THE EFFECT OF CANADIAN GOLDENROD (*Solidago canadensis* L.) AQUEOUS EXTRACT ON THE GROWTH OF PHYTOPATHOGENIC FUNGI

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Original scientific paper
Received: September 26th, 2025
Accepted: October 18th, 2025
HAE-2574
https://doi.org/10.33765/thate.16.3.3

ABSTRACT

The phytopathogenic fungi *Sclerotinia sclerotiorum* and *Botrytis cinerea* pose a significant problem in global agriculture, as they cause substantial yield losses and economic damage. Their threat arises from a broad host range, the ability to survive in soil for long periods, and the emergence of new strains with increased resistance to fungicides. They are most commonly controlled through the application of chemical fungicides; however, their use is associated with negative environmental impacts, the development of resistance, and potential risks to human health. This study aimed to evaluate the antifungal activity of aqueous extracts from leaves and flowers of Canadian goldenrod (*Solidago canadensis* L.) against the growth of these fungi. *In vitro* tests were conducted at concentrations of 75 and 150 mg/mL, and the effectiveness of the extracts was compared with that of commercial fungicides. The highest growth inhibition was observed with the flower extract at the higher concentration (150 mg/mL). In *S. sclerotiorum*, the leaf extract (150 mg/mL) significantly reduced sclerotia formation (91 %). In *B. cinerea*, no sclerotia were formed during the observation period; however, the flower extract (150 mg/mL) was the most effective in inhibiting mycelial growth. The results confirm the antifungal potential of *S. canadensis* extracts in inhibiting mycelial growth and sclerotia formation in *S. sclerotiorum*. However, their effectiveness is still considerably weaker compared to commercial fungicides, suggesting that, for now, they can primarily be considered a supplementary measure or a basis for further development of more effective bioformulations.

Keywords: *plant extracts, S. sclerotiorum, B. cinerea, antifungal activity, inhibition*

INTRODUCTION

Reduction of available arable land and population growth raise the need for sustainable agriculture and effective plant protection methods. Although chemical fungicides remain the primary means of controlling pathogens, their long-term and intensive use has led to soil degradation, environmental pollution, and the development

of resistance in numerous phytopathogens. Consequently, interest is growing in alternative, environmentally friendly strategies that can ensure stable yields without negative effects on the environment and health. Plant diseases still cause significant damage, and in some cases, losses reach up to one-third of total production [1, 2].

Hazardous pathogens belong to the genera *Botryotinia* and *Sclerotinia*. Their species have a broad host range and cause significant losses in agriculture. *Sclerotinia sclerotiorum* (Lib.) de Bary, a pronounced polyphagous species from the family *Sclerotiniaceae*, can cause losses of up to 50 % of total yield and affect crop quality [3]. The genus *Botryotinia* comprises about 22 species. Its anamorphic form is the genus *Botrytis*, with the best-known species being *B. cinerea* Pers., a parasite on more than 230 plant species, including grapevine, strawberry, and tomato [4, 5]. Their harmfulness is further increased by their ability to form sclerotia, resistant structures that remain viable in the soil for years [6 - 8]. Both pathogens produce oxalic acid, which degrades the host's cell walls and facilitates infection [9]. Control is further complicated by their ability to develop resistance to fungicides rapidly. This leads to reduced effectiveness of protection and higher production costs [10, 11].

Due to these limitations, alternative protection methods are increasingly being investigated, including biological control and the application of plant extracts [12]. They contain bioactive compounds that inhibit the growth and development of phytopathogenic fungi [13, 14] and are more and more recognized as a sustainable alternative to chemical fungicides.

