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Testing the efficacy of different substances against *Arion* slugs (Arionidae) under laboratory conditions

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ABSTRACT

In 2008 and 2009 we studied molluscicidal activity of 26 substances in 89 different treatments under laboratory conditions. The experiments in which slugs (*Arion* spp.) were a part took place in two series: 1) with the injection of active substances in slug intestines; and 2) with the application of pellets. After giving the injection we observed 100% mortality of slugs in treatments with *Bacillus thuringiensis* var. *kurstaki* (0.25 ml in 10% concentration/individual), caffeine (0.25 ml in 10% concentration/individual), sodium dodecyl sulphate (0.25 ml in 10% concentration/individual, 0.125 ml in 10% concentration/individual, 0.125 ml in 5% concentration/individual, 0.0625 ml in 10% concentration/individual), and pirimicarb (0.25 ml in 10% concentration/individual, 0.125 ml in 10% concentration/individual, 0.125 ml in 5% concentration/individual, 0.0625 ml in 10% concentration/individual). Meanwhile, the application of pellets resulted in the highest (100%) slug mortality when sodium dodecyl sulphate in 0.5% concentration with caraway as a supplement was used.

Keywords: laboratory experiment, *Bacillus thuringiensis* var. *kurstaki*, caffeine, sodium dodecyl sulphate, pirimicarb, slugs, *Arion* spp., molluscicides, efficacy

IZVLEČEK

PREIZKUŠANJE UČINKOVITOSTI RAZLIČNIH SNOVI ZA ZATIRANJE LAZARJEV (*Arion* spp., Arionidae) V LABORATORIJSKIH RAZMERAH

V letih 2008 in 2009 smo v laboratorijskih razmerah preizkušali limacidno delovanje 26 snovi v 89 različnih obravnavanjih. Poskusi, v katere smo vključili polže lazarje (*Arion* spp.), so potekali v dveh serijah, in sicer z injiciranjem aktivne snovi v prebavilo polžev in z uporabo pelet. Pri injiciranju smo 100 % smrtnost polžev ugotovili v obravnavanju z bakterijo *Bacillus thuringiensis* var. *kurstaki* (0,25 ml v 10 % koncentraciji/osebek), kofeinom (0,25 ml v 10 % koncentraciji/osebek), natrijevim dodecil sulfatom (0,25 ml v 10 % koncentraciji/osebek; 0,125 ml v 10 % koncentraciji/osebek; 0,125 ml v 5 % koncentraciji/osebek; 0,0625 ml v 10 % koncentraciji/osebek), in pirimikarbom (0,25 ml v 10 % koncentraciji/osebek; 0,125 ml v 10 % koncentraciji/osebek; 0,125 ml v 5 % koncentraciji/osebek; 0,0625 ml v 10 % koncentraciji/osebek), medtem ko smo največjo (100 %) smrtnost polžev pri uporabi pelet dosegli z natrijevim dodecil sulfatom v 0,5 % koncentraciji z dodatkom kumine.

Ključne besede: laboratorijski poskus, *Bacillus thuringiensis* var. *kurstaki*, kofein, natrijev dodecil sulfat, pirimikarb, lazarji, *Arion* spp., limacidi, učinkovitost

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1 INTRODUCTION

Terrestrial slugs and snails are destructive agricultural pests that cause economic damage to a wide variety of plants including vegetables, forage crops, tree fruits, shrubs, flowers, ground green cover, and newly sown lawngrasses. Morover, they play an important role in transmitting and spreading disease to cultivated plants (Ohlendorf, 1999). Thus, the use of specific molluscicides is considered one of the most effective measures for terrestrial molluscs control (Barker, 2001).

