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RESEARCH ARTICLE

A method for reducing environmental pollution by using essential oils in rodent pest management program

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Abstract Strong-smelling plant extracts, such as essential oils, have a variety of feeding effects on mammals. Considering current concerns over long-term health issues and environmental effects of chemicals, plant-based products with repellent or antifungal activities may represent good solutions for improvement of rodent pest control programs. The present study was therefore focused on examining the effects of bergamot, lavender, and thyme essential oils as additional bait components on daily intakes of cereal-based baits by wild house mice. Lavender essential oil, containing linalool and linalyl acetate as main components, and thyme essential oil with a prevailing thymol component had no effects on house mice diet. Bergamot essential oil, whose main components were linalool, limonene, and linalyl acetate, showed a repellent effect on house mouse diet.

Keywords Repellent · Essential oils · Bergamot · Lavender · Thyme · House mouse · Bait uptake

Introduction

The house mouse (*Mus musculus* L.) is one of the most widespread commensal rodent species and most important cause of damage of stored food, primarily through consumption and contamination of stored foodstuffs. Much of this loss is due to contamination with droppings and urine, making food unfit for human consumption. House mice may cause damage to feed storage structures and feed transporting equipment (Gorham 1991). They carry a number of zoonotic agents and should be treated as a potential threat to public health (Vujanić et al. 2011).

Pest control is required as soon as mice presence has been observed. Chemicals are the most frequent method of controlling house mice. Excessive use of pesticides, their high toxicity, and slow degradation have negative impact on the environment (Gomez-Canela et al. 2014; Pereira et al. 2015; Donkor et al. 2016). Increasing reports of resistance evolution to anticoagulating rodenticides in rodent populations often lead to rising dosages that fail to provide better control efficacy. Literature data indicate harmful effects of rodenticides on non-target mammals or prey birds (Mendenhall and Pank 1980; Berny et al. 1997; McDonald et al. 1998; Brakes and Smith 2005; Giraudoux et al. 2006). Anticoagulant contents in resistant animals may be up to fivefold the content in susceptible rodents (MacVicker et al. 2005; Berny et al. 2011).

Natural based product are a potentially promising alternative to chemicals. In quite different forms as essential oils, phytochemicals can be used with a wide range of effects on different organisms. Due to their rapid degradation, low mammalian toxicity and low likelihood of developing resistance, essential oils are a good alternative to chemical products (Moharramipour and Negahban 2014). Essential oils are safe to the environment, as well as to human health, and antimicrobial and antifungal properties of some essential oils in formulations of cereal-based rodenticides that have no negative effect on rodent food consumption could prolong their sustainability and efficacy. A repellent effect may be recommended as a useful means for protecting seeding materials, food, and seed commodities in storage. Attractiveness will increase bait acceptance and efficacy of rodenticides (Císarová et al. 2016).

The potentials of several essential oils rich in components that are known to have repellent effects on animals were tested

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on laboratory mice, Swiss strain in a follow-up study after initial experiments (Jokić et al. 2013). The essential oil of bergamot (*Citrus aurantium* ssp. *bergamia*) initially showed repellent activity, while lavender (*Lavandula officinalis*) and thyme (*Thymus vulgaris*) oils had mildly attracting effects on laboratory mouse diet. Based on our previous examination, we checked the effects of the mentioned essential oils on bait acceptance and palatability to wild-born house mice.

Materials and methods

Essential oils used and their analysis

Commercial essential oil products of bergamot (*C. aurantium* ssp. *bergamia*), lavender (*L. officinalis*), and thyme (*T. vulgaris*) used in this trial were manufactured by BeoLab, Belgrade, Serbia.

