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Response of wild *Mus musculus* to baits containing essential oils: II Bromadiolone and difenacoum baits with 0.75% cinnamon oil tested in storages

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SUMMARY

The effects of 0.75 % concentration of cinnamon essential oil on bait attractiveness and total biological efficacy of bromadiolone and difenacoum rodenticides to the house mouse were tested in practice.

The experiments were conducted in storages with stable house mouse populations for which no resistance to anticoagulant rodenticides had been previously reported.

A statistically significant difference was detected between bromadiolone and difenacoum baits. Consumption of bromadiolone baits supplemented with cinnamon essential oil was 74 % higher than the consumption of bromadiolone baits without cinnamon oil. The average efficacy of bromadiolone baits in controlling house mice was 96 %.

Cinnamon essential oil added to difenacoum baits increased bait consumption by 39 %, i.e. it was 119 % higher than the consumption of oil-free baits. The average efficacy of difenacoum baits in controlling house mice was 99.5 %.

Keywords: Rodenticides; Essential oils; Cinnamon; Storages; House mouse; Attractants

INTRODUCTION

The house mouse, *Mus musculus*, is a highly polyphagous species with high reproductive potential which is also highly adaptable to various conditions and types of habitat. It uses almost all man-made products either for food or for shelter. Problems in controlling its populations mainly arise from its specific traits (Hrgović et al., 1991).

There is a range of construction engineering, technologically manipulative and sanitary measures devised to foil mice entry in buildings and to prevent their habitation. Various other methods are available for modifying rodent behavior, such as repellents, high-frequency sonic devices or barriers. However, despite a variety of methods for modifying mouse behaviour or preventing their survival, rodenticides are the most frequently applied method of control of harmful rodents (Hrgović et al., 1991; Buckle & Smith, 1994).

Since their introduction in the middle of the 20th century, anticoagulant rodenticides have been the most widely used chemicals for control of rodents (RRAG, 2012). In our region, bromadiolone has been the active ingredient most frequently used for controlling commensal rodent species (Team of authors, 2016). Bromadiolone is a representative of second generation anticoagulant rodenticides intended for controlling susceptible and warfarin-resistant populations of rodents. First house mouse populations resistant to bromadiolone were found after merely several years of its intensive use (Rowe et al., 1981). It is a highly persistent chemical with extended activity period (Roberts & Hutson, 1999). The oral LD_{50} for the mouse is 1.75 mg/kg (US EPA, 1998). The active ingredient difenacoum then showed good results in controlling bromadiolone-resistant populations (Šćepović, 2015). The properties of difenacoum, another representative of group II of anticoagulant rodenticides, were first reported in 1975 (Hadler et al., 1975). Similar to bromadiolone, it is persistant in natural environments and has an extended activity period (+Roberts & Hutson, 1999). Difenacoum acute oral toxic dose is 0.45-1.18 mg/kg for males, and 1.0-2.75 mg/kg for females (US EPA, 2007). Vitamin K1 (phytomenadion) is the antidote for bromadiolone and difenacoum (Janjić, 2005).

Successful rodent control requires an adequate choice of active ingredient and formulation, but also a good bait attractiveness (Buckle & Smith, 1994). Bait attractiveness is especially important under extreme environmental conditions (humid and warm environments, cellars, etc.) or in rooms with plenty of alternative quality food, such as plant product storages, forage mixing stations, meat industry and pet food production facilities (Hrgović et al., 1991).

Based on the current knowledge of potentials of the essential oil of cinnamon, *Cinnamoumi zeylanicum* (Jokić et al., 2013, Jokić et al., 2018), the present study focused on determining the biological efficacy of bromadiolone and difenacoum baits supplemented with cinnamon essential oil at 0.75 % concentration under practical conditions. Improvements of the acceptability and efficacy of rodenticides in environmentally friendly manner would also be a contribution to rodent pest management control programs by finding an acceptable solution both from the aspect of environment protection and improved quality and safety of food and human health protection. The results should be important for advisory services, producers and rodent control practitioners.

MATERIAL AND METHODS

Bait preparation

Baits were prepared according to EPPO/OEPP (2004a) methodology. Placebo baits and baits containing 0.75 % essential oil of cinnamon (dissolved in 25 ml pure alcohol) were prepared as described in Part I of this article (Jokić et al., 2018). Oil-free baits were made by mixing the appropriate amounts of bromadilone (0.005 %) or difenacoum (0.005 %) bait concentrates with placebo bait and adding 25 ml pure alcohol. Optimal concentrate rates of bromadiolone and difenacoum products supplied by EkoSan d.o.o., Serbia, were also added to the baits containing cinnamon essential oil.

