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Short research contribution

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CONTENT OF COLCHICINE IN CORMS AND EDAPHIC CONDITIONS OF *COLCHICUM AUTUMNALE* L. FROM KACZAWSKIE MOUNTAINS (POLAND)

ABSTRACT: The chemical properties and colchicine content in *Colchicum autumnale* corms from sites localized in Kaczawskie Mountains (Poland) were studied. The colchicine in corms differs significantly and depends on chemical properties of the soils. This dependence is confirmed by the calculated significant correlations between colchicine content and soil pH, Ca, Mn and Fe content.

KEY WORDS: *Colchicum autumnale* L., colchicine, corms, edaphic conditions, Kaczawskie Mountains, Poland

Alkaloids together with terpenoids and phenol compounds are one of the most important secondary plant substances. These nitrogen-containing compounds are present in about 20% of angiosperm species. They are also generally absent in ferns, mosses and gymnosperms (Harborne 1999). Alkaloid characteristics, chemical constitution and biosynthesis has been examined (Mothes and Schuette 1969). Their ecological importance has not been fully explained (Ostroumov 1992). Alkaloids may take part in interactions between plants. In the previous years their allelopathic activity has been widely investigated (Wójcik-Wojtkowiak 1987).

Colchicum autumnale L. (meadow saffron) contains alkaloids, mainly colchicine. It is present in the whole plant, with the highest content in seeds and corms (Burda 1998). Colchicine toxicity (Hood 1994, Roche *et al.* 1994, Panariti 1996), the use in genetics and plant cultivation as well as in therapeutics (Heeger 1989) is known. It was the first substance with recognized anti-neoplastic properities (Sadowska 1995). The French pharmaceutical industry obtains this compound exclusively from (imported) natural raw material as the chemical synthesis is very expensive (Poutaraud and Champay 1995). In Poland, meadow saffron is cultivated for pharmateutical industry (Piwoni 1995, Strzelecka and Kowalski 2000). Until now the factors influencing colchicine content in *C. autumnale* corms have not been well recognized (Gašić 1993, Vicar *et al.* 1993). It is important in both artificial and natural cultivation of this plant to obtain a vegetable material with both maximal and balanced colchicine content. It is necessary in study on the type of interaction with other plant species accompaning directly *C. autumnale* in populations.

The purpose of the present research was to determinate colchicine content and to find the extent of edaphic conditions influence on colchicine content in *C. autumnale* corms.

The study was carried out in the Kaczawskie Mountains (50°54' N, 16°09' E, Poland) (Fig. 1). The mountains are a small mountain range of relative altitude not exceeding 250 meters above sea level; the climatical-vegetal layers are not distinguished (Kondracki 1994). For the research 7 sites of local population have been chosen. In 1996 five trial square areas (the side length – 1 m) were randomly chosen in the acreage of examined sites. The average individual density per 1 m^2 was determined. In every site, in randomly chosen place one experimental locality – square-shaped of 2.25 m^2 area with similar lighting and soil humidity conditions was established. The distance between subpopulations was as follows: from 1 to 2 – 0.8 km; from 2 to 3 – 0.9 km; from 3 to 4 – 1.2 km; from 4 to 5 – 7.2 km; from 5 to 6 – 0.8 km; from 6 to 7 – 0.8 km (Fig. 1) The number of individuals necessary to keep the experimental subpopulation density at the same level as in parent population density was taken randomly from every site. If *C. autumnale* was present in the given square then the number of individuals taken from this site was only so high as to complete the density to the level similar to average density of parent population. The placement of individuals on examined sites was done according to table of random numbers determining coordinates of planting points (Kershaw 1978). The subpopulations have the following densities respectively: 1 and 4 – 5 plants/m^2; 2 – 6 plants/m^2; 3 – 12 plants/m^2, 5 – 11 plants/m^2; 6 and 7 – 8 plants/m^2. *C. autumnale* subpopulations occur in a species-rich meadow unfertilized for some 20 years, on a north-east (1, 2, 6); north-west (4, 7) and south-east (3, 5) facing slope (inclination 8–10°, alitude 300–350 m). The subpopulations can be classified to the *Molinio-Arrhenatheretea*. The subpopulations 1, 2, 4 have a higher share of *Poa pratensis*, *Dactylis glomerata*, *Vicia cracca*, *Achillea millefolium*; 3, 5, 6, 7 have the highest abundance *Festuca pratensis*, *Holcus lanatus*, *Geum rivale*, *Sanguisorba officinalis*, *Polygonum bistorta*. The soils on which *C. autumnale* grows belong to the light (1–4) and medium (5–7) loam types with a low share of sceleton parts. In mid-May of 1999 from every subpopulation corms of individuals in generative stage were taken randomly together with soil samples from their levels. Soil has been dug up by paddle at depth of 30 cm. After which the soil has been left on the paddle and from the layer, which was into direct contact with the corms the soil samples were taken.

