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Effect of selected plant extracts on winter wheat (*Triticum aestivum* L.) plant growth stimulation and flag leaf infection by fungal pathogens

Wpływ wybranych ekstraktów roślinnych na stymulację wzrostu roślin i indeks porażenie liścia flagowego pszenicy ozimej (*Triticum aestivum* L.) przez patogeny grzybowe

Abstract. The aim of the study was to assess the effect of plant extracts from hemp inflorescences (H) as well as a mixture of extracts from hemp inflorescences, sage leaves and tansy leaves (M) on the stimulation of plant growth and the infection index of the flag leaf of winter wheat (*Triticum aestivum* L.) by fungal pathogens in a two-year field experiment. The analysis of selected biometric parameters in both growing seasons (2022/2023 and 2023/2024) showed a beneficial effect of both types of extracts, regardless of the type and concentration of the extract, on the length of ears, fresh weight of the aerial part of plants and the mass of one thousand wheat grains. The study also analyzed the intensity of the occurrence of wheat flag leaf diseases (septoria leaf blotch, tan spot of wheat, and brown leaf rust of cereals), in two growing seasons. In the case of septoria leaf blotch, the lowest infection index (IP) of the flag leaf was recorded after spraying the plants with a mixture of extracts (M) in the 2022/2023 season (4.91%) and after spraying the plants with hemp extract (H) in the 2023/2024 growing season (2.13%). Similarly, in the case of tan spot of wheat, in both growing seasons, the infection of the flag leaf with the pathogen was most effectively limited by hemp extract (H) in the 2022/2023 season: 24.43%; in the 2023/2024 season: 6.23%. In turn, the infection with brown leaf rust of cereals was strongly correlated with weather conditions, and the lowest index of infection with this pathogen was recorded only after the application of chemical protection (F: 2022/2023: 0.33% and 2023/2024: 6.38%). The presented results constitute the basis for the production of a biological preparation that will contribute to the biostimulation of plants and optimize the protection of wheat against fungal diseases.

Keywords: plant protection, plant extracts, biostimulation, tan spot of wheat, septoria leaf blotch, brown leaf rust of cereals

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INTRODUCTION

Wheat (*Triticum* L.) is one of the most important cereal crops worldwide. In Poland, it is considered the most valuable cereal species, requiring good quality soils and a sufficiently long growing season [Dobosz et al. 2023]. The greatest threat to wheat cultivation arises from pathogenic fungi, which can cause substantial and destructive yield losses [Kayim et al. 2022]. Agrochemicals used to date, such as fertilizers and pesticides, have led to serious negative environmental effects, such as water and soil pollution, destruction of soil structure, nutrient depletion and loss of biodiversity [Bastos et al. 2020, Soto-Gómez and Pérez-Rodríguez 2022, Szpunar-Krok et al. 2022]. The reduction of chemical use in agriculture and the growing interest in environmentally friendly protection methods have led to intensified research into the development of eco-friendly plant protection strategies [Rohr et al. 2019, Rouphael and Colla 2020, Szpunar-Krok et al. 2022, Kursa et al. 2024a, 2024b]. Among the main recommendations in wheat cultivation, in addition to the selection of appropriate varieties adapted to environmental conditions, are the protection of the flag leaf and the proper scheduling of agronomic practices [Kayim et al. 2022]. Nutrients supplied by the flag leaf are essential building materials for quality grain filling [Rădoi et al. 2022]. Infection of the flag leaf leads to a reduction in yield and economic losses [Alam et al. 2013, Kayim et al. 2022]. The most threatening fungal diseases affecting wheat leaves include: septoria leaf blotch caused by *Zymoseptoria tritici*, tan spot of wheat caused by *Pyrenophora tritici-repentis*, brown leaf rust of cereals caused by *Puccinia recondita*, and powdery mildew caused by *Blumeria graminis* [Panasiewicz et al. 2008, El Jarroudi et al. 2022, Abdel-Kader et al. 2023, Schierenbeck et al. 2023]. The use of natural biostimulants of plant origin is increasing significantly. Current research confirms the protective and stimulating potential of plant extracts [Baltazar et al. 2021, Kisiriko et al. 2021, Kursa et al. 2024a]. Biostimulants positively affect both the qualitative and quantitative yield parameters, biochemical characteristics of plants and can mitigate abiotic stresses [Zulfiqar et al. 2020, Ma et al. 2022]. Furthermore, the biostimulatory effects of plant extracts have been confirmed to improve plant growth and development, including fruiting, seed germination and emergence, root development, and shoot elongation, as well as increasing nutrient and water uptake [Baltazar et al. 2021, Kisiriko et al. 2021]. The positive influence of biostimulants is attributed to improving soil microbial activity as well as increasing the presence of factors responsible for the solubility of nutrients in the soil. Moreover, the wide-range effects of biostimulants can be attributed to the biosynthesis of phytohormones, enhanced photosynthetic efficiency, improved carbon and nitrogen metabolism, and increased sugar content as a carbon source [Elzaawely et al. 2017, Zulfiqar et al. 2020]. Pannacci et al. [2022] proved that aqueous extract of mugwort (*Artemisia vulgaris* L.) stimulates seed germination and seedling growth in vegetable crops (onion, carrot, tomato, rapeseed, cauliflower and lettuce). Extracts from herbal plants (John's wort, giant goldenrod, common dandelion, red clover, nettle, valerian) positively affect the yield, chemical composition and antioxidant activity of celeriac (*Apium graveolens* L. var. *rapaceum*) [Godlewska et al. 2020]. Ben-Jabeur et al. [2019] confirmed that coating wheat kernels with thyme essential oil improved drought resistance in wheat.

The aim of the present research was to evaluate the effects of selected plant extracts on specific biometric traits of winter wheat (*Triticum aestivum* L.), including the fresh and dry weight of roots and aerial parts, the length and thickness of stem, as well as yield, and the health status of the flag leaf.

