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ORIGINAL PAPER

IMPACT OF FOLIAR APPLICATION OF THE BIOSTIMULATOR Mg-TYTANIT ON THE FORMATION OF WINTER OILSEED RAPE PHYTOMASS AND TITANIUM CONTENT*

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Abstract

The objective of the following three-year small-plot experiments was to determine the impact of a dose and application date of the biostimulator Mg-Tytanit (MgTi) on the formation of winter oilseed rape phytomass and its titanium cont ent. In the trial the biostimulant Mg-Tytanit containing 8.5 g of titanium in 1 liter was used. The experiment consisted of 5 treatments: 0 – control treatment without MgTi; 2xTi0.2 – two applications of MgTi in the dose of 0.2 dm^3 ha^{-1}; 3xTi0.2 – three applications of MgTi in the dose of 0.2 dm^3 ha^{-1}; 2xTi0.4 – two applications of MgTi in the dose of 0.4 dm^3 ha^{-1}; 3xTi0.4 – three applications of MgTi in the dose of 0.4 dm^3 ha^{-1}. The BS was applied in spring during two or three different growth stages: BBCH 50, BBCH 59, BBCH 66. The first plant sampling was carried out shortly before the first application of BS (BBCH 50). The second, third and fourth sampling were taken 2 – 3 weeks after the application of Mg-Tytanit (BBCH 59, BBCH 66, BBCH 71). The results showed that the biostimulator MgTi, regardless of its dose and application date, resulted in a higher weight of the aerial and underground phytomass. All the MgTi applications had impact on the winter oilseed rape yield increase. The seed yield was increased by 0.3 to 0.63 t ha^{-1}. Higher yields were achieved in the treatments where MgTi was applied three times in comparison with the treatments, where it was used twice. The oil content in rape seeds was increased significantly only if MgTi was used in the total doses 0.8 and 1.2 dm^3 ha^{-1} in the single application dose 0.4 dm^3 ha^{-1}. The oil content

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value was increased by 0.94 % and by 0.82 %. The oil production per hectare was increased after each use of MgTi, i.e. regardless of the dose and date of its application. The highest Ti content in the aerial phytomass was 68.5 mg kg^{-1} and in the underground phytomass it was 247.1 mg kg^{-1}.

Keywords: foliar fertilizing, biostimulant, titanium, Mg-Tytanit, winter rape.

INTRODUCTION

The first congress dealing with the plant biostimulators, which was held in Strasbourg in 2013, defined biostimulators to be the substances able to stimulate either phytomass formation or phytomass and contained substances at the same time, or possibly only the substances contained in the phytomass. Biostimulators were also defined as the substances which have the ability to stimulate the defence potential of a plant organism in a nonspecific way, and therefore they help it to adapt to the changing environment and stress better and more promptly. Since the aforementioned congress, biostimulators have been considered to be different not only from organic compounds (Pulkrábek 1995, Šimanský 2010, Sayara et al. 2011, Tejada et al. 2013), but also from plant nutrients or any elements which application stimulates some part of a plant.

Mg-Tytanit is a biostimulator containing *inter alia* 8.5 g Ti in 1 dm^3 of preparation. Its aplication in the experiments of Wysocka-Owczarek (2001), Borkowski, Dyki (2003), Dobromilska (2007) decreased the plant's sensitivity towards stress conditions in the growing environment while increaseing the height of tomato plants, thickness of the stalk, number of leaves and fruit yield. This preparation also restricted the mould development of the plants grown in a greenhouse. In the study of Markiewicz and Kleiber (2014), a significant effect of Tytanit on the biological value of tomato fruits was observed. The lycopene content was the highest in the case of 80 g ha^{-1} Ti, whereas the dose 960 g ha^{-1} Ti induced a significant increase in total acidity of fruits. The nitrate content did not differ significantly. In the experiments of Alcaraz-Lopez et al. (2003), Tlustoš et al. (2005), the leaf nutrition by titanium increased the uptake of some macroelements and microelements and it also contributed to the improved fixation of air nitrogen by fabaceous plants (Ram et al. 1983). Unlike the above positive impacts of the preparations containing titanium on the plant yield parametres, Skawiński et al. (2012) registered in some cases a decrease in grain yield and straw yield of winter wheat after the Mg-Tytanit application. Similarly, Kováčik et al. (2014c) recorded a decrease in the total chlorophyll in wheat leaves in most instances after the Mg-Tytanit application in the growth phase BBCH 55. The negative effects of fertilizers containing titanium are also reported by Kužel et al. (2007). They claim that the unwanted impacts of titanium occured more frequently in soils where Mg content is insufficient, pH is lower than 6 and C_{ox} content is lower than 0.7%.

The plant sensitivity to titanium is different among individual crops. Wallace et al. (1977) recorded chlorotic and necrotic spots on bean leaves when the Ti content in leaves achieved the concentration of about 200 mg kg^{-1}. The damage of cabbage plants was registered when the concentration of titanium was higher than 4 mg kg^{-1} in the inner leaves and 3,000 $mg.kg^{-1}$ higher in roots (Hara et al. 1976). The toxic impacts of titanium on oat plants were evident when the Ti concentration in sprays was higher than 10 mg kg^{-1}, or 12.5 mg dm^{-3} (Kužel et al. 2007). The different sensitivity of crops to the use of substances containing titanium is related to the different tolerance of plants to titanium, or the different requirements of titanium uptake. The titanium content is low with the majority of crops and it usually varies in the interval of 0.1 - 10.0 mg kg^{-1} of dry matter (Tlustoš et al. 2005), or it can even reach about 100 mg kg^{-1} (Czekalski 1987). The titanium content is twice or even 6 times as high in the plant roots as in the aerial phytomass (Kováčik et al. 2016), or 70 even 700 times as high in the latter (Hara et al. 1976).

