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DETERMINATION OF THE CARROT (*DAUCUS CAROTA* L.) YIELDS PARAMETERS BY VERMICOMPOST AND EARTHWORMS (*EISENIA FOETIDA*)

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ABSTRACT
The impact of different types of vermicomposts as well as different species and genera of earthworms on the quantity of the cultivated crops yield has been studied for decades. There is scarce information about the effects of these factors on the quality of plant production. One of the qualitative parameters of vegetables, to which a special attention is paid, is the content of antioxidants (vitamin C, total polyphenols and other substances). The pot experiment carried out in the vegetation cage studied: A) the influence of soil itself, soil mixed with vermicompost in a ratio of 4:1; B) the influence of earthworms number (genus *Eisenia foetida*, 10 and 20 individuals per pot) supplied to soil mixed with vermicompost in a ratio of 4:1 on the weight of radish roots and leaves, to the total chlorophylls content in leaves, to the selected qualitative parameters of the roots and leaves (vitamin C, total polyphenols content, total antioxidant activity). The results showed that the supplementation of vermicompost into soil increased the content of the total chlorophylls in leaves. The carrot roots and leaves yield has also been risen. In the roots the content of vitamin C and content of total polyphenols (TPC) was decreased and the total antioxidant activity (TAA) dropped, too. The increased content of vitamin C and TPC was detected in leaves. The inoculation of soil containing vermicompost by earthworms increased the root yield and TAA in roots. It increased the content of vitamin C and TPC in leaves. From the viewpoint of antioxidant content (vitamin C and total polyphenols) the leaves are more attractive than a root.

Keywords: carrot; antioxidant; vitamin C; total polyphenols; yield

INTRODUCTION
The positive impact of earthworms on soil and plants was monitored several centuries ago **(Kováčik, 2007)**. In spite of it, at present the research of earthworms impact on soil, environment, quantity and quality of the grown yield has been done with more attention **(Milcu et al., 2006; Amador et al., 2006; Xiang et al., 2016)**. The researchers of the whole world study the requirements of the different species of earthworms to soil parameters and substrates in order to create the optimal conditions for their growth and reproduction **(Nuutinen et al., 2001; Gunadi and Edwards, 2003; Arnold and Hodson, 2007)**. They detect which genera (species) of earthworms are optimal for the purposes of the ecological valuation of precisely defined waste produced by chemical, food processing, textile, papermaking or other type of industry **(Nurhidayati et al., 2016; Santos et al., 2017; Bhat et al., 2018)**. The researchers also test which temperature, substrate humidity, salts content, ammonia, total nitrogen, methane and ratio C:N occur in the most effective decay, the transformation of organic "waste" into the high quality vermicompost. They also study the different technological procedures of vermicompost production for its direct usage in the plant production, recultivation and production of vermiextracts **(Scheuerell, 2004; Padmavathiamma et al., 2008; Gutiérrez-Miceli et al., 2008; Kováčik et al., 2015)**.

The vermicomposts are composts in the production of which the process of biological transformation of organic substances takes part the earthworms. For these purposes predominanlty the earthworms of genera *Eisenia foetida* and *Eudrilus eugeniae* **(Lalander et al., 2015; Najjari and Ghasemi, 2018)**, or *Lumbricus terrestris and Eisenia Andrei* **(Rämert et al., 2000; Amossé et al., 2013)**, as well as others, are being used. More scientific knowledge about the impact of vermicomposts on the quantity and quality of the cultivated crops yields has been gathered than the knowledge about the impact of earthworms on the given parameters.

The positive impact of vermicomposts on the quantity of the cultivated field and garden crops, fruit trees and bushes was recorded by **Arancon et al. (2004), Manh and Wang (2014), Khan et al. (2015), Goswami et al. (2017)**.

The positive impact of earthworms (of different genera) on the plant growth, the quantity and quality of yield was recorded by **Brown et al. (2004)**, **Groenigen et al. (2014)** and others. For example, **Xiang et al. (2016)**, taking into consideration the knowledge of meta-analysis, claim that the presence of earthworms in the ecosystem increases crop yield on average by 25%, formation of aboveground phytomass by 23% and underground phytomass by 20%. Zero and negative plant responses to the presence of earthworms were recorded **(Doan et al., 2013; Nurhidayati et al., 2016; Elmer, 2016)**. **Kováčik et al. (2018)** also detected the negative impact of earthworms (*Eisenia foetida*) on the formation of radish roots, however, the positive impact on the content of vitamin C and negative impact on the content of nitrates in radish roots was also recorded.