The fresh corms were cleaned, washed with distilled water, then grinded (particle size: 2–10 mm) and mixed. 20 g of corms was four times extracted with 100 ml of methanol in 115 °C for 1 hour with FexIka 200 extraction apparatus. Then the extract was cooled

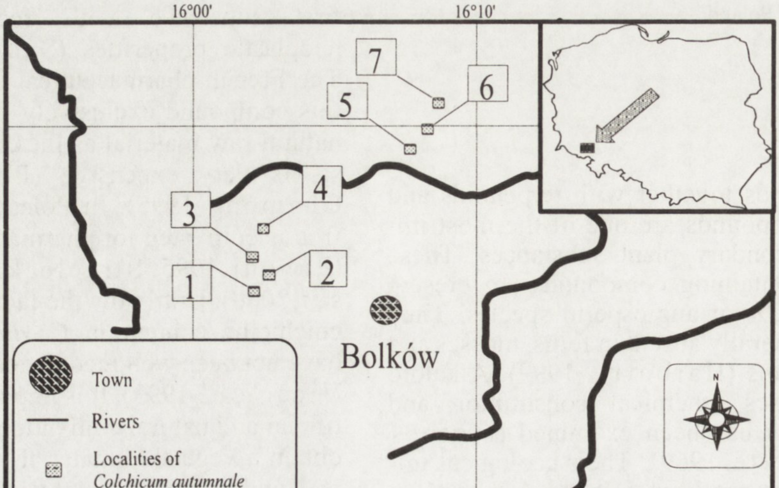

Fig. 1. Localities of *C. autumnale* subpopulations on investigated area

down and filtered through Wathman filter paper. 10 ml of extract has been thickened by evaporation to 5 ml. The extracts were analysed by HPLC LKB version with an UV detector DAP version and automatic fraction collector. The following conditions were applied: column C18RP , particle size 10 m, 250 mm 4.6 mm, mobile phase ACN and H_2O (30:70, 100 l/l H_3PO_4), isocratic elution, flow rate 0.75 ml/min , injection volume at 20 l, the room temperature, detection 243 nm.

The content of available P was determined colometrically with vanadium molybdate; a quantities exchangeable Ca^{+2}, K^+ nad Mg^{+2} was determined with flame photometer (Ca^{+2}, K^+) and AAS (Mg^{+2}) after one hour extraction in 0.03 n acetic acid. Soil total N content was determined by the Kjeldahl method. Soil total S content was determined by Carbo Erba NA-1500 CNS Analyzer. Soil pH (pH_{KCl}) was determined in dry soil after 24 h.

The microelements Mn, Pb, Cu, Cd, Zn, Ni, Fe and Cu were measured with AAS (Perkin Elmer ASS 3300). About 250 mg of samples of soil were weighed into a CEM Teflon vessels and mixture of 5 ml HNO_3 (conc) and 1.5 ml HCl (conc) was added. Teflon vessel was placed in microwave oven (CEM-MARS 5). Heating of the samples was carried out in three cycles with increased time and power of microwave energy. After cooling down it was quantitatively transferred into volumetric flask (20 ml) and diluted to the mark with distilled water. All analyses were done in duplicate.

