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THE EFFECT OF WATER EXTRACTS FROM *ARTEMISIA ABSINTHIUM* L. ON FEEDING OF *LEPTINOTARSA DECEMLINEATA* SAY. LARVAE

Summary

The aim of this study was to determine the effect of water extracts from dry and fresh matter of Artemisia absinthium L. on feeding of Leptinotarsa decemlineata Say. L2 stage larvae. Dried extracts were prepared in concentrations of 2%, 5% and 10%, while the fresh plant in concentrations of 10%, 20% to 30%. Larvae feeding intensity assessment was carried out by dipping leaves of potato in respective solutions of the extracts, and then determining the weight of food eaten by larvae, and changes of larvae body weight once a day. In addition, absolute deterrence index and palatability index were calculated. The results of the experiment show that the extracts prepared from both dry and fresh matter at the highest concentrations (10% and 30% respectively) contributed to the greatest reduction of the larvae feeding and significant decreased their body weight. With increasing concentrations of extracts from both fresh and dry matter, their deterrent effect on the tested larvae was usually increasing while the value of palatability index was decreasing.
***Key words**: water extracts, biological control, Artemisia absinthium, Colorado potato beetle*

WPŁYW WODNYCH WYCIĄGÓW Z BYLICY PIOŁUNU (*ARTEMISIA ABSINTHIUM* L.) NA ŻEROWANIE LARW STONKI ZIEMNIACZANEJ (*LEPTINOTARSA DECEMLINEATA* SAY.)

Streszczenie

Celem przeprowadzonych badań było określenie oddziaływania wodnych wyciągów z suchej i świeżej masy bylicy piołunu (Artemisia absinthium L.*) na żerowanie larw L_2 stonki ziemniaczanej (Leptinotarsa decemlineata* Say.*). Wyciągi przygotowano w stężeniach 2, 5 i 10% dla suchej masy oraz 10, 20 i 30% dla świeżej masy. Ocena intensywności żerowania larw została przeprowadzona poprzez moczenie liści ziemniaków w roztworach odpowiednich wyciągów, a następnie określenie masy pokarmu zjedzonego przez larwy oraz zmiany masy ich ciała, z częstotliwością raz na dobę. Dodatkowo obliczono bezwzględny wskaźnik deterentności i smakowitości. Na podstawie badań stwierdzono, że wyciągi sporządzone zarówno z suchej, jak i ze świeżej masy w najwyższych stężeniach przyczyniły się do największego ograniczenia żerowania larw oraz spowodowały istotny spadek masy ich ciała. Wraz ze wzrostem stężenia wyciągów z suchej oraz ze świeżej masy najczęściej zwiększało się także ich deterentne oddziaływanie w stosunku do analizowanego szkodnika, a także zmniejszała się wartość wskaźnika smakowitości.*
***Słowa kluczowe**: wyciągi roślinne, ochrona biologiczna, bylica piołun, stonka ziemniaczana*

1. Introduction

Colorado potato beetle (*Leptinotarsa decemlineata* Say.) is one of the most dangerous pest of potatoes, but it can also damage other plants of the Solanaceae family like tomatoes and eggplants. Both adults and larvae are harmful because they feed on the above-ground parts of the plants. Their harmfulness is associated primarily with high fertility and voracity. Yield losses caused by feeding of this pest can reach up to 60-90% [1, 2].

Application of chemicals is one of the most popular methods to protect plants against *Leptinotarsa decemlineata* Say. but these pests are often able to immunize to the active substances contained in these products [3, 4]. In addition, due to the risks posed by pesticides for the whole environment, non-chemical methods which may equally limit feeding of these pests are currently sought [5, 6].

The aim of this study was to determine the effect of water extracts from dry and fresh matter of *Artemisia absinthium* L. on the feeding of *L. decemlineata* Say. L2 stage larvae. Furthermore absolute deterrence index and palatability index were calculated.

