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VERMICOMPOST AND *EISENIA FOETIDA* AS FACTORS INFLUENCING THE FORMATION OF RADISH PHYTOMASS

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Many of the world's findings indicate the positive effect of earthworms on soil parameters, and consequently, on the formation of phytomass of cultivated plants. In our experiment we studied: A) the influence of soil itself, soil mixed with vermicompost in a ratio of 9:1; B) the influence of earthworms number (genus *Eisenia foetida*, 10 and 20 individuals per pot) supplied to soil mixed with vermicompost in the ratio of 9:1 on the dynamics of changes in the weight of radish roots, the total chlorophyll content in leaves and the selected qualitative parameters of the roots. The results obtained showed that one tenth proportion of vermicompost from the total weight of soil substrate caused the statistically significant increase in the total chlorophyll content in leaves, the increase in yield of radish roots, the reduction of the vitamin C content and the increase of nitrate content in the roots. The impact of earthworms on the chlorophyll content in leaves and on the root weight was negative. The addition of 10 individuals of earthworms into 20 kg of substrate (soil + vermicompost) resulted in the increased content of vitamin C and the decreased content of nitrates in the radish roots. Twenty earthworms added to vermicompost, compared to vermicompost alone, did not affect the vitamin C content and reduced the nitrate content.

Key words: earthworms, *Eisenia foetida*, radish, vermicompost, vitamin C

Despite the sharp increase in knowledge from the area of plant nutrition optimization and soil protection observed in the recent years, many data have only limited territorial applicability. For this reason, more attention should be paid to obtaining such information, which could be used in the largest possible agricultural area and effectiveness of it would be long-term.

From this aspect plant nutrition through vermicomposts enables biotechnologically and energetically undemanding utilization of by-products or waste products from a wide range of industrial and agricultural activities, which is one of the ways of increasing soil fertility, the use of which has assumptions of applicability on each agricultural land.

The product of composting with help of earthworms is a vermicompost. The vermicomposting is the utilization of the waste products by the technology of earthworm breeding, most frequently from the genus *Eisenia foetida*. The earthworms mix the predigested organic matters with soil in the alimentary tract. They create relatively waterproof aggregates excreted in the form of cylinders that have the positive impact on the soil parameters (Albanell *et al.*

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1988; Jouquet *et al*. 2010). The usage of vermicompost affects the firmness of cells of cultivated plants that are then more resistant to pests (Sinha & Valani 2011). The use of vermicomposts increases the content of total antioxidants, carotenes, lycopene, carbohydrates, vitamin C, proteins, dry matter, iron and zinc in cultivated plants (Gutiérrez-Micely *et al*. 2007; Shankar *et al*. 2009; Sinha *et al*. 2011; Ghosh *et al*. 2013).

The vermicompost production has been recognized for several decades (Chan & Griffiths 1988; Datar *et al*. 1997; Jeyabal & Kuppuswamy 2001). In spite of that, its production will have a significant impact also in the future decades because the contemporary society produces more and more new types of wastes, which have to be involved ecologically and energetically into the matter circulation. Therefore, the research of the effective production of vermicomposts and their further usage is still continuing.

Earthworms in soil are bio-indicators of the soil environment and quality of soils (Smith *et al.* 2008; Boyer & Wratten 2010; Fazekašová & Bobuľská 2012). The activity of earthworms increases the content of available nitrogen, phosphorus and potassium (Tripathi & Bhardwaj 2004; Garg *et al*. 2006; Doan *et al*. 2015), it also improves the soil structure (Adhikary 2012), but their impact on plants has not been examined sufficiently. Zero, positive and negative plant responses to the presence of earthworms were recorded (Eisenhauer *et al*. 2009; Doan *et al*. 2013; Nurhidayati *et al*. 2016). For this reason, the future research needs to be focused on testing the impact of individual species of earthworms on individual plant species.

The aim of our contribution is to give an answer to the question of how the application of the vermicompost without earthworms and with earthworms of the genus *Eisenia foetida* effect the quantity and quality of the radish yield.

