

ARTYKUŁ ORYGINALNY

Charakterystyka fitochemiczna i aktywność supresyjna ekstraktu z liści firletki chalcedońskiej (*Silene chalcedonica*) wobec nicienia *Meloidogyne hapla*

Phytochemical characterization and nematode-suppressive activity of *Silene chalcedonica* leaf extract against *Meloidogyne hapla*

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Streszczenie

Celem badań było określenie profilu fitochemicznego ekstraktu z liści firletki chalcedońskiej (*Silene chalcedonica*) oraz ocena jego aktywności supresyjnej wobec osobników drugiego stadium młodocianego (J2s) nicienia *Meloidogyne hapla* w kontrolowanych warunkach komory fitotronowej. Analiza fitochemiczna wykazała obecność 20-hydroksyekdyzonu (20E), którego zawartość wynosiła 0,19 mg/g suchej masy, przy jednocześnie niskiej zawartości polifenoli ogółem oraz niskiej aktywności antyoksydacyjnej. Zastosowany preparat roślinny istotnie ograniczał tworzenie wyrosli korzeniowych oraz liczbę larw zasiedlających tkanki roślin żywicielskich, szczególnie w niższej spośród badanych temperatur, tj. 15°C w porównaniu z 20°C, wskazując na istotny potencjał supresyjny wobec nicieni. Uzyskane wyniki sugerują, że ekstrakty z liści *S. chalcedonica* mogą stanowić obiecujący, przyjazny dla środowiska element strategii zrównoważonego ograniczania populacji guzaków (*Meloidogyne* spp.).

Słowa kluczowe: 20-hydroksyekdyzon, *Silene chalcedonica*, *Meloidogyne hapla*, ograniczanie liczebności nicieni glebowych

Abstract

The aim of this study was to determine the phytochemical profile of *Silene chalcedonica* leaf extract and to evaluate its nematode-suppressive activity against second-stage juveniles (J2s) of *Meloidogyne hapla* under controlled growth chamber conditions. Phytochemical analysis revealed the presence of 20-hydroxyecdysone (20E) at a concentration of 0.19 mg/g dry weight (DW), accompanied by low total phenolic content and low antioxidant activity. The botanical preparation significantly reduced root gall formation and the number of juveniles established within host plant tissues, particularly at the lower tested temperature of 15°C compared with 20°C, indicating significant suppressive activity against nematodes. The obtained results suggest that *S. chalcedonica* leaf extracts may represent a promising, environmentally friendly component of strategies for the sustainable management of root-knot nematodes (*Meloidogyne* spp.).

Keywords: 20-hydroxyecdysone, *Silene chalcedonica*, *Meloidogyne hapla*, nematode suppression

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Wstęp / Introduction

Root-knot nematodes of the genus *Meloidogyne* are considered to be the most economically important plant parasite nematodes in the world due to their wide range of hosts and their strong impact on agricultural production (Swaruparani and Shanmugam 2024). Among them, *Meloidogyne hapla* Chitwood, 1949 is particularly important in temperate climate regions and greenhouse systems where it causes root galling, disrupted nutrient absorption, growth reduction and significant yield losses in horticultural crops (Moens et al. 2009; Collange et al. 2011; Vestergård 2019). Recent studies show that the economic importance of root-knot nematodes is continuing to increase, highlighting the need for a sustainable and environmentally safe management strategy (Swaruparani and Shanmugam 2024).

For many years, control of plant-parasitic nematodes relied mainly on synthetic nematicides. However, the widespread use of chemical nematicides has raised serious environmental and toxicological concerns and has resulted in the restriction or abolition of many active compounds from agricultural practices (Mwamula et al. 2022). As a result, increasing attention is being paid to bioactive compounds of plant origin (POBCs) as an eco-friendly alternative to nematode management (Swaruparani and Shanmugam 2024; Kamaraj et al. 2025). Botanical extracts are considered promising because they are biodegradable, relatively safe for non-target organisms, and rich in bioactive secondary metabolites (Swaruparani and Shanmugam 2024).

