Environment. Technology. Resources. Rezekne, Latvia
Proceedings of the 15th International Scientific and Practical Conference. Volume I, 177-181

Soil amendments based on forest logging residues on dill (Anethum graveolens L.) productivity and composition

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Abstract. **The purpose of this work was to evaluate the impact of low rates of application of environmentally friendly organo-mineral soil amendments on grown under organic farming conditions dill's green mass yield and it's composition, including the content of polyphenolic compounds. Soil amendments were obtained based on forest logging residues – lignocellulosic biomass, after isolation of polyphenols by water-ethanol extraction and enrichment with silicon (Si)-containing inorganic oligomer in various mass ratios. Lignocellulosic biomass is rich in polyphenols which can damage the functionality of the bacterial cell membranes thus inhibiting the growth of soil microorganisms. Polyphenols can be isolated from the lignocellulosic substrate and evaluated in further studies for their potential to protect plants from pathogenic microorganisms. The influence of the prepared soil amendments on soil microorganisms was tested. Field experiments were carried out in a certified biological field intended for scientific purposes. It was shown that soil amendments have a beneficial effect on the yield (42%) and a slight influence on the composition of dill at low amendment application rates. The addition of soil amendments also contributed to insignificant changes in the amount of polyphenols. The soil amendments didn't influence the soil microorganisms under study. The results confirmed the ability of the soil amendments based on the forest logging residues to activate dill growth.**

Keywords: Forest logging residues, growth activation, organo-mineral soil amendments, silicon

I. INTRODUCTION

In 2021, the EU had an estimated 160 million hectares of forests which on average covered 39% of the EU land area; whereas in Latvia, forest area amounts to 53% of the total area [1]. According to the European Commission terminology, forest logging residues that formed after forestry logging operations include branches with needles or leaves, tops, stumps, roots, and bark left in the forest, as well as small trees from thinning and clearing operations, and un-merchantable stem wood [2]. Forest logging residues comprise at least 11% of the total mass of wood [3], in Europe, they amount to an average of 32% [4].

Large quantities of forest logging residues result in forest degradation, pollution of the environment, and loss of potentially valuable raw material [5]. Until recently, the logging residues were mostly burned, which harmed the environment. Removal of the residuals from the forest, on one side, improves its sanitary condition, but on the other side, leads to the loss of nitrogen (about 40%), phosphorus (55%), and minerals [6]. It was estimated that around 30% of the residues on average should be left in the forest to minimize the depletion of soil fertility, especially in the areas, where the carbon content of the soil is small [7], [8].

Forest residues – lignocellulosic biomass – consist of lignocellulose (cellulose, lignin, hemicellulose), and extractives. Lignocellulose is the major renewable source of organic matter in soil [9]. It was proven that lignin

Print ISSN 1691-5402
Online ISSN 2256-070X
https://doi.org/10.17770/etr2024vol1.7983

contributes substantially to the formation of humic substances [10]. One of the possible alternative ways to increase the soil fertility is to return part of the logging residues back to the forest, but with a more easily accessible form of their organic part, and either enriched with largely missing mineral part or modified for increased uptake of the minerals by the plants. Moreover, lignocellulosic biomass is rich in polyphenols which can damage the functionality of the bacterial cell membranes thus inhibiting the growth of soil microorganisms. Therefore, for preparation of the soil additives, the polyphenols should preferably be preliminary extracted, for further use as antimicrobial agents in agriculture, veterinary or human health care.

In our studies, it was proven that organo-mineral fertilizers on the basis of lignin or lignocellulosic biomass of sea buckthorn, after extraction of the polyphenols, and enriched with silicon, had a positive effect on the plants growth and development [11]. It was shown that silicon increased the plant-available part of phosphorus in the soil, affected the uptake and accumulation of several mineral nutrients in various plants, and it's effect needs further investigation [12].

Thus, this work aimed to prepare, characterize, and test the soil amendments on the basis of forest logging residues. For this purpose the following tasks were set: to modify the forest logging residues after extraction of polyphenols with silicon-containing inorganic oligomer; to test antimicrobial activity of the obtained soil amendments against four pathogenic soil bacteria; and to evaluate the effect of the obtained soil amendments on dill (*Anethum graveolens* L.) productivity and composition.