It is evident that the impact of earthworms on the particular plants has not been explored sufficiently. This statement is related not only to the quantity of yield but also to its qualitative parameters, which are the contents of antioxidants.

Scientific hypothesis

The objective of the presented experiment is to give the answer to the question how the supplementation of vermicompost into soil and soil inoculation enriched by vermicompost of earthworms genus *Eisenia foetida* affect the yield of carrot roots and leaves, the content of total chlorophylls in leaves, the content of antioxidants (vitamin C and total polyphenols) and the total antioxidant activity in carrot roots and leaves.

MATERIAL AND METHODOLOGY

Experimental design and field management

The pot experiment was carried out in the vegetation cage located in the area of the Slovak University of Agriculture in Nitra, Slovakia. 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. The weighted soil (treatment 1) and mixture of soil and vermicompost (treatments 2 – 4) were put into the cylindrical pots 35 cm high with the diameter 35 cm. In the treatment 1, twenty (20) kg of soil (Haplic Luvisol) was used and in the treatments 2, 3 and 4 was put into the pots of 20 kg of mixture of soil and vermicompost. The mixture was created by 16 kg of soil (S) and 4 kg of vermicompost (V), which was the ratio S:V = 4:1, (20% proportion of V). In the treatments 2 – 4 the same soil was used like in the treatment 1. The used soil was taken from the field located in Párovské Háje, (cadaster Nitra, Slovakia), in particular, from the upper horizon of soil 0.0 – 0.3 m. The used vermicompost was produced from cow dung (about 50%), sheep manure (about 10%), green grass (about 10%) and wood chips (about 30%). After 3 to 4 months of fermentation (composting) was prepared a mixture of the new with the old vermicompost in a ratio of 1:1. In the old vermicompost were earthworms and cocoons. Basic agrochemical parameters of soil and vermicompost used in experiment are presented in Table 1.

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 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 experiment was established according to the method of random arrangement of pots in four repetitions. The model crop was carrot (*Daucus carota* L. ssp. *sativus*) cultivar Nantes 3. The sowing was carried out on March 16. Subsequently, the experiment was irrigated to the level of 75% FWC (field or full water capacity). In the following eight weeks all pots were irrigated by the same dose of water containing the minimal quantity of nutrients. During the last days the treatments 2, 3 and 4 were irrigated by a higher dose of water, because the plants in these treatments evaporated more water as a result of the significantly larger leaf area. On Jun 27, 103 days after sowing, samplings of plant material were accomplished. 20 average individuals of carrot were taken from each treatment and repetition, which served for the evaluation of the root and leaf weight, the content of total chlorophylls in leaves, the content of vitamin C, total polyphenols and antioxidant activity in carrot roots and leaves.

Analysis of soil and vermicompost

Were used the following analytical methods for the indication of the agrochemicals parameters of used soil and vermicompost. $N\text{-}NH_4^+$ by Nessler's colorimetric method; $N\text{-}NO_3^-$ by colorimetric method with phenol – 2.4 disulphonic acid, where the extract from soil was achieved by using the water solution 1% K_2SO_4. $N_{min} = N\text{–}NH_4^+ + N\text{–}NO_3^-$. 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 according to Tyurin **(Dziadowiec and Gonet, 1999)**, EC by the method of specific electrical conductivity and pH/KCl (in solution of 1.0 $mol.dm^{-3}$ KCl) potentiometrically.

Determination of total chlorophylls

For the analysis of the pigment content, the last fully developed leaves were used. The segments of the youngest mature leaves were homogenized with using sea sand, $MgCO_3$ and 100% acetone and then extracted with 80% acetone. Extracts were centrifuged 2 minutes. Absorbance (A) of the solution was measured by UV-VIS spectrophotometer, at 647 nm and 663 nm, with correction for scattering at 750 nm; the measurements were done in three repetitions. The concentrations of chlorophyll $\underline{a}$ (Chl $\underline{a}$), chlorophyll $\underline{b}$ (Chl $\underline{b}$) in $mg.L^{-1}$ were determined by using the equations of **Lichtenthaler (1987)**:

$\text{Chl } \underline{a} = 12.25 \times (A_{663} - A_{750}) - 2.79 \times (A_{647} - A_{750}) \times D$

$\text{Chl } \underline{b} = 21.50 \times (A_{647} - A_{750}) - 5.10 \times (A_{663} - A_{750}) \times D$

$\text{Chl } \underline{a}+\underline{b} = 7.15 \times (A_{663} - A_{750}) + 18.71 \times (A_{647} - A_{750}) \times D$

D is the optical thickness of cuvette.