Despite the above reports on the negative effects of the application of titanium containing substances, it is still valid to say that there is less information about phyotoxic impacts of foliar application than about the positive effects.

The titanium nutrition follows the same principles as the nutrition by any other element. Reasonable doses, applied in accordance with the agrochemical rules, have a positive effect. Overdoses or erroneous application or incorrect combination with other fertilizers can have a negative impact (Kováčik 2014).

The objective of the paper is to evaluate the impact of two doses of the biostimulant Mg-Tytanit (0.2 and 0.4 dm^3 ha^{-1}) which were applied two or three times on winter oilseed rape leaves on: dynamics of the underground and aerial phytomass formation, dynamics of the changes of content of total chlorophyll in leaves, of the content of titanium in the underground and aerial phytomass, quantity and quality of winter rape yield.

MATERIAL AND METHODS

The effect of the application of the biostimulant Mg-Tytanit (MgTi) on the creation of aerial and underground phytomass, total chlorophyll content and content of titanium in plants of winter oilseed rape (cv. Chagal) was investigated in a small-plot field trial (20 m^2 per one plot) perfromed on Haplic Chernozem (48°42′ N, 17°70′ E – western Slovakia, near the village Bučany) during three farming years (2009/2010, 2010/2011 and 2011/2012).

The agrochemical parameters of Haplic Chernozem taken from the soil layers 0.0 - 0.3 m and 0.3 - 0.6 m are given in Table 1. They were deter-

Table 1

Soil agrochemical parameters before the foundation of the experiment

Year	Depth (m)	N_{in}	P	K	Ca	Mg	S	$N_{tot.}$	$Ti_{tot.}$	C_{ox}	pH_{KCl}
		(mg kg^{-1})								(%)	
2009/ 2010	0.0 - 0.3	17.00	58.0	250	2,600	415	14.5	1,766	4,305	1.64	6.80
	0.3 - 0.6	7.10	21.0	191	4,550	413	15.5	1,651	4,186	1.34	6.98
2010/ 2011	0.0 - 0.3	15.40	38.0	210	3,850	495	23.8	1,360	4,167	1.60	6.92
	0.3 - 0.6	10.70	8.0	110	11,450	515	45.1	838	3,953	0.90	6.98
2011/ 2012	0.0 - 0.3	26.50	76.3	218	2,300	468	11.9	1,596	4,435	1.65	6.85
	0.3 - 0.6	13.20	20.6	150	6,525	480	7.5	945	4,172	1.11	6.95
Mean	0.0 - 0.3	19.63	57.4	226	2,917	459	16.7	1,563	4,302	1.63	6.86
	0.3 - 0.6	10.33	16.5	150	7,508	469	22.7	1,145	4,104	1.12	6.97

N_{in} – inorganic nitrogen; P, K, Ca, Mg – available phosphorous, potassium, calcium, magnesium; S – water soluble sulphur, $N_{tot.}$ – total nitrogen, $Ti_{tot.}$ – total titanium, C_{ox} – total (oxidizable) carbon

mined by the following methods: N_{in} = N-NH_4^+ + N-NO_3^-, N-NH_4^+ – colorimertically with the Nessler's agent, N-NO_3^- – colorimertically with phenol 2.4 disulphonic acid, P – colorimertically (Mehlich 3 extract – Mehlich 1984), K and Ca – flame photometry (Mehlich 3 extract), Mg – AAS (Mehlich extract), S – spectrometrically ICP OES (water extract – Zbíral et al. 2011), C_{ox} – oxidometrically with the Ťjurin method modified by Nikitin (Dziadowiec, Gonet 1999), N_t – by distilling (Kjeldahl – Bremner 1960), Ti_t – spectrometrically (mixture of HF + $HClO_4$ – Doležal et al. 1966), pH_{KCl} – potentiometrically (1.0 mol dm^{-3} KCl).

The biostimulant used in the trial was Mg-Tytanit (MgTi) containing 8.5 g dm^{-3} Ti. Mg-Tytanit is dark brown liquid with bulk density of 1.36 kg dm^{-3}. Titanium is in the form of titanium ascorbate, whereas sulfur (4%) and magnesium (3%) contained in the biostimulant are in the form of magnesium sulphate ($MgSO_4$).

The winter oilseed rape, cultivar Chagal, was sown in the density of 50 individuals per m^2 in the last ten days of August every year. In spring, during the sampling of plant material, there was 29 individuals per 1 square meter (average of three years).

