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Original Research

Weight of Carrot Phytomass and Content of Vitamin C 100 Days after Seeding in Dependence of Vermicompost Quantity and Earthworms (*Eisenia fetida*) in Soil Substrate

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Abstract

The aim of the study was to evaluate the effect of various amounts of vermicompost (0, 10, 20, 25 and 50%) and *Eisenia fetida* earthworms (0, 10, 20 individuals per pot) on the weight of carrot roots and leaves and their vitamin C content on the 100th day after seeding. The achieved results indicate that along with the increasing content of vermicompost in the soil substrate the weight of roots and leaves was rising and the content of vitamin C was decreasing. The weight of roots was increasing more dynamically than the weight of leaves. The high proportion-even 50% of Vc-in the soil substrate did not have a negative impact on the formation of aboveground and underground phytomass. The earthworms had a positive influence on the formation of carrot roots and leaves. The impact on the roots was stronger. The impact of earthworms on the formation of phytomass depended on the interactive impact of the vermicompost quantity in the substrate and number of earthworms. The smallest percentage increase of roots and leaves yields with both numbers of earthworms was achieved if the earthworms were put into the substrate of the highest 50% proportion of vermicompost.

Keywords: roots, leaves, vitamin C, red earthworms, soil substrates

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Introduction

Carrot (*Daucus carota* L.) is grown successfully around the whole temperate climatic zone. It is an important component of human nutrition. Carrot has a high nutritional value [1], it is rich in antioxidants [2-5] and its consumption acts preventively against several diseases [6-9]. Therefore, its growing is being increased in the whole world. China is the significant world carrot producer. In spite of the positive effects of carrot consumption and also other vegetables in the fresh or processed form on the human health, the area of fields assigned for the vegetables growing represents less than 1% of the area of arable soils in Slovakia.

In the whole world carrots is being grown conventionally and ecologically [10, 11]. Both the commercial and organic fertilizers are used in its cultivation [12-14]. The Slovak farmers have a shortage of organic fertilizers, predominantly manure. The availability of composts is better. However, farmers' confidence in compost is low, especially in compost from municipal solid waste, but also in industrial and domestic compost, in compost produced by cities. In the country, the trust in vermicomposts is significant, which is related to the fact that the components of vermicompost mean the fodder and living place and also reproduction of earthworms. In the case when the vermicompost contains the components unsuitable for earthworms´ life, they either escape from the vermicompost or die [15]. The vermicompost full of earthworms and cocoons indicates its biological appropriateness for agricultural purposes.

The majority of the European population believes that the fertilization by the organic fertilizers is the guarantee of a higher quality of the cultivated products and lower threat for the environment in comparison with the mineral fertilizers. But many scientific publications state that the impact of the organic fertilizers on the yield quantity and quality of the cultivated crops can be positive, neutral, and also negative [16-21]. Similarly, their effect on the soil parameters and environment can be either positive or negative [22-24]. The organic fertilizers are the source of the organic substances, from which soil humus is being formed [25], they increase the sorption capacity and buffering ability of soils [26, 27]. The organic fertilizers decrease the bulk density of soils [28], they have a positive impact on the heat capacity [29] and air regime in soils. The organic fertilizers increase the water infiltration into soil and water capacity, improve the water management of soil [30, 31]. They provide the growth stimulants [32], macro- and microelements, improve the nutrient uptake [33]. They effect the formation of soil aggregates [34, 35] and they increase the soil stability [27]. After their usage there occurs a faster decomposition of the crop residues [36]. They inhibit the intake of heavy metals into the food chain [37]. They vitalize biologically soil [38]. They accelerate the biodegradation of undesirable matters in soil [39]. They increase pH of soil, and also the number of bacteria genus *Azotobacter chroococcum* in soil, which has the positive impact on the health condition of crops, on the formation of aboveground and underground phytomass of plants [15].

