified the fatty acid composition, contributing to a higher content of linoleic acid and calendic acid (∑ α-calendic and β-calendic acids). A nitrogen rate of 100 kg N ha^{-1} proved to be irrational in terms of the quantity and quality of pot marigold yield. A density of 50–70 plants per 1 m^2 resulted in the highest seed and fat yield per unit area. A plant density of 50 plants per 1 m^2 resulted in the highest content of C18 unsaturated acids (stearic acid, oleic acid, linoleic acid) in the oil.

Key words: pot marigold, N fertilization, row spacing, fat yield, fatty acids, calendic acid

INTRODUCTION

Among alternative oilseed crops (milk thistle, bitter melon, white mahlab, white sandalwood, coriander), species producing oil with a special composition of fatty acids (with conjugated double bonds) and unique oil properties attract a great interest. They are particularly useful for the chemical and pharmaceutical industries and are also used to produce medicines and diet supplements (among others, as bioactive components of functional food), but also in the production of plastic films, solvents, detergents, polymers, and high quality paints [Hreczuch et al. 2000, Metzger and Bornscheuer 2006, Biermann et al. 2011, Yuan et al. 2014, Fontes et al. 2017]. One of such potential oilseed crops is the pot marigold (*Calendula officinalis* L.),

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a species that is commonly grown as a medicinal and ornamental plant. Marigold seeds contain 20% of fat, characterized by the presence of conjugated linolenic acid (CLNA) isomers – represented by α-calendic acid and β-calendic acid [Mulley et al. 2009, Biermann et al. 2010, Dulf et al. 2013].

Linolenic acid isomers exhibit biological activity and health-promoting effects. Studies of some authors show that CLNA exhibit anticarcinogenic, antioxidative, anti-inflammatory, and hypotensive effects and can induce the apoptosis of various tumor cells [Kohno et al. 2004, Yasui et al. 2006, Kobori et al. 2008, Saha and Ghosh 2011, Li et al. 2013].

Pot marigold is grown as an oilseed crop, variety selection is important. Varieties with a short flowering period (14–20 days) are the best because long flowering results in uneven seed ripening and seed shattering [Król et al. 2016]. Nevertheless, application of nitrogen with flower pruning can be beneficial in this regard [Estaji et al. 2011, 2016]. The literature shows that an appropriate selection and level of mineral fertilization, in particular nitrogen fertilization, are of vital importance for the quantity and quality of marigold seed yield [Mili and Sable 2003, Król 2011, Szwejkowska and Bielski 2012]. In milk thistle research Estaji et al. [2016] found that the highest yield of seeds and plant height were as the level of N fertilization increased (0, 50 and 100 kg ha^{-1}). In turn, the content of linoleic, arachidonic, linolenic and stearic acids were not affected by N fertilization treatments [Estaji et al. 2011]. An important element of agronomic practices in growing pot marigold is the selection of a proper crop density. An appropriately selected plant density determines the optimal growth of vegetative parts, but also affects yield components. Some studies reveal that the best plant density for pot marigold is 100 plants per 1 m^2 [Martin and Deo 2000], but other researchers report that 25 plants per 1 m^2 is the optimum plant density [Seghatoleslami and Mousavi 2009]. In the opinion of Król [2017], the best plant density for pot marigold grown as an oilseed crop is 60 plants per 1 m^2.

This study hypothesized that the determination of optimum rates of mineral N fertilization and an appropriate density of pot marigold plants would allow the best seed and fat yield structure to be obtained and would also contribute to a high content of valuable linolenic acid isomers. It was assumed that both high N fertilization rates and the highest crop density could result in a deterioration in the chemical composition of plant raw material.

The aim of this study was to evaluate cultural practices (different mineral N fertilization levels and four plant density treatments) on pot marigold yield and seed oil quality.