MATERIAL AND METHODS

The pot experiment was carried out in the vegetation cage located in the area of the Slovak University of Agriculture in Nitra. The size of the cage was 20 m × 20 m × 5 m. On its sides and ceiling there was the metal mesh with the size of a mesh 15 mm × 15 mm, which protected the experiment against birds.

The experiment was established on March 13, 2017. Soil (20 kg – treatment 1) and soil with vermicompost (20 kg) mixed in the rate 9:1, i.e. 18 kg soil and 2 kg vermicompost (treatment 2, 3, 4) was put into the cylindrical pots of the height 35 cm and diameter 35 cm. Ten individuals of adult earthworms (*Eisenia foetida*) were placed to the pots of the treatment number 3, and twenty individuals of earthworms were introduced to the pot of the treatment 4. The used soil (Haplic Luvisol) was taken from the field located in Párovské Háje, (cadaster Nitra), in particular, from the upper horizon of soil 0.0–0.3 m. The weighed out pots were placed into the dishes, which were able to keep 1,000 ml of the leaked soil solution during the period of precipitation. The leaked through solution was returned back to the pots. The agrochemical parameters of the used soil and the soil mixed with vermicompost (VC) are indicated in the Table 1. We used the following analytical methods for the indication of the given parameters: N-NH_4^+ by Nessler's colorimetric method; N-NO_3^- by colorimetric method with phenol – 2,4 disulphonic acid, where the extract from

Table 1

Parameters of the soil substrates used in the experiment

Substrate	N_{min}	P	K	Ca	Mg	S	N_t	C_{ox}	C:N	EC [mS/cm]	pH_{KCl}
	[mg/kg]						[%]				
Soil	13.20	21.90	156	4,250	444	1.3	0.077	0.915	11.88	0.14	6.97
Soil + VC	91.65	87.78	3,925	4,270	966	938	0.367	4.908	13.37	1.23	6.99

VC – vermicompost

soil was achieved by using the water solution 1% K_2SO_4. N_{min}= N–NH_4^+ + N–NO_3^-. The contents of available P, K, Ca, Mg were determined by Mehlich 3 extraction procedure (Mehlich 1984). The content of P was determined by colorimetric method, K by flame photometry, Ca and Mg by atomic absorption spectrophotometry, S spectrophotometrically (in the leachate of ammonium acetate), N_t by distillation after the mineralization of strong H_2SO_4 (Kjeldahl - Bremner 1960), C_{ox} spectrophotometrically after the oxidation (Tyurin 1966), EC by the method of specific electrical conductivity and pH/KCl (in solution of 1.0 mol/dm^3 KCl) potentiometrically (Kováčik 1997).

The experiment was established according to the method of random arrangement of pots with the quadruple repetition. The model crop was radish (*Raphanus sativus* L.) cultivar Granát. The sowing was carried out on March 16. Subsequently, the experiment was irrigated to the level of 75% FWC. In the following three weeks all pots were irrigated by the same dose of water containing the minimal quantity of nutrients. During the last 14 days the treatments 2, 3 and 4 were irrigated by a higher dose of water, because in these treatments the plants vaporized more water as a result of the significantly larger leaf area. During the growth season (April 24, May 3, and May 9, i.e. in 27/39, 36/48 and 42/54 days after germination/sowing) three samplings of plant material were accomplished. (The plants of radish germinated after 12 days from sowing.) 10 average individuals were taken from each treatment and repetition, which served for the evaluation of the root weight.

For the analysis of the pigment content, the last fully developed leaves were used. The photosynthetic pigments were determined in the acetonic extract by spectrophotometric method using the equations of Lichtenthaler (1987). The diameter of the root thickness was measured by the slide calliper. The nitrate content and vitamin C content in the roots of the second and third sampling was detected. The quantity of vitamin C was determined by titration with 2,6-dichlorophenolindophenol. In order to determine nitrates we used ion-selective electrode of the type 07-35 and reference electrode of the type RCE 101 (Monokrystaly Turnov). The second and third samplings were carried out in six days interval because radish is usually harvested in two or more terms.

