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In vitro EFFICACY OF HERBAL PLANT EXTRACTS ON SOME PHYTOPATHOGENIC FUNGI

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ABSTRACT

Crops are subject to yield losses caused by the presence of pests whose species and population diversity are changing with climate warming. The use of agrochemicals is still the most effective method of plant protection against diseases and pests. However, the intensive use of pesticides in some EU countries makes it necessary to search for alternative methods that can be applied in plant protection for consumer and environmental safety. The aim of the study was the laboratory evaluation of the fungistatic effect of extracts of herbal plants occurring in natural state in Poland: horseradish (*Armoracia rusticana* L.), yarrow (*Achillea millefolium* L.) and tansy (*Tanacetum vulgare* L.) on selected polyphagic phytopathogenic fungi (*Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum coccodes* and *Fusarium oxysporum*). In this study, 5%, 10% and 20% concentrations of herbal water-extract extracts were applied to evaluate their effects on linear growth of fungi and inhibition of their growth relative to control. Total content of polyphenols and flavonoids was assessed in the extracts using spectrophotometry, and their antioxidant activity by applying the synthetic DPPH radical. The plant extracts of tansy and yarrow leaves were characterized by a higher content of polyphenols and flavonoids compared to horseradish leaf extracts; they also had a higher antioxidant activity. Plant extracts inhibited the growth of fungi to a different extent, depending on the species of fungus, type of extract, its concentration and duration of action. The strongest fungistatic effect was recorded for tansy and yarrow extracts, while the weakest for the extract of horseradish leaves. Plant extracts showed the weakest effect against *Botrytis cinerea*, inhibiting the development of this fungus only during the first days of the experiment. The present research is a preliminary study that will be used in the further to develop a biological preparation for the protection of agricultural and horticultural plants against fungal pathogens.

Key words: plant extracts, *Armoracia rusticana*, *Achillea millefolium*, *Tanacetum vulgare*, phytopathogenic fungi, flavonoids, polyphenols, DPPH

INTRODUCTION

Climate change and increasing environmental pollution require the introduction of modern and safer agriculture practices [Wrzaszcz and Prandecki 2020]. The new development strategy of the European Union presented in 2019, called the "European Green Deal", is a response to the need to reduce the use of agrochemicals in plant production [Pralińska et al. 2020]. The aim of these activities is to transform European society into a more resource-efficient and competitive economy [Hafner and Raimondi 2020]. The "Green Deal" strategy, apart from ensuring climate neutrality, also specifies the necessity to reduce the agrochemical in agricultural production, while guaranteeing food safety [Pralińska et al. 2020]. Its main assumption is

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to protect the environment against pollution by limiting the use of pesticides, with particular emphasis on reducing particularly hazardous substances by 50% by 2030 [Montanarella and Panagos 2021]. The strategy is to support production with restricted application of pesticides and fertilizers [Wrzaszcz and Prandecki 2020]. It contributes to the search for alternative methods in plant protection e.i the production of natural preparations with a broad spectrum of action on plants, to protect crops against pests and protect the environment [Grzyb et al. 2019].

Climate change affects, *inter alia*, the variability of pest populations, and the emergence of new groups of crop pathogens and pests. Therefore, there is an urgent need to search new methods and measures to protect crops and stored agricultural products. Herbal plants are a natural source of biologically active compounds with a strong antimicrobial (bactericidal and fungicidal) activity [Wyszkowska-Kolatko et al. 2015, Piekutowska 2017, Krzepiłko et al. 2020]. Biologically active components are known as natural pesticides and are present in plants occurring in various climatic zones [Roccioni and Orzali 2011, Lucas et al. 2012, Aleksandrowicz-Trzcińska and Hallmann 2013, Jamiołkowska and Kopacki 2019, Nxumalo et al. 2021]. Currently, over 400,000 plant species have been identified, most of which are flowering plants (369,000), and nearly 2,000 new ones are discovered each year [Nxumalo et al. 2021]. Compounds with biocidal properties present in plants include mainly phenolic compounds, phenolic acids, stilbenes, flavonoids, terpenoids, diterpenes, carotenoids, alkaloids, coumarins, phytosterols and glycosides [Krzepiłko et al. 2020]. They have a direct impact on pathogens by limiting their growth and development (sporulation inhibition, mycelium deformation) and indirectly acting as elicitors of plant defense responses [Horoszkiewicz-Janka and Jajor 2006, Lorens et al. 2017, Jamiołkowska 2020]. Studies have shown the effect of active compounds of plant origin on increasing the biodiversity of the soil and plant microbiome, which also minimizes the risk of dominance of a single pathogenic species [Jamiołkowska 2013]. At present, many bioactive compounds form the basis for the production of biotechnological formulations, which are alternatives to synthetic chemicals used in agriculture. Preparations of natural origin are developed on the basis of herbal plants that are combinations of plant extracts enriched with microelements. A large group of such preparations is products based on native herbal plants such as garlic, onion, horsetail and nettle extracts [Aleksandrowicz-Trzcińska and Hallmann 2013, Jamiołkowska 2013]. The application of natural compounds for pathogen control is very attractive, and the availability of novel applications and molecular techniques open new prospects for plant protection approaches.