MATERIAL AND METHODS

Weather conditions at the study site

Weather conditions were evaluated based on data obtained from the Meteorological Station of the Institute of Plant Protection in Priluki. Throughout the study period (2014–2016), the average air temperatures differed from the long-term average. The year 2014 should be considered to be cold because the average temperature was lower by 0.7°C than the long-term average (1985–2005). April proved to be particularly cold, which slightly delayed the emergence of marigold plants. The second year of the study (2015) was moderately warm (the air temperatures during the growing season of pot marigold fluctuated around the long-term average), the average temperatures recorded in 2016 were higher than the long-term average by 0.8°C, and April, May, and August were particularly warm months. The rainfall distribution varied in 2014–2016 of the study. The total rainfall in 2014 exceeded the average values for this region by about 53.6 mm, with the highest amount of rainfall recorded in August. In 2015 the total rainfall was lower than the long-term average by 24.8 mm, and July was a particularly dry month. In 2016, in turn, the distribution of rainfall during the growing season was similar to the long-term average and hence, in terms of rainfall and temperature conditions, this year should be considered to be optimal for growth and development of pot marigold.

To determine the temporal and spatial variation in weather elements and to evaluate their effect on the growing season of pot marigold during the experiment in Priluki, Selyaninov's hydrothermal coefficient (K) was calculated [Bac et al. 1993], dividing the monthly total rainfall by one tenth of the sum of the average daily air temperatures for a given month.

When we consider the hydrothermal conditions in the individual growing seasons of pot marigold, we notice that during the initial growing period (April – May) these conditions were most favorable in 2016, whereas in the other years there was a slight drought. It was only in 2014 that there was no drought in June. In July, a favorable hydrothermal coefficient was recorded in 2016, while in 2015 a drought occurred. In August (the month when marigold seeds were harvested), there was no drought only in 2014, but in the years 2015–2016 a slight drought was recorded (Tab. 1). To sum up, the 2015 growing season of pot marigold was characterized by the frequent occurrence of a slight drought (and even a drought – July) compared to 2014 and 2016.

Site, field experiment, and cultural practices

A field experiment on growing pot marigold (cv. 'Tokaj') was conducted during the period 2014–2016 in experimental fields of the Institute of Plant Protection in Priluki near Minsk (Belarus). Cultivar 'Tokaj' is characterized by a short flowering period (14–20 days), which predisposes this cultivar to be grown as an oilseed crop (even ripening, lower seed shattering). Moreover, cv. 'Tokaj' is marked by the highest percentage of hooked achenes in seed yield, with a higher thousand seed weight (about 12 g), which translates itself into high seed yield (about 1800 kg ha^{-1}) and fat yield (about 400 kg ha^{-1}) [Król 2016, Król and Paszko 2017]. In most pot marigold cultivars, smaller larva-shaped achenes with lower yield potential are predominant [Wilen et al. 2004, Zanetti et al. 2013].

The experiment was established on slightly sandy loess-derived soil (soil quality class III). The soil content of major nutrients is shown in Table 2.

Considering the data contained in Table 2, we can note that the soil used in the experiment was characterized by medium availability of major nutrients, a medium humus content, and a pH = 5.9–6.1. The experiment was set up as a randomized block design in five replicates, with a single plot area of 2.0 m × 4.0 m (8.0 m^2).

The experiment included the following factors:

I. N fertilization rate (pure N): 1. N = 40 kg ha^{-1}; 2. N = 60 kg ha^{-1}; 3. N = 80 kg ha^{-1}; 4. N = 100 kg ha^{-1}.

II. Plant density per 1 m^2: A. 30; B. 50; C. 70; D. 90.

Table 1. Selyaninov's hydrothermal coefficient (K) in individual months of the pot marigold growing season during the period 2014–2016

Month	Year		
	2014	2015	2016
April	0.85	0.88	1.02
May	0.92	0.95	1.04
June	1.05	0.76	0.79
July	0.81	0.52	1.04
August	1.22	0.78	0.91
Mean	0.97	0.77	0.96

K ≤ 0.5 severe drought; 0.51–0.69 drought; 0.70–0.99 slight drought; K > 1 no drought

Table 2. Characteristics of the soil used in the experiment

Year	pH (in 1 M KCl)	Content			
		P (mg kg^{-1} soil)	K (mg kg^{-1} soil)	Mg (mg kg^{-1} soil)	Humus (%)
2014	5.9	120	164	59	1.25
2015	6.1	127	174	66	1.33
2016	6.0	121	167	62	1.28

