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Scientific paper

Effects of Different Essential Oils on the Acceptability and Palatability of Cereal-Based Baits for Laboratory Mice

Goran Jokić[1], Marina Vukša[1], Suzana Đedović[1], Bojan Stojnić[2], Dragan Kataranovski[3,4] and Tanja Šćepović[5]

[1]Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia (jokicg@ptt.rs)
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia
[3]Institute for Biological Research „Siniša Stanković", Department of Ecology, University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade, Serbia
[4]University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg 16, 11000 Belgrade, Serbia
[5]Grant-holder of the Ministry of Education, Science and Technological Development of the Republic of Serbia

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SUMMARY

The aim of our study was to investigate the possibility of application of essential oils as additives in cereal-based rodenticide baits, at rates which prevent mould development and are applicable in humane medicine. Also, the purpose of these oils would be to extend the utility value of baits and reduce the use of antifungal ingredients that usually affect bait acceptance.

The effects of essential oils of ten different plant species, applied at rates of one and two ml per kg of plain bait, on bait acceptance and palatability in choice feeding tests for Swiss mice were studied under controlled laboratory conditions. Baits were prepared according to relevant EPPO standards (2004). The effects of essential oils on bait acceptance and palatability for Swiss mice were determined in choice feeding tests using a formula by Johnson and Prescott (1994).

There was no significant statistical difference in effects between groups and sexes regarding weight change in the experiment. During the experiment and recovery period, neither change in mice behavior no deaths were observed. Cinnamon and anise essential oils, commonly used as attractants, and clove oil, were most effective because they had no negative effect on bait acceptance and palatability, while fenchel and bergamot oils showed repellent activity that grew as the oil content in baits increased.

Keywords: Rodenticide baits; Essential oils

INTRODUCTION

Rodents are important pests in crop production (Larsson, 1975; Santini, 1986; Sheikher and Jain, 1991; Jokić et al., 2010) both as transmitters of a number of diseases and animals that cause feeding damage and pollution (Hrgović et al., 1991) in warehouses, granaries and grain mills (Pimentel, 1991) and on farms (Meerburg et al., 2004; Pelz and Klemann, 2004). A wide range of precautionary control methods have been used to prevent rodent damage (Jacob, 2003; Brown and Khamphoukeo, 2010; Stuart et al., 2010). However, lethal chemical agents, i.e. rodenticides, remain the most common method of control of rodent pests (Prakash, 1988). The success of rodent eradication with rodenticides depends on their active ingredients and carriers (Prakash et. al., 2003), environmental conditions (Salmon and Dochtermann, 2006) and bait persistance (Marsh, 1988).

As cereals are a worldwide commodity and an important dietary element for a majority of species, they have become the most common carriers for rodenticide baits. In dry environments, cereal-based baits stay in good condition for long periods but moulds develop fast under warm and highly humid conditions (Buckle, 1994).

Under such environmental conditions, moulds rapidly grow, especially fungi of the genera *Fusarium*, *Aspergillus* and *Penicillium,* which produce most mycotoxins (Amadi and Adeniyi, 2009). Moulds cause bait degradation, making bait ineffective, while their products, mycotoxins, cause rodent aversion to bait (Hrgović et al., 1991; Dunlevy et al, 2000).

Preservative materials are incorporated as additives in rodenticide baits to prevent or delay mould development (Brooks, 1962). Preservative materials in baits give us multiple advantages in rodent control management but each one has been found to reduce significantly the acceptance and palatability of baits (Buckle, 1994).

The aim of our study was to investigate the potentials of essential oils as additives in cereal-based rodenticide baits. The contents of essential oils in baits were chosen so as to be non-toxic to humans and to have fungicidal activity that prevents mould development in cereal-based products. The acceptability and palatability of baits containing 0.1 and 0.2% of essential oils for Swiss mice were determined under laboratory conditions.

MATERIAL AND METHODS

Animals

The laboratory trial was approved by the Ethics Committee of the Institute for Biological Research „Siniša Stanković“ (IBISS), Belgrade, (No. 3-04/13) and conducted in compliance with animal ethics regulation.

