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ORIGINAL PAPER

Phytotesting of Liquid Products of Wood Pyrolysis on Seeds of Higher Plants

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Abstract

An effective method for processing lignocellulosic raw materials into marketable products is the pyrolysis method. With this method, a water-soluble fraction is formed, which has a rather narrow range of applications. The purpose of this work was to determine the effect of the water-soluble fraction of the pyrolysis liquid and wood vinegar in different dilutions on seed germination and root length and biomass of seedlings of higher plants. The pyrolysis liquid was obtained using the rapid pyrolysis method. Widespread food crops were selected as test crops: white mustard, arugula, radish, dill, curly parsley, lettuce, seed peas and oats. In dilutions of the water-soluble fraction of the pyrolysis liquid (1:200, 1:400 and 1:900), a statistically significant stimulating effect was observed on the germination of radish seeds, and root growth stimulation in radish seedlings was revealed when using dilutions of 1:200, 1:300, 1:400 and 1:900. The 1:900 dilution of the water-soluble fraction of the pyrolysis liquid contributed to the stimulation of seed germination and root growth of seedlings in oats, as well as to arugula seed germination. A statistically significant stimulating effect of wood vinegar was established on the germination of radish seeds using a 1:100 dilution, as well as on the growth of the roots of seedlings and the accumulation of raw biomass by the roots of lettuce seedlings in dilutions of 1:400 and 1:900.

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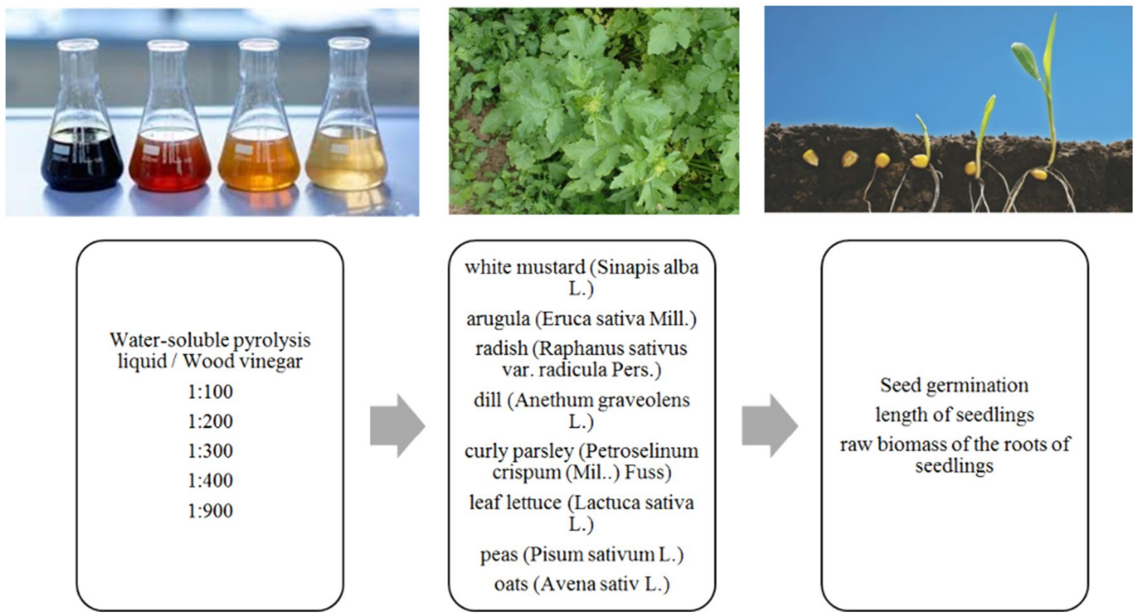
Graphical Abstract



Keywords Pyrolysis liquid · Wood vinegar · Seed germination · Seedling root length · Seedling root biomass · Multivariate discriminant analysis

Highlights

- Sprouting Root Sprouts Using Wood Vinegar
- Pyrolysis of lignocellulosic biomass and application of its products
- Commercialization of the fast pyrolysis process for bio-oil production
- Water-soluble bio-oil fraction and scope

Introduction

An effective method of processing lignocellulosic biomass is pyrolysis, which processes the biomass into liquid products, regardless of its composition. Bio-oil obtained from fast pyrolysis is gaining popularity around the world as a replacement for fossil fuels [1–6]. The presence of oxygen-containing components in bio-oil is the main deteriorating indicator of the extensive use of bio-oil in various industries. With each of the methods of using biooil, a water-soluble fraction (wood vinegar) is inevitably formed, which contains dissolved substances, such as acids, phenols, furans and sugars.

Wood vinegar has long been used as an organic additive [7–11]. Modern research into the use of wood vinegar was first conducted in Japan in the early 1950s. It has been reported to be effective against wheat rosette or green mosaic, sweet potato nematode, tobacco mosaic, leafy vegetable powdery mildew, leaf miner moth and other insect pests. However, due to the advent of agrochemicals and their immediate impact, wood vinegar research has taken a backseat [12].

A decade ago, there was a resurgence of interest in wood vinegar, as the effectiveness and safety of agrochemicals came to the fore. It is now widely used by Japanese farmers and is also rapidly gaining momentum in Taiwan and Korea. However, in the last 2–3 years, the use of wood vinegar for agricultural purposes has increased significantly, as evidenced by numerous publications [13–15]. This is due to the promising use of various pyrolysis methods for processing raw lignocellulosic waste materials. The question arises about the use of a water-soluble fraction obtained by the fractionation of bio-oil.

The use of wood vinegar and biochar improves the soil structure and facilitates the flow of nutrients and water, allowing plants to grow faster and produce higher yields. By promoting potassium absorption, wood vinegar and biochar contribute to strong root systems, leading to healthier plants, preventing soil-borne diseases and damage, especially in horticulture, soilless crops and fruit production [16]. The mechanisms for promoting the plant growth may be attributed to the multiple benefits on the improvement of soil fertility, nutrient supply and translocation to the plant after the biochar and wood vinegar amendment.

