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EFFECT OF WATER EXTRACTS FROM *GERANIACEAE* PLANTS WITH ADJUVANT ADDED ON FEEDING AND DEVELOPMENT OF COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY)

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Abstract: Water extracts from selected *Geraniaceae* plants, to which paraffin oil was added as adjuvant, were tested. It was observed that the plant extracts researched limited Colorado potato beetle feeding and development and adding adjuvant increased the effects. The highest antifeedant activity towards Colorado potato beetles and their larvae was observed in extracts obtained from *Pelargonium* × *hortorum* Bailey and *Geranium pusillum* L. The extract from *Pelargonium* × *hortorum* Bailey added to food showed a negative effect on the development of female reproductive organs and embryo development and showed the highest effectiveness in field conditions.

Key words: Colorado potato beetle, *Geraniaceae*, plant extracts, adjuvant

INTRODUCTION

Potato (*Solanum tuberosum*) is attacked by a number of agrophages, out of which Colorado potato beetle represents those most dangerous. Losses caused by feeding of its larvae and beetles on potato plantations can be up to 10% of the annual yields. A considerable part of insecticides used in agriculture is allocated every year to control the pest (Szafranek et al. 1998), which, as a result, leads to Colorado potato beetle soon becoming resistant to most of the chemical pesticides (Dubis et al. 1995). For that reason it is important to search for new non-chemical methods of control. Natural substances contained in plants seem very promising (Wyrostkiewicz 1995). Research is being carried out with the intention of determining plants whose extracts show an effect on insects. Some promising results were recorded, e.g. for *Geraniaceae* (Wyrostkiewicz 1992). The research reported by Lamparski and Wawrzyniak (2004) showed that secondary metabolites contained in *Geraniaceae* were active towards Colorado potato beetle, however the activity was

too low to compete successfully with chemical pesticides. While searching for methods of increasing their effectiveness, a working hypothesis was developed which assumed that adding adjuvant to plant extracts should increase their activity towards insects.

Adjuvants are supplementary substances which modify and enhance the biological activity of pesticides, increasing the range of their effects (Wachowiak et al. 1996). They limit the effect of unfavourable environmental factors during treatment, and do not affect agrophages adversely (Adamczewski and Matysiak 1997; Kucharski 1999).

The aim of the present research was to define the effect of water extracts from selected *Geraniaceae*, to which paraffin oil was added as adjuvant, on feeding of Colorado potato beetles and larvae and on the development of the pest.

MATERIAL AND METHODS

Research carried out over 1999–2000 covered the laboratory experiments performed in the laboratory of the Department of Applied Entomology as well as field observations – on experimental plots located at Mochełek in the vicinity of Bydgoszcz, of the Experiment Station of the University of Technology and Agriculture. The effect of water extracts of six selected *Geraniaceae* plant species was analyzed: *Pelargonium* × *hortorum* Bailey, *Erodium cicutarium* L., *Geranium pusillum* L., *Geranium sanquineum* L., *Geranium pratense* L., *Geranium robertianum* L.; the paraffin oil was added as adjuvant.

The plants were collected by cutting overground parts (herbage) over flowering from meadows, barren land, boundary strips and road-sides in the vicinity of Bydgoszcz. *Pelargonium* × *hortorum* Bailey was obtained from a horticultural plantation. The plants were dried at room temperature and then ground with WŻ-1 laboratory grinder. The dried plant material was stored in hermetic glass containers.

The ground dried plant material was poured for 24 hours with cold water, 100ml of water per 10 g of dried material, and filtered to obtain the extract (defined as 10%) to which adjuvant, paraffin oil, was added, following the dose recommended (1.5 l of oil per 400 l of water = extract). For each experimental series fresh plant extract was prepared. Potato leaves weighed under laboratory conditions were dipped for 3 seconds in an adequate plant extract, and once they had dried (on the filter paper), they were put onto Petri dishes. Under field conditions the extracts were placed onto plants with Kwazar pressure sprayer (1 liter of extract per 25 sq*m of the plot).

Wintering Colorado potato beetles were sampled from plantations of the Mochełek Experiment Station of the University of Technology and Agriculture. The eggs, larvae and beetles of the summer generation obtained were used in laboratory experiments.

