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VERMICOMPOST AFFECTS GROWTH, NITROGEN CONTENT, LEAF GAS EXCHANGE AND PRODUCTIVITY OF PEPPER PLANTS

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Abstract

The purpose of this study was to investigate the effect of the vermicompost Lumbrical, produced by the Californian earthworm *Lumbricus rubellus*, on growth, nitrogen content, leaf gas exchange and productivity of pepper plants (cv. Gorogled 6). The experiments were carried out in 2007-2009, on experimental field of the Agroecological Centre of the Agricultural University of Plovdiv (Bulgaria). Immediately before planting, two levels of the vermicompost were applied: 50 and 100 cm^3 per plant. Thirty days after planting, some of the plants fertilized with 50 cm^3 Lumbrical (the third variant) were additionally fed with a solution of vermicompost (200 cm^3 per plant). Prior to the experiment, chemical and microbiological analyses were performed on both the soil and vermicompost. During plant vegetation, some biometrical indices characterizing the plant growth were measured. The nitrogen concentration and nitrate reductase activity in leaves of pepper plants were determined. The leaf gas exchange was taken into account. The pepper yield and quality parameters of fruits were analyzed. The results of the present study showed that by feeding the pepper with the vermicompost Lumbrical it was possible to increase the plants' vegetative mass improve the development of their generative organs. Incorporation of the vermicompost into the soil increased the content of N in the root area. It also considerably raised the nitrogen content in leaves of pepper plants. A positive correlation between the content N in leaves and the activity of the enzyme called nitrate reductase was observed. The positive effect of the vermicompost was reflected by improved leaf gas exchange parameters and productivity of plants.

Key words: *Lumbricus rubellus*, *Capsicum annuum* L., *nitrate reductase*, photosynthesis, nitrogen, yielding.

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WPŁYW WERMIKOMPOSTU NA WZROST, ZAWARTOŚĆ AZOTU, WYMIANĘ GAZOWĄ LIŚCI I PLONOWANIE PAPRYKI

Abstrakt

Celem badań było określenie wpływu nawożenia wermikompostem Lumbrical, wyprodukowanym z wykorzystaniem dżdżownicy kalifornijskiej (*Lumbricus rubellus*), na wzrost, zawartość azotu, wymianę gazową liści oraz plonowanie papryki odmiany Gorogled 6. Badania przeprowadzono w latach 2007-2009, na polu doświadczalnym Centrum Agroekologicznego Akademii Rolniczej w Płowdiw (Bułgaria). Bezpośrednio przed sadzeniem roślin zastosowano dwa poziomy nawożenia wermikompostem – 50 i 100 cm^3 na roślinę. Trzydzieści dni po sadzeniu część roślin nawożonych 50 cm^3 Lumbricalu (trzeci wariant) dodatkowo zasilono roztworem wermikompostu (200 cm^3 na roślinę). Przed rozpoczęciem badań wykonano analizy chemiczne i mikrobiologiczne gleby i wermikompostu. W okresie wegetacji badano wybrane wskaźniki charakteryzujące wzrost roślin – zawartość azotu i aktywność reduktazy azotanowej w liściach papryki, wymianę gazową liści. Oceniano wielkość plonu oraz parametry charakteryzujące jakość owoców. Wykazano, że nawożenie wermikompostem Lumbrical wpływa na zwiększenie masy wegetatywnej oraz przyspieszenie rozwoju generatywnego roślin. Wprowadzony do gleby wermikompost znacznie wpłynął na zwiększenie zawartości N w strefie korzeni oraz w liściach badanych roślin papryki. Stwierdzono dodatnią korelację między zawartością N w liściach i aktywnością reduktazy azotanowej. Pozytywny wpływ wermikompostu w uprawie papryki wykazano na podstawie wymiany gazowej liści i plonowania roślin.

Słowa kluczowe: *Lumbricus rubellus*, *Capsicum annuum* L., *reduktaza azotanowa*, azot, fotosynteza, plonowanie.