Bromadiolone and difenacoum contents in bait preparations were checked at the Laboratory of Applied Chemistry of the Institute of Pesticides and Environmental Protection, Belgrade.

Experimental design

Attractiveness and biological efficacy of bromadiolone baits were tested in storage rooms of a mill and a farm. In the mill storages of 950 m^2, packaged products were kept on pallets, while the 600 m^2 storages on the farm kept bulk products.

The attractiveness and biological efficacy of difenacoum baits were determined in two storage facilities on a collective farm. The first facility, intended for storing seeds and seeding materials, as well as packaging, had an area of 1200 m^2, and the products were kept on pallets. In the facility for storing materials of various origin and intended use (e.g. paper or synthetic packaging, forage mix) of 500 m^2 total area, the stored products were in bulk.

Baits were offered in commercial bait boxes as recommended by the EPPO/OEPP (2004b) standard method described in Part I of this article.

Bait attractiveness

To determine the attractiveness and biological efficacy of bromadiolone and difenacoum baits supplemented with cinnamon essential oil to house mice under practical conditions, poisonous baits with or without cinnamon oil were laid simultaneously. A total of 84 bait boxes were used in the mill and an equal number on the farm, i.e. 42 boxes containing bromadiolone baits without cinnamon oil, and 42 boxes with bromadiolone baits containing also 0.75% cinnamon essential oil. Difenacoum baits were laid in the same way in the facilities for storing seeds and seeding materials, and those for storing other miscellaneous materials.

Daily uptake of baits was measured daily per box, and new bait was added as required. Placebo baits were laid at the beginning and the end of each experiment for census assessments.

Data processing

The results were processed using Sokal and Rohlf's (1995) methodology and StatSoft (1997) software. The effects of cinnamon essential oil on the consumption of bromadiolone and difenacoum baits by house mice were assessed using Student's t-test at p=0.05 significance.

Mice numbers were assessed at the beginning and the end of each experiment using a method recommended by Hrgović et al. (1991).

The efficacy of rodenticide baits was calculated according to Richars and Huson's formula (cited by Đukić et al., 2005):

$$\%Rtime = 100 - \frac{Tsend}{TSmax} \times 100$$

where: % Rtime = percent reduction in mice numbers (efficacy);
Tsend = mean of consumed placebo bait in the last three days (end of experiment);
TSmax = maximum consumed placebo bait at the beginning of experiment.

RESULTS

Efficacy of bromadiolone baits

At the beginning of experiment in the mill, the lowest consumption of approximately 179.2 g was measured two and three days after laying the placebo baits, while the highest consumption of 194.1 g was found on the fifth day. Total consumption of placebo baits over the last three days of the experiment was 23.8 g.

In the farm storages, the lowest consumption of placebo baits of 154.2 g was noted on the first day, and the highest on the third day, 169.4 g. Total consumption of placebo baits over the final three days of baiting in the experiment was 19.9 g (Table 1).

Daily consumptions of bromadiolone (B) and bromadiolone+cinnamon essential oil (BC) baits over ten days of baiting in the mill storages showed significant differences (Table 2). Total consumption of B baits was 300.4 g, while the overall consumption of BC baits was 496.1 g. The highest daily consumption of either bait was noted on the first day, while a significant decrease in consumption was found after five days of baiting (Table 3).

Daily consumption of B and BC baits over ten days of baiting in the farm storages were found to differ significantly (Table 2). Total consumption of B baits was 199.8 g, while BC consumption was 365.9 g.

Table 1. Daily consumption of placebo baits at the beginning and the end of a house mouse experiment in mill and farm storages

Days	Placebo baits (g)			
	Mill storages		Farm storages	
	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment
1.	192.8	14.1	154.2	5.9
2.	179.8	10.4	158.3	6.9
3.	178.6	8.7	169.4	6.4
4.	186.9	9.6	158.6	6.4
5.	194.1	5.5	165.3	7.1
∑*	832.2	48.3	805.8	32.7

*Total bait eaten

Table 2. Population assessment at the beginning of experiment and results of total efficacy of baits in house mouse control, and effects of essential oil on the acceptability of bromadiolone and difenacoum baits (t-test, df=9)

Experiment	Population assessment[1]	Total efficacy (%)	t[2]	P[3]
Bromadiolone I[†]	28	95.91	3.2008	0.0108
Bromadiolone II	27	96.08	2.9837	0.0153
Difenacoum I	42	98.67	2.7813	0.0213
Difenacoum II	22	100.00	2.2892	0.0478

* Number of house mouse specimens according to Hrgović et al. (1991) formula; [2] Student's t-test; [3] * Significance at P=0.05; † Bromadiolone I-Experiment in mill storages; Bromadiolone II-Experiment in farm storages; Difenacoum I-Experiment in storages for seeds and seedling materials, and waste packaging; Difenacoum II-Experiment in storage of miscellaneous materials.