The present study was aimed at assessing the fungistatic effect of herbal plant extracts, such as horseradish, tansy and yarrow from natural sites in eastern Poland, on the growth of selected phytopathogenic fungi *in vitro*. One of the research stages was the assessment of the content of selected biologically active compounds in plant extracts and the analysis of their influence on the growth of pathogenic fungi. The results of the research will form the basis for further studies aimed at the development of commercial preparations of natural origin intended for plant protection against fungal phytopathogens.

MATERIALS AND METHODS

Chemical characterization of extracts

Extract preparation. The research material consisted of aerial parts of horseradish (*Armoracia rusticana* L.)**,** yarrow (*Achillea millefolium* L.) and tansy (*Tanacetum vulgare* L.) collected by hand from natural sites located in the Lubelskie Voivodeship (Skrzynice 51.1167°N 22.2500°E, Zemborzyce 51.1833°N 22.5000°E) in the period from June to August 2020. Plant material of the same species from different natural sites was mixed. For each plant species, a total of 1 kg of fresh herb was taken. The harvested plant material was dried at 22°C, in a ventilated and shaded place. The dried plant material was ground, then 100.0 g of pulverized plant material was weighed into round-bottom flasks and 1000 mL of 70% ethanol were added. Extraction was carried out for 6 h under a reflux condenser at the boiling point of 70% ethanol. The resulting extract was filtered through filter paper and concentrated to 100 mL using a rotary evaporator (Heidolph Company).

Total polyphenol analysis. Total polyphenol concentration in the examined extracts was determined using spectrophotometry (λ = 765 nm) according to

the method of Singleton and Rossi [1965]. Phenol content results are expressed in gallic acid equivalents. The results were calculated from the equation of the calibration curve prepared for gallic acid standards in the concentration range of 10–60 mg/L (10, 20, 30, 40, 50, 60 mg/L). Each sample, depending on the starting concentration, was diluted according to the range of the standard curve. All analyses were performed in triplicate.

Flavonoid analysis. The concentration of total flavonoids in the extracts was determined spectrophotometrically according to the procedure described by Karadeniz et al. [2005], using epicatechin as a standard. The results of the assays were converted into the amount of epicatechin in 1 mL of the infusion. For this purpose, 1 mL of the extract with experimentally determined concentration was aliquoted into a 10 mL volumetric flask, and subsequently 5 mL of redistilled water and 0.3 mL of 5% w/w water sodium nitrate (III) solution were added. The resulting solution was stirred and left for 5 min, 0.6 mL of 10% w/w of water aluminum chloride hexahydrate solution was added and remixed. After 5 min, 2 mL of 1 mol/L water NaOH solution was added and filled to the mark with redistilled water. The absorbance of the prepared samples was measured at 510 nm against reagent blank. The results were calculated based on the calibration curve prepared for epikatechin standards in the concentration range of 10–400 mg/L (10, 50, 100, 150, 200, 250, 300 and 400 mg/L). Each sample, depending on the starting concentration, was diluted according to the range of the standard curve. All analyses were performed in triplicate.

Assessment of extract antioxidant activities. The antioxidant activity was determined according to the modified method of Brand-Wiliams et al. [1995] using the synthetic DPPH radical converted to mM Trolox [Wyrostek and Kowalski 2022]. Absorbance of the solutions was measured at $\lambda = 517$ nm. A 0.5-mmol/L alcohol DPPH solution was prepared by dissolving 19.71 mg DPPH (M = 394.32 g/mol) in 100 mL of ethanol. The obtained solution was diluted so that its absorbance at $\lambda = 517$ nm was approximately 0.9. The solution was stored in the dark. Spectrophotometer calibration was performed using ethanol. Absorbance A_0 of the DPPH radical solution was measured by adding 1.5 mL of DPPH and 20 µL of ethanol to the solution. The experimental sample contained 1.5 mL of DPPH solution and 20 µL of the tested antioxidant solution or extract; absorbance (A) was measured 30 min after the start of the reaction. Each measurement was performed in triplicate and the mean absorbance value (A_m) of the solution was calculated. The ability of the tested antioxidant to counteract the oxidation reaction was calculated from the following formula: % inhibition = $100 (A_0 - A_m)/A_0$, where A_m is the mean absorbance of the test solution containing the antioxidant, and A_0 is the absorbance of the DPPH radical solution.

