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In vitro ESTIMATE OF INFLUENCE OF *Silphium perfoliatum* L. LEAVES EXTRACT ON SOME FUNGI COLONIZING THE PEPPER PLANTS

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Abstract. Biological control is a modern, comprehensive and non-polluting approach to the management of diseases. Control of plant pathogen by using biological preparations derived from plants like garlic, mint, thyme, grapefruit, has shown attractive and promising results. Present study aimed at laboratory evaluation the properties of ethanol extract of *Silphium perfoliatum* leaves towards fungi colonizing pepper plants grown in the field. The mycelium of *Alternaria alternata, Botrytis cinerea, Colletotrichum coccodes, Fusarium oxysporum, Penicillium expansum, Trichoderma harzianum* were used in experiment. The *Silphium* extract was applied in two concentrations: 5% and 10%. Leaves were obtained from the three-year *S. perfoliatum* plantation. Tested fungal isolates originated from pepper plants grown in the field. The studies made use of a Petri dishes method recommended for testing fungicides in laboratory conditions. The *Silphium* extracts in two tested concentrations significantly inhibited the growth of tested fungi species, with the exception of *T. harzianum* and *B. cinerea* in 5% concentration extract. Effects of 10% extract were longer than those of 5% one. *A. alternata* and *C. coccodes* were fungi, growth of which was the most strongly inhibited by tested concentrations of *Silphium* extracts.

Key words: antifungal activity, *A. alternata*, *C. coccodes*, biocontrol

INTRODUCTION

The marketable production of pepper (*Capsicum annuum* L.) in Poland is now possible owing to growing demand of food processing companies for pepper fruits and to new cultivars adapted to field cultivation. Recently, the acreage of pepper and the consumption of this vegetable have grown considerably. Pepper is popular due to its taste

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and a high content of vitamin C. It is used mostly as addition to many dishes and as component of spice mixtures. Pepper yielding depends on many factors, including its health status [Jamiołkowska and Buczkowska 2009].

Pepper can be usually infected by *Alternaria alternata* Keiss., *Fusarium oxysporum* Schlecht., *Colletotrichum coccodes* (Wallr.) Hughes, and *Botrytis cinerea* Pers. [Wagner 2004, Jamiołkowska 2009a, b]. *A. alternata* is extremely important for pepper's health. That fungus makes necrotic spots on leaves and fruits [Mesbah et al. 2000]. Its destructive action towards the host-plant is a result of enzymes and toxins production [Rotem 1994]. *F. oxysporum* is also the pepper's and other vegetables' pathogen; it causes plants to fade and die due to colonizing their underground organs or vascular bundles [Nelson et al. 1983]. Similarly *C. coccodes* infects roots and pepper stem's bottom making necrosis of tangential layer. Important patogen causing pre- and post-harvest diseases of pepper fruits and other vegetables grown in field is *B. cinerea* [Jamiołkowska 2009a]. Chemical plant protection can makes not only fungal resistance, environmental pollution, but first of all, it directly influences on human's health [Lingk 1991]. More often biological preparations based on natural substances are applied for protecting plants against diseases. Plant extracts and essential oils are more often used by producers to protect plants against disease-forming fungi both in ecological and integrated production.

Species of *Silphium* L. are interesting group in this aspect. They commonly occur on prairies, fields and open forests of middle and eastern parts of USA and Canada. Chemical composition determinations revealed that contained: phenolic acids, flavonoids, terpenes including triterpene saponins – oleanosides as well as essential oils [Bohlmann and Jakupovic 1979, 1980, Davidjanc and Abubakirov 1992, Pcolinski et al. 1994, El-Sayed et al. 2002, Kowalski 2005, Kowalski and Wolski 2003, 2005, Kowalski et al. 2005, Calabria et al. 2008].

Davidjanc et al. [1997] paid attention to saponins contained in *S. perfoliatum* leaves inhibiting development of some phytopathogenic fungi such as *Dreschlera graminae*, *Rhizopus nodosus* and *Rhizopus nigricans*. Other studies carried out upon the biological activity of extracts achieved from *S. perfoliatum* indicated the action accelerating wound healing [Kujanceva and Davidjanc 1988] and antiarteriosclerotic features [Syrov et al. 1992]. Kowalski and Kędzia [2007] reported that methanol and ethanol extracts made of *S. perfoliatum* were characterized by antibacterial action towards Gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), while essential oils from *S. trifoliatum* and *S. integrifolium* showed antifungal action towards *Candida albicans* and *Malassezia pachydermatis* strains [Kowalski 2008d].