Scientists [17] have demonstrated the effectiveness of the use of wood vinegar obtained during the pyrolysis of urban elm pruning waste against broadleaf weeds. Higher efficiency has also been achieved at low temperature (10 °C) compared to high temperature conditions (20 or 30 °C).

Chinese scientists [18] applied a systematic foliar spray in a field with the 'Huayouza 9' hybrid variety for two years to study the effect of wood vinegar and its compounds on rape seed growth. The results showed that seed yield, leaf area index and number of pods per plant of rapeseed treated with wood vinegar increased by an average of 9.58, 23.45 and 23.80% over two years compared to controls treated with tap water.

De Guzman and Cababaro [13] conducted studies to evaluate the effect of varying levels of wood vinegar as a nutrient availability enhancer in eggplant, and treatment with 1% wood vinegar yielded the best results.

According to some reports, when strongly diluted, the water-soluble fraction of the pyrolysis liquid and wood vinegar can stimulate the growth of wheat roots and its resistance to drought [19], stimulate the growth of rice roots [20], improve and accelerate the germination of parsley and dill seeds [21], and increase the content of dry soluble substances in tomatoes [22].

Wood vinegar promotes rooting and helps regulate the soil nutrient status and microbiological population balance. It kills nematodes directly and multiplies the microbes that feed on them.

Many pesticides and herbicides that negatively affect the useful quality of products, as well as the habituation of weeds, insects and fungi, open a niche for additives that can combat a wide range of problems in the agricultural industry and still be of organic origin. The possibility of using a water-soluble fraction and biochar is very interesting and relevant.

The use of the pyrolysis liquid and wood vinegar in agriculture can be attributed to so-called "green chemistry," since these additives are obtained from renewable plant materials. The use of the pyrolysis fluid and wood vinegar can reduce crop production's dependence on the use of synthetic chemicals. Research in this direction is being conducted in China, Thailand and South Africa. In Russia, such works are rare.

The search for new affordable, cheap and environmentally friendly drugs for use in agriculture is an urgent task, as well as the search for ways to process and dispose of waste.

However, the water-soluble fraction of the pyrolysis liquid and wood vinegar, in addition to biologically active substances and growth stimulants, contain substances that can have a toxic effect on plants if the solution is not sufficiently diluted.

The purpose of this work was to determine the effect of the water-soluble fraction of the pyrolysis liquid and wood vinegar in different dilutions on seed germination and root length and biomass of seedlings of higher plants.

Materials and Research Methods

Production of Pyrolysis Liquid and Wood Vinegar

The pyrolysis liquid was obtained using the FPP02 (Fast pyrolysis plant-02) fast pyrolysis unit, a patented development of Energolesprom LLC, Kazan [23]. Pyrolysis liquid was obtained from crushed birch wood at a temperature of 500 ± 50 °C. Wood moisture was 8 ± 0.5% (ASTM D4442-16); the ash content was 0.3% (ASTM D1102-84); and the size of the wood chips was 0.5–5.0 mm, with an average size of 1.2 mm. The water-soluble fraction was obtained by settling the pyrolysis liquid for 1 h at room temperature. The vacuum distillate of liquid products of rapid pyrolysis of wood was used as the wood vinegar. Vacuum distillation was carried out at a residual pressure of 20 kPa and a temperature of 80 ℃ in a stirred reactor [24].

Composition Analysis

To determine the possibility of using the water-soluble fraction of the pyrolysis liquid and wood vinegar, their composition and properties were investigated. To study the composition, a preliminary extraction of the distillate with diethyl ether (1:1) was carried out and held at room temperature for 15 min. Diethyl ether with extracted organic substances was analyzed by gas chromatography-mass spectrometry (GC/MS) using a Shimadzu GCMS-QP2010 instrument on an HP-1 MS column. The composition of the water-soluble fraction of the pyrolysis liquid and ancient vinegar of organic substances minus diethyl ether is presented in Table 1 (density of wood vinegar, 1.0–1.15 g/cm^3, pH ≈ 2.1; density of the water-soluble fraction of the pyrolysis liquid, 1.1–1.2 g/m^3, pH ≈ 2.3).

Wood vinegar, obtained by vacuum distillation from pyrolysis liquid, consisted only of the most volatile substances that were part of it. It did not contain carbonaceous particles; it contained only traces of phenols, and a significant portion of the dissolved substances was represented by acetic acid.

Preparation of Dilutions

To study the effect on seed germination and the length and biomass of seedling roots, the water-soluble fraction of the pyrolysis liquid and wood vinegar were used in dilutions of 1:100, 1:200, 1:300, 1:400, and 1:900. Dilutions from 1:300

Table 1 Composition of the water-soluble fraction of the pyrolysis liquid and wood vinegar

Chemical name	Mass fraction, %
Wood vinegar	
Acetic acid	1.93–22
Propionic acid	0.05–1.03
Butanoic acid	0.14–0.45
Crotonic acid	0.008–0.05
Furfural	0.43–2.52
Methoxyphenols (for 2-methoxyphenol)	1.63–5.78
Water	75–94
Water-soluble fraction of the pyrolysis liquid	
Aldehydes	0.52
Anhydrosugar	20.63
Heterocyclic compounds	2.66
Ketones	8.1
Carboxylic acids	31.98
Alcohols	3.74
Phenols	12.63
Not identified	19.74
Water	54

to 1:900 were used based on previous work [19, 22]. Dilutions of 1:100 and 1:200 were also used to test their effects on plant seeds and seedlings and to compare them with less concentrated dilutions.

Selection and Preparation of Test Crops

Widespread food crops from the following plant families were chosen as test crops: cruciferous – white mustard *Sinapis alba* L. ('Rainbow'), arugula *Eruca sativa* Mill. ('Dikovina'), radish *Raphanus sativus* var. *Radicula* Pers. ('Ruby'); Umbelliferae – garden dill *Anethum graveolens* L. ('Malachite'), curly parsley *Petroselinum crispum* (Mill.) Fuss ('Vorozheya'); Compositae – leaf lettuce *Lactuca sativa* L. ('Azart'); legume – peas *Pisum sativum* L. ('Alpha'); and cereal – oats *Avena sativa* L. ('Skakun').

