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Soil Improvement Using Earthworm Compost Elutriate

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Abstract
Earthworm compost is known to be of higher benefit for plant nutrition and soil improvement than material deriving from a common thermophilic composting process. Beneficial compost contents, especially soluble nutrients and mesophilic micro-organisms can be elutriated from the solid matrix and applied as aqueous slurry. The composition of the micro-organism population in the elutriate can be influenced by substrate addition during the elution process. The final product has an immediate nurturing effect and is of particular advantage in all those situations where an addition of solid compost is not possible. In a current research project the reason for the described and observed positive effect of compost elutriate has been investigated by means of chemical, microbiological and molecular biological analysis. In parallel, the brewing process (elutriation) has been optimised for a reproducible, stable product. First applied results confirm a change of the micro-organism population in soil and a slightly increased plant growth under normal greenhouse conditions. Studies on soil fertility, plant health and crop yield in field studies will complement the laboratory analysis.

1 Introduction
Generally, compost elutriate (= 'compost tea') is produced by placing a defined quantity of compost into water and mixing it for a definite time. Different types of compost teas are available (Scheuerell and Mahaffee, 2004):

- (actively) aerated compost tea ((A)ACT): no anaerobic periods or zones occur during production
- non-aerated compost tea (NCT): aeration is not provided during the extraction process
- teas with additives: these additives (normally added at the beginning of the brewing process) should increase microbial cell density and activity.
- teas without additives

The production of actively aerated compost teas with additives is recommended (Ingham and Rollins, 2006) to enhance the growth of beneficial microorganisms – too little oxygen (below 6 ppm) would encourage the growth of pathogens.

During the brewing process, microorganisms, together with all soluble nu-

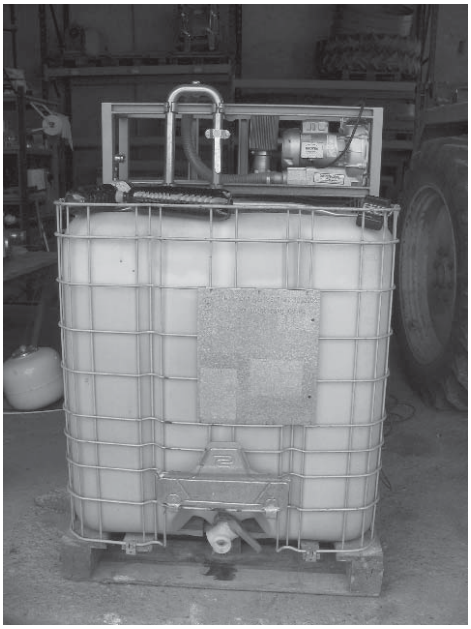


Fig. 1: Compost tea machine used for the experiments (1000 l)

trients from the used compost are extracted into the tea. The number of (beneficial) microorganisms increases during production due to the nutrients supplied and the mesophilic temperature.

For these purposes, special compost tea machines are available on the market (Figure 1). They consist of a container, a 'tea sock' and an aeration system. The compost is put into the sock and hung in the container filled with water. The aeration system provides the necessary oxygen supply and a thorough mixing.

Vermicompost is the product of the digestion of organic material (conventional compost after the thermophilic stage) by earthworms (*Eisenia foetida*, *Eisenia andrei*, *Lumbricus rubellus*, e. g.). The worms excrete casts that are reported to have positive effects on plant growth exceeding those produced by compost or commercial potting mixture amendments (Chaoui et al., 2002). The reasons are not well defined and range from improved physical soil structure, plant growth hormones and highly diverse microbial populations to higher levels of soil enzymes.

Nutrient contents in vermicompost have been shown to be much higher than in traditional compost (Dickerson, 2001), with a high level of phosphorous being available in casts (Le Bayon and Binet, 2006).

Hence, in the production of vermicompost tea, these worm casts are used in obtaining the elutriate. Generally, the elutriate is applied directly to the soil or by spraying on the plant foliage.

Applied to the soil, the tea tends to be directed towards the roots, thus nutrients can be used by the plant and the microorganism population of the rhizosphere (positively) affected (Bess, 2000). The latter probably represents an interaction by several factors (mineral nutrients, microorganisms, plant growth regulators,…) resulting in an improved plant growth following the application of vermicompost teas (Edwards et al., 2006).

Very few controversial scientific papers have been published on this topic. It is for this reason that our institution decided to investigate the issue of compost teas and their effect on plants. This project was co-financed by INTERREG IIIA

and by the government of Lower Austria. The worm elutriate was produced by the company VermiGrand.