The experiment consisted of 5 treatments (Table 2). The samplings of the plant material (aerial and underground oilseed rape phytomass) were carried out in the growth stages BBCH 50 - 52, BBCH 59, BBCH 66 - 67, BBCH 71 (Table 3). After each sampling of the plant material, apart from the last sampling, the application by Mg-Tytanit was used on the same day. Beside the first sampling, the other samplings of the plant material were carried out 2 or 3 weeks after the Mg-Tytanit application. Twenty specimens in the first sampling, 15 in the second sampling and 8 in the the third and fourth sampling were taken from each small field.

Table 2

Treatments of the experiment

Treatment		Growth phase			Total application dosage of Mg-Tytanit	Total dosage of Ti
		BBCH 50-52	BBCH 59	BBCH 66-67		
Number	marking	Mg-Tytanit application dosage (dm^3 ha^{-1})			(dm^3 ha^{-1})	(g ha^{-1})
1	0	0	0	0	0	0
2	2 x $Ti_{0.2}$	0.2	0.2	0	0.4	3.4
3	3 x $Ti_{0.2}$	0.2	0.2	0.2	0.6	5.1
4	2 x $Ti_{0.4}$	0.4	0.4	0	0.8	6.8
5	3 x $Ti_{0.4}$	0.4	0.4	0.4	1.2	10.2

Table 3

Growth stages at sampling of winter oilseed rape and application of the stimulator Mg-Tytanit

Type of treatment			
1st sampling and subsequent 1st Ti spray	2nd sampling and subsequent 2nd Ti spray	3rd sampling and subsequent 3nd Ti spray	4th sampling
growth phase			
From occurrence of the inflorescence to inflorescence BBCH 50 - 52	yellow bud BBCH 59	flowering BBCH 66 - 67	from end of flowering to start of green maturity BBCH 71 - 75

To determine the content of the assimilation pigments (chlorophyll *a*, chlorophyll *b* and total chlorophyll) the last fully developed healthy leaves of ten plants were sampled. The pigments were determined in the acetonic extract by the spectrophotometric method using the equations of LICHTENTHALER (1987).

In order to determine the Ti content in the aerial and underground phytomass during the winter oilsseed rape growing season and in seeds and straw of winter oilseed rape, the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method was used (YANG et al., 2012). The titanium consumption by the green winter oilseed rape phytomass, seeds and straw was calculated from the data of its weight, titanium content and the number of individuals per 1 square metre (29 individuals per m^2).

The acquired results were processed by mathematical and statistical methods, using analysis of variance (ANOVA) and linear regression analysis in the Statgraphics PC programme, version 5.0.

RESULTS AND DISCUSSION

Table 1 shows that in the total titanium content Haplic Chernozem in which the experiment was carried out was 2.7 even 3.6 times as high as the total nitrgen content, and varied in the interval of 3.953 - 4.305 mg kg^{-1}. The above data are comparable with the parametres quoted for the countries of Central Africa (3.000 - 10.000 mg kg^{-1}) and they are approximately twice as high as the average titanium content in soils of Poland, which is the northern neighbour of Slovakia (Kabata-Pendias, Pendias 1993). A higher content of the total titanium in the tested Haplic Chernozem (Slovakia) in comparison with the soils in Poland is related to a lower presence of titanium in sandy soils and a higher presence in the clayey parts of soil (Czarnowska 1965, Borowiec et al. 1977). In Slovakia, sandy soils comprise only 1.6% of the agricultural stock of land, while in Poland they constitute even 25% of farmland (Fulajtár 2006).

In the topsoil (0.0 - 0.3 m) of the used soil, the content of total titanium was on average by 200 mg kg^{-1} higher than in the subsoil (0.3 - 0.6 m), which confirms the data suggesting that the titanium content increases along with the degree of the weathering of minerals (Lupinovich 1965).

The data given in Table 4 show that all three applications of Mg-Tytanit, carried out in the growth phases BBCH 50 - 52, 59 and 66 - 67, stimulated

Table 4

Impact of dose and number of applications of the biostimulator Mg-Tytanit on dynamics of aerial and underground phytomass formation of winter rape (average of three years)

Treatment		Growth stage (BBCH)						
		50 - 52	59	66 - 67	71 - 75	59	66 - 67	71 - 75
No.	marking	weight of one plant at gram (100% d.m.)				(%)		
Aerial phytomass								
1	0	3.47	7.11	15.10	29.01 a [a]	100.0	100.0	100.0
2	2 x $Ti_{0,2}$	3.47	8.57	23.28	42.01 b [b]	120.5	154.1	144.8
3	3 x $Ti_{0,2}$	3.47	8.57	23.28	43.39 b [b]	120.5	154.1	149.6
4	2 x $Ti_{0,4}$	3.47	9.03	21.56	41.55 b [b]	127.0	142.7	143.2
5	3 x $Ti_{0,4}$	3.47	9.03	21.56	44.10 b [b]	127.0	142.7	152.0
$LSD_{0.05}$					5.477			
Undeground phytomass								
1	0	1.49	2.33	2.77	3.89 a [a]	100.0	100.0	100.0
2	2 x $Ti_{0,2}$	1.49	2.72	3.95	5.31 b [b]	116.8	142.6	136.3
3	3 x $Ti_{0,2}$	1.49	2.72	3.95	5.35 b [b]	116.8	142.6	137.4
4	2 x $Ti_{0,4}$	1.49	2.93	4.22	5.15 b [b]	126.1	152.5	132.4
5	3 x $Ti_{0,4}$	1.49	2.93	4.22	5.37 b [b]	126.1	152.5	137.9
$LSD_{0.05}$					0.438			

