

# Using *Trichoderma viride* for Optimization of Vermicomposting Processes to Improve the Quality of Vermicompost and Prolong the Storage Period

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**Abstract** The performed study demonstrates that a preliminary fermentation of organic substrate with *Trichoderma* is promising, since this allows the vermicultivation time to be reduced and the yield and quality of vermicompost to be elevated. Vermicompost obtained after pre-fermentation of organic substrate with *Trichoderma* has the maximal indicators of growth-stimulating activity and the highest level of fungistatic properties in relation to root rot pathogens. This variant of vermicompost maintains the highest level of biological activity even after 8 months of storage. During the storage period growth-stimulating activity of vermicompost changes cyclically. The level of growth promoting activity of vermicompost is greatly influenced not only by period of storage, but also by the stage of organic matter transformation in vermicompost at the end of the process of vermicultivation. Excessive prolongation of vermicultivation causes a decrease in its biological activity.

**Keywords** Vermicompost, Vermicultivation, *Trichoderma viride*, Introduction, Growth-stimulating activity, Fungistatic activity, Storage period

## 1. Introduction

The efficiency of introduction of the active fungal strains belonging to the genus *Trichoderma* into the plant rhizosphere, as well as preplanting inoculation, has been demonstrated in numerous studies [1–3]. Despite the fact that preparations involving *Trichoderma* as the major component is a real alternative to synthetic agrichemicals as antagonists of plant soil diseases and growth stimulators, their commercial application presents certain difficulties. Application of such preparations does not always give stable results, especially under field conditions [4]. Ecological studies have shown that fungal strains belonging to the genus *Trichoderma* can not always be successfully introduced into the soil microbial community due to their low competitiveness and high demand for available sources of carbon. For this reason, their influence as biological control agents is limited to a few weeks after introduction [5].

Besides some technological problems caused by short shelf life of the liquid form of the preparations based on *Trichoderma* frequently take place. Whereas the use of the solid preparation form often causes the problem of autoinhibition of conidia germination. For this reason, there is a need for a careful calculation of the optimal dose for each

particular strain [6].

Therefore, development of multifunctional biological preparations stable in their efficiency, including the preparations involving *Trichoderma*, is among the most important tasks in the system of developing organic crop farming. In order to maintain a stable population of *Trichoderma* in the soil during the growing season, some authors propose to perform the application of *Trichoderma* simultaneously with the introduction of various organic substrates: wheat bran, peat moss, pine bark, compost from cow manure and even waste substrates after oyster culturing [7, 8].

In our opinion, a joint application of *Trichoderma* and vermicompost is promising technological method to raise *Trichoderma* survival in the soil and fungal colonization of the plant rhizosphere. On the other hand, since the quality of vermicompost depends on the content and activity of the agronomically valuable microflora, vermicompost enrichment for the active microbial strains responsible for fungistatic, growth-stimulating, and other beneficial properties can have a positive effect on the efficiency of its use. It is also known that many species of the genus *Trichoderma* are able to accelerate the composting rate of organic substrates due to their ability to transform the lignin–cellulose complex with their cellulolytic enzymes, which, in turn, enriches the final product for nutrients [9].

In accordance with the above arguments the goal of this work was to improve the technology for producing vermicompost by preliminary fermentation of organic

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substrate using the fungus *Trichoderma viride* with the aim to reduce the time required for vermicultivation and to elevate the growth-stimulating and fungistatic activity of vermicompost.

## 2. Experimental

Objects of study were the earthworm *Eisenia andrei* and a pure culture of the saprophytic fungus *Trichoderma viride*, isolated by the authors from pine wood. The growth-stimulating activity of vermicompost was determined by biological tests and vegetation experiments with the wheat cultivar Tulunskaya 12.

The earthworms were cultivated in a peat–manure mixture (PMM) composed of fen peat from the Kandinskoe deposit and cattle litter manure at a ratio of 1: 4 (wt/wt) with 40 mature earthworm individuals (30 g) added per 1 kg substrate. The moisture content in the substrate was maintained at a level of 80% and temperature was maintained in the range of 20–25°C.