The objectives of the study were initially to investigate the principal chemical and microbial characteristics of compost elutriates and their different production methods. Furthermore, the effects on plants and on soil have been studied on a laboratory scale by means of plant growth tests and molecular and biochemical analysis of the soil microorganisms. The main aim was to identify which tea was most effective in promoting plant growth and to establish how this positive effect is produced.

2 Materials and Methods

2.1 Production of compost teas

Compost teas were produced under different conditions with regard to type of compost, quality and type of water (tap and deionized water), type and quantity of additional ingredients, temperature and production time. 850 liters of compost tea were produced from 10 liters (in some cases 20 liters) of compost.

2.2 Stability

To test the stability of the product, one tea was stored at three different temperatures (10 °C, 22 °C, 36 °C) for 15 days. All were aerated during the experiment. Samples were taken at the beginning, after 1, 2, 7 and 15 days for molecular analyses.

2.3 Chemical properties

The pH and electrical conductivity (EC) were determined after the tea production or during the elution process every 6 hours. Absorbance and dry weight (DW) as well as NO_3, NH_4 (photometric test according to Dr. Lange), K, P, Mg (by ICP) of the final product were analyzed.

2.4 Microbial and biochemical analyses

At the end of the brewing process, total germ number (plate count agar) and fungi germ number (YGC agar) were determined and results were calculated in CFU/ml of compost tea. Respiration was analyzed from different soil samples according to Schinner et.al (1995) by measurement of the released CO_2.

2.5 Molecular Analyses

DNA was extracted using the commercial kit 'Power Soil DNA Kit' (MO BIO laboratories, Inc.).

PCR amplification targeting bacterial 16S rDNA was performed by using the primer pair 341 f-GC 5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG-3′ and 907 r CCG TCA ATT CMT TTG AGT TT (Muyzer et al. 2001). For amplification of the fungal DNA, the primers NS1 5′- GTA GTC ATA TGC TTG TCT C - 3′ and

FR1-GC 5′- CCC CCG CCG CGC GCG GCG GGC GGG GGG GCG GGG GCG GGG GCA CGG GCC GAI CCA TTC AAT CGG TAI T - 3′ (Vainio and Hantula, 2000) were used.

The final volume of each reaction mixture was 50 µl and consisted of 32.3 µl PCR-water, 2.5 mM $MgCl_2$, 1x Taq buffer, 0.2 mM/dNTP, 0.2 mg/ml Bovine Serum Albumine, 25 pmol of each primer, 1 U Taq DNA-polymerase (Fermentas Life Sciences) and 1 µl (~ 10 – 20 ng) of template DNA. The PCR-programme for bacteria started with an initial denaturation at 95 °C for 4 min followed by 14 cycles: 95 °C for 30 s, 58 °C for 30 s (the temperature was reduced by 0.5 °C every second cycle), 72 °C for 45 s; 21 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 45 s and ended with a final extension at 72 °C for 10 min. For fungi, the following programme was used: 95 °C for 4 min – 35 x (30 s/95 °C – 45 s/47 °C – 3 min/72 °C) – 72 °C for 10 min.

DGGE was performed using a D GENE DGE-System (Bio-Rad Laboratories, Hercules, California). For bacteria, 6 % acrylamide gels with a gradient of 28 to 65 % and for fungi, 7.5 % gels with a gradient of 20 % to 39 % were used. The gels were electrophoresed at a temperature of 60 °C at 100 V for 16 h and at 58 °C at 180 V for 17.5 h. Subsequent to the run, gels were stained with SYBR Green I nucleic acid stain (Molecular Probes, Eugene OR) at 10,000 dilution in 1 x TAE for 1 h, destained for 30 min and scanned with a Thyphoon imager. Gels were processed and quantified using the programme GelCompar II v. 4.50 (Applied Maths, Belgium)

2.5.1 Determination of the Shannon-Weaver diversity index:

The Shannon Index is widely used, having been often applied to fingerprinting data (Brodie et al., 2003). It was calculated in the following way: $H' = C/N (N \log_{10} N - \sum n_i \log_{10} n_i)$ where C = 2.3, N = sum of band quantity in a given profile, n_i = band intensity of band i and i = number of bands in each profile

2.5.2 Cluster Analysis

For the cluster analysis, the similarity coefficient Dice was used, which considered only the presence/absence of the bands, combined with UPGMA (unweighted pair-group method using averages).

