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Impact of Selected Plant Extracts on Winter Wheat (*Triticum aestivum* L.) Seedlings: Growth, Plant Health Status and Soil Activity

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Abstract: The aim of the study was to assess the impact of plant extracts from hemp inflorescences (H10—10% and H20—20%), as well as a mixture of extracts from hemp inflorescences, sage, and tansy leaves (M10—10% and M20—20%) on phytotoxicity and selected physiological and biometric parameters of wheat seedlings, as well as the biological activity of soil in a growth chamber experiment. In all experimental combinations, a low phytotoxicity of the extracts was observed in the form of leaf tip yellowing, classified as first-degree damage or its complete absence. The plant extracts and their mixtures, except for the H20 extract, had an inhibitory effect on the development of fungal pathogens, especially *Fusarium* spp. The H20 extract increased the fresh and dry weight of root seedlings. The tested extracts also had a positive effect on the chlorophyll content in seedlings. The highest chlorophyll concentrations were recorded for the seedlings sprayed with the M20 extract mixture. The applied plant extracts influenced the activity of soil enzymes. The highest activity of catalase and dehydrogenases was observed after spraying seedlings with M20, while the lowest was recorded after applying H10. Of all the tested groups of soil environment compounds included in the Biolog EcoPlates test, carbohydrates and carboxylic acids were most actively utilized. Conversely, amines and amides constituted the group of compounds utilized the least frequently. The present study demonstrated the high effectiveness of plant extracts on wheat seedlings due to their biocidal action against phytopathogenic fungi and increased biological activity of the soil. This research serves as an initial phase of work, which will aim to verify the results obtained under field conditions, as well as assess the biological stability of the extracts.

Keywords: plant extracts; wheat seedlings; damage index; chlorophyll; catalase activity; dehydrogenase activity; soil biological activity

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1. Introduction

In addition to its many positive aspects, agricultural intensification contributes to a number of adverse environmental phenomena, such as soil, water, air, or food contamination by pesticide residues and heavy metals from mineral fertilizers, leading to soil erosion, a reduction in the biodiversity of soil environment, and landscape impoverishment due to the introduction of monocultures [1,2]. In the last decade, the use of fertilizers and crop-protection products has increased in European Union countries [3]. Overuse of pesticides leads to strong selective pressure and an increase in resistance among agricultural pests [4]. In this regard, efforts should be made to reduce the chemical intensity of agriculture through an increasingly measured and controlled selection of products, as well

as expanding research to search for new products of natural origin. These products, by strengthening plant defense systems, would act as elicitors and growth conditioners [5–7]. Such products mainly originate from herbal plants, which have the ability to synthesize aromatic secondary metabolites, such as phenols, phenolic acids, quinones, flavones, flavonoids, phenolic acids, quinones, tannins, and coumarins, and are highly effective against phytopathogens [8,9]. Natural plant extracts modulate plant growth and modulate plant defense reactions [5]. They are also used as antimicrobial preparations against many fungal phytopathogens, such as *Alternaria solani*, *Aspergillus fumigatus*, *A. niger*, *Trichoderma longibrachiatum*, *A. flavus*, *A. fumigatus*, *Fusarium solani*, *F. oxysporum*, *Bipolaris orzyae*, *Botrytis cinerea*, *Curvularia lunata*, *F. verticilliodies*, and *F. graminearum* [10–15]. In addition, they can be used against plant bacterial pathogens, such as *Pectobacterium carotovorum*, *Pectobacterium atrosepticum*, *Dickeya solani*, and *Agrobacterium tumefaciens* [16]. The effects of natural compounds, such as terpenoids, phenols, and alkaloids, are not specific, and their effects on pathogens are versatile [15]. Natural bioactive compounds applied in plant protection exhibit fungicidal activity or limit the growth of pathogens (fungistatic effect), as well as act as elicitors inducing plant defense responses [5].

The need to protect the natural environment has made it necessary to search for new and safe plant protection products [17], where preparations obtained from plant-derived products represent one of the most promising prospects [18]. These preparations have the potential to stimulate plant growth and development, protecting them from biotic and abiotic stresses [2,7], when they act as biostimulants [19–21]. Plant extracts are a rich source of compounds, including essential and non-essential amino acids, saccharides (glucose, mannose, cellulose), micronutrients (Cu, Fe, Mn, Zn), macronutrients (Ca, K, Mg, N, P), vitamins (e.g., B1, B2, B6, C), and phytohormones (e.g., gibberellins, salicylic acid), among others [22]. They also contain antioxidants and osmoprotectants such as α-tocopherol, ascorbic acid, vitamins from the A and B groups, glutathione, salicylic acid, selenium, amino acids like proline, and soluble sugars, as well as phytohormones like auxins, gibberellins and cytokinins (zeatin type), and nutrients [23,24]. Despite their economic significance, evidence regarding the justified widespread use of plant extracts to replace synthetic products such as pesticides remains scarce.

The objective of the study was to assess the impact of a mixture of extracts from selected herbal plants on:

- health status of wheat seedlings,
- fresh and dry plant weight,
- chlorophyll content in leaves, and
- soil biological activity. The results of the study will form the basis for further field trials to develop the formulation of the natural product and evaluate its effectiveness in plant protection against fungal phytopathogens.

To achieve the intended research goals, the following research hypotheses were formulated:

The null hypothesis (H0) assumes that the mixtures of extracts from selected herbal plants do not affect the health status of wheat seedlings, the fresh and dry weight of plants, the chlorophyll content in leaves, as well as the biological activity of the soil.

The alternative hypothesis (H1) aims to prove that:

I. The use of a mixture of extracts from selected herbal plants will increase the health status of wheat seedlings, the fresh and dry weight of plants, and chlorophyll content in leaves,

II. The use of a mixture of extracts from selected herbal plants will increase the biological activity of the soil.

2. Materials and Methods

The experiment was carried out in a growth chamber at the Department of Plant Protection of the University of Life Sciences in Lublin (Poland). Soil for the pot experiment was collected from a farm located in the Skrzynice, Lublin Voivodeship (Skrzynice—51.1167° N,

22.2500° E) Poland, with the following content of assimilable forms of mineral components: N-NO_3—43.04 mg/kg of dry soil (very low), P_2O_5—13.1 mg/100 g dry soil (average), K_2O—24.1 mg/100 g dry soil (high), Mg—4.7 mg/100 g dry soil (low), and pHH_2O—4.4 (very acidic), which, after the experiment, reached the values presented in Table 1.

Table 1. Content of assimilable forms of mineral components in the soil after plant extract application.