$LSD_{0.05}$ – limit of significant differences at the level $\alpha = 0.05$ (LSD test), different letter superscript corresponds to a statistically significant difference at the level 95.0%

the aerial and underground formation of winter oilseed rape phytomass. The stimulation of the aerial and underground phytomass was not identical, however, it was comparable. There were slightly higher effects detected in percents with the aerial phytomass than with the underground phytomass. After the first application the weight of the aerial phytomass was increased by 20.5 even 27.0% and roots by 16.8 even 26.1%. The second application resulted in the phytomass growth by 15.7% to 33.6% (aerial mass), therefore the difference in comparison with the control after two applications of Mg -Tytanit was 42.7% to 54.1%. The growth of the underground phytomass after the second spraying was 25.8% to 26.3%, therefore the difference in comparison with the control after two applications of Mg-Tytanit achieved 42.6 to 52.5%. The impact of the third spraying on the aerial and underground phytomass was the weakest and it was positive albeit insignificant (tr. 2 versus tr. 3 and tr. 4 versus tr. 5), which does not coincide with the findings of Kováčik et al. (2014a). In their experiments the third spraying by Mg-Tytanit, which was applied in the growth phase BBCH 55, inhibited the formation of aerial phytomass of winter wheat and in some cases resulted in a lower grain yield.

A higher simple dose of Mg-Tytanit (0.4 dm^3 ha^{-1}) always stimulated the root formation more positively than a lower one (0.2 dm^3 ha^{-1}). The aerial phytomass formation was stimulated by the higher simple dose (0.4 dm^3 ha^{-1}) of Mg-Tytanit in the first and third spraying more considerably than the lower one (0.2 dm^3 ha^{-1}).

In the growth phase BBCH 71 - 75, differences in the weight between the oilseed rape phytomass in the treatments with Mg-Tytanit versus the control, not treated with Mg-Tytanit, were highly significant. However, differences in the weight of both aerial and underground phytomasses between the treatments with Mg-Tytanit were insignificant. It is clear that in order to increase significantly the weight of oilseed rape phytomass in the growth phase BBCH 71 - 75, it is unimportant whether a dose of Mg-Tytanit is 0.2 or 0.4 dm^3 ha^{-1} as long as the applications are carried out in the growth phases BBCH 50 - 52 and BBCH 59 or also in BBCH 66 - 67, i.e. if a dose is split into tow or three sprays.

Apart from the second spraying of the dose 0.4 dm^3 ha^{-1} Mg-Tytanit (phytomass measurement in BBCH 66 - 67), all the other applications of the stimulator expanded the ratio between the aerial and underground phytomass, which confirms a more positive effect of Mg-Tytanit on the rape green parts as well as roots (Table 5).

One of the first signs of the excess plant nutrition by titanium is the decreased content of total chlorophyll (Carvajal, Alcaraz 1998, Hrubý et al. 2002). The quantity considered to be a rational and irrational dose of titanium varies depending on the farming conditions and different crops, which encourages constant research into optimal dates and application doses of the biostimulator Mg-Tytanit (Dobromilska 2007, Kováčik et al. 2014*b*).

Table 5

Impact of dose and number of applications of the biostimulator Mg-Tytanit on the dynamics of weight ratio changes of aerial to underground rape phytomass (average of three years)

Treatment		Growth stage (BBCH)			
		50 - 52	59	66 - 67	71 - 75
No.	marking	aerial phytomass : underground phytomass			
1	0	2.33	3.06	5.45	7.45
2	2 x $Ti_{0,2}$	2.33	3.15	5.89	7.91
3	3 x $Ti_{0,2}$	2.33	3.15	5.89	8.09
4	2 x $Ti_{0,4}$	2.33	3.08	5.10	8.06
5	3 x $Ti_{0,4}$	2.33	3.08	5.10	8.25

Table 6

Impact of trial treatments on dynamics of changes of pigment content in leaves of winter oilseed rape (average of three years)

Treatment		Growth stage	Chlor. *a*	Chlor. *b*	Chlor. *a* + *b*		Ratio *a*/*b*
No.	marking		(mg m^{-2})			(%)	
1	0	BBCH 59	303.6*a*	117.3*a*	420.9*a*	100.0	2.59
2 - 3	$Ti_{0,2}$		312.4*a*	115.6*a*	428.0*a*	101.7	2.70
4 - 5	$Ti_{0,4}$		311.7*a*	116.5*a*	428.2*a*	101.7	2.68
$LSD_{0.05}$			12.6	4.7	10.2		
1	0	BBCH 66 - 67	297.9*a*	127.0*a*	424.9*a*	100.0	2.35
2 - 3	$Ti_{0,2}$		307. 9*a*	152.5*b*	460.3*b*	108.3	2.02
4 - 5	$Ti_{0,4}$		307.1*a*	146.8*b*	453.8*b*	106.8	2.03
$LSD_{0.05}$			11.9	8. 8	15.4		
1	0	BBCH 71 - 75	257.8*a*	124.1*a*	381.9*a*	100.0	2.08
2	2 x $Ti_{0,2}$		304.1*c*	147.3*b*	451.4*cd*	118.2	2.07
3	3 x $Ti_{0,2}$		289.6*b*	145.8*b*	435.4*b*	118.7	1.99
4	2 x $Ti_{0,4}$		302.8*c*	153.8*b*	456.6*d*	119.5	1.97
5	3 x $Ti_{0,4}$		289.6*b*	150.0*b*	439.6*bc*	115.1	1.93
$LSD_{0.05}$			12.0	9.3	15. 9		