Carbamates proved to be the most potent class of molluscicides (Miller *et al*., 1988; Radwan *et al*., 1992). Growers and farmers often experience difficulty controlling these pests with conventional bait pellets containing molluscicides such as methiocarb and metaldehyde. For example, in wet conditions the efficacy of these pellets can be very low (Hata *et al*., 1997), and lead to unsatisfactory control levels. Furthermore, poison baits can be toxic to other non-target soil invertebrates, as well as birds and mammals such as shrews and field mice (Hagin and Bobnick, 1991; Purvis, 1996). Clearly, additional molluscicides are needed that are highly selective and toxic for slugs and/or snails with minimal effect on other species.

The development of effective alternatives to conventional molluscicides, particularly those which could be used in an integrated control strategy, would reduce plant losses, improve quality, and offer a sustainable strategy for controlling slug and snail pests with reduced molluscicide input. The development of alternative snail and slug control methods compatible with Integrated Pest Management (IPM) strategies used to control other pests would help satisfy increasing market demands for ornamental plants and edible crops grown with environmentally responsible production methods (Schüder *et al*., 2003).

In biological control the usage of the parasitic nematode *Phasmarhabditis hermaphrodita* (Schneider) has already been confirmed to be efficacious (Wilson and Grewal, 2005). Since the researching of parasitic nematodes hasn't been done in Slovenia yet, the aforementioned species is still on the list of exotic agents and its application is therefore limited to laboratory work. At the moment there are seven molluscicide products on the Slovenian market. There are two products in a catalogue of permitted products in organic agriculture (Ozimič *et al*., 2007), but each of them has some deficiencies which have led researchers, industry, and producers to introduce a better and environmentally-friendly product. The product Carakol (active ingredient metaldehyde, 5%) is environmentally unsafe (toxic to warm-blooded organisms), but it is on the list because there are no more suitable molluscicides on our market. The product Feramol (active ingredient iron [III] phosphate), the second of two previously mentioned products, is environmentally safe but its efficacy is slow and often unsatisfactory.

The scope of our research was: (1) to study the efficacy of different substances according to their molluscicidal activity with injection; and (2) to study the efficacy of different substances in the form of pellets. Verification of satisfactory activity with injection would lead to test substance activity in the form of a pellet. This would further lead to the future testing of selected substances outdoors, and the potential implementation of such a product into the systems of food production in conditions when slugs represent an economically important group of pest organisms.

2 MATERIALS AND METHODS

The investigation was carried out during 2008 and 2009 at the Laboratory of Entomology (University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair of Phytomedicine, Agricultural Engineering, Crop Production, Grassland and Pasture Management) in Ljubljana, Slovenia. To begin, 890 *Arion* spp. slugs were collected at the Experimental Field of Biotechnical Faculty in Ljubljana, Slovenia (46°04'N, 14°31'E, 299 m alt.). The slugs used in the experiments were of different in size and age, and of different species of genus *Arion* – as we wanted to draw near (not sure what 'draw near' means) the conditions in the open.

We included 26 different substances in a trial and tested their potential molluscicidal activity in 89 different treatments (Table 1). All treatments were repeated 10 times, with five replicates where a treated slug had accessible feed (cabbage or salad leaf), and five replicates without feed (only pellets). In each plastic petri dish (150 x 20 mm; producer: Kemomed d.o.o., Kranj, Slovenia) we put one slug and a paper tampon (35 x 11 mm; Tosama d.d., Vir pri Domžalah, Slovenia) which was soaked in water prior to the start of the experiment. We put in each Petri dish around 4 g of pellets. The petri dishes were put in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) without light at 20 ºC and at a relative humidity of 85%. We observed slug mortality and their potential feeding on 1, 2, 3, 4, 5, 6, and 7 DAT (days after treatment). The tampon in the petri dish was wetted daily with distilled water. If mould appeared on pellets they were replaced with fresh material.

In order to determine the toxicity of the substances tested against *Arion* slugs, they were injected on the dorsal side introducing a needle no more than 4 mm and at a 30º angle in relation to the slug's skin to reduce the risk of damaging vital organs (Aguiar and Wink, 2005). For injection we used pen needles (0.36 x 13 mm; B-D Micro-fine IV, Becton Dickinson, U.S.A.), meanwhile pellets (composition: 18% maize meal, 36% wheat meal, 15% wheat flour, 4.6% soybean meal, 4.6% notilac, 0.16% mycosorb, 1.8% lignobond, and 9.5% starch) with selected active ingredients and concentrations were produced by the company Unichem d.o.o. The results of the experiments are shown in Tables from 2 to 15 as a % of mortality in selected treatments for each day after treatment (DAT).