The positive impact of organic fertilizers on the biological, chemical and physical soil parameters [40], it determines positively the quyntitative and qualitative parameters of the cultivated crops. Blouin et al [41] claims that vermicompost brings about average increases of 26% in commercial yield, 13% in total biomass, 78% in shoot biomass, and 57% in root biomass. After the application of vermicompost the content of the total antioxidants, content of carotenes, lycopene, crude fibre, saccharides, Ca, Mg, P, Fe, Zn and other elements was increased in the crops like tomato, pepper, apple, pears, spinach, lettuce, cabbage, turnip, carrots, beetroots, celery, potato lentil [42-44]. The utilization of vermicompost increased the content of proteins and fat in the wheat grains [45]. The yields harvested from plants receiving vermicompost were firmer, characterized by ascorbic acid content and lower acidity, and have attractive colour [46] and better taste [47]. The substitution of mineral fertilizers by vermicompost reduced drastically the incidence of physiological disorders like albinism, fruit malformation and occurrence of grey mould in strawberry, indicating that vermicompost played a significant role in reducing nutrient-related disorders and diseases like Botrytis rot, and thereby increasing the marketable fruit yield up to 58.6% with better quality parameters [46, 48].

The negative impact of organic fertilizers on soil, environment and a man is associated predominantly with their improper usage, when they can lead to the increased content of nitrates in the ground water and surface water and plants [49]. The utilization of organic fertilizers raises the risk of leakage NH_3 into the atmosphere [50]. The threat of organic fertilizers consists also in the fact that they can contain pathogenes harmful for both people and plants [51].

A considerable part of the European population also supposes that the more earthworms are in soil the higher yield and quality of the cultivated crops is. However, the impact of earthworms on the quantity and quality of the cultivated crops depends on several factors, as a result there were recorded the positive effects on plant [52-54], also ambiguous, i. e. positive and negative [55, 56]. The statistically insignificant but also positive and negative plant reactions to the earthworms occurrence were presented by [57, 58].

Individual countries focus the different attention to the study of the impact of the particular species of earthworms on the quantity and quality of yields of the particular plant species. As far any facts have been presented about the number of earthworms of the particular species which can be beneficial for the individual crops and when they are harmful. The utility and harmfulness of earthworms should be evaluated not only form the aspect of the yield quantity, but also from the viewpoint of the yield quality, or the impact on the environment.

The objective of the submitted article is to answer the questions (I) how the different quantities of vermicompost in soil and (II) the presence of earthworms (*Eisenia fetida*) in the soil substrates can influence the carrot root and leaves weight and the content of vitamin C on the 100th day after its seeding.

Material and Methods

Experimental Design and Field Management

The two-year pot experiment (2017 and 2018) was carried out in the vegetation cage located in the campus of the Slovak University of Agriculture in Nitra (48°18'N, 18°05'E). In the experiment, the impact of two factors (I. amount of vermicompost and II. number of red earthworms) were studied on the carrot phytomass production and vitamin C content on the 100th day after carrot sowing.

The total number of treatments was 13. The weighed soil (treatment 1) and the mixture of soil and vermicompost (treatments 2 to 13) were placed in 35 cm high cylindrical pots with a diameter of 35 cm. The treatment 1 was the control one (soil without vermicompost). In the treatments 2-5 the impact of vermicompost quantity in the soil substrates was detected and in the treatments 6-13 the impact of the number of earthworms added to the soil substrates was studied, tested in the treatments 1-5.

