The effect of nitrogen form and dose on yield, chemical composition and antioxidant activity of stinging nettle (*Urtica dioica* L.)

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S u m m a r y

The purpose of the investigation was the assessment of the effect of fertilization with different form and dose of nitrogen as well as harvest term on yield, chlorophyll, carotenoids and polyphenols content, and antioxidant activity of stinging nettle (*Urtica dioica* L.) leaves. The lowest total yield obtained from three-year-lasting plantation resulted from preplant fertilization with lime saltpeter. The highest yield of raw material was provided by nitrogen fertilization in the dose of 150 kg N/ha. Total content of polyphenols in nettle leaves collected in May amounted, counted over gallic acid, 7.13–9.46 mg·g^{-1} d.m. and was by 40–50% higher than that assayed in July. Chlorophylls (7.72–9.63 mg·g^{-1} d.m.) and carotenoids (0.74–0.96 mg·g^{-1} d.m.) content was lower in May by 20–40% than the one in nettle leaves collected in July. In the course of effect of nitrogen preplant fertilization plants accumulated higher amounts of polyphenols when treated with N saltpeter form. Increased nitrogen dose from 50 to 150 kg N·ha^{-1} contributed to the increase in chlorophyll and carotenoids content in nettle leaves and, to a lower degree, to differentiating polyphenols level.
Harvest term did affect antioxidant activity of raw material more significantly than nitrogen form and dose used in the experiment. Raw material collected in May characterized higher antioxidant activity measured according to FRAP and ABTS.

Key words*: stinging nettle nitrogen fertilization, yield, phenolics, antioxidant activity*

INTRODUCTION

Stinging nettle is a valuable medicinal plant, also used as an edible plant and fodder for animals, as well as a source of obtaining chlorophyll. Its fiber has been utilized as yarn raw material for ages [1-3]. Chemical composition of stinging nettle is relatively well known, yet not much attention has been paid to the effect of growing factors on the quality of raw material [1, 4-7].

Therapeutic activities of stinging nettle is multidirectional which results from its rich chemical composition. The extract from leaves is used as a diuretic, in infections of urinary tract, oliguria and, as an adjuvant, in renal calculi and gout. Schilcher et al. [8] in their elaboration classify stinging nettle to a group of a dozen most important agents intensifying urination. Nettle extracts are recommended in renal sand treatment [9], they support elimination of chlorides, urea and bacterial toxins as well as metabolites. The latter activity can be an explanation to nettle property as an agent purifying blood and body fluids and, therefore, this herb is applied as an adjuvant in rheumatic, dermatological and allergic diseases [5, 10]. Nettle products stimulate metabolic processes, serve as a source of vitamins and mineral compounds, intensify excretion of gastric juice and bile which facilitates digestion and absorption of nutrients, as well as soothe inflammatory states of alimentary duct and prevent diarrhea [5]. Lipide-steroid complex extracted from nettle roots is used in prostate hypertrophy treatment [11, 12]. According to Alkiewicz and Lutomski [13], it is noteworthy that in many cases the use of nettle root combined with other phytotherapeutics enables patients avoid surgery and, in a long-term, systematic therapy, can reach a permanent salutary effect.

Alterations in chemical composition of stinging nettle in relation to endo- and exogenic factors have been relatively well researched. Chemical composition of this plant is dependent, among others, on development stage, form and plant organ, site of occurrence, term of harvest, as well as conditions of storage and drying [14, 15].

Stinging nettle is a typical nitrophilic plant, highly dependent on the amount of nitrogen in the soil it grows in [16, 17]. According to the Growing Guide elaborated for this plant by Research Institute of Medicinal Plants in Poznań, the following doses of nitrogen fertilizer in $kg \cdot ha^{-1}$ are recommended: N 60–90, P_2O_5 50–60, K_2O 80–100 [18]. It has been proved that nettle cultivation in nutrients-poor medium, including nitrogen, inhabits aboveground plant parts growth [19, 20]. In his investigation [20], applied combined fertilization with N, P, K ranging from 75 to 375 $kg \cdot ha^{-1}$, reporting that the increase in mineral fertilizers dose influenced on the increase in above-ground parts biomass of nettle and, at the same time, decreased the biomass of its underground parts. The form of nitrogen delivered to the herb is a crucial factor governing plant growth and the quality of raw material. Fodor and Cseh [21] observed that in hydroponic cultivation the kind of nitrogen forms applied into medium effects on plant growth and the amount of chlorophyll in nettle leaves. The solution containing only NH_4 ions or urea resulted in inhibition of plant growth and necrosis. Weiss [22] proved that as N dose increased from 80 to 440 kg N·ha^{-1}, dry matter of shoots and leaves did increase.