2. Material and methods

Fresh leaves of potato and *Leptinotarsa decemlineata* Say. L2 stage larvae used for the analysis at the end of June 2014 were collected. The experiment was carried out in the laboratory, in six replicates. Extracts from dry matter (DM) of *Artemisia absinthium* L. were prepared at concentration conventionally adopted as 2%, 5% and 10% (dried plants + cold double-distilled water in proportions of 2:100, 5:100 and 10:100) and at concentration of 10%, 20% and 30% for fresh matter (FM) (fresh above-ground parts of plants + cold double-distilled water in proportions of 10:100, 20:100 and 30:100). Extracts were stored in the dark at room temperature over a period of 24 hours. After this period they were filtered through filters paper and immediately used to perform the experiments. Potato leaves were dipped for 3 seconds in an adequate plant extracts and in distilled water which was a control object, and then dried at room temperature. The test was carried out in Petri dishes, and as a substrate wet filter paper was used. In each dish two leaves of potato, suitable for a specific object were placed and then two *L. decemlineata* Say. larvae (L2) were introduced.

In determining the effect of extracts of *A. absinthium* L. on *L. decemlineata* Say. food weight eaten by larvae and changes of larvae body weight once a day were established. In addition, values of palatability index as the percentage ratio of the weight of leaves eaten in individual objects to

the percentage weight of leaves eaten in the control were calculated. Furthermore absolute deterrence index which takes into account the relationship between the weight of leaves eaten by larvae in the individual objects and the weight of leaves eaten in the control was established[7]:

$$Bwd = [(K–T) : (K+T)] \cdot 100, \quad (1)$$

where:
Bwd - absolute deterrence index,
K – weight of leaves eaten in control [mg],
T – weight of leaves eaten in individual objects [mg].

The obtained results were then subjected to analysis by STATISTICA 10.0 software. The significance of differences between the means were tested by univariate analysis of variance, and the means were differentiated by Fisher's LSD test at $\alpha = 0.05$.

3. Results and discussion

The use of plant extracts to control or limit the feeding of pests is increasingly important, especially because of the safety of this method for all living organisms and for the environment [6].

In this experiment extract prepared from dry matter of *A. absinthium* L. at the highest concentration (10%) in all terms of observations contributed to a significant decrease in the weight of food eaten by the larvae of *L. decemlineata* Say. (Table 1). After the end of the experiment the weight of leaves eaten by larvae in this object reached 31.4 mg and was over 60% lower than in the control. Similar regularities were also reported in the case of the extract from fresh matter at concentration of 30%, however, statistically significant differences in relation to the control object only in the first two terms of the observations were observed, when the weight of food eaten by larvae was lower by 20 mg and 14.8 mg respectively. Other extracts had no significant effect on the analysed parameter.

Table 1. The effect of extracts from *Artemisia absinthium* L. on the food weight eaten by the larvae of *Leptinotarsa decemlineata* Say. [mg]
Tab. 1. Wpływ wyciągów z bylicy piołunu na masę pokarmu zjedzonego przez larwę stonki ziemniaczanej [mg]

Object	Time			
	8 h	20 h	44 h	68 h
C	33,3 b	36,2 b	68,6 b	85 b
DM 2%	32,9 b	41,5 b	58,9 b	74 b
DM 5%	13,6 ab	25,2 ab	46,0 ab	50,2 ab
DM 10%	9,5 a	15,3 a	24,7 a	31,4 a
FM 10%	42,3 b	54,9 b	67,4 b	83,1 b
FM 20%	36,9 b	49,8 b	68,3 b	71,7 ab
FM 30%	10,3 a	21,4 a	34,7 ab	44,5 ab

C – control, DM – dry matter, FM – fresh matter. Values for individual terms of observations marked by different letters are statistically different ($\alpha = 0.05$)
Source: own work / Źródło: opracowanie własne

Adverse effect of the highest concentrations of the extract of *A. absinthium* L. on the *L. decemlineata* Say. larvae feeding was probably due to the chemical composition of the tested plant. *A. absinthium* L. contains flavonoids, tannins, organic acids, mineral salts, as well as approximately 0.5% of essential oil, which components are thujone, phellandrene, pinene and other compounds [8]. Tabachnik [9] showed that flavonoids are acting as a natural insecticides, limiting feeding of pests. Tannins, in turn, are characterized by a toxic effect against some insects, which is associated with initiation of reactions leading to the formation of large amounts of reactive oxygen forms [10] and with inhibition of the activity of many enzymes [11]. However, it is difficult to determine the significance of the individual compounds because the effect of secondary metabolites on insect does not result from the interaction of the individual substances but they affect the insects as a mixture of all those chemical compounds, as emphasized by many authors [12, 13].