Chlor. – chlorophyll, 2 - 3 = mean of treatments number 2 and 3, 4 - 5 = mean of treatments number 4 and 5, $LSD_{0.05}$ – key under Table 4

Table 6 shows that the application of Mg-Tytanit in the doses of 0.2 and 0.4 dm^3 ha^{-1} in the growth phase BBCH 50 - 52 (analysis in the growth phase BBCH 59) had no significant impact on either the content of chlorophyll *a*, chlorophyll *b* or chlorophyll *a*+*b*. The second spraying, applied in the growth phase BBCH 59 (analysis in the growth phase BBCH 66 - 67), resulted in a more distinct content of the monitored pigments than the first spraying. It had a positive effect on the content of chlorophyll *b* and total chlorophylls (*a*+*b*). The third spraying (tr. 3 versus tr. 2 and tr. 5 versus tr. 4) decreased significantly the chlorophyll *a* content and consequently also the total chlorophyll (*a*+*b*). It has a slight negative impact on the chlorophyll *b* content. These facts are in accord with the data of VICIAN et al. (2012) and KOVÁČIK et al. (2014c), claiming that in winter wheat after the third spraying with Mg-Tytanit, which was applied in the growth phase BBCH 59 - 60, in most cases there was a negative impact on the content of total chlorophyll in wheat leaves.

Despite the well-known fact that the formation of phytomass notably depends on the efficiency of the photosynthetic apparatus, quality and quantity of the pigmental system, quantity of chlorophyll *a*, *b* and total chlorophyll (BAKER 1996), for reliable forecast of the quantity of main product yield, it is important in which phase the analysis of total chloropyll is carried out (KOVÁČIK et al. 2010). It is also essential if the prediction contains data of total chlorophyll or chlorophyll *a* or *b*. Table 7 shows that there was not any positive relationship between the content of chlorophyll *a* and the aerial and

Table 7

Correlation coefficient *r* expressing the relationship between the oilseed rape phytomass and the pigments occurring in rape leaves in particular growth phases (average of three years)

Parameter			Correlation coefficient (*r*)	*n*	*P* - value
Dependent		independent			
Weight of aerial phytomass	v BBCH 59	total chlorophyll	0.9796		0.1300
		chlorophyll *a*	0.9552	12	0.1917
		chlorophyll *b*	- 0.6968		0.5091
	v BBCH 66 - 67	total chlorophyll	0.9996*		0.0179
		chlorophyll *a*	0.9917	36	0.0818
		chlorophyll *b*	0.9999**		0.0084
	v BBCH 71 - 75	total chlorophyll	0.9015*		0.0365
		chlorophyll *a*	0.8571	60	0.0624
		chlorophyll *b*	0.9347*		0.0198
Weight of undeground phytomass	v BBCH 59	total chlorophyll	0.9458		0.2310
		chlorophyll *a*	0.9100	12	0.2720
		chlorophyll *b*	-0.6010		0.5895
	v BBCH 66 - 67	total chlorophyll	0.9392		0.2229
		chlorophyll *a*	0.9689	36	0.1590
		chlorophyll *b*	0.9242		0.2494
	v BBCH 71 - 75	total chlorophyll	0.9107*		0.0315
		chlorophyll *a*	0.8765	60	0.0510
		chlorophyll *b*	0.9272*		0.0232

* statistically significant (at the level 95.0%), ** statistically highly significant (at the level 99.0%)

underground phytomass formation in any growth phase. More significant relations were detected between chlorophyll *b* or total chlorophyll and oilseedd rape aerial phytomass. In the growth phase BBCH 59, no significant relation not determined between the particular monitored pigments and the oilseed rape phytomass formation. On the contrary, in the growth phases BBCH 66 - 67 and BBCH 71 - 75, significant or even highly significant relationships were detected between the content of chlorophyll *b* and the oilseed rape aerial phytomass. The validity of these data implicating a higher dependence between the content of chlorophyll *b* and phytomass than between the content of chlorophyll *a* and phytomass, will require further verification in trials on other crops and in different growth phases.

The titanium content in rape aerial and underground phytomass were falling during the whole monitored period (from growth phase BBCH 50 - 52 to growth phase 71 - 75), see Table 8. The highest titanium contents 68.5 mg kg^{-1}

Table 8

Dynamics of changes in the titanium content in winter oilseed rape plants and uptake of titanium during the growing season (average of three years)