The aim of the research conducted in 2002–2005 was the assessment of the effect of nitrogen form and dose on chemical composition, yielding and antioxidant activity of stinging nettle leaves.

MATERIALS AND METHODS

The investigation was carried out in Research Station in Piastów, on degraded black earth containing 1.8% of humus, pH=7.4.

In two-factorial experiment the effect of nitrogen form and dose on stinging nettle raw material yielding and chemical composition of nettle leaves was investigated. The first factor were nitrogen forms applied in preplant fertilization as ammonium saltpeter, urea, lime saltpeter and ammonium sulfate. The second factor involved total doses of nitrogen used in the course of plant growing period: 50 (50+0), 100 (50+50), 150 (75+75) kg N·ha^{-1}. Top-dressing fertilization was introduced exclusively in the form of ammonium saltpeter in the amount of 0, 50 and 75 kg N·ha^{-1}. Field experiment was established according to randomized split-plot method, in four replications, with plot for harvest sized 1 m^2. Stinging nettle was cultivated from seedlings prepared in 2002–2004 in a greenhouse by sowing seeds at the end of March to multicells of 76.6 cm^3 volume, filled with peat substrate. Ready 7-week-old seedlings were planted every year at the beginning of May on the field previously fertilized according to the mentioned method, with spacing 45x35 cm. In the course of plant growing period the plants were watered and weeded as required.

Yield was estimated on the basis of green matter of harvested herb and leaves from area unit. Herb harvesting took place at the beginning of blossom stage: twice the year of plantation establishing and three times in the following years.

The samples of nettle leaves were collected from two-year-old plants in 2003 and 2004 on 31st May and 24th July and subjected to grinding using Bosch MSM 6700/01 blender. Then, two grams of each plant was extracted in 100 ml of 80% methanol/water by maceration (24 h). In extracts, dry matter was determined by the weight method, according to The Polish Standard PN-90/A-75101/03, chlorophylls and carotenoids content was assayed according to [23]; total phenols were determined by Folin Ciocalteu method [24]. Ferric reducing antioxidant power (FRAP) was measured due to (25), DPPH assay – by [26] and Trolox equivalent antioxidant capacity (TEAC) was estimated with ABTS radical according to [27].

The results of the experiment were presented as mean ±SD of the data obtained. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan multiple range tests. Statistical analysis were done using Statictica 7.0 and correlation coefficients were determined using Microsoft Excel 2000.

RESULTS AND DISCUSSION

The results of the experiment prove that both nitrogen form and dose did significantly influence on herb and leaves yielding in the subsequent years of plantation maintaining, as well as on total yield of nettle herb and leaves obtained from three-year plantation (tab. 1). Stinging nettle in the first year of cultivation negatively reacted to fertilizing with saltpeter form of N, especially to lime saltpeter. Yield of herb and leaves, obtained at pre-planting fertilization with the remaining fertilizers was similar and ranged for herb from 2.59 to 2.67 $kg \cdot m^{-2}$, while for leaves it amounted 0.84–0.86 $kg \cdot m^{-2}$. In the third year of maintaining plantation pre-planting fertilization with lime saltpeter nettle plants gave also the lowest yield. Significantly lowest total yield of herb and leaves was obtained from the treatments fertilized with lime saltpeter, while the other N forms introduced as pre-planting fertilization allowed to obtain more advantageous herb and leaves yield achieving 16.9–16.3 $kg \cdot m^{-2}$ and 7.12–6.44 $kg \cdot m^{-2}$, respectively. During all years of nettle cultivation the plant advantageously responded to increased nitrogen dose producing significantly highest green matter yield in the treatment fertilized with N dose ranging 150 $kg\ N \cdot ha^{-1}$. These results can be also confirmed by those obtained by Biesiada [28] as well as Szewczuk and Mazur [29] who recorded the highest yield of stinging nettle, when plantation was intensively fertilized with nitrogen at the rates dose amounting 200–300 $kg\ N \cdot ha^{-1}$.