Present study aimed at laboratory evaluating the properties of ethanol extract made of *S. perfoliatum* leaves towards fungi achieved from pepper plants grown in the field.

MATERIALS AND METHODS

Chemical characteristics of extracts

Preparation of extracts. The leaves of *S. perfoliatum* originated from three-year-old experimental cultivation (2003) were proportionate by the Department of Analysis and Evaluation of Food Quality University of Life Sciences (Lublin, Poland) in Kazimierzówka near Lublin. Morphological traits of the species were earlier described by Kowalski and Wolski [2001]. Fresh material was frozen and then lyophilized (Labconco lyophilizer) with subsequent grinding.

Extraction. Aliquots of 167.00 g lyophilized and powdered plant material were transferred into the round-bottom flasks and 1000 cm^3 70% was added. Extraction was performed under reflux condenser at ethanol boiling point for 6 hours. Then extract was filtered through filter paper and concentrated in rotational evaporator till 100 cm^3 volume.

Triterpene fraction analysis. Triterpene glycosides isolated from alcoholic extracts were hydrolysed and silanized, and then determined according to previously described procedures [Kowalski 2007b].

Analysis of phenolic compounds of o-dihydroxophenol type. Determinations of phenolic compounds (with conversion for caffeic acid) were made by spectrophotometric means according to modified Singelton and Rossi method [1965].

Flavonoid analysis. Determination of flavonoid content (flavonoles converted for quercetine) was performed by means of spectrophotometry according to modified Polish Pharmacopoeia VI procedure [2002].

GC analysis of extracts composition. The qualitative analysis was carried out on the basis of MS spectra which were compared with the spectra of the NIST library [IST/EPA/NIH 2002], and with data available in literature [Joulain and König 1998, Adams 2001, Kowalski 2005, Kowalski and Wolski 2005, Kowalski et al. 2005, Kowalski 2008a, b, c], and own data (MS for pure reference chemicals). Identity of the compounds was confirmed by their retention indices [Van den Dool and Kratz 1963] taken from literature [Joulain and König 1998, Adams 2001, Kowalski and Wolski 2005, Kowalski et al. 2005] and own data (retention indices for pure reference chemicals). Quantitative analysis was performed by means of internal standard addition method (cholesterol, alkanes C_{12} and C_{19}) according to previously described procedures [Kowalski 2008a].

The identity of the compounds was confirmed by their retention indices (Van den Dool and Kratz 1963)

GC analysis. *GC-MS (triterpene fraction analysis)*: GC-MS: ITMS Varian 4000 GC-MS/MS (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m × 0.25 mm VF-5ms column (Varian, USA), film thickness 0.25 μm, carrier gas He 2.5 ml/min, injector and detector temperature were, respectively, at 280 and 180°C; split ratio 1:10; inject volume 1 μl. A temperature gradient was applied (240°C for 1 minute, then incremented by 20°C/min to 320°C); ionization energy 70 eV; mass range: 100–870 Da; scan time 0.80 s.

GC-MS (analysis of components in Silphium extracts). ITMS Varian 4000 GC-MS/MS (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m × 0.25 mm

VF-5ms column (Varian, USA), film thickness 0.25 μm, carrier gas He 0.5 ml/min, injector and detector temperature were, respectively, at 250 and 200°C; split ratio 1:50; inject volume 1 μl. A temperature gradient was applied (50°C for 1 minute, then incremented by 4°C/min to 250°C, 250°C for 10 minutes); ionization energy 70 eV; mass range: 40–350 Da; scan time 0.80 s.

GC-FID (analysis of components in Silphium extracts). GC Varian 3800 (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m × 0.25 mm DB-5 column (J&W Scientific, USA), film thickness 0.25 μm, carrier gas He 0.5 ml/min, injector and detector FID temperatures were, respectively, at 250 and 260°C; split ratio 1:50; inject volume 1 μl. A temperature gradient was applied (50°C for 1 minute, then incremented by 4°C/min to 250°C, 250°C for 10 minutes).