MATERIAL AND METHODS

Field experiment

The field experiment was conducted between 2022 and 2024 on a farm located in Skrzynice, Lublin Voivodeship (51°11'67"N, 22°25'00"E) on soil with varying levels of available mineral nutrients (Tab. 1). The experiment was set up using a randomized block design in an independent arrangement, with four replicates. The area of the individual experimental plot was 10 m^2 (2 m × 5 m). Winter wheat, cultivar Venecja (Hodowla Roślin Strzelce Sp. z o.o. Grupa IHAR, Strzelce, Poland), was sown at a rate of 270 kg ha^{-1} in the third decade of September (2022/2023 and 2023/2024 growing seasons) on a field previously used for potato cultivation. Soil preparation for winter wheat included a set of post-harvest tillage operations, ploughing, pre-sowing fertilization, and cultivator seed drill preparation, followed by pre-sowing fertilization based on prior soil analysis and in accordance with fertilizer recommendations.

Table 1. Content of bioavailable forms of mineral nutrients in soil during the growing seasons 2022/2023 and 2023/2024

Growing season	pH	Reaction	Assimilable mineral forms							
			Phosphorus – P_2O_5 (mg kg^{-1} soil)		Potassium – K_2O (mg kg^{-1} soil)		Magnesium – Mg (mg kg^{-1} soil)		Nitrogen – N min. (kg ha^{-1})	
			content	abundance	content	abundance	content	abundance	content	abundance
2022/2023	4.7	acidic	77.0	low	90.0	low	43.0	low	95.9	high
2023/2024	6.2	slightly acidic	97.0	low	179.0	moderate	54.0	low	117.4	very high

Preparation and application of plant extracts

The field experiment is a continuation of several years of laboratory research conducted with plant extracts to assess their biocidal activity [Kursa et al. 2022, Kursa et al. 2024a, 2024b]. Plant extracts with the strongest antimicrobial properties such as tansy, sage, hemp were selected for further field research. The plant extracts were prepared on the basis of dried leaves of sage (*Salvia officinalis* L.), tansy (*Tanacetum vulgare* L.) and dried lateral inflorescences of hemp (*Cannabis sativa* L.). The plant extracts were prepared according to the methodology described by Kursa et al. [2022]. The following experimental combinations were used in the study:

C – spraying plants with water (absolute control),

F – spraying plants with a fungicide (Tarcza Łan Extra 250 EW, active ingredient –tebuconazole, dose 250 g/l; relative control),

H – spraying plants with a 20% hemp inflorescence extract,

M – spraying plants with a 20% mixture of extracts from hemp, sage, and tansy.

Potassium silicate (0.17%) and glycerin (1%) were added to each type of plant extract. The mixture of extracts, containing pure (100%) plant extracts, was prepared from the hemp extract, sage extract, and tansy extract in a 2 : 2 : 1 ratio. For all experimental combinations, three foliar applications were performed at a rate of 300 dm^3 ha^{-1} during the following wheat developmental stages: BBCH 29 (the end at tillering stage), BBCH 37–39 (the flag leaf stage), and BBCH 55 (the earring stage). Foliar treatments were conducted using a Kwazar Orion sprayer at a pressure of 0.2 MPa. Other plant protection measures were carried out in accordance with the recommended practices for plant protection for the years 2022–2024 [Program Ochrony Roślin Rolniczych 2022, 2023, 2024].

Weather conditions

Weather conditions are presented based on data from the Institute of Meteorology and Water Management – National Research Institute, sourced from the synoptic station in Radawiec Duży, Konopnica municipality (51°13'00"N, 22°23'35"E, altitude 238 m). Selyaninov's hydrothermal coefficient (k) was used to assess the weather conditions during the growing season of the plants, calculated according to the following formula:

$$k = \frac{P}{0.1 \times \sum t}$$

where: P – total monthly precipitation (mm), Σt – monthly sum of air temperatures >10°C [Kopcińska et al. 2018] according to the scale developed by Skowera and Puła [2004] for the territory of Poland. The analyzed parameters are divided into ten classes based on the values of the k coefficient with the following ranges: $k \leq 0.4$ (ss – extremely dry); $0.4 < k \leq 0.7$ (bs – very dry); $0.7 < k \leq 1.0$ (s – dry); $1.0 < k \leq 1.3$ (ds – fairly dry); $1.3 < k \leq 1.6$ (o – optimal); $1.6 < k \leq 2.0$ (dw – fairly wet); $2.0 < k \leq 2.5$ (w – wet); $2.5 < k \leq 3.0$ (bw – very wet); $k > 3.0$ (sw – extremely wet).

Assessment of fresh and dry weight of plant, internode length and thickness

The fresh and dry weights of the roots as well as aerial parts of the plants were analyzed. The assessment of these parameters was conducted three weeks after the experimental treatments, which corresponded to the BBCH 59–61 developmental stages (end of heading – beginning of flowering). These parameters were evaluated on 50 plants from each experimental combination. The fresh weight of the roots and aerial parts was expressed in g f.w. $plant^{-1}$. The plant material was dried for 7 days in a ventilated room at 23–25°C. It was subsequently weighed, and the results were expressed in g d.w. $plant^{-1}$. The length and thickness of the internode were measured between the first and second nodes, while the thickness was measured above the first node. Measurements were taken using an electronic caliper (Limit CDN-NT IP67).

Assessment of the flag leaf infection index

Wheat health status was assessed at the plant flowering stage (BBCH 61–69) by determining the percentage of the flag leaf area infected by the following pathogens: tan spot of wheat (*P. tritici-repentis*), septoria leaf blotch (*Z. tritici*) and brown leaf rust of cereals (*P. recondita*), using EPPO PP 1/26(4) methodology [EPPO 2012]. Randomly sampled flag leaves were evaluated for each experimental combination (50 randomly selected leaves in 4 replicates, 200 in total). The assessment of the degree of infection of the analyzed leaf area was conducted using a 4-point scale: 1° – 1–25% of the area with infection symptoms; 2° – 26–50% of the area with infection symptoms; 3° – 51–75% of the area with infection symptoms; 4° – 76–100% of the area with infection symptoms. Subsequently, the leaf infection index (IP) was calculated according to the formula:

$$IP = \left[\frac{(n \times 1^0) + (n \times 2^0) + (n \times 3^0) + (n \times 4^0)}{i \times N}\right] \times 100\%$$

where: n = the number of leaves at a given level of infection, N = total number of leaves, i = the highest degree of the scale.

