THESES OF DOCTORAL (PhD) DISSERTATION

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EVALUATION OF COMPOSTABILITY OF POST-EXTRACTION HERBAL WASTE

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1. INTRODUCTION AND AIMS

Herbs are extensively used for their medicinal, antipathogenic, aromatic, and culinary properties by the pharmaceutical, cosmetic, and food industries. Since the essential oil content of these plants is relatively low (around 4-5% of dry biomass), pre-processing, extraction, and/or distillation of active ingredients from the raw materials produce large quantities of herbal residues. Proper management of these wastes has become a social challenge, because they can spoil the aesthetic sense of local habitats as well as induce further environmental issues. Besides, raw herbal residues contain large quantity of soil nutrients, but they cannot be considered for direct land application because of their unknown composition (e.g., pathogens, weed seeds, toxic compounds, etc.). Therefore, alternative methods of herbal waste utilization are needed.

Composting is an effective way to stabilize and reuse post-extraction wastes through microbial decomposition of biodegradable materials under controlled conditions. However, herbal residues can be difficult to biotransform because they are rich in cellulose, hemicellulose, lignins, and other bioactive components (poliphenols, alkaloids, terpenes), that can inhibit nutrient cycling, litter decomposition, and seed germination. Various biological methods, such as the addition of co-substrates (co-composting) or microbial inoculation during composting can be used to overcome these difficulties.

The objective of this study was to produce a stabilized and mature compost by utilizing the post-distillation herbal waste, cattle manure, and barley straw. To achieve the best results co-composting technology and self-developed microbial agent containing lignin- and cellulose-degrading bacteria were used.

The main aim of the pre-experiments was to evaluate the compostability of post-extraction aromatic plant waste supplemented with cattle manure and barley straw with or without microbial inoculants. During the composting period, the effect of herbal residues and four commercial microbial inoculums on the conventional co-composting process, certain physicochemical parameters, and microbial density were investigated.

During the third composting experiment, a mixed culture of *Cellulomonas flavigena* (NCAIM B.01383) and *Streptomyces viridosporus* (NCAIM B.02369) strains was applied to promote the co-composting process of *Lavandula angustifolia* Mill. herbal residues (60% w/w) rich in lignocellulose and improve the quality of the final product. Physical, chemical, and biological properties of the composts, as well as microbial density were monitored in the compost bins.

2. MATERIALS AND METHODS

Outdoor pilot-scale composting trials were conducted at Kisalföldi Agricultural Ltd (Nagyszentjános, Hungary). The chemical and microbiological analysis of the compost samples was performed at the Department of Food Science, Faculty of Agricultural and Food Sciences, Széchenyi István University.

2.1. Co-composting pre-experiments (1st and 2nd experiment)

Co-composting pre-experiments were carried out to determine the optimal ratio of feedstock materials (post-extraction herbal waste, cattle manure, and barley waste). Furthermore, different commercially available inoculants were applied to promote the biodegradation of organic matters and shorten the composting process.

2.1.1. Composting system

Compost bins (1 m^3) were made from pallets and were covered with mesh and insulated with styrofoam. Each bin contained 250 kg of mixed waste material.

2.1.2. Preparation of composts and sampling

The mixed waste consisted of solid residues of extracted herbal waste (30-60%), cattle manure (30-60%), and barley straw (10%) as bulking agent. Commercial microbial inoculums (EM 1, GeoCell-1, BioeGO two-components biofertilizer, and EM-BIO) were mixed to the feedstock materials at d 0 (Table 1).

Table 1: Treatments used for composting of post-extraction herbal waste

Treatment No.	Substrate	Microbial inoculum
	Pre-experiment No. 1	
K_1	Post-extraction herbal waste (30%), cattle manure (60%) barley straw (10%)	-
I_1		EM -1
II_1		GeoCell-1
III_1		BioeGO
IV_1		EM-BIO
	Pre-experiment No. 2	
K_2	Post-extraction herbal waste (60%), cattle manure (30%) barley straw (10%)	-
I_2		EM-1

During the first pre-experiment, on d 0, 7, 14, 28, 56, and 84, subsamples were taken from five representative points in the bins at three different depths. For the second pre-experiment, samples were collected on d 6, 12, 26, and 48. These subsamples were mixed thoroughly to obtain homogeneous samples of approximately 1000 g each.

2.1.3. Physicochemical and microbiological analysis

The temperature and moisture content of the compost piles were monitored at the place of composting (Nagyszentjános). pH values of the diluted samples were also measured. Pour plate method was used to enumerate beneficial microorganisms (e.g., aerobic mesophilic microorganisms, mesophilic cellulose-degrading microorganisms, mesophilic fungi and yeast).