Chemical characterization of the essential oils was done by gas chromatography (GC), using two detector types. An Agilent GC (7890A model) was equipped with a split/splitless injector, flame ionization detector (FID), and HP-5 capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness). Injector and detector temperatures were set to 250 and 300 °C, respectively, while the nitrogen flow rate was 1 ml/min. Column temperature was linearly programed to rise from 50 to 250 °C at 4 °C/min before holding for 10 min. Analyses were performed on the Varian CP-3800/Saturn 2200 model of mass spectrometer (MS) detecting device. The injector temperature and column temperature were the same as for GC-FID analysis, while separation was performed using an Agilent DB-5MS column (30 m, 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas (1 ml/min), and the ion trap and transfer line temperatures were set to 250 and 280 °C, respectively. The mass detector was operated in the electron impact (EI) mode (70 eV; 40–600 m/z range). In both cases, essential oil solutions in n-hexane (1%) were injected in the split mode (1:20).

In order to determine the retention indices (RI), a mixture of n-alkanes (C_6-C_{28}) was analyzed by both GC-FID and GC-MS under identical conditions as the essential oils. Identification of essential oil components was performed using both the Wiley 7.0 mass spectral library and the obtained RI data, while quantitative data were expressed as area percent obtained by the GC-FID analysis. The obtained RI data were compared to those from available literature (Adams 2007). These data used as an additional tool to approve MS findings.

Procedure for testing effects of essential oils on rodent intake

The trial was conducted in compliance with the animal ethic regulation and approved by the Ethics Committee of the Institute for Biological Research "Siniša Stanković", Belgrade, Serbia.

In the trials, we used house mice previously caught from wild populations. The animals were acclimated in laboratory conditions (21–23 °C temperature, 45–70% relative humidity, and 12:12 day:night photoperiod) for at least 3 weeks before starting the trial. The animals were also monitored for possible behavioral changes over a period of 3 weeks after the trial. Individually caged mice adult (n = 20, 10 females and 10 males) were used in each trial.

One day before starting the trial, plain and oil baits (placebo bait with 1.0% of essential oil) were prepared as described in OEPP/EPPO standard methods (OEPP/EPPO 2004). Plain bait was prepared by mixing 1 kg of different crushed grains (maize, wheat, and barley) and 25 ml of pure alcohol (97%). Test baits were prepared by adding 1 ml of each essential oil dissolved in 25 ml of pure alcohol to 1 kg of crushed grain bait. The baits were kept in closed plastic bags in the laboratory before using them.

The trials included choice feeding tests in which the relevant OEPP/EPPO methodology was applied (OEPP/EPPO 2004). Over a period of 4 days, the animals were simultaneously offered plain and oil baits in two symmetrically placed food bowls at the front of their cages. To avoid possible accustomed feeding in the same position, bowl positions were alternated daily. The contents of the food bowls were made up daily to provide an excess of the animals' daily requirement from each bowl. Baits were replaced by fresh ones each day, and intake was measured daily. Standard food for laboratory mice was offered before the trial and after it was over. Water was available ad libitum. The weight of each animal was recorded immediately before the start of each trial.

Statistical analysis

Daily intakes were converted to bait (g)/100 g body weight (BW). Student's t test at 5% level of significance was performed to compare daily intake of oil and plain baits by wild-born house mice in a 4-day test.

Acceptance and palatability of baits with different oils on daily house mouse intake were calculated by Johnson and Prescott's proportion formula (Johnson and Prescott 1994). Effects of the tested oils on daily intake by males, females, and both genders of house mice were determined by one-way factorial analysis of variance. The Tukey-Kramer test was used for post hoc analyses at 0.5% significance level. Comparisons were made in Statistica for Windows 6.0.

Results

Analyses of essential oils

The main components of the tested essential oils are presented in Table 1. Six predominant components of thyme essential oil accounted for 95.17% (*v*/*w*) of total oil mass. Similarly, six compounds of bergamot essential oil made 87.1% of its total mass, while 12 components of lavender oil made 86.1%.

