J. Elem. s. 555–565

DOI: 10.5601/jelem.2011.16.4.05

CONTENT OF MACRONUTRIENTS AND VALUES OF MOLE RATIOS IN LEAVES OF THE WOOLLY FOXGLOVE (*DIGITALIS LANATA* EHRH.) CULTIVATED UNDER DIFFERENTIATED MINERAL FERTILISATION

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Abstract

The Woolly Foxglove (*Digitalis lanata*) is one of the herbal plants cultivated in Poland. It is grown as important plant material for production of cardenolides used in the pharmaceutical industry. The aim of the research has been to determine the chemical composition and mole ratios in leaves of cv. Victoria Woolly Foxglove affected by mineral fertilisation. For this purpose, a strict, one-factor, micro-plot field experiment was set up in randomised blocks with four replications. The experimental factor was NPK mineral fertilisation applied to soil in the following doses per hectare. A_0 – no mineral fertilisation, A_1 – 40 kg N + 17.48 kg P + 49.8 kg K, A_2 – 80 kg N + 34.96 kg P + 99.6 kg K, A_3 – 120 kg N + 52.44 kg P + 149.4 kg K. For chemical analyses, samples of 0.50 kg of leaves were taken from each plot. Dried, melted and wet mineralised in sulphuric(VI) acid, the samples were analysed and concentrations of the following elements were determined: nitrogen by Kjeldahl method, total phosphorus by the colorimetric method with ammonium molybdate, potassium, calcium and sodium by flame photometry, and magnesium by atomic absorption spectrometry (AAS). Mole ratios of the chemical elements in leaves were also calculated. In general, the use of mineral fertilisation induced a significant increase in the content of total nitrogen, total phosphorus and potassium, but reduced the sodium concentration in leaves of the Woolly Foxglove compared to the control. After the applied fertilisation doses, the K:Ca and K:Na ratios in leaves were significantly broadened and the Ca:P ratio was narrowed. Significantly higher values of the K:(Ca + Mg) ratio were observed in leaves after applying the medium (A_2) and highest NPK ha^{-1} (A_3) doses. The Ca:Mg ratio in leaves of the Woolly Foxglove was distinctly narrowed versus the control after using the

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lowest fertilisation dose (A_1). However, after applying the medium dose of NPK (A_2), the K:Mg ratio was broader than in the control treatment.

Key words: Woolly Foxglove, fertilisation, macronutrients, chemical composition, mole ratios.

ZAWARTOŚĆ MAKROELEMENTÓW I WARTOŚCI STOSUNKÓW MOLOWYCH W LIŚCIACH NAPARSTNICY WEŁNISTEJ (*DIGITALIS LANATA* EHRH.) UPRAWIANEJ W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO

Abstrakt

Jedną z roślin zielarskich uprawianych w Polsce jest naparstnica wełnista. Stanowi ona ważny surowiec kardenolidowy wykorzystywany w przemyśle farmaceutycznym. Celem badań była ocena składu chemicznego oraz proporcji molowych w liściach naparstnicy wełnistej odmiany Victoria pod wpływem zróżnicowanego nawożenia mineralnego. W związku z powyższym założono ścisłe jednoczynnikowe doświadczenie mikropoletkowe, metodą losowanych bloków, w czterech powtórzeniach. Czynnikiem doświadczenia było nawożenie mineralne NPK zastosowane doglebowo w następujących dawkach na 1 ha: A_0 – bez nawożenia mineralnego, A_1 – 40 kg N + 17,48 kg P + 49,8 kg K, A_2 – 80 kg N + 34,96 kg P + 99,6 kg K, A_3 – 120 kg N + 52,44 kg P + 149,4 kg K. Do analiz chemicznych pobrano reprezentatywne próby w ilości po 0,50 kg liści z każdego poletka. Po wysuszeniu, zmieleniu i mineralizacji na mokro w kwasie siarkowym(VI) określono w nich zawartości: azotu ogólnego metodą Kjeldahla, fosforu ogólnego metodą kolorymetryczną z molibdenianem amonu, potasu, wapnia oraz sodu metodą fotometrii płomieniowej, a magnezu metodą absorpcyjnej spektroskopii atomowej. Obliczono również stosunki molowe pierwiastków w liściach. Zastosowane nawożenie mineralne, w odniesieniu do obiektu kontrolnego, powodowało na ogół istotny wzrost zawartości azotu ogólnego, fosforu ogólnego, potasu, natomiast zmniejszenie koncentracji sodu w liściach naparstnicy wełnistej. Pod wpływem stosowanych dawek stwierdzono istotne rozszerzenie wartości stosunków K:Ca i K:Na oraz zawężenie Ca:P w liściach. Znaczące rozszerzenie wartości stosunku K:(Ca + Mg) w liściach uzyskano po zastosowaniu dawek: średniej (A_2) oraz najwyższej NPK ha^{-1} (A_3). Wartość stosunku Ca:Mg w naparstnicy uległa wyraźnemu zawężeniu w porównaniu z obiektem kontrolnym, po zastosowaniu dawki najniższej (A_1). Natomiast po aplikacji średniej dawki NPK (A_2) stwierdzono rozszerzenie stosunku K:Mg w odniesieniu do obiektu kontrolnego.

