ASSESSMENT OF MINERAL COMPOUNDS IN DIFFERENT POPULATIONS OF THYME (*THYMUS* L.)

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Abstract. *The mineral profile of thyme, a species so often used as a medicinal plant or spice, represents a permanent and significant research topic for the fields of agronomy, chemotaxonomy, biochemistry. Previous studies have indicated a significant number of organic substances with high antimicrobial potential not only in cultivated but also in spontaneous populations of thyme, revealing the phytobiological value of these species. We are in a permanent search for new resources from the wild flora, necessary either for direct use or introduction into culture. In this context, the study evaluates the mineral profile related to 17 spontaneous populations of thyme identified in the Banat area, compared to a cultivated population, investigating the quality of the biological material analyzed. The content of total mineral substances (ash) in the analyzed populations varied between 1.19% in the population of Th. pannonicus ssp. auctus from the Silagiu area and 10.85% in the cultivated thyme (Th. vulgaris), observing the existence of individual variations depending on the species, but also depending on the harvesting area. The characteristics of the biotope are defining and influence the mineral profile of the thyme populations in natural areas. Among the mineral elements analyzed in the present work, those related to the analysis of the macroelement content are presented. The amount of calcium accumulated in the dry vegetable mass can represent a relevant indication as a species character, if it will be confirmed by other specialized studies.*

Keywords*: thyme, Lamiace (Labiatae), mineral profil, macroelements*

INTRODUCTION

The Lamiaceae family arouses the interest of researchers from different fields due to the large number of genera, important from a medicinal and aromatic point of view. Among them, the genus *Thymus*, is well represented and groups over 200 species, spread all over the world. The species has been cultivated since ancient times, currently there are numerous studies that refer to its chemical composition and biological properties (JAVED *et al.*, 2013; URITU *et al*., 2018).

It is known that the chemical composition of plants is significantly influenced by soil fertility and environmental factors, which are in close and direct correlation. The chemical composition varies within high limits, depending on the genotype, on the time of day or during the vegetation period (STAHL-BISKUP, 1991; KROL *et al*., 2014; JAMALI *et al*., 2013).

According to the latest research, about 6% of cormophytes have been studied for their pharmacological potential and only 15% have been evaluated for their phytochemical potential. (CRAGG &NEWMAN, 2013, ESPINOSA-LEAL *et al*., 2018).

The species of the genus *Thymus* are recognized for the large amount of biologically active compounds (TOHIDI *et al*., 2019; BEICU *et al*., 2019; BORUGĂ *et al*., 2014), products based on thyme extracts find applicability today in different fields, both in human medicine, (CIOBOTARU *et al*., 2017; RAMCHOUN *et al*., 2020), as well as in veterinary medicine (HOVEN *et al*., 2003), as cosmetic and food additives (TAGHOUTI *et al*., 2020).

In this regard, given the economic and pharmaceutical importance of the species of this genus, the clear identification of some spontaneous species, with significant phytomedicinal properties, can open new ways of valorization, by introducing them into culture. That is why the accurate determination of the species in the field, especially in scientific research, is absolutely necessary, in order to compare the data with similar ones from the specialized literature. The accuracy of results and interpretation regarding flora and vegetation studies, are closely related to taxonomic determination. Wild thyme is difficult to determine taxonomically, due to the similarity of the morphological characters of different populations, interspecific hybridization, causing confusion even for specialists. As a result, the development of any tool that brings value in the taxonomy of this species, is useful and necessary. In this sense, the present study aims to provide additional information regarding the chemical characteristics and mineral profile for 17 spontaneous populations of thyme, compared to a cultivated population, all from the Banat area.

MATERIAL AND METHODS

Biological material

The analyzed populations were harvested during the flowering period (May-June, 2019). The taxonomic determination was made on the basis of plant identification books (*Flora of Romania vol. VIII*, GUȘULEAC,1961; *Illustrated Flora of Romania*, CICÂRLAN, 2009; *Vascular plants from Romania*, SÂRBU *et al*., 2013).