2.2. Third composting experiment (3rd experiment)

The objective of this experiment was to produce a mature compost by utilizing the distillation waste of lavender, cattle manure, and barley straw. A mixed culture of *Cellulomonas flavigena* NCAIM B.01383 and *Streptomyces viridosporus* NCAIM B.02369 strains was also applied to enhance the co-composting process of lavender waste and improve the quality of the end product.

2.2.1. Preparation of composts and sampling

The mixed waste consisted of solid residues of extracted lavender (60%), cattle manure (30%), and barley straw (10%) as bulking agent (Table 2). Cellulolytic and lignin-degrading strains of *C. flavigena* (NCAIM B.01383) and *S. viridosporus* (NCAIM B.02369), were used as a bacterial inoculant. The incubated suspensions of *C. flavigena* (7.1×10^8 CFU/mL) and *S. viridosporus* (6.0×10^7 CFU/mL) were mixed at a ratio of 1:1. One part of inoculant was then diluted with 20 parts of water and added to the compost pile at a concentration of 8% (v/w) on d 8 of the composting process.

Table 2: Composition of the compost mixtures

Treatment No.	Substrate	Microbial inoculum
K_3	Cattle manure (90%), barley straw (10%)	-
I_3	Post-extraction herbal waste (60%), cattle manure (30%), barley straw (10%)	-
II_3		*Cellulomonas flavigena* and *Streptomyces viridosporus* (0.5-0.5 l)

On d 0, 8, 15, 21, 42, 56, 78, and 161, subsamples were taken from five representative points in the bins at three different depths. These subsamples were mixed thoroughly to obtain homogeneous samples of approximately 500 g each, which were then divided into two parts. One part was used for chemical analysis after natural air drying, whereas the other part of the fresh sample was used immediately for determination of microbiological properties, pH value, and GI.

2.2.2. Physicochemical and microbiological analysis

Temperature, pH value, moisture content, and microbial density (i.e., viable counts of mesophilic and termophilic microorganisms, fungi and yeast, streptomycetes, cellulose-degrading bacteria, and cellulose degrading fungi) were monitored in the compost bins over a period of 161 d. In addition, organic matter (OM), total organic carbon (TOC), total nitrogen (TN), and acid-insoluble lignin contents, carbon-to-nitrogen (C/N) ratio, germination index (GI) (with *Brassica rapa* subs. *chinensis* seeds), and the presence or absence of potentially

pathogenic enterobacteria (*Escherichia coli*, fecal streptococci, and *Salmonella* spp.) were determined in the final products to evaluate compost maturity. *In vitro* antimicrobial effect of lavender waste and matured compost extracts on certain phytopathogens (*Sclerotinia sclerotiorum* NCAIM F.00746, *Verticillium dahliae* F.00734, *Xanthomonas campestris* B.01466, and *Pectobacterium carotovorum* subsp. *carotovorum* B.01109) was also estimated by using agar well diffusion assay.

3. RESULTS AND DISCUSSION

3.1. First co-composting pre-experiment

The first experiment showed that post-extraction herbal waste (30%) is a suitable substrate for co-composting with cattle manure and barley straw. The applied commercial microbial amendments did not influenced significantly the composting process. Nonetheless, at d 84 the presence of higher levels of mesophilic fungi and cellulose-degrading microorganisms in the control compost implies that cellulose degradation and, thus, compost maturation have not been finished yet.

Based on the results of the microbiological analysis, it is concluded that the addition of EM-1 to I_1 improved the viability of beneficial microbes (i.e., total mesophilic microorganisms, cellulose-degrading microorganisms) throughout the composting process, although EM (Effective Microorganisms) is a mixture of different microbes which are less specifically targeted on the degradation of complex materials.

3.2. Second co-composting pre-experiment

Compared to the first experiment, the larger amount of post-extraction herbal waste (60%) positively influenced the co-composting process through enhancing total density of mesophilic microorganisms at the initial stage of composting and prolonging the thermophilic phase. Nonetheless, this extended thermophilic environment reduced the microbial quantity during the cooling and curing stages.

The used commercial microbial inoculum (EM-1) could not induce positive changes in the co-composting process or the microbial community composition. A possible explanation is that the larger amount of recalcitrant compounds (lignocellulosic fractions) decreased the efficiency of the bioaugmentation.

In order to overcome this issue, a microbial consortium was developed and used as an inoculum to promote the composting process of post-extraction herbal waste, manure, and straw.

3.3. Third co-composting experiment

The results showed that the inoculation with the mixed culture of *C. flavigena* NCAIM B.01383 and *S. viridosporus* NCAIM B.02369 had several positive effects on the composting process (density of beneficial microorganisms, TOC, C/N ratio, Klason lignin content, biopesticide effect) compared to the control compost (K_3).