In vitro evaluation of antifungal extract activities

The following species of phytopathogenic fungi were included in the study: *Alternaria alternata* (Fr.) Keissl. (strain PCL10), *Botrytis cinerea* Pers. (strain CH10), *Colletotrichum coccodes* (Wallr.) S. Hughes (strain P74/2) and *Fusarium oxysporum* Schltdl. (strain ECR4). Fungi used in the study were derived from the collection of the Department of Plant Protection, University of Life Sciences in Lublin. The study assessed the effect of 5%, 10% and 20% ethanol extracts from horseradish (H), yarrow (Y) and tansy (T) on the linear growth of fungi. The study used the poison plate method, which consisted of adding the test extract with a specific concentration to a sterile medium (potato-dextrose agar – PDA, Difco), cooling to 50°C, and inoculating it on the solidified medium of the tested fungal species [Jamiołkowska and Kowalski 2012]. Media with extracts were poured into sterile Petri dishes with a diameter of 9 cm, and subsequently fungal colonies of 3-mm diameter were centrally inoculated onto the solidified medium. Inoculum was derived from 10-day single-spore colonies of *A. alternata*, *B. cinerea*, *C. coccodes* and *F. oxysporum* cultured on PDA. Three replicates were prepared for each extract concentration and fungal strain tested. Colonies of *A. alternata*, *B. cinerea*, *C. coccodes* and *F. oxysporum* fungi, growing on PDA with 5%, 10% and 20% ethanol fractions (ethanol 70%; the total volume of 1000 mL was evaporated to 100 ml in a rotary evaporator under conditions identical to those for the preparation of plant extracts) served as controls. The thus prepared fungal cultures were incubated for 10 days at 25°C. The diameter of fungal colonies (mm) was measured after 4, 6, 8 and 10 days.

The measure of antifungal activity was the inhibition of linear mycelial growth on the medium enriched with *A. rusticana*, *A. millefolium* and *T. vulgare* extracts compared to control. The antifungal efficacy of the extracts was calculated using Abbott's formula: I = [(C – T)/C] × 100%, where: I – inhibition index of fungus linear growth (%), C – diameter of fungus colony in the control sample, T – diameter of fungus colony in the experimental sample containing the test substance in agar [Jamiołkowska and Kowalski 2012].

STATISTICAL ANALYSIS

Data were analyzed by analysis of variance (Duncan's test) at the P ≤ 0.05 significance level using the SAS statistical system [SAS 9.1, 2004].

RESULTS AND DISCUSSION

This study determined the chemical composition of horseradish, yarrow, and tansy extracts. The average contents of flavonoids in the extracts are presented in Table 1. The concentration of flavonoids in the tested extracts ranged from 1.18 to 23.06 mg/mL and differed significantly depending on the plant species. The highest flavonoid content was recorded in yarrow (Y – 23.06 mg/mL) and tansy extracts (T – 22.27 mg/mL). The highest polyphenol content was also found in yarrow (Y – 37.41 mg/mL) and tansy extracts (T – 36.85 mg/mL), while the lowest and statistically different in horseradish extract (H – 20.78 mg/mL). The lowest antioxidant activity was recorded for the extract of horseradish leaves (3.06 mM Trolox), and the highest for tansy (63.08 mM Trolox) – Table 1.

The plant extracts exerted different effects on the growth of the tested fungal strains, depending on the species of fungi, type of plants from which the extract was prepared and concentration extract, as well as the time of action of biologically active compounds. Tansy and yarrow extracts showed the strongest fungistatic effect, while horseradish extracts the weakest (Tabs. 4–6, Fig. 1).

The strongest fungistatic effect against *A. alternata* was observed for tansy extract 20% (T20 19.26–43.65%), and its effect in the following days remained high level (Tab. 2). The fungistatic effect of 20% tansy extract was similar to that of yarrow extract (Y20 3.0–43.46%) (except 6th day) (Tabs. 2 and 3). A strong fungistatic impact on *A. alternata* was also recorded for 5% yarrow and tansy extracts, but only on 8th and 10th days of growth (but, T5 – 33.59% at 6th day). Horseradish extracts were the least active against *A. alternata*. Horseradish extract (5%) even stimulated fungus growth in the first days of experiment, and inhibited it only on 8th and 10th day (Tab. 3). The effect of horseradish extract against *A. alternata* was statistically weaker compared to other extracts (Tab. 3).

The strongest fungistatic effect of plant extracts against *B. cinerea* was noted only in the first days of the experiment (4th day) for 20% yarrow (Y20 – 59.61%) and horseradish extracts (H20 – 53.57%), and their impact was significantly stronger compared to 20% tansy extract (T20 – 37.13%) (Tab. 2, 4, Fig. 1). The 5% extracts slightly inhibited fungus growth (Y5 – 12.3%, T5 – 16.86%) or showed no fungistatic effect (H5 – 0.0%). Due to the strong growth dynamics of the fungus, no effect of plant extracts on *B. cinerea* was noted in the consecutive days of observation (Tabs. 2 and 4).