Treatment		Growth stage	Aerial phytomass			Undeground phytomass		
No.	marking		content Ti	uptake Ti		content Ti	uptake Ti	
		BBCH	(mg kg^{-1})	(mg $plant^{-1}$)	(g ha^{-1})	(mg kg^{-1})	(mg $plant^{-1}$)	(g ha^{-1})
1-5	0Ti	50-52	68.50	0.24	69.60	247.1	0.37	107.3
1	0	59	45.04*a*	0.32	92.8*a*	202.8*a*	0.47	136.3*a*
2-3	$Ti_{0,2}$		53.60*b*	0.46	133.4*b*	210.5*b*	0.57	165.3*b*
4-5	$Ti_{0,4}$		62.86*c*	0.57	165.3*c*	198.6*a*	0.58	168.2*b*
Mean			53.83	0.45	130.50	204.0	0.54	156.6
$LSD_{0.05}$			5.709		15.96	4.7		5.4
1	0	66-67	31.41*b*	0.47	136.3*a*	173.8*a*	0.48	139.2*a*
2-3	$Ti_{0,2}$		28.17*a*	0.66	191.4*b*	185.0*b*	0.73	211.7*b*
4-5	$Ti_{0,4}$		30.70*b*	0.66	191.4*b*	182.5*b*	0.77	223.3*c*
Mean			30.09	0.60	173.0	180.4	0.66	191.4
$LSD_{0.05}$			1.20		15.79	2.9		11.0
$LSD_{0.01}$			1.63		19.95	4.3		13.7
1	0Ti	71-75	20.60*a*	0.60	174.0*a*	161.8*b*	0.63	182.7*a*
2	$2xTi_{0,2}$		22.24*ab*	0.93	269.7*b*	159.4*ab*	0.85	246.5*b*
3	$3xTi_{0,2}$		22.96*b*	1.00	290.0*cd*	157.0*ab*	0.84	243.6*b*
4	$2xTi_{0,4}$		23.10*b*	0.96	278.4*bc*	157.0*ab*	0.81	234.9*b*
5	$3xTi_{0,4}$		23.30*b*	1.03	298.7*d*	156.9*a*	0.84	243.6*b*
Mean			22.44	0.90	262.2	158.4	0.79	230.3
$LSD_{0.05}$			1.88		14.3	4.7		12.7

0Ti = 1-5 – mean of all treatments before application of Mg-Ti, $LSD_{0.05}$ – key under Table 4 (aerial phytomass) and 247.09 mg kg^{-1} (underground phytomass) were detected in the growth phase BBCH 50 - 52. The lowest titanium contents 20.6 mg kg^{-1} in the aerial phytomass and 156.88 mg kg^{-1} in underground phytomass were registered in the growth phase BBCH 50 - 52. These data correspond with the information that under the conditions of temperate climate the content of nutrients is being decreased (IVANIČ et al. 1984) with the majority of annual winter crops and spring crops in the period from February till the technological ripeness.

In the growth phases BBCH 50 - 71 the titanium contents varied around the recorded average values in the rape aerial phyotomass. It was around 68.50 mg kg^{-1} in the growth phase BBCH 50, 53.83 mg kg^{-1} in BBCH 59, 30.09 mg kg^{-1} in BBCH 66 - 67 and 22.44 mg kg^{-1} in BBCH 71 - 75 (Table 8). These data can serve as the basic information for the creation of criteria about the sufficient titanium content in rape plants in the growth phases BBCH 50 - 71. It is well-known that the realization of the corrective measures of the insufficient nutrition by microelements in the growth phases BBCH 50 - 59 is very effective (JAKIENĖ 2013).

After the first application of Mg-Tytanit there was detected the increased titanium content in the plants of winter rape (tr. 2 to 5 versus tr. 1) in both years of experiment. Paradoxally enough, the titanium content was increased more significantly in plant roots with the treatments in which a lower dose of Mg-Tytanit was applied (tr. 2 and 3), in comparison with the treatments where a higher dose of Mg-Tytanit was applied (tr. 4 and 5). The effect of the second spraying by Mg-Tytanit (applied in the growth phase BBCH 59) on the titanium content in the rape plant did not correspond with the effect of the first spraying (carried out in the growth phase BBCH 50). The second spraying caused that the titanium contents in the aerial phytomass in the growth phase BBCH 66 of the plants treated by Mg-Tytanit were lower than the contents of the untreated treatment. The impact of the third spraying (tr. 3 versus tr. 2 and tr. 5 versus tr. 4) was similarly ambiguous like the impact of the first two sprayings. These facts show that in the given experiment in the period from the growth phase BBCH 50 to 71 the unambiguous impact of the application by the fertilizer with titanium content was not detected on the titanium content in the aerial and underground rape phytomass. The assumed reasons of this finding are the relatively low quantities of titanium supplied to plants by the BS Mg-Tytanit. The particular treatments were supplied by this biostimulant from 3.4 to 10.2 g of titanium per hectar of soil, which is less than 0.5 mg per a plant (Table 2).

In spite of the considerable differences in the titanium contents in roots and the aerial mass, the titanium uptakes by the aerial and underground phytomass were comparable (Table 8). In the growth phase BBCH 71 - 75 the average uptake by the green mass was 262.2 g ha^{-1} Ti and by roots 230.3 g ha^{-1} Ti. The whole rape phytomass uptake was on average 492.4 g ha^{-1} Ti. These data show the relatively high biological titanium uptake by rape, which is at the level of microelements.