Słowa kluczowe: naparstnica wełnista, nawożenie, makroelementy, skład chemiczny, stosunki jonowe.

INTRODUCTION

Growing herbs is one of the newest plant production branches. According to the FAO data, about 50 thousand plant species are used by man, including 700 grown by farmers. It has been estimated that about 15-20 thousand species, i.e. 3 to 5% of the global plant resources, are used for medicinal purposes (SEIDLER-ŁOŻYKOWSKA 2009). Poland produces around 50% of the herbal raw material made in Europe, which corresponds to about 20% of the global production. Thus, Poland belongs to the countries with high herbal production potential, verified by high quality of herbal raw material and herbal processing (MIKOŁAJCZYK-GRZELAK 2008). The Woolly Foxglove, an important raw material for cardenolides, is one of the plants cultivated in Poland. It is a biennial plant, which in the first year forms a rosette of lanceolate, dark green leaves that are harvested together with seeds in the following year, making tradable raw material. In the second year of vegetation, a foxglove plant produces a stem which is about 150 cm long. The flower head of *Digitalis lanata* forms a bunch consisting of numerous bell-shaped flowers white and creamy-brown in colour. The plant blooms in late June and early July. Seeds mature gradually from August to the end of September. Cardiac glycosides contained in leaves of the Woolly Foxglove (SUCHORSKA, WĘGLARZ 1988) are used for treating circulatory deficiency by improving the contractility of the heart muscle. Although the literature contains agronomic guidelines concerning the cultivation of this plant, we still lack precise recommendations on how to fertilise it. Moreover, there is no information about mole ratios in leaves of the plant as shaped by mineral fertilisation. Therefore, the present research has been undertaken to determine the chemical composition and mole ratios in leaves of cv. Victoria Woolly Foxglove as modified by mineral fertilisation.

MATERIAL AND METHODS

In 2006-2008, at the research station owned by the Faculty of Agriculture of the University of Technology and Life Sciences in Bydgoszcz and located in Wierzchucinek near Bydgoszcz, a strict, multiple, one-factor, micro-plot experiment was set up in four replications according to the method of randomised blocks. The experimental factor was NPK fertilisation applied to soil in the following doses per hectare:

0 – with no mineral fertilisation,

A_1 – 40 kg N + 17.48 kg P + 49.8 kg K = 107.3 kg NPK;

A_2 – 80 kg N + 34.96 kg P + 99.6 kg K = 214.6 kg NPK;

A_3 – 120 kg N + 52.44 kg P + 149.4 kg K = 321.8 kg NPK.

Phosphorus and potassium fertilisation was applied before seeding in the form of Polifoska 6 (6-20-30). Nitrogen fertilisation was applied in three dosages: before seeding as Polifoska 6, and – in two equal doses – after sprouting and three weeks later as ammonium nitrate (V).

The experiment was conducted on Luvisol typical soil (Pw), good rye complex, characterized by the following physicochemical parameters:

- pH_{KCl} – 6.6,
- content of bio-available forms of phosphorus – 55 P mg kg^{-1} of soil (mean fertility),

– content of bio-available forms of potassium – 55 K mg kg^{-1} of soil (mean fertility),
– content of bio-available forms of magnesium – 49 Mg mg kg^{-1} of soil (mean fertility).