Table 1.

Yielding of stinging nettle as dependent on nitrogen form and dose

treatments	1st year			2nd year			3rd year			total yield from 2002–2006	
	herb	leaves	% of leaves in herb	herb	leaves	% of leaves in herb	herb	leaves	% of leaves in herb	herb	leaves
treatment I – form of nitrogen											
NH_4NO_3	2.19	0.81	37.1	5.09	2.15	42.2	9.58	4.16	43.4	16.9	7.12
$Ca(NO_3)_2$	1.79	0.63	35.3	5.22	2.03	38.9	7.32	3.36	45.9	14.3	6.04
$CO(NH_2)_2$	2.59	0.84	32.3	4.89	1.86	37.9	8.83	3.73	42.2	16.3	6.44
$(NH_4)_2SO_4$	2.67	0.86	32.2	4.98	1.85	37.1	9.01	3.92	43.5	16.7	6.63
$LSD \alpha=0.05$	0.11	0.11		0.18	0.12		0.15	0.14		0.83	0.32
treatment II – dose of nitrogen $kg\ N \cdot ha^{-1}$											
50	1.86	0.61	32.9	4.43	1.71	38.6	7.47	3.29	44.1	13.8	5.61
100	2.38	0.82	34.7	5.09	1.95	38.3	8.70	3.76	43.2	16.2	6.53
150	2.70	0.93	34.9	5.63	2.26	40.1	9.89	4.33	43.8	18.2	7.53
$LSD \alpha=0.05$	0.10	0.10		0.05	0.10		0.10	0.10		0.93	0.39

Phenolic compounds showed the greatest variations between different sampling times. The total phenols contents of the extracts in terms of gallic acid equivalent were between 3,53 and 15,06 (tab. 2) which was in agreement with the results obtained by [30].

Table. 2.

Total phenolics, chlorophylls and carotenoids content in stinging nettle leaves as dependent on nitrogen form

harvest time	form of nitrogen	total phenolics [mg x g d.m.$^{-1}$]	chlorophylls a+b [mg x g d.m.$^{-1}$]	carotenoids [mg x g d.m.$^{-1}$]
May 31st	$(NH_4)_2SO_4$	27.78 B	9.63 A	0.81 B
	$Ca(NO_3)_2$	29.33 A	7.72 C	0.69 D
	$CO(NH_2)_2$	27.13 B	8.45 B	0.74 C
	NH_4NO_3	29.46 A	8.42 B	0.86 A
July 22th	$(NH_4)_2SO_4$	17.79 A	11.04 B	1.31 B
	$Ca(NO_3)_2$	14.72 B	11.16 A	1.40 A
	$CO(NH_2)_2$	16.70 A	8.92 D	1.02 C
	NH_4NO_3	13.53 B	10.28 C	1.27 B

Statistically homogeneous ($p \leq 0.05$) groups are designated with the same letters separately for each harvest term

Phenolic compounds contents in the extracts of stinging nettle leaves were about 30% higher in May than that in July. The highest total phenolic content was observed in plants grown with two forms of nitrate fertilizers in May, and with $(NH_4)_2SO_4$ and $CO(NH_2)_2$ in July. Mudau et al. [31] and Owour et al. [32] also found that polyphenol concentration in tea leaves displayed seasonal differences. Węglarz et al. [33] examined a correlation between plantation age and harvest term regarding the content of flavonoids. They reported no effect of plantation age on flavonoids content as well as diminishing concentration of flavonoids in raw material coming from the subsequent cuts. Polyphenols levels can be influenced by intensity of solar UV radiation and water deficits [34, 35]. According to Andersen [36], UV light induced synthesis of polyphenols with higher hydroxylation level rather than general increase of phenolic compounds and depends on plant species and developmental stage.

Chlorophylls and carotenoids contents increased according to the length of plant growing period and was by 25% and 40 higher in July than in May, respectively. Similar results were observed by [37] in *Echinacea purpurea* leaves collected in July and September.

Nitrogen dose differentiated polyphenols content in nettle leaves only to a low degree, while the level of carotenoids and chlorophylls did considerably increase according to N dose (tab. 3).

