

EFFECT OF AQUEOUS EXTRACT OF ST. JOHN'S WORT (*HYPERICUM PERFORATUM* L.) ON THE COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) BEHAVIOUR

Summary

The object of the laboratory experiment was to investigate the effect of aqueous extracts of dried St. John's Wort (*Hypericum perforatum* L.) in concentrations of 2%, 5% and 10%, and fresh parts of this plant, in concentrations of 10%, 20% and 30% on feeding of larvae of Colorado potato beetle (*Leptinotarsa decemlineata* Say) at L3 and L4 stages. We examined the mass of eaten potato leaves treated with different extracts after 24, 48 and 60 hours from the start of the experiment. Additionally, reactions of adult potato beetle individuals to the scent of the above mentioned plant was tested with the use of an olfactometer. Aqueous extracts from St. John's wort exhibit inhibitory effect on feeding of potato beetle larvae at a concentration of at least 5% and 10% for dried plant and 20-30% for fresh plant weight and the effect is stronger for L3 larvae than L4 larvae. However, the comparatively low values of the absolute deterrence index suggest limited possibility to use aqueous extracts from the plant for the inhibition of potato beetle larvae feeding. Tests with the use of olfactometer did not exhibit a deterrent reaction of aromatic substances from St. John's wort to adult individuals of potato beetle (both females and males).

Keywords: *Leptinotarsa decemlineata* Say, biological control, *Hypericum perforatum* L., plant extracts

WPLYW WODNEGO WYCIĄGU Z DZIURAWCA ZWYCZAJNEGO (*HYPERICUM PERFORATUM* L.) NA ZACHOWANIE STONKI ZIEMNIACZANEJ (*LEPTINOTARSA DECEMLINEATA* SAY)

Streszczenie

Celem doświadczenia laboratoryjnego było zbadanie wpływu wodnych wyciągów z suszu dziurawca zwyczajnego (*Hypericum perforatum* L.) w stężeniach 2, 5 i 10% oraz ze świeżych części tej rośliny w stężeniach 10, 20 i 30% na żerowanie larw L3 i L4 stonki ziemniaczanej (*Leptinotarsa decemlineata* Say). Oceniano masę zjadanych przez larwy stonki ziemniaczanej liści ziemniaka potraktowanych poszczególnymi wyciągami w 24, 48 i 60 godzinie od założenia doświadczenia. Ponadto przy użyciu olfaktometru zbadano reakcje postaci dorosłych stonki ziemniaczanej na zapach wyżej wymienionej rośliny. Wodne wyciągi z dziurawca pospolitego wykazują działanie ograniczające żerowanie larw stonki ziemniaczanej przy stężeniu co najmniej 5 i 10% dla suszu oraz 20-30% dla świeżej masy roślinnej, przy czym efekt jest silniejszy wobec larw L3, aniżeli larw L4. Stosunkowo niskie wartości bezwzględne wskaźnika deterentności sugerują jednak ograniczone możliwości wykorzystania wodnych wyciągów z tej rośliny dla hamowania żerowania larw stonki ziemniaczanej. Badania z wykorzystaniem olfaktometru nie wykazały reakcji odstraszałej substancji zapachowych pochodzących od dziurawca zwyczajnego w stosunku do chrząszczy stonki ziemniaczanej.

Słowa kluczowe: *Leptinotarsa decemlineata* Say, ochrona biologiczna, *Hypericum perforatum* L., wyciągi roślinne

1. Introduction

The search of plant protection products based on natural substances constitutes an activity that may bring measurable benefits for organic farms and it fits in the current method of integrated plant protection against pests. As example of such products can serve plant extracts, which possess numerous advantages, such as: ease of obtaining of the raw material (the reference material includes common herbaceous plants and even certain weed species), simplicity of preparation and the associated low production costs and safety of use [14, 25]. They are safe for the human health, do not contaminate the environment via accumulation in soils, water and atmosphere, do not cause agrophage immunization, do not require maintaining of waiting period and re-entry period, do not leave harmful compounds in crops and do not have phytotoxic effect [6].