20 kg of soil (Haplic Luvisol) was applied into the pots in the treatment 1 and 20 kg of the mixture consisting of soil and vermicompost was applied into the pots in the treatments 2-5. In the treatment 2 the mixture was formed by 18 kg of soil (So) and 2 kg of vermicompost (Vc), which was the ratio So:Vc = 9:1, (10% proportion Vc). In the treatment 3 the mixture was created by 16 kg of soil and 4 kg of vermicompost, which was the ratio So:Vc = 4:1 (20% proportion Vc). In the treatment 4 the mixture contained 15 kg of soil and 5 kg of vermicompost, which was the ratio So: Vc = 3:1 (25% proportion Vc). In the treatment 5 the mixture was formed by 10 kg of soil and 10 kg of vermicompost, which was the ratio So:Vc = 1:1 (50% proportion Vc). In the treatments 2-5 the same soil was used for the preparation of mixture like in the treatment 1 (Haplic Luvisol). The used soil was taken from the field located in Párovské Háje, (cadaster Nitra), in particular, from the upper horizon of soil 0.0-0.25 m. The basic chemical parameters of the used soil (So) and vermicompost (Vc) are indicated in the Table 1. The vermicompost, which was used in the experiment, was produced by the company Vermivital Ltd. In the treatments 6-13 the same soil substrate were used like in the treatments 2-5, whereby there were added earthworms (*Eisenia fetida*). The pairs of treatments 6 and 7, 8 and 9, 10 and 11, 12 and 13 contained the identical substrates as the treatments 2, 3, 4 and 5, whereby 10 individuals of red worm was added to the treatments of the even number (8, 10, 12) and 20 individuals of red worm was put into the treatments of the of the odd number (9, 11, 13) (Table 2-5).

Before putting the soil substrates into the pots, the plastic net was placed at the bottom of all pots to prevent the earthworms (EW) to escape. The pots with the weighted substrate were located on the saucers which were able to capture 1,000 ml of the leaked soil solution in the period of precipitation. The leaked 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. In both years the sowing took place on 16 March. Subsequently, the experiment was irrigated to the level of 75% FWC (field or full water capacity). In the following 70 days all the pots were irrigated by the same dose of water containing the minimal quantity of nutrients. During the last 30 days of experiment the treatments 2 to 13 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. Twenty days after emergence of plants, they were unitized by thinning down their number to ensure the same number per pot (repetition). During the growth season (24 June on the 100th day after sowing) sampling of plant material was accomplished. 10 average individuals were taken from each treatment and repetition, which served for the evaluation of the root and leaves weight. The contents of vitamin C were determined in roots and leaves.

Analysis of Soil and Vermicompost

Were used the following analytical methods for the indication of the agrochemicals parameters of used soil

Table 1. Parameters of the soil and vermicompost used in the experiment.

Subs.	N_{in}	P	K	Ca	Mg	S	N_{tot}	C_{ox}	C:N	EC	pH_{KCl}
	(mg/kg)						(g /kg)			(mS/cm)	
So	9.20	17.80	173	3,100	452	4.40	0.70	9.00	11.88	0.12	6.35
Vc	310.1	3,085	8,763	5,135	3,252	2,068	29.70	198.9	5.53	4.98	7.33

Subs. – substrate, So - soil, Vc – vermicompost

Table 2. Impact of vermicompost and earthworms on weight of fresh carrot roots.

Treatment		(g/10 individuals)	(%)				
n.	Designation						
1	So	31.53 [a]	100.00				
2	$SoVc_{9:1}$	109.48 [b]	347.22	100.00			
3	$SoVc_{4:1}$	189.45 [e]	600.86	173.05	100.00		
4	$SoVc_{3:1}$	207.85 [f]	659.21	189.85	109.71	100.00	
5	$SoVc_{1:1}$	218.13 [g]	691.81	199.24	115.14	104.95	100.00
6	$SoVc_{9:1}+EW_{10}$	135.03 [c]	428.26	123.34	71.27	64.97	61.90
7	$SoVc_{9:1}+EW_{20}$	160.97 [d]	610.53	147.03	84.97	77.45	73.80
8	$SoVc_{4:1}+EW_{10}$	208.38 [f]	660.89	190.34	109.99	100.26	95.53
9	$SoVc_{4:1}+EW_{20}$	219.05 [gh]	694.74	200.08	115.62	105.39	100.42
10	$SoVc_{3:1}+EW_{10}$	221.44 [hi]	702.32	202.27	116.89	106.54	101.52
11	$SoVc_{3:1}+EW_{20}$	223.06 [i]	707.45	203.75	117.74	107.32	102.26
12	$SoVc_{1:1}+EW_{10}$	231.02 [j]	732.70	211.02	121.94	111.15	105.91
13	$SoVc_{1:1}+EW_{20}$	234.32 [k]	743.17	214.03	123,68	112.74	107.42
$LSD_{0.05}$		3.296					
1-13		183.82	-	-			
2-5		181.23	100.00	-			
6-13		204,16	112.65	-			
6,8,10,12		198,97	109,79	100.00			
7,9,11,13		209,35	115,52	108.46			