Extracts prepared from both dry and fresh matter at the highest concentrations (10% and 30% respectively) in all terms of observations resulted in a significant decrease in larvae body weight compared to the control object (Table 2). Subzero values of the analysed parameter in these objects indicate that the weight of larvae body got lower in the particular terms of observation. In addition, it was observed that after 44 hours from the setting up the experiment, extract from dry matter at concentration of 5% and from fresh matter at concentration of 20% also contributed to a significant decrease in the value of the selected parameter.

Studies on the effect of water extracts from herbal plants for *L. decemlineata* Say. larvae feeding were also conducted by other authors. Lamparski and Wawrzyniak [14] found that extracts from dry matter of *Geranium pusilum* L., *Geranium robertanium* L. and *Pelargonium x hortorum* Bailey at concentration of 10% resulted in weight body loss of larvae by almost 70% after 48 hours. Extract from dry matter of *A. absinthium* L. at concentration of 10% used in our experiment also caused weight body loss of larvae by nearly 64% after 44 hours. In other studies Lamparski and Wawrzyniak [11] also confirmed that water extracts from *Pelargonium x hortorum* Bailey at concentration of 10% caused a reduction in body weight of *L. decemlineata* Say. larvae of about 23 mg compared to the control object.

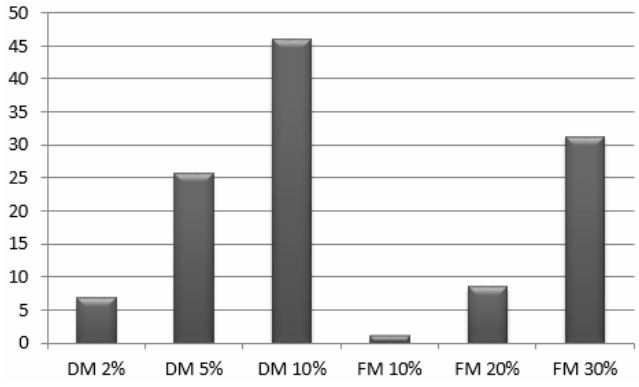
Table 2. The effect of extracts from *Artemisia absinthium* L. on changes in larvae body weight [mg]
Tab. 2. Wpływ wyciągów z bylicy piołunu na zmiany masy ciała larw stonki ziemniaczanej [mg]

Object	Time			
	8 h	20 h	44 h	68 h
C	8,2 b	6,8 b	13,4 c	9,7 b
DM 2%	6,4 b	6,4 b	5,3 bc	3,9 ab
DM 5%	0,6 ab	2,6 ab	0,2 ab	1,1 ab
DM 10%	-0,2 a	-1,0 a	-0,4 a	-1,6 a
FM 10%	4,0 ab	7,4 b	3,8 bc	4,5 b
FM 20%	5,2 ab	2,3 ab	1,6 b	3,1 ab
FM 30%	-0,2 a	-1,2 a	-0,8 a	-1,2 a

C – control, DM – dry matter, FM – fresh matter. Values for individual terms of observations marked by different letters are statistically different ($\alpha = 0.05$)
Source: own work / Źródło: opracowanie własne

All extracts used in our experiment were characterized by inhibitory effect on feeding of analysed pest as evidenced by the positive values of absolute deterrence index (Fig. 1). The highest values of this index after the application of extract from dry matter at concentration of 10% and extract from fresh matter at concentration of 30% were obtained (46.0 and 31.3 respectively). With increasing con-