In all experimental treatments, the rates of other mineral (phosphorus and potassium) fertilizers were set at the same level (pure ingredient): P = 50 kg ha^{-1}, K = 60 kg 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 – 30%) and potassium (potassium salt – 50%) fertilization, respectively, was applied in all objects in autumn. The previous crop for pot marigold was lacy phacelia (a very popular crop in Belarus) in all years of the study. After harvest of the previous crop (the third 10 days of August), post-harvest treatments were carried out (plough skimming, double harrowing). In autumn (the second 10 days of October), autumn ploughing and harrowing were done as well as phosphorus fertilizers (30% granulated triple superphosphate) and potassium fertilizers (50% potassium salt) were applied. Nitrogen fertilizers (34% ammonium nitrate) were applied in spring in two doses: ½ of the dose before seeding and ½ of the dose after crop thinning.

Pot marigold seeds were sown using a precision seed drill directly in the field in the second 10 days of April, at a row spacing of 30 cm and using a seeding rate of 9 kg ha^{-1}. After emergence, the pot marigold crop was thinned, leaving for further growth 30 plants (treatment A), 50 plants (treatment B), 70 plants (treatment C), and 90 plants (treatment D), respectively. During the further growing period, crop management involved soil loosening in the interrows (hand hoe) and mechanical (manual) weed removal. Pot marigold seeds were harvested when about 65% of seeds were ripe (the first/second 10 days of August).

Measurements and determinations

Before harvest of the marigold crop, 30 plants were randomly selected from each plot. The plant height and the length of the flowering zone (measured from the setting of the first inflorescence to the setting of the height inflorescence) were measured as well as the number of flower heads was determined. After harvest, seed threshing and cleaning were done; subsequently, the seeds were dried (to moisture content of 10%) and seed yield was determined.

Seeds from each treatment were ground separately in a stainless steel mill, and then fat (from a 5 g sample) was extracted in a Soxhlet apparatus for 8 h using n-hexane. Fatty acid methyl esters were obtained using BF_3-CH_3OH [AOCS 1997]. Chromatographic analysis was performed on a Varian GC 3800 chromatograph (Walnut 123 Creek, CA USA) equipped with an autosampler and a flame ionization detector (FID). Separation of fatty acid methyl esters was performed on a UltiMetal™ UCP-WAX 52CB capillary column with the stationary phase in the form of polyethylene glycol. The carrier gas was helium at a flow rate of 1.4 cm^3 min^{-1}. The initial column temperature was 120°C, whereas the maximum temperature 210°C. The analysis duration was 125 min. The injector and detector temperature was set at 160°C. Fatty acid ester peaks were identified based on Supelco 37 Component FAME Mix and LGC reference materials. On the basis of the obtained results, the theoretical fat yield was calculated on a per hectare basis (marigold seed yield × seed fat content).

Statistical analyses

All study results were statistically analyzed by analysis of variance (ANOVA), and the significance of differences was determined using Tukey's test at a significance level of p = 0.05.

RESULTS

Mineral nitrogen fertilization at the rates of 60, 80, and 100 kg N ha^{-1} stimulated the growth of marigold plants, including their flowering zone length, thus affecting the number of inflorescences per plant, compared to an N rate of 40 kg ha^{-1}. The study also found increased nitrogen rates to have a positive effect on 1000 seed weight (TSW) of pot marigold (statistically significant in the case of the rates of 60–80 kg N ha^{-1}) – Table 3.

It was proved that a density of 30–50 plants per 1 m^2 contributed to the formation of the longest flowering zone length on the stem, which in turn resulted in obtaining the highest number of flower heads per plant and the highest TSW. It was observed that with increasing density of marigold plants up to 70–90 plants per 1 m^2, the plant height increased, but the flowering zone shortened as well as the number of inflorescences per plant and 1000 seed weight decreased (Tab. 3).