The St. John's wort (*Hypericum perforatum* L.) is a ruderal plant, common in the whole of Poland, Europe and central Asia, America and Australia. It is used on a large scale in the treatment of numerous diseases of the respiratory, urinary and gastrointestinal tracts and also in neurologi-

cal and mental diseases, including depression [3, 4]. In the flowering time, *H. perforatum* contains approximately 30% of hypericins, and the highest concentration is found in buds (approx. 48%), flowers (approx. 30%) and lower in leaves (approx. 17%) [4]. The active ingredient content in the plant depends i.a. on the harvest time, its habitat and herb drying methods [5, 22]. Furthermore, *H. perforatum* is rich in flavonoids (their content is 2–5%) [7]. Additionally, proanthocyanidins (catechins and epicatechins in the form of dimers, trimers, tetramers and polymers) play important role and constitute approximately 12% of raw material d.w. [3, 4]. The St. John's wort also contains phenolic acids, xanthones (characteristic of *Hypericaceae*) and other acids (e.g. nicotinic), as well as carotenoids, choline, vitamin C, nicotinamide, pectins, b-sitosterole, saturated hydrocarbons (C₁₆-C₃₀) and alcohols (C₂₄-C₂₈) [3, 12]. Essential oils also play important role from the point of view of plant protection against pests and they can have a deterrent effect. The St' John's wort contains, i.a.: n-nonane, n-undecane, 2-methyldecane, a- and b-pinene, a-terpineol, geraniol, b-caryophyllene, humulene and traces of monoterpenes (myrcene, limonene) [3].

Colorado potato beetle (*Leptinotarsa decemlineata* Say) is an omnivorous beetle of the leaf beetle family (Coleoptera, Chrysomelidae), and it is the most dangerous pest of potato crops in the world [2, 15, 16]. The mode of feeding of the larvae and adult individuals consists in the complete stripping of potato leaves, leading to a considerable drop of yields [1, 9]. The available literature provides no information on the possibility of using the aqueous extracts from St. John's wort in the Colorado potato beetle control, as well as on the impact of the scent of this plant on the behavior of the pest. A few studies on the insecticidal properties of this plant refer to alcoholic extracts [17] or essential oils [23]. The water extracts are easier to prepare and cheaper, so it is advisable to test their capabilities.

The objective of the study was to determine the effect of aqueous extracts from fresh and dry mass of the St. John's wort on the Colorado potato beetle feeding on potato leaves (*Solanum tuberosum* L.). Additionally, reactions of adult potato beetle individuals to the scent of the above mentioned plant was tested with the use of an olfactometer.

2. Materials and methods

Fresh leaves of potato of the Bellarosa cultivar were used in the laboratory experiment, collected from the second level of the stem below the apex in order to eliminate the effect of leaf age on the intensity of the pest feeding. The obtained plant material was treated with aqueous extracts from the St. John's wort. They were prepared from dried and fresh fragments of the plant (leaves, young stems and flowers). 2 g, 5 g and 10 g of dried St. John's wort was weighed (conventionally assumed as 2%, 5% and 10% concentrations) and 10 g, 20 g and 30 g of fresh parts of the plant (concentration 10%, 20% and 30%) and then 100 ml of cold redistilled water was added. Extracts were stored for 24 hours in darkness in room temperature. Then, the obtained solutions were filtered through filter papers and were immediately used in experiment. The potato leaves were immersed for 3 seconds in the appropriate extract or in redistilled water (control) and then dried in room temperature, and then placed on Petri dishes lined with moist filter paper. Then, 2 potato beetle larvae were placed in each Petri dish. The experiment was conducted in 6 replications, separately for L3 and L4 larvae. During the experiment, filter paper was moistened in the dishes in order to prevent leaf drying. In 20, 48 and 60 hours of the experiment, the amount of food consumed by potato beetle was weighed in order to determine the changes in their feeding intensity.

After conclusion of the conducted observations, the following values were calculated:

- palatability index - the ratio of the percentage of the consumed leaf weight on individual objects to the percentage of consumed weight in control;
- absolute deterrence index, which included the relationship between the weight of consumed food on the analyzed objects and the weight of leaf consumed in control [11]; $Adi = [(K-T) : (K+T)] \cdot 100$, where: K - leaf weight consumed in control [mg], T - leaf weight consumed on analyzed object [mg].