In the laboratory experiments the effect of plant extracts was tested on:

1. feeding of Colorado potato beetles and larvae. 1 couple of winter generation beetles and 10 L2 stage larvae were placed onto each Petri dish (as a replication). Based on the differences in the weight of food consumed by insects on Petri dishes (4 replications mean), the following were calculated: weight of the food

consumed by a beetle couple or larva, increase in the larva body weight, relative deterrence index (for beetles and larvae) – bwd (Kiełczewski et al. 1979).

2. on the development of insects:
   – oviposition. The total number of eggs laid by 1 couple of winter generation Colorado potato beetles, fed with potato leaves treated with plant extracts, was compared with the number of eggs laid by beetles fed with non-treated leaves (control defined as 100%).
   – course of larvae hatch. The egg deposits were treated with selected extracts placed with a brush. After 5–7 days the hatch of larvae was evaluated (in %).
   – pupation of larvae. Summer generation Colorado potato beetles which emerged after pupation were weighed and counted.
   – gonads development. Ovarial tubes were counted and 10 randomly selected tubes each, obtained from geranium-extract-fed insects, were measured (Błażejewska 1965), with stereoscopic microscope equipped with measuring eyepiece.

   The following were defined in field experiments:
   – Effectiveness of extracts (following the Henderson–Tilton formula (Sas-Piotrowska 1992) towards beetles and larvae observed 2 and 6 days after the treatment.

$$Sk = \left(1 - \frac{K_1 x T_2}{K_2 x T_1}\right) \times 100$$

   where: Sk – effectiveness of extracts
   K_1 – number of insects on control field prior to treatment
   K_2 – number of insects on control field after treatment
   T_1 – number of insects on test plot prior to treatment
   T_2 – number of insects on test plot after treatment
   – Oviposition. The number of egg deposits noted on plants treated with extracts after 2 and 6 days after treatment against the number of egg deposits prior to treatment (in %).
   – Wintering of imagines fed with geranium extracts. The number of beetles emerging in spring was compared with the number prior to their coming down to soil for the period of winter diapause.

The results obtained were statistically verified with single-factor variance analysis method as completely randomized design. The significance of the differences between means for respective factor levels was defined with the Tukey confidence semi-intervals at $\alpha = 0.05$.

RESULTS

Laboratory experiments

1. Effect of water extracts on feeding of Colorado potato beetles and larvae.

1.1. In the tests in which extracts from *Geranium sanquineum* L., *Erodium cicutarium* L. and *Pelargonium* × *hortorum* Bailey were used, imagines were only slightly biting the leaves and stopped feeding. The greatest weight of the food consumed was ob-

served in the treatment with the extract from *Geranium pusillum* L. (88mg, which accounts for 18.5% of the weight of the food consumed in the control) (Table 1). The differences between weight of the food consumed in respective treatment and the control were shown to be significant. In all the tests (Fig. 1) very high bwd values were obtained (70–97).

1.2. The lowest weight of the food consumed over 48 hours (which accounted for 35–36% of the control) was observed in the experimental variant in which the potato leaves were treated with *Geranium pusillum* L. and *Pelargonium* × *hortorum* Bailey extracts. In all the tests were recorded lower larva body weight gains than in the control. The lowest values were noted following the treatment of potato leaves with *Geranium robertianum* L., *Geranium pusillum* L. and *Pelargonium* × *hortorum* Bailey (30.4–32.6% of the larva body weight gain as compared with the control gain) (Table 1). The highest value of the absolute deterrence index was obtained in the tests in which the larvae were treated with leaves with *Pelargonium* × *hortorum* Bailey and *Geranium pusillum* L. extracts: 51.8 and 56.4 (Fig. 1).

2. Effect of extracts on the development of Colorado potato beetle

2.1. Oviposition. The number of eggs laid by beetles ranged from 54% against the eggs laid in the control (in the experimental variant in which the beetles were fed with potato leaves treated with *Geranium pratense* L. extract) to 122% (with the *Geranium pusillum* L. extract) (Table 2).

2.2. Course of larvae hatch. The lowest number of larvae was recorded in the experimental variant in which *Geranium pusillum* L. and *Pelargonium* × *hortorum* Bailey extracts were used (larvae hatched from 55% of eggs) (Table 2).

2.3. Pupation. The lowest number of beetles coming out from the ground was observed in the tests in which larvae were fed with potato leaves with *Erodium cicutarium* L. and *Geranium sanquineum* L. extracts (35.3 and 38.2% as compared with the control). The imagines body weight obtained in respective tests ranged from 67 mg (*Geranium robertianum* L.) to 81 mg (*Erodium cicutarium* L.) and 102 mg (control) (Table 2).