Weather conditions varied between years and had a significant impact on the growing season of pot

Table 3. Selected morphological characteristics and yield components of pot marigold

Factor	Plant height (cm)	Flowering zone length (cm)	Number of inflorescences per plant	1000 seed weight (g)
Mineral N fertilization rate (kg ha^{-1})				
N = 40	65.7	27.3	33	10.24
N = 60	70.5	30.3	46	11.79
N = 80	71.4	31.0	43	11.61
N = 100	72.2	31.6	38	11.07
$LSD_{0.05}$	4.21	1.62	4.2	0.771
Plant density (plants m^{-2})				
30	67.8	28,1	48	11.97
50	70.7	31.5	45	11.90
70	71.2	30.3	41	11.66
90	71.8	26.2	32	10.83
$LSD_{0.05}$	3.15	1.83	4.7	0.572
Year				
2014	70.3	31.2	45	11.74
2015	68.5	27.0	40	10.70
2016	71.6	31.6	47	12.01
$LSD_{0.05}$	2.92	1.74	3.8	0.953

marigold. Substantial water deficits during the 2015 growing season had a negative effect on plant growth and development as well as on the investigated morphological characteristics of pot marigold; the TSW values were also significantly lower than in 2014 and 2016 (Tab. 3).

In the present experiment, an increase in the rate of mineral N fertilization by 60 kg ha^{-1} caused a significant increase in pot marigold seed yield compared to the lowest rate of 40 kg ha^{-1}. Application of N at rate of 100 kg ha^{-1} had a minimal effect on increasing theachene yield per unit area (by 2%) compared to 80 kg ha^{-1} N rate (thus, this fertilization level was irrational). The fat content in marigold seeds was significantly the highest when 60 kg ha^{-1} N was applied. An N rate of 40 kg ha^{-1} also promoted a high seed fat content. Application of the higher N fertilization rates (80 and 100 kg ha^{-1}), on the other hand, contributed to a gradual, statistically decrease in seed fat content. Significantly the highest fat yield (in kg ha^{-1}) was obtained at the N rates of 60–80 kg ha^{-1} (Tab. 4).

Plant density caused significant differences in pot marigold seed yields. By far the lowest yields were obtained from the treatments where pot marigold grew at a density of 30 plants per 1 m^{-2}. On average, over the 3-year study period, the highest yields were obtained at a density of 70 plants per 1 m^2, followed by a density of 50 plants per 1 m^2. The increase in density to 90 plants per 1 m^2 resulted in a decrease in seed yield (statistically significant compared to the densities of 50 and 70 plants per 1 m^2). Plant density per plot had a significant effect on seed fat content. A density of 50 plants per 1 m^2 promoted the highest fat accumulation. On the other hand, the lowest seed fat content was found at density of 90 plants per 1 m^2. Nevertheless, a density of 50–70 pot marigold plants per 1 m^2 provided significantly the highest fat yield per 1 ha (Tab. 4).

Pot marigold yields were also significantly modified by weather conditions in the individual growing seasons. The highest seed yield and the highest fat yield were found in the year 2016, which was optimal for growing pot marigold in terms of rainfall and temperature conditions, while the lowest yields were

Table 4. Seed yield, seed fat content, and fat yield of pot marigold

Factor	Seed yield (kg ha^{-1})	Fat content (%)	Fat yield (kg ha^{-1})
Mineral N fertilization rate (kg ha^{-1})			
N = 40	1583.4	20.7	324.8
N = 60	1691.2	21.8	368.7
N = 80	1764.3	19.8	349.3
N = 100	1798.5	18.6	334.5
$LSD_{0.05}$	104.54	1.05	19.52
Plant density (plants m^{-2})			
30	1561.2	20.8	324.7
50	1775.0	21.6	383.4
70	1801.3	20.2	363.8
90	1672.4	19.5	326.1
$LSD_{0.05}$	100.63	0.95	20.72
Year			
2014	1764.2	21.5	379.3
2015	1654.8	19.6	324.3
2016	1825.6	21.0	383.4
$LSD_{0.05}$	106.22	0.92	19.93

Table 5. Fatty acid composition in pot marigold seed oil

Factor	16 : 0 palmitic acid	18 : 0 stearic acid	18 : 1 oleic acid	18 : 2 linoleic acid	CLNA ∑ α-calendic and β-calendic
Mineral N fertilization rate N (kg ha^{-1})					
N = 40	3.90	3.03	6.73	33.19	48.11
N = 60	4.10	3.10	6.67	34.22	49.82
N = 80	4.16	2.95	6.16	34.36	49.84
N = 100	4.02	2.88	6.09	34.02	49.21
$LSD_{0.05}$	n.s.*	n.s.	0.456	0.924	1.646
Plant density (plants m^{-2})					
30	3.92	2.72	6.21	33.15	47.16
50	4.20	3.13	6.84	35.80	49.01
70	4.18	2.98	6.62	34.22	48.64
90	3.98	2.81	6.30	34.54	48.25
$LSD_{0.05}$	n.s.	0.312	0.474	1.123	1.655
Year					
2014	3.96	3.04	6.69	34.13	48.54
2015	3.70	2.91	6.51	33.21	47.36
2016	4.11	3.25	6.88	35.77	49.85
$LSD_{0.05}$	0.394	0.305	n.s.	0.992	1.775