Solidago canadensis L., known as Canadian goldenrod, is a perennial plant from the *Asteraceae* family native to North America. It was introduced to Europe in the 19th century as an ornamental and beekeeping species, but it quickly became naturalized and is now widely distributed in Europe and Asia, where it is considered an invasive species that suppresses native flora and alters ecosystems [15, 16]. The presence of *S. canadensis* near arable land may also have unfavourable consequences, as it serves as a host for aphids that transmit plant viruses [15]. In addition to its negative environmental impact, this species has attracted attention due to its rich phytochemical composition. Complexes isolated from its flowers contain flavonoids, phenolic acids, polysaccharides, and proteins, and show biological activity in pharmacological tests [17]. These groups of compounds are known for their antimicrobial and antifungal properties, which further justifies the interest in investigating the antifungal potential of *S. canadensis* extracts. Therefore, this species is increasingly perceived as both a threat to biodiversity and a potential resource for the development of natural plant protection products [16].

Previous studies confirm the potential of *S. canadensis* plant biomass as an alternative measure in plant protection. Extracts and essential oils of numerous plants, including *S. canadensis*, have demonstrated significant inhibition of *S. sclerotiorum* and *B. cinerea* growth, particularly at higher concentrations and with extended incubation periods [18 - 21].

In view of the above, this study aimed to evaluate the effect of aqueous extracts of *S. canadensis* on the growth of the pathogens *S. sclerotiorum* and *B. cinerea*. Special emphasis was placed on the inhibitory potential of the extracts and their comparison with the effectiveness of commercial fungicides.

MATERIALS AND METHODS

The study of the antifungal effect of leaf and flower extracts of *S. canadensis* was conducted at the Central Agrobiotechnical Analytical Unit of the Faculty of Agrobiotechnical Sciences Osijek (Croatia), in the Laboratory of Phytopathology. Isolates of pure cultures of the pathogens *S. sclerotiorum* and *B. cinerea* were selected from the collection of isolates of the Department of Phytopathology.

Preparation of plant extracts

Leaves and flowers of *S. canadensis* were collected during the flowering stage in the summer of 2022, dried at 40 °C, and ground. For extract preparation, 15 g of dry material was combined with 100 mL of distilled water. After 24 hours of extraction, the samples were filtered and prepared at concentrations of 75 mg/mL and 150 mg/mL.

Experimental setup

Pathogen cultures were grown on potato dextrose agar (PDA) medium at 22 ± 2 °C under a 12 h light/12 h dark cycle. Seven-day-old cultures were used for inoculation. The study included six treatments in four replicates:

1. Leaf extract 75 mg/mL
2. Leaf extract 150 mg/mL
3. Flower extract 75 mg/mL
4. Flower extract 150 mg/mL
5. Negative control (PDA without treatment)
6. Positive control (commercial fungicides: pyrimethanil 0.2 % for *B. cinerea* and fenhexamid 0.075 % for *S. sclerotiorum*).

Each pathogen (*B. cinerea* and *S. sclerotiorum*) was tested separately, with six treatments and four replicates.

One millilitre of plant extract was applied to the surface of the PDA medium and evenly spread with a sterile rod. After the medium had dried, a 4 mm disc of actively growing pathogen mycelium was placed in the centre of the Petri dish. The plates were incubated at 25 °C.

Evaluation of antifungal efficacy

The diameter of mycelial growth was measured for 48, 96, and 120 hours after inoculation in two perpendicular directions. The percentage of inhibition was calculated according to the formula [22]:

$$I\,(\%) = \frac{C - T}{C - 4} \times 100 \qquad (1)$$

where: C is mycelial growth diameter in the control (mm), and T is mycelial growth diameter in the treatment (including the initial 4 mm inoculum disc).

The number of sclerotia was recorded on the final day of measurement (144 hours after inoculation), and the results were expressed as the percentage of sclerotia inhibition per treatment. The percentage of sclerotia formation inhibition was calculated according to Goussous et al. [23]:

$$\%\ \text{Inhibition} = \frac{C - T}{C} \times 100 \qquad (2)$$

where: C is average number of sclerotia in the negative control (without treatment), and T is average number of sclerotia in the treatment.

Statistical analysis of data

Statistical analysis was performed using SAS Software 9.1.4 (SAS Institute Inc., 2003). The results were analysed by one-way analysis of variance (ANOVA) to determine significant differences among treatments. Mean inhibition values were expressed as arithmetic means (AM) ± standard error of the mean (SE), based on four replications (n = 4). Duncan's multiple range test was used to compare the means at a significance level of $p \leq 0.05$. Mean values sharing the same letter within the same column were not significantly different.