Evaluation of antifungal activity of extracts

Study involved fungi *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum coccodes*, *Fusarium oxysporum*, *Penicillium expansum*, *Trichoderma harzianum*. The experiment aimed at examining the influence of 5% and 10% *S. perfoliatum* extracts on above fungi species growth. The Petri dish method was applied, which is recommended to test chemical agents under laboratory conditions according to Thanassoulopoulos et al. [1971]. The method consists in adding the tested substance into the sterile potato-dextrose agar (PDA) medium cooled to 50°C and inoculation onto solidified medium of tested fungus species. Medium and *S. perfoliatum* extract was poured onto sterile Petri dishes of 9 cm diameter, and then the medium's surface of inoculated with fungi colonies of 3 mm diameter. Inoculum originated from 10-day-old single-spore colonies of *A. alternata*, *B. cinerea*, *C. coccodes*, *F. oxysporum*, *P. expansum*, *T. harzianum* grown on PDA culture medium. The isolates were selected on the basis of macro- and microscopic properties of fungi isolated from sweet pepper plants growing in the field. Five replications of tested biological substance at particular concentration considered as an object and particular fungus were made. The control consisted of *A. alternata*, *B. cinerea*, *C. coccodes*, *F. oxysporum*, *P. expansum*, *T. hamatum*, colonies grown on PDA culture medium with addition of 5% and 10% of the solution fraction (ethanol 70% in total volume of 1000 ml was evaporated to 100 ml in rotational evaporator under identical conditions as for making the *Silphium* extracts). Such prepared dishes were stored in thermostat for 14 days at 25°C. After 7, 14, and 21 days, diameter of tested fungi colonies was measured. Inhibition of mycelium growth on the medium enriched with *S. perfoliatum* extract in relation to that on control medium was the measure of antifungal activity.

The antifungal efficiency of *Silphium* extract was calculated from Abbot's formula:

$$I = [(C - T)/C] \times 100\%$$

where: I – fungus linear growth inhibition index (percentage),
C – fungus colony diameter in the control combination,
T – fungus colony diameter in combination containing a tested substance concentration in the agar [Jamiołkowska 2011].

Data were analyzed by analysis of variance (Duncan's test) at 5% significance level using the SAS statistical system [SAS Version 9.1, SAS Inst., Cary, N.C., U.S.A.].

RESULTS AND DISCUSSION

Chemical composition of *S. perfoliatum* leaves extracts was characterized in the experiments (tab. 1, 2). Alcoholic extracts contained of glycoside-bonded: oleanolic acid and ursolic acid at levels of about 18 and 17 mg/ml. Among phenolic-type substances, concentration of o-dihydroxyphenols converted to caffeic acid (tab. 1) and flavonoids contents converted to quercetin (Table 1) were determined in ethanol *Silphium* extract. It was found that extract contained about 28 mg/ml of o-dihydroxophenols, whereas flavonoids concentrations were at the level of 7 mg/ml. Earlier studies reveal that caffeic acid that occurs in its free and bonded forms is the main phenolic acid in phenolic fraction prepared from *Silphium* genus [Kowalski and Wolski 2003, Kowalski 2004, 2007a]. Other phenolic acids identified in *Silphium* are: p-coumaric ferulic protocatechuic, p-hydroxobenzoic, vanillic, salicylic, and chlorogenic. Caempherol and quercetin occurring as dominating glycoside forms were identified in flavonoic fraction of *Silphium* [El-Sayed et al. 2002, Kowalski 2004, 2007a]. The following compounds dominated in the extract from leaves of the *S. perfoliatum*: (E)-caryophyllene, spathulenol, caryophyllene oxide, salvial-4(14)-en-1-one, τ-cadinol, α-pinene, humulene epoxide II, silphiperfol-6--en-5-one, khusinol, methyl palmitate, β-amyrine, α-amyrine, and germacrene D (tab. 2). Amyrines are transitional products on the course of oleanolic and ursolic acids synthesis as well as corresponding saponins that are glycosides of these acids. Moreover, these compounds are characteristic for plants producing resins and milky juice [Kowalski 2005].