2.6 Plant Growth Test

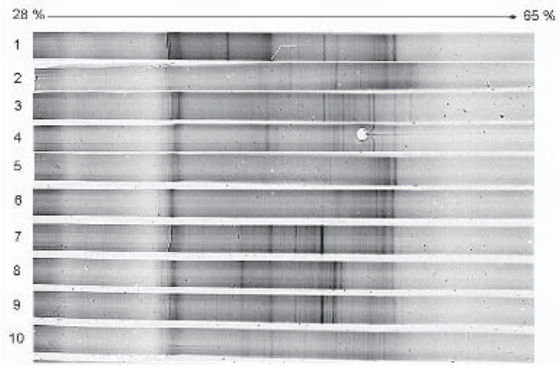
The test species cress (*Lepidium sativum*) and tomatoes (*Solanum lycopersicum*) were used for plant growth tests. The test was performed in an air-conditioned room with a relative humidity of 60 % and a temperature of 25 °C, using standard soil as blind. After plants germinated, they were either watered with compost tea or with deionized water by means of a glass tube. Fresh biomass was determined at the end of the test runtime.

3 Results and Discussion

3.1 Characterization of compost elutriates produced under different

conditions

Brewing time for the first series of elutriates was first established in 48 h. There were no remarkable differences in germ numbers (see Table 1), varying from 10^6 to 10^8 for bacteria and from 10^4 to 10^6 for fungi per ml. The diversity index for bacteria ranged between 2.73 and 3.39. Teas 6 - 7 and 8 – 9 were produced in the same way, but 7 and 9 with 48 h extended brewing time respect to 6 and 8. In both cases, the bacterial diversity index was raised after this longer elutriation time. For tea number 7, a considerable rise in fungal germ number was also observed. Figure 2 shows the bacterial DGGE band patterns for these 10 samples. It can be seen that the different elutriates were characterized by very different bacterial populations, displaying different band patterns. A clear change was observed between the 48 h and the 96 h teas, indicating the development of other dominating bacteria. Chemical parameters (Table 2) partly showed marked differences in



nutrient contents. For the longer production times, a significant reduction of NO_3, compared to the original teas, could be observed. Nitrate reducing bacteria may be the main reason for this phenomenon. Potassium showed high fluctuations between the elutriates.

Fig. 2: DGGE profile of 10 differently produced compost teas

Tab. 1: Germ numbers and bacterial diversity index for teas produced under different conditions

Batch	Special production condition	Total (CFU/ml)	Fungi (CFU/ml)	Shannon-index bact.
1	polenta, flour, silica	$1.0 * 10^8$	$4.0 * 10^5$	2.85
2	polenta, flour, silica, deionized water	$7.5 * 10^7$	$6.7 * 10^5$	2.96
3	polenta, oat bran, compost leachate	$5.0 * 10^8$	$2.2 * 10^5$	2.95
4	wheat flour, oat bran, sunflower press cake, compost leachate	$2.4.0 * 10^8$	$4.0 * 10^5$	2.98
5	compost leachate, flour and oat bran	$3.1 * 10^8$	$1.9 * 10^6$	2.87

6	citric acid	$6.1 * 10^7$	$2.0 * 10^5$	2.73
7	citric acid, 96 h	$2.8 * 10^7$	$2.1 * 10^6$	3.08
8	green leaf compost, of oat bran	$7.3 * 10^7$	$2.2 * 10^5$	3.09
9	green leaf compost, of oat bran, 96 h	$8.8 * 10^7$	$7.1 * 10^5$	3.39
10	compost leachate and oat bran	$2.7 * 10^6$	$4.6 * 10^4$	3.36

Tab. 2: Chemical parameters of compost teas produced under different conditions

Sample	ABS	pH	EC [mS/cm]	DW [%]	NO_3 [mg/l]	NH_4 [mg/l]	K [mg/l]	P [mg/l]	Mg [mg/l]
1	0.072	8.00	1.46	0.45	5.85	1.50	70.40	13.30	92.00
2	0.201	7.55	1.36	0.51	50.80	0.24	305.00	23.20	30.30
3	0.905	8.05	2.46	0.47	57.30	5.36	405.00	13.20	93.50
4	0.652	8.14	2.17	0.40	39.80	0.60	324.00	11.00	88.50
5	0.149	8.25	1.47	0.35	5.17	0.29	94.50	4.60	78.50