RESULTS AND DISCUSSION

The effects of aqueous extracts obtained from the leaves and flowers of *S. canadensis* on the inhibition of mycelial growth of phytopathogenic fungi were monitored after 48, 96, and 144 hours of incubation. The results are presented as the percentage of mycelial growth inhibition. The data indicate that *S. canadensis* extracts, particularly at higher concentrations, exhibit a specific

antifungal effect, although it is weaker compared to the commercial fungicide. As expected, the highest inhibition was achieved with the fungicide, which almost completely suppressed mycelial growth throughout the experiment.

Effect of aqueous extract of *S. canadensis* on the inhibition of *S. sclerotiorum* mycelium

In the case of *S. sclerotiorum*, clear differences in the effectiveness of aqueous extracts of *S. canadensis* were observed depending on the plant part and concentration (Table 1). The best results were obtained with extracts at the higher concentration (150 mg/mL), while treatments at the lower concentration (75 mg/mL) showed weak effects. After 48 hours of incubation, the most effective treatment was the fungicide, with complete inhibition (100 %). Among the plant treatments, the leaf extract at 150 mg/mL had the strongest effect (37.5 %), while the flower extract was somewhat weaker (24.6 %). Extracts at the lower concentration (75 mg/mL) exhibited very weak antifungal potential (< 10 %) and did not differ statistically from the negative control.

After 96 hours, the fungicide still maintained high inhibition (94.4 %), which was significantly different from all plant treatments ($p \leq 0.05$). Flower (43.8 %) and leaf (38.6 %) extracts at the higher concentration (150 mg/mL) showed significantly stronger effects compared to the lower concentrations, although they did not differ from each other. At the lower concentration (75 mg/mL), the flower extract (10.7 %) was more effective than the leaf extract (3.0 %), with only the leaf extract at this concentration not statistically different from the negative control (Figure 1).

After 144 hours of incubation, the leaf extract at 75 mg/mL showed no antifungal effect and did not differ statistically from the negative control. In contrast, the flower extract at the same concentration achieved 9.7 % growth inhibition, which was statistically significantly higher than that of the leaf extract. At the higher concentration (150 mg/mL), both plant extracts exhibited a more pronounced antifungal effect - growth inhibition was 38.4 % for the flower and 29.8 % for the leaf, with the flower being statistically significantly more effective. The highest growth inhibition was obtained with the commercial fungicide (91.9 %), which differed significantly from all other treatments.

Table 1. Effect of *S. canadensis* aqueous extracts on mycelial growth inhibition (%) of *S. sclerotiorum* at different incubation periods (h)

Aqueous extract	**Concentration (mg/mL)**	**Inhibition (%) (Mean ± SE)**		
		48 h	96 h	144 h
***S. canadensis* L. (leaf)**	75	7.83 ± 2.55 d	3.01 ± 0.35 d	0.00 ± 0.00 e
	150	37.47 ± 1.89 b	38.55 ± 9.53 b	29.80 ± 9.27 c
***S. canadensis* L. (flower)**	75	8.27 ± 2.54 d	10.69 ± 2.34 c	9.74 ± 1.63 d
	150	24.57 ± 4.39 c	43.82 ± 3.42 b	38.37 ± 5.75 b
Positive control (fungicide)	0.075	100.00 ± 0.00 a	94.38 ± 0.67 a	91.86 ± 0.54 a
Negative control (PDA)	-	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 e

* *Values within the column represent mean inhibition (%) values calculated from four replicates (n = 4) ± standard error of the mean (SE). Results were analysed by analysis of variance (ANOVA). Mean values with different letters within the same column are statistically significantly different according to Duncan's multiple range test ($p \leq 0.05$).*