Evaluation of ear length and thousand-grain weight (TGW)

Ear length was measured in 50 plants from each experimental combination during the BBCH 89–91 developmental stages (fully ripe of grain). Thousand-grain weight (TGW) was assessed by analyzing 200 ears from each experimental combination at the BBCH 89–91 developmental stages.

Statistical analysis

The collected data were analyzed using Statistica software version 13.3 (1984–2017 TIBCO Software INC, Palo Alto, CA, USA). A one-way analysis of variance (ANOVA) was conducted, and the significance of differences was assessed using Tukey's post hoc test and Kruskal-Wallis test at a significance level of $p = 0.05$.

As part of the assessment of the linear relation between variables (quantitative traits):

- flag leaf infection index (IP%) and ear length (cm), and thousand-grain weight (g) of wheat,
- weather conditions (coefficient k) vs. flag leaf infection index (IP%).

Pearson's linear correlation coefficient (r) was determined, and the interpretation was based on the following Matyja [2014] scale: negligible correlation: $0 < r < |0.1|$; weak correlation: $|0.1| \leq r < |0.3|$; moderate correlation: $|0.3| \leq r < |0.5|$; strong correlation: $|0.5| \leq r < |0.7|$; very strong correlation: $|0.7| \leq r < |0.9|$; nearly perfect correlation: $|0.9| \leq r < |1|$.

RESULTS

Weather conditions

High variability in weather conditions was recorded throughout the experiment. Weather conditions had a significant impact on the development of flag leaf diseases in winter wheat. In the 2022/2023 growing season, the average monthly temperatures from March to July were close to the long-term averages (Fig. 1). In the corresponding period of the 2023/2024 season, the average monthly temperature exceeded the long-term average by 1–4°C. At the same time, the average monthly temperatures during the autumn and winter months (September–February) of both growing seasons were generally higher than the long-term average. The distribution of rainfall during the two-year study period was uneven. In the first year, autumn rainfall (September–November) was lower than the long-term average, while in the winter months (December–February), it was higher, and in the spring and summer, it was close to the long-term averages (except for April and June). In contrast, the following growing season was characterized by significant variability. From October to March, as well as in June and July, precipitation levels were higher compared to the long-term average, whereas low rainfall was recorded in April, May, and August (Fig. 1)

Assessment of fresh and dry plant weight, internode length and thickness

The fresh and dry weight of plant included an analysis of the roots and aerial parts. Root dry weight was higher in the 2022/2023 season than in the 2023/2024 season. The plants treated with a 20% mixture of plant extracts (M) consistently had the highest dry and fresh root weight each year. However, only in the second year of the study were these differences statistically significant compared to the values obtained in the control trials (C, F) – Table 2. The average fresh weight of the aerial part was highest in the 2022/2023 growing season for plants treated with a 20% hemp extract (H – 8.50 g plant^{-1}). This value

Table 2. Fresh and dry weight of wheat roots and aerial parts

Experimental combination	Fresh weight (g plant^{-1}) ±SD				Dry weight (g plant^{-1}) ±SD			
	root		aerial part		root		aerial part	
	2022/2023	2023/2024	2022/2023	2023/2024	2022/2023	2023/2024	2022/2023	2023/2024
Absolute control	0.52 ±0.25^{a}	0.40 ±0.15^{bc}	6.50 ±1.54^{c}	6.87 ±1.51^{b}	0.14 ±0.07^{a}	0.17 ±0.08^{bc}	2.50 ±1.24^{a}	2.50 ±0.41^{b}
Spraying with fungicide	0.49 ±0.29^{a}	0.32 ±0.17^{c}	7.10 ±1.49^{bc}	7.11 ±1.30^{ab}	0.14 ±0.07^{a}	0.15 ±0.08^{c}	2.70 ±1.28^{a}	2,.8 ±0.52^{ab}
Spraying with hemp flower extract	0.45 ±0.24^{a}	0.54 ±0.22^{ab}	8.50 ±1.24^{a}	8.13 ±1.31^{a}	0.13 ±0.07^{a}	0.23 ±0.10^{ab}	3.00 ±1.18^{a}	3.19 ±0.75^{a}
Spraying with a mixture of plant extracts	0.65 ±0.28^{a}	0.58 ±0.23^{a}	8.20 ±1.89^{ab}	7.28 ±1.62^{ab}	0.18 ±0.10^{a}	0.28 ±0.13^{a}	3.00 ±1.43^{a}	3.06 ±0.86^{a}

a, b, c – values in columns marked with the same letter do not differ significantly at a significance level of $p \leq 0.05$

was significantly different from the control combinations (C – 6.50 g plant^{-1} and F – 7.10 g plant^{-1}). Similarly, in the second year of the study, the highest value of fresh weight for the aerial part was recorded for plants treated with hemp extract (H – 8.13 g plant^{-1}), although this value differed significantly only from the absolute control (C – 6.87 g plant^{-1}). The dry weight of the aerial plant part was higher in the 2022/2023 season than in the 2023/2024 season. The narrowest statistically significant values compared to the control were obtained for plants treated with the mixture of plant extracts (M). They were significantly higher only compared to the absolute control (C) and did not differ significantly from the combination treated with hemp extract and fungicide (Tab. 2). The studies also indicated that the plant extracts applied as a spray had a beneficial effect on the length and thickness of wheat internode. The longest internodes were obtained in both growing seasons following the application of the plant extract mixture (M: 2022/2023 – 8.32 cm; 2023/2024 – 9.08 cm), with their length being statistically greater than that of the control group (C: 2022/2023 – 7.01 cm; 2023/2024 – 7.95 cm) – Table 3. Spraying the plants with hemp inflorescence extract also increased internode length, but the values obtained were not significantly higher compared to the control (Tab. 3). The influence of plant extracts on internode thickness was observed only in the first year of the study, and only in the case of spraying the plants with the mixture of extracts (M – 4.68 mm) – Table 3. This trend was not confirmed in the second year of the study, although the results did not differ significantly between the experimental combinations.