Adult males and females of the Swiss mouse (obtained from the Military Medical Academy, Belgrade, Serbia), weighing 20-25 g, were used in the study. The animals were kept individually in plastic cages and under a 12 h photoperiod, 21-24°C temperature and 45-70% relative humidity. Water was available *ad libitum*. The weight of Swiss mice did not vary significantly between treatment groups in assays with the lower ($F_{9,90}$ = 1.66; P > 0.05) and higher concentration of essential oils per 1 kg bait ($F_{9,90}$ = 0.35; P > 0.05).

Baits

Plain baits were prepared according to the relevant EPPO standard procedure (2004) by mixing 90% of coarse cereal, 5% of corn oil (cholesterol-free) and 5% of medium-ground oatmeal. The tested „oils“ baits were made by adding one or two ml of alcohol-diluted essential oil to each kilogram of plain bait. Previously, essential oils were dissolved in 25 ml/kg of pure alcohol. The same amount of pure alcohol (25 ml) was added to the plain baits. All baits were prepared 24 hours before treatment.

We used the essential oils of ten different plant species: anise (*Pimpinella anisum*), bergamot orange (*Citrus aurantium* ssp. *bergamia*), clove (*Eugenia caryophyllata*), cinnamon (*Cinnamomi zeylanicum*), eucalyptus (*Eucalyptus globulus*), fenchel (*Foeniculum vulgare*), lavender (*Lavandula officinalis*), rosemary (*Rosmarinus officinalis*), Scots pine (*Pinus silvestris*) and thyme (*Thymus vulgaris*).

Rodenticide baiting tests

In the pre-test period, the animals were given a diet for laboratory mice produced by the Veterinary Institute Subotica, Serbia. At the beginning of each assay, all mice were weighed individually.

Ten animals (five females and five males) were used in each assay. The animals were offered plain and oil baits in bowls placed at the opposite cage ends over a period of 96 h. In each bowl, 10 g of bait was offered daily. After each bait measurement, the location of bowls was switched. The daily amount of bait eaten by each animal

was recorded. During the assays, ordinary food was taken out of the cages. At the end of the exposure period, oil baits were removed and untreated feed was added.

Computation and statistical analyses

One-way analysis of variance (ANOVA) was used to compare the weights of individual animals between groups before treatment. The variance in body weight during assays was calculated according to the formula ((initial weight – final weight) / initial weight) × 100, as proposed by Guidobono et al. (2009) and compared by one-way analysis of variance. All comparisons were done using the software package Statistica for Windows 6.0 (Stat Soft Italia, 1997).

The effects of essential oils on bait acceptance and palatability for Swiss mice were determined in a choice feeding test according to a formula by Johnson and Prescot (1994):

$$\text{Bait acceptance (\%)} = \frac{\text{total weight (g) of rodenticide bait eaten (T)}}{\text{total weight (g) of control bait (C) and rodenticide bait (T) eaten}} \times 100$$

$$\text{Palatability ratio} = \frac{\text{total weight (g) of rodenticide bait eaten (T)}}{\text{total weight (g) of control bait eaten (C)}}$$

RESULTS

Cinnamon, anise and clove oils showed the highest level of acceptance (Table 1 and 2). With increasing cinnamon oil content, a growing trend of bait acceptance was recorded from 51.1% to 59.0%, while palatability correspondingly grew from 1.04 to 1.44. With an increase in anise oil content, the level of bait acceptance decreased from 49.5% to 41.6%, as did palatability, from 0.98 to 0.71. Also, with an increase in clove oil content in baits from one to two ml/kg of plain bait, bait acceptance grew from 47.0 to 50.4, and palatability from 0.88 to 1.01. The lowest level and declining trend in bait acceptance and palatability with increased content of oil in baits was recorded for fenchel, from 26.2% to 8.0%, and from 0.35 to 0.08, respectively, and for bergamot, from 9.0% to 2.6% and from 0.09 to 0.03.

