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Bartosz PIECHOWICZ[1*], Kinga STAWARCZYK[1] and Michał STAWARCZYK[1]

INSECTICIDE AND FOOD CONSUMPTION OF SPANISH SLUG (*Arion lusitanicus* Mabille 1868)

INSEKTYCYDY A KONSUMPCJA POKARMU PRZEZ ŚLINIKA LUZYTAŃSKIEGO (*Arion lusitanicus* Mabille 1868)

Abstract: In the years 2007 and 2011, research was carried out on the impact of: pyrethroid group agents (beta-cyfluthrin, lambda-cyhalothrin, deltamethrin, esfenvalerate, alpha-cypermethrin, bifenthrin) benzoylphenyl ureas (teflubenzuron), derivatives of pyridine (pyriproxyfen), organophosphorus (diazinon) and neonicotinoid insecticides (acetamiprid) on the food consumption by Spanish slug (*Arion lusitanicus* Mab). The quantity of food consumed by animals treated by plant protection agents, the quantity of food treated by insecticides consumed and food preferences of *A. lusitanicus* individuals were analysed. The slugs were made available a selection between food with an addition of insecticide and without it. The results obtained indicate that the preparations which contained lambda-cyhalothrin and deltamethrin with which the animals were treated increase the quantity of food consumed by the Spanish slug. It was also shown that the food treated with lambda-cyhalothrin and alpha cypermethrin is consumed in a larger amount than the food not treated by this preparation. Deltamethrin and lambda-cyhalothrin and pyriproxyfen probably constitute food attractants for *A. lusitanicus* individuals and also alpha-cypermethrin, bifenthrin, beta-cyfluthrin and esfenvalerate are additive, which reduces the attractiveness of food for slug.

Keywords: Spanish slug, *Arion lusitanicus*, insecticides, attractants

Arion Lusitanicus Mabille 1868 is a slug, which naturally occurs on the Iberian Peninsula and the Azores [1]. Since more than 50 years the significant expansion of this species to other areas of Europe has occurred [1-6] which results in finding its populations in such climate-varied regions as Scandinavian Peninsula [7] or British Isles [8].

In Poland *A. lusitanicus* first appeared in the 1980 s. Initially it was observed only in the proximity of Albigowa Village in Podkarpacie [9], at the moment, it is present in the whole country [10]. As indicated by research of Kałuski et al [11] individual populations of this species, occurring in Poland are of different origin.

Arion lusitanicus together with *Deroceras reticulatum* and *Arion Rufus* are the most important pest among slugs, causing significant losses, especially in the cultivation of cruciferous family, although this polyphagic species feeds also on fruit, herb and meat

[1] Department of Ecotoxicology, Institute of Applied Biotechnology and Basic Sciences, University of Rzeszow, Werynia 502, 36-100 Kolbuszowa, Poland, phone +48 17 872 32 54
[*]Corresponding author: bpiechow@univ.rzeszow.pl

[12-16]. It also acts as a vector in transmission of diseases, the parasites of domestic animals included [17, 18]. It occurs most commonly in highly antropogenically degraded environments, preferring urban areas [19, 20] and its occurrence is limited by such factors as soil pH, its humidity and lime contents of the ground [21].

The problem that can be found together with Iberian slug invasion is the choice of adequate crop protective chemicals. It turns out that biocides used at present against pest in case of *A. lusitanicus* can have the opposite effect, not only do they not harm it, but they may be the alimentary attractant.

The purpose of the research carried out was to establish if commonly used insecticides of different spectrum of toxic action applied by contact and by ingestion influence the intensity of Iberian slug (*Arion lusitanicus*) feeding.

Materials and methods

Animals: The adult individuals of Iberian slug (*Arion lusitanicus*) collected in the area of Sanok and Werynia villages (province Podkarpackie) were used. Species identification was carried out on a representative sample of 21 individuals. Only the individuals without apparent signs of diseases were used. Altogether 384 *A. lusitanicus* individuals of an average body weight of 4.13 g were used for testing. Before each test, the animals were starved for 24 hours.