* not significant difference

recorded in the year 2015, which was characterized by the frequent occurrence of drought (Tab. 4).

Nitrogen fertilization at the rates of 60 and 80 kg ha^{-1} contributed to a significantly higher percentage of linoleic acid and in consequence a higher CLNA content in the marigold oil compared to an N rate of 40 kg ha^{-1}. At the same time, the higher N fertilization rates of 80 and 100 kg ha^{-1} resulted in a significant decrease in the oleic acid content in the seed oil compared to the rates of 40–60 kg N ha^{-1}. The contents of the other fatty acids determined were not statistically significantly modified under the influence of nitrogen fertilization (Tab. 5).

A density of 50 plants per 1 m^2 contributed to significantly the highest content of C18 fatty acids in the marigold oil, compared to a density of 30 plants per 1 m^2. A higher plant density per 1 m^2 (70–90 plants) did not modify substantially the fatty acid composition (Tab. 5).

Pot marigold oil quality was significantly affected by weather conditions in the individual growing seasons. In the dry season in 2015, the lowest significant content of palmitic, stearic, linoleic, and calendic acids (total CLNA) was found compared to the most favorable growing season in 2016 (in which the air temperature and rainfall averages were similar to the respective long-term averages). Among fatty acids, the usefulness and use of pot marigold oil are determined by linolenic acid isomers, i.e. α-calendic and β-calendic acids. Their content (termed as total CLNA) was 47.36–49.85% over the study period. Regardless of the experimental factors, among other polyunsaturated acids the highest content of linoleic acid was found (33.21–35.77%), among monounsaturated acids – oleic acid (6.51–6.88), while as regards saturated acids – palmitic acid (3.70–4.11%) and stearic acid (2.91–3.25%) – Table 5.

DISCUSSION

In this study, the achene yield of pot marigold throughout the experiment ranged 1600–1800 kg ha^{-1}. The information in the literature relating to pot marigold yield is very diverse, depending on agro-climatic conditions, cultivar, and agronomic factors. Pot marigold seed yields vary within a wide range from 500 kg ha^{-1} [Jevdović et al. 2013] to more than 2000 kg ha^{-1} [Cromack and Smith 1998]. Forcella et al. [2012], Gesch [2013], and Król [2016] obtained similar achene yields as in this experiment.

Among agronomic factors, nitrogen fertilization is of major significance in determining the quality characteristics of oil seeds [Dordas 2010, Estaji et al. 2011, Jiang et al. 2014]. Król [2017a, 2017b] argues that when growing pot marigold as an oilseed crop, application of nitrogen at rate of 60–90 kg ha^{-1} results in best oil production. The above observation is confirmed in the present study because the highest seed yield of pot marigold was obtained when N fertilization was applied at the rates of 60–80 kg ha^{-1}.

In this study, application of the high rates of mineral N fertilization caused extended flowering and seed ripening; in consequence, this resulted in a decrease in TSW and total achene yield per unit area. Such relationships are confirmed in studies of other authors [Chauhan and Kumar 2007, Johnson and Gesch 2013, Król 2017a] who note that particularly high N rates contribute to extended flowering of pot marigold and shattering of seeds before harvest. Nitrogen by far, is the most needed nutrient elements by many crops [Souri and Hatamian 2019]. These authors notice that in various studies, higher yield and quality as well as higher concentration of nutrient elements have been obtained by application of aminochelates rather than simple chemical fertilizers. These compounds claiwed to be more natural and safer forms of chelating agents, with higher use efficiency and without environmental side effects. Many growth and reproductive characteristics as well as quality factors of plant products are influenced by fertilization particularly nitrogen application. Souri et al. [2019] they notice that using N fertilization (urea pellet) caused significant improvement in growth parameters of SPAD value, leaf area, plant height, shoot fresh weight and yield in sweet basil raw material.