The samples taken were collected from different areas of Banat: samples P1 (*Th. glabrescens*) and P2 (*Th. pannonicus ssp. auctus*) come from Silagiu (Timiș); samples P3 (*Th. comosus* – Dobraia) and P4 (*Th. praecox ssp. janke* – Domogled Cruce) come from Domogled-Valea Cernei National Park (Caraș-Severin); samples P5 (*Th. dacicus* – Ostrov), P6 (*Th. dacicus* – Coronini Mila 1039), P7 (*Th. praecox ssp. janke* – Coronini Gaura cu Muscă), P8 (*Th. dacicus* – Lescovița), P9 (*Th. pulegioides ssp. chamaedrys* – Pojejena), P10 (*Th. pulegioides ssp. montanus* – Pojejena), from the Iron Gate Natural Park (Caraș-Severin); samples P11 (*Th. praecox ssp. polytrichus* – Semenic Peak), P12 (*Th. praecox ssp. polytrichus* – Gozna Peak), P13 (*Th. pulegioides ssp. pulegioides* – Văliug), P14 (*Th. pulegioides ssp. chamaedrys* – Carșova) , P15 (*Th. pulegioides ssp. montanus* – Carașova), P16 (*Th. pulegioides ssp. pulegioides* – Carașova), P17 (*Th. pulegioides ssp. pulegioides* – Nermet), come from Semenic-Cheile Carașului National Park; sample P18 is the sample from the culture (*Th. vulgaris*) from Lovrin (Timiș). Samples voucher for each population were stored after identification in the herbarium of the Botany Department within the University of Life Sciences "King Mihai I" from Timișoara.

Determination of total mineral content

The analysis of the plant from the ash is a simple, safe and cheap method compared to the mineralization method with a mixture of HNO3 – HClO4 (CRISTA&RADULOV, 2009).

Samples of dry biological material were weighed for each studied population (500mg), placed in numbered ceramic vessels. The ceramic vessels were placed in a calciner (Nabertherm LE2/11) at 600ºC for 10 hours to obtain ash. The resulting ash should have a uniform color (white or gray) and should not contain black coal dots.

Determination of mineral elements by atomic absorption spectroscopy (SAA)

In order to determine the macroelements by atomic absorption spectroscopy, the ash resulting from the calcination of the samples was treated with 10 mL of 20% HCl (SC Chimreactiv SRL, Bucharest).

The macroelements (potassium, calcium, magnesium) were determined by atomic absorption spectrophotometry (Varian 220 FAA spectroscopic equipment) through an official method certified by the AOAC (Association of Official Analytical Chemists) at the wavelength specific to each element. The calibration standard (ICP Multielement Standard Solution IV CertiPUR) was purchased from Merk. The reagents used were of analytical purity. Three determinations per mineral element were performed for each sample. The results were expressed in g/kg dry plant mass.

RESULTS AND DISCUSSIONS

For each plant sample collected, the calcined mass was calculated, expressed in grams. The percentage of total mineral substances, relative to the dry plant sample, was determined. The average concentration for the 18 populations studied was 6.33±2.24% (table 1).

Table 1

Percentage of total mineral substances in the analyzed *Thymus* populations

Sample	Population	Harvesting place	Sample mass (g)	Calcined sample mass (g)	Total ash (%)
P1	*Th. glabrescens*	Silagiu (Timiș)	0.541	0.037	6.839
P2	*Th. pannonicus ssp. auctus*	Silagiu (Timiș)	0.506	0.006	1.186
P3	*Th. comosus*	Dobraia (Caraș-Severin)	0.536	0.032	5.970
P4	*Th. praecox ssp. janke*	Domogled (Caraș-Severin)	0.561	0.026	4.635
P5	*Th. dacicus*	Ostrov (Caraș-Severin)	0.532	0.03	5.639
P6	*Th. dacicus*	Coronini (Caraș-Severin)	0.538	0.038	7.063
P7	*Th. praecox ssp. janke*	Coronini (Caraș-Severin)	0.815	0.046	5.644
P8	*Th. dacicus*	Lescovița (Caraș-Severin)	0.512	0.03	5.859
P9	*Th. pulegioides ssp. chamaedrys*	Pojejena (Caraș-Severin)	0.561	0.023	4.100
P10	*Th. pulegioides ssp. montanus*	Pojejena (Caraș-Severin)	0.535	0.048	8.972
P11	*Th. praecox ssp. polytrichus*	Gozna (Caraș-Severin)	0.525	0.027	5.143
P12	*Th. praecox ssp. polytrichus*	Semenic (Caraș-Severin)	0.512	0.029	5.664
P13	*Th. pulegioides ssp. pulegioides*	Valiug (Caraș-Severin)	0.746	0.043	5.764
P14	*Th. pulegioides ssp. chamaedrys*	Carașova (Caraș-Severin)	0.538	0.026	4.833
P15	*Th. pulegioides ssp. montanus*	Carașova (Caraș-Severin)	0.512	0.04	7.813
P16	*Th. pulegioides ssp. pulegioides*	Carașova (Caraș-Severin)	0.553	0.052	9.403
P17	*Th. pulegioides ssp. pulegioides*	Nermet (Caraș-Severin)	0.522	0.045	8.621
P18	*Th. vulgaris*	Lovrin (Timiș)	0.562	0.061	10.854

The content of total mineral substances (ash) in the analyzed populations varied between 1.19% in the population of *Th. pannonicus ssp. auctus* from the Silagiu area and 10.85% in the cultivated thyme (*Th. vulgaris*), depending on the sample, and falls within the limits of the values found for the genus *Thymus* in the specialized literature (TOMESCU *et al.*, 2015).