Fig. 1: Tested substances with potential molluscicidal activity

Substance and treatment	ml of liquid /slug (injection) or g pellets/slug (pellets)	Form of application	Experiment
Bacillus thuringiensis var. *kurstaki* (Delfin 10%)	0.25; 0.125; 0.0625	I	8; 9; 11
Bacillus thuringiensis var. *kurstaki* (Delfin 5%)	0.125 4	I, P	10 6
Bacillus thuringiensis var. *kurstaki* (Delfin 1%)	4	P	7
Bromadiolone 10%	4	P	5
Diatomaceous earth 10%	4	P	1; 2; 3
Diatomaceous earth 5%	4	P	1; 2; 3
Diatomaceous earth 1%	4	P	1; 2; 3
Glyphosate (Boom efekt 10%)	0.25	I	8
Glyphosate (Boom efekt 4%)	4	P	5; 6
Glyphosate (Boom efekt 1%)	4	P	7
HDK 2.5%	0.125	I,	14
HDK 1.25%	0.125	I	14
Carboxylic acid 0.2%	4	P	1; 2; 3
Caffeine 10%	0.25; 0.125; 0.0625	I	8; 9; 11
Caffeine 5%	0.125 4	I, P	10 6
Caffeine 1%	4	P	7
Control	0.25; 0.125; 0.0625	I, P	8; 9; 11; 14 1; 2; 3; 4; 5; 6; 7; 10; 12; 13
Caraway (milled seed) 10%	0.25; 0.125; 0.0625 4	I, P	8; 9; 11 2; 3
Caraway (milled seed) 5%	0.125 4	I, P	14 2; 3
Lactose 10%	0.125	I	14
Lactose 5%	0.125	I	14
Limonene 10%	0.125 4	I, P	14 1; 2; 3
Limonene 5%	0.125 4	I, P	14 1; 2; 3
Natren 10%	0.125	I	14
Natren 5%	0.125	I	14
Niclosamide 10%	0.25	I	8
Niclosamide 5%	0.125 4	I, P	14 6
Niclosamide 1%	4	P	7
Pirimicarb (Pirimor 10%)	0.25; 0.125; 0.0625	I	8; 9; 11
Pirimicarb (Pirimor 5%)	0.125 4	I, P	10 6
Pirimicarb (Pirimor 1%)	4	P	7
Pirimicarb (Pirimor 0.25%)	4	P	12
Pirimicarb (Pirimor 0.125%)	4	P	12
Pirimicarb (Pirimor 0.25%) + caraway	4	P	12
Pirimicarb (Pirimor 0.125%) + caraway	4	P	12
Pirimicarb (Pirimor 0.25%) + malt	4	P	12
Pirimicarb (Pirimor 0.125%) + malt	4	P	12