The model experiment comprised several variants, namely, (1) traditional vermicultivation on PMM for 33 days (VC-1); (2) vermicultivation on PMM after preliminary solid-phase cultivation of the fungus *T. viride* for 11 days, with the period of vermicultivation per se of 22 days (VC-2); (3) vermicultivation on PMM for 33 days with concurrent introduction of the fungus *T. viride* into the substrate (VC-3); (4) PMM composting with introduction of the fungus without vermicultivation (PMM<sub>Tr</sub>).

During vermicultivation the efficiency of the initial substrate conversion into compost by earthworms was determined in all experimental variants. For this purpose, the fractions of coprolite were separated using 3- and 5-mm sieves to calculate the percent rate of each fraction.

In the vegetation experiment, peat supplemented with 25% of PMM was used as a control. The experimental soil variants were composed of the peat supplemented with 25% of the corresponding vermicompost variants (VC-1, VC-2, or VC-3) or 25% of the PMM<sub>Tr</sub> relative to the total weight of the prepared substrate. The experiment was performed in seven replicates using plastic containers (500 ml) with six wheat grains planted in each. The wheat was grown at a temperature of 25 ± 1°C in a phytochamber with daylight of 15 h.

Biological activity of vermicompost was assessed according to the increase in wheat green mass (dried to a constant weight) expressed as percent of the control. Growth-stimulating activity was determined by biological testing of wheat seeds soaked for 5 min in an acid extract (pH 4–4.5) of vermicompost. Stimulatory effect was assessed according to the difference between the dry weights of green shoots and roots in the experimental and control (with distilled water) variants expressed as percent of the control.

*T. viride* enrichment culture was produced on wheat grain preliminary boiled to softness in a small amount of water. The grain was placed into bottles; the bottles were stopped

and twice sterilized in an autoclave at 1 atm for 30 min with an interval of 1 day. Then, the tubes with the grain inoculated with *T. viride* were kept in a thermostat at a temperature of 28–30°C until fungal mycelium completely covered the grain. The produced enrichment culture was introduced into the substrate in an amount of 2% of the PMM weight in the container. Since the fungal population on the PGA (potato–glucose agar) medium was reduced due to active growth of the colonies and competition for the substrate, the *T. viride* during vermicultivation was counted on MPA (meat–peptone agar) [10].

Vermicomposts and initial organic substrate were chemically assayed for the following components: nitrate–nitrogen using phenol disulfonic acid (state standard GOST 26488-85), ammonium nitrogen using the Nessler reagent (GOST 26489-85), labile phosphorus according to Kirsanov (GOST 26207-91), and exchange potassium according to Kirsanov using flame photometry (GOST 26207-91).

To assess the impact of the storage period on the vermicomposts quality (growth-stimulating and fungistatic activity) the model experiment with the cultivation of wheat seedlings in peat containing soil was conducted after 3 months of storage. All variants of vermicompost were stored at 5°C. In each experimental variant the soil was supplemented with 25% of previously obtained corresponding vermicompost variants (VC-1, VC-2, VC-3). As a control we used the soil supplemented with 25% of peat–manure mixture (PMM). To evaluate the level of fungistatic activity of vermicomposts the infectious load was artificially modeled in the experiment by adding mycelium and spores of *Bipolaris sorokiniana* into the soil as a part of the chopped agar medium Capek. The model experiment include the following variants: (1) Soil + VC-1; (2) Soil + VC-1 + *Bipolaris sorokiniana*; (3) Soil + VC-2; (4) Soil + VC-2 + *Bipolaris sorokiniana*; (5) Soil + VC-3; (6) Soil + VC-3 + *Bipolaris sorokiniana*. The experiment was performed in five replicates using plastic containers (500 ml) with six wheat grains planted in each. The wheat was grown at a temperature of 25 ± 1°C in a phytochamber with daylight of 15 h.