Seed Germination and Seedling Growth Measurements

Seed germination was determined according to GOST 12038-84 [25]. Before the start of the experiment, all plant seeds were pre-sorted by size, discarding empty, very small, too large, ugly, etc. seeds. In sterilized Petri dishes (diameter 10 cm), a double layer of filter paper was placed and pre-moistened in the tested concentrations of dilutions of the pyrolysis liquid and wood vinegar. Distilled water was used as a control. The sowing rate in each parallel determination was 50 seeds. The number of repetitions for each option was threefold. Petri dishes with planted seeds were kept in a thermostat (thermostatic cabinet) in dark conditions at an air temperature of 20 °C or 25 °C, depending on the requirements of each crop for germination according to GOST 12038-84.

The determination of seed germination of tested crops, the length of seedlings and the raw biomass of seedlings was carried out considering their botanical characteristics. For radish and oats, experimental data were collected on the 3rd day, for white mustard, arugula, leaf lettuce and peas, on the 4th day, for dill, on the 8 day, and for curly parsley, on the 10 day.

The roots of all germinated seeds were measured, and the length of the longest root in each repetition, determined with an accuracy of 1 mm, was included in the final calculation. The raw biomass of the roots of seedlings was determined immediately after measuring their length with an accuracy of 0.0001 g. The dry phytomass of the seedling roots of each repetition was determined after drying in a thermostat in metal weighing bottles to a constant weight at a temperature of 65 °C with an accuracy of 0.0001 g.

In accordance with existing methods (MP 2.1.7.2297-07 [29], FR.1.39.2006.02264 [26]) if the root length of the plants of the tested crops exceeds the control values, the tested substances have a stimulating phytoeffect. The decrease in seed germination of the tested crops and the inhibition of the growth of seedling roots by more than 20% compared to the control options indicate the phytotoxic effect of the tested substances.

Data Analysis

Statistical data processing was carried out using the standard packages Microsoft Excel 2013 and Statistica 10. The significance of the differences between the mean values was assessed by Student's t-test ($p < 0.05$). The establishment of wet and dry biomass of seedling roots in the tested cultures was carried out as an average value from germinated seeds.

Results

Seed Germination of Tested Cultures Using Dilutions of the Pyrolysis Liquid and Wood Vinegar

According to the diagram shown in Fig. 1, the negative effect was shown by seed treatment of white mustard and dill when treated with wood vinegar and a water-soluble fraction of pyrolysis liquid in all dilutions.

Arugula showed a slight increase in seed germination when diluting the water-soluble fraction 1:900 and wood vinegar 1:100, 1:300 and 1:900. Also, a slight improvement in the germination of curly parsley seeds was observed when

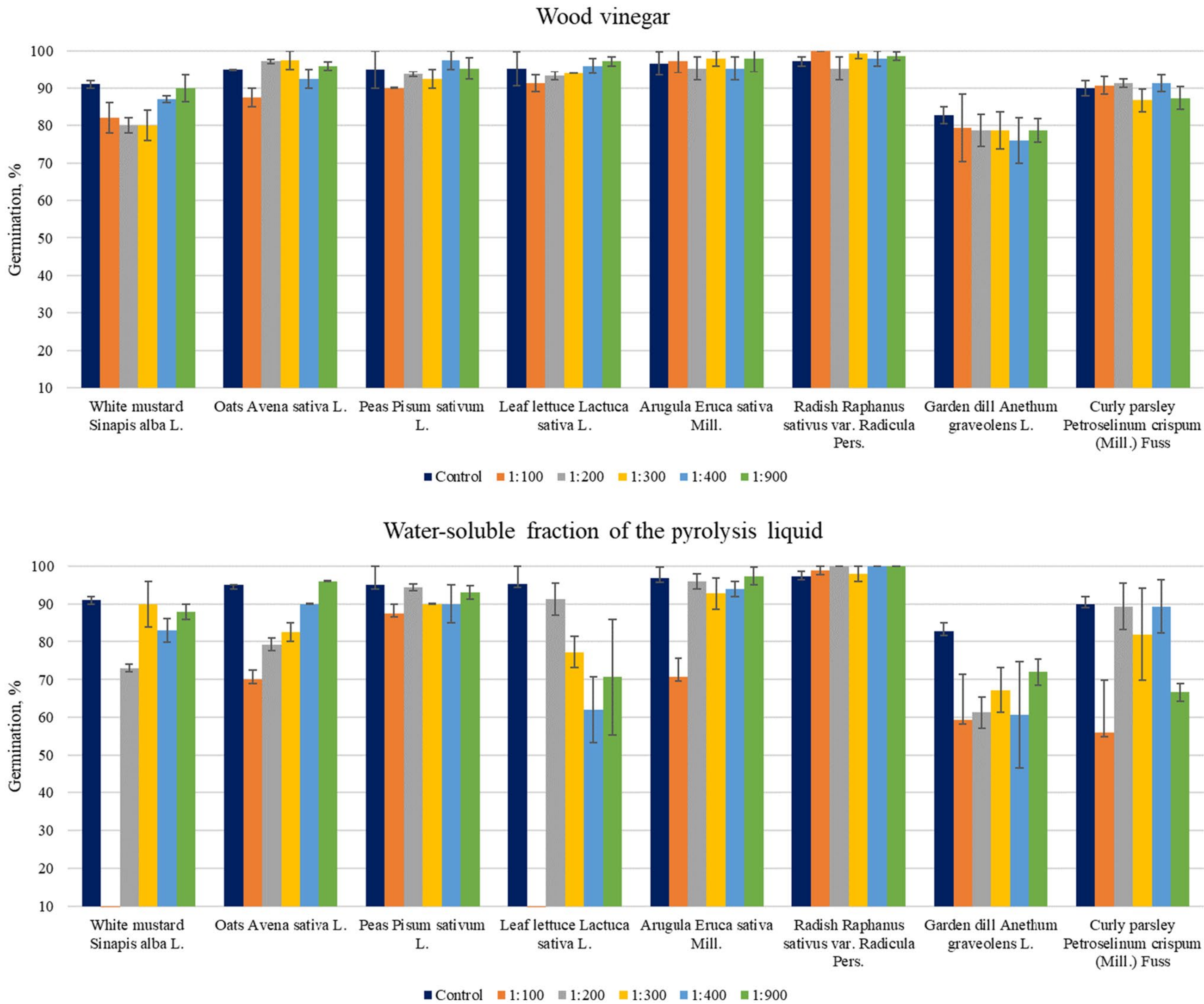


Fig. 1 Seed germination of the tested crops

diluting wood vinegar 1:100, 1:200 and 1:400, with all dilutions of the water-soluble fraction of the pyrolysis liquid, the opposite effect was observed. These indications of improvements vary in the range of no more than 1.3%.

