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ARTYKUŁ ORYGINALNY

Odpowiedź olfaktometryczna wybranych szkodników roślin uprawnych na zapach pokrzywy zwyczajnej (*Urtica dioica* L.) oraz wpływ wodnych ekstraktów roślinnych na ich zachowanie

Olfactometric response of selected crop pests to the scent of common nettle (*Urtica dioica* L.) and the influence of aqueous plant extracts on their behavior

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Streszczenie

W badaniach analizowano wpływ wodnych ekstraktów z *Urtica dioica* L. oraz świeżych części pokrzywy na zachowanie żywieniowe *Sitona lineatus* L., przeżywalność *Aphis fabae* Scop. na liściach bobu oraz aktywność pokarmową larw (L4) *Leptinotarsa decemlineata* Say. Zbadano również reakcje węchowe owadów na zapach pokrzywy. Wykazano, że 10, 20 i 30% stężenia wodnych ekstraktów ze świeżej pokrzywy istotnie hamowały żerowanie samic *S. lineatus*. Z kolei 10% ekstrakt z suszonej pokrzywy miał najwyraźniejszy wpływ na samce *S. lineatus*. Wyższe stężenia ekstraktów z pokrzywy wpływały na wzrost śmiertelności samic i larw *A. fabae*, przy czym świeże ekstrakty wykazywały silniejszy wpływ. Masa ciała larw *L. decemlineata* nieznacznie wzrosła po zastosowaniu wszystkich ekstraktów, szczególnie przy 30% stężeniu wyciągu ze świeżej pokrzywy. Pomimo że wszystkie ekstrakty ograniczały żerowanie larw *L. decemlineata* nie zaobserwowano zauważalnych różnic w zależności od stężenia czy metody przygotowania ekstraktu. Badania z użyciem olfaktometru wykazały odstraszający wpływ zapachów pokrzywy na samice *L. decemlineata*, nie zaobserwowano jednak istotnego wpływu na zachowanie *S. lineatus* i uskrzydlone samice *A. fabae*.

Słowa kluczowe: *Aphis fabae* Scop., ochrona biologiczna, *Leptinotarsa decemlineata* Say., olfaktometr, *Sitona lineatus* L., ekstrakty wodne

Abstract

The study examined the impact of aqueous extracts from *Urtica dioica* L. and fresh nettle parts on the feeding behavior of *Sitona lineatus* L., the survival of *Aphis fabae* Scop. on broad bean leaves, and the feeding of L4 larvae of *Leptinotarsa decemlineata* Say. Olfactory responses of the insects to nettle were also investigated. Results revealed that 10, 20 and 30% concentrations of aqueous extracts from fresh nettle significantly inhibited feeding in *S. lineatus* females. Conversely, the 10% extract from dried nettle had the most pronounced effect on *S. lineatus* males. Higher concentrations of common nettle extracts increased mortality in *A. fabae* females and larvae, with fresh extracts exhibiting a more potent impact. The body weight of *L. decemlineata* larvae slightly increased with all extracts, particularly the 30% concentration of fresh nettle. While all extracts reduced food consumption by *L. decemlineata* larvae, no discernible differences were observed based on concentration or preparation method. Olfactometer research indicated a repellent response of *L. decemlineata* females to common nettle odors, but no significant influence on the behavior of *S. lineatus* and winged *A. fabae* females was observed.

Key words: *Aphis fabae* Scop., biological control, *Leptinotarsa decemlineata* Say., olfactometer, *Sitona lineatus* L., water extracts

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Wstęp / Introduction

The common nettle (*Urtica dioica* L.) is a versatile plant with a well-established history of diverse uses. It serves as a source of edible, cosmetic, fodder, fibrous, and coloring materials. The beneficial effects of extracts from this plant on the human organism are extensively recognized (Dhouibi et al. 2020; Bhusal et al. 2022). Furthermore, extracts from both fresh and dried nettle leaves exhibit insect-repelling properties, suppress the development of various plant diseases, and are considered safe for use (Taadaouit et al. 2012; Thakur et al. 2012; El-Aswad et al. 2023). While alcoholic extracts and essential oils typically demonstrate higher insecticidal efficacy compared to water extracts, the former are more complex and expensive to prepare (Baran et al. 2020; Bhardwaj et al. 2020; Suteu et al. 2020). Water extracts, particularly those based on cold water, represent a simpler and more cost-effective method that can be easily prepared by the farmer.

The olfactory stimulus plays a pivotal role in guiding herbivores to locate a host plant. Disrupting the reception of this stimulus, such as through the presence of other plants in proximity to the host plant, may serve as a natural method for protecting crops against plant pests (Belda and Riudavets 2010; Chen et al. 2012; Finlay-Doney and Walter 2012; Al-Joary et al. 2021; Zhang et al. 2022). Analyzing the response of insects to plants growing in the vicinity of the host plant enables inferences about potential plant selection to achieve the desired effect in mixed crops or when organizing the surroundings of the crop (Xie et al. 2012; Maharjan and Jung 2016; Islam et al. 2017). The existing literature lacks information regarding nettle odor stimuli concerning pests.