Quackgrass 12.4 mg /250 g dry matter	4	P	1; 2; 3
Quackgrass 40 mg /250 g dry matter	4	p	1; 2; 3
Castor oil plant oil 10%	0.125	I	14
Castor oil plant oil 5%	0.125	I	14
Sodium dodecyl sulphate 10%	0.25; 0.125; 0.0625	I	8; 9; 11
Sodium dodecyl sulphate 5%	0.125 4	I, P	10 6
Sodium dodecyl sulphate 1%	4	P	7
Sodium dodecyl sulphate 0.25%	4	P	12
Sodium dodecyl sulphate 0.125%	4	P	12
Sodium dodecyl sulphate 0.25% +Caraway (milled seed)	4	P	12
Sodium dodecyl sulphate 0.125% + caraway (milled seed)	4	P	12
Sodium dodecyl sulphate 0.5% + caraway (milled seed)	4	P	13
Sodium dodecyl sulphate 0.5% + malt	4	P	13
Sodium dodecyl sulphate 0.5% + caraway (milled seed) + bran	4	P	13
Sodium dodecyl sulphate 0.5% + malt + bran	4	P	13
Sodium dodecyl sulphate 0.25% + malt	4	P	13
Sodium dodecyl sulphate 0.125% +malt	4	P	13
Salt 10%	0.25 4	I P	8 1; 2; 3
Coated salt 5%	4	P	1; 2; 3; 6
Coated salt 1%	4	P	1; 2; 3; 7
Thymol 10%	0.25	I	8
Thymol 5%	4	P	6
Thymol 1%	4	P	7
Yew (milled needles) 10%	0.25	I	8
Yew (milled needles) 5%	4	P	5; 6
Yew (milled needles) 1%	4	P	7
Ureaformaldehyde 10%	0.125	I	14
Ureaformaldehyde 5%	0.125	I	14

Legend: I- injection; P-pellets

3 RESULTS

In the first experiment (beginning of the trial: June 17, 2008) we studied molluscicidal activity of eight different substances included in 17 treatments in pellets. The highest slug mortality (100%) was determined in commercial products Mesurol (active ingredient methiocarb) and Terminator (a.i. metaldehyde) already by the second day after treatment. From the rest of the substances (caraway 1%, 5%, and 10%; limonene 1%, 5%, and 10%; diatomaceous earth [DE] 1%, 5%, and 10%; salt 1%, 5%, and 10%, quackgrass extract [12.4 mg/ 250 g dry matter, 40 mg / 250 d dry matter]), 0.2% carboxylic acid) we observed only 20% mortality on the 6th day with the latter. In the second experiment (Table 2), which lasted for four days and in which we tested the efficacy of nine substances included in 19 treatments, the fastest activity (100% mortality in 24 hours after the exposure) and highest mortality rate of slugs was observed in the application of pellets Arion (a.i. metaldehyde), followed by carboxylic acid with 40% slug mortality after the first day after treatment (DAT) and with caraway 1% on third and fourth DAT with 60% slug mortality.

In the third experiment (beginning of the trial: July 14, 2008) treatments from the second experiment were repeated and we concluded that mortality was attained with the commercial product Arion + (a. i. metaldehyde), while carboxylic acid was not effective. Caraway with 1% caused only a 20% slug mortality only by the four DAT.

In the fourth experiment (beginning of the trial: August 19, 2008) we decided for a combination of seven active ingredients in 15 different treatments: 1) DE and limonene, 2) DE and carawy, 3) DE and boletus, 4) DE and starch, 5) salt and caraway, 6) salt and boletus, 7) salt and starch, 8) salt, DE and caraway, 9) salt, DE, caraway and boletus, 10) salt, DE, caraway and starch, 11) caraway and malt, 12) salt and malt, 13) DE and malt, 14) caraway and starch, and 15) caraway and

boletus), where we noted 20% slug mortality only at four DAT in treatments with caraway and starch as an additive in pellets.

In the fifth experiment (Table 3) we tested the activity of nine active ingredients. On the fourth DAT we determined 60% mortality only in the treatment glyphosate, while the highest mortality (100%) was registered on the seventh DAT in the same treatment. Fruits of yew gave only 60% slug mortality. We also found out that slugs fed on additional cabbage in the majority of the treatments, except in treatments when pellets contained yew (milled needles), salt+caraway+diatomaceus earth and salt+caraway.

In the sixth experiment (Table 4) we studied the efficacy of nine active ingredients in 18 different treatments. After seven days 20% slug mortality was determined in treatments pirimicarb (pirimor 5%) and sodium dodecyl sulphate 5%, while other treatments did not result in death to the slugs.