Table 1. Concentration of flavonoids (epicatechin equivalent, mg/mL), polyphenols (gallic acid equivalent, mg/mL) and antioxidant activity in the basal extract (100%)

Plant extract	Flavonoids (mg/mL) ±SD	Polyphenols (mg/mL) ±SD	Antioxidant activity	
			% inhibition ±SD	mM Trolox ±SD
A. rusticana (H)	1.18 ±0.032 b	20.78 ±0.756 b	4.55 ±0.478 b	3.06 ±0.754 b
A. millefolium (Y)	23.06 ±0.547 a	37.41 ±0.495 a	40.28 ±9.244 a	59.40 ±14.57 a
T. vulgare (T)	22.27 ±0.348 a	36.85 ±2.810 a	42.62 ±3.011 a	63.08 ±4.747 a

H – horseradish; Y – yarrow; T – tansy; a, b, c – values in columns marked with the same letter do not differ significantly at a significance level of P ≤ 0.05

Table 2. Colony diameter (mm) of fungi growing on potato-dextrose agar (PDA) with different plant extract concentrations (5, 10 and 20%)

Fungus species	Days	Experimental combination											
		control 5	H5	Y5	T5	control 10	H10	Y10	T10	control 20	H20	Y20	T20
Alternaria alternata	4th	21.7 ±1.2bc	34.0 ±0.8a	22.0 ±2.8bc	22.5 ±3.1bc	25.0 ±0.8b	24.7 ±0.5bc	21.7 ±0.9bc	21.0 ±3.3bc	29.5 ±0.4ab	33.0 ±0.0a	22.7 ±2.1bc	18.0 ±2.4c
	6th	45.7 ±3.3ab	48.7 ±1.9a	37.7 ±2.1bc	30.3 ±2.9c	32.3 ±1.7bc	37.0 ±2.2bc	31.2 ±1.0c	30.7 ±3.3c	32.3 ±1.7bc	40.0 ±0.0b	31.3 ±1.2c	26.0 ±0.8c
	8th	59.2 ±6.2a	57.7 ±0.2a	41.7 ±2.4bc	37.0 ±2.4bc	36.0 ±2.9cd	38.8 ±1.8bc	38.2 ±0.6bc	35.3 ±5.0d	51.0 ±0.8ab	47.3 ±2.9b	40.0 ±0.0bc	39.0 ±0.8bc
	10th	79.0 ±7.8a	73.7 ±2.6a	52.7 ±2.5b	56.0 ±2.2b	56.7 ±9.0b	54.7 ±0.5b	51.3 ±0.6b	50.7 ±4.7b	79.0 ±0.8a	47.7 ±2.7b	44.7 ±0.5b	44.5 ±1.2b
Botrytis cinerea	4th	89.3 ±0.9a	90.0 ±0.0a	78.3 ±1.7ab	74.3 ±6.8b	90.0 ±0.0a	74.7 ±8.8b	75.3 ±2.6b	70.0 ±1.6b	88.3 ±1.2a	41.0 ±0.8d	35.7 ±0.9d	55.5 ±2.0c
	6th	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a
	8th	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a
	10th	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a	90.0 ±0.0a
Colletotric hum coccodes	4th	34.3 ±0.9a	33.7 ±0.5a	24.7 ±1.7c	26.7 ±0.5bc	28.7 ±0.9b	22.0 ±2.2c	24.7 ±0.5c	22.7 ±0.5c	28.7 ±0.9b	24.0 ±0.8c	22.0 ±0.0c	23.5 ±0.4c
	6th	50.3 ±0.5a	49.0 ±1.4a	40.7 ±0.5c	45.0 ±0.0b	40.0 ±0.0c	35.0 ±0.0de	40.0 ±0.0c	40.7 ±0.9c	40.0 ±0.0c	39.8 ±0.6c	36.7 ±1.2d	33.8 ±1.3e
	8th	58.3 ±0.6a	56.0 ±1.4ab	51.5 ±0.4b	56.7 ±1.7ab	44.8 ±1.6c	37.3 ±0.5d	46.3 ±1.2bc	45.0 ±0.8bc	48.0 ±0.0bc	46.5 ±5.3bc	47.7 ±0.5bc	47.3 ±0.5bc
	10th	80.7 ±0.9a	77.5 ±0.4ab	71.0 ±2.2b	71.7 ±1.2ab	64.7 ±3.9bc	56.8 ±1.3c	67.0 ±1.6bc	66.0 ±0.8bc	64.7 ±3.9bc	52.0 ±6.5c	59.0 ±0.0c	58.0 ±0.0c
Fusarium oxysporum	4th	33.0 ±0.9b	37.3 ±1.2ab	31.0 ±1.4bc	31.0 ±0.8bc	30.3 ±2.1bc	26.0 ±5.0c	28.0 ±1.6bc	26.7 ±2.5bc	42.5 ±0.4a	31.2 ±0.2c	32.0 ±0.0bc	32.0 ±0.0bc
	6th	46.0 ±0.8bc	59.0 ±0.8a	48.0 ±2.8b	49.0 ±1.4b	45.0 ±1.6bc	40.7 ±0.9c	47.0 ±2.2bc	41.7 ±3.1c	45.0 ±1.6bc	45.5 ±1.6bc	44.0 ±0.8bc	41.5 ±0.4c
	8th	54.0 ±1.5b	70.2 ±5.7a	58.8 ±4.9ab	60.5 ±6.0ab	45.0 ±1.6bc	41.3 ±1.9c	53.7 ±1.2b	53.0 ±5.7bc	53.7 ±1.2bc	65.0 ±0.0ab	60.0 ±0.0a	60.0 ±0.0a
	10th	81.3 ±1.9b	88.7 ±1.9ab	82.3 ±3.8ab	83.7 ±2.6ab	80.0 ±0.0bc	70.0 ±0.0c	78.0 ±2.2bc	73.0 ±5.0c	90.0 ±0.0a	80.0 ±0.0bc	72.0 ±0.0c	72.0 ±0.0c