n. – number, So - soil, 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%

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_{in} was calculated as a sum of $N\text{-}NH_4^+ + N\text{-}NO_3^-$ ($N_{in} = N–NH_4^+ + N–NO_3^-$). The contents of available P, K, Ca, Mg were determined by Mehlich 3 extraction procedure [59]. 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 method) [60], C_{ox} spectrophotometrically after the oxidation according to Tiurin [61], EC by the method of specific electrical conductivity and pH/KCl (in solution of 1.0 mol/dm^3 KCl) potentiometrically.

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 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.

Statistical Analysis

The acquired results were processed by the mathematical and statistical method, by the multifactorial analysis of variance (ANOVA). The differences between the treatments were evaluated by LSD test (Tukey's test) at the significance level α = 0.05.

Table 3. Impact of vermicompost and earthworms on weight of fresh carrot leaves.

Treatment		(g/10 individuals)	(%)				
n.	Designation						
1	So	25.29 [a]	100.00				
2	$SoVc_{9:1}$	69.02 [b]	272.91	100.00			
3	$SoVc_{4:1}$	101.55 [c]	401.54	147.13	100.00		
4	$SoVc_{3:1}$	109.03 [cd]	431.12	157.97	107.37	100.00	
5	$SoVc_{1:1}$	119.30 [ef]	471.73	172.85	117.48	109.42	100.00
6	$SoVc_{9:1}$+EW_{10}	73.83 [b]	291.94	106.97	72.71	67.72	61.89
7	$SoVc_{9:1}$+EW_{20}	75.00 [b]	296.56	108.66	73.86	68.79	62.87
8	$SoVc_{4:1}$+EW_{10}	107.21 [cd]	423.93	155.33	105.57	98.33	89.87
9	$SoVc_{4:1}$+EW_{20}	113.20 [de]	447.61	164.01	111.47	103.82	94.89
10	$SoVc_{3:1}$+EW_{10}	114.02 [de]	450.85	165.20	112.28	104.58	95.57
11	$SoVc_{3:1}$+EW_{20}	115.57 [def]	456.98	167.44	113.81	106.00	96.87
12	$SoVc_{1:1}$+EW_{10}	122.17 [ef]	483.08	177.01	120.31	112.05	102.41
13	$SoVc_{1:1}$+EW_{20}	124.27 [f]	491.38	180.05	122.37	113.98	104.17
$LSD_{0.05}$		9.760					
1-13		97.66	-	-			
2-5		99.73	100.00	-			
6-13		105.66	105.95	-			
6,8,10,12		104.31	104.59	100.00			
7,9,11,13		107.01	107.30	102.59			

n. – number, , So - soil, 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%

The PC statistical program "Statgraphics, version 5.0" was used.

Results and Discussion

Weight of Underground Phytomass

Along with increasing the content of vermicompost in the soil substrate (tr. 2-5) the weight of carrot roots was rising (Table 2) as a result of the increasing content N_{in} but also other macroelements in the substrate, because Vc contained severalfold higher contents of macroelements in the available form than soil (Table 1). The increasing contents Vc in the soil substrate (10, 20, 25 and 50%) increased the yield of carrot roots compared with the treatment without Vc 3.47, 6.00, 6.59 and 6.92 times (Table 2). This finding did not correspond with the facts of [62], who monitored the rise of roots weight in radish cultivation only up to the level 20% of proportions Vc in the soil substrate. The contents Vc in the soil substrate above the level 20% (25 and 50%) resulted in the fall of roots weight. The different reaction of the carrot plant and radish plants to the quantity of vermicompost in the substrate depends probably on the different duration of the experiment. The experiment with radish took 52 days and the experiment with carrot 100 days. The weight increase of carrot roots in consequence of the increasing quantity Vc the soil substrate approved the fact that the crop yields depend on the quantity of available nutrients in soil [63].