Although the addition of lavender waste influenced the maturity indicators (C/N ratio, GI) negatively, bacterial inoculation was capable of mitigating the adverse effects of inhibitory residues, because the supplementation of the mixed culture of *C. flavigena* NCAIM B.01383 and *S. viridosporus* NCAIM B.02369 improved the efficiency of biodegradation (K_b: 0.53; TOC%: 42.33; OM%: 87.27) and preserved the nitrogen content (Table 3).

Table 3: Major chemical properties (on dry weight basis) of different types of composts at the beginning and end of the composting process

Parameter	Day	K_3	I_3	II_3
Biodegradability coefficient (K_b)		0.35	0.46	0.53
Carbon-to-nitrogen ratio[1]	Initial	32.43 ± 2.45[a]	32.99 ± 0.34[a]	32.99 ± 0.34[a]
	Final	17.60 ± 0.15[b]	19.05 ± 0.06[a]	16.91 ± 0.18[c]
Acid-insoluble lignin (%)[1]	Initial	15.05 ± 0.58[a]	15.60 ± 0.74[a]	15.60 ± 0.74[a]
	Final	14.09 ± 0.33[ab]	14.16 ± 0.68[a]	12.66 ± 0.99[b]

K_3: Control compost; I_3: Control lavender waste compost; II_3: Lavender waste compost with bacterial inoculum.
[1] Values are means ± SD, based on three observations.
[a-c] Means within a row without a common lowercase superscript differ ($p < 0.05$).

The results of microbiological analysis show, especially in the cooling and curing stages, that the used inoculum elevated beneficial microorganisms counts, such as mesophilic cellulolytic bacteria, streptomycetes, total fungi, and cellulolytic fungi, respectively (Fig. 1). Morover, II_3 compost containing lavender waste and microbial inoculum showed notable antibacterial and antifungal activities against tested microorganisms (*Pectobacterium carotovorum* subsp. *carotovorum, Xanthomonas campestris*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*).

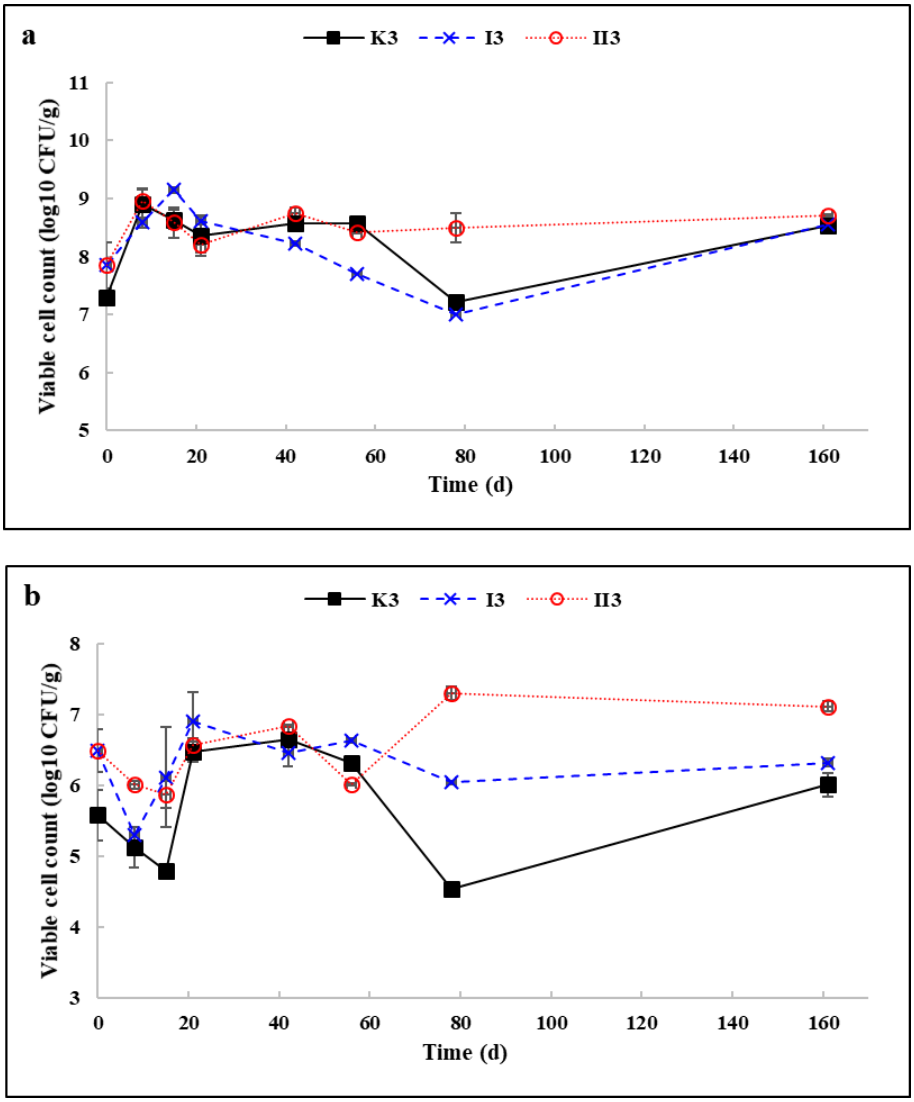

Fig. 1: Changes in viable counts of various microbial groups in different types of composts