Data were statistically processed using Statistica v. 5.5 and 6.0 software packages [11]. In the paper, the data are presented as a mean value with a confidence intervals for variance and standard deviation calculated using Student's test ( $p < 0.05$ ). Data on wheat growth in the vegetation experiment were compared according to the nonparametric Mann–Whitney test ( $p < 0.05$ ).

## 3. Results and Discussion

Determination of the efficiency of organic substrate conversion by earthworms have demonstrated that preliminary fermentation of the substrate with *T. viride* (VC-2) allowed the yield of vermicompost to be elevated by 8.5% as compared with traditional vermicultivation (VC-1)

as early as day 11 (Table 1).

By the end of the experiment, preliminary fermentation (VC-2) provided for 88.7% yield of vermicompost, which is approximately 4.0% higher compared with traditional vermicultivation. Thus, in this case the period of vermicultivation alone providing a high percent yield of vermicompost can be reduced to 11 days.

For comparison in variant with traditional vermicultivation (VC-1) it takes at least 22 days. The total period of the organic substrate conversion by *T. viride* and earthworms takes no more than 22 days (since the periods when fungi and earthworms are used overlap).

Decrease in the period of vermicultivation alone as the most energy- and resource-consuming process can decrease the net cost of vermicompost and increase the overall economic efficiency of vermicultivation.

Consequently, solid-phase bioconversion of the substrate, which is a modern and promising approach not requiring considerable expenses, makes it possible to more rationally utilize vermicultivation facilities and provide the necessary conditions for both the growth of *Trichoderma* and bioconversion of the substrate. Such effect was earlier described for wastewater sediments [12].

The results of the vegetation experiment and data of the biological test demonstrate that decrease in the period of vermicultivation did not have a negative effect on the quality of the produced vermicompost, since all the mean values of growth-stimulating characteristics in the variant with preliminary composting of the substrate by *T. viride* (VC-2) were higher compared with traditional vermicultivation (VC-1) (Table 2). However, differences between the variants

were statistically insignificant.

Results of the vegetation experiment with wheat demonstrate that the use of vermicomposts VC-2 and VC-3, as well as the PMM inoculated with *T. viride* (PMM<sub>Tr</sub>), led to a statistically significant increase in wheat green mass relative to the control variant of experiment (without VC or PMM<sub>Tr</sub>). In addition, variants VC-3 and PMM<sub>Tr</sub> demonstrated a statistically significant positive effect as compared with variant VC-1 (by 20 and 31%, respectively) (see Table 2).

The most pronounced positive effect in the vegetation experiment was observed in the variant with PMM<sub>Tr</sub>. However, this effect was unobservable in the biological test with PMM<sub>Tr</sub> acid extract and the total content of mineral nitrogen (N-NH<sub>4</sub> + N-NO<sub>3</sub>) in the variant with PMM<sub>Tr</sub> was minimal (Figure 1, Table 3). Presumably, this suggests that the formation of a certain fungal hyphosphere in the root-inhabited layer caused the plant stimulation rather than the substances produced by *Trichoderma* that accumulated in the substrate.

As is known, hyphae during their growth release into the rhizosphere various amino acids, vitamins, organic acids, enzymes, and other compounds, thereby constantly stimulating plant growth [13].

However, this variant of compost in both the agrochemical characteristics and results of biological test displayed a considerably lower efficiency as compared with vermicomposts (see Figure 1 and Table 3). In addition, the *T. viride* population in the variant of compost without earthworms was higher than in the vermicomposts (see below).