INTRODUCTION

Composting organic waste with various types of earthworms is an intriguing problem from both a scientific and a practical point of view (Romero Lima et al. 2000, Manna et al. 2003, Sallaku et al. 2009). Several types of worms have become important in practice: *Eisenia fetida, Lumbricus rubellus, Amyanthes diffrigens and Eudrillus engineac.* They feed on different types of organic manure and other organic waste. While being digested, this raw material undergoes physical and chemical modifications. Around 60% of the food assimilated by worms is released as excrements. The new product (vermicompost) improves soil fertility (Harris et al. 1990, Karbauskiene 2000) and has a stimulating effect on the growth and development of plants (Atiyeh et al. 2000 a,b,c, Domini et al. 2000, Makulec 2002, Edwards, Arancon 2004, Arancon et al. 2004).

The nutritional value of vermicompost depends on its origin. Golchin et al. (2006) report that vermicompost made from animal manure has a higher nutritional value than that extracted from organic public waste. For example, vermicompost from cattle and pig manure as well as from food waste can improve the germination, growth and flowering rate of a larger number of ornamental and vegetable crops than vermicompost from other sources (Atiyeh et al. 2000 a,b,c). Compared to natural fertilizer, vermicompost contains between 40 and 60% more humic substances and exceeds the universal compost in quality (DOMINGUEZ et al. 1997, MAKULEC 2002).

MATERIAL AND METHODS

The field experiments were carried out in 2007-2009, at the Agroecological Centre of the Agricultural University in Plovdiv, Bulgaria. The experiments were carried out on the Gorogled 6 pepper cultivar, which is grown for ground red pepper. In March, seeds for the production of seedlings were planted at a density of 12-15 g m^{-2}. The seedlings were grown in a polythene greenhouse, on cold seed-beds with tunnel covers above them. The seedlings were transplanted onto a permanent site at the beginning of June, after a lasting increase in the soil temperature to over 16°C. They were planted in long furrows, spaced 60 × 15 cm.

For the purpose of the experiments, vermicompost produced by the Californian earthworm *Lumbricus rubellus* (Lumbrical) was used. The worms were fed with cattle manure. Lumbrical was placed at the bottom of 6-8 cm deep furrows directly before planting the seedlings. During the vegetative season, the peppers, fertilized with 50 cm^3 in the third variant, were treated with Lumbrical extract (30 days after the first nutrition). The extract consisted of aqueous solution of Lumbrical in the 1:10 ratio. The solution was left to mature for at least 24 hours and was periodically stirred. The clear part of the sediment was decanted. The plants were treated with the extract in a dose of 200 cm^3 per plant.

The experiments were carried out under the following scheme: 1) control sample – soil without the vermicompost; 2) soil fertilized with 50 cm^3 of the vermicompost Lumbrical per plant before planting; 3) soil fertilized with 50 cm^3 of the vermicompost per plant before planting and 200 cm^3 of Lumbrical extract per plant after planting; 4) soil fertilized with 100cm^3 of Lumbrical per plant before planting. Each of the tested variants had four replications, each including 24 plants.

The peppers were harvested once in mid-October, when over 80% of the fruits had reached full botanical maturity. The unripe fruits were left to season.

Prior to the experiment, some chemical characteristics and microbiological analyses were performed both on the soil and on the vermicompost. The organic matter was determined using the Tiurin method for determination of organic carbon content. The determination of pH was performed potentiometrically. Solutions used for the determination of pH were prepared with carbon-dioxide-free water R. The total nitrogen was determined titrometrically, after ashing in sulfuric acid (H_2SO_4) and subsequent distillation in a Parnas-Wagner apparatus. The total content of phosphorus was determined colorimetrically with the Egner-Riem. The total quantity of potassium was determined in hydrochloric acid extract (2 M HCl) with the modified method of Milcheva in a flame photometer. The counts of bacteria, spore-forming bacteria, actinomycetes, microscopic fungi and cellulose decomposing bacteria were determined by the dilution agar plate method.