Older plants featured higher chlorophylls levels. Yet it was not the kind of fertilizer to be responsible for significantly increasing or decreasing chlorophylls and carotenoids levels in plants in both harvest terms. Among plants harvested in May, the highest chlorophylls level characterized the samples supplied with $(NH_4)_2SO_4$ and, regarding carotenoids – those with NH_4NO_3.

Table 3.

Total phenolics, chlorophylls and carotenoids content in stinging nettle leaves as dependent on nitrogen dose

harvest time	dose of nitrogen [kg N·ha^{-1}]	total phenolics [mg x g d.m.$^{-1}$]	chlorophylls a+b [mg x g d.m.$^{-1}$]	carotenoids [mg x g d.m.$^{-1}$]
May 31st	50	31.10 B	5.83 C	0.57 C
	100	32.92 A	7.07 B	0.67 B
	150	32.43 A	8.42 A	0.75 A
July 22th	50	18.73 A	7.57 C	0.68 C
	100	16.89 B	9.02 B	0.90 B
	150	17.59 B	10.28 A	1.30 A

Statistically homogeneous ($p \leq 0.05$) groups are designated with the same letters separately for each harvest term

Plants collected in July and fertilized with $Ca(NO_3)_2$ had higher levels of chlorophylls and carotenoids than those grown with the use of other fertilizers. Plants harvested in May and fertilized with $Ca(NO_3)_2$ showed the lowest level of chlorophylls and carotenoids and in July the lowest amount of those components were in plants grown with $CO(NH_2)_2$. There was no consistent change among all plants grown in a particular year with some fertilizers, similarly to the results reported by Kalt et al. [38]. Our findings suggested a seasonal influence on components formation. The samples harvested in May had higher level of phenolic compounds and plants collected in July featured higher level of chlorophylls and carotenoids than in May. Seasonal differences are also reported by Mercandante et al. [39].

Free radicals are involved in many disorders. Antioxidants, through their scavenging power are known to exhibit beneficial pharmacological properties. Some of these properties have been related to phenolic compounds ability of radical scavenging, metal chelating activity and quenching of singlet and triplet oxygen. Activity of phenolic compounds is correlated to their structure. The compounds such as flavonoids, which contain hydroxyls, are responsible for radical scavenging effect in plants [40]. According to our study, high contents of these phytochemicals in stinging nettle can justify its radical scavenging activity.

The effect of antioxidants on DPPH and ABTS radicals scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical. The FRAP method can be used to determine ferric reducing ability and calculate total antioxidant power according to antioxidants or reducing agents present in a sample. Basing on the data obtained from this study, it is possible to state that nettle extracts exhibited free radical scavenging activity and reducing power. Plants harvested in different terms characterized markedly variable antioxidant activity (tab. 4).

Table 4.

Antiradical activity and reducing power of stinging nettle as dependent on nitrogen form

harvest term	form of nitrogen	FRAP [μM Fe/1g d.m.]	DPPH [μM DPPH/1g d.m.]	ABTS [μM Trolox/1g d.m.]
May 31st	$(NH_4)_2SO_4$	266.9 A	0.056 B	13.11 B
	$Ca(NO_3)_2$	282.4 A	0.271 A	18.07 A
	$CO(NH_2)_2$	263.0 A	0.014 B	10.08 B
	NH_4NO_3	256.3 A	0.338 A	15.17 A
July 22th	$(NH_4)_2SO_4$	117.72 A	0.484 A	11.27 A
	$Ca(NO_3)_2$	87.01 C	0.259 C	9.46 B
	$CO(NH_2)_2$	99.82 B	0.354 B	9.94 B
	NH_4NO_3	77.99 C	0.093 D	8.07 C

Statistically homogeneous ($p \leq 0.05$) groups are designated with the same letters separately for each harvest term

There was observed a range of seasonal variation from 0.014 to 0.48 μmol DPPH·g^{-1} d.m. and 8-18 μmol Trolox·g^{-1} d.m. in ABTS assays. The type of fertilizer influenced antiradical activity. In the samples collected in May higher activity was observed regarding the plants grown with $Ca(NO_3)_2$ and NH_4NO_3. In plants harvested in July scavenging effect and reducing power were higher for those grown with $(NH_4)_2SO_4$, and the lowest values were recorded for plants fertilized with NH_4NO_3 and $Ca(NO_3)_2$. In previous studies by Biesiada et al. [41, 42] with coneflower and pot marigold the highest antioxidant activity was provided by urea and ammonium sulfate in both FRAP and ABTS method. The same research proved that antioxidant activity was the highest in treatment fertilized with nitrogen in dose of 100 kg N·ha^{-1}, whereas in our experiment with stinging nettle nitrogen dose insignificantly differentiated antioxidant activity of this plant (tab. 5).