Table 1. Content of triterpene aglycones, o-dihydroxyphenol type phenolic compounds (converted to caffeic acid), flavonoids (flavonoles converted to quercetine) in *S. perfoliatum* leaves ethanol extract

Tabela 1. Zawartość aglikonów triterpenowych, o-didydroksyfenoli typu związków fenolowych (w przeliczeniu na kwas kawowy), flawonoidów (flawonole w przeliczeniu na kwercetynę) w ekstrakcie etanolowym z liści *S. perfoliatum*

Group of compounds Grupy składników	Concentration – Zawartość ($mg \cdot ml^{-1}$)
Flavonoids – Flawonoidy	7.40 ± 0.21
Phenolic acids – Kwasy fenolowe	28.04 ± 0.75
Oleanolic acid – Kwas oleanolowy	18.18 ± 0.80
Ursolic acid – Kwas ursolowy	16.60 ± 0.63

The 10% *S. perfoliatum* extract completely inhibited the growth of tested fungi after 7 days. After 14 days the colony inhibition varied from 70.0% to 95.7% as compared to the control depending on the fungus species, while after 21 days – from 11.1% to 81.4%. *T. harzianum* was the exception; its growth was not inhibited after 21 days of experiment (tab. 4, fig. 1). The 5% *Silphium* sp. extract inhibited fungal growth from

65.5% to 93.8% after 7 days, from 0.7% to 79.81% after 14 days, whereas from 20.0% to 52.2% after 21 days. *B. cinerea* and *T. harzianum* were the exceptions, because 5% *Silphium* extract did not inhibit fungal growth, but their sporulation as compared to the control sample (tab. 3, 4, fig. 1).

Table 2. Concentrations of particular components in ethanol extract made of *S. perfoliatum* leaves

Tabela 2. Stężenia poszczególnych składników w ekstrakcie etanolowym z liści *S. perfoliatum*

No Nr	Compound Składniki	RI	Concentration (μg/ml) Stężenie (μg/ml)
1	α-pinene	938	2.33 ± 0.17
2	camphene	949	1.19 ± 0.08
3	β-pinene	975	0.88 ± 0.06
4	verbenone	1217	0.72 ± 0.05
5	α-copaene	1375	0.66 ± 0.05
6	β-bourbonene	1380	1.16 ± 0.11
7	β-cubebene	1382	1.22 ± 0.11
8	(E)-caryophyllene	1420	606.37 ± 16.26
9	β-copaene	1424	0.37 ± 0.07
10	trans-α-bergamotene	1430	0.87 ± 0.12
11	α-humulene	1455	225.12 ± 5.56
12	γ-muurolene	1470	0.31 ± 0.06
13	α-amorphene	1475	0.82 ± 0.14
14	γ-amorphene	1478	0.74 ± 0.11
15	germacrene D	1480	287.24 ± 10.20
16	δ-amorphene	1513	0.18 ± 0.03
17	γ-cadinene	1515	0.30 ± 0.06
18	(E)-nerolidol	1560	1.08 ± 0.13
19	spathulenol	1578	761.19 ± 13.02
20	caryophyllene oxide	1582	2058.30 ± 32.41
21	salvial-4(14)-en-1-one	1596	1611.72 ± 17.41
22	humulene epoxide II	1610	884.70 ± 12.49
23	silphiperfol-6-en-5-one	1630	1337.43 ± 16.55
24	τ-cadinol	1644	579.49 ± 11.14
25	khusinol	1680	2019.50 ± 15.70
26	oplopanone	1750	376.30 ± 3.87
27	methyl palmitate	1926	939.76 ± 21.38
28	methyl linoleate	2097	131.75 ± 5.11
29	β-amyrine	n.d.	683.06 ± 17.94
30	α-amyrine	n.d.	588.87 ± 12.37

RI – non-ishothermal Kovats retention indices (from temperature-programming, using definition of Van den Dool and Kratz 1963) for series of n-alkanes C6-C31, n.d – non determined

RI – nieizotermiczne indeksy retencji Kovats'a (dla programu temperaturowego przy użyciu wzoru Van den Doola i Kratza 1963) dla serii n- alkanów C6-C31, n.d – nie określono

Table 3. Colonies diameter (cm) of fungi growing on potato-dextrose agar (PDA) with different concentrations of *S. perfoliatum* extract
Tabela 3. Średnica kolonii (cm) grzybów rosnących na pożywce ziemniaczano-glukozowej (PDA) przy różnych stężeniach ekstraktu *S. perfoliatum*