Experimental Combination	pH	Reaction	Assimilable Mineral Forms							
			Phosphorus—P_2O_5 [mg/100 dry g soil]		Potassium—K_2O [mg/100 g dry soil]		Magnesium—Mg [mg/100 g dry soil]		Nitrogen—N min. [kg/ha]	
			Content	Abundance	Content	Abundance	Content	Abundance	Content	Abundance
C	6.1	Slightly acidic	26.1	Very high	37.0	Very high	7.5	Moderate	30.60	Very low
H10	4.3	Very acidic	11.2	Moderate	9.7	Low	4.6	Low	43.90	Very low
H20	4.8	Acidic	14.9	Moderate	16.8	Moderate	5.6	Low	38.60	Very low
M10	5.1	Acidic	18.3	High	21.0	High	6.1	Moderate	42.90	Very low
M20	6.1	Slightly acidic	28.2	Very high	38.6	Very high	8.0	Moderate	35.90	Very low

2.1. Plant Material

Winter wheat seedlings (*Triticum aestivum* L.), cultivar 'Venecja' from Hodowla Roślin Strzelce Sp. z o.o. Grupa IHAR, Strzelce, Poland, were used in the experiments. Seeds of wheat were surface-disinfected with 0.1% sodium hypochlorite for 1 min, and subsequently rinsed three times in distilled water [25]. The seeds were germinated on glass plates filled with sterile filter paper. Germinating winter wheat grains were subjected to a short vernalization stage (10 days) at a temperature of +1 to +3 °C, with access to moisture and air. Then the seedlings were planted in 1 L plastic pots filled with soil. Seedling plants were placed in a growth chamber at 22 ± 1 °C and 85% relative air humidity with a 14 h photoperiod, and watered with sterile water as needed.

2.2. Extract Preparation

The plant extracts were prepared using dried lateral inflorescences of hemp (*Cannabis sativa* L.), dried leaves of common sage (*Salvia officinalis* L.), and dried leaves of tansy (*Tanacetum vulgare* L.). The plant extracts used in the experiment were prepared according to the methodology described by Kursa et al. [26]. The herbal material used in the experiment was purchased from hemp producer Bartosz Michalski (Polskie CBD, Lublin, Poland).

2.3. Biological Assay

After seven days (1 week) of seedling growth in the pots (BBCH 10), the seedlings were sprayed once with plant extracts (50 mL of solution per pot) in the following experimental combinations:

(1) seedlings sprayed with 10% hemp extract (H10),
(2) seedlings sprayed with 20% hemp extract (H20),
(3) seedlings sprayed with a 10% mixture of extracts from hemp, common sage, tansy (M10),
(4) seedlings sprayed with a 20% mixture of extracts from hemp, common sage, tansy (M20),
(5) control seedlings sprayed with water (C).

Five replicates (pots) were used for each experimental combination. Twenty-five germinated wheat seedlings were planted in each pot (resulting in a total of 125 seedlings for each experimental combination). Fifty mL of plant extract was applied to each pot. An adjuvant in the form of a 1% glycerin solution was added to the spraying formula for better adherence of the extracts to the leaf surface.

2.4. Estimation of Phytotoxicity Index (PI)

The phytotoxicity of extracts (phytotoxicity index—PI) was assessed three weeks after extract application to plants using a five-point scale: 0°—no symptoms; 1°—symptoms present on less than 25% of the plant; 2°—symptoms present on 25–50% of the plant; 3°—symptoms present on 50–75% of the plant, 4°—symptoms present on 75–100% of the plant, according to the European and Mediterranean Plant Protection Organization standard—PP 1/135 (4) [27]. The phytotoxicity index was estimated for each replication using the following formula:

The phytotoxicity index (PI) = ([(a × 0.25) + (b × 0.5) + (c × 0.75) + (d × 1])/n, where a is the number of plants with first-degree symptoms, b is the number of plants with second-degree symptoms, c is the number of plants with third-degree symptoms, d is the number of plants with fourth-degree symptoms, and n is the total number of examined plants multiplied by the highest index of the numerical scale (fourth degree). The degree of phytotoxicity was expressed by an index on a 0–1 scale.

2.5. Seedling Mycological Analysis

Mycological analysis of the seedlings was performed 3 weeks after application of the extracts (tillering stage, BBCH 23). For analysis, 25 randomly selected seedlings were sampled from each experimental combination. Cleaned plant fragments (aerial part and roots) were placed on a mineral medium according to the methodology described by Jamiołkowska [25] and incubated at 21 °C for seven days in the dark. Ten replicates (Petri dishes) were prepared for each experimental combination, and plant parts were analyzed. The resulting fungal colonies were transferred to a potato-dextrose medium (PDA, Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and identified to the species based on microscopic features and available monographs.

2.6. Assessment of Seedling Fresh and Dry Weight

The analysis was carried out four weeks after the application of the extracts to the plants (BBCH 23). From each experimental combination, 50 seedlings (10 plants from each replicate/pot) were collected. The seedlings were cleaned of soil debris and weighed. The measurement results were expressed in g fw^{-1}. After drying the plants (in a ventilated room at a temperature of 23–25 °C for five days), the experimental material was weighed and the obtained results were expressed in g fw^{-1}.

2.7. Analysis of Leaf Chlorophyll Content

Three weeks after the extracts were applied to the plants, the chlorophyll a, b, a + b contents of winter wheat seedlings were analyzed according to the methodology described by Blamowski and Borowski [28]. From each experimental combination, 1 g of leaves was weighed, cut into small pieces (<0.5 cm), and ground in a mortar with the addition of 5 g of calcium carbonate ($CaCO_3$) and 5 cm^3 of cooled acetone solution (80%). The mixture was ground until an intense green color was obtained. The green solution was filtered through filter paper into a 50 cm^3 measuring flask. To the mass remaining in the mortar, another 5 cm^3 of acetone was added, ground, and the solution was transferred to the flask. These steps were repeated until the macerated leaf tissue was completely colorless (the missing amount of solution was replenished with acetone). The prepared solutions were protected from sunlight. The flasks were refrigerated until analysis. The contents of each flask were thoroughly mixed before spectrophotometric measurements. The control was an 80% acetone solution. Measurements were performed at the following wavelengths: for chlorophyll a—663 nm, chlorophyll b—645 nm, and chlorophyll a + b—652 nm. The pigment content was then calculated according to the following formulas:

$$\text{Chlorophyll a} = (12.7D663 - 2.7D645) \times V/(1000 \times m)\ [\text{mg/g f.w.}]$$

$$\text{Chlorophyll b} = (22.9D645 - 4.7D663) \times V/(1000 \times m)\ [\text{mg/g f.w.}]$$

$$\text{Chlorophyll a + b} = 27.8D652 \times V/(1000 \times m)\ [\text{mg/g f.w.}],$$

where D—absorbance at a given wavelength, V—total volume of extract [cm^3], m—sample mass [g].