In the study on the olfactometric reaction of adult potato beetle females and males, a glass Y-tube olfactometer was used. This olfactometer is commonly used for the determination of olfactory preferences of insects [19, 21]. Y-tube olfactometer consisted of one arm referred to as release

arm, and two test arms. The arms consisted of glass pipes, each with 250 mm length and 12 mm diameter. Test arms were aligned relative to each other at 70° angle. The angle between the release arm and each of the test arms was 145°. Air filtered via carbon filter was fed with the use of a pump (Power Cab, DC Power Supply 3050) to each of the test arms. Air flow was established at 900 ml/min/arm and controlled with a rotameter (Kytala Instruments, Muurame, Finland, EK-2MR-H) separate for each arm. Then, the air stream flowed through the source of the smell, i.e. a glass container (height 120 mm, 70-mm diameter) containing respectively 30 g of *H. perforatum* fresh mass with a disk of filter paper moistened with distilled water (to ensure the appropriate air moisture) or only moistened filter paper (control). Test insect was placed at the exit of the release arm pipe, and for the subsequent 10 minutes its behavior was observed, recording the number of entries to each individual fields (arms) of the olfactometer. Each arm was cleaned with distilled water, and then ethanol after testing each 2 specimens. In the meantime, position of the arms was changed in order to prevent the influence of visual effect. During the experiment, Y-tube was placed in a cardboard container with walls painted in black and illuminated from above with scattered light. The experiment, divided into males and females, was conducted in 12 replications.

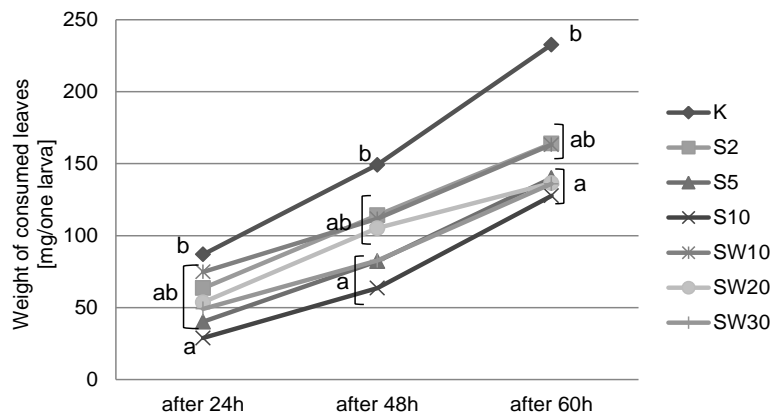
Statistical analysis of the obtained results was conducted using the Statistica 12.5 PL software. In the experiment on the influence of *H. perforatum* extracts on the feeding of the studied pests, the significance of the differences between means was tested via conduct of a single variant variance analysis (the study factor was extract type - prepared from fresh matter or dry matter and at different concentration) and the means were differentiated using the NIR Fisher test at the significance level of $\alpha = 0.05$. In the reference to the results obtained with the use of olfactometer, a t-Student test was used for independent samples (the grouping variable was the presence or absence of the supply of St. John's wort scent).

3. Results

The experiment demonstrated restrictive effect of aqueous extracts from the St. John's wort on the feeding intensity of potato beetle L3 larvae, yet the differences became more visible with the passage of time (Fig. 1). The 5 and 10% extracts from dried wort and 30% extract from fresh matter of the plant exhibited the strongest (statistically significant) effect. After 60 hours of the experiment, a significant effect was also reported for the extract of fresh matter used in the 20-100 ratio (20%).

Changes in the loss of potato leaf weight consumed by L4 larvae of potato beetle were significantly different from the control only after 48 and 60 hours of the experiment (Fig. 2). However, in comparison to the L3 stage, the observed effect was weaker. The strongest effect was in this case observed for the extracts of wort fresh matter at 20% and 30% concentration.

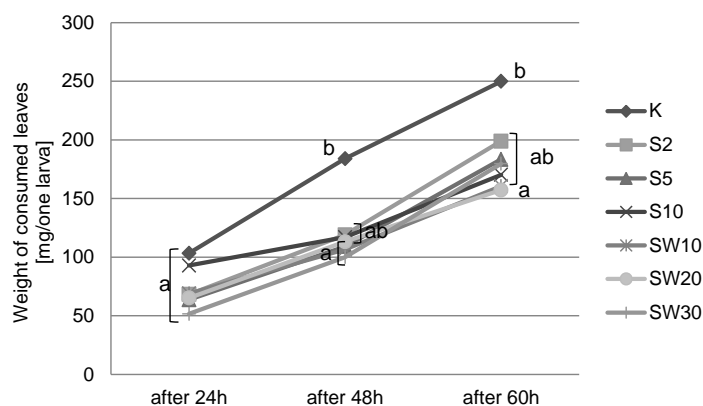
All used extracts of the St. John's wort were characterized by inhibitory effect for the L3 larvae feeding on potato leaves, which is demonstrated by the positive values of the absolute deterrence index (Fig. 3). Normally, the higher was extract concentration, the stronger was the effect of feeding inhibition and the extracts from dried plant had slightly stronger effect than extracts from fresh matter. The palatability index for L3 larvae was lowest after the use of extracts from fresh matter of St. John's wort with concentrations of 20% and 30% (Fig. 3).