The acquired results were processed by mathematical and statistical method, by analysis of variance (ANOVA) and linear regression analysis using Statgraphics PC program, version 5.0. The differences between the treatments were evaluated subsequently by *LSD* test at the significance level α = 0.05.

RESULTS AND DISCUSSION

Growing of radish sown in the soil with 10% content of vermicompost (Trt. 2) in comparison with the soil without vermicompost (Trt. 1) increased considerably root weight. The positive impact of VC was measurable from April 24, i.e. 27 days from the beginning of germination (Table 2). On the 36th day of the growth season (May 3) there was the most

T a b l e 2

Impact of vermicompost on the dynamics of the changes in the weight of the radish roots

Treatment		24. IV.		3. V.		9. V.	
number	mark	[g/10ind.]	[%]	[g/10ind.]	[%]	[g/10ind.]	[%]
1	control	1.95	100.00	20.03^a	100.00	27.83^a	100.00
2	VC	2.83	145.13	148.70^c	742.39	185.80^c	667.62
3	VC + EW_{10}	1.87	95.90	106.13^b	529.86	141.95^b	510.06
4	VC + EW_{20}	1.53	78.46	95.15^b	475.04	127.88^b	459.50
$LSD_{0.05}$				12.686		20.527	

[g/10ind.] – g/10 individuals; VC – vermicompost; EW_{10} – ten earthworms; $LSD_{0.05}$ – least significant difference at the level α = 0.05

considerable difference. In this period of the growth season the root yield was 7.42 times higher in the treatment with 10% content of VC than in the control treatment. On the 42^{nd} day of the growth season (May 9) the highest root yields were achieved in all treatments, however, the differences of the yields between the treatments were decreased.

The diameter of roots in the unfertilised treatment was so low that those roots were unmarketable, similarly on 36^{th} as well as on 42^{nd} day of the growth season. The unmarketability was related to the achieved root diameter. According to the Slovak Technical Standards 46 3120 the radish roots grown in the field conditions must have the diameter bigger than 2.0 cm. The average root size in the control treatment was smaller than 2.0 cm and it achieved from 1.44 to 1.68 cm (Table 3).

During the whole radish growth season the presence of earthworms in soil had the negative impact on the root weight (Trt. 2 versus Trt. 3 and Trt. 3 versus Trt. 4 – Table 2). The negative impact of earthworms was evident especially at the beginning of the growth season (April 24), when the root weight in the treatments 3 and 4 was lower compared to the treatment 1. The smallest root mass was in the treatment containing the most worms (Trt. 4). This fact is probably the consequence of the attack of the earthworms at root hair, which appears more considerably in younger plants.

In the period of technological ripeness (second and third sampling) the radish roots were 4.6 to 5.1 times heavier in the treatments with vermicompost and earthworms (Trt. 3 and 4) than in the control treatment (Trt. 1), however, the roots were smaller than in the treatment with vermicompost without earthworms (Trt. 2). The differences in root mass between treatments which contained earthworms and treatment without earthworms (Trt. 3 and 4 versus Trt. 2) were significant. The differences between the treatments with the earthworms (Trt. 3 versus Trt. 4) were not significant. Nevertheless, it can be stated that with the growth of the number of earthworms in the soil, the weight of the roots decreased. The recorded differences in the weight of the roots between the treatments with the number of 10 and 20 individuals of earthworms (Trt. 3 versus Trt. 4) were in the second sampling of 10.3% and in the third sampling at 9.9%. The discovered facts are not consistent with the major opinion of public and scientists on the positive impact of earthworm on the growth of plants (Friberg *et al*. 2005; Groenigen *et al*. 2014) but they are consistent with the opinion of Ayuke *et al*. (2017) who are stating that the mechanism of action of earthworms is not still well known. Our findings are consistent with the findings of Doan *et al*. (2013) who along with the positive effects of earthworms noted also the negative effects of earthworms on plants. Ayuke *et al*. (2017) did not notice the influence of earthworms on the formation of rapeseed phytomass (*Brassica napus*).