During the growth and development of the pepper, some biometric parameters of the plants were taken (30 and 60 days after the application of the vermicompost). The leaf area was measured with a digital area meter NEO-2 (TU, Sofia, Bulgaria). The nitrate reductase activity (NRA, EC 1.6.6.1) was determined *in vivo* by the method of KAISER and LEWIS (1984) in the first leaf under the first node (30 days) and in the first leaf under the second node (60 days). The concentration of nitrite was calculated by drawing a calibration curve of nitrite. The enzymatic activity was expressed as µg NO_2^- g^{-1} FW h^{-1}. The leaf gas exchange (portable photosynthetic system LCA-4, ADC, England) was reported. Ground red pepper was prepared from the fruit pericarp. The total pigment content was determined with the ASTA-19 method, modified by MANUELYAN (1979), immediately after grinding pepper fruits.

Statistical analyses were carried out by one-way ANOVA using the Tukey's test to validate the significance of differences at $p = 0.05$.

RESULTS AND DISCUSSION

The soil used for the experiment had a low mineral nitrogen content (Table 1). At the beginning of the experiment, the soil contained 22.4 mg NO_3^- kg^{-1} and 5.6 mg NH_4^+ kg^{-1}. The ammonium content of the vermicompost exceeded that of the soil by 8.4 mg kg^{-1} and the nitrate content was 134.4 mg kg^{-1} higher. Furthermore, the amount of mineral nitrogen in vermicompost exceeded that of the soil by 142.8 mg kg^{-1}. The soil was moderately rich in the phosphorus content and had a high quantity of potassium.

The microbiological analysis showed that the count of bacteria mineralizing organic nitrogen compounds and the count of actinomycetes were higher in vermicompost than in the soil. Irrespective of the fact that the number of nitrogen-immobilizing bacteria was reduced in the vermicompost, the balance between mineralization and immobilization was maintained and the bacteria decomposing organic nitrogen compounds dominated. This led to the accumulation of accessible mineral nitrogen compounds in the vermicompost. There were fewer microscopic fungi and cellulose-decomposing bacteria in the vermicompost than in soil. A possible reason was a shortage of oxygen in the vermicompost, limiting counts of the above aerobic microorganisms.

Table 1

Chemical and microbiological analyses of the soil and vermicompost at the beginning of the experiment

Specification	Soil	Vermicompost
Chemical analysis		
pH (H_20)	7.6	7.4
Organic matter (%)	2.3	32.6
NH_4^+ (mg kg^{-1})	5.6	14.0
NO_3^- (mg kg^{-1})	22.4	156.8
Total N (mg kg^{-1})	28.0	28.0
P_2O_5 (mg kg^{-1})	99.0	325.0
K_2O (mg kg^{-1})	430	498
Microbiological analysis		
Bacteria mineral. org. N compounds	0.4 x 10^6	4.2 x 10^6
Spore-forming bacteria	2.8 x 10^4	6.9 x 10^4
Bacteria transf. mineral. N compounds	7.1 x 10^5	5.2 x 10^5
Actinomycetes	2.2 x 10^5	2.7 x 10^5
Microscopic fungi	2.7 x 10^4	2,5 x 10^4
Cellulose decomposing bacteria	0.6 x 10^4	0.5 x 10^4

Research shows that vermicompost improves the growth of many agricultural crops (ARANCON et al. 2004, GUTIERREZ-MICELI et al. 2007). A better growth of vegetative organs is accompanied by an increased development of generative organs (HASHEMIMAJD et al. 2004). At the same time, GUTIÉRREZ--MICELI et al. (2007) report that vermicompost in greenhouse production of tomatoes leads to plants growing much taller but has no significant effect on the number of leaves. Positive results have been reported from a turnip growing experiment (*Brassica napa* L.). Plants fed with vermicompost are characterized by a higher number of leaves and larger leaf mass compared with the control (CLASSEN et al. 2007).