(K_3: Control compost; I_3: Control lavender waste compost; II_3: Lavender waste compost with bacterial inoculum ;

a: mesophilic cellulolytic bacteria; b: mesophilic fungi)

Overall, the bacterial inoculant reduced the final C/N ratio (16.91) and enhanced the GI of the herbal compost (GI: 97.1%). Thus, the mixed culture of *Cellulomonas flavigena* and *Streptomyces viridosporus* accelerated the decomposition of herbal wastes, shortened the time of composting and, eventually, resulted in a better quality product. Therefore, co-composting herbal wastes with this bacterial inoculum has both economic and environmental benefits if the process is properly controlled and carried out.

4. NEW SCIENTIFIC RESULTS

1. Post-extraction herbal waste is suitable for co-composting with cattle manure and barley straw.

2. The applied commercial inoculum had no additional effect on the co-composting process when the feedstock material contained higher amount of herbal waste (60%).

3. The use of herbal waste (60%) affected the composting process beneficially through extending the thermophilic phase, elevating the initial counts of useful microorganisms, and accelerating the degradation of organic matter; however, adverse effects were also observed, including decreased nitrogen content and germination index. This resulted in a lower-quality end product.

4. Composts containing herbal waste showed notable biopesticide activities against four plant pathogens (*Pectobacterium carotovorum* subsp. *carotovorum* B.01109, *Xanthomonas campestris* B.01466, *Sclerotinia sclerotiorum* NCAIM F.00746, *Verticillium dahliae* F.00734).

5. The developed microbial inoculum was found to improve the efficiency of biodegradation. On day 161, the final composting product was mature and free from potential enteropathogens.

5. SCIENTIFIC PUBLICATIONS AND PRESENTATIONS ON THE TOPIC OF THE PRESENT DISSERTATION

Peer-Reviewed Papers

In English:

1. **Greff, B.,** Szigeti, J., Varga, Á., Lakatos, E., Sáhó, A., Varga, L. (2021). Co-composting of lavender (*Lavandula angustifolia* Mill.) waste and cattle manure with and without bacterial inoculation. 3 Biotech. 11:306. DOI: 10.1007/s13205-021-02860-2 *[IF(2019): 1,798]*
2. **Greff, B.,** Lakatos, E., Szigeti, J., Varga, L., (2021). Co-composting with herbal wastes: Potential effects of essential oil residues on microbial pathogens during composting. *Critical Reviews in Environmental Science and Technology*. 51:457-511. DOI: 10.1080/10643389.2020.1732780 *[IF(2019): 8,302]*

In Hungarian:

1. **Greff, B.,** Hanczné Lakatos, E., Szigeti, J. (2019). Extrahált gyógynövények komposztálási lehetőségeinek vizsgálata. *Acta Agronomica Óváriensis*. 60:16-30.

Patent Application

1. **Greff, B.,** Lakatos, E., Varga, L., Ásványi, B., Szigeti, J., Molnár, Z., Sáhó, A. Mikrobiológiai anyag és eljárás gyógynövényhulladékok komposztálására. P2000287 patent application.

Papers Published in Conference Proceedings

In Hungarian:

1. **Greff, B.** (2019). Nehezen komposztálható gyógynövény maradványok biológiailag aktív vegyületeinek hatása a lebontási folyamatot végző főbb mikroorganizmusokra. Új Nemzeti Kiválósági Program 2018/2019 Tanulmánykötet. Széchenyi István Egyetem. Győr [ISSN 2630-8134]
2. **Greff, B.**, Varga, Á., Hanczné, Lakatos E. (2018). Gyógynövények hatóanyagainak kinyerése után visszamaradt extrakciós maradványok komposztálhatóságának vizsgálata. XXXVII. Óvári Tudományos Napok, 2018. november 9-10. Fenntartható agrárium és környezet, az Óvári Akadémia 200 éve - múlt, jelen, jövő. Széchenyi István Egyetem, Mezőgazdaság- és Élelmiszertudományi Kar. Mosonmagyaróvár [ISBN978-615-5837-15-9]