2.8. Analysis of Soil Catalase and Dehydrogenase Activity

Three weeks after spraying the seedlings, soil samples were collected from each pot from a depth of 5–20 cm (rhizosphere zone). Soil samples were averaged for each experimental combination. To determine the catalase activity in the soil, the method described by Johnson and Temple [29] was used, which involved incubating the soil with the addition of hydrogen peroxide (natural enzymatic substrate). Catalase activity was examined in fresh soil material sieved through a 1 mm mesh. The remaining hydrogen peroxide (H_2O_2), not degraded by catalase, was titrated with potassium permanganate in an acidic environment. The activity analyses for each sample were repeated three times, and the averaged results were reported in units of catalase activity, i.e., mg H_2O_2 g^{-1} dm min^{-1}. Soil dehydrogenase activity was assessed using the method described by Casida et al. [30]. The procedure is based on the use of 2,3,5-triphenyltetrazolium chloride (TTC), which acts as an artificial hydrogen and electron acceptor. Enzyme activity was measured spectrophotometrically at 485 nm. Activity analyses for each sample were repeated three times, and the averaged results were expressed as μg TPF g^{-1} dw d^{-1} equivalents.

2.9. Assessment of Soil Functional Biodiversity Using Biolog EcoPlates

Soil functional biodiversity was assessed using Biolog EcoPlatesTM (Biolog, Inc., Hayward, CA, USA) according to the methodology described by Jamiołkowska et al. [31]. One gram of each soil sample was suspended in 99 mL of sterile water and vortexed for 20 min at room temperature. The suspension was allowed to settle for 30 min at 4 °C [32]. Each well was inoculated with 120 μL of suspension and incubated at 25 °C for 7 days in an OmniLog® ID System multiplate reader (Biolog, Inc., Hayward, CA, USA). Plates were prepared in triplicate for each experimental sample. After 24, 72, and 96 h of incubation, very intense metabolic activity was observed for 31 carbon sources on the plates. Microbiological activity was observed in five groups of compounds (amines and amides, amino acids, carbohydrates, carboxylic acid and polymers). Cluster analysis and PCA were performed on standardized data using the mean absorbance values after 96 h (Biolog EcoPlates).

2.10. Statistical Analysis

The data were subjected to statistical analysis using Statistica version 13.3 software (1984–2017 TIBCO Software INC, Palo Alto, CA, USA). The significance of differences in the results was assessed based on the Tukey's multiple comparison test and Kruskal–Wallis test at $p \leq 0.05$.

Cluster analyses (agglomeration, area and feature clustering, and SANN) were conducted using data standardized relative to the mean absorbance values at 24, 72, and 96 h after inoculation (Biolog EcoPlates). In addition, the results were subjected to principal component analysis (PCA).

3. Results

3.1. Phytotoxicity Index (PI)

The hemp extracts (H10 and H20) and mixtures of plant extracts (M10 and M20) used in the experiment at concentrations of 10% and 20% exerted negligible phytotoxicity to wheat seedlings. In all experimental combinations, slight yellowing of the leaf tips was observed (classified as first-degree damage) (Chart 1). At this stage of seedling development under controlled growth conditions, more pronounced symptoms of phytotoxicity did not occur in any of the combinations tested (Figure 1). A slightly higher value of PI was recorded after spraying wheat seedlings with 20% hemp extract (H20) and a mixture of plant extracts

(M20) (PI = 0.02), but these values did not significantly differ from the phytotoxicity indices of the other experimental combinations (Table 2).

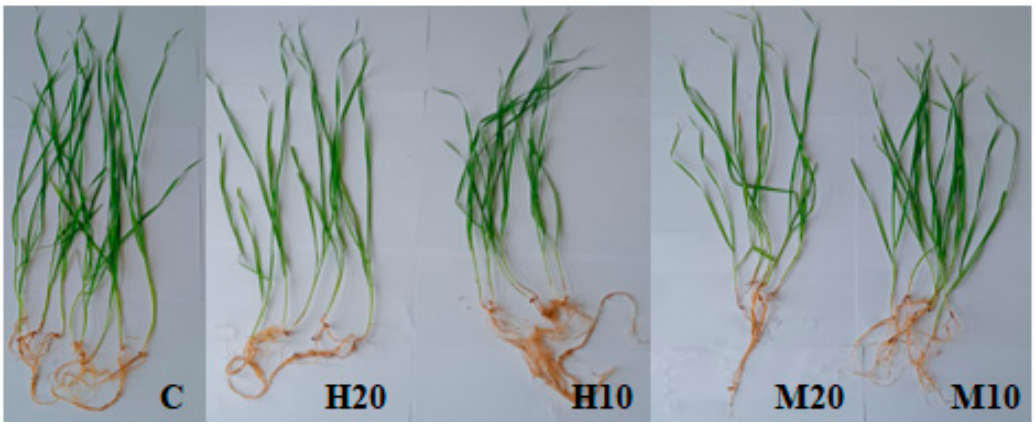

Chart 1. Winter wheat seedlings sprayed with plant extracts and in the control; C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy.

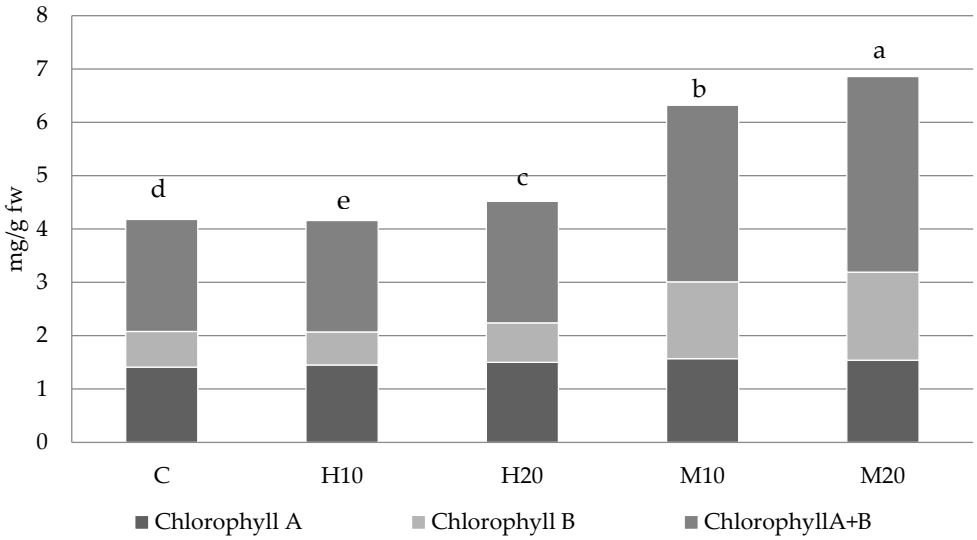

Figure 1. Total content of selected plant pigments in wheat seedling leaves after the application of plant extracts. C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy; a, b, c, d, e—values in columns marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

Table 2. Phytotoxicity of wheat (*Triticum aestivum* L.) seedlings after the application of hemp extracts (H10, H20) and extract mixtures (M10, M20).