The results presented in Table 2 showed that the vermicompost Lumbrical accelerated the vegetative growth of the plants. On the 30th day after feeding with Lumbrical the plants' height and the diameter of their crowns were larger in the fed plants. The number, size and mass of leaves considerably increased in comparison with the control. The formed photosynthesizing surface was by 45.6% larger compared with the control. This was a result of the improved nutrition of plants, which accelerated the growth

Table 2

Effect of Lumbrical vermicompost on some growth parameters of pepper plants 30 days after vermicompost applied

Parameters \ Variants	1*	2	3
Plant height (cm)	33.6 *a***	37.6 *b*	36.6 *b*
Crown diameter (cm)	25.0 *a*	27.3 *a*	27.2 *a*
Number of fully expanded leaves	10.1 *a*	12.6 *ab*	14.8 *b*
Area of mature leaves (dm^2)	1.94 *a*	2.80 *b*	2.86 *b*
Leaf fresh weight (g)	8.9 *a*	13.1 *b*	13.3 *b*
Flower buds number	10.50 *a*	19.10 *b*	20.5 *b*
Flower buds fresh weight (g)	0.31 *a*	0.59 *b*	1.03 *c*

* 1 – control, soil without vermicompost; 2 – soil fertilized with vermicompost, 50 cm^3 per plant before planting; 3 – soil fertilized with vermicompost, 100 cm^3 per plant before planting ;
**Values in columns followed by the same letter do not differ significantly at $p = 0.05$.

and the accumulation of biomass of photosynthesizing organ. The accelerated vegetative growth of the fed plants may have been achieved owing to a considerable quantity of assimilated nitrogen, as well as to the biologically active components of the vermicompost (Canellas et al. 2002). Other causes could be the improved structure and mechanical properties of the vermicompost-enriched soil (Wang et al. 2010). The vermicompost also influenced the generative development of the plants. The number and mass of formed leaf buds were much higher in the fed plants. The stimulating effect of the vermicompost on the plants' growth continued until the end of the reported period (Table 3). The additional enrichment of the soil with the vermicompost extract had a positive effect on the growth indices. They improved but were lower than determined for the plants fed once with a higher level of Lumbrical.

The results show (Table 4) that on the 30th day after feeding with Lumbrical, the content of the N-element was within the optimal range. This means that the plants (including the control) were grown under favourable conditions, which could have been expected considering the fact that the preceding crop was wheat. On the 60th day, the nitrogen content fell beyond the optimal range in leaves of both the control and the fed plants. Regardless of the fluctuations in the N balance, a positive effect of Lumbrical was observed throughout the entire reported period. The additional enrichment of the soil with the vermicompost extract increased the nitrogen level in the fed plants. Similar results were achieved by other authors as well. The

Table 3

Effect of Lumbrical vermicompost on some growth parameters of pepper plants 60 days after vermicompost applied

Parameters \ Variants	1*	2	3	4
Plant height (cm)	41.8 *a***	45.5 *b*	45.5 *b*	45.8 *b*
Crown diameter (cm)	28.7 *a*	35.3 *b*	39.1 *c*	39.8 *c*
Leaf number	79.0 *a*	126.0 *b*	137.2 *bc*	154.0 *c*
Leaf area (dm^2)	7.7 *a*	20.0 *b*	20.2 *b*	24.1 *c*
Leaf weight (g)	24.7 *a*	60.7 *b*	61.9 *b*	73.1 *c*
Ovarium number	6.8 *a*	7.8 *a*	12.7 *b*	15.7 *c*
Ovarium weight (g)	1.0 *a*	1.6 *ab*	2.8 *ab*	3.0 *b*
Fruits number	11.8 *a*	13.5 *a*	16.5 *b*	18.3 *b*
Fruit weight (g)	92.0 *a*	270.3 *b*	269.8 *b*	304.9 *c*

*1 – control, soil without vermicompost; 2 – soil fertilized with vermicompost, 50 cm^3 per plant before planting; 3 – soil fertilized with vermicompost, 50 cm^3 per plant before planting and vermicompost extract, 200 cm^3 per plant after planting; 4 – soil fertilized with vermicompost, 100 cm^3 per plant before planting;
** Values in columns followed by the same letter do not differ significantly at $p = 0.05$.