The best germination rate was observed when processing radish seeds in all dilutions except for the dilution of wood vinegar 1:200. The best germination (100%) of radish seeds was revealed when diluting the water-soluble fraction of pyrolysis liquid 1:200, 1:400 and 1:900 and when diluting wood vinegar 1:100.

As for leaf lettuce and seed peas, the best germination rates of up to 2.6% are achieved when diluting wood vinegar 1:400 and 1:900, with all dilutions of the water-soluble fraction of the pyrolysis liquid, the results are negative. Sowing oats with dilutions of wood vinegar from 1:200 to 1:900 had the highest result in seed germination, in contrast to the control by about 2.6%. Also, when diluting the water-soluble fraction of pyrolysis liquid 1:900, the germination of oat seeds increased by 1%.

Given the tendency of the negative effect of the water-soluble fraction of the pyrolysis liquid on seed germination, it can be assumed that the composition of the liquid has a more aggressive composition (phenols, ketones and anhydrous sugar) and has a phytotoxic effect on the seeds of test crops.

Seedling Root Length of Tested Cultures Using Dilutions of the Pyrolysis Liquid and Wood Vinegar

The seedling root length was a more sensitive indicator of the toxic and stimulating effects of various substances than germination [27, 28].

Studies have shown that the length of the roots of mustard and pea seedlings increases with an increase in dilution of wood vinegar solution to 1:400 (Fig. 2). It was found that the length of pea roots increases with the dilution of wood vinegar 1:400 by 6.7%, with other dilutions, deterioration from 5 to 40% was observed. When germinating mustard seeds using a solution of a water-soluble fraction of pyrolysis liquid in a 1:100 dilution, the seeds did not germinate, therefore the root length was not measured.

The tested solutions, a water-soluble fraction of pyrolysis liquid and wood vinegar, at a dilution of 1:900, improve the length of oat roots by 6% and 13%, respectively.

It was found that the length of the roots of lettuce seedlings increases with increasing dilution of the wood vinegar solution from 1:100 to 1:900 (Fig. 2).

We found that all dilutions of the water-soluble fraction of the pyrolysis liquid have a phytotoxic effect on the seedlings of lettuce, white mustard, seed peas, arugula, garden dill and parsley, which indicates the direct toxic effect of the water-soluble fraction of the pyrolysis liquid. And also with all dilutions of wood vinegar, the phytotoxic effect is on arugula and dill seeds.

The stimulating effect of wood vinegar solutions on curly parsley seedlings has not been statistically reliably established, however, in dilution variants 1:100, 1:300, 1:400 an increase in the lengths of the roots of seedlings was recorded by 5.5%, 8.9% and 6.1%, respectively, in dilutions of 1:200 and 1:900, the length of the roots of seedlings does not differ from benchmarks.

Unlike the rest of the crops studied by us, solutions of the water-soluble fraction of pyrolysis liquid and wood vinegar have a predominantly stimulating effect on radish seedlings, however, there is no statistically confirmed, pronounced phytotoxic effect of solutions of the wood pyrolysis liquid on the test culture seedlings. The maximum length of the roots of radish seedlings exceeding the control values by 14.1% was