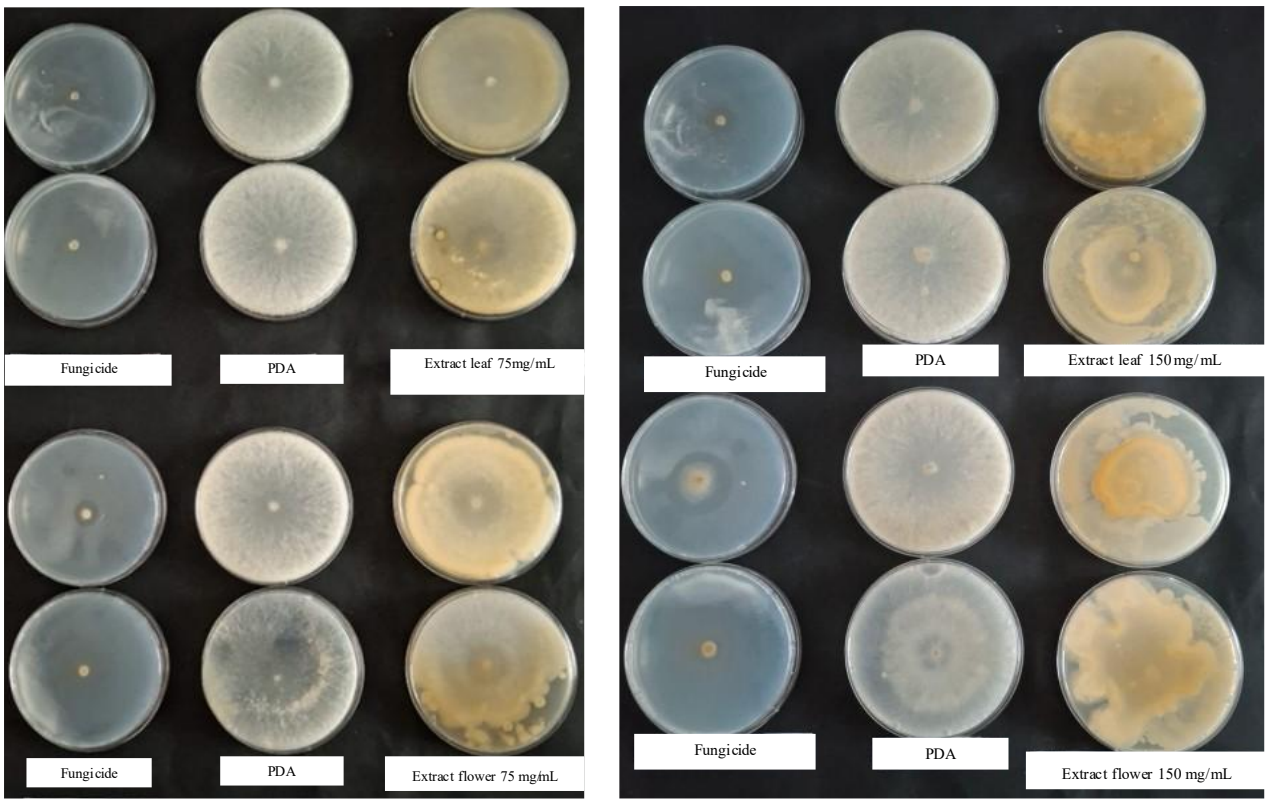

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Figure 1. Effect of aqueous extracts at concentrations: a) 75 g/100 mL, b) 150 g/100 mL on *S. sclerotiorum* 96 h after inoculation (Source: Doboš M.)

The obtained results are consistent with previous studies, which confirm that plant extracts exhibit stronger antifungal effects at higher concentrations. Plant extracts and phenolic compounds may have pronounced antimicrobial activity, with hydroxytyrosol highlighted as a key component with potential antibacterial and antifungal effects [24]. Garlic husk extract has also been found to exhibit strong antifungal activity and reduce the risk of resistance development in pathogens [25]. Similarly, stronger inhibition of *S. sclerotiorum* and other pathogens has been observed at higher extract concentrations [20, 26]. The obtained results are further supported by reports of stronger effects of plant preparations at higher concentrations [27, 28]. Extracts such as *Calotropis procera* (Aiton) W.T.Aiton and *Eucalyptus globulus* Labill. can also significantly reduce the mycelial growth of *S. sclerotiorum*, although complete inhibition was not achieved [29].