Insecticides: the insecticides tested contained different active substance of different chemical groups. They were used in doses recommended by producers of cruciferous plant protective chemicals, in particular:

- Bulldock 25 EC - an insecticide from the group of pyrethroids (active substance): beta-cyfluthrin - 25 g in 1 dm^3 of the agent). The concentration of the usable liquid: 0.5 cm^3 / 1 dm^3 H_2O,
- Karate-Zeon 050 CS an insecticide from the group of pyrethroids (a.s.: lambda-cyhalothrin - 50 g in 1 dm^3 of the agent). The concentration of the usable liquid: 0.5 cm^3 / 1 dm^3 H_2O,
- Decis 2.5 EC - an insecticide from the group of pyrethroids (a.s.: deltamethrin - 25 g in 1 dm^3 of the agent). The concentration of the usable liquid: 0.6 cm^3 / 1 dm^3 H_2O,
- Sumi-Alpha 050 EC - an insecticide from the group of pyrethroids (a.s.: esfenvalerate - 50 g in 1 dm^3 of the agent). The concentration of the usable liquid: 0.5 cm^3 / 1 dm^3 H_2O,
- Fastac 100 EC - an insecticide from the group of pyrethroids (a.s.: alpha-cypermethrin - 100 g in 1 dm^3 of the agent). The concentration of the usable liquid: 0.24 cm^3 / 1 dm^3 H_2O,
- Talstar 100 EC - an insecticide from the group of pyrethroids (a.s.: bifenthrin - 100 g in 1 dm^3 of the agent). The concentration of the usable liquid: 0.5 cm^3 / 1 dm^3 H_2O,
- Nomolt 150 EC - insecticide from the group of benzoylphenyl urea derivativee (a.s.: teflubenzuron - 150 g in 1 dm^3 of the agent). The concentration of the usable liquid: 0.5 cm^3 / 1 dm^3 H_2O,
- Mospilan 20 SP - insecticide from the group of neonicotynoids (a.s.: acetamiprid - 200 in 1 kg of the agent). The concentration of the usable liquid: 160 mg / 1 dm^3 H_2O,

- Admiral 100 EC - an insecticide from the group of derivatives of pyridine (a.s.: pyriproxyfen - 100 g in 1 dm^3 of the agent). The concentration of the usable liquid: 1.25 cm^3 / 1 dm^3 H_2O,
- Diazol 500 EW - insecticide from the group of organophosphorus (a.s.: diazinon - 500 g in 1 dm^3 of the agent). The concentration of the usable liquid: 0.83 cm^3 / 1 dm^3 H_2O.

Feed: Tested slugs were fed with apples originating from an orchard, where no chemical procedures were carried out during the years of research. In the research, the apple pulp formed in cubes of known weight and dimensions of 20 x 20 x 5 mm were used.

Analyses conditions: Tests were carried out in the laboratory conditions in the temperature of 18±0.5ºC with the L/D cycle of 16/8 and with humidity of 75±5%. During testing, all animals were placed singly in a plastic see-through container with a constant access of fresh air and a piece of apple prepared adequately, depending on the type of test.

Food consumption of the insecticide intoxicated animals: Each of the analysed preparation (water was used in the control group) was applied in the area of the mantle of twelve individuals of *A. lusitanicus* at a dose of 0.02±0.004 cm^3 and after every 12 hours, the remaining food and the animals were weighed. Every twelve hours, the animals also received an additional portion of fresh food. The results of testing are reflected in Figure 1.

The consumption of food treated with insecticides: Each of the analysed preparations (water used in the control group) was evenly distributed with the use of atomiser on the surface of the apple section at an amount of 0.06±0.01 cm^3. The remaining food and animals were weighed at twelve-hour intervals and also new pieces of insecticide-treated apples were added to the containers. Each chemical was tested on a group of twelve animals. The results of the tests are shown in Figure 2.

Feeding preferences: Tested animals were placed singly in the containers, where 2 pieces of apple were to be found. One piece of apple was treated evenly on the whole surface with 0.06±0.01 cm^3 of water, the other piece in the analogous way with the same volume of insecticide. In twelve-hour time intervals, the weight of the pieces of apples and of slugs was analysed. Every twelve hours, the animals received a fresh portion of food. Results of tests are presented in Figure 3 and Table 1.