Experimental Combination	Mean Phytotoxicity Index ± SD
C	0.00 ± 0.00 a
H10	0.01 ± 0.00 a
H20	0.02 ± 0.01 a
M10	0.01 ± 0.01 a
M20	0.02 ± 0.03 a

C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy; a—values in columns marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

3.2. Seedling Mycological Analysis

Mycological analysis of winter wheat seedlings allowed us to obtain a total of 469 fungal isolates representing 10 species. The dominant species were fungi of the genera *Trichoderma* (*T. hamatum*—53.51%, *T. harzianum*—17.06%), *Gliocladium* spp. (*G. catenulatum*—18.7%) and *Fusarium oxysporum* (6.18%) (Table 3). Mycological analysis of the roots showed that after the application of plant extracts, there was an increase in the total number of fungal species colonizing the roots, especially *T. hamatum* (Table 3). After spraying the seedlings with plant extracts, there was also an increase in the overall number of fungi on the aerial parts of the seedlings, where saprotrophic species such as *T. hamatum* and *G. catenulatum* were predominant. Seedlings sprayed with plant extracts were less colonized by *F. oxysporum* compared to control seedlings, except for plants from the H20 combination. The lowest number of *F. oxysporum* colonies was isolated from seedlings sprayed with 10% hemp extract, while the highest was obtained from the control (Table 3).

Table 3. Fungi isolated from the roots and aboveground parts of wheat seedlings after plant extract application.

Species of Fungi	Number of Isolates										Total (%)
	Roots					Aboveground Part					
	C	H10	H20	M10	M20	C	H10	H20	M10	M20	
Aspergillus niger Tiegh.	-	-	-	-	-	-	2	-	-	-	2 (0.43)
Chaetomium spp.	-	-	-	-	-	-	-	-	-	2	2 (0.43)
Fusarium oxysporum Schltdl.	-	-	-	-	-	9	1	12	4	3	29 (6.18)
Giocladium catenulatum J.C. Gilman & E.V. Abbott	-	-	-	-	-	19	38	11	1	19	88 (18.76)
Giocladium roseum Bainier	-	2	-	-	-	-	-	-	-	-	2 (0.43)
Penicillium spp.	-	-	-	-	-	-	-	-	5	-	5 (1.07)
Trichoderma hamatum (Bonord.) Bainier	26	35	46	22	33	10	10	17	41	11	251 (53.51)
Trichoderma harzianum Rifai	22	8	3	29	18	-	-	-	-	-	80 (17.06)
Trichoderma koningii Oudem.	-	1	-	-	-	-	-	-	-	-	1 (0.21)
Trichoderma polysporum (Link) Rifai	-	-	-	-	-	-	-	-	-	9	9 (1.92)
Total	48	46	49	51	51	38	51	40	51	44	469 (100)

C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy.

3.3. Fresh and Dry Weight of Seedlings

Plant biomass was expressed as the dry and fresh weight of seedlings. The results of the measurements were expressed in grams per individual seedling and are presented in Table 4. Wheat seedlings sprayed with plant extracts showed varied increases in fresh and dry weight. The highest and statistically significantly different fresh weight of the roots was observed in the seedlings sprayed with 20% hemp extract (H20—0.214 g/seedling). On the other hand, the application of 10% hemp extract and 10% extract mixture resulted in a reduction in the fresh weight of seedling roots compared to other experimental combinations (H10—0.132 g/seedling, M10—0.105 g/seedling), although the differences were not statistically significant (Table 4). The highest dry weight of root seedlings was recorded after spraying with 20% hemp extract (H20—0.028 g/seedling), but it was not significantly different from the remaining experimental combinations (Table 4). The highest fresh weight of aboveground parts was recorded for seedlings sprayed with 20% hemp extract (H20—0.59 g/seedling) and 20% plant extract (M20—0.589 g/seedling), where these values did not differ statistically from the control, nor from M10 combination

(M10—0.483 g/seedling), but were significantly higher compared to the H10 combination (H10—0.439 g/seedling). Similarly, the dry weight of the aboveground parts of the seedlings was highest in the control and in the H20 and M20 combinations (C—0.09 g/seedling, H20—0.087 g/seedling, M20—0.088 g/seedling), but these differences were not statistically significant (Table 4).

Table 4. Fresh and dry weight of the roots and aboveground parts of wheat seedlings (g/seedling) after spraying with hemp extracts (H10, H20) and plant extract mixtures (M10, M20).

Experimental Combination	Fresh Weight (g/seedling) ±SD		Dry Weight (g/seedling) ±SD	
	Root	Aboveground Part	Root	Aboveground Part
C	0.140 ± 0.04 b	0.633 ± 0.11 a	0.024 ± 0.01 a	0.090 ± 0.02 a
H10	0.132 ± 0.04 b	0.439 ± 0.05 c	0.023 ± 0.00 a	0.070 ± 0.01 a
H20	0.214 ± 0.03 a	0.590 ± 0.09 ab	0.028 ± 0.00 a	0.087 ± 0.01 a
M10	0.105 ± 0.03 b	0.483 ± 0.03 bc	0.020 ± 0.00 a	0.075 ± 0.01 a
M20	0.146 ± 0.02 b	0.589 ± 0.06 ab	0.025 ± 0.00 a	0.088 ± 0.01 a

C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy; a, b, c—values in columns marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

3.4. Chlorophyll Content in Seedling Leaves

Seedling leaves exhibited varying levels of plant pigments (chlorophyll a, chlorophyll b, total chlorophyll a + b) depending on the applied spraying (Table 5, Figure 1). The highest chlorophyll contents were obtained for combinations of plant extract mixtures. Seedlings sprayed with a 10% mixture of plant extracts (M10) exhibited the highest chlorophyll a content (1.57 mg/g f.w.). Conversely, the highest chlorophyll b and total chlorophyll (a + b) contents were recorded after spraying with a 20% mixture of plant extracts (M20), reaching 1.65 mg/g f.w. and 3.67 mg/g f.w., respectively. (Table 5). The control combination (C) had the lowest chlorophyll a content (1.41 mg/g f.w.), while the combination utilizing 10% hemp extract (H10) showed the lowest chlorophyll b and total chlorophyll (a + b) contents (0.62 mg/g f.w. and 2.09 mg/g f.w., respectively) (Table 5, Figure 1).