To provide a more straightforward overview of temporal changes and differences among treatments, the results are presented as a heat map (Figure 2). A gradual decrease in inhibition was observed up to 144 hours, which may be attributed to the evaporation of volatile active compounds, their chemical degradation during incubation, or the pathogen's possible adaptation to the presence of natural antifungal substances. The results indicate statistically significant changes over time within individual treatments. Duncan's test revealed that the differences were most pronounced between the initial (48 h) and later incubation phases (96 and 144 h). The flower extract at the higher concentration stood out in particular, as its effect remained relatively stable throughout the entire period. In contrast, the leaf extract at the same concentration showed a more pronounced decline in effectiveness. In contrast to the plant extracts, the fungicide maintained high inhibitory activity throughout the entire observation period.

A similar trend was observed with bergamot essential oil (*Citrus bergamia* Risso), where

antifungal efficacy decreased gradually over a six-day period [30]. The authors attributed the decline in efficacy to the evaporation and chemical degradation of volatile compounds, since the inhibitory effect of the oil against *Aspergillus niger* Tiegh. and *Penicillium expansum* Link continuously decreases over time.

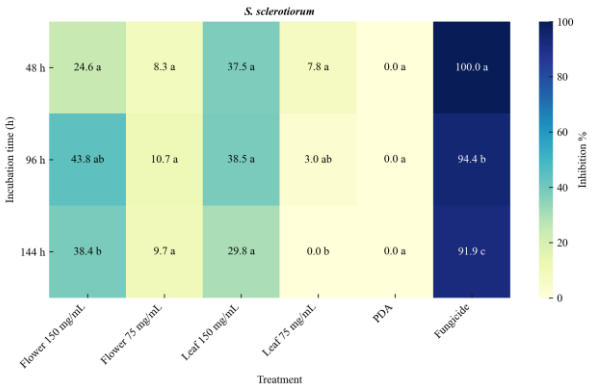

Figure 2. Inhibition (%) of *S. sclerotiorum* under the influence of aqueous extracts of *S. canadensis* (150 and 75 mg/mL). Different letters indicate significant differences during incubation time within the same treatment, as determined by Duncan's multiple range test ($p < 0.05$)

Effect of aqueous extract of *S. canadensis* on the inhibition of *B. cinerea* mycelium

In this part of the study, the effect of aqueous extracts from the leaves and flowers of *S. canadensis* on the inhibition of *B. cinerea* mycelial growth was analysed. Inhibition was monitored at three time points (48, 96, and 144 hours of incubation), and the results are presented in Table 2. After 48 hours of incubation, all plant extracts showed a low level of inhibition of *B. cinerea* mycelial growth, with values below 20 %. The highest inhibition among the plant treatments was achieved by the flower extract at 75 mg/mL (14.9 %), which was statistically significantly different from the negative control. Other extracts had lower values (6 - 10 %), with no significant differences between the leaf extract concentrations. After 96 hours, the highest inhibition was recorded with the flower extract at 150 mg/mL (49.6 %), which differed significantly from all other treatments. Leaf extracts (35.6 - 36.7 %) and the flower extract at the lower concentration (34.0 %) showed weaker effects and did not differ significantly from each other (Figure 3). After 144 hours, the effects again varied among treatments. The weakest result was observed with the leaf extract at 75 mg/mL (14.8 %), which did not differ from the negative control. The flower extract at 150 mg/mL achieved the highest inhibition (43.3 %), significantly greater than that of the leaf (29.2 %) and the flower at the lower concentration (25.0 %). The fungicide maintained almost complete inhibition of growth throughout the entire period (97.9 - 100 %), significantly higher than all plant treatments.