H – horseradish; Y – yarrow; T – tansy; 5 – 5% extract, 10 – 10% extract, 20 – 20% extract; a, b, c – values in the rows marked with the same letter do not differ significantly at a significance level of $P \leq 0.05$

Table 3. Linear growth inhibition index (±SD) of *Alternaria alternata* relative to control after application of plant extracts (%)

Experimental combination	Number of day			
	4th	6th	8th	10th
H 5	0.00 ±0.00 b	0.00 ±0.00 b	2.53 ±8.126 c	6.71 ±12.83 c
Y 5	0.00 ±0.00 b	17.5 ±11.391 ab	29.56 ±13.335 ab	33.29 ±4.228 ab
T 5	0.00 ±0.00 b	33.59 ±13.484 a	37.5 ±11.354 a	29.11 ±9.923 ab
H 10	1.2 ±4.441 b	0.00* ±0.00 b	0.00 ±0.00 c	3.52 ±10.56 c
Y 10	13.13 ±7.931 b	3.45 ±4.718 b	0.00 ±0.00 c	9.52 ±12.47 c
T 10	16.0 ±16.108 ab	4.95 ±9.221 b	1.94 ±13.684 c	10.58 ±19.65 c
H 20	0.00 ±0.00 b	0.00 ±0.00 b	7.22 ±5.994 c	39.69 ±3.353 ab
Y 20	23.05 ±9.81 ab	3.00 ±3.031 b	21.55 ±1.539 abc	43.46 ±0.374 a
T 20	39.09 ±9.138 a	19.26 ±8.025 ab	23.54 ±0.462 abc	43.65 ±2.612 a

H – horseradish; Y – yarrow; T – tansy; 5 – 5% extract, 10 – 10% extract, 20 – 20% extract; * – stimulation of fungus growth; a, b, c – values in the columns marked with the same letter do not differ significantly at a significance level of P ≤ 0.05

Table 4. Linear growth inhibition index (±SD) of *Botrytis cinerea* relative to control after application of plant extracts (%)

Experimental combination	Number of day			
	4th	6th	8th	10th
H 5	0.00 ±0.000 e	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a
Y 5	12.30 ±2.85 de	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a
T 5	16.86 ±8.33 d	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a
H 10	17.04 ±11.98 d	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a
Y 10	16.30 ±3.57 d	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a
T 10	22.22 ±2.22 cd	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a
H 20	53.57 ±1.745 a	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a
Y 20	59.61 ±1.58 a	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a
T 20	37.13 ±3.89 bc	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 a

H – horseradish; Y – yarrow; T – tansy; 5 – 5% extract, 10 – 10% extract, 20 – 20% extract; a, b, c – values in the rows marked with the same letter do not differ significantly at a significance level of P ≤ 0.05

Strong antifungal effect of plant extracts against *C. coccodes* was recorded for all extracts and concentrations in the first days of the experiment (exception H5 – 1.9%), and the degree of inhibition of fungal growth relative to control ranged from 13.89 to 28.20% (Tab. 5). The strongest inhibition of fungus growth was observed already on the 4th day, followed the application of 5% yarrow (Y5 – 28.20%) and tansy extracts (T5 – 22.25%), and their effect was stronger compared to 10% and 20% concentrations, but not statistically different (Tab. 5, Fig. 1). The effect of plant extracts significantly decreased in the following days (Tab. 5). The least impact on *C. coccodes* were horseradish extracts (5%), whose fungistatic effect on 10th day was the weakest and significantly lower compared to other extracts (Tabs. 2 and 5).