In the seventh experiment (beginning of the trial: July 7, 2009) we repeated the treatments from the sixth experiment, only we lowered the concentration of active ingredients in the pellets (Boom-effect 1%, coated salt 1%, Delfin 1%, sodium dodecyl sulfate 1%, niclosamide 1%, Pirimor 1%, thymol 1%, yew meal 1%, caffeine 1%; all treatments with and without cabbage leaf as an additional food source). None of the treaments killed slugs although slugs fed on pellets and the enclosed cabbage.

In the eighth experiment (beginning of the trial: July 10, 2009) we injected 0.25 ml of nine active ingredients in 10% solution into the slugs. The highest mortality we determined already on the first day in treatments *Bacillus thuringiensis* var. *kurstaki* (product Delfin), pirimicarb (product Pirimor), caffeine and sodium dodecyl sulphate, while the 33% slug mortality in pellets with added salt was determined only on the second day. In the rest of the treaments (Boom-effect [a.i. glyphostae], niclosamide, thymol, and yew meal) slugs fed with the enclosed cabbage, and their death was not confirmed until the end of the experiment.

In the ninth experiment (Table 5) we employed substances which proved to be effective in the previous experiment, only we now injected half of the dose into slugs (0.125 ml of 10% suspension) of the active ingredient. Pirimicarb as well as sodium dodecyl sulphate provoke the death of all the slugs already in the first few days, while bacteria and caffeine were less efficient.

The 10th experiment (Table 6) was a repetition of the ninth, only we injected 0.125 ml of 5% suspension of active ingredients into each slug. Pirimicarb and sodium dodecyl sulphate acted with the same efficacy as in the preceding experiment. In the meantime, the activity of *Bacillus thuringiensis* var. *kurstaki* and caffeine was somewhat worse.

In the 11th experiment (Table 7) we again used active ingredients from the ninth and 10th experiments, only this time we injected 0.0625 ml of suspension in 10% solution. Pirimicarb and sodium dodecyl sulfate again demonstrated the best molluscicidal activity. Meanwhile, the other two substances were only 33% effective after seven days.

In the 12th experiment (beginning of the trial: August 4, 2009) we tested the activity of four active ingredients in 24 different treatments. Despite feeding on pellets, all slugs survived. Therefore, we redoubled the concentrations in pellets in the 13th experiment (Table 8), and in combination with sodium dodecyl sulphate in 0.5% and caraway, and attained 100% mortality of slugs already after the first day of the experiment. Lower molluscicidal activity also showed a combination of sodium dodecyl sulphate and malt, but only on the fifth day of the beginning of the experiment. In the rest of treatment slugs survived, even though they fed on pellets.

In the 14th experiment (Table 9) we injected seven different active ingredients in 14 treatments into slugs. Sufficient molluscicide activity was determined only at 5% concentration of limonene and caraway, while slug mortality in the rest of the treatments was distinctively lower.

Table 2: Mortality (%) of *Arion* slugs after pellet treatment (beginning of the trial: July 7 2008).

Treatment	Days after treatment			
	1	2	3	4
Control	0	0	20	20
Carboxylic acid 0. 2%	40	40	40	40
Coated Salt 5%	0	0	40	40
Arion	0	0	40	80
Salt 10%	0	0	0	0
Salt 5%	0	0	0	0
Salt 1%	0	0	20	20
Coated salt – new 1%	0	0	20	20
Caraway 10%	20	20	40	40
Caraway 5%	0	0	0	20
Caraway 1%	0	40	60	60
Arion +	100	100	100	100
Limonene 10%	0	20	20	20
Limonene 5%	20	20	20	20
Limonene 1%	0	0	0	0
Diatomaceus earth 10%	0	20	20	20
Diatomaceus earth 5%	0	0	40	40
Diatomaceus earth 1%	20	20	20	60
Quickgrass extract 40 mg	0	0	0	0
Quickgrass extract 12.4 mg	20	20	40	40