Thymol was the dominant component of thyme essential oil. This monoterpene phenol made 60.0% of total oil mass. Other components included γ-Terpinene (19.0%) and p-Cymene (7.1%) as monoterpene hydrocarbons, linalool (4.7%) as oxygenated monoterpene, carvacrol (2.3%) as monoterpene phenol, and 3-Octanone (2.0%) as ketone.

The predominant component of bergamot essential oil was limonene (39.0%) as a monoterpene hydrocarbon, and the next were linalool (12.5%) and linalyl acetate (27.0%) as oxygenated monoterpenes. All other components listed in Table 1 belong to monoterpene hydrocarbons (α-Pinene, sabinene, and β-Pinene), and they represent 8.6% of total oil mass.

In the essential oil of lavender, oxygenated monoterpenes represented 69% of total oil mass and linalyl acetate (28.8%) and linalool (27.1%) were their dominant components. Monoterpene and sesquiterpene hydrocarbons made 9.2 and 6.8%, respectively, while 3-Octanone as a ketone made 1.1% of total oil mass.

Bait intake

Total intakes of plain and oil baits by house mouse males, females, and both genders showed significant statistical difference (Table 2). The lowest total average intake by both genders of house mice was associated with the bergamot oil, around 15/100 g BW, while the highest intake of 49.33/100 g BW was recorded for the plain bait eaten by males in the lavender oil trial. Overall intake by house mice in the lavender trial was the highest, 69.9/100 g BW, while the consumption of bergamot and thyme baits was 56.43/100 g BW and 61.65/100 g BW, respectively. No change in body weight was detected in any of the test groups during the trial.

Acceptance and palatability of the oil baits differed significantly for male mice, being $F_{2,24} = 5.33$; $P = 0.0121$ and $F_{2,24} = 3.99$; $P = 0.0318$, respectively (Table 3). There was no statistical difference in bait acceptance considering all animals, while significant differences were detected for palatability, $F_{2,51} = 4.47$; $P = 0.0161$.

Discussion

A comparison of our results with literature data revealed a considerable consistency. Nikolić et al. (2014) and

Table 1 Chemical composition of essential oils tested

Component	RI_E[a]	RI_L[b]	Content (%)		
			Bergamot	Lavender	Thyme
α-Thujene	923	924	0.2		0.1
α-Pinene	932	932	1.2		0.5
Camphene	948	946		0.4	0.2
Sabinene	970	969	1.0		0.2
β-Pinene	974	974	6.4		0.1
1-Octen-3-ol	976	974		0.7	
3-Octanone	981	979		1.1	2.0
Myrcene	989	988	0.6	0.7	0.1
α-Phellandrene	1005	1002	0.5		0.2
Hexyl acetate	1006	1007		0.6	
δ-3-Carene	1010	1008		1.6	
α-Terpinene	1012	1014	0.4	0.6	0.3
p-Cymene	1022	1020			7.1
Limonene	1029	1024	39.0	0.7	0.3
1,8-Cineole	1025	1026		0.7	0.2
(Z)-β-Ocimene	1030	1032	0.6	5.0	
(E)-β-Ocimene	1040	1044	0.5	2.6	0.2
γ-Terpinene	1052	1054	0.7		19.0
cis-Linalool oxide	1066	1067		0.7	
Linalool	1096	1095	12.5	27.1	4.7
Camphor	1140	1141		0.8	0.1
Borneol	1163	1165		1.4	0.2
Lavandulol	1167	1165		1.2	
Terpinene-4-ol	1184	1174	0.4	5.2	0.2
α-Terpineol	1187	1186	0.7	0.6	
Nerol	1228	1227	0.7		
Neral	1238	1235	0.6		
Hexyl isovalerate	1242	1241		0.7	
Geraniol	1250	1249	0.5		
Linalyl acetate	1252	1254	27.0	28.8	
Geranial	1266	1264	0.5		
Bornyl acetate	1284	1284		0.8	
Lavandulyl acetate	1287	1288		5.3	
Thymol	1289	1289			60.0
Carvacrol	1294	1298			2.3
Terpinen-4-ol acetate	1298	1299	0.5		
Thymol acetate	1348	1349			0.2
Neryl acetate	1358	1359	0.6	0.6	
Carvacrol acetate	1370	1370			0.1
Geranyl acetate	1377	1379	0.4		
Daucene	1378	1380		0.6	
(Z)-α-Bergamotene	1409	1411	0.2		
β-Caryophyllene	1416	1417	0.5	4.6	0.2
(E)-α-Bergamotene	1431	1432	0.3		
(Z)-β-Farnesene	1442	1440		2.2	
α-Humulene	1451	1452			0.2
γ-Muurolene	1480	1478	0.1		0.1