**Table 1.** Yield of vermicompost at different stages of vermicultivation

Variants of experiment	Period of cultivation*, days		Yields of vermicompost fractions (coprolite, fraction 3 + 5 mm), % of initial PMM
	Trichoderma	Earthworms	
Traditional vermicultivation on PMM (VC-1)	—	11	67.8
	—	22	84.5
	—	33	85.0
Vermicultivation on PMM after preliminary <i>T. viride</i> cultivation for 11 days (VC-2)	11	—	—
	22	11	76.2
	33	22	88.7
Vermicultivation on PMM with concurrent <i>T. viride</i> inoculation (VC-3)	11	11	67.2
	22	22	82.5
	33	33	87.3

Note: (—) without *T. viride* inoculation into vermicompost;

\* For variant VC-2, the total cultivation time is not equal to the sum of fungus and earthworm cultivation times (see Experimental).

**Table 2.** Effect of vermicomposts on the formation of wheat green mass

Variants of experiment	Wheat green mass, mg	Increase in green mass relative to control, %	Increase in green mass relative to VC-1, %	Number of plants with four leaves, % of total number of leaves
Control (peat + PMM)	33.28 ± 1.62	—	—	6.06
Peat + VC-1	36.54 ± 2.50	9.8	—	9.75
Peat + VC-2	40.81 ± 1.98	22.62	11.68	12.50
Peat + VC-3	43.87 ± 1.91	31.82	20.06	30.95
Peat + PMM <sub>Tr</sub>	47.92 ± 3.55	44.02	31.14	54.76

**Table 3.** Content of nutrient elements in the composts produced in different variants of experiment (see Experimental), mg/kg of absolutely dry substrate

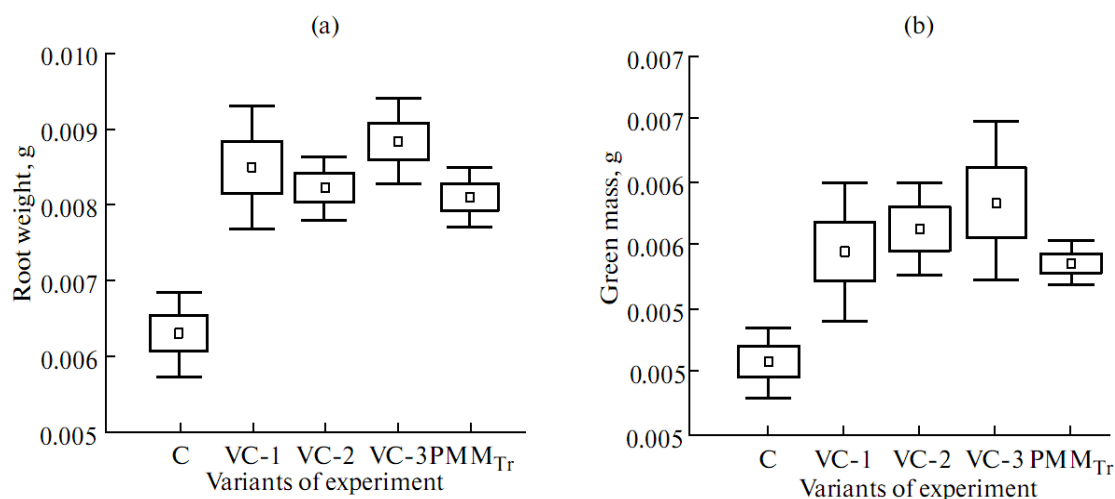
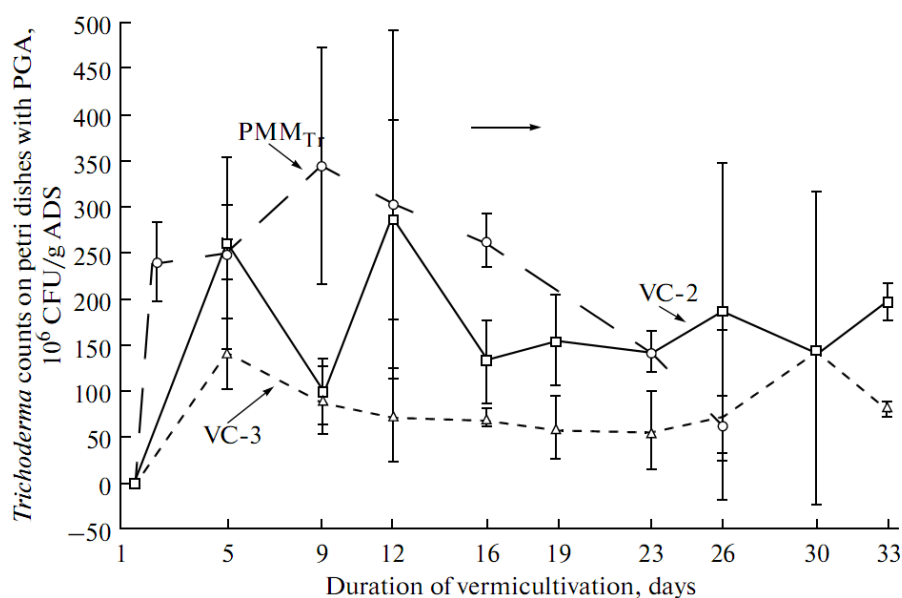
Variants of experiment	N-NH <sub>4</sub>	N-NO <sub>3</sub>	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
Initial PMM	262	120	8556	4850
VC-1	137	869	9954	6120
VC-2	91.5	1554	13447	8027
VC-3	194	1170	8671	6558
PMM <sub>Tr</sub>	212	470	10620	6511