The mean air temperature in the years when the research was conducted, during the vegetation period of the Woolly Foxglove (from March to September), did not diverge from the multi-year mean. However, some differences appeared in the precipitation (Figures 1). The highest rainfall during the plant's growth occurred in 2007 (on average 428.2 mm). In June and July, the mean total precipitation was by 50.6 mm and 33.6 mm higher, respectively, than the multi-year mean for these months. In contrast, June and July in 2006 had the lowest total precipitation (by 33.1 mm and 46.6 mm less, respectively, than the multi-year mean). The lowest precipitation was recorded in 2008, especially in May and June, when the mean total precipitation was lower than the multi-year mean by 31.5 mm and 39.4 mm, respectively.

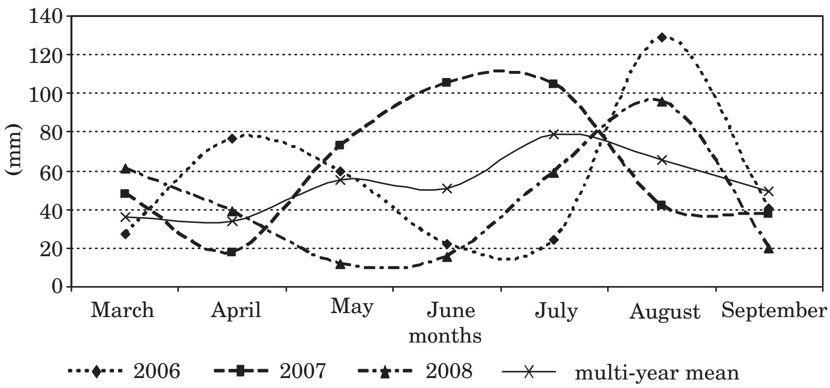

Fig. 1 Rainfall in the vegetation period of the Woolly Foxglove

Digitalis lanata, preceded by spring barley as forecrop, was sown every year. Its leaves were gathered manually from the 3 m^2 plots in the first decade of September. Representative samples of 0.50 kg of leaves were taken from each plot to make chemical analyses. Dried, melted and wet mineralised in sulphuric(VI) acid, the samples were analysed to determine the content of: nitrogen by Kjeldahl method, total phosphorus by the colorimetric method with ammonium molybdate, potassium, calcium and sodium by flame photometry, and magnesium by atomic absorption spectrometry (AAS). The mole ratios of chemical elements in leaves were also calculated.

The findings were statistically processed by the one-way analysis of variance (ANOVA), and the differences between the means were estimated by Tukey's test at the significance level α = 0.05.

RESULTS AND DISCUSSION

The research revealed that the applied doses of nutrient components generally had a significant effect on the chemical composition of the Woolly Foxglove leaves (Table 1). The fertilisation affected most strongly the content of total nitrogen in leaves, which ranged on average from 18.54 g kg^{-1} to 23.29 g kg^{-1}. In response to the subsequently higher NPK rates, the content of this element in leaves rose considerably, i.e. by 12.3%, 20.8%, and 25.6%, respectively, compared to the control treatment. It should be emphasized that a significant increase in the content of total phosphorus in leaves of *Digitalis lanata* was observed every year during the research, in all the fertilised treatments except A_1 in 2007. The highest amount of total nitrogen (on average 23.89 g kg^{-1}) was determined in the leaves gathered in 2006, when it significantly surpassed the results obtained in the other years. The lowest amounts of total nitrogen were observed in the leaves harvested in 2007, which was also the driest year (the lowest total precipitation). Nitrogen belongs to elements which are easily transported in the plant (labile elements). The deficiency of this element retards the growth of plants and causes chlorosis on the oldest leaves. Proteins are products of the synthesis of nitrogen, which is the basic structural material in plants. This element participates in stimulating the synthesis of growth regulators, i.e. auxins and gibberellins. It is also essential for the biochemistry of herbal plants because some alkaloids and natural protective substances contain nitrogen (Pozsonyi 2002, Kordana et al. 1998).

The total phosphorus content in the Woolly Foxglove's leaves was also significantly modified by fertilisation. On average, it reached 2.65 g kg^{-1}. Most phosphorus (2.85 g kg^{-1}) was accumulated by leaves gathered from the plots fertilised with the highest dose (object A_3). The increase was 21.8% with reference to the control. Under the medium dose of NPK (object A_2), an increase in the total phosphorus content was slightly lower – 18.8% more than the control. The NPK fertilisation considerably increased the mean content of this macronutrient in leaves of *Digitalis lanata* in particular years of the research. The mean content of total phosphorus in leaves was significantly higher in 2007 than in the other years of the research.