Mili and Sable [2003] as well as Król and Paszko [2017] found that number of flower heads and seed size (TSW) are the yield components that largely determine marigold seed yield. In this experiment, with increasing crop density, pot marigold plants produced fewer flower heads and seeds were smaller (with increasing plant density, the TWS decreased), which is confirmed in the study by Król [2017c]. Seghatoleslami and Mousavi [2009] report that a density of 25 plants per 1 m^2 is optimal for growth and yield of pot marigold. Martin and Deo [2000], in turn, obtained the highest yield of pot marigold flower heads at a density of 100 plants per 1 m^2.

In the present study, the seed yields and fat yields were modified to a large extent by weather conditions during the growing season. In the year 2015, characterized by a lower amount of rainfall than in the other study years, significantly lower yields were obtained compared to the years 2014 and 2016 (by 7% and 10%, respectively). The impact of humidity and temperature conditions on seed and oil yield of pot marigold has also been confirmed in other studies [Ruiz de Clavijo 2005, Joly et al. 2013, Król 2017a, 2017b, 2017c]. It can therefore be stated that under the agro-climatic conditions of central-eastern Belarus, weather conditions with possibly evenly distributed rainfall during the period of intensive plant growth and moderate temperatures during the seed ripening period are most favorable for growing pot marigold for seed. Król and Paszko [2017] report that seed and oil yield is dependent on number of marigold flower heads, percentage of specific seed types, and humidity and temperature conditions. This information has a bearing on agricultural practice; growing pot marigold for oil production purposes, we should select a region most favorable in terms of agro-climatic conditions.

Shakib et al. [2010] claim that in the cultivation of pot marigold plant density per plot does not have a significant effect on seed fat content. In the present study, the highest fat content in pot marigold seeds was found at a plant density of 30–50 plants per 1 m^2, while the highest fat yield at a density of 50 plants per 1 m^2. A density of 50 pot marigold plants per 1 m^2 also had the most beneficial effect on fatty acid composition.

In this experiment, linolenic acid (CLNA) isomers were predominant in the fatty acid structure of the marigold oil, accounting for about 47–49% of total fatty acids. The percentages of individual fatty acids were modified by weather conditions and the experimental factors used. The literature of the subject shows that the CLNA content in pot marigold oil varies within a wide range (29–59%) and that it is higher under temperate climate conditions than in Mediterranean climate conditions [Janssens and Vernooij 2001, Dulf et al. 2013, Król 2017a]. Thus, a higher CLNA content in the fatty acid composition is noted at moderate temperatures (which was confirmed in this study) than at high temperatures [Angelini et al. 1997, Król et al. 2016, Król and Paszko 2017]. Ahmad and Abdin [2000], Jiang et al. [2013], and Król [2017a], among others, found fatty acids to be modified under the influence of nitrogen fertilization. Estaji et al. [2011] they did not find changes in the content of fatty acids under the influence of different doses of N in the case of milk thistle. Król [2017c], on the other hand, did not find any relationship between different densities of pot marigold plants and the percentages of individual fatty acids in the oil composition.

CONCLUSIONS

During the three-year study period, high seed yield of pot marigold and fat yield were obtained, whereas the herbal raw material had a favorable fatty acid composition, with a large proportion of calendic acid in it. Varying weather conditions throughout the study period affected plant growth, seed yield as well as fat yield and its quality. The best quantitative and qualitative parameters of pot marigold raw material were found during the growing season rainfall was evenly distributed and the temperatures were moderate. Nitrogen fertilization of pot marigold at rates of 60–80 kg ha^{-1} had a significant positive effect on plant height, flower stem length, and flower head production, and in consequence also on seed and fat yield. The above specified nitrogen rates also contributed to a higher content of linolenic acid isomers ($\sum$ α-calendic and β-calendic). Plant density had a significant effect on the morphological characteristics of pot marigold, seed yield, and fat yield, and it also modified the fatty acid composition of the marigold oil (a higher content of C18 unsaturated fatty acids). A plant density of 50 or 70 plants per 1 m^2 proved to be most favorable.

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