Numerous studies have demonstrated that plant secondary metabolites, including phenolic compounds, flavonoids, alkaloids, tannins, terpenoids, and saponins, may exhibit strong nematocidal activity against *Meloidogyne* species (Naz et al. 2013; D'Addabbo et al. 2022; Kamaraj et al. 2025). These compounds may affect nematode survival, mobility, egg hatching, and reproductive potential (Naz et al. 2013). Data specifically concerning *M. hapla* are also available. Lee et al. (2011) reported that extracts from several herbal plants, including *Daphne genkwa*, *Eugenia caryophyllata*, *Quisqualis indica*, and *Zingiber officinale*, showed strong nematocidal activity against second-stage juveniles (J2s) of *M. hapla* and inhibited egg hatching. Essential oils and crude plant extracts obtained from medicinal and aromatic plants were previously reported to significantly reduce the viability of second-stage juveniles (J2s) of *M. hapla* under laboratory conditions (Jeon et al. 2016). Recently, Dobosz et al. (2023) demonstrated that *Vicia sativa* L. seed diffusates impaired the motility of second-stage juveniles (J2s) of *M. hapla* and altered the expression levels of selected *hsp* genes, indicating that plant-derived compounds may affect both nematode behavior and stress-response mechanisms. Furthermore, bioactive phytochemicals can penetrate or disintegrate the nematode's lipid layer and block their nervous system, causing continuous paralysis that leads to eventual death (Keerthiraj et al. 2021).

Silene chalcedonica (L.) E.H.L. Krause, a perennial species belonging to the Caryophyllaceae family, is widely known as an ornamental and medicinal plant (Bilal and Kutluay 2026). Species of the genus *Silene* are recognized as valuable source of biologically active compounds, including phytoecdysteroids, triterpenoid saponins, flavonoids, and phenolic acids (Mamadaliyeva et al. 2014, 2024; Mamedov et al. 2017; Bilal and Kutluay 2026). In *S. chalcedonica*, flavonoids such as isovitexin, neovitexin, and schaftoside have previously been identified (Zibareva et al. 2023), as well as phytoecdysteroids such as 20-hydroxyecdysone and polypodine B (Plotnikov et al. 2017). Previous phytochemical and pharmacological studies have demonstrated that *Silene* species possess various biological activities, including antimicrobial, antioxidant, enzyme inhibitory (e.g. tyrosinase), anti-inflammatory, cytotoxic, and insecticidal effects. In particular, some *Silene* species have been reported to exhibit repellent activity against mosquitoes and have been traditionally used in preparations applied to the hair to kill lice, while phytoecdysteroids present in these plants may function as natural deterrents against phytophagous insects (Mamadaliyeva et al. 2019; Bilal and Kutluay 2026). Despite extensive phytochemical investigations of several *Silene* species, information concerning the nematode-suppressive potential of *S. chalcedonica* remains very limited.

Therefore, the aim of the present preliminary study was to determine the phytochemical profile of *S. chalcedonica* leaf extract and to evaluate its nematode suppression activity against second-stage juveniles (J2s) of *M. hapla* under controlled growth chamber conditions. The obtained results may contribute to the development of environmentally friendly plant-based alternatives for sustainable nematode control. However, their practical use would require further efficacy, safety, and regulatory assessment.

Materiały i metody / Materials and methods

Przygotowanie materiału roślinnego i ekstraktu / Plant material and extract preparation

Four-month-old seedlings of *S. chalcedonica* were obtained from a commercial supplier. The leaves were harvested, frozen, and subsequently lyophilized to ensure the stability of the phytochemical constituents. The extraction was performed using a triple-cycle heat-assisted method to ensure exhaustive recovery of the target compounds. Briefly, 1.0 g of the lyophilized and powdered leaf material was mixed with 30 ml of 40% ethanol (v/v) in four repetitions. The mixture was heated in a water bath at 55°C for 3 hours. After each heating cycle, the extract was filtered through a 0.45 µm polyethersulfone (PES) filter, and the solid plant residue was collected and re-extracted with a fresh 30 ml portion of 40% ethanol under the same conditions. This heating and filtration procedure was repeated three times

in total. The resulting filtrates from all three cycles were combined and subsequently evaporated to dryness using a vacuum concentrator at 50°C. The obtained precipitate was dissolved in Milli-Q water to obtain a final concentration of 100 mg/ml.