The content of potassium in the air-dried leaves of *Digitalis lanata*, except the 3rd year of the research, was significantly modified by the doses of fertiliser. Generally, an increase in the content of this element in the dried mass of leaves was observed in all of the fertilized treatment. Significantly less potassium was accumulated by the leaves gathered in 2006 compared with 2007 and 2008. The differences were 6.5 and 7.9%, respectively. On average, during the three-year research period, the highest amount of potassium (20.21 g kg^{-1}) was accumulated by leaves of the Woolly Foxglove fertilised by the A_2 dose, where the increment was 4.6% compared to the control. A slightly lower content of potassium (19.72 g kg^{-1}) in leaves was

Table 1

The content of macronutrients in leaves of the Woolly Foxglove

Nutrient	Years	Fertilisation treatment				Mean
		A_0	A_1	A_2	A_3	
Total nitrogen (g kg^{-1})	2006	20.53	23.92	25.64	25.46	23.89
	2007	16.25	17.38	18.79	20.74	18.29
	2008	18.85	21.17	22.77	23.68	21.62
	mean	18.54	20.82	22.40	23.29	21.26
	LSD: for years – 0.626 for fertilisation – 0.382			for fertilisation in years: 2006 – 2.041 2007 – 1.352 2008 – 1.233		
Total phosphorous (g kg^{-1})	2006	1.92	2.23	2.47	2.56	2.29
	2007	2.72	2.98	3.05	3.10	2.96
	2008	2.37	2.66	2.82	2.89	2.68
	mean	2.34	2.62	2.78	2.85	2.65
	LSD: for years – 0.109 for fertilisation – 0.048			for fertilisation in years: 2006 – 0.106 2007 – 0.168 2008 – 0.155		
Potassium (g kg^{-1})	2006	18.75	18.76	19.48	18.29	18.82
	2007	19.25	20.03	20.28	20.61	20.04
	2008	19.95	20.37	20.88	20.10	20.31
	mean	19.32	19.72	20.21	19.67	19.73
	LSD: for years – 0.298 for fertilisation – 0.354			for fertilisation in years: 2006 – 0.595 2007 – 0.679 2008 – n. s.		
Calcium (g kg^{-1})	2006	5.96	5.85	5.98	5.79	5.89
	2007	4.48	4.31	4.35	4.31	4.36
	2008	5.07	4.93	5.02	4.90	4.98
	mean	5.17	5.03	5.12	5.00	5.08
	LSD: for years – 0.217 for fertilisation – 0.133			for fertilisation in years: 2006 – 0.187 2007 – n. s. 2008 – 0.165		
Magnesium (g kg^{-1})	2006	3.72	3.62	3.30	3.13	3.44
	2007	3.64	4.01	4.09	4.18	3.98
	2008	4.60	4.77	4.62	4.57	4.64
	mean	3.99	4.13	4.00	3.96	4.02
	LSD: for years – 0.085 for fertilisation – 0.116			for fertilisation in years: 2006 – 0.283 2007 – 0.317 2008 – n. s.		
Sodium (g kg^{-1})	2006	4.96	4.76	4.41	4.38	4.63
	2007	2.35	2.24	2.04	2.01	2.16
	2008	4.57	4.38	3.88	3.69	4.13
	mean	3.96	3.79	3.44	3.36	3.64
	LSD: for years – 0.192 for fertilisation – 0.081			for fertilisation in years: 2006 – 0.221 2007 – 0.141 2008 – 0.265		

achieved after applying the lowest fertilisation dose. Potassium content in herbal plants varies greatly depending on the region of cultivation (AJASA et al. 2004, SHEDED et al. 2006, WOJCIECHOWSKA 2008), type of raw material (SINGH, GARG 1997) and soil fertility (SHEDED et al. 2006, ZARĘBA, BŁONIARZ 2008).

The mean content of calcium in leaves of *Digitalis Lanata* was significantly modified by fertilisation. The increasing level of mineral fertilisation induced a lower content of this chemical element in the air-dried matter of leaves, which proved significant for the treatments A_1 and A_3 with reference to the control combination (2.7% and 3.3% less, respectively). In 2007, no significant effect of the applied fertilisation on the calcium content in the Woolly Foxglove's leaves was observed. In 2007, the concentration of this element was significantly lower (by 35.1 and 14.2%) than in leaves collected in 2006 and 2008. Calcium in plants is responsible for the rate of the uptake of mineral components and their transport. Its deficiency in soil may disturb the uptake of ions. The content of calcium in plants depends considerably on the type of fertilisation, physicochemical characteristics of soil, air temperature and the amount of precipitation during the plant vegetation period, as well as on the age of the plant (WÓJCIK 1998).