Table 3. Daily consumption of bromadiolone baits with and without cinnamon essential oil supplement in house mouse experiment in mill and farm storages

Days	Bait consumption (g)			
	Mill storages		Farm storages	
	B†	BC††	B	BC
1.	76.6	99.6	44.7	91.5
2.	61.7	98.4	51.8	78.3
3.	38.5	94.3	26.7	61.4
4.	44.2	81.6	31.9	65.3
5.	29.1	54.3	15.7	32.9
6.	22.6	34.6	12.1	16.2
7.	13.9	18.7	7.3	7.4
8.	6.6	8.4	2.2	6.9
9.	3.8	3.2	4.3	3.4
10.	3.4	3.0	3.1	2.6
Σ*	300.4	496.1	199.8	365.9

* Total bait eaten; † Bromadiolone baits with no essential oil supplement; †† Bromadiolone baits with cinnamon essential oil supplement

Table 4. Daily consumption of placebo baits at the beginning and the end of the house mouse experiment in storages for seeds and seedling material, and miscellany

Days	Placebo baits (g)			
	Seed and seedling storages		Miscellany storages	
	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment
1.	246.1	5.8	162.3	2.6
2.	248.9	5.4	188.7	0
3.	256.5	6.1	184.1	0
4.	258.3	1.0	153.5	0
5.	249.9	3.2	174.6	0
Σ*	1259.7	20.5	674.6	2.6

*Total baits eaten

On the first day of baiting, house mice consumed the highest amount of BC baits, i.e., 91.5 g, while B baits were most consumed on the second day, 51.8 g. A decrease in daily consumption was noted on the fifth day of baiting (Table 3).

Mouse counts were assessed to be 32 animals in the mill storages, and 28 animals in the farm storages. Total efficacy of bromadiolone in storages on both locations was 95.9 % and 96.1 %, respectively (Table 2).

Efficacy of difenacoum baits

At the beginning of the experiment in storages for seeds and seedling materials, the lowest consumption of placebo baits of 246.1 g was noted on the first day of baiting, while the highest consumption was found on the fourth day, 258.3 g. Over the final three days of the experiment, total consumption of placebo baits was 10.3 g (Table 4).

In the facility for miscellaneous materials, the lowest consumption of placebo baits of 153.5 g was found on the fourth day of baiting, while the highest consumption of 188.7 g was noted on the second day. Placebo baits were not consumed at all in the last four days of the experiment (Table 4).

Daily consumption of difenacoum baits (D) and difenacoum baits supplemented with cinnamon essential oil (DC) was significantly different in the facilities for storing seeds and seedling materials over the ten-day baiting period (Table 2). Total consumption of D baits was 259.8 g, while total consumption of DC baits was 568.6 g. Daily consumption of both baits was highest on the first day, and it decreased from the fourth day onwards (Table 4).

Daily consumption of D and DC baits over the ten-day bating period in miscellany storages was found to differ significantly (Table 2). Total consumption of D baits was 265.1 g, while the consumption of DC baits was 368.4 g.

Table 5. Daily consumption of difenacoum baits with and without cinnamon essential oil supplement in the house mouse experiment in storages for seeds and seedling material, and for miscellany

Days	Bait consumption (g)			
	Seed and seedling storages		Miscellany storages	
	D†	DC††	D	DC
1.	92.3	162.7	67.6	115.3
2.	63.7	154.8	74.1	88.4
3.	42.3	111.3	54.5	66.1
4.	21.4	65.2	35.4	44.7
5.	16.8	39.6	23.1	36.2
6.	10.8	23.2	7.8	12.5
7.	6.1	4.3	2.1	3.9
8.	3.6	5.1	0.0	1.3
9.	1.8	2.4	0.5	0.0
10.	1.0	0.0	0.0	0.0
Σ*	259.8	568.6	265.1	368.4

* Total baits eaten; † Difenacoum baits with no essential oil supplement; †† Difenacoum baits with cinnamon essential oil supplement

On the first day of baiting, house mice consumed the largest amount of DC baits, 115.3 g, while D baits were consumed in the largest amount of 74.1 g. Daily consumption was found to decrease from the sixth day onwards. Over the final three days of baiting, daily consumption of the tested D and DC baits was 0.5 and 1.3 g, respectively (Table 5).