Depending on the agrotechnical conditions of plant cultivation and weather conditions during the growth season, the pigment contents usually increase, but the declines are recorded too (Vician *et al*. 2012). In this experiment on the 48^{th} day after sowing the radish had a higher content of chloro-

T a b l e 3

Impact of vermicompost on the change in the diameter of the radish roots and on qualitative parameters of radish roots

Treatment		Roots diameter		Viatmin C		$NaNO_3$	
		3. V.	9. V.	3. V.	9. V.	3. V.	9. V.
number	mark	[mg/kg]				[cm]	
1	control	1.44^{a}	1.68^{a}	114.88^{c}	161.84^{c}	738^{a}	570^{a}
2	VC	3.13^{c}	3.29^{c}	91.92^{a}	115.00^{ab}	1,649^{d}	1,480^{c}
3	VC + EW_{10}	2.77^{bc}	2.80^{bc}	101.92^{b}	127.52^{b}	1,032^{b}	815^{b}
4	VC + EW_{20}	2.77^{b}	2.56^{b}	91.92^{a}	110.00^{a}	1,131^{c}	816^{b}
$LSD_{0.05}$		0.411	0.530	7.667	14.549	87.636	55.831

VC – vermicompost; EW – earthworms; $LSD_{0.05}$ – least significant difference at the level α = 0.05; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%

phyll than the plants on the 54th day from sowing (Table 4).These discovered findings confirmed that after the period of growth, in the senescence period, the leaves of plants were losing gradually chlorophyll. The decrease in chlorophylls content was from 4.02% to 10.7% but the decrease in carotenoids content was more pronounced. It reached the values from 10.3% to 19.4%. It has been found out that the carotenoid contents in comparison with the total chlorophyll contents react more considerably to the ageing process of the radish leaves.

The vermicompost application increased significantly the content of total chlorophylls and did not affect the carotenoid contents in the radish leaves. However, the opposite conclusions were recorded by Ali (2007), who determined the decrease in chlorophyll content with an increasing proportion of vermicompost in the substrate. The significant decrease occurred only when the cultivation substrate consisted solely of vermicompost. Adding earthworms to vermicomposts reduced the total chlorophylls content. The size of the drop depended on the num-

T a b l e 4

Impact of vermicompost on pigments formation in radish leaves

Treatment		3. V. (48 days a.s)	9. V. (54 days a.s)	3. V.	9. V.
		Total chlorophyll		Carotenoids	
number	mark	[mg/m^2]			
1	control	281.3^a	270.0^a	64.3^c	51.8^a
2	VC	335.0^c	299.3^c	59.7^b	51.8^a
3	VC + EW_{10}	325.0^{bc}	292.0^{bc}	59.2^{ab}	53.2^a
4	VC + EW_{20}	315.0^b	286.0^b	58.7^a	52.5^a
$LSD_{0.05}$		16.426	12.991	0.717	1.735

VC – vermicompost; EW – earthworms; $LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%; days a.s – days after sowing

T a b l e 5

Correlation coefficient r expressing the relationship between the radish quantitative and qualitative yield parameters and the total chlorophyll content occurring in the last fully developed leaves in the particular terms

Parameter		Correlation coefficient (r)	n
dependent	independent		
Weight of roots	total chlorophyll (3. V.)	0.935^{++}	16
	total chlorophyll (9. V.)	0.832^{++}	16
	total chlorophyll (3.V. and 9. V. together)	0.482^{++}	32
Vitamin C	total chlorophyll (3. V.)	–0.666^{++}	16
	total chlorophyll (9. V.)	–0.632^{++}	16
	total chlorophyll (3.V. and 9. V. together)	–0.719^{++}	32
$NaNO_3$	total chlorophyll (3. V.)	0.814^{++}	16
	total chlorophyll (9. V.)	0.712^{++}	16
	total chlorophyll (3.V. and 9. V. together)	0.753^{++}	32
Roots diameter	total chlorophyll (3. V.)	0.875^{++}	16
	total chlorophyll (9. V.)	0.796^{++}	16
	total chlorophyll (3.V. and 9. V. together)	0.609^{++}	32