Table 5. Chlorophyll content in wheat leaves after spraying seedlings with plant extracts (mg/g f.w.).

Experimental Combination	Chlorophyll a	Chlorophyll b	Chlorophyll a + b
C	1.41 e	0.67 d	2.10 d
H10	1.45 d	0.62 e	2.09 e
H20	1.50 c	0.74 c	2.28 c
M10	1.57 a	1.44 b	3.31 b
M20	1.54 b	1.65 a	3.67 a

C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy; a, b, c, d, e—values in columns marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

3.5. Soil Biological Activity

The results indicated that the applied plant extracts affected the activity of soil enzymes. The highest catalase activity was observed after spraying with a 20% mixture of extracts, M20 (0.040 g H_2O_2 g^{-1} dm min^{-1}). For the remaining combinations, catalase activity was statistically lower: after spraying with a 10% mixture of extracts, M10 (0.022 H_2O_2 g^{-1} dm min^{-1}), 20% hemp extract, H20 (0.024 g H_2O_2 g^{-1} dm min^{-1}), and for soil in the control sample (0.026 g H_2O_2 g^{-1} dm min^{-1}), as well as 10% hemp extract, H10 (0.030 g H_2O_2 g^{-1} dm min^{-1}) (Table 6).

Table 6. Catalase (CAT) activity (mg H_2O_2 g^{-1} dm min^{-1}).

Experimental Combination				
C	**H10**	**H20**	**M10**	**M20**
0.026 b	0.030 b	0.024 b	0.022 b	0.040 a

C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy; a, b—values in lines marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

The highest dehydrogenase activity, statistically different from the other experimental combinations, was observed in the control soil (C—139.82 ugTPF/g d.w. soil/24 h) and in the soil treated with 20% extract mixtures (M20—137.74 ugTPF/g d.w. soil/24 h). High dehydrogenase activity was also recorded in the soil sampled from the combinations treated with 20% hemp extract (H20—113.33 ugTPF/g d.w. soil/24 h). Meanwhile, the lowest values were recorded for the combinations with 10% hemp extract (H10—86.57 ugTPF/g d.w. soil/24 h) and 10% extract mixtures (M10 96.19 ugTPF/g d.w. soil/24 h) (Figure 2).

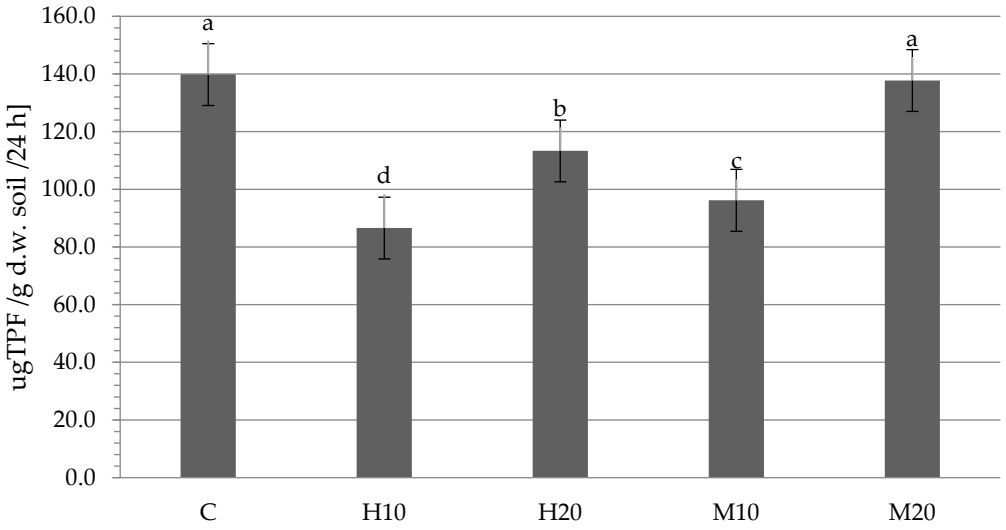

Figure 2. Soil dehydrogenase activity after spraying wheat seedlings with plant extracts. C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy; a, b, c, d—values in columns marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

3.6. Biolog EcoPlates Analysis

Carbohydrates and carboxylic acids were the most actively utilized of all the groups of compounds tested in the Biolog EcoPlates test. Amines and amides, on the other hand, were the group of compounds used the least frequently (Figure 3, Table 7). This relationship persisted throughout the study, and the differences in the utilization of the compound groups in individual combinations decreased over time compared to the control (Figure 3). Different activity in the utilization of individual carbon compounds was observed in all soil samples analyzed (after 24, 72, and 96 h). The highest utilization activity of the tested carbon compounds was observed after 96 h of incubation (Table 7). The utilization of carboxylic acids was the highest in the M20 sample (28.32%). The lowest utilization of amino acids and amines/amides was observed in the H10 sample (6.39%), while carbohydrates were most optimally utilized in this sample (H10 = 36.53%). However, the observed differences were not statistically significant (Table 7).