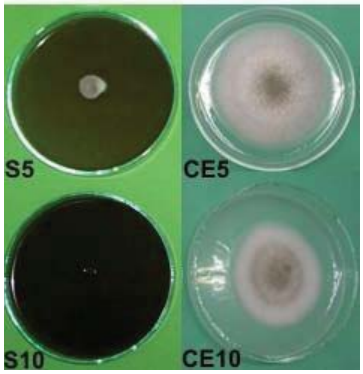
Fungus species Gatunek grzyba	Days dni	Diameter of colony (cm) – Średnica kolonii (cm)							
		CE5		S5		CE10		S10	
		average ± SD średnica ± SD	median mediana	average ± SD średnica ± SD	median mediana	average ± SD średnica ± SD	median mediana	average ± SD średnica ± SD	median mediana
Alternaria alternata	7	7.30 ± 0.29Ad	7.40	0.60 ± 0.55Cl	0.90	4.78 ± 0.59Bf	5.00	0.00 ± 0.00Dd	0.00
	14	9.00 ± 0.00Aa	9.00	1.82 ± 0.26Bjk	1.90	8.40 ± 0.65Ab	8.60	0.74 ± 0.71Ccd	1.00
	21	9.00 ± 0.00Aa	9.00	4.48 ± 1.20Be	4.20	9.00 ± 0.00Aa	9.00	3.64 ± 1.83Bb	3.50
Botrytis cinerea	7	9.00 ± 0.00Aa	9.00	0.56 ± 0.52Cl	0.40	4.60 ± 0.32Bf	4.70	0.00 ± 0.00Dd	0.00
	14	9.00 ± 0.00Aa	9.00	8.16 ± 1.47Ab	9.00	9.00 ± 0.00Aa	9.00	1.88 ± 3.57Bbcd	0.00
	21	9.00 ± 0.00Aa	9.00	9.00 ± 0.00Aa	9.00	9.00 ± 0.00Aa	9.00	3.60 ± 4.93Bb	0.00
Colletotrichum coccodes	7	4.54 ± 0.13Af	4.60	0.40 ± 0.37C	0.60	2.46 ± 0.21Bh	2.40	0.00 ± 0.00Dd	0.00
	14	7.84 ± 0.19Ac	7.90	2.30 ± 0.62Cij	2.10	5.66 ± 0.38Be	5.70	0.24 ± 0.54Dd	0.00
	21	8.44 ± 0.09Ab	8.50	5.84 ± 0.36Bd	6.00	8.38 ± 0.36Ab	8.50	1.56 ± 1.49Cbcd	2.00
Fusarium oxysporum	7	5.78 ± 0.25Ae	5.80	1.12 ± 0.11Ckl	1.10	4.68 ± 0.55Bf	4.40	0.00 ± 0.00Dd	0.00
	14	8.42 ± 0.18Ab	8.40	3.90 ± 0.68Bef	3.80	7.56 ± 0.77Ac	7.00	2.08 ± 0.81Cbcd	2.10
	21	9.00 ± 0.00Aa	9.00	7.20 ± 0.12Bc	7.20	9.00 ± 0.00Aa	9.00	6.70 ± 0.10Ca	6.70
Penicillium expansum	7	4.08 ± 0.28Ag	4.20	1.38 ± 0.08Ck	1.40	3.14 ± 0.32Bg	3.10	0.00 ± 0.00Dd	0.00
	14	5.66 ± 0.42Ae	5.80	2.84 ± 0.15Chi	2.80	4.86 ± 0.21Bf	4.90	1.40 ± 0.20Dbcd	1.40
	21	7.36 ± 0.21Ad	7.40	3.72 ± 0.13Cfg	3.70	7.00 ± 0.00Bd	7.00	3.18 ± 0.19Db	3.10
Trichoderma harzianum	7	9.00 ± 0.00Aa	9.00	3.08 ± 0.42Aa	3.10	9.00 ± 0.00Aa	9.00	0.00 ± 0.00Cd	0.00
	14	9.00 ± 0.00Aa	9.00	8.94 ± 0.13Aa	9.00	9.00 ± 0.00Aa	9.00	2.70 ± 1.83Bbc	3.20
	21	9.00 ± 0.00Aa	9.00	9.00 ± 0.00Aa	9.00	9.00 ± 0.00Aa	9.00	8.00 ± 1.61Aa	9.00