Results were also recalculated in $mg.m^{-2}$ using the volume of solution and the area of leaf segments: $mg.m^{-2}$ =

V/1000 x 1/A, when V is volume of 80% acetone and A is area of leaf segments.

Determination of L-ascorbic acid

Three grams of homogenized fresh samples were stabilized with 10 mL of acid solution prepared as follows: 10% perchloric acid and 1% orthophosphoric acid in ultra pure water. The mixture was thoroughly vortexed for 1 minute. This solution was diluted to 50 mL with HPLC mobile phase. The sample was filtered with 0.45 μm filter.

L-ascorbic acid was determined by HPLC Agilent 1260 with quaternary solvent manager coupled with degasser, sample manager, column manager and DAD detector. All analyses were performed on C18 end capped column. Mobile phases consisted of methanol (B) and 0.1% H_3PO_4 (C). The isocratic elution was as follows: 0 – 6 min (20% B and 80% C) and 3 minutes post-run. The mobile phase flow was 1 mL.min^{-1} and the sample injection was 20 μL. Column thermostat was set to 30 °C and the samples were kept at 4 °C the sampler manager. The detection wavelength was set at 256 nm. The spectral data were collected and processed using Agilent OpenLab ChemStation software for LC 3D Systems.

Determination of total polyphenols

Total polyphenols content (TPC) was determined by the method according to **Lachman et al. (2003)** and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolic content determination because a wide spectrum of phenolic compounds. The total polyphenol content was determined using Folin-Ciocalteau reagent. 2.5 mL of Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 μL of extract. The content was mixed and 5 mL of a sodium carbonate solution (20%) was added after 3 min. Then the volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min and the absorbance was measured at 765 nm of wave length against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Determination of total antioxidant activity

Total antioxidant activity (TAA) was measured according to **Brand-Williams et al. (1995)**. The method is based on using DPPH (2.2-diphenyl-1-picrylhydrazyl). DPPH was pipetted into the cuvette number 1 (3.9 ml) and the absorbance was measured using the spectrophotometer at wavelength 515.6 nm. The measured value corresponds to the initial concentration of DPPH solution at the time A0. Then 0.1 cm^3 extract was added to start measuring dependence A = f x (t). The content of cuvette number 2 was mixed and the absorbance was measured at 1, 5 and 10 minutes in the same way as DPPH solution. The percentage of inhibition expresses how antioxidant compounds are able to remove DPPH$^{\cdot}$ radical at the given period of time.

Inhibition (%) = (A0 - At/A0) x 100.

Statistical analysis

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

RESULTS AND DISCUSSION

Vermicompost in soil (var. 2) increased significantly the content of total chlorophylls in carrot leaves (Table 2). The increase of total chlorophylls contents was pursued via the increase of contents of chlorophyll *b*. The content of chlorophylls *a* was decreased. The increase of contents of chlorophylls *b*, which was recorded in all treatments with vermicompost (trt. 2, 3 and 4), according to **Razzaq et al. (2017)** was the consequence of higher content of mineral nitrogen in soil of the given treatments compared with the treatment 1 (Table 1). A higher statistical dependence is recorded very often between the content of chlorophylls *b* and the yield of cultivated crops than between the contents of chlorophylls *a* and yield **(Kováčik, et al., 2016)**. These data emphasize the practical significance of finding the

Table 1 Parameters of the soil substrates used in the experiment.

Subs.	N_{min} (mg.kg^{-1})	P (mg.kg^{-1})	K (mg.kg^{-1})	Ca (mg.kg^{-1})	Mg (mg.kg^{-1})	S (mg.kg^{-1})	N_t (%)	C_{ox} (%)	C:N	EC (mS.cm^{-1})	pH_{KCl}
Soil	13.2	21.9	156	4,250	444	1.3	0.077	0.915	11.88	0.14	6.97
V	313.8	351.3	19,000	4,350	3,052	4,688	3.775	20.880	5.53	5.58	7.06

Note: Subs. – substrate, V – vermicompost.

Table 2 Impact of vermicompost and earthworms on chlorophyl *a*, chlorophyl *b* and total chlorophylls content in carrot leaves.

Treatment		Chl. *a*	Chl. *b*	Chl. *a*+*b*
number	mark	(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1})
1	S	0.616 b	0.562 a	1.178 a
2	SV	0.608 a	0.781 b	1.389 b
3	SV+EW_{10}	0.607 a	0.824 b	1.431 b
4	SV+EW_{20}	0.605 a	0.836 b	1.441 b
$LSD_{0.05}$		0.00645	0.06525	0.06941

Note: Chl. *a* – chlorophyll a; Chl. *b* – chlorophyll b; S – soil; SV – soil + vermicompost, EW_{10} – ten individuals of 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%.

increase of contents of chlorophyll b in crops cultivated on soils where vermicompost was supplied.