Table 1 (continued)

Component	RI_E[a]	RI_L[b]	Content (%)		
			Bergamot	Lavender	Thyme
Germacrene D	1483	1484	0.1	0.6	0.2
β-Bisabolene	1508	1505	0.4	0.7	
γ-Cadinene	1514	1513		0.6	
δ-Cadinene	1521	1522			0.1
Elemol	1546	1548			0.1
Caryophyllene oxide	1583	1582		0.5	
Epi-α-cadinol	1636	1638		0.4	
α-Bisabolol	1684	1685	0.1	0.5	
Total			97.2	98.6	99.2

[a] RI_E—Retention indexes experimentally determined (calculated relative to C6-C28 n-alkanes on the HP-5 column)

[b] RI_L—Retention indexes-literature (Adams 2007)

Pekmezović et al. (2015) reported that thymol, p-Cymene, and γ-Terpinene were the main components of thyme essential oil originating from Serbia. On the other hand, a thyme oil originating from Italy had carvacrol and p-Cymene as its main components, while an oil from Brazil contained mainly borneol and α-Terpineol and an Indian oil was mainly rich in thymol, eucalyptol, and terpinene-4-ol (Pesavento et al. 2015; Kohiyama et al. 2015; Ghrairi and Hani 2015).

Chemical characterization of bergamot and lavender essential oils originating from various world regions revealed significantly less difference regarding their dominant components than thyme oil. For example, limonene and linalyl acetate were the main components of bergamot oil originating from Italy, Turkey, and Tunisia (Kirbaslar et al. 2000; Romano et al. 2005; Costa et al. 2010; Nabiha et al. 2010). Similarly, linalyl acetate and linalool were the predominant compounds in lavender essential oils originating from Argentina, Australia, Turkey, and Poland (Danh et al. 2013; Kara and Baydar 2013; Śmigielski et al. 2013; Martucci et al. 2015). All of these data are consistent with the composition of the essential oils tested in our present study.

In contrast to previous trials with a laboratory strain of house mice, when lavender and thyme essential oils showed slightly attractant effects, the tested oils had no effects on diet of the wild house mice used in our present study. This indicates the importance of using wild rodent populations in experiments for adequate assessment of test compounds in practical conditions.

The effect of lavender oil with linalyl acetate as its prevailing component was not significantly different from those of the other two tested oils. Thymol, the main component of our thyme oil, is used as a repellent for pet or wild animal protection, mostly in sprays or creams (EPA 1993). Our data show that although thymol content was predominating (60%), thyme essential oil cannot be used as a reliable house mice repellent. Cereals make the predominant carriers of rodenticide active ingredients. However, baits tend to mold under some environmental conditions and become prone to infection with mold-causing fungi, primarily of the genera *Fusarium*, *Aspergillus*, and *Penicillium* (Buckle and Smith 1994). Molds developing on baits produce toxins, which significantly reduce their acceptability to targeted rodents. Also, baits that are only weakly acceptable or fully unacceptable to rodents turn into a considerable environmental hazard. With toxicity intact, they increase the risk of accidental poisoning of humans or other warm-blooded organisms (Dunlevy et al. 2000; Berentsen et al. 2014). The antifungal activity (Zabka and Romana 2013; Houicher et al. 2016; Divband et al. 2017) and low toxicity of thymol (LD_{50} = 1800 mg/kg) make it an eco-friendly component of rodenticides. As thymol has no negative impact on food consumption of the house mouse, its incorporation in baits would prevent or postpone mold development, thus, improving the quality of rodenticide baits, their persistence, and acceptability to rodents. The use of such