The differences in the roots weight (weight growth) between the treatments 1 and 2, 2 and 3, 3 and 4, 4 and 5 were being decreased gradually. The biggest weight increase 3.47 times was detected between the treatments 1 and 2, and the lowest one only 1.05 times occurred between the treatments 4 and 5 (Table 2). This fact emphasizes that along with the increasing quantity of the applied vermicompost into soil the efficiency of its usage decreased. However, 6.92 times higher weight of carrot roots in the treatment with 50% proportion Vc compared with the control treatment without Vc pointed out the possibility to use Vc in the relatively high doses without the risk of the yield decrease. It is important to bear in mind that the application of high doses of vermicompost into soil should be carried out only in the reasonable cases (low content of available nutrients

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

Treatment		(mg/100 g)	(%)				
n.	Designation						
1	So	14.94 ^{c}	100.00				
2	$SoVc_{9:1}$	13.42 ^{bc}	89.83	100.00			
3	$SoVc_{4:1}$	12.00 ^{ab}	80.32	89,42	100.00		
4	$SoVc_{3:1}$	11.73 ^{ab}	78.51	87,41	97.75	100.00	
5	$SoVc_{1:1}$	10.67 ^{a}	71.42	79,51	88.92	90.96	100.00
6	$SoVc_{9:1}+EW_{10}$	12.69 ^{abc}	84.94	94,56	105.75	108.18	118.93
7	$SoVc_{9:1}+EW_{20}$	12.22 ^{ab}	81.79	91,06	101.83	104.18	114.53
8	$SoVc_{4:1}+EW_{10}$	10.67 ^{a}	71.42	79,51	88.92	90.96	100.00
9	$SoVc_{4:1}+EW_{20}$	10.52 ^{a}	70.41	78,39	87.67	89.68	98.59
10	$SoVc_{3:1}+EW_{10}$	10.58 ^{a}	70.82	78,84	88.17	90.20	99.16
11	$SoVc_{3:1}+EW_{20}$	10.48 ^{a}	70.15	78,09	87.33	89.34	98.22
12	$SoVc_{1:1}+EW_{10}$	10.53 ^{a}	70.48	78,46	87.75	89.77	98.69
13	$SoVc_{1:1}+EW_{20}$	10.39 ^{a}	69.54	77,42	86.58	88.58	97.38
$LSD_{0.05}$		2.424					
1-13		11.60	-	-			
2-5		11.96	100.00	-			
6-13		11.01	92.06	-			
6,8,10,12		11.12	92.98	100.00			
7,9,11,13		10.90	91.14	98.02			

n. – number, So - soil, 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%

in soil - Table 1), in particular, in the areas where there is not risk of deterioration of quality of ground water and worsening of the parameters of environment.

The earthworms had the positive impact on the formation of carrot roots. When comparing the average roots weight of all treatments with earthworms and the average roots weight in the treatments without earthworms, it is obvious that the earthworms increased the roots weight by 12.65% (tr. 6 - 13 vs tr. 2 - 5). The positive effect of earthworms (different genera) on the phytomass formation of the grown crops was recorded by Brown et al. and Nweke [52, 54]. Based on the meta-analysis [64] claim that the presence of earthworms elevated significantly the underground biomass by 29%.

The average difference in carrot root yield between the treatments with 20 and 10 earthworms in the substrate (tr. 7, 9, 11, 13 vs. tr. 6, 8, 10, 12-Table 2) was 8.46% in favour of a higher number of earthworms. In the majority of cases the increases of roots weight as a result of a higher number of earthworms were statistically significant. Nurhidayati et al. [55] detected that along with the increasing number of earthworms genus *Pontoscolex corethrurus* in the growing medium there occurs the increase as well as decrease of cabbage yield. On the contrary, the opposite results were presented by Kováčik et al. [56], who recorded the decrease of radish root yield with the rising number of earthworms in the soil substrate. The presented different effect of earthworms on the carrot roots in comparison with the impact on the radish roots is the consequence of the fact that carrot has a longer growing season than radish. A longer growing season allows the carrot plants to cope with the initial attack of earthworms of root hairs better, and at the same time it allows carrot plants to use better the positive effect of earthworms on several soil parameters [56].