Source: own work / Źródło: opracowanie własne

Fig. 1. The weight of potato leaves consumed by the larvae L3 of Colorado potato beetle *Leptinotarsa decemlineata* Say, after applying an aqueous extract from dried St. John's wort at a concentration of 2% (S2), 5% (S5) and 10% (S10), and fresh parts of the plants in concentrations of 10% (SW10), 20% (SW20) and 30% (SW30) compared to the control (K), in a series of 3 observations at of 24-, 48- and 60 hour intervals. Means marked with the same letters in particular time do not differ significantly at $\alpha = 0.05$

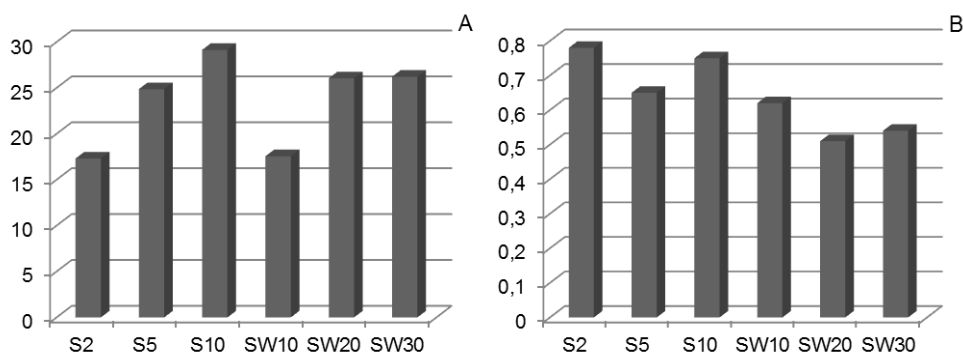
Rys. 1. Masa liści ziemniaka zjedzonych przez larwy L3 stonki ziemniaczanej (*Leptinotarsa decemlineata* Say), po zastosowaniu wodnego wyciągu z suszu dziurawca zwyczajnego w stężeniach 2% (S2), 5% (S5) i 10% (S10) oraz świeżych fragmentów tej rośliny w stężeniach 10% (SW10), 20% (SW20) oraz 30% (SW30) w odniesieniu do obiektu kontrolnego (K), w cyklu 3 obserwacji w odstępach 24-, 48- i 60-godzinnych. Średnie oznaczone takimi samymi literami dla danego terminu nie różnią się od siebie istotnie przy $\alpha = 0,05$



Source: own work / Źródło: opracowanie własne

Fig. 2. The weight of potato leaves consumed by the larvae L4 of Colorado potato beetle *Leptinotarsa decemlineata* Say, after applying an aqueous extract from St. John's wort. See figure 1 for explanations

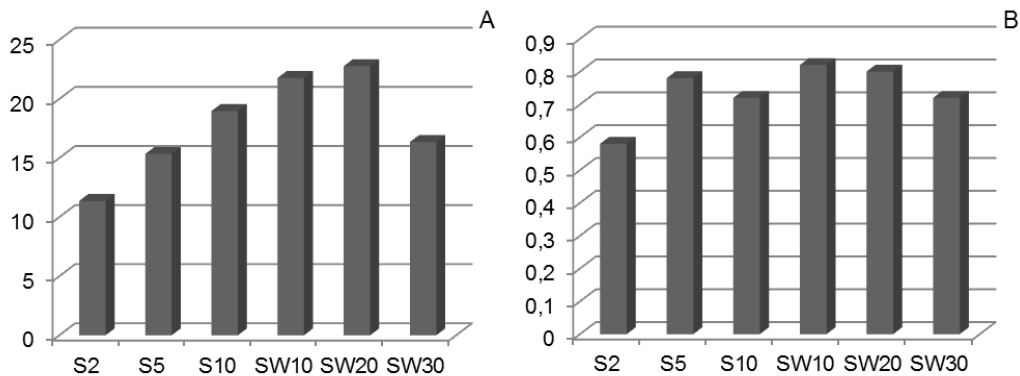
Rys. 2. Masa liści ziemniaka zjedzonych przez larwy L4 stonki ziemniaczanej (*Leptinotarsa decemlineata* Say) po zastosowaniu wodnego wyciągu z dziurawca zwyczajnego. Objasnienia jak na rys. 1