Magnesium, a basic component of chlorophyll and an element that activates many enzymatic processes during the syntheses of proteins, nucleic acids, lipids and hydrocarbons, plays a key role in the plant metabolism. It has been demonstrated that after the fertilisation the mean content of magnesium in *Digitalis lanata* was the highest in the treatment fertilised with the lowest dose of NPK (A_1). The content of magnesium in the air-dried matter of leaves from the other treatments was similar to the control. Most magnesium was accumulated in leaves gathered in 2008.

The physiological role of sodium is less thoroughly recognised than that of the other macronutrients. Based on the response to fertilisation with this element, the plants are divided into natriophytes and non-natriophytes. The content of sodium in plants usually varies from 0.1 to 5 g kg^{-1} but in natriophytes it reaches from 15 to 25 g kg^{-1} (ZARĘBA, BŁONIARZ 2008). As seen from the above, the Woolly Foxglove does not belong to natriophytes and the content of sodium in its leaves may be modified by mineral fertilisation (Table 1). In comparison to the control, a significant decrease in the content of sodium in leaves of the Woolly Foxglove was observed after applying the experimental fertilisation doses. The lowest amounts of sodium were in leaves gathered from the plot fertilised with the highest NPK dose (A_3), where the decrease was 15.2%. The lowest sodium concentrations were typical of leaves gathered in 2007 (2.16 g kg^{-1}). Compared to 2006 and 2008, the differences were significant and equalled to 2.47 g kg^{-1} and 1.97 g kg^{-1}, respectively.

The optimum content of mineral components in herbal plants ensures proper quantitative ratios between the elements in herbal raw material and is beneficial for the bio-availability and efficacy of products extracted from

Table 2

The values of mole ratios in leaves of the Woolly Foxglove

Ratio	Year	Fertilisation object				Mean
		A_0	A_1	A_2	A_3	
1	2	3	4	5	6	7
Ca:P	2006	2.44	2.08	1.92	1.80	2.06
	2007	1.27	1.12	1.10	1.07	1.14
	2008	1.65	1.43	1.38	1.32	1.44
	mean	1.79	1.55	1.47	1.40	1.55
	LSD: for years – 0.133; for fertilisation – 0.037			for fertilisation in years: 2006 – 0.171; 2007 – 0.008; 2008 – 0.121		
Ca:Mg	2006	0.99	1.01	1.13	1.15	1.07
	2007	0.75	0.65	0.65	0.63	0.67
	2008	0.67	0.63	0.66	0.65	0.65
	mean	0.80	0.76	0.81	0.80	0.80
	LSD: for years – 0.066; for fertilisation – 0.037			for fertilisation in years: 2006 – 0.109; 2007 – n. s.; 2008 – n. s		
K:Ca	2006	3.17	3.21	3.26	3.16	3.20
	2007	4.42	4.77	4.80	4.91	4.72
	2008	4.05	4.24	4.27	4.21	4.19
	mean	3.88	4.07	4.11	4.10	4.04
	LSD: for years – 0.266; for fertilisation – 0.053			for fertilisation in years: 2006 – n. s.; 2007 – 0.255; 2008 – 0.123.		
K:Mg	2006	3.13	3.23	3.68	3.64	3.42
	2007	3.30	3.11	3.09	3.07	3.14
	2008	2.70	2.66	2.82	2.74	2.73
	mean	3.04	3.00	3.20	3.15	3.10
	LSD: for years – 0.069; for fertilisation – 0.122			for fertilisation in years: 2006 – 0.368; 2007 – n. s.; 2008 – n. s.		
K:Na	2006	2.23	2.32	2.60	2.46	2.40
	2007	4.82	5.26	5.85	6.02	5.49
	2008	2.57	2.73	3.17	3.21	2.92
	mean	3.20	3.44	3.87	3.90	3.60
	LSD: for years – 0.161; for fertilisation – 0.097			for fertilisation in years: 2006 – n. s.; 2007 – 0.410; 2008 – 0.313		