The results of the chemical analyses of plant material and soil were tested with ANOVA. Mean content of micro- and macroelements and pH differentiation in soil from the subpopulations was determined by analysis of variance. The relationship between measured soil parameters and colchicine content in corms was tested by two variables correlation (Łomnicki 1999).

The content of colchicine in *C. autumnale* corms is presented in Table 1. The colchicine in corms differs significantly. The highest content of colchicine is present in corms from subpopulation 6, the lowest – in corms from subpopulation 1.

The chemical properties of subpopulations soil are presented in Table 2. The soils pH and examined macro- and microelements content differ significantly. pH varies from 4.3 to 6.7. The soils were very acidic (5), acid (6, 7), sub-acid (1–3) and neutral (4). The highest content of N and the lowest of Ca and Mg was found in soil from subpopulation 5, while the lowest content of N, P, K and S and the highest Cu and Ni was found in soil from subpopulation 7. Soil from subpopulation 4 has the highest Pb, Cd and Zn content, from subpopulation 1 – the lowest Cu and Ni content. In the present study, the following relationships between pH, macro- and microelements content in soils and colchicine content in *C. autumnale* corms were found: soil pH and Ca content are negatively correlated with corms colchicine content. Mn and Fe content in soil is positively correlated with corms colchicine content (Table 3).

The content of colchicine in seeds, corms and flowers is different (Burda 1998) and changes during vegetation period (Vicar *et al.* 1993). The maximum of colchicine content in seeds appears in the middle of May and in the following period decreases as a result of glycosylation (Poutaraud and Champay 1995). The content of colchicine in *C. autumnale* corms from examined subpopulations is different. The lowest colchicine content is smaller than nominal value while the highest one does not equals maximum value for this plant given by Hayashi *et al.* (1987). Gašić (1993) stated that content of alkaloids in *C. autumnale* collected from different localities in Vojvodina province is closely related to the contents of Cu, Mn and Zn in the soil. Present study proved the existence of statistically significance positive correlation between Mn content in soil and corms colchicine content (Table 3). Colchicine originates from alanine, tyrosine and methionine (Mothes and Schuette 1969). Its synthesis, probably the same as in other alkaloids, proceeds parallely to proteins

Table 1. Mean (n = 3) content of colchicine in *C. autumnale* corms from different subpopulations and significance of the difference

Subpopulation**	Colchicine μg/g f.wt.
1	135.00
2	312.50
3	262.50
4	208.20
5	331.25
6	368.75
7	237.50
$F_{0.05}$	1230*

*significant at $P < 0.05$, F_{tab} 2.85

** see Fig.1

Table 2. pH and mean (n = 3) concentration (mg/kg) of macroelements and microelements in soil from different subpopulations and significance of the differences (*significant at $P < 0.05$, F_{tab} 2.85)

		Subpopulation							$F_{0.05}$
		1	2	3	4	5	6	7	
Macroelements	pH	6.4	6.4	6.2	6.7	4.3	4.5	5.2	2056*
	N	3164	3010	2786	3472	3934	3066	2450	41.3*
	P	42	53.7	36.5	49	36.5	26	22.5	31.7*
	K	31.5	33.2	27	39.3	35.9	17.3	8.5	189.6*
	Ca	3179	3502.3	3878.2	3404.6	810.9	1216.8	1036.4	98.2*
	Mg	534.9	398.1	531.3	629.3	117.8	629.3	167.8	47.5*
	S	378.2	425.1	340.5	443.2	328.2	268.2	245	6.3*
Microelements	Mn	721.3	734.7	728	620	836	780	752	21.9*
	Pb	75.2	69.1	77.6	121.6	68.8	78.4	72.8	2592.7*
	Cu	20.8	21.9	24.8	26.4	33.6	32	46.4	486.3*
	Cd	2.1	2.4	2.8	3.6	1.8	1.9	1.8	110.8*
	Zn	80.4	91.6	83	164	59	77.8	73.9	140.5*
	Ni	26.8	27.1	28.4	32.6	53.6	52.8	91.4	8667.7*
	Fe	25280	23920	45440	20320	34720	44720	24160	3.4*