Table 3: Mortality (%) of *Arion* slugs after pellet treatment (beginning of the trial: October 8 2008).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)*	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Yew – milled needles 1%	0	0	0	0	0	40	40
Yew – fruits 1%	0	0	0 (20)	20 (25)	20 (50)	20 (50)	60 (100)
Starch + caraway + coated salt 10%	0	0	20	20	20 (25)	20 (25)	20 (25)
Bromadiolone 10% + starch	0	0	0	0 (20)	0 (20)	0 (20)	20 (25)
Salt 10% + caraway 10%	0	0	20	20 (25)	40 (33)	40 (66)	40 (66)
Salt 10% + caraway + diatomaceus earth 10%	0	0	0	0	0	0	0
Salt 10% + caraway 5%	0	0	40	40	40	40	100
Boom-efect (glyphosate) 4%	0	0	20 (75)	60 (100)	60 (100)	80 (100)	100

* Number in parenthesis means % of slugs, eating additional food

Table 4: Mortality (%) of *Arion* slugs after pellet treatment (beginning of the trial: May 18 2009).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Boom-efect 4% (Glyphosate)	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Boom-efect 4% (Glyphosate) (cabbage)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Coated salt 5%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Coated salt 5% (cabbage)	0 (100, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Delfin 5% (*Bacillus thuringiensis* var. *kurstaki*)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Delfin 5% (*Bacillus thuringiensis* var. *kurstaki*) (cabbage)	0 (80, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Sodium dodecyl sulfat 5%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Sodium dodecyl sulfat 5% (cabbage)	0 (100, 60)	0 (100, 80)	0 (100, 100)	0 (100, 100)	20 (100, 100)	20 (100, 100)	20 (100, 100)
Niclosamide 5%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Niclosamide 5% (cabbage)	0 (80, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Pirimor 5% (pirimicarb)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Pirimor 5% (pirimicarb) (cabbage)	0 (100, 80)	20 (100, 75)	20 (100, 75)	20 (100, 75)	20 (100, 75)	20 (100, 100)	20 (100, 100)
Thymol 5%	0 (60)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Thymol 5% (cabbage)	0 (100, 40)	0 (100, 60)	0 (100, 80)	0 (100, 80)	0 (100, 80)	0 (100, 80)	0 (100, 80)
Yew 5%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Yew 5% (cabbage)	0 (60, 60)	0 (60, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Caffeine 5%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Caffeine 5 % (cabbage)	0 (80, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)

* First number in parenthesis means % of slugs, eating additional food, second number reffers to % of slugs, eating pellets.

Table 5: Mortality (%) of *Arion* slugs after inject treatment (beginning of the trial: July 13 2009).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Delfin (*Bacillus thuringiensis* var. *kurstaki*)	66	66	66	66	66	66	66
Pirimor (pirimicarb)	100	100	100	100	100	100	100
Caffeine	66	66	66	66	66	66	66
Sodium dodecyl sulfate	100	100	100	100	100	100	100

* 0.125 ml of 10% suspension was injected in every slug. The number in parenthesis means % of slugs, eating additional food.

Table 6: Mortality (%) of *Arion* slugs after inject treatment (beginning of the trial: July 13 2009).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Delfin (*Bacillus thuringiensis* var. *kurstaki*)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)
Pirimor (pirimicarb)	100	100	100	100	100	100	100
Caffeine	33 (50)	66 (100)	66 (100)	33 (50)	33 (50)	66 (100)	66 (100)
Sodium dodecyl sulfate	100	100	100	100	100	100	100

* 0.125 ml of 5% suspension was injected in every slug. The number in parenthesis means % of slugs, eating additional food.

Table 7: Mortality (%) of *Arion* slugs after inject treatment (beginning of the trial: July 15 2009).

Treatement	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Delfin (*Bacillus thuringiensis* var. *kurstaki*)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)
Pirimor (pirimicarb)	100	100	100	100	100	100	100
Caffeine	0	33 (50)	0	33 (50)	0	33 (50)	0
Sodium dodecyl sulfate	100	100	100	100	100	100	100

* 0.0625 ml of 10 % suspension was injected in every slug. The number in parenthesis means % of slugs, eating additional food.