Time of analysis: Assumed time of testing in each case was 60 hours.

Presentation of results: The results obtained were presented in the form of a chart generated by Microsoft Excel 2010 Software. Statistical analysis was generated by Statistica 7.0 (Tukey's test).

Results

While testing the influence of the plant protection chemicals of different groups in the doses recommended for protection of cruciferous plants, on the feeding activity of the *Arion Lusitanicus* slugs in the assumed time of testing, no death of any animals used for research was noted.

Results of measurement of an amount of food consumed in the period of 60 hours by the individuals of *A. lusitanicus* treated with preparations of the pyrethroid group (Fig. 1) indicate that the animals treated with deltamethrin$^{(C)}$ - ($P < 0.001$), and lambda-cyhalothrin$^{(B)}$ ($P < 0.01$) were eating much more than animals from the control group$^{(K)}$. Significant divergences in the amount of food ingested were also observed

between the animals treated with deltamethrin[C] and beta-cyfluthrin[A] ($P < 0.05$), deltamethrin[C] and bifenthrin[F] ($P < 0.05$), deltamethrin[C] and acetamiprid[H] ($P < 0.01$), deltamethrin[C] and esfenvalerate[D] ($P < 0.01$), deltamethrin[C] and pyriproxyfen[I] ($P < 0.05$), deltamethrin[C] and diazinon[J] ($P < 0.05$), lambda-cyhalothrin[B] and acetamiprid [H] ($P < 0.05$), lambda-cyhalothrin[B] and esfenvalerate[D] ($P < 0.05$).

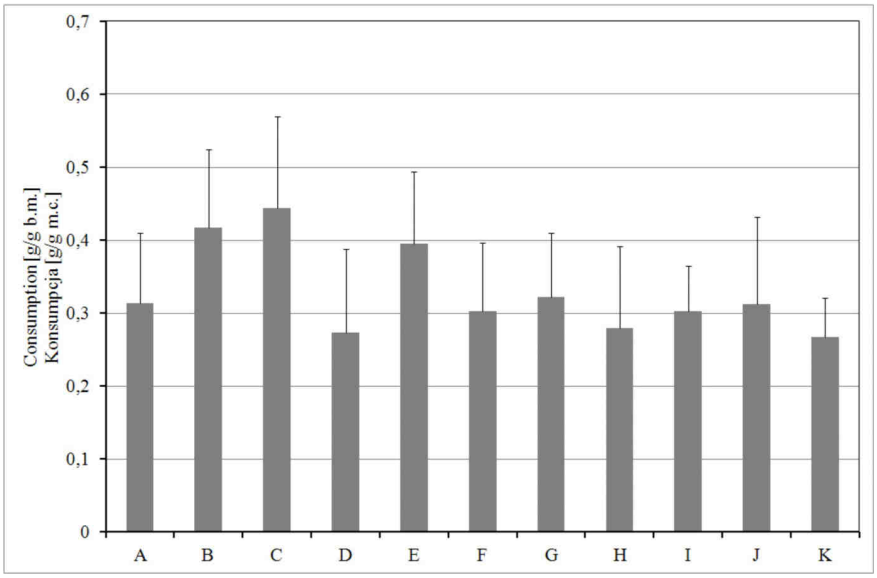

Fig. 1. Average mass of food consumed during the whole research by *A. lusitanicus* individuals treated by insecticides. A - beta-cyfluthrin, B - lambda-cyhalothrin, C - deltamethrin, D - esfenvalerate, E - alpha-cypermethrin, F - bifenthrin, G - teflubenzuron, H - acetamiprid, I - piriproxyfen, J - diazinon, K - control