Mineral elements are of great interest from a biological point of view, as they have numerous functions in carrying out biochemical processes in plant cells (SOETAN *et al.*, 2010).

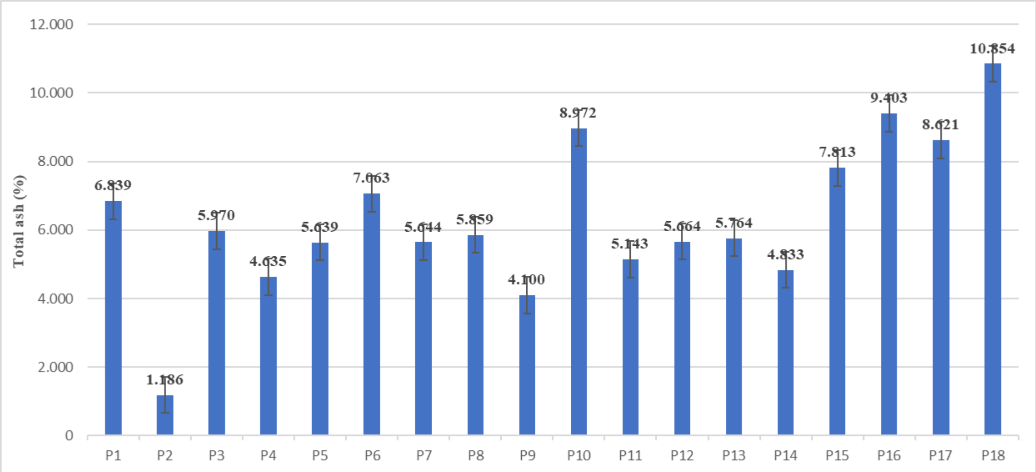

Figure 1. Individual variations ash content, by species and harvest area

Table 2

Content of macroelements in the analyzed *Thymus* populations

Sample	Population	Harvesting place	K (g/kg)	Ca (g/kg)	Mg (g/kg)
P1	*Th. glabrescens*	Silagiu (Timiș)	6.915	21.665	4.978
P2	*Th. pannonicus* ssp. *auctus*	Silagiu (Timiș)	7.379	17.034	4.119
P3	*Th. comosus*	Dobraia (Caraș-Severin)	14.927	27.274	3.864
P4	*Th. praecox* ssp. *janke*	Domogled (Caraș-Severin)	3.294	3.526	1.319
P5	*Th. dacicus*	Ostrov (Caraș-Severin)	2.398	15.994	3.733
P6	*Th. dacicus*	Coronini (Caraș-Severin)	10.253	53.039	6.491
P7	*Th. praecox* ssp. *janke*	Coronini (Caraș-Severin)	9.567	46.357	5.930
P8	*Th. dacicus*	Lescovița (Caraș-Severin)	8.793	40.057	6.580
P9	*Th. pulegioides* ssp. *chamaedrys*	Pojejena (Caraș-Severin)	4.428	17.713	3.982
P10	*Th. pulegioides* ssp. *montanus*	Pojejena (Caraș-Severin)	3.619	13.170	2.886
P11	*Th. praecox* ssp. *polytrichus*	Semenic (Caraș-Severin)	15.084	8.905	2.381
P12	*Th. praecox* ssp. *polytrichus*	Gozna (Caraș-Severin)	13.002	6.479	3.459
P13	*Th. pulegioides* ssp. *pulegioides*	Valiug (Caraș-Severin)	9.715	32.105	6.034
P14	*Th. pulegioides* ssp. *chamaedrys*	Carașova (Caraș-Severin)	6.110	21.032	4.699
P15	*Th. pulegioides* ssp. *montanus*	Carașova (Caraș-Severin)	6.432	14.311	4.826
P16	*Th. pulegioides* ssp. *pulegioides*	Carașova (Caraș-Severin)	2.092	17.972	4.083
P17	*Th. pulegioides* ssp. *pulegioides*	Nermet (Caraș-Severin)	5.213	18.454	4.255
P18	*Th. vulgaris*	Lovrin (Timiș)	13.454	16.157	5.082

It is important to state that the mineral profile of a *Thymus* population is influenced by the characteristics of the area of origin (soil type, climate, thermal regime, precipitation regime, etc.). A heterogeneous distribution of macroelements is noted in the 18 *Thymus* populations studied (figure 1). The distribution of macroelements by species is in accordance with the data from the specialized literature (IMBREA *et al*., 2016; ARCEUSZ *et al*., 2010).