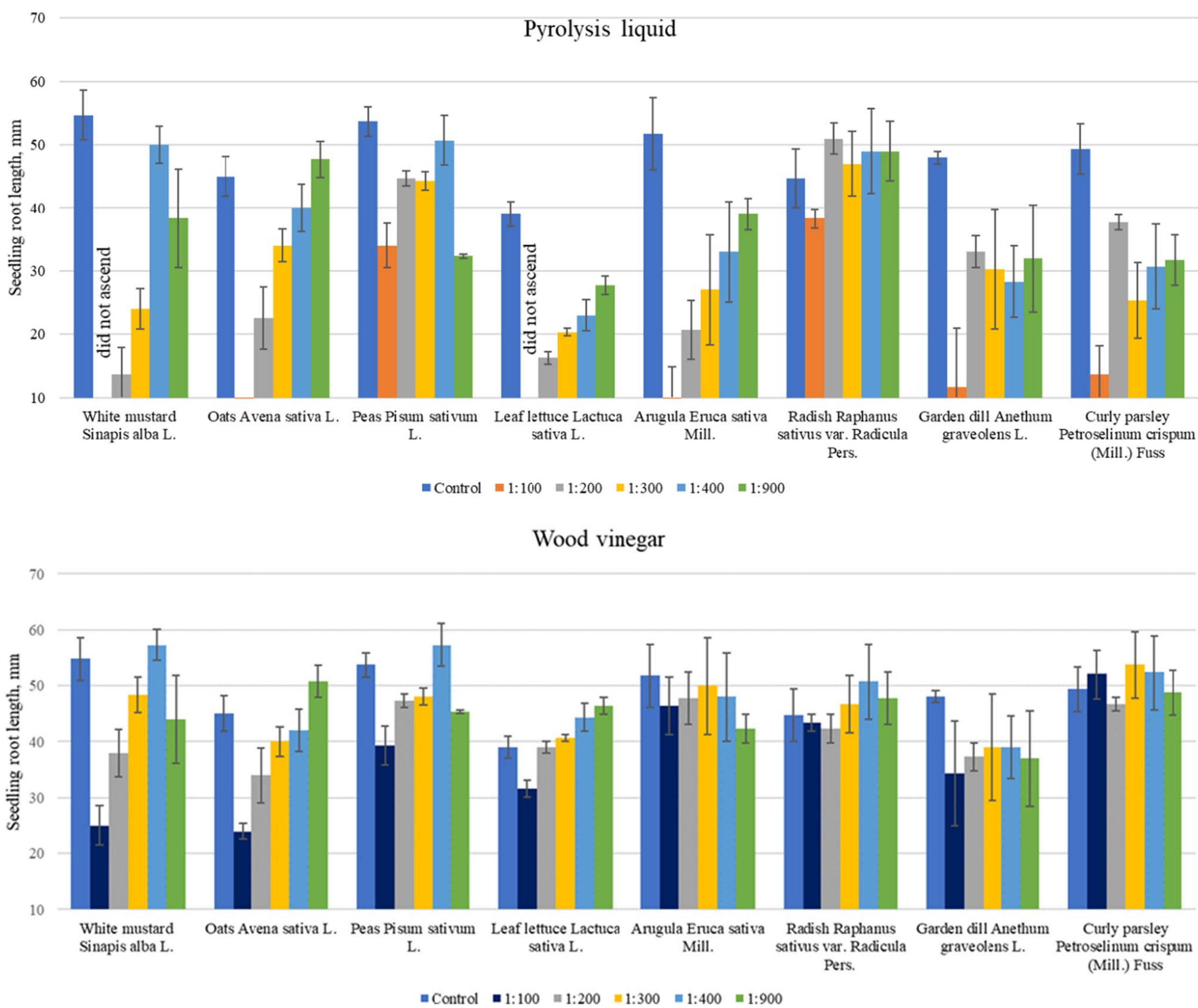


Fig. 2 Root length of seedlings of the tested cultures

recorded in the dilution variant of the water-soluble fraction of pyrolysis liquid 1:200, as well as in the 1:400 solution of wood vinegar, the length of the roots of test culture seedlings in which is 13.4% higher than in the control.

Thus, the results obtained on the change in the length of the roots of the studied test plants, depending on the use of various dilution options for wood pyrolysis liquid, vary greatly, which does not allow us to establish pronounced general patterns in their effect on test crops. In general, it can be stated that the water-soluble fraction of the pyrolysis liquid has a more toxic effect on most seedlings of the plants under consideration compared with the solution of wood vinegar.

Biomass of Seedling Roots When Using Dilutions of the Pyrolysis Liquid and Wood Vinegar

In order to determine the most sensitive parameter to the effect of liquid pyrolysis products on higher plants, in addition to taking into account the germination of plant seeds and the root length of test crop seedlings, we have established the raw and dry biomass of the roots of the test plant seedlings.

The results obtained showed that the use of the solutions of the water-soluble fraction of pyrolysis liquid during germination of all seeds of the test crops in all dilutions leads to a statistically significant decrease in the crude biomass of the roots of seedlings, which indicates a pronounced phytotoxic effect (Fig. 3). In particular, for lettuce, a decrease in the crude biomass of the roots of the seedlings in dilutions of the water-soluble fraction of the pyrolysis liquid 1:100, 1:200, 1:300, 1:400, 1:900 is amounted to 100% (the seeds did not germinate), 45.1%, 46.3%, 38.0%, 41.4%, for arugula similar indicators were 44.8%, 66.4%, 48.8%, 47.2%, 31.2%, for radishes, the corresponding values are – 48.0%, 47.7%, 39.2%, 37.3%, 36.2%, for garden dill – 75.8%, 32.3%, 49.5%, 53.7%, 24.2%, for the parsley curly – 68.6%, 46.4%, 54.5%, 45.5%, 46.8%, respectively.

It is noted that the indicators of the dry biomass of the roots of the test crops seedlings in the considered dilutions of the water-soluble fraction of the pyrolysis liquid differ in the presence of a small number of significant differences with the control values (Fig. 3).

The effect of the wood vinegar solution on the accumulation of raw and dry biomass by the roots of test crop seedlings is very different. We have recorded an increase in the raw and dry biomass of the roots of lettuce seedlings with an increase in the degree of dilution of the wood vinegar solution with distilled water (Fig. 3). In the 1:400 and 1:900 wood vinegar solution, there is a statistically significant excess of the crude biomass of the roots of lettuce seedlings by 27.2% and 40.1% of the control values, respectively, indicating a pronounced stimulating phytoeffect of the wood vinegar solution of these dilutions. In addition, the 1:400 and 1:900 wood vinegar dilutions tend to stimulate the accumulation of dry biomass by the roots of lettuce seedlings, contributing to its increase by 10.8% and 18.9%, respectively, compared with the control. It was revealed that a solution of wood vinegar with a dilution of 1:100 has a statistically significant phytotoxic effect, dilutions of wood vinegar 1:200 and 1:300 do not have statistically significant differences from the control for the raw and dry biomass of the roots of lettuce seedlings.

The effect of wood vinegar solutions on the accumulation of raw and dry biomass by the roots of arugula seedlings has not been statistically confirmed (Fig. 3). Nevertheless, there is a tendency to stimulate the accumulation of dry biomass by the roots of test culture seedlings in dilution variants 1:100, 1:200, 1:300, 1:400 and 1:900, the values in which are higher than the benchmarks by 6.5%, 16.1%, 9.7%, 6.5% and 6.5%, respectively. In addition, it was found that the accumulation of crude biomass by the roots of arugula seedlings is positively influenced by solutions of wood vinegar in dilutions of 1:200 and 1:300, the values in which exceed the control values by 17.6% and 5.6%, respectively.

There is also a tendency to stimulate the accumulation of raw biomass by the roots of dill and curly parsley seedlings in dilution variants 1:100, 1:200, 1:300, 1:400 and 1:900, the values in which exceed the control values from 0.9% to 14.5%, however, the indicators of the dry biomass of the roots of garden dill seedlings are lower than the control ones in all variants of dilutions of wood vinegar. A reliably confirmed phytotoxic effect, manifested in a decrease in the dry biomass of the roots of curly parsley seedlings by 28.8% compared with the control parameters, was established for a solution of wood vinegar with a dilution of 1:400.