Table 3. Average internode length (cm) and thickness (mm)

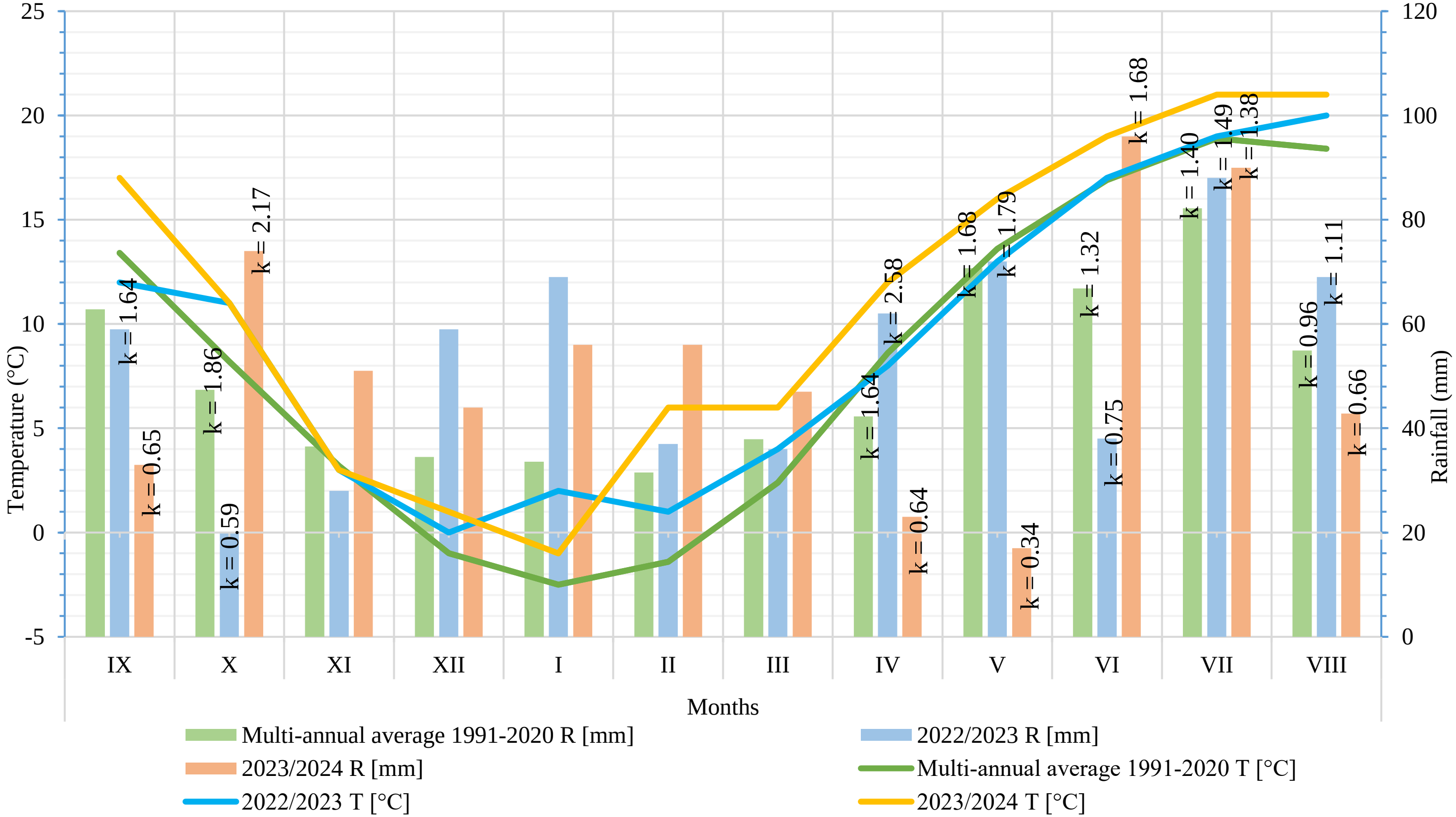
Experimental combination	Internode length (cm) ±SD		Internode thickness (mm) ±SD	
	2022/2023	2023/2024	2022/2023	2023/2024
Absolute control	7.01 ±1.42[b]	7.95 ±0.76[b]	3.83 ±0.49[b]	4.60 ±0.45[a]
Spraying with fungicide	7.60 ±1.41[ab]	8.03 ±1.26[ab]	4.00 ±0.51[b]	4.15 ±0.65[a]
Spraying with hemp flower extract	7.91 ±1.20[ab]	8.15 ±0.75[ab]	4.10 ±0.64[b]	4.25 ±0.68[a]
Spraying with a mixture of plant extracts	8.32 ±1.51[a]	9.08 ±1.44[a]	4.68 ±0.59[a]	4.23 ±0.91[a]

a, b – values in columns marked with the same letter do not differ significantly at a significance level of $p \leq 0.05$

Flag leaf infection index

Analyses conducted between 2022 and 2024 revealed the presence of septoria leaf blotch (*Z. tritici*), tan spot of wheat (*P. tritici-repentis*), and brown leaf rust of cereals (*P. recondita*) on flag leaves (Fig. 2.). The intensity of these diseases on the flag leaf varied depending on the protective treatments applied and weather conditions during the growing season (Fig. 3, Tab 4). The most severe infection of the flag leaf by *P. triticina* was recorded in the 2023/2024 season for the control combination (C – 74.75%), while the lowest infection was observed in the 2022/23 season for the fungicide combination (F – 0.33%) and the plant extract mixture (M – 3.66%) – Figure 3. Pearson's linear correlation coefficient (r) showed a very high relationship between disease intensity and prevailing weather conditions ($r = –0.77$) – Table 4.