Wheat plants grown on vermicomposts modified with *T. viride* (VC-2 and VC-3) exceeded, in dry weight, the plants grown on traditional vermicompost (VC-1) (see Table 2). In addition, the stimulatory effect of Trichoderma in these variants appeared in the maximal number of plants with four

completely developed leaves as compared with the control and VC-1 (see Table 2).

Comparison of three vermicompost variants has demonstrated the most pronounced positive effect of VC-3, produced by concurrent inoculation with *T. viride* and earthworm cultivation (see Table 2). The stimulatory effect of Trichoderma, observed in the experiment, agrees with the data of several researchers [1, 14–16].

According to the data of the biological test shown in Fig. 1, treatment with acid extracts prepared from vermicomposts and PMM<sub>Tr</sub> provided for a statistically significant increase in the wheat green mass and root weight relative to the control. However, the differences between the experimental variants were statistically insignificant except for the wheat green mass in variants VC-3 and PMM<sub>Tr</sub> (see Figure 1a).

**Figure 1.** Efficiency of acid extracts derived from the vermicompost variants (VC-1, VC-2, VC-3) and compost produced by *T. viride* fermentation (PMM<sub>Tr</sub>) on the root weight (a) and green mass (b) of wheat seedlings**Figure 2.** Dynamics of *T. viride* population in the vermicompost variants (VC-1, VC-2, VC-3) and compost produced by fermentation of organic substrate with *T. viride* (PMM<sub>Tr</sub>)

Despite the fact that statistically significant differences between the vermicompost variants in vegetation experiment and bioassay were not obtained the increase in green mass of wheat seedlings in the sequence VC-1 < VC-2 < VC-3 was observed in both experiments, probably due to the increase in the total positive impact of the joint effect of earthworms and *Trichoderma* (see Figure 1b).

The greatest stimulating effect in the biological test was obtained by treating wheat seeds with the acid extract of VC-3 (see Figure 1), suggesting that this particular vermicompost variant accumulated the largest amount of biologically active substances produced during vermicultivation.

Thus, the growth-stimulating activity of vermicompost logically increases with the duration of earthworm and *T. viride* cultivations (see Figure 1). In this process, a synergistic effect of earthworms, the microflora associated with them, and fungi on the formation of vermicompost growth-stimulating characteristics is observed.