$^+$statistically significant ($P < 0.05$); $^{++}$statistically high significant ($P < 0.01$); n – number of measurements ber of added earthworms. Ten earthworms per 20 kg of cultivation substrate had an insignificant impact. Twenty earthworms had a significantly negative impact. It can be assumed that the earthworms by consuming amino acids, organic nitrogenous substances found in the growing substrate, at various degrees of degradation, limited the amount of inorganic nitrogen but also other nutrients entering the plant. The relationship between the N content but also Mg and other nutrients and the amount of chlorophyll in the plant is generally known (Evans 1983; Bojovič & Markovič 2009; Liu *et al*. 2012; Saberioon *et al.* 2014; Gholizadeh *et al.* 2017). Earthworms did not affect the amount of carotenoids in the leaves.

The diameter of radish roots grown in the treatments with earthworms complied with the requirements of traders. It was more than 2.0 cm (Table 3). The small diameters and weights of radish roots detected in the treatment 1 emphasize the fact that radish grown in the soil with the relatively sufficient supply of N_{min}, K, Ca and Mg (Table 1), without the application of nutrients (inorganic or mineral fertilisers) did not create the prerequisites for the growing marketable yield (commercially realizable yield).

The influence of earthworms on the diameter of the radish roots was identical with the effect on chlorophyll content in leaves and on the weight of the roots. It is evident that the amount of chlorophyll influenced significantly the diameter of the radish roots, and consequently, the weight of the radish roots. This finding is supported by the values of the correlation coefficient *r* between the amount of total chlorophyll and the diameter of the root between the amount of total chlorophyll and the weight of the roots (Table 5). Similarly, Kim *et al*. (2015) have noticed a strong interaction between the morphology of tea tree root and earthworms.

The analysis of roots related to two qualitative parameters – content of vitamin C and content of nitrates - confirmed that there is the negative correlation between these two parameters (Kováčik 2014; Wang *et al.* 2017). The presence of vermicompost in the substrate (Trt. 2) increased the content of nitrates and decreased the content of vitamin C (Table 3). The effect of the earthworms added to the vermicompost depended on the number of earthworms supplied. Ten earthworms significantly increased vitamin C content and reduced nitrate content. Twenty earthworms had a negative effect on vitamin C content (Trt. 4 versus Trt. 3). It reduced the content. The effect on nitrate content was negative on the 48th day from sowing (increased the content) and on the 54th day it was insignificant. Similar findings were obtained by Nurhidayati *et al*. (2016), who by testing of three species of vermicomposts discovered that in the most cases with the growing number of earthworms in the cultivaiting substrate, the cabbage plants decreased both the vitamin C content and the sugar content.

The content of vitamin C in the treatment with 20 earthworms (Trt. 4) was the same as in the treatment with vermicompost without earthworms (Trt. 2) and the nitrate content in treatment 4 was lower than in treatment 2. In both treatments with earthworms (Trt. 3 and 4), there were significantly less nitrates in the radish roots than in the comparable treatment 2 without earthworms. The data from the Table 3 also show that on 54th day after sowing (which is the last suitable date for harvest) there were determined less nitrates and more vitamin C than 6 days ago, i.e. on 48th day after sowing. Based on this finding, we can say that the older plants contained less nitrates and more vitamin C than younger ones.

CONCLUSIONS

The impact of earthworms on the chlorophyll content in radish leaves and on the root weight and its diameter was negative. The addition of 10 individuals of earthworms (*Eisenia foetida*) into 20 kg of substrate (soil + vermicompost) resulted in the increased content of vitamin C and the decreased content of nitrates in the radish roots. Twenty earthworms added to vermicompost, compared to vermicompost free of earthworms, did not affect the vitamin C content and reduced the nitrate content in roots. The tenth ratio of vermicompost out of the total weight of soil substrate caused a statistically significant increase in the total chlorophyll content in leaves, the increase in yield of the radish roots, reducing the vitamin C content and increasing the nitrate content in the roots.

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