The aim of the study was to elucidate the influence of aqueous extracts from common nettle on the feeding behavior and mortality rates of specific arable crop pests, namely pea leaf weevils (*Sitona lineatus* L.), black bean aphids (*Aphis fabae* Scop.), and Colorado potato beetles (*Leptinotarsa decemlineata* Say.). Additionally, an olfactometric response, specifically the reaction to olfactory stimuli, of the aforementioned pests to common nettle was assessed.

Materiały i metody / Materials and methods

The Y-tube olfactometer is a specialized apparatus designed for investigating the behavioral responses of insects to olfactory stimuli. It typically comprises a Y-shaped glass tube, wherein one arm functions as a control or blank arm, while the other serves as the arm dispensing the odor stimulus. Insects are introduced at the base of the Y-tube and are presented with a choice between the two arms. Subsequently, the insects' responses and preferences towards specific odors or stimuli are systematically observed and quantified. This apparatus provides a controlled experimental setting for the in-depth exploration of insect olfactory behavior and preferences. Scientifically, the Y-tube olfactometer is a valuable tool for elucidating the chemotactic behavior of insects, enabling researchers to discern intricate details regarding odor perception, discrimination, and selection. The controlled design of the Y-tube facilitates the isolation of variables, contributing to the precision of behavioral studies involving insect olfaction. The olfactometric investigation on the response of the mentioned insects was carried out using a glass Y-tube olfactometer for pea leaf weevil beetles and Colorado potato beetles, while a 4-arm olfactometer arena, employed with multiple-choice tests, was utilized for winged females of the black bean aphid. Entomological studies using an olfactometer were conducted according to the methodology described in the works of Islam et al. (2017) and Zhang et al. (2022). The utility of such olfactometers for assessing insects' olfactory preferences has been established in various studies (Ninkovic et al. 2013; Marler and Marler 2018; Masui et al. 2020; Mensah-Bonsu et al. 2020). The Y-tube olfactometer consisted of one incoming arm and two testing arms, each comprising glass tubes with dimensions of 250 mm in length and 12 mm in diameter. The testing arms were positioned at an angle of 70 degrees in relation to each other, while the angle between the incoming arm and each testing arm was 145 degrees. The olfactometer arena, constructed from a transparent Plexiglass plate, featured a central cuboid field measuring 65 mm × 65 mm × 55 mm (L × W × H) and four testing arms with dimensions of 160 mm × 65 mm × 55 mm (L × W × H), extending from each of the four sides of the central field. The bottom of the central field had a 5 mm diameter opening serving as an air outlet. The top wall of the cuboid was a removable plate. Pre-treated air, filtered through a carbon filter, was pumped using a Power Cab pump (DC Power Supply 3050) and directed to each testing arm. The air flow was set at 900 ml/min/arm and controlled with a separate rotameter for each arm (Kytala Instruments, Muurame, Finland, EK-2MR-H). The air stream passed through the scent source, which was a glass container (height 120 mm, diameter 70 mm) containing 30 g of fresh *U. dioica* mass and a ring with water-dampened filtering tissue-paper (to maintain proper air humidity) or only dampened filtering tissue-paper (control) in the case of the Y-tube. For the 4-arms' arena, two arms received air flowing through separate containers with common nettle and damp tissue-paper, while the subsequent two arms contained only damp filtering tissue-paper serving as the control. A test insect was placed at the tube outlet (incoming arm for Y-tube or middle part of the central field for 4-arms' arena), and its behavior was observed for 10 minutes, with counts of entries into respective fields (arms) of the olfactometer recorded. After every two specimens, each arm and the entire arena were washed with distilled water and then ethanol to prevent visual effects, with

the positioning of the arms altered accordingly. Throughout the experiments, both the arena and Y-tube were enclosed in a cardboard container with black-painted walls and illuminated from above with diffused light. Each experiment, segregated by pest and gender, was conducted in 12 repetitions.

The study utilized fresh leaves of the Bartek variety broad bean (*Vicia faba* L.) and potatoes of the Bellarosa variety (*Solanum tuberosum* L.). Leaves from the second layer below the top were subjected to treatment with water extracts derived from common nettle, prepared from both dried and fresh plant parts, and were then placed in Petri dishes. The extracts were prepared by weighing 2, 5 and 10 g of dried common nettle (corresponding to conventionally assumed concentrations of 2, 5 and 10%) and 10, 20 and 30 g of fresh nettle fragments (corresponding to concentrations of 10, 20 and 30%). Subsequently, 100 ml of cold re-distilled water was added, and the mixture was left undisturbed for 24 hours. The extracts were stored in darkness at room temperature. After the 24-hour period, the prepared extracts were filtered through a tissue-paper filter and immediately utilized in laboratory experiments [in accordance with the methodology outlined in Arshad et al. (2019)].