Table 2. Effect of *S. canadensis* aqueous extracts on mycelial growth inhibition (%) of *B. cinerea* at different incubation periods

Aqueous extract	**Concentration (mg/mL)**	**Inhibition (%) (Mean ± SE)**		
		48 h	96 h	144 h
***S. canadensis* L. (leaf)**	75	10.14 ± 4.86 b	35.57 ± 2.20 c	14.83 ± 8.71 d
	150	6.24 ± 5.84 bc	36.71 ± 1.60 c	29.22 ± 5.49 c
***S. canadensis* L. (flower)**	75	14.87 ± 6.62 b	33.96 ± 5.52 c	25.00 ± 6.36 cd
	150	8.73 ± 7.48 bc	49.62 ± 5.84 b	43.31 ± 5.39 b
Positive control (fungicide)	0.2	100.00 ± 0.00 a	98.36 ± 0.40 a	97.93 ± 0.87 a
Negative control (PDA)	-	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 e

** Values within the column represent mean inhibition (%) values calculated from four replicates (n = 4) ± standard error of the mean (SE). Results were analysed using analysis of variance (ANOVA). Mean values with different letters within the same column are statistically significantly different according to Duncan's multiple range test (p ≤ 0.05).*

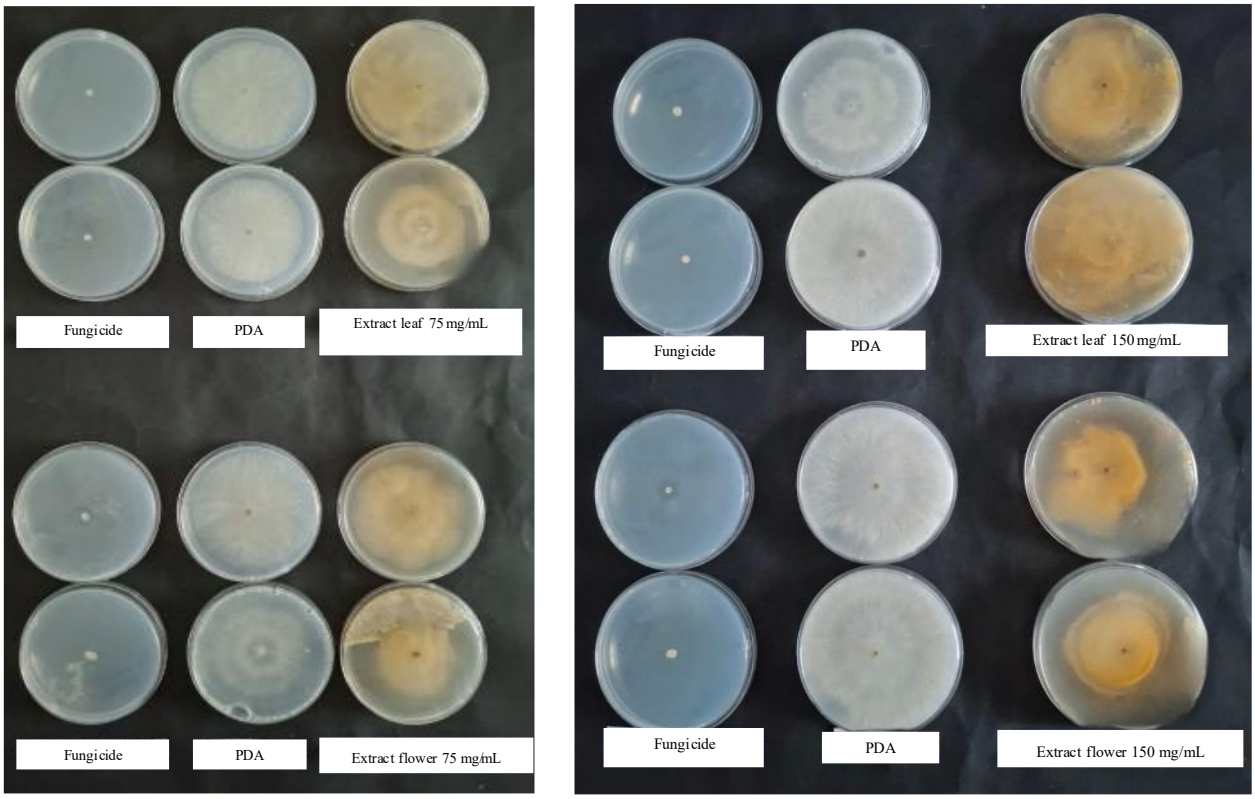

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Figure 3. Effect of aqueous extracts at concentrations: a) 75 g/100 mL, b) 150 g/100 mL on *B. cinerea* 96 h after inoculation (Source: Doboš M.)