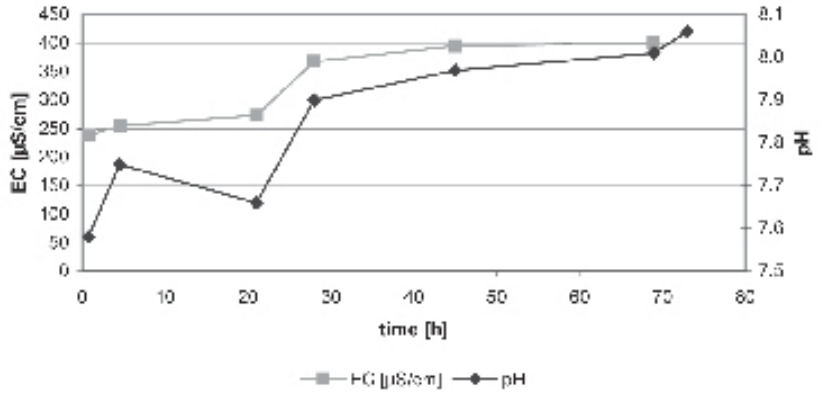


Fig. 3: Typical development of pH and electrical conductivity during a 73 h production process

6	0.130	6.54	1.35	0.40	96.70	0.16	93.60	18.90	99.50
7	0.050	8.81	1.63	0.37	3.42	5.26	95.40	11.20	93.00
8	0.103	8.41	1.43	0.30	26.00	0.11	81.60	1.60	72.00
9	0.060	8.46	1.37	0.31	3.63	0.09	82.20	1.90	72.50
10	0.503	8.73	2.11	0.34	87.60	0.95	288.00	4.20	80.00

In Figure 3, a typical development of electrical conductivity and pH is shown. For this specific tea, oats (approximately 200 g) alone were added at the beginning of the brewing process, carried out at a temperature of 17 to 18 °C. Subsequently, values were generally seen to be raised. After approximately 20 h, EC and pH

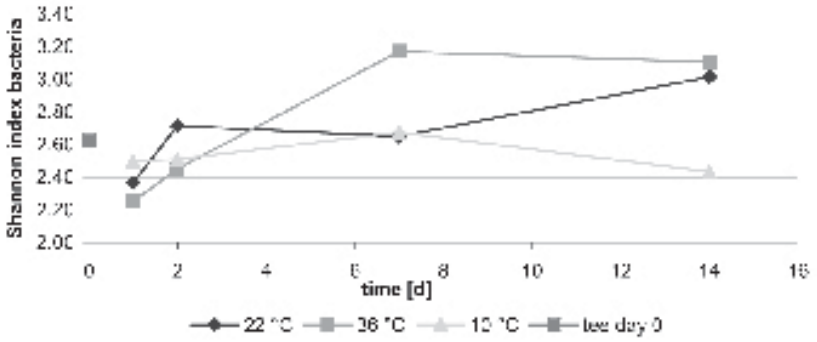


Fig. 4: Shannon diversity index for bacteria in the course of tea storage at three different temperatures (high numbers indicate a diverse population structure)

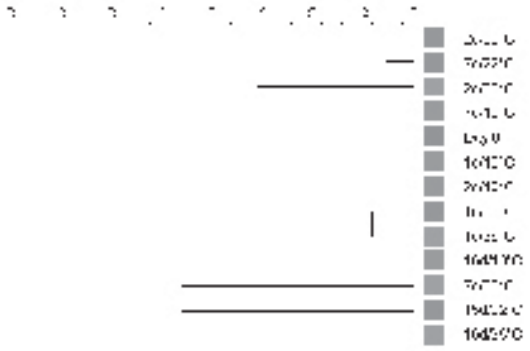


Fig. 5: Cluster analysis of DGGE results from samples stored at different temperatures

decreased, to be subsequently followed by a considerable increase.

3.2 Stability

Molecular analyses showed an initial decrease in bacterial diversity after 24 hours (Figure 4). A possible explanation could be that very fast growing bacteria suppressed others to an undetectable limit, resulting in a decline in diversity index. Later it increased at all three temperatures (22 °C, 36 °C, 10 °C), the most rapid

increase being manifested at 36 °C. At 10 °C bacterial changes were lowest, indicating an increased stability of the tea. Moreover, composition of the microbial population appeared similar on observation of the band pattern (not shown). The most outstanding change of bacterial population was manifested during storage at 22 °C and 36 °C after 7 days – the population structure built its own cluster (see Figure 5), displaying a completely different aspect to that of the original sample. With regard to these results, it was not clear whether the microorganisms in the tea were already at a stationary phase after 48 hours, due to the fact that nutrients were still undergoing degradation.

Nevertheless, compost elutriates can be processed for at least 2 to 3 days when stored at 10 °C. After longer storage and/or at higher temperatures, a clear change in microbial population is observed, although to date it is not clear whether this would imply a positive effect.