Source: own work / Źródło: opracowanie własne

Fig. 3. Absolute deterrence index (A) of extracts from dry and fresh matter of St. John's wort in different concentrations (from 2-10% and 10-30%) for larvae L3 Colorado potato beetle, calculated according to the formula: $Adi = [(K-T) : (K+T)] \cdot 100$, where: K - leaf weight consumed in control [mg], T - leaf weight consumed on analyzed object [mg] and palatability index (B), calculated as the ratio of the percentage of the consumed leaf weight on individual objects to the percentage of consumed weight in control. S - dry matter, SW - fresh matter

Rys. 3. Bezwzględny wskaźnik deterentności (Bwd) (A) wyciągów z suchej i świeżej masy dziurawca zwyczajnego w różnych stężeniach (od 2–10% oraz 10–30%) dla larw L3 stonki ziemniaczanej, wyliczony wg wzoru: $Bwd = [(K-T) : (K+T)] \cdot 100$, gdzie: K – średnia masa zjedanego pokarmu w kontroli [mg]; T – średnia masa zjedanego pokarmu w badanym obiekcie [mg] oraz wskaźnik smakowitości (B), wyliczony jako stosunek procentu zjedzonej masy liścia w poszczególnych obiektach do procentu masy zjedzonej w kontroli. S – sucha masa, SW – świeża masa



Source: own work / Źródło: opracowanie własne

Fig. 4. Absolute deterrence index (A) of extracts from dry and fresh matter of St. John's wort for larvae L4 Colorado potato beetle and palatability index (B). See figure 3 for explanations

Rys. 4. Bezwzględny wskaźnik deterentności (A) wyciągów z suchej i świeżej masy dziurawca zwyczajnego dla larw L4 stonki ziemniaczanej oraz wskaźnik smakowitości (B). Objaśnienia jak na rys. 3

All used *H. perforatum* extracts were also characterized by inhibitory effect on the feeding of L4 larvae of potato beetle (Fig. 4). In this case, higher values of the absolute deterrence index were reported for extracts prepared from fresh matter. On objects, where dried plant was used, these values increased with the increase of concentration of a given extract, but no such tendency was observed for extracts from fresh matter. On the other hand, the palatability index attained similar values on individual objects. These results correspond to the experiment, which tested the effect of aqueous extracts from absinthium toward larvae and adult individuals of the potato beetle [20].

No significant reaction of adult potato beetle (expressed by the number of entries in selected field of olfactometer) to aromatic stimuli from the side of wort were observed (Table 1). *L. decemlineata* females visited the field with flowing scent of the St. John's wort slightly more frequently than the control field, whereas males' response was reversed, but in both these cases the differences were not statistically proven.

Table 1. Responses of *Leptinotarsa decemlineata* Say imagines to odors derived from *Hypericum perforatum* L. fresh matter expressed as a number of entrance per one insect into selected areas of Y-tube olfactometer. Control – without flowing scent, *Hypericum perforatum* L. – with flowing scent

Tab. 1. Odpowiedź imago *Leptinotarsa decemlineata* Say wobec substancji zapachowych pochodzących od świeżego dziurawca (*Hypericum perforatum* L.), wyrażona liczbą wejść w poszczególne pola olfaktometru Y w przeliczeniu na 1 osobnika. Kontrola – bez dopływu zapachu, *Hypericum perforatum* L. – z dopływem zapachu

Pest	Control	<i>Hypericum perforatum</i> L.	t	P
Females	0,250	0,375	-0,509	0,619
Males	0,500	0,375	0,475	0,642

Source: own work / Źródło: opracowanie własne

4. Discussion

The conducted experiment demonstrated, that aqueous extracts of St. John's wort at concentrations of at least 5-10% for dried plant and 20-30% for fresh matter inhibit the feeding activity of primarily L3 larvae of potato beetle. Older larvae also foraged to a lesser extent on the plants treated with extracts, but in this case significant changes were determined only for the extracts from fresh matter at