Plant extracts inhibited the growth of *F. oxysporum* only in the first days of observation (4th and 6th days) (the exception was H5), and only 20% extracts (H20 – 26.66%; Y20, T20 – 24.7%) (at only for 4th day, not for

Table 5. Linear growth inhibition index (±SD) of *Colletotrichum coccodes* relative to control after application of plant extracts (%)

Experimental combination	Number of day			
	4th	6th	8th	10th
H 5	1.90 ±1.65 c	2.61 ±4.53 c	3.97 ±3.99 bc	3.92 ±0.88 a
Y 5	28.20 ±4.66 a	19.20 ±1.06 a	11.71 ±0.35 ab	11.96 ±3.88 a
T 5	22.25 ±3.80 ab	10.59 ±1.02 b	3.43 ±2.75 bc	11.15 ±2.08 a
H 10	23.41 ±6.11 ab	12.50 ±0.00 ab	16.63 ±3.37 a	11.82 ±6.51 a
Y 10	13.89 ±2.99 bc	0.00 ±0.00 d	0.72 ±1.25 c	0.48 ±0.82 a
T 10	20.79 ±5.08 ab	0.00 ±0.00 d	1.45 ±2.51 bc	1.90 ±3.29 a
H 20	16.27 ±1.818 b	0.83 ±1.44 cd	6.60 ±8.86 abc	18.75 ±17.78 a
Y 20	23.17 ±3.02 ab	8.33 ±3.82 bc	0.69 ±1.20 c	8.45 ±6.47 a
T 20	17.98 ±1.96 ab	15.42 ±4.02 ab	1.39 ±1.20 bc	10.00 ±6.36 a

H – horseradish; Y – yarrow; T – tansy; 5 – 5% extract, 10 – 10% extract, 20 – 20% extract; a, b, c – values in the rows marked with the same letter do not differ significantly at a significance level of P ≤ 0.05

Table 6. Linear growth inhibition index (±SD) of *Fusarium oxysporum* relative to control after application of plant extracts (%)

Experimental combination	Number of day			
	4th	6th	8th	10th
H 5	0.00 ±0.00 b	0.00 ±0.00 a	0.00 ±0.00 a	0.00 ±0.00 c
Y 5	1.08 ±1.86 b	0.74 ±1.28 a	0.00 ±0.00 a	2.78 ±4.81 c
T 5	1.08 ±1.86 b	0.72 ±1.22 a	0.93 ±1.62 bc	0.00 ±0.00 c
H 10	17.06 ±16.68 ab	9.44 ±6.45 a	22.77 ±1.22 a	12.50 ±0.00 ab
Y 10	7.47 ±6.81 ab	1.42 ±2.45 a	0.6 ±1.03 c	2.5 ±3.31 c
T 10	13.74 ±11.91 ab	7.48 ±5.62 a	6.43 ±5.67 b	8.75 ±7.60 bc
H 20	26.66 ±0.44 a	2.48 ±4.29 a	0.00 ±0.00 c	11.11 ±0.00 ab
Y 20	24.70 ±0.88 a	2.84 ±4.91 a	0.00 ±0.00 c	00.00 ±0.00 c
T 20	24.70 ±0.88 a	7.64 ±4.71 a	0.00 ±0.00 c	00.00 ±0.00 c

H – horseradish; Y – yarrow; T – tansy; 5 – 5% extract, 10 – 10% extract, 20 – 20% extract; a, b, c – values in the rows marked with the same letter do not differ significantly at a significance level of P ≤ 0.05

6th day) exhibited a significant fungistatic effect. On subsequent days, the antifungal activity of the plant extracts decreased or was absent, except for the effect (22.77%) of H10 in 8th day (Tab. 6).

The present study has shown a different fungistatic effect of plant extracts on phytopathogenic fungi. The strongest antifungal activity was shown by tansy (T) and yarrow (Y) extracts. Tansy occurs in Europe and Asia in temperate regions [Mitich 1992]. It is a valuable source of biologically active compounds such as tannins, organic acids and sugars, sesquiterpene lactones, flavonoids and essential oil [Zawiślak and Nurzyńska-Wierdak 2017]. Due to its rich chemical composition, yarrow exhibits antibacterial, antioxidant, cytotoxic and antifungal properties, and the strength of its effect on microorganisms depends on the chemical composition of the essential oil [Coté et al. 2017, Devrnja et al. 2017]. Flavonoids are a large