According to the results of microbiological analysis, the number of *Trichoderma* conidia in PMM<sub>Tr</sub> over the entire composting period was higher compared with variants VC-2 and VC-3, which could further lead to a more pronounced growth-stimulating effect, evident from Table 2. This assumption is confirmed by L.V. Kolombet [1], who demonstrated that the growth-stimulating effect depends on the amount of *Trichoderma* conidia introduced into the soil mixture.

Data shown in Figure 2 demonstrate that the fungal population in the variant with PMM<sub>Tr</sub> was maintained at a high level for the first 16 days and then (by day 26) decreased fourfold (from  $2.9 \times 10^8$  to  $0.7 \times 10^8$  CFU/g ADS (absolutely dry substrate), presumably due to a decrease in the amount of accessible substrate. Correspondingly, decrease in *Trichoderma* population can be used as a criterion indicating completion of the preliminary substrate fermentation and its readiness for further vermicultivation.

Agrochemical analysis (see Table 3) has demonstrated that the contents of labile potassium and phosphorus species increased in all the examined vermicompost variants and PMM<sub>Tr</sub>, presumably, due to production of acids and enzymatic activity of the microorganisms in the organic substrate and earthworm digestive tract [17]. Note that the vermicompost obtained from substrate previously fermented by *Trichoderma*, VC-2, displayed maximal contents of labile potassium and phosphorus. This fact suggests a deeper transformation of the lignocellulose complex of the converted substrate in the case of its preliminary fermentation with *Trichoderma*. This phenomenon can be explained by the higher number of the fungus in the initial stages of processing in the absence of earthworms as a rule eating fungal mycelium that provide the highest number of *Trichoderma* in this variant of vermicompost (VC-2).

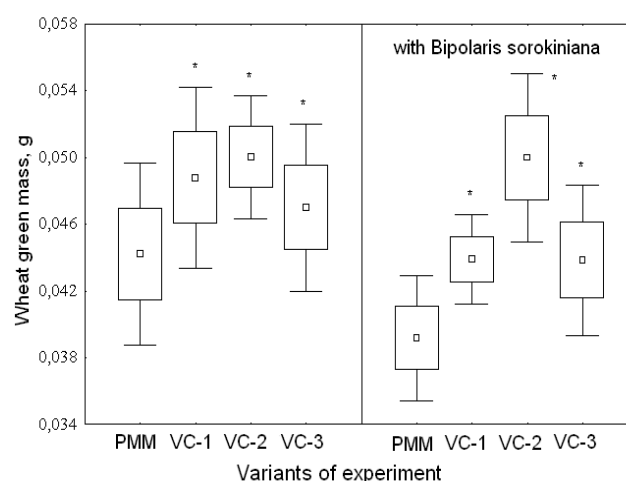
As is evident from Table 3, the content of nitrate nitrogen in variants VC-2 and VC-3 was higher as compared with VC-1, respectively, by almost 79 and 35%, presumably, owing to a higher level of degradation of the organic material

by the fungus *T. viride*. This agrees with the data of P. Pramanik [18], who observed an increase in the content of total nitrogen in the vermicompost produced using inoculation of the substrate with ligninolytic fungi (*T. viride* and *Phanerochaete chrysosporium*).

For the practical vermicompost application as biologically active fertilizer one of the most important tasks is the need to control its quality throughout the storage period. Publications analysis has shown that this question is still poorly understood. To assess the effect of storage period on the fungistatic and growth-stimulating activity of vermicomposts the model experiment with the cultivation of wheat seedlings in peat containing soil was conducted after 3 months of storage.

The results have shown that all three variants of vermicompost kept a high level of growth-stimulating activity: their use as an organic fertilizer provided a significant increase in wheat green mass relative to the control even after 3 months of storage.

Note that the difference with the control was most noticeable in the variants with infectious load (*B. sorokiniana*). The greatest effect was obtained by using VC-2, previously obtained by pre-fermentation with *T. viride*. Without infection load statistically significant differences between the vermicompost variants was not found (Figure 3).