Table 3. Relations between content of colchicine in *C. autumnale* corms and chemical properties of soil (n =21)

Relations	r_{est}	P
Colchicine in corms and soil pH	–0.67	0.0008
Colchicine in corms and calcium in soil	–0.46	0.035
Colchicine in corms and manganese in soil	0.58	0.006
Colchicine in corms and iron in soil	0.44	0.048

synthesis (Harborne 1999). Manganese activates various enzymes, such as those taking part in proteins metabolism. In case of its lack the proteins content drops down and the elongation growth and side roots formation are set back (Kopcewicz and Lewak 1998). In present study, the relationships between Cu and Zn in soil and concentration of colchicine in corms were not found. However, in the soil containing the lowest levels of Cu and Ni, *C. autumnale*s growings have the lowest concentration of colchicine in their corms (subpopulation 1) (Tables 1 and 2).

The negative correlation between Ca content in soil and corms colchicine content and the positive correlation between Fe content in soil and corms colchicine content has been stated (Table 3). Hayashi *et al.* (1988) reported that formation of colchicine in suspension cultured is inhibited by high Ca^{+2}, Fe^{+2} and PO_4^{-3} concentration: respectively 10 mM, 1 mM and 2.5 mM. The authors stated that an increase of colchicine content occurs when Fe^{+2} concentration equals 100 μM. Addition of SO_4^{-2} (20 mM) markedly increased the formation of colchicine. Calcium takes part in physiological organization of cell and influences many biochemical reactions. Calcium influences the plants through the soil, improving its physical, chemical and biological properties. It improves phosphorus and molybdenum availability and weakens iron, aluminium and manganese availability (Dobrzański and Zawadzki 1981). Iron is probably connected with proteins metabolism. Its deficiency causes a protein fraction decrease and an increase of the soluble organic nitric compounds fraction (Mengel and Kirkby 1983). In this investigation, in subpopulation 6 the highest and in subpopulations 1 the lowest content of colchicine in corms were found (Table 1). The soil from these subpopulations contained low (6) and high (1) levels of P and S (Table 2). According to Hayashi *et al.* (1988), colchicine concentration seems to be associated with concentration of P and S in soil.

The soils from examined subpopulations have high content of nitrogen, low content of phosphorus, calcium, magnesium and sulphur and a very low content of potassium in comparison with values for various Polish soils according to Lityński and Jurkowska (1982). These soils have an increased amount of Fe, Mn, Pb, Cd, Zn (1–7) and Ni (7) comparing to mean natural content of Polish soils (Kabata-Pendias and Pendias 1993). The Pb, Cd, Zn and Ni contamination is caused by fertilizers, pesticides and sewage (Kabata-Pendias and Pendias 1993). All sites are placed in close vicinity of human settlements. The soil from subpopulation 4 contained the highest level of Pb, Zn and Cd (twice as much as in relation to all examined subpopulations) and the corms from this subpopulation contained low level of colchicine. It can be supposed that the relatively low concentration of colchicine in the corms from this subpopulation is probably due to the contamination of Pb, Cd and Zn of the soil (Tables 1 and 2).

C. autumnale subpopulations from Kaczawskie Mountains differ significantly in soil chemical properties and *C. autumnale* growings there have significantly different corm colchicine content. This differentiation of corms colchicine content is connected with the chemical properties of the soils what was proved by the correlation between soil pH, soil Ca, Mn and Fe content and corms colchicine content. The high concentration of colchicine in corms of *C. autumnale* seems to be related to acidic soil, very poor of Ca and relatively rich of Mn and Fe.

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