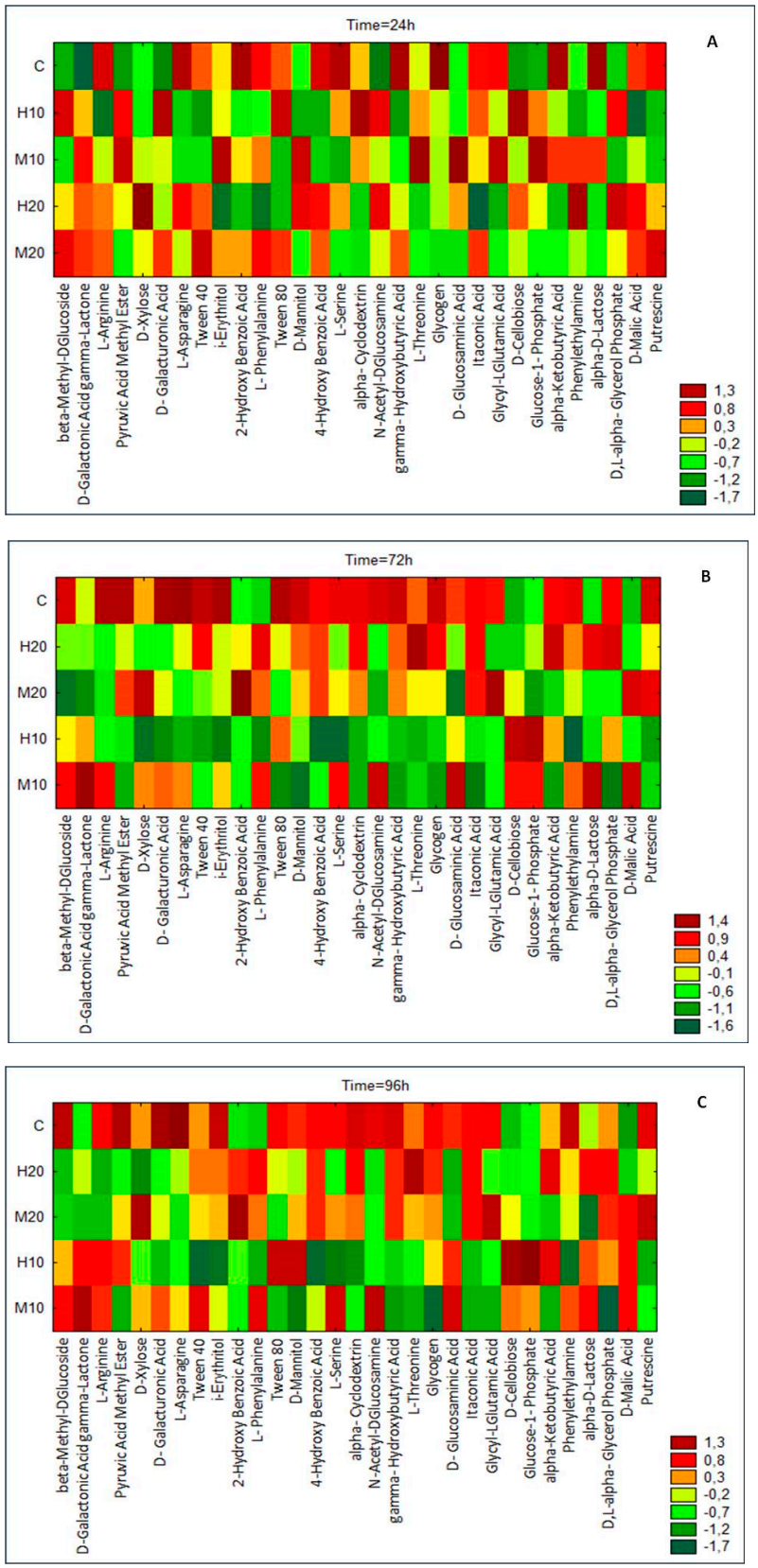

Figure 3. Heat map based on the analysis of 31 carbon sources after 24 (**A**), 72 (**B**), and 96 h (**C**) of incubation on Biolog EcoPlates. C-control; H10-10% extract from hemp inflorescences, H20—20% extract from hemp inflorescences; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy.

Table 7. Utilization of primary carbon sources (%) and their total quantity in soil after 96 h incubation.

Experimental Combination	Amines/Amides (%)	Amino Acids (%)	Carboxylic Acids (%)	Carbohydrates (%)	Polymers (%)	Total Quantity
C	7.50 ± 0.56 a	18.33 ± 2.31 a	27.01 ± 1.79 a	34.05 ± 1.60 a	14.32 ± 0.79 a	56.91 ± 0.45 a
H10	6.39 ± 0.43 a	17.87 ± 1.27 a	25.92 ± 1.57 a	36.53 ± 0.14 a	14.22 ± 1.90 a	52.35 ± 1.16 a
H20	6.79 ± 0.85 a	18.46 ± 2.42 a	27.07 ± 2.02 a	33.15 ± 1.02 a	14.10 ± 0.89 a	56.00 ± 4.15 a
M10	7.10 ± 0.61 a	18.57 ± 2.10 a	26.42 ± 0.92 a	35.59 ± 1.82 a	13.10 ± 0.23 a	52.52 ± 0.57 a
M20	7.32 ± 0.34 a	18.20 ± 1.99 a	28.32 ± 1.64 a	33.04 ± 1.70 a	13.38 ± 0.42 a	56.77 ± 3.52 a

C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy; a—values in columns marked with the same letter are not significantly different at a significance level of $p \leq 0.05$.

Data for all microbiological properties tested were used to perform cluster analysis using the agglomeration method: 31 compounds serving as carbon sources after 96 h of incubation on Biolog EcoPlates, considering the activity of tested soil dehydrogenases and catalase, are shown in Figure 4 and Table 8. The control (C) and M20 samples were found to be the most similar in terms of microbiological properties, forming the first group of most similar soil environments. The second group consisted of the H10, M10, and H20 samples. Additionally, the importance of the analyzed parameters in the clustering process was assessed using the NIPALS algorithm (as part of principal component analysis, PCA). The greatest significance (strength) was attributed to itaconic acid (96.13%), Tween 80 (92.03%), D-galacturonic acid (91.71%), L-serine (90.94%), and dehydrogenase activity (90.01%), while D-galactonic acid gamma-lactone (4.05%), alpha-cyclodextrin (2.67%), L-asparagine (0.75%), total polymer content (0.26%), and L-arginine (0.15%) were considered the least significant.