The macroelements identified in the 18 *Thymus* populations studied, were the following: potassium, with an average value of 7,926 ± 4,198 g/kg, calcium, with an average value of 21,736 ± 13,384 g/kg and magnesium, with an average value of 4,372 ± 1,394g/kg.

The individual values detected in the thyme populations studied, are shown in table 2, and the distribution diagram in the following figures: figure 2, figure 3 and figure 4.

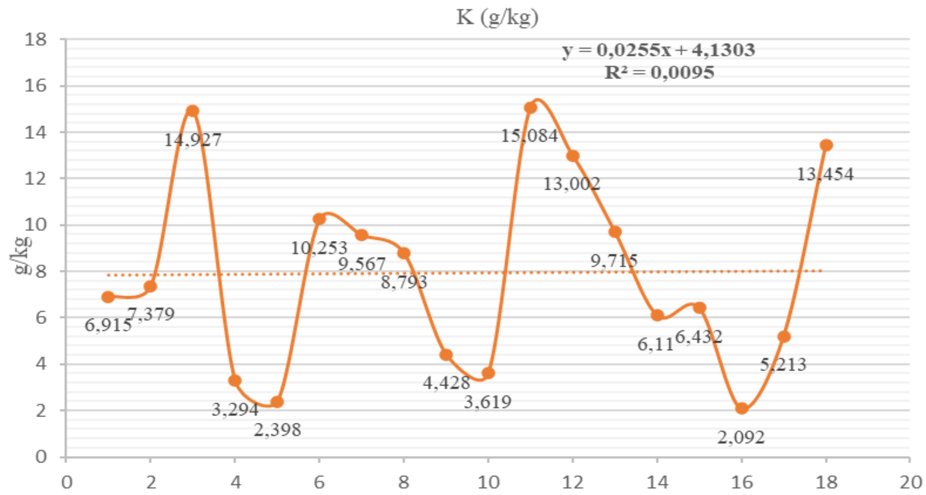

Figure 2. Distribution of potassium in the analyzed *Thymus* populations

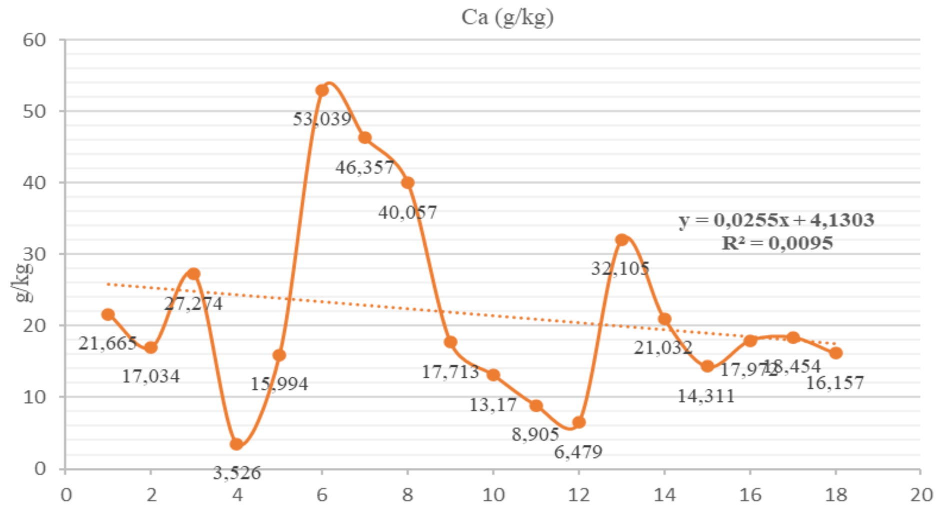

Figure 3. Distribution of calcium in the analyzed *Thymus* populations

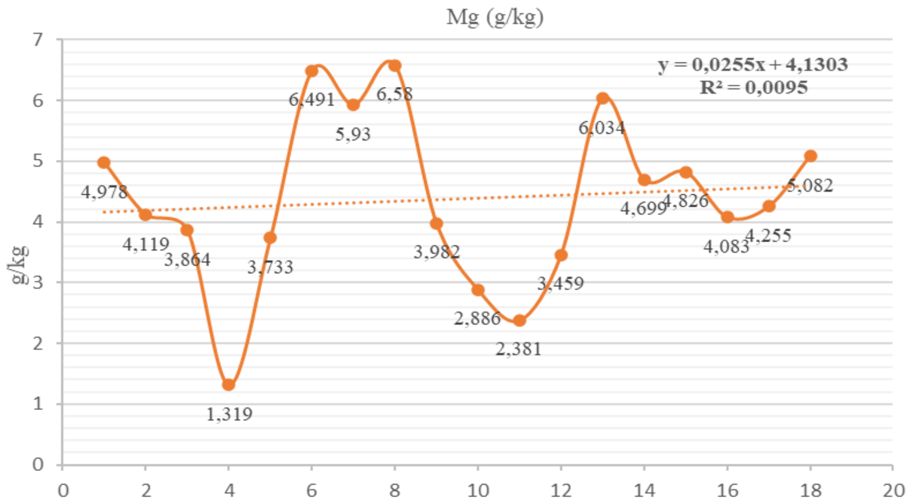

Figure 4. Distribution of magnesium in the analyzed *Thymus* populations

At the individual level, the maximum amount of potassium (Figure 2) was detected in the population of *Th. praecox ssp. polytrichus* from Semenic Peak (15.084 g/kg), and the minimum amount was detected in the population of *Th. pulegioides ssp. pulegioides* (2.092 g/kg) from the Carașova area. Elevation values of potassium, close to the maximum value, were recorded in the endemic species *Th comosus* collected from the Dobraia area (Iron Gate Natural Park). The potassium content values, are similar to the data obtained in other studies (IMBREA *et al*., 2016; KADIFKOVA-PANOVSKA *et al*., 1997; KUCUKBAY&KUYUMCU, 2010).

The maximum amount of calcium (Figure 3) was detected in the population of Th. dacicus from the Coronini area (53.039 g/kg), and the minimum amount was detected in the population of Th. praecox ssp. janke from the Domogled Cruce area (3.526 g/kg). In the specialized literature, the values reported for this macroelement vary around 18-20 g/kg.

Regarding the analysis of magnesium content (Figure 4), the maximum values were recorded in the population of *Th. dacicus* from the area of Lescovița (6.580 g/kg), followed by the population belonging to the same species *Th. dacicus*, from the Coronini area (6.491 g/kg). The minimum amount was detected for the population of *Th. praecox ssp. janke* from the Domogled area (1.319 g/kg). The results are similar to the data reported in the specialized literature for the genus *Thymus*.