Table 4

Effect of vermicompost on nitrogen (N) concentration (g kg^{-1}) and nitrate reductase activity (µg NO_2^- g^{-1} h^{-1}) in the first leaf under the first node (30 days) and 1st leaf under the second node (60 days) of pepper plants

	N (g kg^{-1})		NR (µg NO_2^- g^{-1} h^{-1})	
Variants	30 days	60 days	30 days	60 days
1*	36.7 *a*	22.4 *a*	82.0 *a*	60.0 *a*
2	38.5 *a*	24.1 *a*	94.0 *b*	61.3 *a*
3	-	26.1 *a*	-	77.5 *ab*
4	42.2 *a*	29.9 *a*	114.0 *b*	81.0 *b*

*Key: see Table 3

liquid vermicompost extract increases the nitrogen content in leaves of tomato plants (TEJADA et al. 2007). SAINZ et al. (1998) indicate that addition of vermicompost to soil leads to increasing the content of the element in substrate and higher N concentrations in cucumber leaves.

Nitrate reductase is the first enzyme which limits nitrogen absorption by plants (CAMPBELL 1999). Its activity is often used as an indicator of plants' ability to utilize NO_3^- from soil (BARFORD, LAJTHA 1992). It was found that in leaves of the fed plants the activity of nitrate reductase increased parallel to the increasing levels of Lumbrical. On the 60th day, this stimulating effect was weaker, probably due to a decrease in the nitrate content in leaves. The additional enrichment of the soil with the vermicompost extract influenced the activity of nitrate reductase. The activity of the enzyme increased by 29.2%. This corresponded to higher nitrogen levels reported in leaves of the additionally fed plants. The higher activity of nitrate reductase suggests that these plants were better at utilizing nitrate ions of the soil. Similar results have been reported in tomatoes by DODDEMA et al. (1986), manioc (CRUZ et al. 2004) and coffee (DAMATTA et al. 1999), fed with organic products.

When comparing the nourishment regime at the beginning of vegetation (Table 1) and 30 days after the soil had been enriched with Lumbrical (Table 5), it appears that the nitrogen content in the plants is slightly reduced (28.0-22.6 mg kg^{-1}). After the incorporation of the vermicompost, the level of N in the soil increased by 102.5% compared with the control. The increased level of the element was mostly achieved by nitrate nitrogen. A higher level of nitrogen than in the control variant continued during the entire reported period.

A considerable increase in the nitrogen level in the soil enriched with Lumbrical was most probably induced by the active influence of nitrogen-fixing bacteria contained in the vermicompost (ANSARI 2008a, b). This confirmed the results obtained by PATTIL (1993), who reports that eight weeks after enrichment of soil with the vermicompost Lumbrical, it contained 75 mg kg^{-1} nitrate nitrogen (67% higher compared with the control).

A comparison of the nitrogen values at the beginning and at the end of the reported period shows certain reduction in N quantities, which can be explained by some specific features of the pepper and its yields. Pepper belongs to annual plants whose vegetative growth continues parallel to the flowering and fruiting. This is why absorption of mineral elements (including nitrogen) continues almost throughout the entire vegetation period. The additional application of the vermicompost had a positive effect on the nitrogen content in the soil. The higher content of N-element in the soil enriched with the vermicompost (Table 5) as well as its higher concentration in leaf tissue of the fed plants (Table 4) allow us to suggest that the improved plant growth may be directly related to the nutritional effect of the vermicompost (TOMATI et al. 1988).

The results on the influence of Lumbrical on the photosynthetic rate (Table 6) showed that the vermicompost had a positive effect on this process. On the 30th day after the enrichment of the soil, the rate of net photosynthesis increased by 17.3-22.2%. An increase in the transpiration rate was not mathematically proven. On the 60th day after the application of the

Table 5

Dynamics of nitrogen in the soil (mg kg^{-1} soil)

Variants	Mineral nitrogen (mg kg^{-1} soil)					
	30 days			60 days		
	NH_4^+	NO_3^-	total	NH_4^+	NO_3^-	total
1*	3.6 *a*	19.0 *a*	22.6 *a*	6.6 *a*	9.9 *a*	16.5 *a*
2	3.9 *a*	39.0 *b*	42.9 *b*	15.0 *bc*	22.8 *b*	37.8 *b*
3	-	-	-	14.1 *b*	26.1 *c*	40.2 *b*
4	4.8 *a*	43.8 *c*	48.6 *c*	17.4 *c*	22.8 *b*	40.2 *b*