cont. Table 2

1	2	3	4	5	6	7
K:(Ca+Mg)	2006	1.57	1.61	1.73	1.69	1.65
	2007	1.88	1.88	1.88	1.89	1.88
	2008	1.62	1.63	1.70	1.66	1.65
	mean	1.69	1.71	1.77	1.75	1.73
	LSD: for years – 0.053 for fertilisation – 0.043			for fertilisation in years: 2006 – 0.106 2007 – n. s. 2008 – 0.069		
(K+Na):(Ca+Mg)	2006	2.28	2.30	2.39	2.38	2.34
	2007	2.27	2.24	2.20	2.20	2.23
	2008	2.25	2.23	2.23	2.17	2.22
	mean	2.27	2.26	2.28	2.25	2.26
	LSD: for years – 0.078 for fertilisation – n.s.			for fertilisation in years: 2006 – n. s. 2007 – n. s. 2008 – n. s.		

such plants. The present study has proven that the effect of mineral fertilisation doses on mole ratios in leaves of *Digitalis lanata* was not unidirectional (Table 2). The values of the Ca:P ratio varied from 1.40 to 1.79 and tended to narrow down as the doses of applied fertiliser rose. The optimum mole Ca:P ratios in crop plants, acc. to Filipek (1987), ought to be 1.5:1; the present research has showed similar values for treatments A_1 and A_2. It is worth noticing that mole ratios in herbal plants are rarely discussed in the literature

The use of NPK dose A_1 significantly narrowed the Ca:Mg ratio versus the control. Further increase in the fertiliser dose did not alter the ratio between these elements in leaves. The effect of the applied doses of fertiliser on the chemical composition of the Woolly Foxglove's leaves was also confirmed by changes in the K:Ca ratio. The research showed that the applied fertilisation significantly increased the above ratio in leaves of *Digitalis lanata* relative to the control, which resulted from a rise in the potassium content and a drop in the level of calcium in leaves when the NPK dose was higher. Assuming that the optimum mole ratios of these elements in crops are 2:1 (Panak, Wojnowska 1982), the K:Ca ratio in leaves of the Woolly Foxglove should be recognised as high.

A significant increase in the K:Mg ratio with reference to the control was observed after using the medium dose of NPK (A_2). It should be emphasized that this dose is recommended for *Digitalis lanata*. The fertilisation induced a statistically confirmed enlargement of the K:Na ratio from 3.20 in the control to 3.90 in the plants fertilised with the highest dose of NPK (A_3).

A significant growth in the ratio of potassium to the total of calcium and magnesium with reference to the object without mineral fertilisation was observed after using the highest (1.75) and the medium NPK dose (1.77). In 2006 and 2008, the mean value of this ratio significantly increased compared to the control. Mineral fertilisation did not change significantly the ratio of the total sum of monovalent ions to the total sum of bivalent ions compared with the control. The mean value of the above ratio was 2.26. Analogously, no such changes were observed in the particular years of the research. The value of total monovalent to total bivalent ion ratio is shaped by antagonisms, for example between calcium and potassium, which affect the plant's water management. The effect of calcium on transpiration may also depend on the proportion between the content of this element and the content of potassium and sodium.

The research has clearly demonstrated that the weather conditions, different during the vegetation season in the consecutive years, affected mole ratios of the elements in leaves of the Woolly Foxglove. The fact that the changes in the calculated ratio values were not unidirectional (Table 2.) was mainly the result of the unequal distribution and amount of precipitation.

In the above research, the highest NPK dose (A_3), exceeding the recommend one, caused significant changes in the chemical composition of leaves of *Digitalis lanata*, a development which was also reflected by changes in proportions of mineral compounds in leaves. This finding may imply that higher quality raw material can be obtained by optimised fertilisation.

CONCLUSIONS

1. The tested mineral fertilisation generally induced a significant increase in the content of total nitrogen, total phosphorus, and potassium, but depressed the sodium concentration in leaves of the Woolly Foxglove compared to the control.

2. After applying 107.3 kg NPK ha^{-1}, the content of calcium and magnesium increased, while the ratio between these chemical elements decreased in leaves of *Digitalis Lanata* in comparison to the control.

3. The increased mineral fertilisation significantly widened the ratios of K:Ca and K:Na, and narrowed the Ca:P ratio in leaves.

4. A significantly broader K:(Ca+Mg) ratio in leaves was observed after applying the medium and the highest NPK doses.

5. After applying 214.6 kg NPK ha^{-1}, an increase in the K:Mg ratio in leaves was observed versus the control.

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