To assess the impact of common nettle extracts on the feeding behavior of pea leaf weevil beetles, broad bean leaves were immersed for 3 seconds in solutions containing the respective extracts or in re-distilled water (control). Subsequently, the leaves were air-dried at room temperature and arranged in Petri dishes lined with moistened filter paper. One specimen of pea leaf weevil was introduced into each dish, with separate experiments conducted for females and males. The study encompassed 42 female specimens and 42 male specimens of *S. lineatus*. At 8-hour intervals, the extent of leaf damage was measured fifteen times. All observations were carried out in six repetitions [in accordance with the methodology outlined in Arshad et al. (2019)]. Accurate measurements of leaf surface losses were performed using the *ImageJ* computer program.

The experiment evaluating the impact of common nettle extracts on the viability of the black bean aphid was conducted separately for apterous females (6 specimens per dish) and aphid larvae (10 specimens per dish). Each observation was conducted in 6 repetitions, following the methodology outlined in Arshad et al. (2019). Observations with *A. fabae* were performed in six repetitions. In the experiment, 36 apterous females and 60 larvae of the black bean aphids were used. The larvae utilized for the experiments were of the same age (6 days old). The insects were collected at a single moment from a shoot infested with an aphid colony – observation at the time of plant colonization to ensure age uniformity of the insects. Broad bean leaves with aphid specimens were subjected to spraying with the appropriate common nettle extract or re-distilled water (control). Spraying was conducted using a laboratory sprayer, applying 1 ml of solution per dish from a distance of 20 cm. Fifteen observations were carried out at 8-hour intervals.

The investigation into the influence of common nettle extracts on the Colorado potato beetle was conducted similarly to the experiment involving pea leaf weevils. In each dish, two larvae at the L4 stage were introduced. At 24-hour intervals, the extent of damage to potato leaves caused by these pests was assessed, with the amount of consumed food weighed each time. Additionally, changes in the body weight of the larvae forms of these insects were monitored. The observations were repeated five times. For insect weight measurements, an automatic laboratory scale, the XPR106DUHQ Balance model, was used for automatic weighing.

The statistical analysis of the obtained results was conducted using Statistica 13.1 PL software. The significance of differences between means was assessed employing one-way analysis of variance (ANOVA), and mean separations were determined through Fisher's LSD test at a significance level of $\alpha = 0.05$. Regarding the results derived from the olfactometer, the t-Student test was employed for analysis.

Wyniki i dyskusja / Results and discussion

A noticeable aversive reaction to olfactory substances emanating from the fresh mass of common nettle was observed among female *L. decemlineata* ($t = 2.69$, $P = 0.018$) (tab. 1). These insects exhibited a preference for the olfactometer field where common nettle served as the scent source significantly less frequently than the control field, selecting it over three times less often. *L. decemlineata* males and *S. lineatus* females also displayed a higher frequency of visits to the control field compared to the field scented with common nettle; however, in this instance, the differences were not statistically significant. No significant response to the presence of *U. dioica* olfactory substances was observed among winged females of the black bean aphid and males of *S. lineatus*.

The observation of the changing rate of surface cavities in the broad bean leaf blade as a result of feeding of the females of *S. lineatus* beetles demonstrates the significant differences between the objects treated with the respective common nettle extracts in relation to the control (fig. 1). The highest suppression of the feeding was observed in the objects in which the extracts of the fresh fragments of *U. dioica* were used in 10, 20 and 30% concentrations. The dried common nettle extracts demonstrated a weaker suppressing effect. A significant difference in relation to the control was noted after 24 hours of the observation.

In the experiment with males of *S. lineatus*, the statistical analysis also demonstrated significant differences in the objects in which the respective extracts of common nettle

were used as compared to the control object (fig. 2). However, the rate of consuming the broad bean leaf blade was not so clearly supressed as in the case of the females. The highest suppression of feeding of *S. lineatus* males – visible clearly after 40 hours of the observation, was recorded in the objects where the extracts of the fresh fragments of nattle were used in 30% concentration and dried leaves in 10% concentration (although statistically significant differences were found already after 8 hours of observation). Whereas, the extract of the fresh fragments of *U. dioica* in 10% concentration had a stimulating effect on the feeding of *S. lineatus* males (the effect visible from the 48th hour to the end of the experiment).

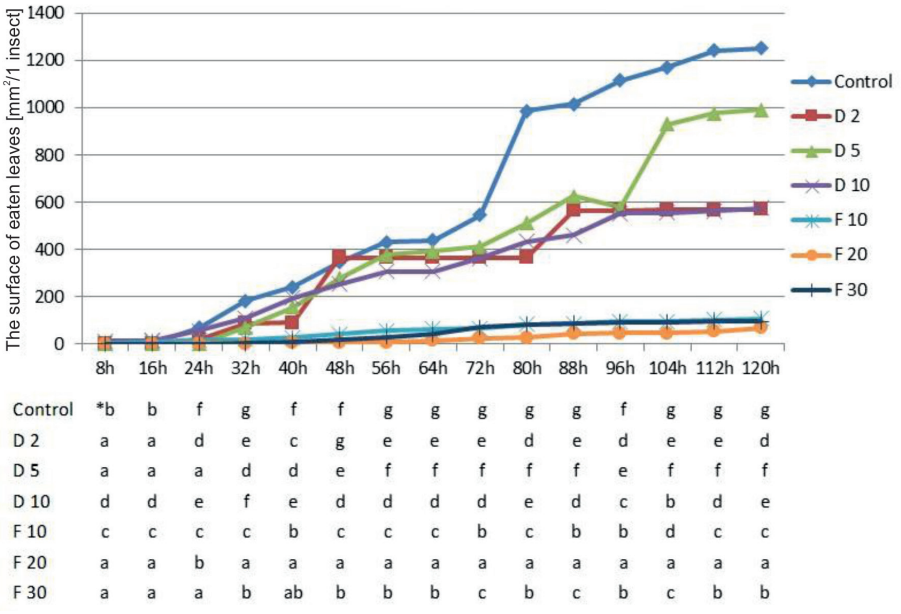
Testing the influence of the common nettle extract on the mortality rate of *A. fabae* aphid larvae demonstrated significant differentiation in relation to the control object.