The results of the study revealed a statistically significant phytotoxic effect of the solutions of wood vinegar in dilution of 1:100 and 1:300 on radish seedlings (Fig. 3). When using a solution of wood vinegar 1:400, a stimulating effect on the accumulation of raw and dry biomass by the roots of radish seedlings is observed, but not statistically reliable. Changes in the accumulation of raw and dry biomass by the roots of test culture seedlings in other variants of wood vinegar dilutions have also not been statistically confirmed.

Multivariate Discriminant Data Analysis

Since the data obtained throughout this study on seed germination and the root length and biomass of the seedlings of the considered higher plants, depending on the influence of various dilutions of the water-soluble fraction of the pyrolysis liquid and wood vinegar, were very contradictory, we carried out a multivariate discriminant analysis considering the indicators for all plants (Tables 2 and 3).

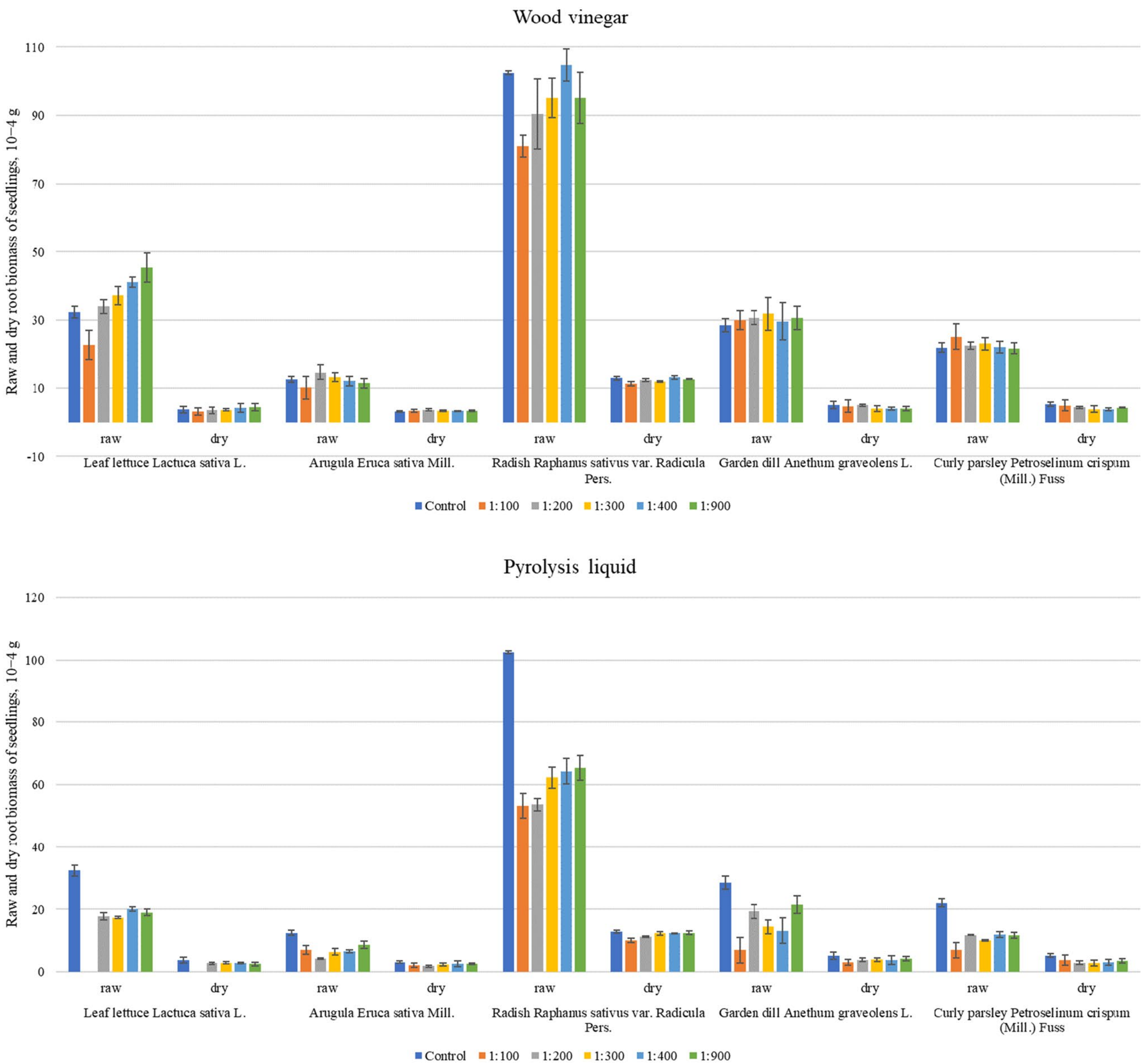


Fig. 3 Raw and dry biomass of seedling roots of the tested cultures

As a result of the discriminant analysis, which considered the indicators of seed germination and the seedling root length of the studied cultures, a statistically significant difference was established between the control and all dilutions of the water-soluble fraction of the pyrolysis liquid (Table 2). With an increase in the dilution of the water-soluble fraction of the pyrolysis liquid, the squared Mahalanobis distance between the centroids of the control and tested dilutions decreased. The pattern indicates a significant decrease in the phytotoxic effect exerted by the water-soluble fraction of the pyrolysis liquid on seed germination of seeds and root growth of the seedlings with an increase in the dilution. According to the square of the Mahalanobis distance, the lowest phytotoxic dilutions were 1:900 and 1:400; the most toxic dilution for plants was 1:100. Considering the statistical values of Wilks' lambda and partial Wilks' lambda, the most informative indicator determining the differences was the root length of the seedlings ($p < 0.0000001$); the seed germination of tested plants was less informative ($p < 0.004$).