The titanium uptakes by the aerial and underground rape phytomass were being increased along with the following growth phases, and in each growth phase the lowest uptake was detected as statistically significant in the control treatment. The mutual differences of the titanium uptake by roots were insignificant between the treatments treated by Mg-Tytanit. It was detected that during the uptakes by aerial phytomass in the growth phase BBCH 71 - 75 the most quantity of titanium was uptaken by the treatments 5 and 3, i.e. the treatments in which Mg-Tytanit was applied three times (Tables 8 and 9). More uptaken titanium by the aerial phytomass in the treatment 3 than in the treatment 4 highlights the well-known fact that in the process of plant nutrition the usage of nutrients is higher if they are applied more frequently and in lower doses than less frequently and in higher doses.

Table 9

The ratio between Ti content in underground and aerial phytomass and titanium uptake by whole winter oilseed rape phytomass

Treatment		Growth stage	Real ratio	Ratio as men of growth stage	Ti uptake by whole phytomass
No.	marking				($g\ ha^{-1}$)
1-5	0Ti	BBCH 50	3.61 : 1	3.61 : 1	176.9
1	Ti	BBCH 59	4.50 : 1	3.86 : 1	229.1
2-3	$Ti_{0.2}$		3.93 : 1		298.7
4-5	$Ti_{0.4}$		3.16 : 1		333.5
1	Ti	BBCH 66 - 67	5.53 : 1	6.01 : 1	275.5
2-3	$Ti_{0.2}$		6.57 : 1		403.1
4-5	$Ti_{0.4}$		5.94 : 1		414.7
1	0	BBCH 71 - 75	7.86 : 1	7.08 : 1	356.7
2	2 x $Ti_{0.2}$		7.17 : 1		516.2
3	3 x $Ti_{0.2}$		6.84 : 1		533.6
4	2 x $Ti_{0.4}$		6.80 : 1		513.3
5	3 x $Ti_{0.4}$		6.73 : 1		542.3

0Ti = 1-5 – mean of all treatments before application of Mg-Ti, $LSD_{0.05}$ – key under Table 4

Along with the increasing growth phase the ratio between the titanium content in the roots and aerial phytomass rose, which is linked with more significant decrease of titanium content in the aerial phytomass in comparison with the decrease of titanium content in the underground phytomass related to the plant age (Table 9).

Each application of Mg-Tytanit, carried out in three different growth phases of rape, increased the titanium uptake by the aerial phytomass. The titanium uptake by the underground phytomass was increased only after the first and second application of the biostimulator Mg-Tytanit. The third spraying did not have impact on titanium uptake by roots (Table 8). The titanium uptake by the whole phytomass was increased after each application of the biostimulator. The growth of the uptaken quantity of titanium exceeded several times the quantity of titanium supplied by biostimulator Mg-Tytanit. This fact was the consequence of the above mentioned significant weight increase of the whole rape phytomass after the application of Mg-Tytanit.

The applications of the BS Mg-Tytanit in the total doses of 0.6, 0.8 and 1.2 dm^3 ha^{-1} increased statistically significantly the yield of oilseed rape seed (tr. 3, 4, 5 – Table 10). The increases varied in the interval 0.4 - 0.63 t ha^{-1},

Table 10

The impact of treatments on the yield parameters of winter oilseed rape (average of three years, 100% dry mater)

Treatment		Yield		Ratio	Oil	Oil production	TKW (95 % dry mater)
		seeds	straw	straw /seeds			
No.	marking	(t ha^{-1})			(%)	(t ha^{-1})	(g)
1	0	3.81*a*	6.62*a*	1.73	40.58*a*	1.55*a*	5.07*a*
2	2 x $Ti_{0,2}$	4.11*b*	6.48*a*	1.58	40.98*a*	1.68*b*	5.04*a*
3	3 x $Ti_{0,2}$	4.44*c*	7.07*b*	1.59	40.73*a*	1.81*b*	5.16*b*
4	2 x $Ti_{0,4}$	4.21*bc*	6.69*a*	1.59	41.52*b*	1.75*b*	5.06*a*
5	3 x $Ti_{0,4}$	4.30*bc*	6.74*a*	1.57	41.40*b*	1.78*b*	5.08*a*
Mean		4.17	6.52	1.61	41.04	1.72	5.08
$LSD_{0.05}$		0.27	0.31		0.41	0.10	0.06

TKW – thousand kernel weight, $LSD_{0.05}$ –key under Table 4

which meant a 10.5 to16.5% rise. The highest yield (4.4 t ha^{-1}) was achieved in treatment 3. The differences in seed yield between treatments 3, 4 and 5 were insignificant. Higher yields were achieved in the treatments where Mg-Tytanit was applied three times (tr. 3 and 5) than in the treatments where it was applied twice (tr. 2 and 4). In the treatments where Mg-Tytanit was applied three times, in the growth phase BBCH 72 - 75, the highest aerial and underground phytomass was recorded (Table 4). On the contrary, the higest content of total chlorophyll was determined in the treatments where Mg-Tytanit was applied twice (Table 8). These data are in accordance with the facts given by Kmeťová, Kováčik (2014), who found out a higher correlation coefficient between the weight of aerial phytomass and grain yield than between the content of total chlorophyll and grain yield in the experiments on maize.

In all the treatments including Mg-Tytanit, straw yield was increased, albeit insignificantly except for treatment 3. The recorded insignifcant impact of Mg-Tytanit on the oilseed rape straw formation is in contrast with the findings regarding the impact of this preparation on wheat straw (Kováčik et al. 2014*a*). A lower impact of Mg-Tytanit on oilseed rape straw than on its seeds is also confirmed by the fact that in all the treatments with Mg-Tytanit the ratio between the phytomass of straw and grain was lower than in the control treatment (Table 10).