Analiza HPLC 20-hidroksyekdyzonu (20E) / HPLC analysis of 20-hydroxyecdysone (20E)

Qualitative and quantitative analysis of 20E in the plant extract was performed using a high-performance liquid chromatography (HPLC) method based on a previously described procedure for ecdysteroid analysis, with minor modifications (Kayser and Eilinger 1999). The analysis was carried out using a Dionex UltiMate 3000 HPLC system equipped with a Dionex RS variable-wavelength UV-Vis detector (Thermo Fisher Scientific, Germering, Germany). Chromatographic separation of 20E was achieved using a Nucleosil C18 column (Dr. Maisch GmbH, Ammerbuch, Germany). The mobile phase consisted of water (solvent A) and methanol (solvent B). The gradient profile was as follows: 39% B from 0 to 2 min, 60% B from 2.01 to 77 min, and 100% B from 77.01 to 87 min. Elution was carried out at a constant flow rate of 1 ml/min, with a sample injection volume of 10 µl. Detection was performed at 245 nm using the variable wavelength UV-Vis detector. The concentration of 20E in the extracts was determined based on a calibration curve generated from 20E standard (PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany).

Oznaczanie całkowitej zawartości fenoli i aktywności antyoksydacyjnej / Determination of total phenolic content and antioxidant activity

Total phenolic content (TPC) was determined using the Phenolic Compounds Assay Kit (Sigma-Aldrich, St. Louis, MO, USA), which utilizes the coupling of phenolic compounds with diazonium salts under alkaline conditions to form a stable diazo chromophore. Absorbance was recorded at 480 nm using a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, Waltham, MA, USA), and results were expressed as catechin equivalents (CE) based on a catechin standard curve. Simultaneously, the total antioxidant capacity (TAC) was evaluated using the Total Antioxidant Capacity Assay Kit (Sigma-Aldrich, St. Louis, MO, USA), based on the reduction of Cu²⁺ ions to Cu⁺ and their subsequent chelation with a colorimetric probe. Absorbance was measured at 570 nm using the same microplate reader, and the values were calculated as Trolox equivalents (TE) using a Trolox standard curve.

Biotest supresji nicieni przeciwko *M. hapla* / Nematode suppression bioassay against *M. hapla*

The evaluation of the nematode suppression of the botanical preparation against the northern root-knot nematode, *M. hapla* was conducted at the Institute of Plant Protection

– National Research Institute in Poznań, Poland. The nematode population was maintained on lettuce (*Lactuca sativa* L. cv. Attraktion) in phytotron chambers under controlled conditions of 20/18°C day/night temperature, 65% relative humidity, and a 16/8 h day/night photoperiod. Plants were grown in KRONENR soil substrate mixed with gravel at a 1 : 1 ratio and watered with a complete nutrient solution containing (mg/l): 226 N, 55 P, 370 K, 180 Ca, 40 Mg, 45 S, 17 Na, 52 Cl, 2.5 Fe, 0.6 Mn, 0.35 B, 0.3 Zn, 0.15 Cu, and 0.05 Mo (Florovit). Infective second-stage juveniles (J2s) were obtained by manual extraction of egg masses and subsequent incubation in distilled water at 20°C, only individuals hatched within the last 48 hours were utilized. Lettuce seedlings at the 3–4 true leaf stage, corresponding to BBCH 13–14 according to the BBCH scale for leafy vegetables, were planted in 0.5 l pots containing a soil-grit mixture (1 : 1 ratio). Before transplanting, the substrate was thoroughly mixed with plant extract at three dosages: 6.25 l/ha (Dose 1), 0.625 l/ha (Dose 2), and 0.065 l/ha (Dose 3). Oxamyl, a systemic carbamate pesticide commonly used as a nematicide, was applied at 10 kg/ha and served as the positive control (K-N), while untreated soil served as the negative control (K). Substrates were pre-incubated for 48 hours at 15°C and 20°C. Each seedling was inoculated with 120 J2s and maintained for six weeks in phytotron chambers at the respective experimental temperatures of 15°C and 20°C. Each treatment combination was performed in four replicates. Root systems were evaluated for the number of root galls per plant using an Olympus SZ-6045 stereomicroscope (Olympus, Tokyo, Japan) at 4× magnification. Subsequently, root tissues were stained with acid fuchsin in lactoglycerine according to Hooper (1986), and the number of J2s established within the tissues was counted using the same stereomicroscope. Data were analyzed using ANOVA and Fisher's LSD test at $p < 0.05$.