Tabela 1. Reakcje szkodników na zapach pochodzący ze świeżej masy pokrzywy zwyczajnej, przedstawione jako liczba wejść do wybranych obszarów olfaktometru Y-tube (*Sitona lineatus* L. i *Leptinotarsa decemlineata* Say.) oraz 4-ramiennej areny olfaktometru (*Aphis fabae* Scop.) przypadająca na jednego owada

Table 1. Responses of pests to odors derived from common neetle fresh matter expressed as a number of entrance per one insect into selected areas of Y-tube olfactometer (*Sitona lineatus* L. and *Leptinotarsa decemlineata* Say.) or four-armed arena (*Aphis fabae* Scop.)

Szkodnik – Pest	Kontrola Control	Pokrzywa zwyczajna Common neetle *Urtica dioica* L.
Aphis fabae Scop. – samice uskrzydlone – winged females	1.63	1.13
Sitona lineatus L. – samice – females	1.00	0.56
Sitona lineatus L. – samce – males	0.58	0.42
Leptinotarsa decemlineata Say. – samice – females	1.37*	0.38
Leptinotarsa decemlineata Say. – samce – males	1.10	0.72

*różnice istotne przy α = 0,05, w pozostałych przypadkach różnice nie były istotne – significant differences at α = 0.05, in other cases insignificant differences

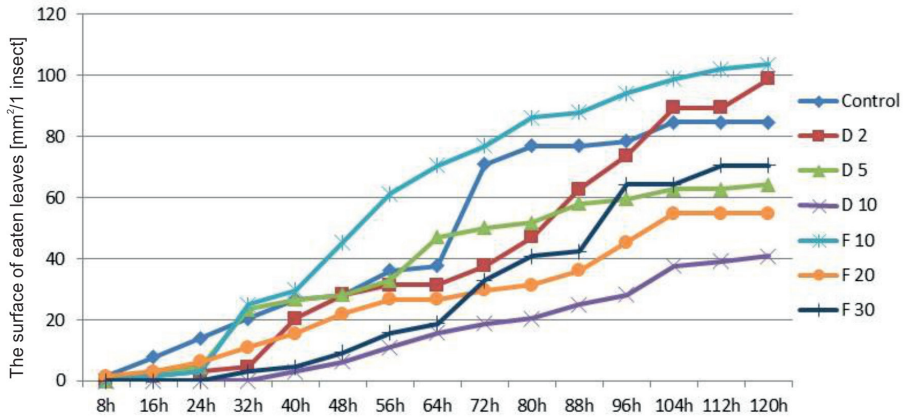


Control	*b	b	f	g	f	f	g	g	g	g	g	f	g	g	g
D 2	a	a	d	e	c	g	e	e	e	d	e	d	e	e	d
D 5	a	a	a	d	d	e	f	f	f	f	f	e	f	f	f
D 10	d	d	e	f	e	d	d	d	d	e	d	c	b	d	e
F 10	c	c	c	c	b	c	c	c	b	c	b	b	d	c	c
F 20	a	a	b	a	a	a	a	a	a	a	a	a	a	a	a
F 30	a	a	a	b	ab	b	b	b	c	b	c	b	c	b	b

*oznaczenia literowe w kolumnach nie różnią się istotnie statystycznie przy poziomie istotności α = 0,05 – means in columns marked with the same letters are not statistically different at α = 0.05

Rys. 1. Dynamika żerowania samic chrząszczy *Sitona lineatus* L. na liściach bobu w cyklu 15 obserwacji w 8-godzinnych odstępach, po zastosowaniu wodnego ekstraktu z suszonej pokrzywy w stężeniach 2% (D 2), 5% (D 5) i 10% (D 10) oraz świeżych części rośliny w stężeniach 10% (F 10), 20% (F 20) i 30% (F 30) w porównaniu z kontrolą

Fig. 1. The dynamic of female *Sitona lineatus* L. beetles feeding on broad bean leaves, in cycle of 15 observations at 8-hour intervals, after application of the aqueous extract of dried nettle in concentrations of 2% (D 2), 5% (D 5) and 10% (D 10), and parts of fresh plants in concentrations of 10% (F 10), 20% (F 20) and 30% (F 30) compared to the control