CONCLUSIONS

The results of the study indicate variations in the recorded values, both according to the analyzed genotype and also according to the biotope conditions. The content of total mineral substances (ash), for the wild populations analyzed, varied between 1.19% in the population of *Th. pannonicus ssp. auctus* from the Silagiu area and 9.403% for *Th. pulegioides ssp. pulegioides* from the Nermet area. The highest value, was recorded for the cultivated population of *Th. vulgaris*, harvested from Timiș area (Lovrin) by 10.854%.

The potassium content varied widely, being between 2 g/kg and 15 g/kg, depending on the species and location. In the cultivated species, the potassium value was 13.454 g/kg, among the wild populations, only two exceeded this value, *Th. comosus* (Coronini) and *Th. praecox ssp. polytrichus* (Semenic).

Regarding the calcium content, we recorded variations between 3 g/kg and 53 g/kg, over 60% of the analyzed wild populations, exceeded the calcium content of the cultivated thyme. Also, significantly higher values were recorded, compared to those reported in the specialized literature for the genus *Thymus*. Regarding this aspect, three spontaneous populations, with a calcium content of over 40 g/kg were highlighted (*Th. dacicus* - Lescovița, *Th. praecox ssp. janke* - Coronini, *Th. dacicus* - Coronini). It should be noted that, within the species *Th. dacicus*, we observe two of the highest values, while the species from Ostrov records much lower values, similar to the cultivated population. Similar content variation, depending on the harvesting area, we also observe in the case of the population *Th. praecox ssp. janke*, the sample collected from Coronini was reaching a value close to the maximum content reported, while the sample from Domogled was determined as the minimum value.

The magnesium content values, were recorded between 1 g/kg and 6 g/kg, being close to the data reported in other studies. Less variation in the content of this macroelement is observed, both within the genus and also according to the location.

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