Note: \* – significant difference from PMM (by Mann-Whitney test), 95% significance

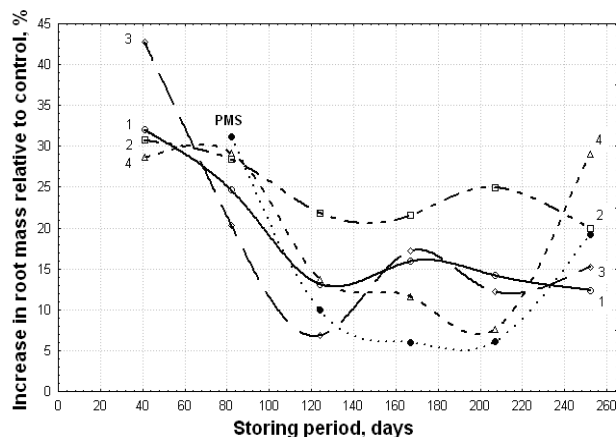
**Figure 3.** Effect of vermicomposts (VC-1, VC-2, VC-3) on the formation of wheat green mass after 3 months of storage

The results of experiment have demonstrated that all the investigated variants of vermicompost even after 3 months of storage were possessed of fungistatic activity against *B. sorokiniana* in a different degree. Number of plants with symptoms of *Helminthosporium* lesions decreased by 12.0, 99.5 and 41% respectively compared with the control (soil fertilized with PMM). Microbiological analysis (culturing on Czapek) has shown a high degree of conservation of *T. viride* in all variants of vermicompost. The number of *T. viride* propagules in the variant with VC-2 was  $15.9 \times 10^6$  CFU / g, in the variant with VC-3 –  $11.8 \times 10^6$  CFU / g.

According to some data it is necessary to maintain a titer of *Trichoderma* strains not less than  $10^5$ – $10^7$  CFU / g of the soil for high efficacy of preparations based on fungi of the genus *Trichoderma* [19, 20]. While the lower number of *Trichoderma* propagules does not always provide suppression of phytopathogenic fungi. For example, the reason for the low efficiency of strain T382 in the field experiment was the actual number of *Trichoderma* corresponding  $10^3$  CFU / g of the soil [21].

Thus, the results of the modeling vegetation experiment suggests that maintaining a high level of vermicompost fungistatic activity during the first 3 months of storage is greatly influenced by vermicomposting technology. The greatest values of fungistatic and growth-stimulating activity in the case of using VC-2 obtained by pre-fermentation an organic substrate by *Trichoderma* indicate that the introduction of *T. viride* into vermicompost has a positive effect.

In addition to the vegetation experiment, the level of growth-stimulating activity of 3 different vermicomposts (VC-1, VC-2, VC-3) and compost based on *Trichoderma* (PMM<sub>Tr</sub>) was investigated by soaking wheat seeds in slightly acidic extracts from studied kinds of vermicomposts (according to our preliminary studies [22], the main growth stimulants in vermicompost are just products of acid hydrolysis and not only the substance of the humic nature). Investigations were carried out in the dynamics by taking samples to obtain extracts every 6 weeks (since the beginning of storage). Wheat seeds were treated by soaking in a five-minute extract. As a control, distilled water was used. The results have shown that the dynamics of the activity of acid extracts, both in relation to the green mass and to the weight of the roots of wheat seedlings was cyclical (Figure 4, Figure 5).