The intensity of earthworms` impact on the weight of roots depended on the interactive effect of the quantity of vermicompost in the substrate and number of earthworms. The root weight was increased by 23.34% with quantity 10% of Vc in the soil substrate under the influence of 10 pieces of earthworms per a pot, and by 47.03% under the influence of 20 pieces (tr. 6 and 7 vs. tr. 2). The weight of roots was increased by 9.99% with the quantity 20% Vc in the substrate under the influence of 10 pieces of earthworms, and by 15.62% under the influence of 20 pieces (tr. 8 and 9 vs. tr. 3). The root weight was increased by 6.54% with

Table 5. Impact of vermicompost and earthworms on the content of vitamin C in fresh carrot leaves.

Treatment		(mg/100 g)	(%)					L:R
n.	Designation							
1	So	32.24 ^{a}	100.00					2.16
2	SoVc$_{9:1}$	41.35 ^{cde}	128.26	100.00				3.08
3	SoVc$_{4:1}$	41.03 ^{cde}	127.26	99.23	100.00			3.42
4	SoVc$_{3:1}$	39.73 ^{cde}	123.23	96.08	96.83	100.00		3.39
5	SoVc$_{1:1}$	34.54 ^{ab}	107.13	83.53	84.18	86.94	100.00	3.24
6	SoVc$_{9:1}$+EW$_{10}$	42.73 ^{e}	132.54	103.34	104.14	107.55	123.71	3.37
7	SoVc$_{9:1}$+EW$_{20}$	47.43 ^{f}	147.12	114.71	115.60	119.38	137.32	3.88
8	SoVc$_{4:1}$+EW$_{10}$	42.25 ^{de}	131.05	102.18	102.97	106.34	122.32	3.96
9	SoVc$_{4:1}$+EW$_{20}$	46.59 ^{f}	144.51	112.67	113.55	117.27	134.89	4.43
10	SoVc$_{3:1}$+EW$_{10}$	40.04 ^{cde}	124.19	96.83	97.59	100.78	115.92	3.78
11	SoVc$_{3:1}$+EW$_{20}$	41.88 ^{de}	129.91	101.28	102.07	105.41	121.25	4.00
12	SoVc$_{1:1}$+EW$_{10}$	37.93 ^{bc}	117.65	91.73	92.44	95.47	109.81	3.60
13	SoVc$_{1:1}$+EW$_{20}$	39.14 ^{cd}	121.40	94.66	95.39	98.51	113.32	3.77
LSD$_{0.05}$		3.508						
1-13		40.53	-	-				
2-5		39.16	100.00	-				
6-13		42.25	107.89	-				
6,8,10,12		40.74	104.03	100.00				
7,9,11,13		43.76	111.75	107.41				

n. – number, So - soil, 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%

quantity 25% of Vc under the influence of 10 pieces of earthworms, and by 7.32% under the influence of 20 pieces (tr. 10 and 11 vs. tr. 4). The weight of roots rose by 5.91% with quantity 50% of Vc in the soil substrate under the influence of 10 pieces of earthworms, and by 7.42% under the influence of 20 pieces (tr. 12 and 13 vs. tr. 5). These facts prove that the highest percentage yield increase was achieved if earthworms were put into the substrate with the lowest quantity of vermicompost. This finding is related to the fact that the positive impact of earthworms on the bulk density of soil and consequently on the rise of roots yield is falling along with the decreasing bulk density of soil, the increasing content of organic matter in soil [15].

Weight of Aboveground Phytomass

The carrot leaves reacted to the presence of vermicompost and red worms in the substrate similarly like the roots, however less markedly. Along with the increasing content of vermicompost in the soil substrate (tr. 2 - 5) the leaves weight was being increased (Table 2). With 10, 20, 25 and 50% of Vc content in the soil substrate the leaves weight was increased compared to the treatment without VC 2.72, 4.02, 4.31 and 4.71 times (Table 2). The registered more dynamic growth of the roots weight than leaves in the treatments with vermicompost approved the fact that the fertilization by nitrogen usually determines more considerably the growth of roots than leaves [65].