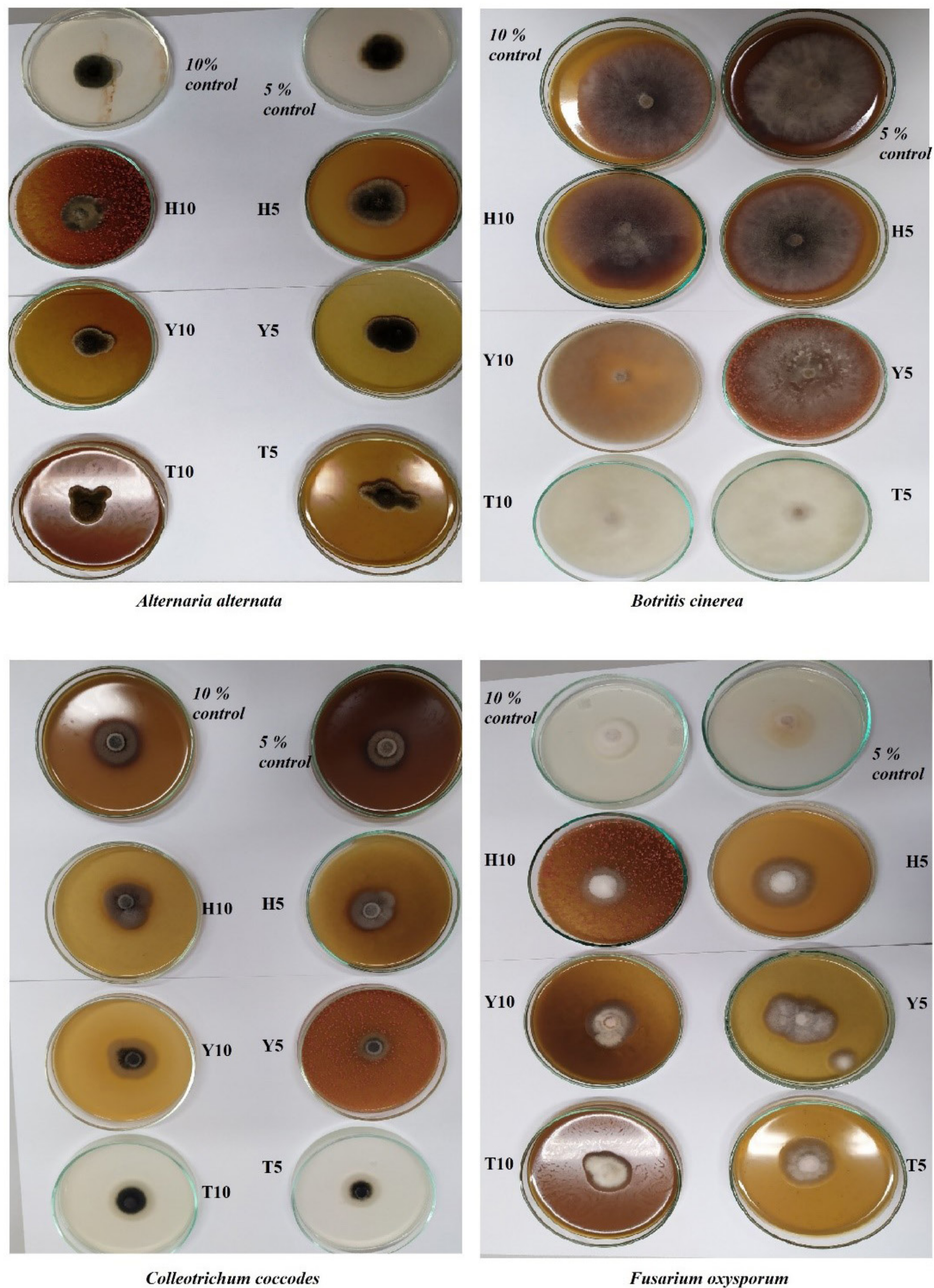

Fig. 1. Linear growth of fungal colonies on PDA with the addition of horseradish (H), yarrow (Y) and tansy (T) extracts; 5 – 5% extract concentration; 10 – 10% extract concentration; day 6 of growth

group of secondary metabolites and play a key role in plant defense against fungal and bacterial pathogens [Jamiołkowska and Kopacki 2019]. Polyphenolic compounds are widely present in many plant species, including yarrow flowers [Devrnja et al. 2017, Ivănescu et al. 2018, Mot et al. 2018, Kaczorová et al. 2021]. The current study demonstrated a high fungistatic activity of tansy extracts, which, due to the high content of flavonoids, allows to consider tansy a plant with very high antimicrobial potential. Similar studies were carried out by Burgieł and Moliszewska [1999], who showed that water tansy extract applied at a concentration of 50% inhibited the development of *Colletotrichum lindemuthianum*, and 70% concentration of the extract reduced plant infection by *Sphaeroteca xantii*. Not only flavonoids, but also essential oils and their composition determine the antimicrobial effect of tansy plant [Vilhelmova et al. 2020]. Chlorogenic [Ivănescu et al. 2018] and cichoric acids [Bączek et al. 2017] are the main phenolic compounds found in tansy extracts, and the variation in the composition of bioactive compounds in the extracts and essential oils depends on the geographical area [Mot et al. 2018]. High content of polyphenolic compounds in alcoholic extracts of herbal plants is correlated with their high antioxidant capacity [Bączek et al. 2015, Devrnja et al. 2017]. This was demonstrated by our experiments carried out using the free radical DPPH method, which showed high antioxidant activity of the extracts from tansy and yarrow, which was also strongly correlated with the highest polyphenol contents, as previously confirmed by Devrnja et al. [2017].