The considerable increase of oil in oilseed rape seed after the application of Mg-Tytanit was detected only in treatments 4 and 5 (Table 11). In the given variants the oil content was higher by 0.9% and by 0.8%. The proportional growth of the oil content in comparison with the control was 2.3% and 2.0%. In these treatments, the highest total doses of Mg-Tytanit were applied (0.8 and 1.2 dm^3 ha^{-1}), or Mg-Tytanit was applied two or three times with the simple dose of 0.4 dm^3 ha^{-1}. The oil production per hectare was increased significantly after Mg-Tytanit application in all treatments. The increases varied in the interval 8.4 - 16.8%. Mg-Tytanit did not have a significant impact on the weight of one thousand of seeds, except for one treatment.

Table 11

The impact of treatments on the titanium content in seeds and straw and on titanium uptake by seeds and straw (average of three years)

Treatment		Content Ti		Uptake Ti			Uptake Ti by the yield of one tonne of seeds and the respective amount of straw
		seeds	straw	seeds	straw	together	
No.	marking	(mg kg^{-1})		(g ha^{-1})			(g t^{-1})
1	0	1.23*a*	7.86*a*	4.69*a*	52.00*a*	56.70	14.88
2	2 x $Ti_{0,2}$	1.58*b*	9.82*b*	6.50*b*	63.65*b*	70.15	17.07
3	3 x $Ti_{0,2}$	1.69*bc*	11.27*d*	7.46*bc*	79.64*d*	87.20	19.64
4	2 x $Ti_{0,4}$	1.99*cd*	11.09*cd*	8.38*cd*	74.19*c*	82.67	19.64
5	3 x $Ti_{0,4}$	2.13*d*	10.76*c*	9.15*d*	72.56*c*	81.86	19.04
Mean		1.72	10.16	7.31	68.41	75.72	18.05
$LSD_{0.05}$		0.32	0.35	1.64	3.47		

$LSD_{0.05}$ – key under Table 4

The titanium content in oilseed rape seeds as a three-year average of all treatments achieved the level of 1.72 mg kg^{-1}. Straw contained on averge 5.91-fold more titanium than seeds. The titanium uptake by oilseed rape seeds did not exceed 10 g ha^{-1}. The highest recorded Ti uptake by straw was 79.64 g ha^{-1}. These figures show that the yield of one tonne of rape seeds and straw takes up on average around 18 grams of titanium (Table 11).

At the average yield of 4.17 t of seeds (Table 10), 75.72 g of titanium from field (uptake by agricultural yield) was taken up by seeds and straw in total. At the given yield 4.17 t ha^{-1} of seeds, the biological uptake of titanium by the aerial phytomass was on the level of 262.16 g (Table 8), which is about 3.5-fold more than the uptake by agricultural yield. Similarly, the biological uptake was 3.5-fold higher than the uptake of potassium by agricultural yield of winter wheat (IVANIČ et al. 1984).

CONCLUSIONS

The use of the biostimulator Mg-Tytanit resulted in a higher weight of the aerial and underground phytomass, independently of the dose and date of its application. Two or three weeks after the last application of Mg-Tytanit, differences in the weight of phytomass between the treatments with Mg -Tytanit were insignificant.

The effect of the Mg-Tytanit application on the content of total chlorophyll in oilseed rape leaves was dependent on the date of the application. The positive impact was detected only if the application was carried out in the growth phase BBCH 59.

In the growth phases BBCH 66-67 and BBCH 71-75, statistically significant positive relations were determined between the content of chlorophyll *b* and the aerial oilseed rape phytomass. The relations between the content of chlorophyll *a* and the aerial phytomass were insignificant.

All the tested applications of Mg-Tytanit resulted in an increased yield of oilseed rape seeds. Higher yields were achieved in the treatments where Mg-Tytanit was applied three times than in the treatments where it was used twice. The oil content in rape seeds was increased considerably only if Mg-Tytanit was used in the total doses 0.8 and 1.2 dm^3 ha^{-1} with the simple application dose 0.4 dm^3 ha^{-1}. The oil production per hectare was increased after each application of the biostimulator Mg-Tytanit, i.e. independently of the date and dose of its application.

In the period from the growth phase BBCH 50 to growth phase BBCH 71, no unambiguous impact of the application of Mg-Tytanit on the titanium content in the aerial and underground phytomass was detected. The data about the titanium content in the aerial oilseed rape phytomass can serve as the basic data for the creation of criteria serving an evaluation of the titanium content in oilseed rape plants. The titanium content in the oilseed rape phytomass decreased from the growth phase BBCH 50 to growth phase 75. The highest titanium content in aerial phytomass was 68.5 mg kg^{-1} and in underground phytomass it equlled 247.09 mg kg^{-1}.

In oilseed rape seeds and straw, there was less titanium than in the oilseed rape organs active physiologically. The biological titanium uptake by oilseed rape achieved the values of the uptake of microelements. Winter oilseed rape plants take up about 18 g of titanium by the yield of one tonne of seeds and the respective amount of straw.

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