	8h	16h	24h	32h	40h	48h	56h	64h	72h	80h	88h	96h	104h	112h	120h
Control	*d	d	e	e	e	c	f	d	f	f	f	f	e	e	e
D 2	c	c	b	c	d	c	d	c	d	d	e	e	f	f	f
D 5	b	b	c	f	e	c	e	e	e	e	d	c	c	c	c
D 10	ab	a	a	a	a	a	b	a	a	a	a	a	a	a	a
F 10	a	c	b	g	f	d	g	f	g	g	g	g	g	g	g
F 20	a	a	d	d	c	a	a	a	b	b	b	b	b	b	b
F 30	a	a	a	b	b	b	c	b	c	c	c	d	d	d	d

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Rys. 2. Dynamika żerowania samców chrząszczy *Sitona lineatus* L. na liściach bobu, w cyklu 15 obserwacji w 8-godzinnych odstępach, po zastosowaniu wodnego ekstraktu z suszu pokrzywy w stężeniach 2% (D 2), 5% (D 5) i 10% (D 10) oraz świeżych fragmentów rośliny w stężeniach 10% (F 10), 20% (F 20) i 30% (F 30) w porównaniu z kontrolą

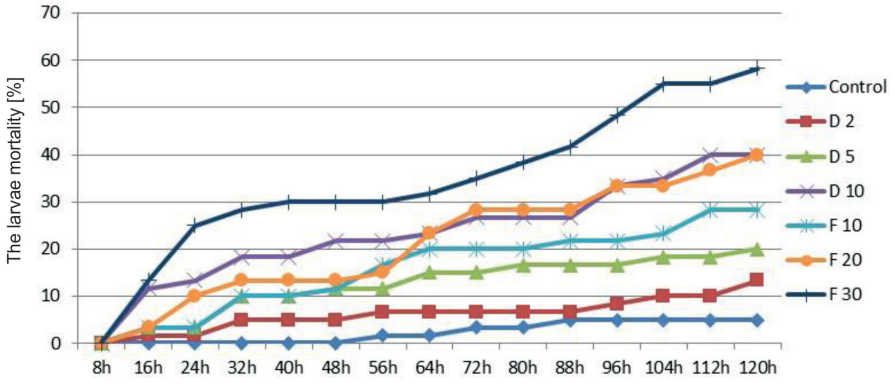
Fig. 2. The dynamic of male *Sitona lineatus* L. beetles feeding on broad bean leaves, in cycle of 15 observations at 8-hour intervals, after application of the aqueous extract of dried nettle in concentrations of 2% (D 2), 5% (D 5) and 10% (D 10), and parts of fresh plants in concentrations of 10% (F 10), 20% (F 20) and 30% (F 30) compared to the control

All the types and concentrations of common nettle caused the increased mortality rate of the black bean aphid larvae (fig. 3). The fresh fragments of *U. dioica* extract in 30% concentration had the strongest effect. The mortality rate after 120 hours of the experiment in this object was at the approximate level of 60%, and in the control it was approximately 5%. In the initial observation phase, also the extract of dried *U. dioica* in 10% concentration had a strong toxic effect. In the subsequent measurements, the 20% extract of the fresh fragments demonstrated a similar effect. The lowest mortality rate was noticed in the objects where lower concentrations of the dried plant were used (2 and 5%).

The observation of the toxicity of *U. dioica* water extracts in relation to the adult apterous females of black bean aphid demonstrated that the highest number of dead specimens was recorded from the 72nd hour of the observation, in the objects where 20% and 30% concentration extracts of the fresh plant fragments were used and 10% from the dried plant and this state continued to the end of the experiment (fig. 4). As a consequence, at the end of the experiment, the death of approximately half the specimens used in the experiment was recorded in these objects.

The observation of the dynamics in the body weight changes among the Colorado potato beetle larvae (L4 stage) demonstrated statistically significant differences compared to the control object in the 48th, 72nd and 96th hour of the experiment (fig. 5). The first measurement (after 24 hours from starting the experiment) did not reveal any statistically significant differences with reference to the control object. After 2 days from the beginning of the experiment, a drop in the body weight was observed among the Colorado potato beetle larvae in all the objects in which leaves were treated with the extracts. The differences were proven statistically in comparison to the control object. The highest drop in the larvae body weight was recorded in the objects with the extracts of the fresh fragments of common nettle (10, 20 and 30% concentration). The 5% extract of the dried plant had a similar effect. The subsequent observations demonstrated a minor rebuilding in the larvae body weight in the objects treated with the extracts. Nevertheless, this tendency was not demonstrated by the object in which the 30% extract of the plant fresh fragments was used and in which a regular decrease in the body weight of the Colorado potato beetle larvae was observed.

The mass of the potato leaf blade consumed by the larvae (L4 stage) of *L. decemlineata* is presented in figure 6. The observations demonstrated the significant limiting influence of the common nettle extracts (in all the concentrations) on the mass of the food consumed by larvae. However, no statistically significant differences were found between the respective concentrations and the types of extracts.