CE5 – control, concentration of solvent 5%; S5 – concentration of extract 5%; CE10 – control, concentration of solvent 10%; S10 – concentration of extract 10%; values designated with the same letters (A, B, C…) within line do not significantly differ at 5% error, values designated with the same letters (a, b, c…) within columns do not significantly differ at 5% error

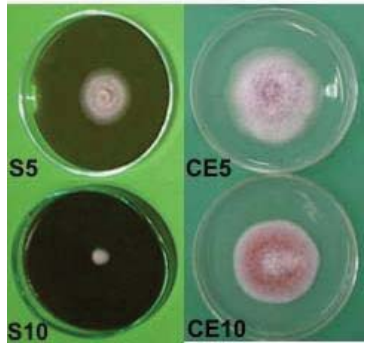
CE5 – kontrola, 5% stężenie rozpuszczalnika; S5 – 5% stężenie ekstraktu; CE10 – kontrola, 10% stężenie rozpuszczalnika; S10 – 10% stężenie ekstraktu; wartości w wierszach oznaczone tą samą literą (A, B, C…) nie różnią się istotnie na poziomie istotności 5%, wartości w kolumnach oznaczone tą sama literą (a, b, c…) nie różnią się istotnie na poziomie istotności 5%

The 10% *S. perfoliatum* extract significantly inhibited the growth of tested fungi species also in comparison to the 5% extract. *T. harzianum* at the 21st growing day was the exception (tab. 3). Action of 10% extract was more prolonged than that of 5% one. After 21 days of experiment, 10% extract the most strongly inhibited *C. coccodes*, *B. cinerea*, *A. alternata*, and *P. expansum* growth (from 54.6% to 81.4%) (tab. 4). The 5% extract strongly inhibited the growth of only *A. alternata* (50.2%), while growth of other tested fungi was inhibited insignificantly or was not inhibited at all after 21 days. *A. alternata* and *C. coccodes* growth was significantly inhibited by tested concentrations of *Silphium* sp. extracts. These fungi are important pepper's pathogens [Jamiołkowska 2009a, b]. The inhibitive effects of *Silphium* extracts on the growth of tested fungi species makes opportunities to use the plant as a material for producing a potential bioagent protecting pepper plants against pathogenic fungi. Species whose growth was inhibited by tested concentrations of *Silphium* sp. extracts to a minimum extent was *T. harzianum* (tab. 3, 4). Fungi from *Trichoderma* genus occur in the soil, on roots, and plant stem's bottom. According to many authors [Ahmed et al. 2000, Pięta and Patkowska 2003, Suarez-Estrella et al. 2007], they are antagonists of plant's pathogens. The lack of inhibiting effects of *Silphium* extracts on *T. harzianum* growth is a satisfactory result. That species is a myco-parasite and its growth should not be reduced, but stimulated.

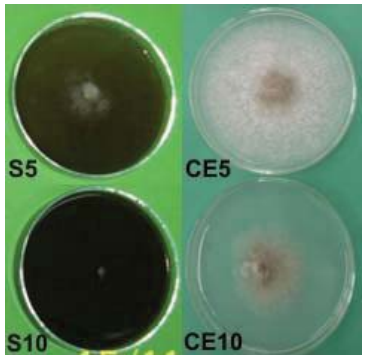
Plants exposed to various pathogens develop a spectrum of protective mechanisms, shape particular morphologic traits, and producing secondary metabolites [Copping 1996]. Synthesis of chemicals of secondary metabolite group is extremely intensified when a pathogen or a parasite invades a plant. These substances may be also useful for a plant itself. Unprocessed plants have been utilized for medicinal purposed by a man for ages. At present, particular substances or whole fractions are isolated from plants and they are used for different purposes, among others for plant protection. Following groups of chemical compounds are important in that respect: amino acid analogues, sugar analogues, cyanogenic glucosides, glucosinolates, furanocoumarins, poliphenolics, volatile terpenes and saponins [Copping 1996]. Analyses of chemical composition of *Silphium* extracts makes possible to distinguish numerous components of above groups. Phenolic and polyphenolic compounds, including phenolic acids, caffeotanoids and flavonoids, triterpenoid glycosides, and volatile terpenes are specific constituents of antibacterial properties. Triterpene saponins present in extracts made of *Silphium*, that probably influenced on fungi development inhibition in present experiment are worth special interests. Positive action of purified saponins fractions from *S. perfoliatum* towards other phytopathogenic fungi was earlier described [Davidjanc et al. 1997]. Physiological functions of triterpenoid saponins in plants as resistance factors against pathogenic fungi, nematodes, molluscs and insects are now well-documented. Moreover, there is evidence that saponins may play a specific role in the interrelations between various plant species, i.e. to act as allelopathic substances [Kalinowska et al. 2005]. There is currently considerable interest in bioengineering commercially important plant species for better resistance against pathogens and herbivores or for improved production of commercially valuable plant metabolites, such as pharmaceuticals or raw materials for industrial purposes. In this context, triterpenoid glycosides are one of the most interesting groups of secondary plant products. In some cases an increased ability