Table 5.

Antiradical activity and reducing power of stinging nettle as dependent on nitrogen dose

harvest term	dose of nitrogen [kg N·ha^{-1}]	FRAP [μM Fe·1g^{-1} d.m.]	DPPH [μM DPPH·1g^{-1} d.m.]	ABTS [μM Trolox·1g^{-1} d.m.]
May 31st	50	231.4 A	0.298 B	12.06 B
	100	252.9 A	0.311 B	12.09 B
	150	256.3 A	0.338 A	15.17 A
July 22th	50	69.85 A	0.423 A	10.45 A
	100	74.46 A	0.134 B	7.79 B
	150	70.20 A	0.096 C	8.13 B

Statistically homogeneous ($p \leq 0.05$) groups are designated with the same letters separately for each harvest term

Differentiation of antiradical activity of extracts from plants collected in different harvest terms and grown with different fertilizers might have resulted from the production of some other undetermined antioxidants in plants treated with different types of fertilizers or from production of phenolic substances with higher antioxidant activities [36, 43]. The accessibility of nitrogen can effect on synthesis of phenolic antioxidants and soluble solids.

CONCLUSIONS

1. The lowest total yield obtained from three-year-lasting plantation resulted from preplant fertilization with lime saltpeter. The highest yield of raw material was provided by nitrogen fertilization in the dose of 150 kg $N\cdot ha^{-1}$.
2. The highest quantity of polyphenols accumulated nettle leaves harvested in May, while those harvested in July contained higher amount of carotenoids and chlorophylls.
3. In the course of intensive effect of nitrogen preplant fertilization plants accumulated higher amounts of polyphenols when treated with N saltpeter form. Increased nitrogen dose from 50 to 150 kg $N\cdot ha^{-1}$ contributed to the increase in chlorophyll and carotenoids content in nettle leaves and, to a lower degree, to differentiating polyphenols level.
4. Harvest term did more significantly affect antioxidant activity of raw material than nitrogen form and dose used in the experiment. Raw material collected in May characterized higher antioxidant activity measured according to FRAP and ABTS.

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WPŁYW FORMY I DAWKI AZOTU NA PLON, SKŁAD CHEMICZNY I AKTYWNOŚĆ ANTYOKSYDACYJNĄ POKRZYWY ZWYCZAJNEJ (*URTICA DIOICA* L.)

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S t r e s z c z e n i e

Celem badań była ocena wpływu formy i dawki azotu na plon, zawartość polifenoli, chlorofilu i karotenoidów w liściach pokrzywy zwyczajnej. Najniższy plon całkowity z trzech lat trwania plantacji zapewniło przedwegetacyjne nawożenie saletrą wapniową. Największy plon ziela uzyskano stosując dawkę 150 kg N/ha. Poziom polifenoli w liściach pokrzywy zbieranej w maju w przeliczeniu na kwas galusowy wyniósł 7,13–9,46 mg/g s.m. i był o 40–50% większy niż w lipcu. Zawartość chlorofilu i karotenoidów w liściach pokrzywy zbieranej w maju była o 20–40% mniejsza niż w lipcu. Rośliny nawożone przedwegetacyjnie saletrą wapniową gromadziły więcej polifenoli. Wzrastająca dawka azotu wpłynęła dodatnio na poziom chlorofilu i karotenoidów w liściach, miała jednak mniejszy wpływ na zawartość polifenoli w pokrzywie. Termin zbioru pokrzywy wywarł większy wpływ na aktywność antyoksydacyjną surowca niż forma i dawka azotu użyte w doświadczeniu.

Słowa kluczowe: *pokrzywa zwyczajna, nawożenie azotem, plon, polifenole, aktywność antyoksydacyjna*