During the two-year field trials, flag leaves were the least affected by septoria leaf blotch. The disease occurred in both growing seasons at a relatively low level (IP: 2–9%). An exceptionally high infection index was recorded in the 2023/2024 season for the control combination (C – 22.99%). After spraying the plants with hemp extract and a mixture



T – mean daily air temperature, R – monthly precipitation, k – Selyaninov’s hydrothermal coefficient

Fig. 1. Average monthly temperatures (°C) and total precipitation (mm) for 2022–2024, compared with the long-term averages from 1991–2020

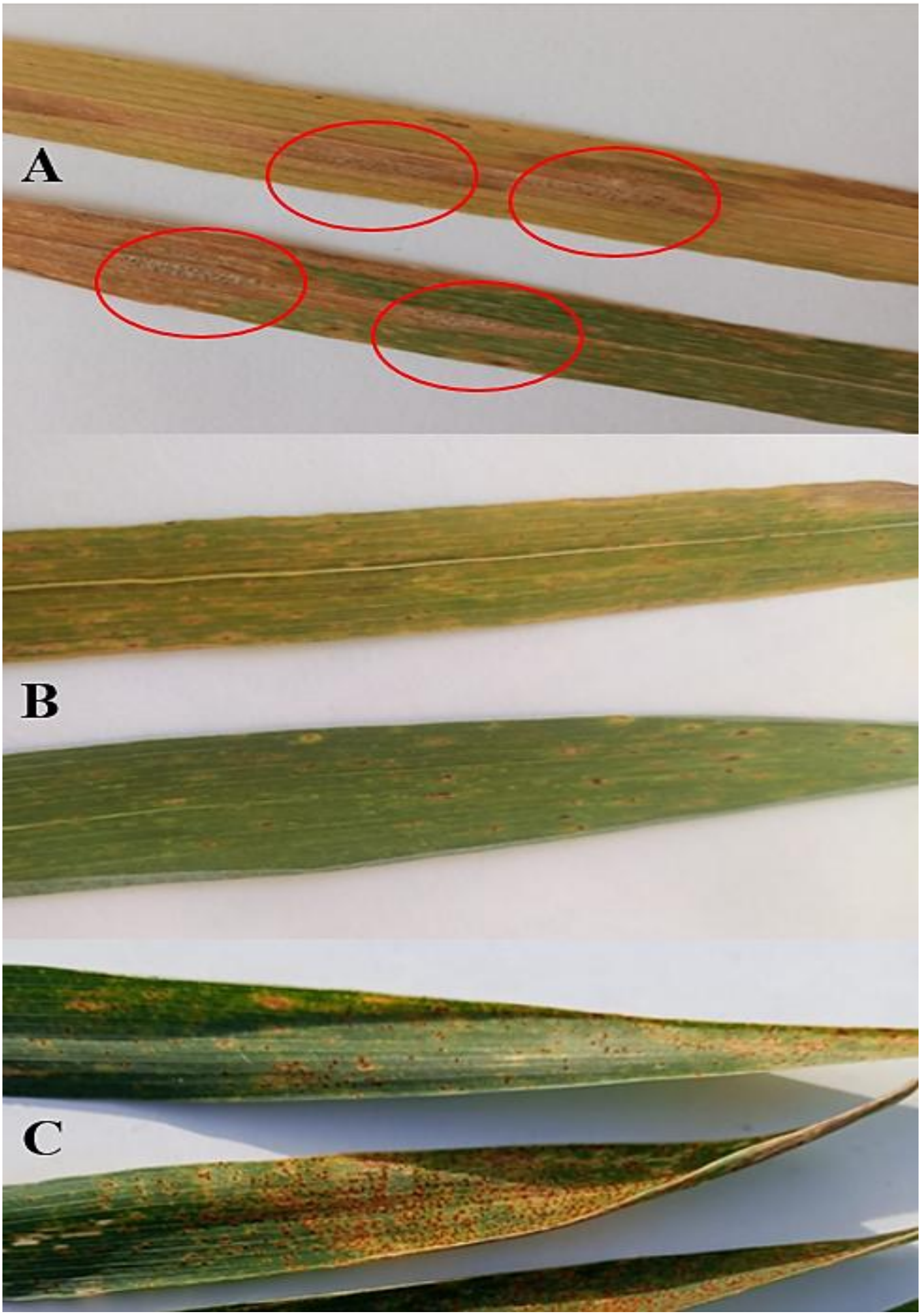
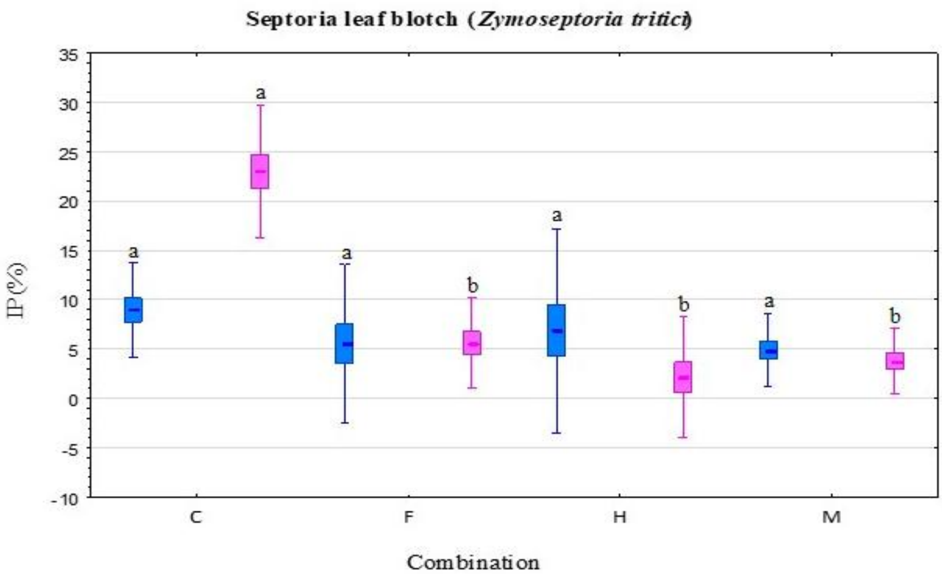
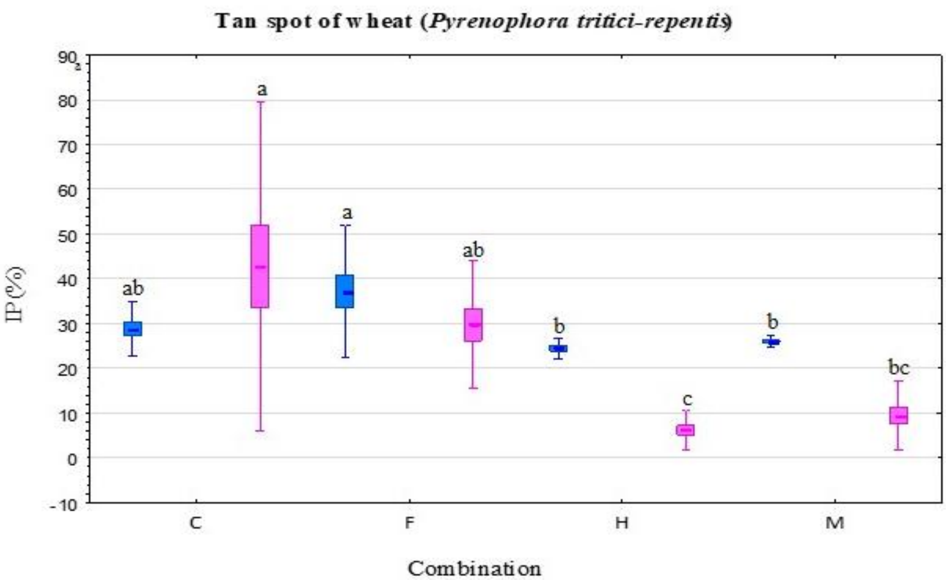
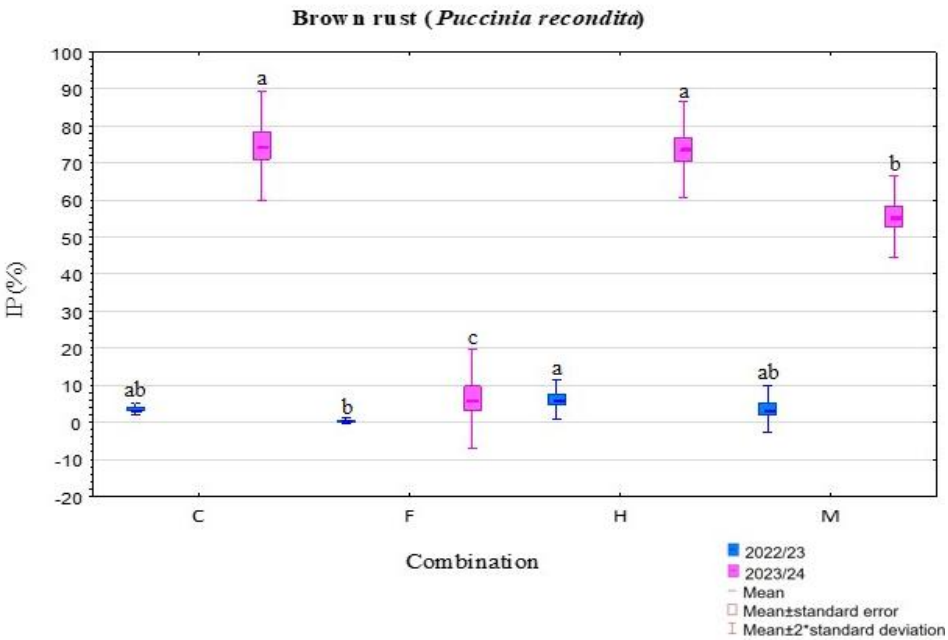


