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Original scientific paper

Response of wild *Mus musculus* to baits containing essential oils: I - Cinnamon and clove tested in storage facilities

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SUMMARY

The effect of cinnamon (*Cinnamomi zeylanicum*) and clove (*Eugenia caryophyllata*) essential oils on the acceptability of cereal-based baits to house mice in storage facilities was examined. The effects of three concentrations, 0.5, 0.75 and 1 %, were tested for optimization purposes. The experiments were performed in a mill storage, and in another storage for seeds and seedlings.

Attractiveness of the examined concentrations of cinnamon and clove essential oils was statistically significant, compared to placebo bait, within seven days of the experiment. On the seventh day, the average consumption of baits containing 0.75 % and 1 % concentrations of cinnamon oil was 46 % in the mill storage, i.e. 35.5 % more than placebo baits were consumed. Fourteen days after the beginning of the experiment, no significant difference was detected in the consumption of baits offered in the mill storage. On the other hand, a significant difference was revealed regarding the consumption of examined baits in the seed and seedling storage. The average consumption of baits containing 0.75 % and 1 % cinnamon oil was 47 % and 51 % higher after 14 days than the consumption of placebo bait. Compared to the other test baits, those containing cinnamon essential oil at 0.75 % and 1 % concentration demonstrated better attractiveness without mutual statistically significant differences.

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

INTRODUCTION

The house mouse, *Mus musculus*, is one of the most important commensal rodent species in human environment. The species may cause significant damage

to plant materials and products, as well as to electrical wiring, machines and electronic devices (Đukić et al., 2005). The activity of house mice raises the cost of product storage both through direct damage and contamination of primary and processed plant products.

Storage costs also rise due to mandatory rodent control measures and compensation for economic damages caused by rodents (Hrgović et al., 1991; Buckle & Smith, 1994).

Cereals, due to their widespread cultivation, nutritive value and resulting importance, are the most common carriers of rodenticide active ingredients for most rodents. Under dry conditions, such baits are stable over long periods of time. However, their persistence under humid and warm conditions depends on the rapidness of mold development, which spoils the quality of bait, and leads to ultimate reduction in efficacy (Buckle & Smith, 1994). Various additives serve to prevent and slow down mold development, and so improve bait quality and persistence (Brooks, 1962). On the other hand, despite improving bait stability, many additives degrade their attractiveness (Buckle & Smith, 1994).

Essential oils are widely used in human and veterinary medicine, and for protection against insects, mites and pathogens. The essential oils of cinnamon (*Cinnamomum zeylanicum*) and clover (*Eugenia caryophyllata*) are used against insects (Plata Rueda et al., 2018), mites (Rezaei et al., 2014) and pathogens (Božik et al., 2017). Antifungal activity of cinnamon and clover essential oils on mold has been confirmed (Ju et al., 2018). Also, laboratory tests have confirmed the attractiveness of cinnamon and clover essential oils in food to the laboratory mouse, Swiss strain (Jokić et al., 2013).

The present study aimed to test possible improvements of bait acceptability for the house mouse by adding essential oils, which is an acceptable solution both from the aspect of environmental protection, quality and safety of food and human health protection. The results should be meaningful to experts in extension services, to manufacturers and to staff providing rodent control treatments.

MATERIAL AND METHODS

Bait preparation

Test baits were prepared according to the relevant OEPP/EPPO (2004a) methodology. Non-poisonous, placebo baits were prepared by mixing coarse grain of wheat, barley and maize at 30:30:40 ratio. Total ratio of the cereal mixture, wheat flower and maize oil was 90:5:5. Baits containing cinnamon or clover essential oils were prepared by adding specific amounts of essential oil, diluted in 25 ml alcohol, to placebo baits. The placebo baits in experiments assessing the effects of essential oils on house mouse food consumption were supplemented with equal amounts of pure alcohol. The test baits were prepared

to contain 0.5 %, 0.75 % and 1 % essential oils of either cinnamon or clover, and the oils used in the testing were manufactured as commercial products by BeoLab, Serbia.

Experimental design

The attractive activity and effects of essential oils on bait acceptability, and their optimal concentrations, were determined in trials conducted in a mill storage (950 m²) and a storage for seeds and seedlings (1200 m²). The products were stored in sacks or boxes and elevated on pallets. In the facility for storing seeds and seedlings, a small part of the material was stored in bulk.

The experiments were performed according to the relevant OEPP/EPPO (2004b) methodology. Baits were given in commercial bait boxes with sideways openings 2 cm in diameter to allow entry to mice while barring other warm-blooded organisms (Figure 1). The boxes were positioned in concentration sequence at 1-2 m distance along mice routes, and in the vicinity of damaged material. Daily offer included 20 g of bait per box.