Mice presence in the facility for storing seed and seedling materials was estimated at 32 animals, and in the miscellany storages at 31 animals. Total efficacy of difenacoum in the former and latter facilities was assessed at 98.7 % and 100 %, respectively (Table 2).

DISCUSSION

Based on characteristic signs of mice presence, such as feces, bites and foorprints, and on examination of animals caught during bromadiolone and difenacoum experiments, the presence of house mice was confirmed in all experimental locations. All storages were detached and no rodenticides had been applied in them for at least four months before the start of the experiment. Biological efficacy of the anticoagulant rodenticides was not found to have decreased on those locations.

Differences in bait consumption between bromadiolone supplemented with cinnamon essential oil, and without it, were confirmed in an analysis of data from Student's t-test. In the mill storage facility, where the effect of 0.75 % concentration of cinnamon essential oil on daily bromadiolone bait consumption was tested, the consumption of oil-supplemented bait was found to be around 65.0 % higher than the consumption of bait without the oil. In the farm storages, the consumption of oil-containing baits was 83 % higher than the consumption of baits without the oil.

Differences in the consumption of difenacoum baits with and without cinnamon oil were significant. In the facility for storing seed and seedling material and waste packaging, daily consumption of difenacoum baits by house mice was 119.0 % higher when bait contained the 0.75 % concentration of cinnamon essential oil, compared to bait without the oil. In the miscellany storage, cinnamon oil was found to increase the consumption of bait by 39 %.

The average efficacy of bromadiolone and difenacoum baits in house mouse control tests under different environmental conditions was 96 % and 99.5 %, respectively. The results of the present study are consistent with various other published data. Rowe and Bradfield (1976) reported an efficacy of bromadiolone of 60.4-100 % in controlling house mice, i.e. 92.4 % on the average, while difenacoum efficacy ranged 70.2-100 %, which was 96 % on the average. Similar results in controlling house mice with bromadiolone were also reported by Brooks and Rowe (1987). In their study, the efficacy of interior and exterior use of a bromadiolone product against house mice ranged 75-100 %. Advani (1995) reported 89-94 % efficacy of bromadiolone baits. In a study by Vukša et al. (2006), the efficacy of bromadiolone in controlling house mice in agricultural storages was 93-97%. Jokić et al. (2008) found the efficacy of different bromadiolone formulations in controlling house mice to range 89.2-91.7 %. Difenacoum efficacy in controlling house mice was found to range 89-100 % in a series of different other tests (Bull, 1976).

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Uticaj etarskih ulja na ishranu jedinki domaćeg miša: II - mamci na bazi bromadiolona i difenakuma sa dodatkom 0.75% etarskog ulja cimeta u praktičnim uslovima primene

REZIME

Utvrđen je uticaj etarskog ulja cimeta, u koncentraciji 0.75 %, na ispoljavanje atraktivnosti mamaca, kao i ukupna biološka efikasnost aktivnih materija bromadiolona i difenakuma za domaćeg miša u praktičnim uslovima primene.

Eksperimenti su izvedeni u skladišnim objektima, sa stabilnim populacijama domaćeg miša, za koje nije bila utvrđena rezistentnost na antikoagulantne rodenticide.

Utvrđena je statistički značajna razlika između ispitivanih mamaca na bazi bromadiolona i ispitivanih mamaca na bazi difenakuma. Mamci na bazi bromadiolona, sa sadržajem eteričnog ulja cimeta, prosečno su bili konzumirani za 74 % više od mamaca na bazi bromadiolona u kojima nije bilo dodatka ulja. Prosečna ukupna efikasnost ovih mamaca u suzbijanju domaćeg miša bila je 96 %.

Povećanje konzumacije mamaca na bazi difenakuma sa dodatim eteričnim uljem cimeta u ovom eksperimentu bila je za 39 %, odnosno 119 % viša u odnosu na mamce bez dodatka eteričnog ulja. Prosečna ukupna efikasnost ovih mamaca bila je 99,5 % u suzbijanju domaćeg miša.

Ključne reči: Rodenticidi; Etarska ulja; Cimet; Skladišta; Domaći miš; Atraktanti