The growths of leaves weight between the treatments 1 and 2, 2 and 3, 3 and 4, 4 and 5 were as following: 2.73, 1.47, 1.07 and 1.09 times (Table 3). This result proves that along with the increasing quantity of vermicompost in soil the effect of Vc on the leaves growth was falling. The curves, expressing the impact of N fertilizers on the plant growth, have the different shape depending on the growing conditions. Their course has usually the sigmoid shape. Along with the growth of dose N first the phytomass rises dynamically, later the growth is being slowed down, and it can even result in the decrease of the phytomass formation.

4.71 times higher weight of carrot leaves in the treatment with 50% proportion of Vc in comparison with the control treatment without Vc does not indicate the depressive impact of the high proportion of Vc in the soil substrate on the carrot plants, which is often evident with high application doses of the fertilizers containing nitrogen [66, 67].

In the treatments 6-13 the earthworms increased the leaves weight on average only by 5.95% (tr. 6 - 13 vs tr. 2 - 5), which is the considerably lower effect compared with the impact on the roots weight (Table 2 and 3). The rate of increase was significantly lower also in comparison with the data presented by van Groenigen [53], who state that earthworms in the different ecosystems increase the formation of the aboveground phytomass by 23 %.

The number of earthworms did not influence considerably the carrot leaves weight, nevertheless, it tended to increase it. The average growth of the carrot leaves weight between the treatments with 20 and 10 earthworms in the substrate (tr. 7, 9, 11, 13 vs. tr. 6, 8, 10, 12-Table 3) was at the level of 2.59% in favour of a higher number of earthworms.

The impact of earthworms on the leaves weight determined the interactive effect of the quantity of vermicompost in the substrate and number of earthworms. The quantity 10% Vc in the soil substrate under the influence of 10 pieces of earthworms per a pot increased the leaves weight by 6.97% and under the influence of 20 pieces by 8.66% (tr. 6 and 7 vs. tr. 2). The quantity 20% Vc in the substrate under the influence of 10 pieces of earthworms increased leaves weight by 5.57% under the influence of 20 pieces by 11.47% (tr. 8 and 9 vs. tr. 3). The quantity 25% Vc under the influence of 10 pieces of earthworms increased the roots weight by 4.58% and under the influence of 20 pieces by 6.00% (tr. 10 and 11 vs. tr. 4). The quantity 50% Vc under the influence of 10 pieces of earthworms increased the roots weight by 2.41% and under the influence of 20 pieces by 4.17% (tr. 12 and 13 vs. tr. 5). The positive interactive effect of earthworms and vermicompost on the formation of phytomass of the cultivated crops was presented by Brown et al. [52].

The lowest percentage growths of leaves weight with both numbers of earthworms were detected in the treatments where was the highest - 50% proportion Vc in the soil substrate. This information corresponds with already presented interactive effect between the quantity of vermicompost in the substrate and the number of earthworms on the roots weight (Table 2). But the impact on the leaves was less strong.

Vitamin C Content

In all treatments of the experiment the average value of vitamin C (11.6 mg/kg) was considerably higher than [68, 9] claim and lower than [69, 70] state. The highest content of vitamin C in the carrot roots (14.94 mg/kg) was detected in the control treatment, where the soil substrate did not involve Vc. Along with the increasing quantity Vc in the soil substrate (tr. 2 - 5) the content of vitamin C was falling (Table 4). The reason was the generally known negative correlation between the content N_{in} in soil and the content of vitamin C in a plant, because with the rising content Vc in the substrate the content N_{in} in the substrate was increasing. Under some growing conditions the increase of quantity Vc in soil does not lead to the decrease of the content of vitamin C in plants [55]. The differences in contents of vitamin C between 1 and tr. 2-5 varied in the interval from 1.52 to 4.27 mg/100g, whereby the differences between tr. 1 and tr. 3-5 were significant.