Fig. 2. Symptoms of diseases on winter wheat leaves: A – septoria leaf blotch (*Z. tritici*), B – tan spot of wheat (*P. tritici-repentis*), C – brown leaf rust of cereals (*P. recondita*)

of plant extracts, the flag leaves were less affected by *Z. tritici*. However, significant differences in the infection index compared to the control were only observed in the 2023/2024 season (H – 2.13%, M – 3.8%) – Figure 3. Pearson's linear correlation coefficient (r) showed a weak association with prevailing weather conditions and disease in the analyzed growing seasons (Tab. 4).







C – absolute control, F – spraying with fungicide, H – spraying with hemp flower extract, M – spraying with a mixture of plant extracts

a, b, c – values marked with the same letter do not differ significantly at a significance level of $p \leq 0.05$

Fig. 3. Infection index (IP%) of wheat flag leaves by septoria leaf blotch (*Z. tritici*), tan spot of wheat (*P. tritici-repentis*), and brown leaf rust of cereals (*P. recondita*)

The flag leaves were also affected by *P. tritici-repentis*, with the infection index ranging between 6.23% and 42.80%. Stronger pathogen infection was observed in the first year of the study in all experimental combinations, except for the absolute control (C). Spraying plants with plant extracts did not result in significantly lower infection rates (H – 24.43%, M – 26.05%) compared to the control (C – 28.92%), but it was significantly lower than after fungicide application (F – 37.27%). In the second year of the study, the highest infection index for flag leaves caused by tan spot of wheat was recorded in the control combination (C – 42.80%), as well as after fungicide application (F – 29.75%). On the other hand, plants sprayed with plant extracts (H – 6.23%, M – 9.50%) had a lower flag leaf infection index compared to the control plants. However, only after the application of hemp extract did the degree of leaf infection differed significantly from both controls (C and F), while the use of the extract mixture reduced infection only in comparison to the absolute control (C) – Figure 2, Table 4. Pearson's linear correlation coefficient (r) between the flag leaf infection index due to tan spot of wheat and weather conditions indicated a weak relationship between these factors (r = 0.26) – Table 4.