Wyniki i dyskusja / Results and discussion

The extraction process yielded 2.9 g of dry extract from 8.08 g of lyophilized *S. chalconica* leaves. HPLC analysis revealed that the concentration of 20E was 0.53 mg per gram of the dry extract. Taking into account the extraction yield, the final content of 20E in the plant material was determined to be 0.19 mg/g of dry weight (DW). The total phenolic content (TPC) of the *S. chalconica* leaf extract was determined spectrophotometrically using a catechin calibration curve ($y = 0.10025x - 0.1475$, $R^2 > 0.99$). For the quantification an aliquot of 30 µl of the reconstituted extract was analysed, yielding a concentration of 0.1862 nmol/µl (mM) of catechin equivalents. After converting molar values to mass and accounting for the initial plant biomass weight, the final total phenolic content in the dried leaves was determined to be 0.096 mg CE/g of dry weight (DW). The total antioxidant capacity (TAC) of the

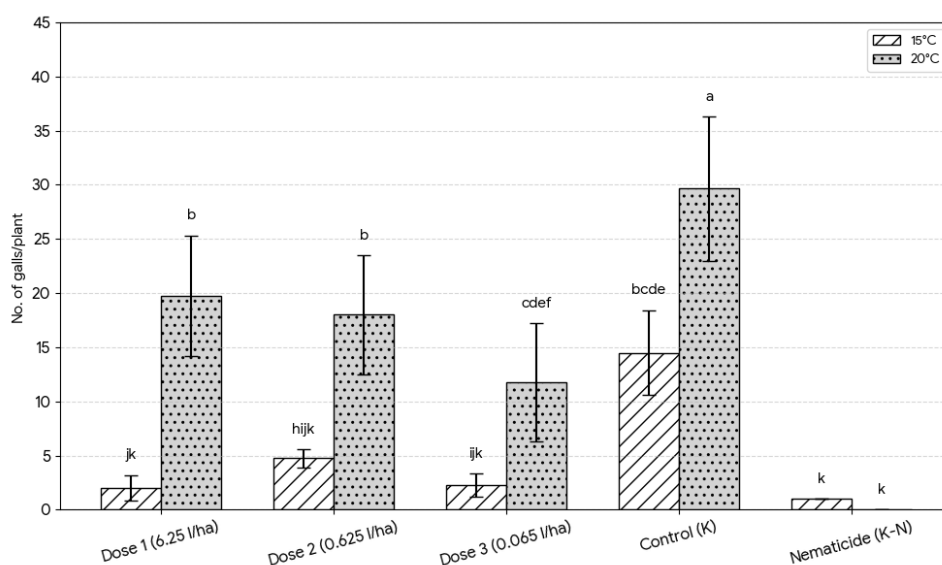
S. chalconica leaf extract was expressed as Trolox equivalents (TE). Based on the linear operating range of the Trolox standard curve ($y = 0.0423x + 0.005$, $R^2 > 0.95$), the minimum potential antioxidant capacity of the extract was estimated at 0.4 mM TE. Consequently, the total antioxidant capacity in the dried leaves was determined to be at least 0.177 mg TE/g of dry weight (DW).