Table 1. Effect of water extracts from Geraniaceae plants with adjuvant added on feeding of Colorado potato beetle and larvae in laboratory conditions

Plants	Weight of food consumed by 1 couple of beetles		Weight of food consumed by larvae		Larvale body weight gain	
	mg	% of control	mg	% of control	mg	% of control
Pelargonium × *hortorum*	15.0 a	3.2 a	54.0 a	36.5 a	15.0 a	32.6 a
Erodium cicutarium	14.0 a	2.9 a	92.0 b	62.2 b	43.0 c	93.5 c
Geranium pusillum	88.0 a	18.5 a	52.0 a	35.1 a	14.0 a	30.4 a
Geranium sanquineum	6.0 a	1.3 a	66.0 ab	44.6 ab	29.0 b	63.0 b
Geranium pratense	30.0 a	6.3 a	73.0 ab	49.3 ab	37.0 bc	80.4 bc
Geranium robertianum	55.0 a	11.2 a	79.0 ab	53.4 ab	14.0 a	30.4 a
Control	476.0 b	100.0 b	148.0 c	100.0 c	46.0 c	100.0 c
LSD	101.2	21.3	34.5	23.3	10.9	23.7

Means followed by the same letters in columns did not differ significantly at $\alpha = 0.05$

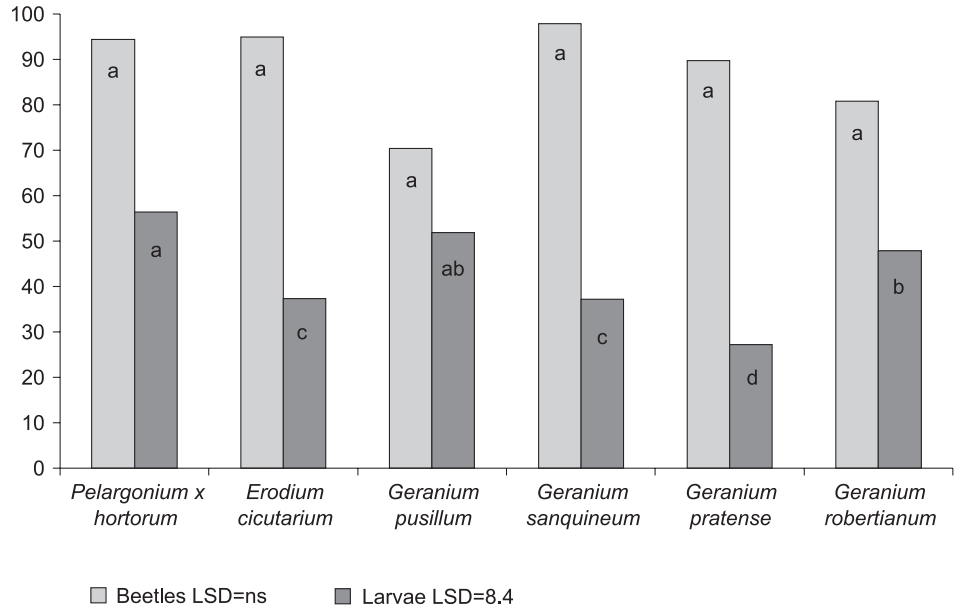

Fig. 1. Absolute deterrence index (bwd)

Table 2. Effect of water extracts from Geraniaceae plants with adjuvant added on Colorado potato beetle oviposition, larvae hatching and pupation

Plants	Oviposition		Larvae hatching			Pupation		
	Number of eggs laid by 1 couple	% of control	Number of eggs	Number of larvae	%	Number of beetles	% of control	Beetle weight mg
Pelargonium × hortorum	412.0 a	87.1 a	31.0	17.0 a	54.8 a	5.5 a	64.7 a	74.0 a
Erodium cicutarium	269.0 a	56.9 a	26.5	19.5 a	73.6 a	3.0 a	35.3 a	81.0 a
Geranium pusillum	58.0 a	122.6 a	29.5	16.3 a	55.1 a	4.3 a	50.0 a	68.0 a
Geranium sanguineum	330.0 a	69.8 a	20.0	19.0 a	95.0 a	3.3 a	38.2 a	70.0 a
Geranium pratense	258.0 a	54.5 a	20.5	18.0 a	87.8 a	3.8 a	44.1 a	72.0 a
Geranium robertianum	347.0 a	73.4 a	19.5	13.0 a	66.7 a	4.0 a	47.1 a	67.0 a
Control	473.0 a	100.0 a	29.0	23.0 a	79.3 a	8.5 b	100.0 b	102.0 b
LSD	ns	ns	–	ns	ns	3.1	36.47	18.6