Table 1. Acceptance and palatability of baits containing 1 ml of essential oil per 1 kg for Swiss mice in choice feeding test

Essential oils	Weight b.t.*		Weight a.t.†		Bait acceptance (%)	Palatability
	MS	SE	MS	SE		
Cinnamon	23.21	0.53	26.60	0.65	51.07	1.04
Anise	23.89	0.35	28.32	0.51	49.55	0.98
Clove	23.01	0.58	27.37	0.72	46.97	0.88
Eucalyptus	22.87	0.41	26.40	0.53	40.32	0.67
Lavender	23.43	0.26	27.69	0.34	36.74	0.58
Rosemary	22.35	0.56	26.47	0.60	34.86	0.54
Scots pine	22.12	0.42	26.37	0.61	36.44	0.57
Thyme	22.06	0.52	26.56	0.51	32.66	0.48
Fenchel	22.82	0.48	27.34	0.74	26.15	0.35
Bergamot orange	23.26	0.35	28.13	0.59	9.02	0.09

* average weight of individuals before treatment; † average weight of individuals after treatment

Table 2. Acceptance and palatability of baits containing 2 ml of essential oil per 1 kg for Swiss mice in choice feeding test

Essential oils	Weight b.t.*		Weight a.t.†		Bait acceptance (%)	Palatability
	MS	SE	MS	SE		
Cinnamon	22.93	0.50	26.75	0.71	59.02	1.44
Anise	23.00	0.39	26.77	0.48	41.64	0.71
Clove	23.04	0.51	26.78	0.73	50.36	1.01
Eucalyptus	23.17	0.46	27.00	0.72	30.28	0.43
Lavender	23.39	0.50	27.00	0.53	21.46	0.27
Rosemary	23.26	0.35	27.48	0.47	26.75	0.36
Scots pine	22.99	0.38	26.57	0.51	17.14	0.21
Thyme	22.43	0.40	26.22	0.41	26.25	0.35
Fenchel	23.13	0.32	27.06	0.39	8.03	0.08
Bergamot orange	22.78	0.28	26.66	0.41	2.64	0.03

* average weight of individuals before treatment; † average weight of individuals after treatment

Table 3. ANOVA parameters for main effects and associated interactions of essential oils and sexes on weight change between the beginning and the end of trial for Swiss mice in choice feeding test

Main effect	df	1 ml oil/kg bait		2 ml oil/kg bait	
		F	P	F	P
Intercept	1	1446.21	< 0.05	27778.03	< 0.05
E. oil	9	1.76	> 0.05	0.53	> 0.05
Sex	1	0.35	> 0.05	1.97	> 0.05
E. oil x sex	90	0.88	> 0.05	1.18	> 0.05

Total df = 80

No change in mice behavior was observed during the experiment and the recovery period and no deaths occurred.

After treatment, the weight of Swiss mice did not vary significantly between treatment groups in assays with the lower ($F_{9,90} = 1.59$; $P > 0.05$) and the higher concentration of essential oils per 1 kg bait ($F_{9,90} = 0.37$; $P > 0.05$).

Changes in animal weight between the beginning and the end of the experiment with baits containing 0.1% of essential oil ranged from 7.5% in the assay with anise oil to 32.0% in the assay with eucalyptus oil. In assays with baits containing 0.2% of essential oil, minimum weight change was detected in the assay with thyme oil, 8.9%, while maximum change of 23.0% was detected in the assay with fenchel oil.

There was no significant statistical difference between groups and sexes regarding the effects on weight change in the experiment (Table 3).