II. Materials and Methods

A. Plant Material

Forest logging residues were collected in summer 2023, from Jelgava county of Latvia, Cenu parish. The residues were dried at room temperature and ground in a mill (Cutting Mill SM100, Retsch, Haan, Germany) until the particle size of < 2mm.

B. Preparation of Soil Additives

Soil additives were obtained on the basis of hardwood chip biomass after extraction of polyphenols, by modifying the biomass after extraction with a Si-containing component (further in the text – Si) in various mass ratios.

C. Field Experiments

The field experiments were carried out in 2023 at a certified biological field (56º 69.275′ Z, E 25º 14.173′) intended for scientific purposes. The test crop was the dill "Thalia". The field experiments with soil additives were carried out in 4 options in 4 repetitions:

1. Reference plot (without soil additive);
2. Soil amendment SA1 (biomass after extraction + 15% Si on DM);
3. Soil amendment SA2 (biomass after extraction + 10% Si on DM);
4. Soil amendment SA3 (biomass after extraction + 5% Si on DM).

The green mass of dill was harvested 63 days after sowing, according to the regulations of MK no. 461 "Requirements for food quality schemes, their implementation, operation, monitoring and control procedure" [13]. Total yield of green mass was calculated as kg m^{-2} (Fig. 1).

Fig. 1. Field experiments a certified biological field.

D. Dill Green Biomass Characterization. Py-GC/MS/FID Analysis

Analytical pyrolysis of dill samples was performed on Frontier Lab Micro Double-shot Pyrolyser Py-2020iD directly coupled with gas chromatography-mass spectrometry Shimadzu GC/MS/FID-QP ULTRA 2010 (Shimadzu, Kyoto, Japan), as described in [14]. Identification of the individual compounds was performed based on GC/MS using Library MS NIST 11 and NIST 11s, whereas the relative area of the peak of individual compounds was calculated using Shimadzu software based on GC/FID data.

E. Preparation and Yield of the Extracts

Hydrophilic extracts of dill green mass were isolated in two ways: 1) at 50 °C, 30 min using ethanol-water solution (1:1, v/v) or 50% EtOH; 2) at 50 °C, 30 min using distilled water. The ethanol-containing extracts after ethanol evaporation and water extracts were freeze-dried to yield dry extracts. The yield of the dry extract is presented as a percentage based on the dry mass (DM) of biomass. The CI for the results did not exceed 3% at α=0.05.

F. Chemical Characterization of the Extracts

The total content of polyphenols in the hydrophilic dry extracts was quantified by the Folin-Ciocalteu method using gallic acid as a reference compound according to Janceva et al. [15]. The total content of flavonoids was measured by a colorimetric assay using rutin as a reference compound according to Andersone et al. [16] The total content of condensed tannins in the hydrophilic extracts was measured by the butanol-HCl assay using procyanidin dimer B2 as a reference compound as described in Andersone et al. [16].

G. Determination of the Number of Functional Groups in the Biomass

The functional groups: aliphatic hydroxyl groups OH (aliphatic), phenolic hydroxyl groups OH (phenol), and carboxyl groups (COOH) were determined using the potentiometric and conductometric titration (InoLab level 3, Wissenachftlich-Technische Werkstatten GmbH & Co. KG, Weilheim, Germany), according to Zakis [17], [18].

H. Antimicrobial Properties

Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of the soil amendments, mg mL^{-1}, were tested on the pathogenic soil bacteria *Pseudomonas syringae* pv. *syringae* MSCL 894, *Erwinia rhapontici* MSCL 651, and fungi *Fusarium culmorum* MSCL 1628, and *Verticillium dahliae* MSCL 863.

I. Statistics

All measurements were conducted in triplicate, except for field trials where four replicates were done. The results are presented as the mean value. Statistical analysis was made using Microsoft Excel 2016. Confidence intervals for a mean using Student's T distribution were calculated at a significance level of 5% ($\alpha = 0.05$).
The analysis of variance by R-studio was used for statistical analysis of the experimental data. Bonferroni test was used for the comparison of means at $p<0.05$. Significantly different values were labelled with different letters in superscript ([a]).

III. Results and Discussion

A. The Yield of Dill Green Mass

The yield of dill green mass was 0.90 – 1.32 kg m^{-2}

(Table 1).