The obtained results partially align with previous findings, which reported a low effect of plant extracts after 48 hours of incubation, followed by a gradual increase in later phases [19]. A similar trend was observed in this study, where the differences between leaf and flower extracts became more pronounced only after 96 and 144 hours. In contrast, no significant antifungal activity of plant extracts was observed even at very high concentrations (500 - 1000 µL/mL) [18]. However, they did report disruption of fungal cell membrane integrity. Their results partially correspond to the results of this study, as *S. canadensis* extracts at lower concentrations also did not show strong antifungal activity. On the other hand, it has been demonstrated that increased concentration had a positive effect on the antifungal activity of essential oils, which is consistent with observations in this study - the higher concentration of the flower extract (150 mg/mL) was the most effective against both pathogens [31]. The more substantial antifungal effect of the flower compared to the leaf is also supported by findings reporting a significantly higher proportion of flavonoids in flower extracts (76 % of total flavonoids) than in leaves. Flavonols, such as quercetin and kaempferol, have shown antifungal activity, which may explain the greater effectiveness of *S. canadensis* flowers [32].

The obtained results are also in line with previous findings, where the highest inhibition of *B. cinerea* was achieved by the positive control with the fungicide (fenhexamid), while aqueous extracts showed weaker effects depending on the plant species [10]. This confirms the limited but existing antifungal activity of plant preparations, as well as the need for further research into their potential applications.

Changes in the antifungal effect of the extracts were also observed over the incubation period (Figure 4). After 48 hours, all plant extracts showed a low level of inhibition, below 15 %, while the fungicide completely suppressed mycelial growth. After 96 hours, the effectiveness of the extracts increased, with

the most pronounced effect achieved by the flower extract at 150 mg/mL (49.6 %), which differed significantly from the other treatments. By 144 hours, a decline in the effectiveness of all extracts was recorded, most notably for the leaf extract at 75 mg/mL (14.8 %). However, the flower extract at the higher concentration maintained the strongest antifungal potential (43.3 %). In contrast to the plant extracts, the fungicide maintained almost complete inhibition of mycelial growth (98 - 100 %) throughout the entire experiment, with no significant fluctuations.

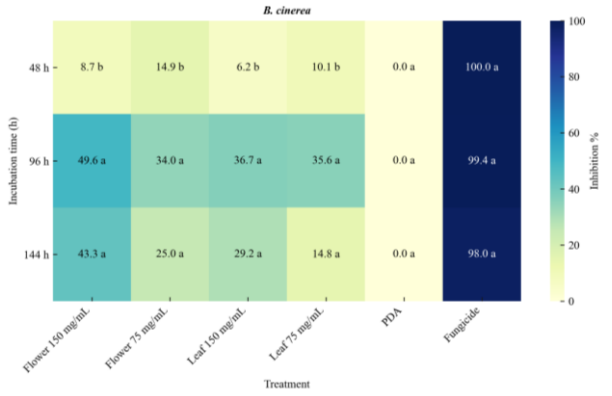

Figure 4. Inhibition (%) of *B. cinerea* under the influence of aqueous extracts of *S. canadensis* (150 and 75 mg/mL). Different letters indicate significant differences during incubation time within the same treatment, as determined by Duncan's multiple range test ($p < 0.05$)

Effect of *S. canadensis* L. aqueous extracts on sclerotia formation

The obtained results show that leaf and flower extracts of *S. canadensis* significantly affect the number of sclerotia produced by *S. sclerotiorum*, with the effectiveness being strongly dependent on the concentration. The lowest number of sclerotia was recorded with the leaf extract at 150 mg/mL (2.75), while lower concentrations, particularly of the flower extract, showed weaker effects.