The evaluation of the botanical preparation revealed a clear relationship between temperature conditions and the suppression of *M. hapla*. This controlled growth chamber study focused on assessing how different application rates and ambient temperatures influence the product's capability to suppress nematode development and host plant infection. However, the results did not indicate a consistent dose-dependent response. The results indicated an interaction between temperature conditions and the efficacy of the tested extract. Overall, the botanical product reduced both gall formation and nematode establishment, particularly at the lower experimental temperature of 15°C. Regarding root galling at this lower temperature, tested extract significantly limited gall formation across all tested dosages, keeping mean values between 2.00 ± 1.2 and 4.75 ± 0.83 compared to 14.5 ± 3.94 in the untreated control. At temperature 20°C, root gall formation was higher in the untreated control group, reaching 29.65 ± 6.65 , whereas Dose 3 (0.065 l/ha) significantly reduced the gall count to 11.75 ± 5.45 and was assigned to the significance group "cdef". Nevertheless, no clear dose-dependent pattern was observed (fig. 1).

Furthermore, the botanical preparation significantly reduced J2 infectivity at 15°C. At this temperature, it reduced

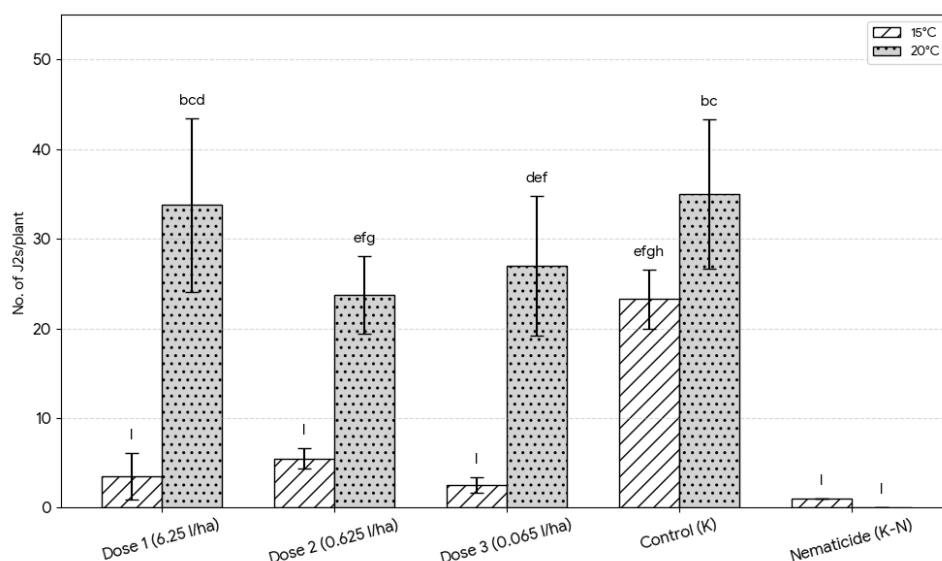
the internal J2 population in roots to a level statistically equivalent to the chemical nematicide, with all application rates sharing the significance letter "l". Specifically, plants treated with tested extract harboured only 2.5 ± 0.87 to 5.5 ± 1.12 J2s, while the untreated control reached 23.25 ± 3.26 . Finally, at temperature 20°C, Dose 2 and Dose 3 significantly limited nematode infectivity to 23.75 ± 4.32 and 27.00 ± 7.81 in comparison to the untreated control value of 35.00 ± 8.34 , suggesting that the formulation may suppress nematode colonization under different temperature conditions. However, as observed for root gall formation, the reduction in J2 infectivity did not follow a clear dose-dependent pattern (fig. 2).