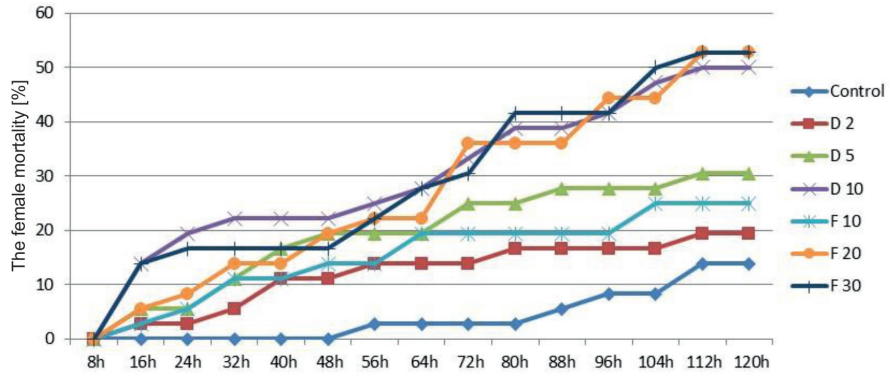


Control	*a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
D 2	a	a	a	ab	ab	ab	ab	ab	a	ab	a	ab	ab	a	ab
D 5	a	a	ab	bc	bc	bc	bc	bc	ab	bc	ab	ab	ab	ab	ab
D 10	a	b	c	c	c	d	de	cd	bcd	cd	b	c	c	cd	c
F 10	a	a	ab	bc	bc	bc	cd	cd	bc	c	b	bc	bc	bc	bc
F 20	a	a	bc	bc	bc	c	bcd	cd	cd	cd	b	c	c	c	c
F 30	a	b	d	d	d	e	e	d	d	d	c	d	d	d	d

*oznaczenia literowe w kolumnach nie różnią się istotnie statystycznie przy poziomie istotności α = 0,05 – means in columns marked with the same letters are not statistically different at α = 0.05

Rys. 3. Śmiertelność larw mszycy burakowej (*Aphis fabae* Scop.) żerujących na liściach bobu, w cyklu 15 obserwacji w 8-godzinnych odstępach, po zastosowaniu wodnego ekstraktu z suszu pokrzywy w stężeniach 2% (D 2), 5% (D 5) i 10% (D 10) oraz świeżych fragmentów rośliny w stężeniach 10% (F 10), 20% (F 20) i 30% (F 30) w porównaniu z kontrolą

Fig. 3. The mortality of black bean aphid larvae (*Aphis fabae* Scop.) feeding on the broad bean leaves, in the cycle of 15 observations at 8-hour intervals, after application of the aqueous extract of dried nettle in concentrations of 2% (D 2), 5% (D 5) and 10% (D 10), and parts of fresh plants in concentrations of 10% (F 10), 20% (F 20) and 30% (F 30) compared to the control

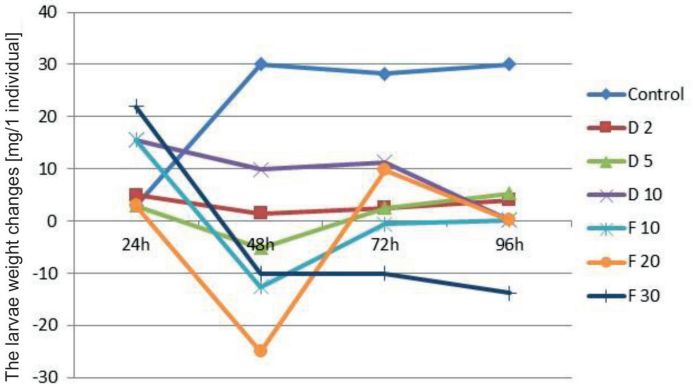


Control	*a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
D 2	a	a	a	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab
D 5	a	ab	a	abc	b	b	b	bc	bcd	bc	bc	bc	b	b	b
D 10	a	b	c	c	b	b	b	c	cd	cd	c	cd	c	c	c
F 10	a	a	a	abc	ab	ab	ab	bc	bc	b	ab	ab	b	ab	ab
F 20	a	ab	ab	abc	ab	b	b	bc	d	cd	c	d	c	c	c
F 30	a	b	bc	bc	b	b	b	c	cd	d	c	cd	c	c	c

*oznaczenia literowe w kolumnach nie różnią się istotnie statystycznie przy poziomie istotności α = 0,05 – means in columns marked with the same letters are not statistically different at α = 0.05

Rys. 4. Śmiertelność bezskrzydłych samic mszycy burakowej (*Aphis fabae* Scop.) żerujących na liściach bobu, w cyklu 15 obserwacji w 8-godzinnych odstępach, po zastosowaniu wodnego ekstraktu z suszu pokrzywy w stężeniach 2% (D 2), 5% (D 5) i 10% (D 10) oraz świeżych fragmentów rośliny w stężeniach 10% (F 10), 20% (F 20) i 30% (F 30) w porównaniu z kontrolą

Fig. 4. The mortality of wingless female black bean aphid (*Aphis fabae* Scop.) feeding on the broad bean leaves, in cycle of 15 observations at 8-hour intervals, after application of the aqueous extract of dried nettle in concentrations of 2% (D 2), 5% (D 5) and 10% (D 10), and parts of fresh plants in concentrations of 10% (F 10), 20% (F 20) and 30% (F 30) compared to the control