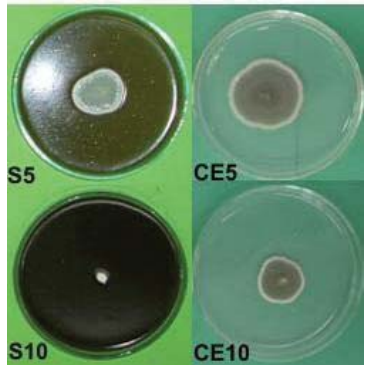
Alternaria alternata



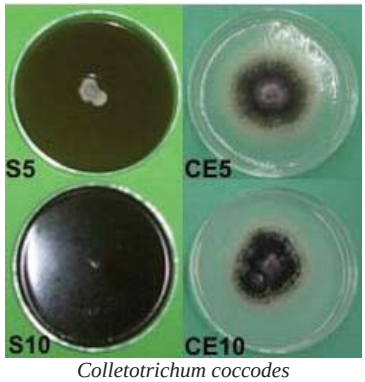
Fusarium oxysporum



Botrytis cinerea



Penicillinum expansum



Colletotrichum coccodes

Trichoderma harzianum

Fig. 1. Effect of leaves extract from *S. perfoliatum* on the growth of fungi on potato-dextrose agar after 14 days 9 (photo by M. Kopacki). S5 – concentration of extract 5%; S10 – concentration of extract 10%; CE5 – control, concentration of solvent 5%; CE10 – control, concentration of solvent 10%

Rys. 1. Wpływ ekstraktu z *S. perfoliatum* na wzrost grzybów na pożywce ziemniaczano-glukozowej po 14 dniach (photo by M. Kopacki). S5 – 5% stężenie ekstraktu; S10 – 10% stężenie ekstraktu, CE5 – kontrola, 5% stężenie rozpuszczalnika; CE10 – kontrola, 10% stężenie rozpuszczalnika

Table 4. Index of growth inhibition (%) of fungus colony on potato-dextrose agar (PDA) with different concentrations of *S. perfoliatum* extract after 7, 14, 21 days

Tabela 4. Indeks hamowania wzrostu (%) kolonii grzybów rosnących na pożywce ziemniaczano-glukozowej (PDA) przy różnym stężeniu ekstraktu *S. perfoliatum* po 7, 14, 21 dniach

Fungus species Gatunek grzyba	Percentage of growth inhibition (%) Procent hamowania wzrostu (%)								
	CE5, CE10			S5			S10		
	7 days dni	14 days dni	21 days dni	7 days dni	14 days dni	21 days dni	7 days dni	14 days dni	21 days dni
Alternaria alternata	0.0	0.0	0.0	91.8	79.8	50.2	100.0	91.2	59.6
Botrytis cinerea	0.0	0.0	0.0	93.8	9.3	0.0	100.0	79.1	60.0
Colletotrichum coccodes	0.0	0.0	0.0	91.2	70.7	30.8	100.0	95.7	81.4
Fusarium oxysporum	0.0	0.0	0.0	80.6	53.7	20.0	100.0	72.5	25.5
Penicillium expansum	0.0	0.0	0.0	66.2	49.8	49.4	100.0	71.2	54.6
Trichoderma harzianum	0.0	0.0	0.0	65.5	0.7	0.0	100.0	70.0	11.1