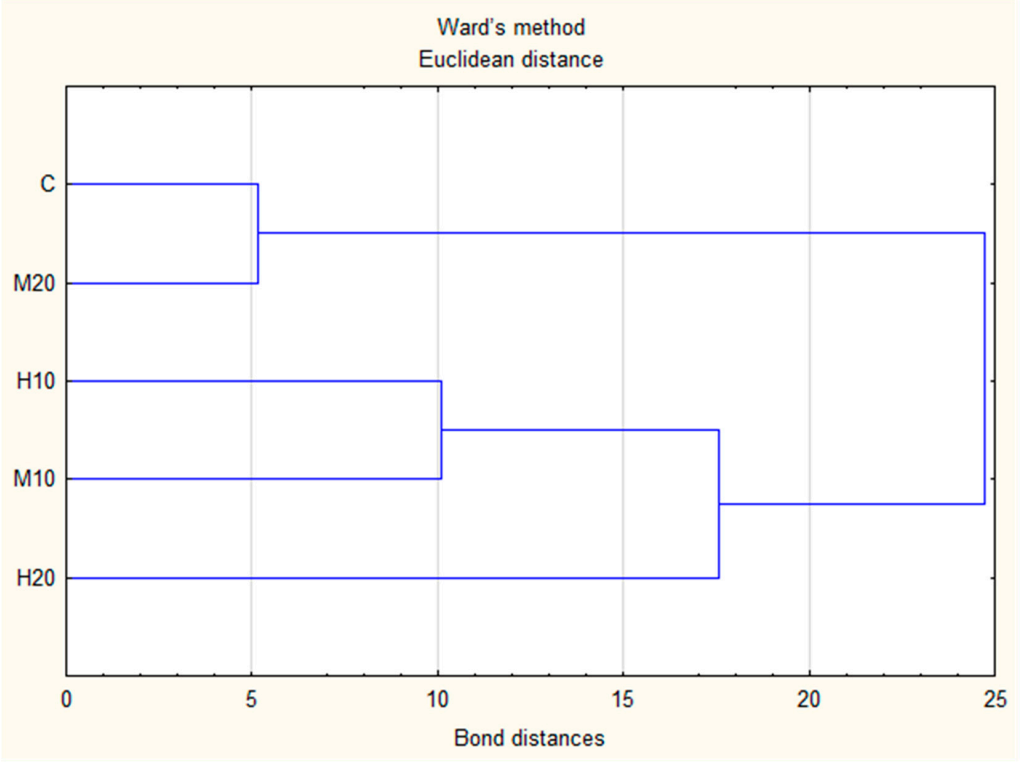

Figure 4. Hierarchical dendrogram of samples utilizing the Ward clustering method with linkage distances after 96 h of incubation: C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy; M20—20% mixture of extracts from hemp, sage, tansy.

Table 8. Functional diversity indices evaluated based on substrate utilization after 96 h in Biolog EcoPlates calculated for the dataset covering all 31 carbon sources (±SD).

Combination	Average Well Color Development	Shannon–Wiener (H′)	Evenness (E)	Richness (R)
C	1.859 ± 0.037 a	3.380 ± 0.012 abc	0.994 ± 0.004 a	30.000 ± 0.000 a
H10	1.709 ± 0.072 a	3.375 ± 0.009 bc	0.987 ± 0.007 a	30.500 ± 0.577 a
H20	1.825 ± 0.109 a	3.414 ± 0.016 a	0.994 ± 0.005 a	31.000 ± 0.000 a
M10	1.715 ± 0.019 a	3.344 ± 0.013 c	0.983 ± 0.004 a	30.000 ± 0.000 a
M20	1.851 ± 0.100 a	3.409 ± 0.012 ab	0.993 ± 0.003 a	31.000 ± 0.000 a

C—control; H10—10% hemp inflorescence extract; H20—20% hemp inflorescence extract; M10—10% mixture of extracts from hemp, sage, tansy M20—20% mixture of extracts from hemp, sage, tansy; different letters in columns indicate significant differences between combinations ($p \leq 0.05$).

Functional diversity indices of soil were calculated based on the utilization of 31 carbon sources in the Biolog EcoPlates method after 24, 48, 72, and 96 h of incubation. The highest degree of utilization of the tested carbon sources was observed after 96 h of incubation (Table 8). Soils from samples C (AWCD = 1.859) and M20 (AWCD = 1.851) exhibited the highest values of the average well color development (AWCD), indicating the highest levels of functional diversity compared to the H10 samples (AWCD = 1.709) and M10 (AWCD = 1.715). The Shannon–Wiener index (H′) revealed significant differences among the tested soil samples in terms of carbon source utilization. Regarding the evenness (E) and richness (R) indices, there were no statistically significant differences observed between the samples.

4. Discussion

Modern agriculture, employing intensive pesticide protection, has significantly reduced the population of crop pests. However, the widespread and prolonged use of chemical agents has led to a rapid increase in resistance among these organisms [33]. In light of this phenomenon, the use of preparations of natural origin, including biologically active compounds derived from plants, is of great importance. Among them are many preparations referred to as biostimulants. According to Regulation (EU) 2019/1009, a plant biostimulant is defined as a product that stimulates plant nutrition processes independently of its nutrient content, with the sole aim of improving one or more of the following characteristics of the plant or plant rhizosphere: (i) nutrient use efficiency; (ii) tolerance to abiotic stress; (iii) quality traits; and (iv) availability of limited nutrients in the soil or rhizosphere [34]. Preparations based on plant extracts constitute a new generation of products and are an environmentally friendly complement to widely applied chemicals. The native environment serves as a source of numerous valuable compounds that can and should be utilized to protect plants from indigenous pests [2,7,17,18,35,36].

The present study demonstrated the beneficial impact of the tested plant extracts on the health status of wheat seedlings. Plants sprayed with extracts were more heavily colonized by saprotrophic fungi (mainly *Trichoderma harzianum*) and to a lesser extent by pathogenic fungi such as *Fusarium oxysporum*. The mixture of plant extracts positively affected the biodiversity of seedling mycobiota, which was particularly noticeable when seedlings were sprayed with a mixture of plant extracts. The effectiveness of using plant extracts in protection against pathogenic fungi stems from their phytochemical potential, especially the presence of bioactive compounds such as flavonoids and polyphenols, and their high antioxidant potential. Kursa et al. [26] indicated a positive effect of plant extracts from sage, tansy, yarrow, and wormwood on the growth of fungi of the genus *Fusarium* spp. The extracts inhibited the growth of *Fusarium* spp. significantly better at a higher concentration (20%) compared to lower extract concentrations (5%, 10%). They also demonstrated that the greater fungistatic effect of plant extracts depended on the higher content of secondary metabolites (polyphenols and flavonoids) and their high antioxidant

activity. The conducted experiment also allowed for the assessment of the suitability of plant extracts as products influencing the biostimulation of winter wheat seedlings, considering their impact on the content of plant pigments (chlorophyll a, b, a + b), as well as the dry and fresh weight of the seedlings. The mixture of extracts from hemp, sage, and tansy significantly increased the chlorophyll content in seedling leaves. In addition, it should be noted that spraying the plants with the extracts did not significantly increase the phytotoxicity of the seedlings, even at higher concentrations. Only the 20% hemp extract statistically exerted a stronger effect compared to water used as control. These results confirmed the findings of Suteu et al. [37], who emphasized the low toxicity of plant extracts even against non-target microorganisms. The safety of the application of plant extracts is also due to their biodegradable nature, as well as the desirable interactions of individual components in terms of plant health, especially with regard to extract mixtures [35,36]. Current literature reports varying effects of plant extracts on plant dry and fresh weight. Many authors have confirmed the effective action of crop protection agents based on plant components as biostimulants for plant growth and development. Extracts from marine algae are excellent examples, as they significantly enhance shoot and root weight [38–40]. The positive or negative impact of plant extracts depends on the type and concentration of bioactive compounds present in the extracts [41,42]. In the present study, only 20% hemp extract and 20% mixture of plant extracts significantly influenced the growth of dry weight of seedling roots, while after spraying with these plant extracts, there was no increase in the fresh weight of the seedlings; rather, there was even a slight decrease in this parameter compared to the control. Stawińska and Matysiak [39] have emphasized that a decrease in fresh weight (to a greater extent than dry weight) signifies a reduced water content of plants. The decrease in water content may be due to impaired water uptake by the roots, which in turn could be a consequence of toxicity of bioactive compounds present in the extracts [43,44]. On the other hand, a reduction in plant dry weight may correlate with a limited photosynthetic activity [39,45]. This relationship was not confirmed by the current research. The plant extracts did not reduce the content of photosynthetic pigments; on the contrary, they increased the levels of the chlorophyll a, b, and a + b, which positively affected photosynthesis intensity. Chlorophyll content in the fresh weight of the leaves of various plants increases when fertilized with macronutrients, especially nitrogen [46]. This is because nitrogen and magnesium are essential for the proper synthesis of chlorophyll [47,48].