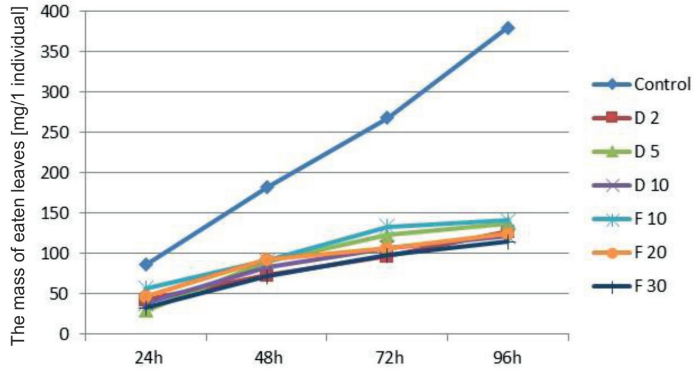


Control	*a	d	b	c
D 2	a	bc	a	ab
D 5	a	bc	a	b
D 10	a	c	ab	ab
F 10	a	ab	a	ab
F 20	a	a	ab	ab
F 30	a	abc	a	a

*oznaczenia literowe w kolumnach nie różnią się istotnie statystycznie przy poziomie istotności α = 0,05 – means in columns marked with the same letters are not statistically different at α = 0.05

Rys. 5. Dynamika zmian masy ciała larw *Leptinotarsa decemlineata* Say. (stadium L4), wyrażona jako różnica w stosunku do wagi początkowej, po zastosowaniu wodnego ekstraktu z suszu pokrzywy w stężeniach 2% (D 2), 5% (D 5) i 10% (D 10) oraz świeżych fragmentów rośliny w stężeniach 10% (F 10), 20% (F 20) i 30% (F 30) w porównaniu z kontrolą, w cyklu 5 obserwacji w odstępach 24-godzinnych

Fig. 5. The dynamic of changes in the body weight of larvae *Leptinotarsa decemlineata* Say. (L4 stage), expressed as a difference with respect to the initial weight, after application of the aqueous extract of dried nettle in concentrations of 2% (D 2), 5% (D 5) and 10% (D 10), and parts of fresh plants in concentrations of 10% (F 10), 20% (F 20) and 30% (F 30) compared to the control, in the cycle of 5 observations at 24 hours intervals



Control	*b	b	b	b
D 2	a	a	a	a
D 5	a	a	a	a
D 10	a	a	a	a
F 10	a	a	a	a
F 20	a	a	a	a
F 30	a	a	a	a

*oznaczenia literowe w kolumnach nie różnią się istotnie statystycznie przy poziomie istotności α = 0,05 – means in columns marked with the same letters are not statistically different at α = 0.05

Rys. 6. Masa liści ziemniaka zjadanych przez larwy *Leptinotarsa decemlineata* Say. (stadium L4), po zastosowaniu wodnego ekstraktu z suszu pokrzywy w stężeniach 2% (D 2), 5% (D 5) i 10% (D 10) oraz świeżych fragmentów rośliny w stężeniach 10% (F 10), 20% (F 20) i 30% (F 30) w porównaniu z kontrolą, w cyklu 5 obserwacji w odstępach 24-godzinnych

Fig. 6. The mass of potato leaf eaten by larvae (L4 stage) *Leptinotarsa decemlineata* Say., after application of the aqueous extract of dried nettle in concentrations of 2% (D 2), 5% (D 5) and 10% (D 10), and parts of fresh plants in concentrations of 10% (F 10), 20% (F 20) and 30% (F 30) compared to the control, in the cycle of 5 observations at 24 hours intervals

Among sixty extracts from 54 different plant species, the extract from *U. dioica* leaves was identified as having the most potent antifeedant effect on Colorado potato beetle larvae (Heisey 2001; Kutas and Nádasy 2005; Tălmaciu et al. 2015; Garmendia et al. 2018), aligning with our findings. The potato leaf mass consumed by larvae after 96 hours from the moment of spraying with extracts was nearly three times lower than in the control treatment. Our research also documented a distinct negative response to olfactory substances from fresh common nettle mass among female *L. decemlineata*. Although males visited the olfactometer control field more frequently than the field with common nettle scent, the differences were not statistically significant in this case. Olfactory responses play a crucial role in the host plant search process among insects (Grazosi and Rieske 2013; Carrasco et al. 2015; Peng et al. 2021). Our results suggest the potential usefulness of having nettle in the vicinity of potato crops.

The conducted experiment established that water extracts derived from common nettle, obtained from both fresh and dried plant masses, exert inhibitory effects on the pea leaf weevil feeding on broad beans. Additionally, these extracts significantly curtail feeding activity and hinder the increase in body weight of Colorado potato beetle larvae on potato leaves. Moreover, a notable reduction in the survival rate of black bean aphids on broad beans was observed at both the larval stage and among adult apterous females. The efficacy and potency of the influence on the tested cultivated plant pests were found to be contingent on the concentration and type of water extract, as well as the developmental stage and species of the insect. The feeding mechanism of a given pest also played a crucial role. Organisms with chewing mouthparts, such as the pea weevil and Colorado potato beetle, exhibited robust responses to substances present in the common nettle extract applied directly to the host plant leaves. In contrast, the black bean aphid on broad beans was subjected to the common nettle water extract through spraying, encompassing the pests. This approach was informed by the distinct feeding behavior of this group of insects. In this scenario, offering leaves sprayed with the extract to the pests was unlikely to yield a significant effect, as aphids extract plant juices from the deeper tissues of the plant.