TABLE 1 DILL GREEN MASS YIELD DEPENDING ON SEED TREATMENT SUBSTRATE, KG M^{-2}

Sample	**Total yield of green mass, kg m^{-2}**	**The above-ground part of dill green mass from the total mass of the plant, %**
Control without soil additives	0.93	86.3
SA1 (biomass after extraction + 15% Si on DM)	1.32[a]	88.2
SA2 (biomass after extraction + 10% Si on DM)	0.96	87.2
SA3 (biomass after extraction + 5% Si on DM)	0.90	86.4

Compared to the control variant (without treatment), the yield of green mass increased significantly only after soil treatment with SA1 ($RS_{0.05}$ = 0.27 kg m^{-2}) the yield of green mass at other treatment options (SA2 and SA3) was within the confidence interval.

To determine the soil amendments effect on dill chemical composition, the following studies were further carried out for all dill samples: analytical pyrolysis (Py-GC/MS/FID); quantitative analysis of functional groups of biomasses; extraction to isolate hydrophilic extractives to determine the content of total polyphenols, flavonoids, and condensed tannins.

B. Analytical Pyrolysis (Py-GC/MS/FID) of Dill Biomass

The Py-GC/MS/FID data represent volatiles formed from cellulose, hemicellulose, lignin, proteins, and extractives. Aliphatic acids and esters, aliphatic alcohols, aliphatic aldehydes, ketones, furan and pyran derivatives, cyclopentane derivatives, and sugars attributed to carbohydrates-derived volatiles represented 50.5-59.6% of the total dill biomass volatile organic products (Fig. 2).

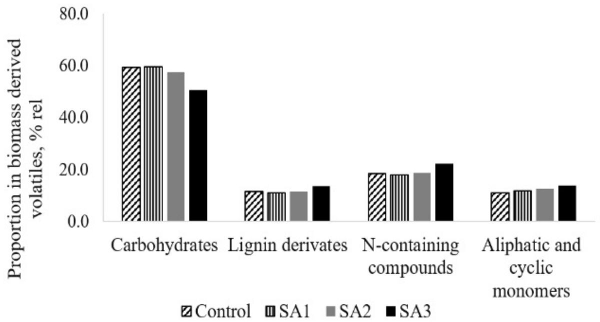

Fig. 2. Py-GC/MS/FID data of dill biomass-derived organic volatiles.

Besides the carbohydrates-derived, dill samples also contained N-containing volatiles, showing the presence of proteins and other nitrogen-containing compounds. According to Py-GC/MS/FID, nitrogen-containing compounds in dill were 17.8-22.1% of the total organic volatiles (TOV). The content of aliphatic and cyclic monomer derivates ranged from 11.0 to 13.9%/TOV. Relative proportion of guaiacyl-type derivatives attributed to lignin-derived volatiles was 0.9-1.1 %/TOV (Fig. 3).

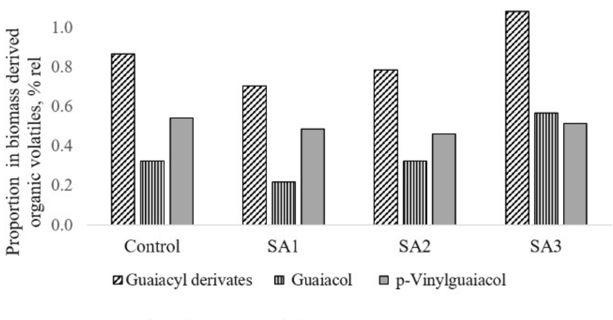

Fig. 3. Py-GC/MS/FID data of dill biomass-derived lignin-derived volatiles.

The total polyphenol-derived volatiles content in dill biomass composition ranged from 11 to 14 %/TOV.

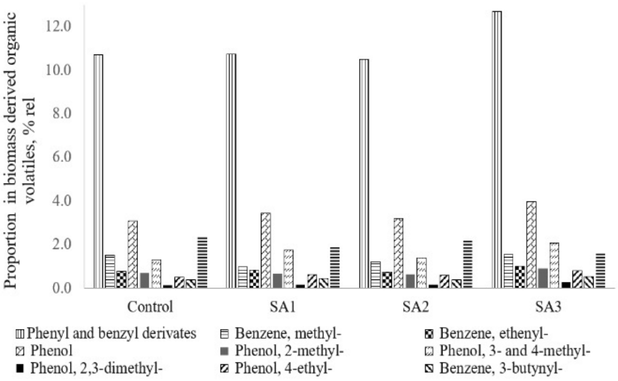

Fig. 4. Py-GC/MS/FID data of dill biomass-derived henyl and benzyl-derived volatiles.