The measurement of the amount of food ingested by *A. lusitanicus* treated with insecticides (Fig. 2) indicate that apples treated with alpha-cypermethrin[E] ($P < 0.05$) and lambda-cyhalothrin[B] ($P < 0.05$) were ingested in the bigger amount than those treated just with water[K]. Significant divergences in the amount of food ingested were also observed between the animals treated with alpha-cypermethrin[E] and acetamiprid[H] ($P < 0.01$), alpha-cypermethrin[E] and esfenvalerate[D] ($P < 0.05$), lambda-cyhalothrin[B] and esfenvalerate[D] ($P < 0.05$), lambda-cyhalothrin[B] and bifenthrin[F] ($P < 0.05$), lambda-cyhalothrin[B] and deltamethrin[C] ($P < 0.05$), acetamiprid[H] and pyriproxyfen[I] ($P < 0.05$), acetamiprid[H] and lambda-cyhalothrin[B] ($P < 0.001$) acetamiprid[H] and diazinon[J] ($P < 0.05$).

Figure 3 shows the results of food choosing by individuals of *A. lusitanicus*. Weight (g) of ingested apples treated both with water and insecticide is shown in Table 1. The results obtained indicate that animals with a constant access to food with added deltamethrin[C] ($P < 0.01$) and pyriproxifen[I] ($P < 0.01$) preferred apples with insecticide. On the contrary, animals having a choice between water and alpha-cypermethrin[E] ($P < 0.001$), bifentrin[F] ($P < 0.001$) beta-cyfluthrin[A] ($P < 0.05$) or esfenvalerate[D] ($P < 0.05$) were choosing more often the food without added biocides.

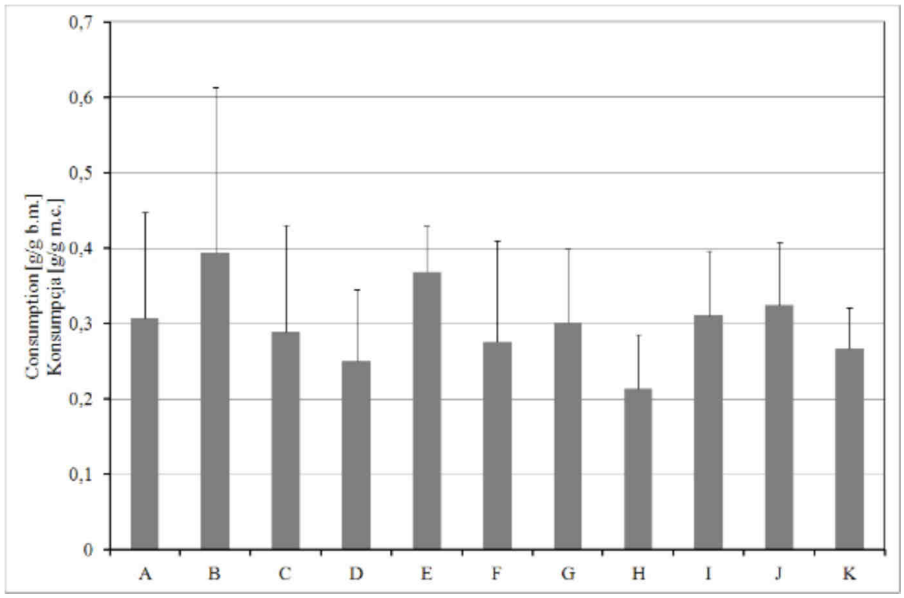

Fig. 2. Average mass of food treated by insecticides consumed during the whole research by *A. lusitanicus* individuals. A - beta-cyfluthrin, B - lambda-cyhalothrin, C - deltamethrin, D - esfenvalerate, E - alpha-cypermethrin, F - bifenthrin, G - teflubenzuron, H - acetamiprid, I - piriproxyfen, J - diazinon, K - control