Means followed by the same letters in columns did not differ significantly at $\alpha = 0.05$
n.s. – not significant

2.4. Gonads development. Based on the calculations, it was stated that the number of ovarioles and their length in the Colorado potato beetle females tested, obtained in larvae fed with potato leaves treated with geranium water extracts with adjuvant, were significantly lower than in the control (Table 3).

Field experiments

3. Effectiveness of extracts towards Colorado potato beetles and larvae

3.1. In both research years, the significantly strongest effect on beetles was observed for *Geranium sanquineum* L. and *Pelargonium × hortorum* Bailey extracts (68.9

Table 3. Effect of water extracts from *Pelargonium* × *hortorum* with adjuvant added on the development of gonads in Colorado potato beetle females

Gonad development	*Pelargonium* × *hortorum*	Control	LSD
Number of ovarioles	29.8 a	39.8 b	3.7
Length of ovarioles [μm]	589 a	696 b	50

Means followed by the same letters in lines did not differ significantly at $\alpha = 0.05$

and 68.5%, respectively). The extracts researched also showed a high effectiveness towards larvae (Fig. 2). The strongest effect was observed in the *Geranium pusillum* L. extract tested (61%).

3.2. Oviposition. In the experimental variant in which *Pelargonium* × *hortorum* Bailey, *Erodium cicutarium* L. and *Geranium robertianum* L. extracts were applied, both 2 and after 6 days after treatment, Colorado potato beetle females did not lay eggs (Table 4).

3.3. Wintering of imagines. The observations did not show a significant effect of the *Pelargonium* × *hortorum* Bailey extract on Colorado potato beetles wintering (non-significant differences, as compared with the control). 32.5% of the beetles in the control sample survived the winter diapause, however after the application of water extract with adjuvant added – 24.32%.

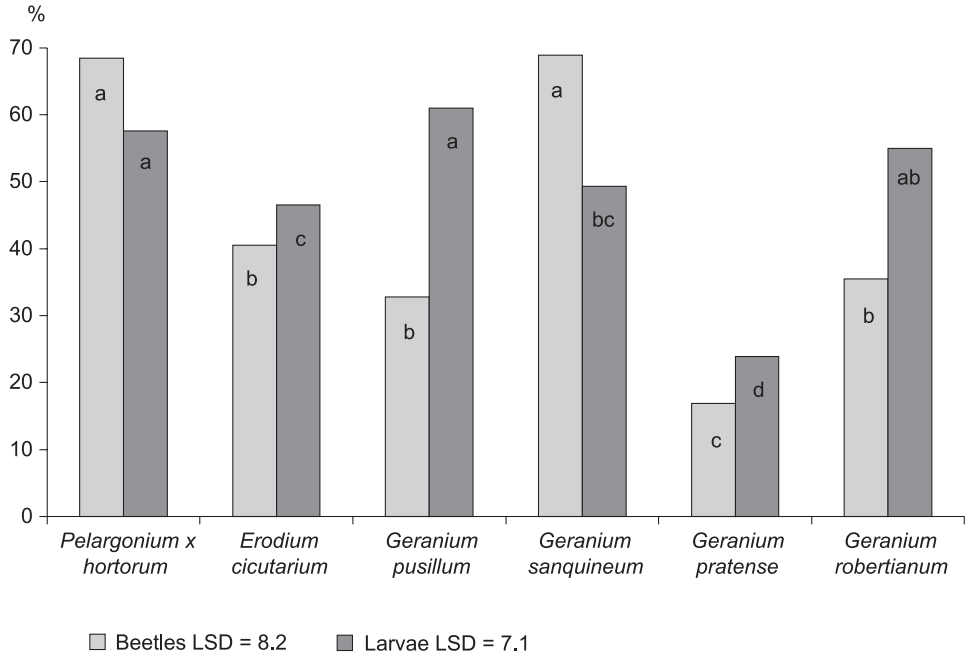

Fig. 2. Effectiveness of water extracts from Geraniaceae plants with adjuvant added towards winter generation of Colorado potato beetle and larvae (2-year mean)