Table 2 Total intake of oil and plain baits (bait (g)/100 g body weight) by wild-born house mice in a 4-day test

Gender	Bergamot		Lavender		Thyme	
	Plain bait	Oil bait	Plain bait	Oil bait	Plain bait	Oil bait
Male	42.11 ± 2.21[†]	14.61 ± 2.28	49.33 ± 2.59	22.67 ± 1.76	37.54 ± 2.33	25.15 ± 2.81
t test	6.5667**		6.7088**		2.5461**	
Female	40.64 ± 2.36	15.51 ± 1.96	46.68 ± 2.83	20.85 ± 2.73	40.28 ± 2.91	20.92 ± 4.29
t test	6.5122**		5.1631**		2.9461**	
Male + female	41.37 ± 1.57	15.06 ± 1.46	48.08 ± 1.83	21.82 ± 1.51	38.94 ± 1.80	22.71 ± 2.52
t test	9.4756**		8.5939**		3.8803**	

Data were compared by Student's *t* test at 1% level of significance

[†] Mean value ± standard error. Daily intakes were converted to bait (g)/100 g body weight

Table 3 Acceptance and palatability of baits to wild-born house mice

Gender	Bergamot		Lavender		Thyme	
Bait acceptance (MS ± SE)						
Male	25.61 a*	± 3.71	31.68 ab	± 2.73	39.81 b	± 4.11
Female	27.67 a	± 3.45	30.68 a	± 3.79	32.46 a	± 6.24
Male + female	26.64 a	± 2.47	31.21 a	± 2.22	35.94 a	± 3.82
Palatability (MS ± SE)						
Male	0.3682 a	± 0.0605	0.4836 ab	± 0.0624	0.7158 b	± 0.1003
Female	0.4083 a	± 0.0678	0.4751 a	± 0.0849	0.5888 a	± 0.1335
Male + female	0.3882 a	± 0.0443	0.4796 ab	± 0.0501	0.6489 b	± 0.0839

*Values marked by the same letter in a row are not statistically significant ($P < 0.05$; Tuckey test)

baits would thus reduce environmental pollution, as well as risks to non-target species.

Bergamot oil had the lowest acceptability and palatability, 26.64 and 0.38, respectively, which agrees with the results of our trials on the laboratory strain of house mice (Jokić et al. 2013). Similar data had been reported by Kalandakanond-Thongsong et al. (2010) and Kalandakanond-Thongsong et al. (2011) who found bergamot oil to have a repellent effect on male adults of the laboratory rat, Wistar strain.

Limonene, one of the main components of our bergamot oil, was in 1983 registered as a repellent to be used for protection of pet dogs and cats (EPA 1994). The other two most dominant components of our bergamot oil, linallol and linalyl acetate, have been used in various commercial rodent repellent products designed for indoor use (Warberg 2002). Our data indicate a necessity to evaluate the effects of individual components (limonene, linallol, and linalyl acetate), as well as their synergistic effects on house mice food intake. It should reveal the most dominant component or the best combination of components with repellent activity, which may improve the non-toxic program for prevention of mice-caused damage. Floor treatment with repellents near or directly on transport packages would reduce possibilities for rodent presence and resulting economic losses. Similar effects could be expected concerning the protection of electrical wiring in buildings and protection of machines and devices. Further research under different environmental conditions are required to evaluate their cost-efficacy and efficacy in practice.

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