Table 4. Pearson's linear correlation coefficient (r) and interpretation of the relationship

Specification	Flag leaf infection index (IP%)		
	septoria leaf blotch	tan spot of wheat	brown leaf rust of cereals
Weather conditions: Coefficient k	–0.15 weak	0.26 weak	–0.77* very high

r – correlation coefficient between the flag leaf infection index, weather conditions and experimental combinations; results significant at a significance level of $p \leq 0.05$ are denoted by *

Ear length and thousand-grain weight (TGW)

Ear length and thousand-grain weight are presented in table 4. Additionally, the correlation coefficient (r) was determined between the specified parameters and the assessed diseases of the flag leaf (Tab. 5). The study showed that the plant extracts (M, H) applied in the form of foliar sprays had a beneficial effect on ear length and thousand-grain weight. In both growing seasons, the longest ears were observed in plants sprayed with hemp extract (2022/2023: H – 10.32 cm; 2023/24: H– 10.92 cm), with these measurements significantly differing from the control group (2022/2023: C – 9.64 cm; F – 9.75 cm; 2023/2024: C – 9.89 cm; F – 10.24 cm). Spraying the plants with a mixture of extracts (M) in both study years, also increased ear length, and the values obtained were significantly different from the absolute control (C), but did not differ from the fungicide trial (F) (Tab. 5). The highest thousand-grain weight in both growing seasons was recorded for the treatment sprayed with a 20% mixture of plant extracts (M) (2022/2023 – 40.45 g; 2023/2024 – 44.13 g). However, only in the second year of the study were these values statistically

Table 5. Ear length (cm) and thousand-grain weight (TGW)

Experimental combination	Ear length (cm) ±SD		TGW (g) ±SD	
	2022/2023	2023/2024	2022/2023	2023/2024
Absolute control	9.64 ±0.95[c]	9.89 ±1.12[c]	39.83 ±0.59[ab]	42.44 ±0.98[bc]
Spraying with fungicide	9.75 ±1.02[bc]	10.24 ±0.72[bc]	39.32 ±0.47[b]	43.98 ±0.60[ab]
Spraying with hemp flower extract	10.32 ±0.88[a]	10.92 ±0.97[a]	40.16 ±0.51[ab]	41.16 ±0.53[c]
Spraying with a mixture of plant extracts	10.16 ±0.84[ab]	10.40 ±0.88[ab]	40.45 ±0.36[a]	44.13 ±1.19[a]

a, b, c – values marked with the same letter do not differ significantly at a significance level of $p \leq 0.05$

higher than the control (C – 42.44 g) – Table 5. Pearson's linear correlation coefficient (r) showed that the flag leaf infection with tan spot of wheat had the greatest negative effect on yield parameters (Tab. 6). In the 2022/2023 season, an increase in *P. tritici-repentis* infection decreased ear length (r = –0.51) and thousand-grain weight (r = –0.49). In the 2023/2024 season, it was only associated with a decrease in ear length (r = –0.61). A negative correlation was also observed between septoria leaf blotch and the analyzed parameters during both growing seasons. The strongest (high) correlation of this type occurred in the 2023/2024 season for ear length (r = –0.57). On the other hand, the occurrence of brown leaf rust of cereals (*P. triticina*) showed a weak correlation with yield parameters in both growing seasons, except for thousand-grain weight in the 2023/2024 season, where the correlation was high (r = –0.58) – Table 6.

Table 6. Pearson's linear correlation coefficient (r) and interpretation of the relationship

Flag leaf diseases (IP%)	2022/2023		2023/2024	
	ear length	TGW	ear length	TGW
Septoria leaf blotch	–0.38 moderate	–0.18 weak	–0.57* high	–0.08 negligible
Tan spot of wheat	–0.51* high	–0.49* moderate	–0.61* high	0.11 weak
Brown leaf rust of cereals	0.19 weak	0.39 moderate	0.14 weak	–0.58* high

r – correlation coefficient between the flag leaf infection index and yield parameters
TGW – thousand-grain weight
* results significant at $p \leq 0.05$

DISCUSSION

European herbaceous plants are a rich source of numerous biologically active compounds and contain numerous phenolic compounds, terpenes and alkaloids [Acheuk et al. 2022, Szparaga 2023].

The biostimulatory effects of these compounds are analyzed in terms of their impact on plant growth, physical traits, and yield [Drobek et al. 2019]. The literature reports on the biostimulatory properties of plant extracts on crops, while also highlighting the selectivity of their action [Pannacci et al. 2022, Szparaga 2023]. Maksoud and coauthors [2023] also confirmed the biostimulatory effect of extract from the leaves of henna (*Lawsonia inermis*) on the yield, growth of shoots and root systems, as well as certain biochemical traits of winter wheat. The biostimulatory effect of extract from dodder (*Cuscuta reflexa*) on the growth and yield of wheat seeds under stress conditions has been attributed to increased seed germination enzyme activity and enhanced antioxidant defense mechanisms [Ali et al. 2020]. Nevertheless, there is a limited number of studies on the use of plant extracts in the cultivation of winter wheat under European conditions. Baltazar et al. [2021] highlighted the need to identify the molecular mechanisms of biostimulants, as well as to define their functional roles in cultivated plants. Our research confirmed the limited scientific reports in this area, indicating that extracts from herbaceous plants based on hemp, sage, and tansy act as biostimulants, improving the growth and yield of winter wheat.

Gupta et al. [2022] also indicated the potential application of biostimulants in alleviating biotic stress in plants. Secondary metabolites contained in plants are the main group of bioactive compounds characterized not only by biostimulatory effects, but also by bioprotective properties [Mrid et al. 2021].

Gebashe et al. [2021] emphasized the role of biostimulants in controlling plant diseases. This creates a need to evaluate the effectiveness of these substances for their antimicrobial properties. Szparaga [2023] reported a high biocidal potential of water extracts (decoctions, infusions, and macerates) from the roots of burdock and lovage, flax seeds, mugwort leaves, and verbascum flowers against the fungi *Botrytis cinerea* Pers., *Fusarium avenaceum* (Fr.) Sacc, *F. culmorum* (Wm.G. Sm.) Sacc., *F. sambucinum* (Fuckel), *F. solani* (Mart.) Sacc., *Rhizoctonia solani* (J.G. Kühn), *Sclerotinia sclerotiorum* (Lib. de Bary) and *Thielaviopsis basicola* (Berk. i Broome). Kursa et al. [2022] also confirmed the high effectiveness of sage and tansy plant extracts at a concentration of 20% in inhibiting the growth of *Fusarium* isolated from cereal grains (especially on *F. avenaceum*, *F. culmorum*, *F. graminearum, F. sporotrichioides*) in *in vitro* tests.