Agrochemicals used in plant cultivation also affect soil microorganisms and their enzymatic activity. In the search for new solutions in plant protection, it becomes necessary to study the effect of substances of natural origin on the biological activity of soils. It has been proven that preparations of natural origin act as elicitors of plant defense reactions and can stimulate plants to increase the production of secondary metabolites, contributing to the enhancement of adaptive capabilities to the environment [5]. The precise characterization of the mechanism induced by plant extracts containing various bioactive compounds, such as secondary metabolites, essential oils, inorganic compounds, signaling molecules, or compounds from the homogenates of plant tissues, which determine the complex structure of these extracts, is very challenging. Analysis conducted in this study confirms the beneficial impact of the applied extracts and their mixtures on the activity of soil microorganisms in the rhizosphere of wheat seedlings. The assessment involved the determination of the activity of catalase, which protects cells from the toxic effects of H_2O_2, and dehydrogenases, which play crucial roles in the transformation processes of organic carbon compounds and are also used to determine the ecological toxicity index [49]. The highest activity of catalase and dehydrogenases was observed after spraying with M20, while the lowest was recorded after the application of the H10 extract, indicating the positive effect of plant (natural) extracts on soil biological activity, as opposed to the use of chemical plant protection agents. Research indicates that chemical plant protection agents, such as fungicides, have a negative impact on the microbial balance of the soil [50,51]. They limit the proliferation of microorganisms, as well as the secretion of enzymes, and can even

directly interact with enzymes (binding to enzyme active sites) [52,53]. Fungicides directly attack by reacting with enzymes or by inhibiting the proliferation of soil microorganisms that produce soil enzymes, especially dehydrogenases [54]. They also contribute to reducing the activity of catalase in the soil, which protects cells from toxic H_2O_2 produced in the cytoplasm and peroxisomes, and which is sensitive to various environmental stressors [55,56]. Additionally, fungicides may affect soil acidification, most likely due to their chemical composition or via chemical reactions occurring in the soil, but also disruptions in the biological balance of the soil [57]. Numerous studies have reported the reduction in enzyme activity, especially dehydrogenases, but also catalase, urease, alkaline phosphatase, and protease, with increasing soil acidification [58–61]. Thus, plant extracts can indirectly interact with soil microorganisms, inducing changes in soil physicochemical properties depending on their composition. Recent research on seaweed-based biostimulants has indicated that they may induce beneficial changes in the soil physicochemical properties, such as pH or moisture, and alter the soil microbial community [62]. The present study demonstrated that as early as 3 weeks after the application of plant extracts, the utilization rate of different carbon sources by soil microorganisms varied. Carbon compounds were utilized most rapidly in the control soil, while plant extracts delayed the effective utilization of carbon compounds by soil microorganisms, except for the combination with the M20 extract mixture, where the use of carbon sources by microorganisms forming in the soil was also effective and similar to the control. The obtained results indicate changes occurring in the soil microbial community and their activity under the indirect influence of plant extracts. Hellequin et al. [62] also demonstrated a delayed but positive effect of the biostimulant on active soil microorganisms. It is widely known that plants shape the soil microbial community through root exudates, which act as signaling molecules that alter soil properties, as well as influence its nutrient resources and soil microbiome [63].

5. Conclusions

Plant extracts rich in bioactive compounds constitute one of the most promising groups of botanical biopesticides. Their antimicrobial activity has been repeatedly confirmed, and furthermore, their ability to induce defense reactions and even systemic resistance has been demonstrated [35,36,64,65]. The present work complements the existing literature on the effects of plant-derived extracts on plants as well as on soil microbiomes. The current results will serve as the basis for further field trials, which are essential for confirming the fortifying and biocidal effects of the plant extracts as potential bio-preparations for crop protection, with particular emphasis on organic farming. Preparations based on plant extracts are a promising element of the modern integrated pest management system (IPM). They have a comprehensive effect, both biocidal against phytopathogens and biostimulating plants for better growth and development. The integration of methods based on plant components with other non-chemical control methods (agronomic methods, plant breeding) will allow for the effective management of modern plant protection. Such a comprehensive approach is the right strategy that effectively eliminates pathogen resistance, and the use of a safe and new generation of biocidal products protects the agroecosystem.

As a result of the conducted research, the following conclusions should be drawn:

1. The potential of tested plant extracts based on biologically active compounds confirms their effective action as biostimulants. Hemp extracts (H20) and a mixture of sage, hemp, tansy extracts at a concentration of 20% (M20) influenced the increase in the fresh and dry weight of seedling roots, while showing no phytotoxicity to the tested plants. Moreover, it was shown that spraying seedlings with a mixture of plant extracts (M10, M20) increased the chlorophyll content in seedling leaves.
2. Mycological analysis of plants showed that the tested plant extracts, mainly mixture components (M10, M20), limited the development of *Fusarium oxysporum*., while contributing to an increase in the number of saprotrophic fungi such as *Trichoderma* spp.
3. The research confirmed the beneficial effect of the mixture of plant extracts on the increase in biological activity soil.

These studies are the basis for field tests to verify the obtained results on a macro scale, as well as to assess the biological stability of the extracts used. The above research constitutes the basis for further research aimed at determining the comprehensive effects of plant extracts, such as their phytotoxicity, biocidal activity, impact on yield and determining their biological stability. The preparation of such products involves the careful selection of plant ingredients and precise determination of doses/concentrations to obtain the desired protective and biostimulating effect on plants. The results of field tests will allow the development of a precise composition of the extract mixture, which will be the basis for the production of a natural biostimulant and biocidal preparation media used in the cultivation of cereal crops.

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