Table 8: Mortality (%) of *Arion* slugs after pellet treatment (beginning of the trial: August 25 2009).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Controla	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Pirimor 0.5% (pirimicarb) + malt	0 (20)	0 (80)	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)
Pirimor 0.5% (pirimicarb) + malt (cabbage)	0 (20, 40)	0 (100, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Pirimor 0.5% (pirimicarb) + caraway	0 (60)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Pirimor 0.5% (pirimicarb) + caraway (cabbage)	0 (60, 20)	0 (80, 60)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Sodium dodecyl sulfate 0.5% + malt	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Sodium dodecyl sulfate 0.5% + malt (cabbage)	0 (20, 80)	0 (40, 100)	0 (60, 100)	0 (60, 100)	20 (75, 100)	20 (75, 100)	20 (75, 100)
Sodium dodecyl sulfate 0.5% + caraway	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Sodium dodecyl sulfate 0.5% + caraway (cabbage)	0 (80, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Sodium dodecyl sulfate 0.5% + malt + bran	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Sodium dodecyl sulfate 0.5% + malt + bran (cabbage)	0 (60, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Sodium dodecyl sulfate 0.5% + caraway + bran	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Sodium dodecyl sulfate 0.5% + caraway + bran (cabbage)	0 (80, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)

* First number in parenthesis means % of slugs, eating aditional food, second number reffers to % of slugs, eating pellets.

Table 9: Mortality (%) of *Arion* slugs after injection treatment (beginning of the trial: September 30 2009). We injected 0.125 ml of active ingredient of related concentrations into slugs.

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	40 (100)	40 (100)
HDK 2.5%	0 (20)	0 (40)	100	100	100	100	100
HDK 1.25%	0	0 (20)	20 (50)	20 (50)	20 (75)	20 (75)	40 (66)
Caraway 10%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	20 (100)	20 (100)
Caraway 5%	20 (75)	20 (100)	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)
Lactose 10%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Lactose 5%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	20 (100)	20 (100)
Castor oil plant oil 10%	0 (100)	0 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
Castor oil plant oil 5%	0 (80)	0 (100)	20 (100)	20 (100)	20 (100)	40 (100)	40 (100)
Ureaformaldehyde 10%	0 (40)	0 (80)	0 (100)	0 (100)	0 (100)	20 (100)	20 (100)
Ureaformaldehyde 5%	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
Natren 10%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Natren 5%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Limonene 10%	0 (100)	0 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
Limonene 5%	80	80 (100)	80 (100)	80 (100)	80 (100)	80 (100)	100

***** First number in parenthesis means % of slugs, eating additional food, second number reffers to % of slugs, eating pellets.

4 DISCUSSION

In our research we included substances that have been used in the research of other authors as potentially or actually efficient when controlling different species of slugs. But no aditional information was acquired about their molluscicide activity on slugs from the genus *Arion*, which tend to be the most noxious group of these animals on vegetables in Slovenia (Laznik and Trdan, 2009).

A quackgrass (*Agropyron repens* L. Beauv.) extract fraction containing phenolic glycosides showed in some previous research both dermal and gastrointestinal toxicity toward two slug species, *Deroceras reticulatum* (Müller) and *Deroceras leave* (Müller) (Hagin, 1989; Hagin and Bobnick, 1991). However, testing this substances in our experiment as pellet treatment with different concentrations of the active ingridient (*Agropyron* extract, carboxylic acid) did not show any molluscicidal effect on the *Arion* slugs.

The study of Kumar and Singh (2006) indicated that dried seed powder of *Carum carvi* L. is an important source of botanical molluscicides. It has been also reported that limonene found in *C. carvi* seed is metabolized into more toxic carvone and causes death of the snail *Lymnaea acuminata* Lamarck after 24 hours of exposure with very low LC_{50} value. However, in our experiment these substances with different concentrations of the active ingridients (powder of the *C. carvi* seeds, limonen) as a pellet treatment did not show any molluscicidal effect on *Arion* slugs.