Due to the necessity to reduce pesticide usage in the European Union [Directive 2009/128/EC, European Commission 2019], numerous attempts are being made to find alternative methods for plant protection [Montanarella and Panagos 2021, Kursa et al. 2024b]. Many studies still focus on assessing the biocidal effects of plant extracts under laboratory conditions [Fierascu et al. 2015, Korpinen et al. 2021, Kursa et al. 2022, Wens and Geuens 2022, Szparaga 2023]. Recent scientific studies indicate that plant extracts are more effective against fungal phytopathogens than synthetic preparations. It has been demonstrated, among other findings, that extracts from clove, ginger, and cinnamon were more effective than the fungicide Amistar 250 SC (active ingredient: azoxystrobin) in controlling wheat stem rust, and that cinnamon bark extract outperformed Mancozeb 75 WG (active ingredient: mancozeb) against *Rhizoctonia solani* [Singh et al. 2019, El-Gamal et al. 2022]. The increasing phenomenon of pathogen resistance to pesticides also underscores the necessity of reducing fungicide application. Chen et al. [2021] documented a high resistance of field populations of *Fusarium graminearum* to tebuconazole. In this context, it is worth noting the promising prospect of alternating the use of synthetic fungicides with products of natural origin [Szczygieł et al. 2024]. Da Silva Santana and colleagues [2022] also highlighted the role of plant extracts as inducers of plant resistance, attributed to the presence of bioactive compounds such as ethylene, jasmonic acid, salicylic acid, abscisic acid, brassinosteroids, gibberellins, auxins, and cytokinins, which are involved in the plant defensive response [Bari and Jones 2009, Mukherjee and Patel 2020]. Jamiołkowska [2020] and El-Gamal et al. [2022] described the beneficial effect of plant extracts on the total phenolic content and oxidative enzymes, which are responsible for inducing plant resistance to pathogenic factors.

Field trials confirmed the beneficial effect of the tested plant extracts in reducing the infection index of the winter wheat flag leaf caused by septoria leaf blotch, tan spot of wheat, and brown leaf rust of cereals. Until now, research on the effects of active substances on crop health, both in Poland and worldwide, has focused primarily on chemical substances [Panasiewicz et al. 2008, Tsialtas et al. 2018]. Only in recent years has there been increased interest in the use of substances of natural origin, most of which, however, focused on their biostimulatory effects [Carvalho et al. 2021, Łozowicka et al. 2022, Pannacci et al. 2022, Szpunar-Krok et al. 2022]. The results of the conducted research demonstrate that the intensity of fungal diseases in wheat cultivation is determined not only by weather conditions but also by the protective treatments applied using a mixture of plant extracts based on hemp, sage, and tansy. In many cases, the application of plant extracts was more effective than the chemical protection based on tebuconazole used in the experiment. It was also found that the application of a mixture of plant extracts was more effective in protecting the flag leaf than the application of a single-component hemp extract. It can be assumed that the positive effects of using plant extracts demonstrated in the current study were also related to the addition of a small amount of silicon to the mixture. As a micronutrient, silicon enhances plant resistance to biotic stress. Sakr [2016] reported that the saturation of cell walls with silicon increased plant resistance to fungal diseases by forming a physical barrier that hindered pathogen penetration into plant cells (preventive action as a physical protection mechanism). Additionally, silicon actively participates in the expression of natural defense mechanisms. The role of silicon in reducing wheat diseases (tan spot of wheat and *Fusarium* head blight) has been confirmed by numerous researchers [Zamojska et al. 2018, Pazdiora et al. 2021]. Dallagnol et al. [2020] described the influence of silicon on stimulating plant defense mechanisms not only against fungal pathogens but also against abiotic factors. The development and health of the wheat flag leaf are undoubtedly influenced by the weather conditions during the growing season. Hýsek et al. [2017] reported that temperature and rainfall from March to June strongly correlated with the prevalence of fungal pathogens. Brown leaf rust of cereals (*P. recondita*) is a disease strongly correlated with weather conditions, such as high temperatures and humidity, sunny and dry conditions in spring and early summer, and the presence of dew at night and/or before rainfall. This correlation has been documented in previous studies [Kolmer 2013, Caubel et al. 2017, Rodríguez-Moreno et al. 2020, Pietrusińska-Radzio and Żurek 2024] and is further confirmed by our own research. Simultaneously, the observed reduction in the duration of developmental phases during the second year of the study may have contributed to increased pathogen pressure on the plants [Simón et al. 2020].

CONCLUSIONS

This study described the effects of hemp, sage and tansy extracts on the growth, yield and health of winter wheat leaf flag under field conditions. The two-year field trial has indicated that the mixture of extracts under study can be applied as a potential biopreparation for wheat protection. The comprehensive effects of plant extracts demonstrated in the present study may address the needs of modern agriculture, which aims to reduce pesticide use and counteract the issue of pathogen resistance. The research conducted allows to formulate the following conclusions:

– plant extracts based on hemp, sage, and tansy (particularly their mixture) can be utilized as a biostimulant in winter wheat cultivation (stimulating fresh and dry weight, length and thickness of stem, ear length, and thousand-grain weight),
– plant extracts reduced the development of septoria leaf blotch (*Z. tritici*) and tan spot of wheat (*P. tritici-repentis*) on the flag leaf of winter wheat,

– the tested extracts had no significant effect on limiting the development of brown leaf rust of cereals (*P. recondita*), and the progression of this disease was determined by the prevailing weather conditions.

The present research represents the first stage of analyses conducted as part of a two-year field trial. Mycological analysis of plants, assessment of soil biological activity, chlorophyll content and morphological analysis of leaves will allow a more comprehensive evaluation of the effects of plant extracts on wheat plants and its cultivation environment. Comprehensive analyses will enable the determination of the composition of the extract mixture, which will form the basis for developing a biostimulant and biocidal product for use in cereal cultivation, particularly in organic farming systems.

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