3.3 Reproducibility

The reproducibility of compost tea production was quite good (Table 3). 5 Teas were produced under the same conditions. Only one remarkable outlier could be found for tea C – in this case NO_3 was found to be considerably higher than in the other elutriates. Fungal germ number varied for one decimal power.

Tab. 3: Chemical and microbial parameters for 5 teas produced under the same conditions

Sample	ABS	pH	EC [mS/cm]	DW [%]	NO_3 [mg/l]	NH_4 [mg/l]	total MOs (CFU/ml)	Fungi (CFU/ml)	Shannon bacteria
A	0.094	8.17	1.49	0.31	3.07	0.11	$1.7 * 10^8$	$1.5 * 10^5$	2.45
B	0.129	7.81	1.52	0.42	2.78	0.14	$3.8 * 10^8$	$2.8 * 10^4$	2.44
C	0.083	7.69	1.50	0.40	15.40	0.08	$1.6 * 10^8$	$6.6 * 10^4$	2.39
D	0.173	7.45	1.56	0.41	5.32	0.16	$2.9 * 10^8$	$3.3 * 10^4$	2.23
E	0.099	7.16	1.54	0.39	4.25	0.08	$2.7 * 10^8$	$1.7 * 10^5$	2.46

3.4 Plant growth test

Plant growth tests were carried out with cress and tomatoes. Normally, for determination of compost quality, short-time tests are made, but in order to demonstrate significant effects of compost tea, these tests were not suitable. Figure 6 illustrates the tomato and cress tests run for one month. In the first row, the control which was only watered with deionized water is shown. A 62% increase in plant growth for tomatoes and 27 % for cress was recorded when watering the plants with compost tea.

To evaluate how the compost tea influenced the microbial soil population, molecular analyses (DGGE) were made. Changes were observed, as shown in the cluster analysis (Figure 7), although these were of varying entities and were not

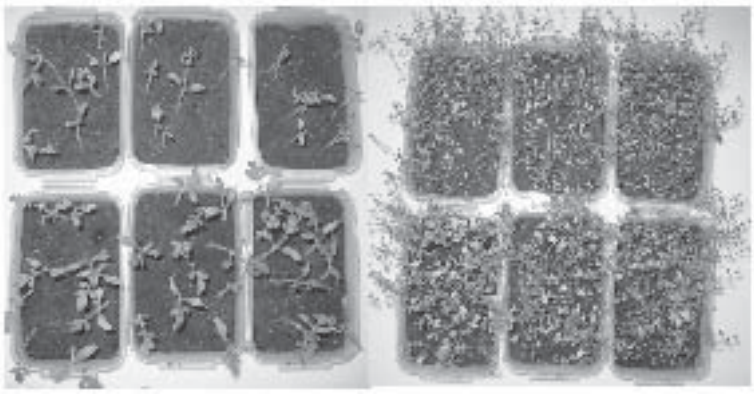


Fig. 6: Plant growth test with tomatoes (left) and cress (right). Top row: control; bottom row: compost tea

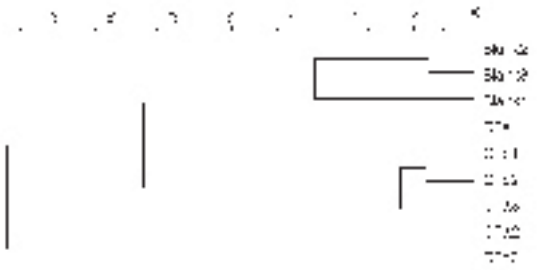


Fig. 7: Cluster analysis for soil samples of plant tests analyzed by DGGE

particularly marked.

An elevated microbial activity was found in soil samples of plant tests watered with compost tea compared to those irrigated using water alone (Table 4). This strengthens the assumption that the increased plant biomass is linked to the changed microbial activity caused by the compost elutriate.

Tab. 4: Glucose induced and basic respiration for soil samples of plant tests

Sample		Glucose induced respiration [mg CO_2/g*4h]	Basic respiration [mg CO_2/g*24h]
Tomato	Compost tea	0.108	0.341
	Blank	0.091	0.320
Cress	Compost tea	0.238	0.471
	Blank	0.108	0.333

4 Conclusion

Vermicompost tea was found to be beneficial for plant growth and biomass production in laboratory experiments, correlating with microbiological parameters. All parameters can be influenced by selection of specific brewing and storage conditions. The reproducibility of compost tea was good and it can be stored for at least 2 to 3 days at 10 °C before application. Final optimization of the elutriation has not yet been performed. Field experiments are planned for the next project stage to quantify the beneficial effect of vermicompost tea under agricultural production conditions.

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