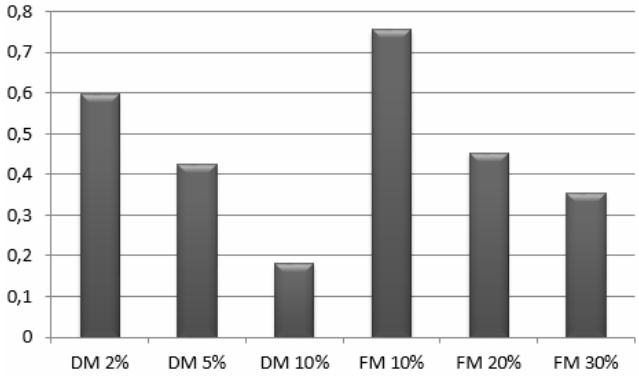
centrations of extracts from both fresh and dry matter, their deterrent effect on the tested larvae usually rose. Similar results were obtained by Wawrzyniak and Lamparski [15], who studied the effects of extracts from herbal plants on feeding of *L. decemlineata* Say. These authors found that increase of extracts' concentration from *Ocimum basilicum* L. and *Origanum majorana* L caused a reduction of adult beetles feeding, while extracts from *Saponaria officinalis* L. and *Thymus vulgaris* L. – a reduction of larvae feeding. These same authors in other studies [11] found that extracts from *Pelargonium x hortorum* Bailey and *Geranium sanquineum* L. at concentration of 10% inhibited mostly the *L. decemlineata* Say. larvae feeding, and calculated values of absolute deterrence index were approximately 50.



Source: own work / Źródło: opracowanie własne

Fig. 1. Absolute deterrence index
Rys. 1. Bezwzględny wskaźnik deterentności

Palatability index in all analysed cases was lower than 1, which indicates that the loss of leaf area caused by *L. decemlineata* Say. larvae feeding was lower in objects with extracts from *A. absinthium* L. comparing to the control object (Fig. 2).



Source: own work / Źródło: opracowanie własne

Fig. 2. Palatability index
Rys. 2. Wskaźnik smakowitości

The highest value was observed in the object, in which extract from fresh matter at concentration of 10% was used. It indicates that this extract the least restricted feeding of larvae. Meanwhile, the lowest value of palpability index was registered after applying the extract from dry matter at concentration of 10% (0.18). Dankowska [16], Dankowska and Robak [17] found that the increasing concentration of brews from the leaves of *Rhododendron* sp, *Abies alba* Mill. and *Lycopersicon esculentum* Mill. from 5% to 10% reduced the value of this index by about 0.2 in relation to *Deroceras laeve* Müll. In our experiment it was also found that with increasing concentrations of the analysed extracts the value of palpability index decreased.

There have been only a limited number of studies conducted to the date on the effect of water extract from *A. absinthium* L. on feeding of pests. The literature shows that only the effect of essential oils extracted from this plant has been evaluated. However essential oils are characterized by a higher concentration of the compounds than conventional water extracts. Essential oils from *A. absinthium* L. at concentration of 0.04 $mg \cdot cm^{-2}$ influenced mortally on *Tetranychus urticae* Koch [18], and their effect was dependent on the rate and the method of their extraction. Other authors found that essential oil at rate $9\mu l \cdot l^{-1}$ of air after 48 hours contributed to 80-90% mortality of *Sitophilus granarius* L. [19]. The essential oils of other plants belonging to the Asteraceae family are characterized by the strong toxicity against storage pests [20-23], *Aphis gossypii* Glover and *Thrips tabaci* Lindman [24].

4. Conclusions

1. Extracts prepared from both dry and fresh matter of *A. absinthium* L. at the highest concentrations (10% and 30% respectively) contributed to the greatest reduction of the *L. decemlineata* Say. larvae feeding and significantly decreased their body weight.
2. With increasing concentrations of extracts from both fresh and dry matter, their deterrent effect on the tested larvae usually increased while the value of palatability index decreased.
3. The use of plant extracts may be an effective way to reduce feeding of pests, however, due to still limited resource of available literature research in this area should be continued.

5. References

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