Inclusion in the multivariate discriminant analysis of parameters for wet and dry root biomass of the seedlings (Table 2) contributed to a statistically significant increase in the squared Mahalanobis distance between the centroids of the control and tested dilutions of the water-soluble fraction of the pyrolysis liquid and their greater divergence in the coordinate plane of two canonical discriminant

Table 2 Values of the squared Mahalanobis distance between the centroids of the dilution for the water-soluble fraction of the pyrolysis liquid

Dilution	Control	1:100	1:200	1:300	1:400	1:900
Control	–	10.1*	3.5*	2.5*	1.1*	1.1*
		27.5*	8.0*	10.8*	7.6*	5.4*
1:100	10.1*	–	3.5*	3.5*	4.7*	4.9*
	27.5*		7.6*	5.0*	6.9*	9.3*
1:200	3.5*	3.5*	–	0.1	0.9	0.8*
	8.0*	7.6*		0.3	0.2	0.7
1:300	2.5*	3.5*	0.1	–	0.4	0.3
	10.8*	5.0*	0.3		0.3	1.2
1:400	1.1*	4.7*	0.9*	0.4	–	0.0
	7.6*	6.9*	0.2	0.3		0.2
1:900	1.1*	4.9*	0.8*	0.3	0.0	–
	5.4*	9.3*	0.7	1.2	0.2	

*Difference is statistically significant at $p < 0.05$

Note: above the line – values for discrimination based on seed germination and root length of test plant seedlings, below the line – values for discrimination based on seed germination, seedling root length, wet and dry biomass of test plant seedling roots

Table 3 Values of the squared Mahalanobis distance between the centroids of dilution options for wood vinegar solution

Options	Control	1:100	1:200	1:300	1:400	1:900
Control	–	2.2*	0.8*	0.2	0.0	0.2
		0.7	0.3	0.5	0.4	0.2
1:100	2.2*	–	0.4	1.4*	2.5*	1.2*
	0.7		0.2	1.6*	1.7*	0.7
1:200	0.8*	0.4	–	0.4	1.0*	0.2
	0.3	0.2		0.8	0.9	0.2
1:300	0.2	1.4*	0.4	–	0.2	0.1
	0.5	1.6*	0.8		0.0	0.2
1:400	0.0	2.5*	1.0*	0.2	–	0.3
	0.4	1.7*	0.9	0.0		0.3
1:900	0.2	1.2*	0.2	0.1	0.3	–
	0.2	0.7	0.2	0.2	0.3	

*Difference is statistically significant at $p < 0.05$

Note: above the line – values for discrimination based on seed germination and root length of test plant seedlings, below the line – values for discrimination based on seed germination, seedling root length, wet and dry biomass of test plant seedling roots

axes, indicating a statistically significant contribution of the parameters of wet and dry seedling root biomass in revealing differences in the effect of water-soluble fraction of the pyrolysis liquid on plant seedlings. According to the values obtained for the square of the Mahalanobis distance (Table 2), the 1:900 dilution of the water-soluble fraction of the pyrolysis liquid had the least phytotoxic effect; the dilution of 1:100 was the most toxic for plants. Considering the statistical values of Wilks' lambda, as well as partial Wilks' lambda, the informative indicators that determined the differences were in the following order: seedling root length ($p < 0.0000001$) > dry seedling root biomass ($p < 0.0000001$) > wet seedling root biomass ($p < 0.0000001$). Seed germination did not make a significant contribution to discrimination.

According to the discriminant analysis, considering the seed germination and root length of the seedlings, a statistically significant difference was established between the control and wood vinegar dilutions of 1:100 and 1:200. Dilutions 1:300, 1:400 and 1:900 were not significantly different from the control (Table 3). The differences indicate the presence of a predominantly phytotoxic effect of the 1:100 and 1:200 dilutions of wood vinegar, and no effect in dilutions of 1:300, 1:400 and 1:900. When using wood vinegar at dilutions of 1:300, 1:400 and 1:900, a phytostimulating effect can be expected with a high degree of probability. According to the squared Mahalanobis distance, the most toxic dilution for plants was 1:100. Considering the statistical values of Wilks' lambda and partial Wilks' lambda, the most informative indicator that determines the revealed differences was the root length of seedlings ($p < 0.000002$). Seed germination did not make a statistically significant contribution to discrimination.

The addition of parameters for wet and dry root biomass of seedlings to the multivariate discriminant analysis (Table 3) did not lead to significant differences between the control and tested wood vinegar dilutions. The effect of wood vinegar dilutions on the accumulation of wet and dry root biomass of seedlings was not as significant as that of the water-soluble fraction of the pyrolysis liquid. According to the statistical values of Wilks' lambda and partial Wilks' lambda, the most sensitive parameter in assessing the effect of wood vinegar on plants was the root length of the seedlings of tested crops ($p < 0.05$).

Discussion

The results indicate that the water-soluble fraction of the pyrolysis liquid is more toxic to the seedlings of the studied cultures than wood vinegar. Apparently, the less volatile components of the water-soluble fraction of the pyrolysis liquid, which were separated during distillation (for example, phenols), were the most toxic to plants. The stimulating effect of the wood vinegar noted for some plants was most likely due to the content of carboxylic acids, sugars and other natural components that are involved in the mineral nutrition of plants and are used by plants as an energy source.

Our results are consistent with the data of Wang Y and co-authors, who noted the stimulating effect of pyrolysis liquid in a 1:900 dilution on the growth of wheat roots [19]. Wheat belongs to cereal plants. The only cereal crop we used in the experiment was oats. A significant stimulating (compared with the control) effect of a solution of the pyrolysis liquid in a dilution of 1:900 on the length of the roots of oats seedlings is noticeable (Fig. 2). Wang Y and co-authors attributed the best root growth to the fact that the roots of wheat seeds, pre-impregnated with pyrolysis liquid, can initiate an early protective mechanism to mitigate stress, that is, the production of an appropriate group of proteins [19]. At higher concentrations, such a hormesis effect was not observed. Apparently, the concentration of phenols, aldehydes and other toxic substances contained in the pyrolysis liquid at lower dilutions turns out to be too high. Kulkarni and co-authors [20], in experiments with rice seeds and seedlings, obtained a similar result at a dilution of 1:500.