Yarrow (*Achillea millefolium* L.) occurs in meadows, pastures and wastelands from lowlands to mountain regions of Poland [Nowak et al. 2010]. It is characterized by a wealth of bioactive compounds [Vitalini et al. 2011, Georgieva et al. 2015, Afshari et al. 2018]. Our research showed that the fungistatic effect of yarrow extracts on phytopathogenic fungi was similar to that of tansy extracts. The antimicrobial activity of yarrow plant against *Aspergillus niger* and *Candida albicans* was also shown by other authors [Candan et al. 2003, Stojanović et al. 2005]. The strong growth inhibition of *A. alternata*, *B. cinerea*, *C. coccodes* and *F. oxysporum* depended, among others, on flavonoid content of the plant extracts, of which the highest quantities were found in yarrow and tansy plants. Naringin is the flavonoid compound present in the highest amounts in yarrow [Kaser et al. 2013]. Essential oils also show antimicrobial (antifungal and antibacterial) properties. The minimum content of essential oils in the plant should be 0.20% [Bączek et al. 2015]. Rahimmalek and co-authors [2009] specified an essential oil content of 0.1 to 2.7% as effective, while other estimates stated that this value should fall between 0.16 and 0.22% [Giorgi et al. 2005]. It should be noted that the composition of essential oil in plants also depends on the geographical location, and there are forms rich in this compound in eastern Poland [Bączek et al. 2015]. Since phenolic compounds exhibit the most potent antioxidant and antiradical properties among all secondary metabolites [Fierascu et al. 2015, Jovanović et al. 2017], *Achillea* spp. extracts, due to the high content of polyphenols, limit the development of pathogenic microorganisms, which was also demonstrated in our study.

Biologically active compounds contained in horseradish plants are characterized by significant antimicrobial activity and cytotoxicity [Dekić et al. 2017, Petrović et al. 2021]. Many studies have described plant extracts of horseradish (*Armoracia rusticana*) as one of the most active against microorganisms [Hać-Szymańczuk et al. 2012]. This effect has not been confirmed in our study. Horseradish leaf extract showed weak antifungal activity against the tested phytopathogens. Tedeschi and co-authors [2011] reported that horseradish in 10% ethanol solution showed fungistatic activity only against *Fusarium oxysporum*, *F. culmorum* and *Sclerotium rolfsii*, while it did not inhibit the growth of *Botrytis cinerea* and *Trichoderma longibrachiatum*. Horseradish is a rich source of many bioactive compounds like glucosinolates (GLS) and their degradation products [Agneta et al. 2013]. The biological activity of horseradish is determined by glucosinolates, but they are present in the highest amounts in the root [Jafernik et al. 2019]. Although glucosinolates do not inhibit fungal growth, their degradation products, which include isothiocyanates, already exhibit strong antifungal and antibacterial activity and are able to destroy fungal and bacterial pathogens [Biller et al. 2019, Plaszkó et al. 2020]. However, horseradish contains small amounts of flavonoids [Petrović et al. 2021] compared to yarrow and tansy extracts, which was confirmed in the present study.

The main flavonoids present in *A. rusticana* include rutoside and quercitin [Jafernik et al. 2019]. However, the low content of flavonoids and polyphenols in the extract of horseradish leaves determines its low fungicidal activity. The results of the present experiments are a valuable source of information for further field research and can be used as preliminary studies in the development of a biological preparation for the protection of agricultural and horticultural plants against pathogens.

CONCLUSIONS

1. The plant extracts of tansy and yarrow leaves were characterized by a higher content of polyphenols and flavonoids compared to horseradish leaf extracts, and also showed a higher antioxidant activity.

2. The fungistatic effect of the plant extracts depended on the species of fungus and the concentration of the extract. Plant extracts in the concentration of 20% showed the strongest anti-fungal activity.

3. The extracts of tansy and yarrow leaves exhibited the strongest fungistatic activity against *Alternaria alternata, Botrytis cinerea*, *Colletotrichum coccodes* and *Fusarium oxysporum*, while horseradish leaf extract the weakest.

4. The least potent fungistatic effect of plant extracts was recorded against *Botrytis cinerea*, as the extracts inhibited the linear growth of this fungus only during the first days of the experiment.

5. Plant extracts were most effective during the first days of the experiment, and their antifungal activity decreased over time.

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