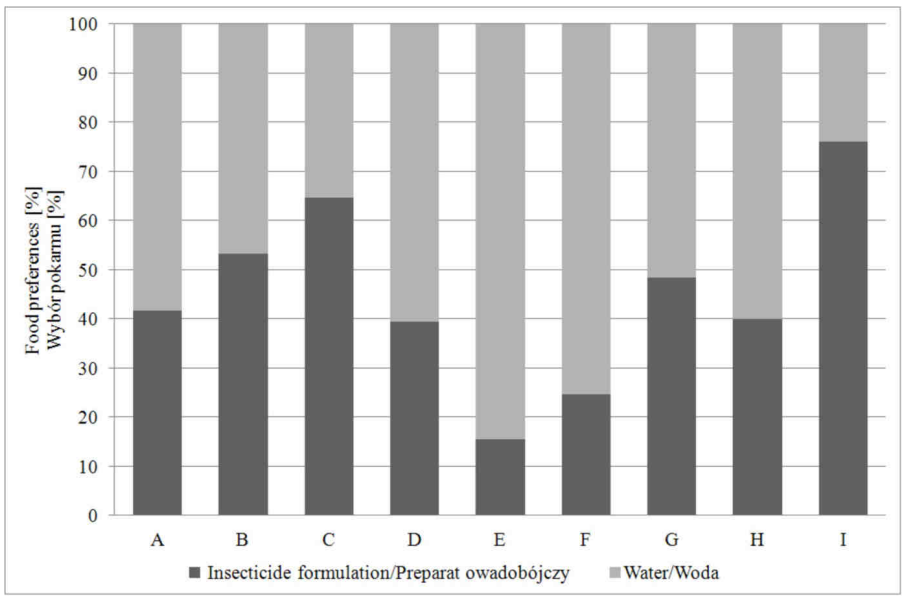

Fig. 3. Food preferences of *A. lusitanicus*. Selection between food treated with insecticides and water. A - beta-cyfluthrin/H_2O, B - lambda-cyhalothrin/H_2O, C - deltamethrin/H_2O, D - esfenvalerate/H_2O, E - alpha-cypermethrin/H_2O, F - bifenthrin/H_2O, G - teflubenzuron/H_2O, H - acetamiprid/H_2O, I - piriproxyfen/H_2O, J - diazinon/H_2O

Table 1

Food preferences of *A. lusitanicus*. Selection between food treated with insecticides and water. A - beta-cyfluthrin/H_2O, B - lambda-cyhalothrin/H_2O, C - deltamethrin/H_2O, D - esfenvalerate/H_2O, E - alpha-cypermethrin/H_2O, F - bifenthrin/H_2O, G - teflubenzuron/H_2O, H - acetamiprid/H_2O, I - piriproxyfen/H_2O, J - diazinon/H_2O. Results are presented calculated per one gram of the animal body mass

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
food treated with insecticide [g/g b.m.] pokarm z insektycydem [g/g m.c.]	0.20	0.47	0.42	0.14	0.13	0.14	0.18	0.13	0.54	0.26
standard error błąd standardowy	0.06	0.49	0.17	0.07	0.08	0.06	0.09	0.09	0.39	0.16
food treated with water [g/g b.m.] pokarm z wodą [g/g m.c.]	0.28	0.41	0.23	0.21	0.73	0.43	0.19	0.20	0.17	0.27
standard error błąd standardowy	0.11	0.20	0.15	0.09	0.22	0.22	0.07	0.08	0.05	0.04

Discussion

Insecticides act to limit the numbers of harmful insects by numerous ways. Benzoylphenyl urea derivatives and partially the derivatives of pyridine act on animals mainly by impairing the moulting process, which results in the organism being unable to finish effectively the process of transformation when it dies in a larval or pupal stage [22, 23].

Neonicotinoids preparations, pyridine derivatives, pyrethroids and organophosphorus insecticides are the chemicals which, acting in different ways, affect both neural conduction in the synapsis and ionic channels located in the body of the neuron, leading to excessive, fatal in result excitation of the nervous system [23-26]. Independently of the mechanism of action, the main role of all insecticides is to limit losses caused by pest insects in the cultivations.

The results obtained show that employed compounds in the dosages recommended to combat harmful insects in the cruciferous plants, which are also most commonly attacked by *Mollusca*, are not lethal for *A. lusitanicus* individuals. Paradoxically - in case of deltamethrin (Fig. 1), lambda-cyhalothrin (Figs. 1 and 2) and alpha-cypermethrin (Fig. 2), the increase in the amount of ingested food after its contact with preparation was noted and in case of lambda-cyhalothrin, this phenomenon occurred independently of the fact whether the preparation was applied directly on the mantle of the slug or on the food.