The presence of earthworms in soil (trt. 3 and 4 versus trt. 2) had impact neither on the content of total chlorophylls nor its particular components (chorophyll a and chlorophyll b).

Weight of aboveground and underground phytomass

Vermicompost in soil (trt. 2) became evident by the considerable growth of aboveground and underground carrot phytomass (Table 3). The positive impact of vermicompost on the growth of carrot roots and leaves is the result of its agrochemical parameters (significant content of available nutrients – mineral nitrogen, P, K, Mg and S).

Soil inoculation treated by vermicompost with earthworms (trt. 3 and 4) had a positive impact on the weight of roots. The weight of leaves was not determined by the presence of earthworms in soil. The differences were significant between the root weight of the treatment without earthworms and with 10 individuals of earthworms in soil (trt. 2 versus trt.3) and also between the treatments with 10 and 20 individuals of earthworms in soil (trt. 3 versus trt. 4). This finding does not correspond with our knowledge **(Kováčik et al., 2018)** about the impact of earthworms on the growth of radish roots, where during the whole growing season the negative impact of earthworms on the roots weight was recorded. The different impact of earthworms on the root weight of carrot and radish was caused by the different reaction of these crops to the attack of earthworms at the root hair of young plants. The different reaction of carrot and radish was caused by the different duration of growing season of these crops. Carrot has a longer growing season therefore the monitored attack of earthworms at the root hair of germinated plants did not have the negative impact on the roots yield. A longer growing season of carrot created a longer space-time for the regeneration of the young plants, which had been attacked by earthworms, also for the positive impact of earthworms on physical, chemical and biological soil parameters **(Garg et al., 2006; Jouquet et al., 2010; Spurgeon et al., 2013)** and on the yield formation **(Friberg et al., 2005; Groenigen et al., 2014)**.

The positive finding is that the vermicompost and earthworms influenced more significantly the roots weight than leaves weight, because in Slovakia the consumed organ is a root. The vermicompost and earthworms in soil increased the ratio between the roots weight and leaves weight (Table 3).

Vitamin C content

20 % proportion of vermicompost in soil (trt. 2) decreased the content of vitamin C in carrot roots almost by o 40 % (Table 4). The drop was the natural consequence of the well-known negative correlation between the content N_{min} in soil and content of vitamin C in plants. The negative impact of vermicompost (rich in the available nutrients – Table 1) on the content of vitamin C in carrot roots was statistically insignificantly decreased by the soil inoculation with earthworms (trt.3 and 4 versus trt.2). Vermicompost (trt. 2 versus trt. 1) had the opposite impact on the content of vitamin C in leaves than on the content in carrot roots. The content of vitamin C rose significantly.

The impact of earthworms on the content of vitamin C in radish leaves was unlike the impact of the content of vitamin C in roots significantly positive (trt. 3 versus trt. 2), or (trt. 4 versus trt.2).

In leaves there was considerably more vitamin C than in roots (Table 4). Similarly, in leaves there was a higher content of the total polyphenols and also the total antioxidant capacity was higher in leaves than in roots, which supports the idea that the countries with the food

Table 3 Impact of vermicompost and earthworms on weight of carrot roots and leaves (fresh phytomass).

Treatment		Root	Leaf	Ratio R/L
number	mark	(g)	(g)	
1	S	4.70 a	11.64 a	0.40
2	SV	89.50 b	45.98 b	1.95
3	$SV+EW_{10}$	109.62 c	48.92 b	2.24
4	$SV+EW_{20}$	120.69 d	50.48 b	2.39
$LSD_{0.05}$		8.832	5.002	

Note: S – soil; SV – soil + vermicompost, EW_{10} – ten individuals of 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%.

Table 4 Impact of vermicompost and earthworms on content of vitamin C in carrot roots and leaves.

Treatment		Root		Leaf		Ratio L/R
number	mark	($mg.kg^{-1}$)	(rel. %)	($mg.kg^{-1}$)	(rel. %)	
1	S	245.89 b	100.00	714.88 a	100.00	2.90
2	SV	150.80 a	61.33	842.72 b	117.88	5.59
3	$SV+EW_{10}$	167.23 a	68.01	994.83 c	139.16	5.95
4	$SV+EW_{20}$	167.37 a	68.07	972.71 c	136.07	5.81
$LSD_{0.05}$		24.730		59.574		

Note: S – soil; SV – soil + vermicompost, EW_{10} – ten individuals of 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%

sufficiency could adopt some eating habits from those countries which suffer from the food shortage (Table 5 and Table 6).