If we consider the results of seed germination, the best indicator was the radish test culture, which, with dilutions of the water-soluble fraction of pyrolysis liquid 1:200, 1:400 and 1:900 and with dilution of wood vinegar 1:100, reached a germination rate of 100%. Considering that the pyrolysis liquid has a strong smell of smoke, which contains (3-methyl-2H-furo-[2,3-c]piran-2-one), which in turn stimulates and accelerates the percentage of germination of radish seeds. These results confirm researches by other scientists [30, 31]. Given the tendency of the negative effect of the water-soluble fraction of the pyrolysis liquid on seed germination, it can be assumed that the composition of the liquid has a more aggressive composition (phenols, ketones and anhydrous sugar) and has a phytotoxic effect on the seeds of test crops. This statement is confirmed by a number of scientists [32]. Luo et al. claimed that higher concentrations of wood vinegar were explained by the presence of phenols that reduce the pH of the solution [33].

As for the results of studies on the length of the roots of the test crop seedlings, it can be assumed that the water-soluble fraction of pyrolysis liquid has a phytotoxic effect on the seeds, however, wood vinegar showed more positive results. These results are confirmed by a number of researchers who have shown that very low concentrations of woody acid (0.002% and 0.02%) improve the growth of seedlings and shoots; this was due to increased availability of nutrients due to the slow release of active acid and phenolic components [33–35].

The best indicators are presented in Table 4, which show that the 1:900 dilution of wood vinegar improves the characteristics of lettuce leaves: the volume of wet biomass by 40%, dry biomass by 19%, seed germination by 3%, root length by 18%, that are impressive positive results.

When switching from experiments on filter paper to another soil, a positive effect can be observed at different concentrations of the pyrolysis liquid solution, which is confirmed by the experiments of Zulkarami and co-authors [36], who studied the effect of pyrolysis liquid on melon growth on peat soil.

Wood vinegar is purified from many components present in the pyrolysis liquid, for example, phenols (Table 1), in connection with it its toxic effect on plants is manifested to a lesser extent. The negative effect of wood vinegar on seedlings may be due to an acidic reaction of the medium.

Presumably, the effect of the pyrolysis liquid on germination of seeds, seedling root growth, and the accumulation of wet and dry root biomass of the tested cultures was due to the plant species characteristics. Therefore, when assessing the impact of the water-soluble fraction of pyrolysis liquid and wood vinegar on the plant, it is necessary to be guided by the results obtained in the experiment with a particular plant species. Also, changing the substrate can lead to a change in the results, as shown by J. Abrego and co-authors using the example of biochar [37]. We have not seen any work on changing the effect of pyrolysis liquid and wood vinegar on plants when changing the substrate.

Table 4 The best dilutions of wood vinegar for test crops

Indicator	The best dilution effect relative to the control sample							
	White mustard *Sinapis alba L*	Oats *Avena sativa L*	Peas *Pisum sativum L*	Leaf lettuce *Lactuca sativa* L	Arugula Eruca sativa Mill	Radish *Raphanus sativus* var. *Radicula* Pers	Garden dill *Anethum graveolens* L	Curly parsley *Petroselinum crispum* (Mill.) Fuss
	Wood vinegar							
Seed germination	Negative effect	1:300	1:400	1:900	1:300 and 1:400	1:100	Negative effect	1:200 и 1:400
		2.6%	2.6%	3%	1.3%	2.8%		1.5%
The length of the roots	1:400	1:900	1:400	1:900	Negative effect	1:400	Negative effect	1:300
	4.8%	12.7%	6.7%	18%		13.4%		8.9%
Dry biomass	It was not measured	It was not measured	It was not measured	1:900	1:200	1:400	Negative effect	Negative effect
				19%	17.6%	2.1%		
Raw biomass	It was not measured	It was not measured	It was not measured	1:900	1:200	1:400	1:300	1:100
				40%	16.1%	1.6%	11.9%	14.6%
Water-soluble fraction of pyrolysis liquid								
Seed germination	Negative effect	1:900	Negative effect	Negative effect	1:900	1:200. 1:400 and 1:900	Negative effect	Negative effect
		1.1%			0.6%	2.8%		
The length of the roots	Negative effect	1:900	Negative effect	Negative effect	Negative effect	1:400 and 1:900	Negative effect	Negative effect
		6%				9.6%		
Dry biomass	It was not measured	It was not measured	It was not measured	Negative effect	Negative effect	Negative effect	Negative effect	Negative effect
Raw biomass	It was not measured	It was not measured	It was not measured	Negative effect	Negative effect	Negative effect	Negative effect	Negative effect

Conclusion

The water-soluble fraction of pyrolysis liquid was more toxic to the seedlings of the studied crops than wood vinegar. The multivariate discriminant analysis showed that the phytotoxic manifestation of the water-soluble fraction of pyrolysis liquid for most plants weakened starting from a dilution of 1:400 and for wood vinegar starting from a dilution of 1:300.

The most informative indicators when phytotesting the water-soluble fraction of pyrolysis liquid were the root length and the dry and raw root biomass of the roots of the seedlings. When phytotesting wood vinegar, the maximum information content is the length of the roots of test crop seedlings, the germination parameter of test plant seeds is uninformative in both cases.

It has been proven that when diluting wood vinegar 1:900, the characteristics of lettuce leaves improve: the volume of wet biomass by 40%, dry biomass by 19%, seed germination by 3%, root length by 18%. That are impressive positive results. As for the water-soluble fraction of the pyrolysis liquid, it generally has a phytotoxic effect on the selected test cultures in any dilutions.

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Availability of Data and Material (Data Transparency) The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors have no other competing interests.

Ethics Approval (Include Appropriate Approvals or Waivers) Humans and their biomaterials have not been obtained as a result of research.

Consent to Participate (Include Appropriate Statements) Informed consent was obtained from all individual participants included in the study.

Consent for Publication (Include Appropriate Statements) The authors confirm that the study participants gave informed consent to the publication of articles with all images and tables.

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