Table 4. Effect of water extracts from Geraniaceae plants with adjuvant added on the course of oviposition by winter generation Colorado potato beetle (2-year mean)

Plants	Egg deposits				
	Number prior to treatment	After 2 days		After 6 days	
		Number	% of the total before treatment	Number	% of the total before treatment
Pelargonium × hortorum	5.4	5.4	0.0 a	5.4	0.0 a
Erodium cicutarium	8.1	8.1	0.0 a	8.1	0.0 a
Geranium pusillum	8.3	9.1	9.6 a	9.1	9.6 a
Geranium sanguineum	3.3	3.3	0.0 a	5.3	60.6 b
Geranium pratense	6.4	12.1	89.1 b	12.7	98.4 c
Geranium robertianum	5.9	5.9	0.0 a	5.9	0.0 a
Control	7.2	16.9	134.7 c	16.9	134.7 c
LSD	–	–	30.2	–	37.1

Means followed by the same letters in columns did not differ significantly at $\alpha = 0.05$

DISCUSSION

The present research showed that selected *Geraniaceae* plant water extracts tested, to which paraffin oil was added as adjuvant, limited both Colorado potato beetle and larvae feeding and Colorado potato beetle development. Both under controlled conditions and in field experiments a greater biological activity of the extracts tested towards the pest was observed, as compared with *Geraniaceae* plant water extracts without adjuvant added (Lamparski and Wawrzyniak 2004).

The inhibition of the Colorado potato beetle imagines feeding intensity towards *Geraniaceae* plant water extracts was also observed by Wyrostkiewicz (1992). Disturbances in feed absorption were also caused by *Polygonaceae* plant extracts (Wenda-Piesik and Wyrostkiewicz 1997; Piesik and Wyrostkiewicz 1999), as well as plant extracts obtained from common tansy – Schearer (1984) and Hough-Goldstein (1989). However, following the application of extracts obtained from *Fagara* spp. and *Azadirachta indica*, a considerable decrease in the Colorado potato beetle females fecundity was recorded (Ginesta et al. 1994; Schmutterer and Ascher 1987). Inhibiting the Colorado potato beetle larvae feeding by hogweed and wood angelica seed extracts was observed by Muckensturm et al. (1981), white oak leaf extracts – by Drummond and Casagrande (1985) and *Daphne gnidium* leaf extracts – by Perez et al. (1992), Perez and Ocete (1994).

The activity of extracts depends on chemical composition of the plants researched, and especially on the presence of secondary metabolites, e.g. flavonoids, polyphenols and tannins which fall into that group and which are considered to be most important as far as the behaviour of insects is concerned during food intake (Dreyer and Jones 1981; Kohlmünzer 1985; Hanczakowski 1988). They affect the insects as a mixture of all those chemical compounds (Leszczyński 1987; Wyrostkiewicz 1995).

In Poland it is recommended to apply adjuvants with numerous herbicides (Adamczewski and Matysiak 1997). There is also a growing interest in adding ad-

juvants to fungicides and insecticides. In Colorado potato beetle control it was much more effective to use pyretroids to which adjuvant was added (Wachowiak et al. 1996).

Geraniaceae extracts with paraffin oil added cannot completely replace chemical insecticides in Colorado potato beetle control, however their application is ecologically-justified and it can constitute an element of integrated control of the pest.

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POLISH SUMMARY

WPŁYW WYCIĄGÓW WODNYCH Z ROŚLIN BODZISZKOWATYCH (*GERANIACEAE*) Z DODATKIEM ADIUWANTU NA ŻEROWANIE I ROZWÓJ STONKI ZIEMNIACZANEJ (*LEPTINOTARSA DECEMLINEATA* SAY)

Testowano wodne wyciągi z wybranych roślin z rodziny bodziszkowatych (*Geraniaceae*) do których dodawano olej parafinowy jako adiuwant. Stwierdzono, że badane wyciągi roślinne ograniczają żerowanie i rozwój stonki ziemniaczanej, a dodawanie adiuwantu zwiększa aktywność ich oddziaływania. Najwyższą aktywnością antyfidantną w stosunku do chrząszczy i larw stonki, wyróżniały się wyciągi z pelargonii pasiastej i bodziszka drobnego. Wyciąg z pelargonii pasiastej dodany do pokarmu niekorzystnie wpłynął na rozwój narządów rozrodczych samic i rozwój embrionalny oraz wykazał najwyższą skuteczność działania w warunkach polowych.