The earthworms did not determine the content of vitamin C in roots statistically significantly. On average in the treatments 6-13 they decreased the content of vitamin C only by 0.95 mg/kg (tr. 6 - 13 vs tr. 2 - 5). The differences in the contents of vitamin C between all the relevant treatments (tr. 2 and tr. 6, 7, tr. 3 and tr. 7 and 8, tr 4 and tr. 9 and 10, tr. 5 and tr. 11 and 12) were minimal, statistically insignificant. The insignificant impact of earthworms on the content of vitamin C in the fruits of papaya (*Carica papaya* L.) was presented by [71].

The rise of number of earthworms from 10 individuals to 20 ones per a pot did not have a considerable impact on the content of vitamin C in carrot roots. In spite of this fact, it is possible to state that the increase of the number of earthworms tended to decrease the content of vitamin C in roots. The average decrease of the content of vitamin C in the treatments 7, 9, 11 and 13 compared with the treatments 6, 8, 10 and 12 achieved cca 2% (Table 4).

The content of vitamin C in carrot leaves was 2.16 even 4.00 times higher than in the roots (Table 5). The effect of vermicompost on the content of vitamin C in the leaves was partially different from the impact on the roots. In all treatments with vermicompost the content of vitamin C was higher than in the control treatment, however, this content was diminishing with the rising quantity of Vc in the soil substrate. The lowest content of vitamin C detected in the treatment without Vc (tr.1) was the consequence of the fact that the plants react to the insufficiency of nutrients by more rapid ageing of leaves and the faster degradation of vitamin C.

The earthworms influenced the content of vitamin C in leaves more significantly than the content in roots, thanks to predominantly the treatments with a higher number of earthworms in the substrate. The effect of earthworms on the content of vitamin C was positive. On average in the treatments 6-13 the increased the content of vitamin C in leaves by 7.89% (tr. 6 - 13 vs tr. 2 - 5). 10% quantity of Vc in the soil substrate with 10 pieces Ew per a pot increased the content of vitamin C in leaves by 3.34% and by the impact of 20 pieces Ew by 14.71% (tr. 6 and 7 vs. tr. 2). The quantity 20% Vc in the substrate with 10 pieces of earthworms increased the content of vitamin C in leaves by 2.97% and by the influence of 20 pieces by 13.57% (tr. 8 and 9 vs. tr. 3). The quantity 25% Vc in the substrate with 10 pieces of earthworms increased the content of vitamin C in leaves by 0.78% and by the influence of

20 pieces by 5.41% (tr. 10 and 11 vs. tr. 4). The quantity 50% Vc in the substrate determined the increases at the level of 9.81% and 13.32% (tr. 12 and 13 vs. tr. 5). 20 pieces of earthworms in comparison with 10 pieces of earthworms (tr. 7, 9, 11, 13 vs. tr. 6, 8, 10, 12) increased the content of vitamin C on average by 7.41%. The rise of the content of vitamin C in carrot roots after the inoculation of earthworms into soil was recorded by Kováčik et al. [72].

Conclusions

Along with the increase of the content of vermicompost in the soil substrate the weight of roots and leaves was increased and the content of vitamin C was falling. The roots weight was rising more dynamically than the leaves weight. A high-50% proportion of Vc in the soil substrate did not have the negative effect on the formation of the aboveground and underground phytomass. The use of Vc in soils with a low content of available nutrients in high doses (25 and 50% in the substrate) does not pose a risk of reduced yields. This finding creates a precondition for the use of year-on-year growing compost production in European countries.

The earthworms (10 and 20 individuals/pot) have the positive imapct on the formation of carrot roots and leaves. The imapct on roots was stronger. A higher yield of roots was achieved in the treatments with 20 earthworms than in the treatments with 10 earthworms per a pot. The highest percentage increases of the roots yield with both numbers of earthworms were achieved if the earthworms were placed into the substrate with the lowest quantity of vermicompost. The earthworms did not determine the content of vitamin C in carrot roots.

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Conflict of Interest

The authors declare no conflict of interest.

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