Common nettle leaves encompass a diverse array of chemical constituents, including xanthophylls (0.12%), protoporphyrins (0.1–0.6%), glucokinins, and tannins. Additionally, trace amounts of essential oil (antofen) and formic acid have been detected in the leaves. Notably, the stinging hairs of nettle contain compounds akin to resin acids, encompassing acetylcholine, histamine, and serotonin (Bhusal et al. 2022). Tannins, recognized for their insecticidal properties, induce reactions leading to the generation of reactive oxygen forms in most insects (Barbehenn and Constabel 2011; Wafa et al. 2022). Furthermore, tannins curtail enzymatic activity and facilitate the production of compounds deleterious to insects (Feeny 1975).

The existing body of research on the impacts of plant water extracts has yielded intriguing findings. However, the available literature lacks studies on the effects of nettle extracts specifically on chewing pests, such as pea leaf weevil beetles. Previous investigations on the influence of water extracts derived from other herbaceous plants on the feeding behavior of this insect indicate a decrease in feeding by females following the application of extracts from dry matter of wormwood (*Artemisia absinthium* L.) at concentrations of at least 5%, whereas only extracts from dry matter with concentrations of no less than 10% were effective against males (Rusin et al. 2016). Similar trends were observed with extracts from common sage (*Salvia officinalis* L.) (Biniaś et al. 2016) and peppermint (*Mentha piperita* L.) (Biniaś et al. 2017). In contrast to the aforementioned studies, our research demonstrated a higher efficacy of extracts based on fresh nettle mass, but notably, only in the case of females. The results underscored distinct differences between male and female pea leaf weevils. A significantly stronger suppressive effect on feeding was observed among females, despite their higher consumption of food. This discrepancy may be attributed to the greater body weight of these specimens and the breeding period of the beetles during which the observations were conducted, a phase known for substantial energy expenditure. In olfactometric response tests among these insects, *S. lineatus* females visited the olfactometer control field considerably more frequently than the field with nettle scent compared to males, suggesting their heightened sensitivity to the proximity of nettles. Nevertheless, these differences did not reach statistical significance. St.Onge (2017) demonstrated that both male and female pea leaf weevil adults, tested individually in a 4-way olfactometer for their response to four natural odor sources, especially pea plants, exhibited similar responses across three physiological states: newly eclosed, recently overwintered, and reproductively active.

The existing literature offers diverse insights into the effectiveness of water extracts from nettle against aphids. Ahmadi et al. (2022) conducted a study on the impact of nettle extract on the black bean aphid, wheat aphid and cowpea aphid. El-Aswad et al. (2023) investigated the biological activity of tannin extracts from *U. dioica* on the aphid *Melaphis chinensis* (Aphididae). Bozsik (1996) found that an undiluted cold water extract of stinging nettle with a 24-hour extraction time, similar to our research conditions, had no significant impact on *Hyalopterus pruni* and *Cryptomyzus ribis* aphids. However, Bozsik (1996) suggested the extract's suitability as an aphid repellent. In the context of natural grapevine protection methods, Czulak (2011) enumerated the toxic, masking, and repellent effects of water extracts and nettle slurry on aphids. According to Dutka (2013), essential oils obtained from nettle may significantly reduce aphid populations. Factors such as the concentration of antifeedant substances, their persistence on the plant, and the mode of action on insects are crucial considerations. Sur-

prisingly, the available literature does not provide information on the response of aphids to nettle olfactory stimuli. Our research, however, revealed the absence of any significant effect of nettle-derived scent on winged females of *A. fabae*.

Wnioski / Conclusions

1. The water extract of the fresh fragments of nettle in 10, 20 and 30% concentration limited, to the largest extent, the feeding of *S. lineatus* L. females. Whereas, the 10% extract of the dried common nettle had the strongest effect on *S. lineatus* L. males.
2. The mortality rate of *A. fabae* Scop. aphid females and larvae under the influence of the extracts of common nettle was increased. Generally, it increased as the extract concentration grew, whereas, the extract of the fresh plant fragments demonstrated a stronger effect than the one prepared from the dried plant.
3. All the used extracts caused limited increase in body weight of Colorado potato beetle larvae; whereas, the stronger influence was demonstrated by the extract of the fresh nettle fragments in 30% concentration.
4. All the used extracts reduced the amount of food consumed by the Colorado potato beetle larvae but no differentiation was determined depending on the extract concentration and the manner of its preparation.
5. The research with the use of the olfactometer determined a repelling reaction of olfactory substances from the common nettle in relation to the Colorado potato beetle females; whereas, its effect on the behaviour of the pea leaf weevils and the females of the winged black bean aphid was not confirmed.

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