*Key: see Table 3

Table 6

Gas exchange in leaves of pepper plants;1st leaf under the first node (30 days) and 1st leaf under the second node (60 days)

Variants	P_N (µmol $m^{-2}s^{-1}$)		E (mmol $m^{-2}s^{-1}$)		g_s (mol $m^{-2}s^{-1}$)	
	30 days	60 days	30 days	60 days	30 days	60 days
1*	17.56 *a*	14.19 *a*	2.83 *a*	3.01 *a*	0.11 *a*	0.14 *a*
2	20.60 *b*	20.03 *b*	3.29 *a*	3.36 *a*	0.18 *b*	0.25 *a*
3	-	19.58 *b*	-	4.35 *a*	-	0.18 *b*
4	21.45 *b*	20.36 *b*	3.25 *a*	3.65 *a*	0.19 *b*	0.30 *b*

P_N – net photosynthesis; E – transpiration rate; g_s – stomatal conductivity
*Explanations: see Table 3

vermicompost, the same regularities were observed as in the earlier period. The amount of solar energy and CO_2 assimilated by the plants depends on the photosynthetic leaf area and on the rate of CO_2 fixation, i.e. on the photosynthetic rate (JAIN et al. 1999). Our results (Tables 2-3 and Table 6) showed that in this respect the vermicompost Lumbrical had a positive effect on the plants.

Table 7 shows that the yield of the plants treated with the vermicompost Lumbrical was higher than that of the control sample. A tendency for increase in the average fruit mass and the pericarp thickness (the main material for preparation of grinded pepper) was observed. Higher yields under the influence of vermicompost have been recorded in other crops: aubergine and carrots (TOMAR et al. 1998), tomatoes (TRINGOVSKA et al. 2005) and potatoes (MRINAL-SAIKIA et al. 1998). According to some authors (ARANCON et al. 2004), there is no positive correlation between the yield and content of mineral nitrogen in the vermicompost or in the plant. However, the observed increase

Table 7

Pepper yield (raw material for drying) and quality parameters of fruits

Variants	Total yield (kg ha^{-1})	Number of fruits per plant	One fruit weight (g)	Pericarp thickness (mm)	Absolute dry substance in the material (%)	Pigment content (ASTA units)
1*	8898 *a*	11.0 *a*	15.2 *a*	1.9 *a*	9.95 *a*	148 *ab*
2	9619 *b*	13.5 *ab*	15.3 *a*	2.5 *ab*	10.62 *a*	142 *a*
3	10 635 *c*	13.9 *b*	16.8 *ab*	2.4 *ab*	11.13 *a*	165 *ab*
4	11 202 *d*	14.4 *b*	16.9 *ab*	2.7 *ab*	11.27 *a*	170 *b*

*Key: see Table 3

in the yield may be explained by a richer population of useful soil microorganisms and the increased intensity of the processes in which they are involved, which in its turn, preconditions better plant nutrition.

The content of absolute dry substance and the content of pigment in plant material are important indices of the quality of red pepper for grinding (TODOROVA et al. 1999). In the current study, dry substance in raw material increased by 13.3% and the pigment content rose by 14.9% in response to a single treatment of plants with a higher level of the vermicompost.

CONCLUSIONS

1. Feeding pepper plants with the vermicompost Lumbrical increased their vegetative mass and leaf area and leads to enhanced development of their generative organs.

2. Incorporation of the vermicompost Lumbrical in the soil increased the content of N-element in the root area.

3. Feeding with the vermicompost considerably increased nitrogen content in the leaves of pepper plants, and there was a positive correlation between the incorporated dose and the activity of the enzyme *nitrate reductase*.

4. The positive effect of the vermicompost on the functional activity of the photosynthetic apparatus was expressed through an improved leaf gas exchange parameters.

5. The yield was positively influenced by the vermicompost Lumbrical. A clear increasing tendency in the average fruit mass and the pericarp thickness (the main material for preparation of grinded pepper) was observed. The absolute dry substance and pigment content was higher in the variants treated with the vermicompost.

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