The content of phenolic and benzyl derivatives formed from various phenolic extractives had similar content and proportions of chemical compounds between the treated and control dill samples within the confidence interval.

C. *Total Content of Polyphenols and Functional OH Groups in Dill Biomass*

Previous studies have shown that plant secondary metabolites, polyphenolic compounds, have a range of biological activities, such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic and other [19], [20], [21], and, thus, are important compounds to be evaluated. The total polyphenol content in 50% EtOH extracts ranged from 60.1 to 65.0 mg GAE per gram of dry extract. The total polyphenol content in water extracts was slightly lower comparing to the extracts isolated by 50% EtOH (42.3 – 52.8 mg GAE/g extract). The total flavonoid content in 50% EtOH extracts ranged from 42.7 to 46.8 mg RU per gram of dry extract. The condensed tannins in the dill extracts were not found.

When comparing dill samples with each other, the composition of functional groups differed slightly. The effect of fertilizers on the content of total hydroxyl groups (OH) in the composition of dill was similar to their influence on the amount of total polyphenols. The total content of OH groups was slightly lower in the control sample. An increased content of acetyl groups and aliphatic OH groups in dill grown on treated soil (SA1-SA3) could indicate to an increased content of fatty acids and aliphatic compounds in dill composition (Fig. 5).

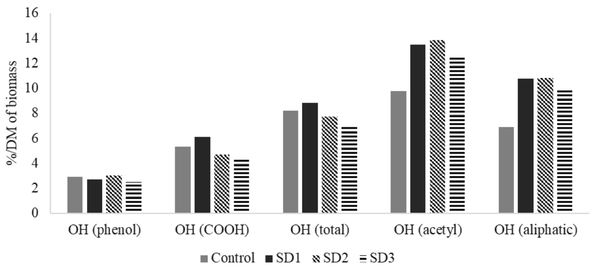

Fig. 5. Soil additive effect on dill functional group composition.

These results are consistent with the Py-GC/MS/FID data.

D. *Antimicrobial Properties of Soil Amendments*

The analysis of the anti-microbial properties of soil amendments obtained from forest logging biomass after extraction of polyphenolic compounds showed that they do not have detected antimicrobial activity (or MIC >50 mg mL^{-1}) against the pathogenic soil bacteria *Pseudomonas syringae* pv. *syringae* MSCL 894, *Erwinia rhapontici* MSCL 651, and fungi *Fusarium culmorum* MSCL 1628, and *Verticillium dahliae* MSCL 863.

IV. Conclusions

The results of the study showed the positive influence of the soil amendment with higher Si concentration (15% of Si on DM of biomass after extraction) on the yield of dill green mass, the green mass total yield increased 1.4 times in comparison with the control. The soil amendments with less Si concentrations (5% and 10% on DM) didn't influence the yield within the confidence interval.

The content of polyphenolic compounds in dill samples with or without treatments was similar; slight differences in the content of functional OH groups could indicate a bigger amount of fatty acids and aliphatic compounds in the composition of dill.

The antimicrobial activity of the studied soil amendments against four pathogenic bacteria and fungi was not detected (MIC >50 mg mL^{-1}). This may be indirect confirmation that polyphenols, which had proven antimicrobial activity in our previous studies, and which were separated from the forest biomass residues before preparation of the soil amendments, have a key role in antimicrobial effect. At the same time, such absence of antimicrobial activity of the studied soil additives could be considered positive for the beneficial soil microorganisms. The investigations of the effect on soil microbiota will be continued.

It can be concluded that the soil amendments based of the forest logging residues could be used for the creation of organo-mineral fertilizers and have positive effect on the yield of dill. Slight changes in the content of polyphenolic compounds of dill could be connected with the less stress of the plants growing on the enriched soil. Since the work with plants demands many repetitions, and depends also on the environmental changes, the studies have to be further continued in order to collect statistically significant amount of plants for making conclusions of the stable effect of the soil amendments in different weather conditions.

Acknowledgement

Rural Support Service of the Republic of Latvia, European Agricultural Fund for Rural Development (EAFRD), Measure "Cooperation", project No. 23-00-A01612-000008 "Wasteless application of residual logging biomass for obtaining of environmentally friendly plant protection products and soil additives". Authors acknowledge Mg.Sc. V.Jurkjane for performing Analytical Pyrolysis analysis.

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