DISCUSSION

The amount of essential oils in baits applied in this study was similar to the quantity of attractants added during preparation of rodenticide baits. It is known that the addition of cinnamon and aniseed during preparation of rodenticide baits can improve bait acceptance by rodents (Marsh, 1988; Buckle, 1994). The results of our study show that cinnamon, anise and clove essential oils at the tested rates do not act as attractants, but also do not negatively affect bait acceptance and palatability for Swiss mice. In previous studies, Lee et al. (1999) had detected repellent properties of five percent ethanol solutions of a methanol extract of *Cinnamomum cassia* bark-derived components to laboratory mice. Yun et al. (1998) observed a potent repellent activity of methanol extracts of *C. cassia* bark and *Pinus densiflora* leaves for laboratory mice. Our research confirmed the repellent properties of *Pinus silvestris*. With the content of Scots pine essential oil increasing from 1 to 2 ml per 1 kg bait, acceptance and palatability dropped 50% and 63%, respectively.

Given that variation in body weight between the beginning and the end of experiment was uniform, especially in tests with 1 ml essential oil per 1 kg bait, we inferred that the essential oils applied at the tested rates had no negative impact on the growth of Swiss mice.

By now, the antifungal activity of cinnamon and clove essential oils against *Aspergilus flavus*, *Fusarium graminearum* and *Penicilium ssp.* (Marin et al., 2004; Aldred et al., 2008; Bluma and Etcheverry, 2008) and of anise oil (Bluma and Etcheverry, 2008) against *Aspergilus flavus* has been confirmed. It is also known that by adding paranitrophenol and dehydroacetic acid or paraffin it is possible to delay or fully prevent mould development, but at the same time they frequently reduce rodent bait acceptance (Buckle, 1994).

Based on the results of our study, cinnamon, anise and clove essential oils applied at the tested rates will not have a negative impact on acceptance and palatability of cereal-based baits. Also, based on findings in other studies, we believe that by adding these oils, mould development on baits can be delayed or prevented.

As the level of acceptance and palatability of baits with fenchel and bergamot essential oils was very low, especially in the assay testing the application rate of 2 ml oil per 1 kg bait, we inferred that those oils had repellent properties for Swiss mice. It may be useful to continue testing the repellent properties of bergamot and fenchel essential oils with the aim of protecting seeds and other material from rodent pests. Laboratory and field studies with rodent pests are required to confirm these results and to refine or optimize the use of some of these oils in rodenticide baits as attractants, or as repellents or preservatives, in order to improve rodent pest management control programs.

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Uticaj različitih esencijalnih ulja na prihvatljivost i palatabilnost mamaca na bazi žitarica za laboratorijskog miša

REZIME

Namera istraživanja je bila da se utvrdi mogućnost primene ispitivanih esencijalnih ulja u rodenticidnim mamcima kao potencijalnih dodataka, u količinama kojima bi se onemogućio razvoj plesni i koje su primenjive u humanoj medicini. Na taj način, primenom ovih esencijalnih ulja produžila bi se upotrebna vrednost mamaka i redukovala količina primene konzervanasa, koji najčešće negativno utiču na prihvatljivost mamaka. Pod kontrolisanim laboratorijskim uslovima, u testu sa izborom hrane, utvrđivan je uticaj esencijalnih ulja deset različitih vrsta biljaka, u količinama od 1 i 2 ml/kg placebo mamca na prihvatljivost i palatabilnost za jedinke domaćeg miša, soja Swiss. Ispitivani mamci su pripremljeni u skladu sa metodom EPPO (2004) standarda. Uticaj esencijalnih ulja na prihvatljivost i palatabilnost mamaka je izračunat na osnovu proporcije koju su predložili Johnson i Prescott (1994).

Nisu utvrđene statistički značajne razlike u težinama između slučajno odabranih grupa eksperimentalnih jedinki. Tokom eksperimenta i u periodu oporavka nisu zabeležene promene u ponašanju eksperimentalnih jedinki, kao ni uginuća. Esencijalna ulja cimeta i anisa, koji se i inače koriste kao atraktanti, i ulje karanfilića, ispoljili su najbolje efekte, odnosno nisu negativno uticali na prihvatljivost i palatabilnost, dok su ulja morača i bergamota ispoljili repelentno dejstvo, koje je bilo izraženije povećavanjem sadržaja ulja u mamcima.

Ključne reči: Mamci rodenticida; etarska ulja