Inhibition rates confirmed these trends: the highest inhibition was achieved with the leaf extract at 150 mg/mL (91 %), while lower concentrations were considerably less effective (53 % for the leaf and 44 % for the flower). Increasing the concentration significantly enhanced the antifungal effect, especially in the flower extract, where the higher concentration (150 mg/mL) resulted in 88 % inhibition (Figure 5).

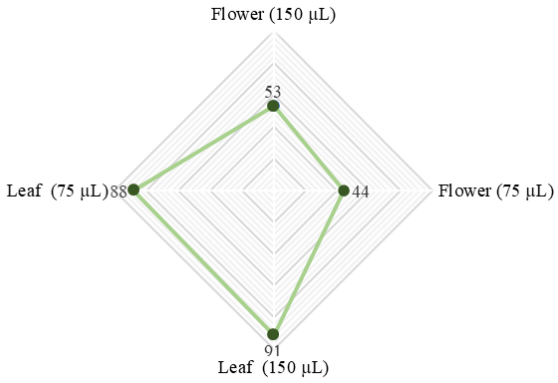

Figure 5. Inhibition of *S. sclerotiorum* sclerotia formation after treatment with aqueous extracts of *S. canadensis*

Similar values have been reported in other studies on biological preparations against *S. sclerotiorum*. For example, sage (*Salvia officinalis* L.) essential oil at 15 µL reduced the number of sclerotia to 3.75 after five days of incubation, which is comparable to the results of this study for the flower extract at 150 mg/mL [33]. The same author noted that treatment with clove (*Syzygium aromaticum* L.) resulted in 18.5 sclerotia after eight days, corresponding to the results of this study for the flower extract at 75 mg/mL. Rosemary extract at 2.5 mg/mL was found to reduce the number of sclerotia to 1 - 1.4, while higher concentrations completely prevented their formation [23]. Changes in the morphology of *B. cinerea* mycelia and conidia after treatment with plant extracts have also been reported, indicating that the effect depends not only on growth inhibition but also on the disruption of reproductive structure development [34]. Similarly, in this study, the application of the leaf extract (150 mg/mL) reduced the number of sclerotia to only 2.75. These results confirm that increasing the concentration of plant preparations can significantly enhance antifungal efficacy and nearly completely suppress sclerotia formation.

In the case of *B. cinerea*, no sclerotia were recorded, which was expected given the duration of the experiment (144 h). It has been reported that sclerotia formation occurs between the 12th and 15th day of incubation [35], although other findings suggest their appearance as early as the 10th day [36]. Since final measurement in this study was conducted after 144 hours of incubation, the absence of sclerotia was anticipated.

CONCLUSION

This study demonstrated that leaf and flower extracts of *S. canadensis* possess antifungal properties against the pathogens *S. sclerotiorum* and *B. cinerea*. The highest effectiveness was observed with the flower extract at 150 mg/mL, particularly in suppressing *B. cinerea*, while weaker effects were recorded at lower concentrations and with leaf extracts. In the case of *S. sclerotiorum*, the extracts were more effective in the earlier stages of incubation. On the other hand, the strongest inhibition of sclerotia formation was achieved with the leaf extract at a concentration of 150 mg/mL (91 %). At the same time, the flower extract at the same concentration also showed high efficacy. Lower concentrations of both leaf and flower extracts, however, were more prone to sclerotia formation.

The antimicrobial properties of this invasive species have not been disputed so far, although their effectiveness may vary depending on the plant part used and the type of solvent. The results of this study further confirm the potential of *S. canadensis* as a source of bioactive compounds for the development of alternative plant protection measures, while also highlighting the need for further *in vivo* research to validate its effectiveness under agroecological conditions.

In conclusion, although *S. canadensis* extracts cannot fully replace commercial fungicides, their selective antifungal activity, especially at higher concentrations, suggests the potential of this invasive species as a sustainable and natural alternative in plant protection.

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