The phytochemical screening of the *S. chalconica* leaf extract revealed the presence of 20E at a concentration of 0.19 mg/g dry weight (DW), while total polyphenol content and antioxidant activity remained relatively low. This profile suggests that phytoecdysteroids may contribute to the biological effects observed, although the involvement of other metabolites cannot be excluded. Phytoecdysteroids are well-known plant defense compounds, and species of the genus *Silene* are recognized as rich sources of these metabolites (Dinan 2001; Bilal and Kutluay 2026). Because nematodes belong to the Ecdysozoa clade and rely on steroid-regulated molting processes, exposure to phytoecdysteroids has been proposed to disrupt cuticle formation, molting, and juvenile development. Consistent with this hypothesis, 20E and related compounds have previously been shown to reduce the mobility and infectivity of root-knot nematodes (Soriano et al. 2004). Although the concentration of 20E de-



Rys. 1. Wpływ dawki preparatu roślinnego oraz temperatury na liczbę wyrosli wywołanych *Meloidogyne hapla* na korzeniach *Lactuca sativa*. Słupki przedstawiają średnie wartości $\pm$ odchylenie standardowe (SD) ($n = 4$). Średnie oznaczone tą samą literą nie różnią się istotnie statystycznie ($p < 0.05$)

Fig. 1. Effect of botanical product dosage and temperature on root gall formation caused by *Meloidogyne hapla* on *Lactuca sativa* roots. Bars represent mean values $\pm$ standard deviation (SD) ($n = 4$). Means followed by the same letter are not statistically different ($p < 0.05$)



Rys. 2. Wpływ dawki preparatu roślinnego oraz temperatury na liczbę osobników J2 *Meloidogyne hapla* zasiedlających korzenie *Lactuca sativa*. Słupki przedstawiają średnie wartości $\pm$ odchylenie standardowe (SD) ($n = 4$). Średnie oznaczone tą samą literą nie różnią się istotnie statystycznie ($p < 0,05$)

Fig. 2. Effect of botanical product dosage and temperature on the number of *Meloidogyne hapla* J2s colonizing *Lactuca sativa* roots. Bars represent mean values $\pm$ standard deviation (SD) ($n = 4$). Means followed by the same letter are not statistically different ($p < 0.05$)

tected in the present study was lower than that reported for several other *Silene* species, significant biological activity was nevertheless observed, suggesting possible synergistic effects among multiple metabolites present in the extract.

In the present study, application of the extract significantly reduced both root galling and the number of *M. hapla* juveniles established within host tissues. These results indicate that compounds present in the extract may interfere with nematode establishment and infection success in the rhizosphere. Similar nematode-suppressive effects have been reported for plant-derived extracts containing bioactive secondary metabolites such as saponins, flavonoids, and terpenoids (D'Addabbo and Avato 2021; Mwamula et al. 2022). Furthermore, the maintained efficacy observed after pre-incubation at 15°C suggests that the active constituents remain sufficiently stable under cooler conditions typical of early-season horticultural production, where soil temperature strongly influences nematode activity and development (Ploeg and Maris 1999).

Although 20E was identified in the extract, the present results do not allow the observed nematode suppression to be attributed exclusively to this compound. Other naturally occurring constituents of *S. chalcidonica* may also contribute to the biological activity. Therefore, further studies are required to identify the compounds responsible for nematode suppression, clarify their mode of action, and evaluate their persistence in soil and effects on non-target organisms. Nevertheless, the results indicate that *S. chalcidonica* re-

presents a promising source of bioactive metabolites with potential applications in sustainable nematode management.

Wnioski / Conclusions

1. *Silene chalcidonica* leaf extract demonstrated a selective phytochemical profile characterized by the presence of 20E and relatively low levels of total phenolics and antioxidant activity.
2. The tested botanical preparation significantly reduced root galling and the infectivity of second-stage juveniles (J2s) of *M. hapla*, particularly at lower tested temperature, indicating nematode suppression potential.
3. The obtained results suggest that extracts from *S. chalcidonica* leaves warrant further investigation as potential components of sustainable nematode management strategies.

Podziękowanie / Acknowledgements

The authors would like to thank doctor Renata Dobosz of the Institute of Plant Protection – National Research Institute in Poznań, Poland, for conducting the evaluation of the efficacy of plant-derived products in reducing host root infection by the northern root-knot nematode, *Meloidogyne hapla* Chitwood, 1949.

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