It is well known that the 'salt effect,' causes slug and snails to emit copious amounts of slime, which leads to dehydration of the animals (Hagin and Bobnick, 1991; Ester and Molendijk, 2003). The method is limited by the requirement to spray the material directly on the slugs. In our experiment we wanted to bring salt to the slug and to determine if salt can act also in the stomach. We did not get any slug mortality using this method. Similarly, as salt and also diatomaceous earth act abrasively, and when in contact with a slug it harms its exoskeleton (Sibley and Thompson, 2004). However, the results in our experiment did not show any molluscicidal effect after trying to feed the *Arion* slugs with diatomaceous earth pellets. Mushroom, starch, and molasses extracts were used as attractants in pellets. These extracts are also known to attract slugs (Keller and Snell, 2002).

Yew extracts have hormonal activity in insects and mammals (Reddy *et al*., 2001), meanwhile researchers as yet have not determined any molluscicide activity of yew. Our research demonstrated that neither extracts from milled yew needles nor from fruits have satisfatory molluscicidal activity on slug from the genus *Arion*. Pirimicarb is a carbamate insecticide used to control aphids on vegetable, cereal, and orchard crops by inhibiting acetylcholinesterase activity (McGregor, 2006). In our experiment, when injected into slugs, pirimicarb also showed molluscicidal activity, while its molluscicidal activity in the continuation with pellets was not confirmed.

Sodium dodecyl sulphate (SDS) is a surfactant, which is widely used as an emulsifier in agricultural chemicals. Tseng *et al*. (unpublished) found that sodium dodecyl sulphate (SDS) was an effective molluscicide, when used on its own at a concentration of 100 ppm, for the semi-aquatic golden apple snail, *Pomacea canaliculata* (Lamarck). SDS was used in this case as an aqueous solution applied on the water surface. These researchers believed that the molluscicidal activity of SDS appeared to be due to "dermal" absorption, rather than as an "oral" (stomach) poison. Also in our experiment, when conducting the injection of SDS, we determined molluscicidal activity. When added to pellets, SDS showed molluscicidal activity only with 0.5% caraway as an additive. Niclosamide is a chlorinated salicylanilide pesticide principally used against aquatic vertebrates and crustaceans. In the research of Dai *et al.* (2008) molluscicidal activity of niclosamide against adult slugs of *Oncomelania hupensis* (Gredler) was confirmed, and at the same time we concluced that niclosamide does not have molluscicidal activity on slugs from the genus *Arion*.

Ester and Nijënstein (1995) reported that *Bacillus thuringiensis* significantly reduced the attack by slug *Deroceras reticulatum* (Müller) in winter wheat after two days, but after seven days this substance was no longer effective. In our experiment the product Delfin (a.i. *Bacillus thuringiensis* var. *kurstaki*) in treatment with pellets showed no molluscicidal activity, while an injection treatment with the highest concentration of suspension managed to kill slugs 100% of the time. In related research Hollingsworth *et al*. (2003) tested the activity of caffeine and discovered molluscicidal activity to some species of slugs. But our results confirmed such caffeine attribute only when injected into slugs, similar to bacteria *B. thuringiensis* var. *kurstaki*. When slugs fed on pellets treated with caffeine we could not make similar conclusions.

In contrast to our research, in which we conclude that thymol does not result in molluscicidal activity against slugs from the genus *Arion,* El-Zemity (2006) in his research establised that thymol has a potential molluscicidal activity in controlling snails from the species *Helix aspersa* Müller. Some preceding research have shown that some herbicides have molluscicidal activity too (El-Fiki and Mohamed, 1978; Zidan *et al*., 1998), but this was not confirmed in our example when testing the active ingredient glyphosate.

The results of our research indicate that the highest molluscicidal potential had sodium dodecyl sulfate in combination with extract of caraway, but future work will be needed to optimize the use of this substance in practical use as a pellet treatment. In several injection treatments we attained sufficient results, yet in the future we have to pay more attention to the production of appropriate pellets which can enable higher consumption rate by slugs from the genus *Arion*.

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