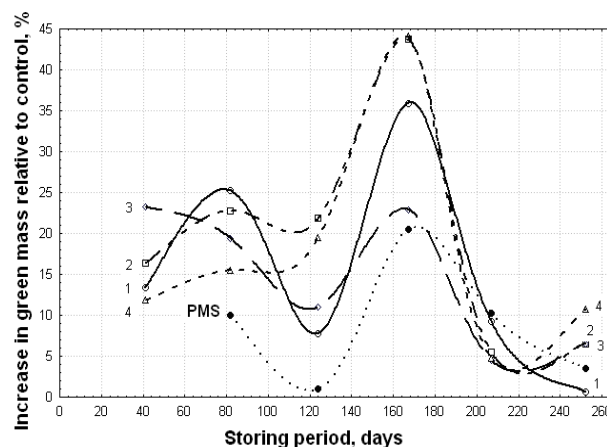
**Figure 4.** Dynamics of the activity of acid extracts derived from vermicomposts (1 – VC-1, 2 – VC-2, 3 – VC-3) and compost produced by *T. viride* fermentation (PMM<sub>Tr</sub>) in relation to the roots of wheat seedlings compared with the activity of acid extracts derived from initial peat-manure substrate (PMS) within 260 days of storage

The cyclical dynamics of growth-stimulating activity in vermicomposts most likely related to the cyclical processes of the metabolic activity of the microflora carrying out the

mineralization of biologically active substances (BAS) and at the same time the producing BAS, which are directly responsible for the growth-stimulating properties of vermicompost.

Synthesis and degradation processes occur in a certain regular sequence and lead to fluctuations in the level of growth-stimulating activity. However, since vermicompost added to the soil as fertilizer is contacted with the plants for a long time cyclical dynamics of growth-stimulating activity doesn't reflect negatively on the effectiveness of its application. For all the growing season plants have time to go through some peaks of growth-stimulating activity.

Decrease in the activity of acid extracts for 17 and 31 weeks of storage is probably a consequence of the gradual utilization of low molecular weight organic matter which are readily available for microorganisms and include growth stimulants. Almost the same impact of acid extracts on wheat green mass in variants with compost of *Trichoderma* and VC-2 (vermicompost obtained by *Trichoderma* pre-fermentation) indicates that the great role in maintaining the growth-stimulating activity play the metabolism of *Trichoderma* which is also capable to produce biologically active substances as well as the microflora of the vermicompost.



**Figure 5.** Dynamics of the activity of acid extracts derived from vermicomposts (1 – VC-1, 2 – VC-2, 3 – VC-3) and compost produced by pre-fermentation of substrate with *T. viride* (PMM<sub>Tr</sub>) in relation to the wheat green mass compared with the activity of acid extracts derived from initial peat-manure substrate (PMS) within 260 days of storage

Among other vermicompost variants VC-2 was demonstrating the highest rates of growth-stimulating activity for the whole study period probably due to the shorter period of vermicomposting and maintaining the high level of BAS (see Figure 4 and Figure 5).

These data allow proposing that unreasonably long processing substrate by earthworms not only promotes the degradation of high molecular compounds but also causes mineralization of low molecular weight compounds having a growth promoting activity. On this basis, we can conclude that there is a need to identify the completion point of vermiculture to maintain the high quality of the finished product – vermicompost.

## 4. Conclusions

Thus, a preliminary composting of peat–manure mixture using *Trichoderma viride* is a promising technological method for accelerating the conversion of organic substrate during vermicultivation, which enhances an increase in production efficiency. An increase in the rate of organic substrate conversion does not decrease the quality of the produced vermicompost but, on the contrary, enhances its enrichment for nutrients and increase in its growth-stimulating activity.

High level of fungistatic activity of vermicompost persists for at least 3 months from the beginning of the storage period. The maximum level of resistance of wheat seedlings against root rot (*Helminthosporium*) has VC-2 obtained after preliminary fermentation substrate by *Trichoderma*.

Growth-stimulating activity of vermicompost remains high, at least for 260 days from the beginning of the storage period. During the storage period growth-stimulating activity of vermicompost changes cyclically.

The level of growth promoting activity of vermicompost is greatly influenced not only by period of storage, but also by the stage of organic matter transformation in vermicompost at the end of the process of vermicultivation. Definition and strict adherence to the optimal period of vermicultivation ultimately provides a production of vermicompost characterized by high level of growth-stimulating activity and high stability during period of storage. Excessive prolongation of vermicultivation causes a decrease in its biological activity.

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