concentration of 20 and 30%. The available literature provides little information on the influence of *H. perforatum* in the aspect of natural plant pest management, and the results obtained by scientists are inconsistent, depending on the type of extract used, as well as the species of pest. Pavela [17] conducted study on the level of discouragement of *L. decemlineata* Say larvae to feed via the use of alcoholic extract from i.a. *H. perforatum* flowers. A dosage of 500 µg/cm² of potato leaf exhibited 98.5% efficiency, with ED₅₀ doses of 94 µg/cm² and ED₉₀ of 117 µg/cm². On the other hand, a 40% alcoholic extract (on the basis of methanol, distilled water and 10% acetone) from *H. perforatum* used for spraying L4 *L. decemlineata* larvae turned out to be ineffective in comparison to other plant extracts (the efficiency of the extract was 2.2% at 34.9% larvae mortality with the use of the extract from goosefoot (*Chenopodium album* L.)) [8]. However, alcoholic extract from St. John's wort (100 g of ground dried plant/ 500 ml of methanol, stored for 24 hours) exhibited high efficiency (92.3%, LD₅₀=496 ppm, LD₉₀=980 ppm) in controlling mosquitoes *Culex quinquefasciatus* Say. (Diptera, Culicidae) [18]. Essential oils possess considerably stronger toxic effect towards insects. An application of 5, 10 and 20 µl of oil was conducted (corresponding to 1.25; 2.5 and 5 µl/l) to filter paper disk placed in a Petri dish. Dosage of 20µl of oil from above-ground parts of *Hypericum scabrum* L. caused 100% mortality of *Bruchus dentipes* Baudi already in 36th hour of experiment [23].

Numerous studies have been conducted on the management of potato beetle with the use of extracts from other herbaceous plants. In comparison to the results of these studies, the aqueous extract of St. John's wort tested by us is characterized by relatively low potential of inhibiting feeding of potato beetle larvae. 10% aqueous extracts from plants of the *Geraniaceae* family used for L3 *L. decemlineata* larvae significantly limited their feeding, and the strongest effect was observed for the extract from *Pelargonium x hortorum* L., which is reported by Lamparski and Wawrzyniak [14]. The absolute deterrence index was in this case over two times higher (Adi = 56) in comparison to our result for 10% extract from dried *H. perforatum* (Adi for L3 larvae = 29.1). In the above study [14] it was observed, that under the influence of extracts, the L3 larvae consumed considerably lower amounts of food than in control (assumed as 100%), i.e. from 28.7% for *Pelargonium x*

hortorum L. to 55.3% for *Erodium cicutarium* L. These results correspond to the results obtained in the present study, in which on all objects decrease in the weight of consumed potato leaves was obtained in comparison to control, however, these differences were not that significant (from 54.9% for the 10% extract from dried plant to 70.5% for 2% extract from dried *H. perforatum*). In the study of Wawrzyniak and Lamparski [24], the highest values of the absolute deterrence index toward L3 potato beetle larvae were exhibited by the 10% aqueous extracts from caraway (*Carum carvi* L.) (Adi = 46) and fennel (*Foeniculum capillaceum* Gilib.) (Adi = 43).

The efficiency of numerous measures (including the use of natural plant protection products) is strongly dependent on the developmental stage of the insect. In the papers of Lamparski and Wawrzyniak [14, 24], the efficiency of the analyzed extract was considerably higher for beetles of Colorado potato beetle, which is demonstrated by considerably higher values of the absolute deterrence index than in the case of the larvae. On the other hand, in our study values of the absolute deterrence index than in the case of the larvae. On the other hand, in our study values of the absolute deterrence index suggest higher susceptibility to the effect of aqueous extracts from St. John's wort in the case of younger potato beetle larvae.

Olfactory reactions of agrophages also have significant influence on their feeding intensity [10, 13]. In our study, *H. perforatum* did not exhibit a significant effect on the reaction to aromatic stimuli of the adult forms of potato beetle. As a comparison, the study of Rusin et al. [20] demonstrated with the use of olfactometer a strong deterrent reaction of aromatic substances from absinthium for males of potato beetle.

5. Conclusions

1. Aqueous extracts from St. John's wort exhibit inhibitory effect on feeding of potato beetle larvae at a concentration of at least 5% and 10% for dried plant and 20–30% for fresh plant matter and the effect is stronger for L3 larvae than L4 larvae.
2. However, the comparatively low values of the absolute deterrence index suggest limited possibility to use aqueous extracts from the plant for the inhibition of potato beetle larvae feeding.
3. Tests with the use of olfactometer did not exhibit a deterrent reaction of aromatic substances from St. John's wort to adult individuals of potato beetle (both females and males).

6. References

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The study implemented within the framework of the specific grant for conducting scientific study or development works and the associated tasks, contributing to the development of young scientists and participants of doctoral studies of the Agricultural University, financed by means of competition in 2015 – topic no. 4171 and by the Ministry of Science and Higher Education of the Republic of Poland.