Total polyphenols content (TPC) and total antioxidant activity (TAA)

The roots of carrot variety Nantes contained the content of polyphenols on average 2.5 higher than the content of polyphenols in garlic **(Lenková et al., 2018)**, 1.7 times higher than in the young green peas **(Hegedűsová et al., 2018)** and 1.3 times higher than in flesh-raw of sweet potato **(Musilová et al., 2017)**. Despite the detected relatively high content of the total polyphenols in carrot roots compared with other vegetables, the content of the total polyphenols in roots was 5.11 even 11.8 times lower than in carrot leaves. The total antioxidant activity detected in leaves was 3.26 even 7.84 times higher that in carrot roots (Table 5 and Table 6). The polyphenols are known for their antioxidant and antiradical activity. They protect the animal organism from the oxidation stress. The application of vermicompost into soil (trt. 2) decreased the content of the total polyphenols in carrot roots and also the value of the total antioxidant activity, which is the negative effect, because polyphenols have the positive impact on our health **(Oszmianski et al., 2013; Ražná et al., 2018)**. The decrease of content of vitamin C, the total polyphenols and consequently also monitoring the decrease of the total antioxidant activity in carrot roots after the application of vermicompost shows the evidence that the fertilization by the fertilizers containing nitrogen, leads mostly to the growth of yield, on the other hand, the decrease of the quality of the cultivated crops.

The impact of vermicompost on TPC in carrot leaves was significantly positive and the content of TAA in carrot leaves was not influenced by vermicompost.

The soil inoculation by earthworms (trt. 3 and 4 versus trt. 2) increased the content of total polyphenols and also the total antioxidant activity of the whole carrot phytomass. However, the impact of earthworms on TPC and TAA in roots and leaves was not equally significant. In the consumed part of carrot – in roots – the increase of TPC was insignificant and TAA significant. On the contrary, in leaves the increase of TPC was significant and TAA insignificant.

CONCLUSION

The supplemantation of vermicompost into soil led to the increase of yield of carrot roots, but the content of vitamin C and total polyphenols was decreased in roots, also the total antioxidant activity dropped. The inoculation of soil containing vermicompost with earthworms inhibited the negative impact of vermicompost on the content of vitamin C and the total polyphenols in carrot roots. The impact of earthworms on the total antioxidant activity in leaves was positive.

The supplementation of vermicompost into soil increased the leaves yield and it increased the content of total chlorophylls, content of vitamin C and total polyphenols in leaves. Earthworms had impact neither on the content of total chlorophylls in leaves, leaves yield nor TAA in leaves. They had the positive impact on the content of vitamin C and TPC in leaves. Carrot leaves contain several times more vitamin C and total polyphenols than roots. Similarly, the total antioxidant activity in leaves is several times higher than in carrot roots.

Table 5 Impact of vermicompost and earthworms on content of total polyphenols of aboveground and underground carrot phytomass.

Treatment		Root		Leaf		Ratio
number	mark	(mg.kg^{-1})	(rel. %)	(mg.kg^{-1})	(rel. %)	L/R
1	S	2,385 b	100.00	12,183 a	100.00	5.11
2	SV	1,157 a	48.50	12,773 b	104.84	11.04
3	SV+EW$_{10}$	1,200 a	50.32	13,292 c	109.10	11.08
4	SV+EW$_{20}$	1,241 a	52.03	13,583 d	111.49	10.95
LSD$_{0.05}$		242.497		341.847		

Note: S – soil; SV – soil + vermicompost, EW$_{10}$ – ten individuals of 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%.

Table 6 Impact of vermicompost and earthworms on content of total antioxidant activity of aboveground and underground carrot phytomass.

Treatment		Root		Leaf		Ratio
number	mark	(%)	(rel. %)	(%)	(rel. %)	L/R
1	S	27.40 d	100,00	89.46 a	100.00	3.26
2	SV	11.38 a	41,53	89.25 a	99.77	7.84
3	SV+EW$_{10}$	13.21 b	48,21	89.00 a	99.49	6.74
4	SV+EW$_{20}$	14.74 c	53,80	89.06 a	99.55	6.04
LSD$_{0.05}$		0.913		0.525		

Note: S – soil; SV – soil + vermicompost, EW$_{10}$ – ten individuals of 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%.

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