An extension of the research was an analysis of the choice of food treated with plant protective chemicals or not. The data obtained in this part of the experiment show that in case of deltamethrin and pyriproxifen, the animals were eating more food treated with insecticide than apples treated with water only. Portions of apples with the addition of beta-cyflutrin and esfenvalerate and especially alpha-cypermethrin and bifenthrin turned out to be less attractive than food without them, however, even in this case, it is impossible to say if food was completely unattractive for slugs (Fig. 3, Table 1).

The impulse to carry out the research came from the observation of gardeners from Kolbuszowa area in Podkarpackie, suggesting that cultivations where plants protection chemicals were used were visited by slugs more often. The research carried out confirms

that some of the insecticides used are digestive attractants for the adult individuals of *A. lusitanicus*. The tests of this type should be repeated in the natural conditions and in a longer period of time, since a short period tests do not allow to draw unambiguous conclusions related to the behaviour of the organisms in the environment. It could also be recommended to test particular compounds included in the composition of biocide preparations.

The results obtained during these tests should encourage changes to the plant protection procedures being in force at the moment.

Conclusions

1. Synthetic pesticides in the doses recommended in instructions for use to protect the cruciferous plants against agrophages were not lethal for any of the *Arion lusitanicus* individuals used for testing.
2. Animals treated by contact with preparations containing lambda-cyhalothrin and deltamethrin during the planned period of measuring ate more than animals from the control group.
3. Animals fed with food containing lambda-cyhalothrin and alpha-cypermethrin during the assumed period of measuring ate more than animals from the control group,
4. Apples with addition of deltamethrin and pyriproxifen were more attractive to slugs than apples treated just with water.
5. Sections of apples treated with alpha-cypermethrin, bifenthrin, beta-cyflutrine and esfenvalerate were less attractive for animals, than food without these insecticides.

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INSEKTYCYDY A KONSUMPCJA POKARMU PRZEZ ŚLINIKA LUZYTAŃSKIEGO (*Arion lusitanicus* Mabille 1868)

Zakład Ekotoksykologii, Instytut Biotechnologii Stosowanej i Nauk Podstawowych, Uniwersytet Rzeszowski

Abstrakt: Badania wpływu wybranych substancji aktywnych z grupy pyretroidów (beta-cyflutryna, lambda-cyhalotryna, deltametryna, esfenwalerat, alfa-cypermetryna, bifentryna), pochodnych benzoilomocznika (teflubenzuron), pochodnych pirydyny (piryproksyfen), insektycydów neonikotynowych (acetamipryd) i fosfoorganicznych (diazynon) na konsumpcję pokarmu przez ślinika luzytańskiego (*Arion luisitanicus* Mab.) przeprowadzono w latach 2007 i 2011. Przenalizowano: ilość spożywanego pokarmu przez zwierzęta traktowane środkami ochrony roślin, ilość spożywanego pokarmu traktowanego insektycydami oraz preferencje pokarmowe osobników *A. lusitanicus*, którym umożliwiono wybór pomiędzy pokarmem z dodatkiem preparatu owadobójczego i bez niego. Otrzymane wyniki wskazują, że preparaty zawierające deltametrynę i lambda-cyhalotrynę, którymi potraktowano zwierzęta, zwiększają ilość spożywanego przez ślimaki pokarmu. Wykazano również, że pokarm traktowany lambda-cyhalotryną i alpha-cypermetryną jest zjadany w większej ilości, niż nie traktowany tymi preparatemi. Deltametryna, lambda-cyhalotryna i piryproksyfen mogą stanowić atraktant pokarmowy dla osobników *A. lusitanicus*, a alfa-cypermetryna, bifentryna, beta-cyflutryna i esfenwalerat są dodatkami, które zmniejszają atrakcyjność pokarmu.

Słowa kluczowe: ślinik luzytański, *Arion lusitanicus*, insektycydy, atraktanty