CE5 – control, concentration of solvent 5%; CE10 – control, concentration of solvent 10%; S5 – concentration of extract 5%; S10 – concentration of extract 10%

CE5 – kontrola, 5% stężenie rozpuszczalnika; CE10 – kontrola, 10% stężenie rozpuszczalnika; S5 – 5% stężenie ekstraktu; S10 – 10% stężenie ekstraktu

for the synthesis of a particular glycoside or glycosides may be desirable, e.g. for medicinal use, drug production or increased resistance against pathogens. On the other hand, in plants containing triterpenoid glycosides with toxic or anti-nutritional properties, a reduction of the synthesis of these compounds may be beneficial, at least in these plant parts which are used for food production or as feeding stuffs for domestic animals [Copping 1996]. Referring to remaining compounds, components of the volatile fraction that can be determined by means of GC technique and contained in essential oils should be also mentioned. A large number of reports upon essential oils confirm antifungal properties of these secondary metabolites [Lahlou 2004].

Summing up, it can be concluded that ethanol extract made of *S. perfoliatum* leaves could be applied in practice for protecting pepper and other plants plantations infected by fungal pathogens such as *A. alternata*, *B. cinerea*, *C. coccodes*, and *F. oxysporum*, being an alternative for chemical protection that is not permissible in organic farms. Moreover, it was observed that *S. perfoliatum* extract did not significantly inhibit the growth of antagonistic fungi such as *P. expansum* and *T. harzianum.* Presence of this fungi in a community is required, because they secrete antibiotics reducing growth of pathogenic fungi, colonize the environment, and directly parazite on pathogenic fungi [Papavizas 1985]. Here achieved results make to conduct further studies for extracts achieved from other *Silphium* species in a wider spectrum of fungal and bacterial pathogens taking into account the field conditions, which is going to be the goals of future research.

CONCLUSIONS

1. Extract of *Silphium perfoliatum* leaves was effective in inhibition the growth of tested fungi.

2. *A. alternata* and *C. coccodes* were fungi, growth of which was the most strongly inhibited by tested concentrations of *Silphium* extracts.

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OCENA WPŁYWU EKSTRAKTU Z LIŚCI *Silphium perfoliatum* L. W WARUNKACH *in vitro*, NA NIEKTÓRE GRZYBY ZASIEDLAJĄCE ROŚLINY PAPRYKI

Streszczenie. Ochrona biologiczna jest nowoczesną, bezpieczną i niezagrażającą środowisku formą walki z chorobami roślin uprawnych. Badania prowadzone przy użyciu naturalnych substancji pochodzenia roślinnego, takich jak czosnek, mięta, tymianek, grejpfrut, stosowanych w ochronie roślin przed patogenami są bardzo obiecujące. Celem badań była laboratoryjna ocena wpływu ekstraktu etanolowego z liści *Silphium perfoliatum* na niektóre grzyby występujące na papryce. W doświadczeniu użyto izolaty *Alternaria alternata, Botrytis cinerea, Colletotrichum coccodes, Fusarium oxysporum, Penicillium expansum, Trichoderma harzianum* wyosobnione z roślin papryki. Ekstrakt z *Silphium* stosowano w stężeniach 5% i 10%. Liście pochodziły z trzyletniej plantacji *S. perfoliatum*. Badane izolaty grzybów uzyskano z korzeni i części nadziemnej papryki uprawianej w polu. W badaniach zastosowano metodę szalkową zalecaną do testowania fungicydów w warunkach laboratoryjnych. Badane ekstrakty *Silphium* istotnie ograniczały wzrost testowanych gatunków grzybów względem kontroli, z wyjątkiem *T. harzianum* i *B. cinerea* przy 5% stężeniu ekstraktu. Działanie 10% stężenia ekstraktu było dłuższe niż 5%. *A. alternata* i *C. coccodes* to grzyby wobec, których odnotowano najsilniejszy efekt hamujący przez badane stężenia *Silphium*.

Słowa kluczowe: aktywność antygrzybowa, *A. alternata, C. coccodes*, ochrona biologiczna

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