Figure 1. Bait boxes

Bait attractiveness

Placebo baits containing only the nutrient carrier, and baits with different contents of cinnamon and clover essential oils, were laid in bait boxes in acceptability (attractiveness) trials (Figure 2). A total of 84 bait boxes were laid in each storage facility, providing the following arrangement:

- 12 boxes containing placebo bait;
- three concentrations of cinnamon oil:
3 × 12 boxes = 36 boxes;
- three concentrations of clover oil:
3 × 12 boxes = 36 boxes.

Consumed bait was measured daily per box, and fresh bait was added to each as needed. The baits were offered over a total period of 14 days. The effects were assessed 1, 2, 3, 7 and 14 days after the baits were first offered.



Figure 2. Baits laid

Data processing

The results were processed according Sokal and Rohlf (1995) using StatSoft software (1997). One-way analysis of variance (ANOVA) was employed to assess the effects of different concentrations of cinnamon and clover essential oils on house mouse feeding in the mill and in the facility for storing seeds and seedling material. Bait consumption means were compared using Duncan’s test at P=0.05 significance level.

The house mouse populations were assessed at the beginning and the end of each experiment using a method proposed by Hrgović et al. (1991). Mice numbers were calculated based on daily consumption of placebo baits over several consecutive days using the formula:

$$N = \frac{H}{h \times n}$$

where

N = assessed number of animals;

H = total amount of food (g) consumed over the entire experimental period (n);

h = average daily food requirement of the rodent species per animal (g), i.e. 6 g/day (six, h=6) for the house mouse, according to Hrgović et al. (1991);

n = duration of baiting period (days).

RESULTS

Table 1 shows the daily amounts of eaten placebo baits over a five-day baiting period in the mill storage. House mouse population was assessed to be 40 animals at the beginning, and 39 animals at the end of the experiment.

Table 1. Daily placebo bait consumption in the mill storage (facility I)

Baiting duration	Placebo bait consumption per day (g)				
	1 st day	2 nd day	3 rd day	4 th day	5 th day
Beginning of experiment	192.5	255.8	273.2	237.4	245.6
End of experiment	237.8	241.3	232.6	229.8	240.8

Figures 3-7 show the average mouse consumption of placebo bait and baits containing different concentrations of cinnamon and clover essential oils. The symbols used in the figures should read: PI= Placebo; C0.5= Cinnamon 0.5 %; C0.75= Cinnamon 0.75 %; C1= Cinnamon 1 %; K0.5= Clover 0.5 %; K0.75= Clover 0.75 %; K1= Clover 1 %. The data are means for eaten (consumed) bait per box, according to Duncan’s test (p=0.05). Symbols marked with the same letter indicate non-significant statistical difference.

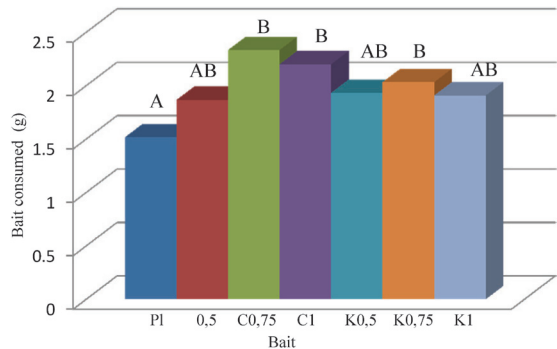


Figure 3. Average consumption of placebo bait and baits with different contents of cinnamon and clover essential oils by house mice on the 1st (first) day of experiment in the mill storage

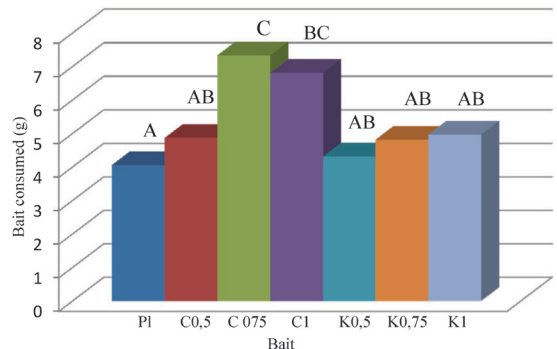


Figure 4. Average consumption of placebo bait and baits with different contents of cinnamon and clover essential oils by house mice 2 (two) days after the beginning of experiment in the mill storage

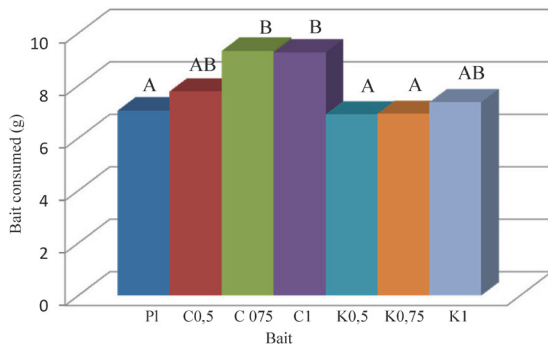


Figure 5. Average consumption of placebo bait and baits with different contents of cinnamon and clover essential oils by house mice 3 (three) days after the beginning of experiment in the mill storage

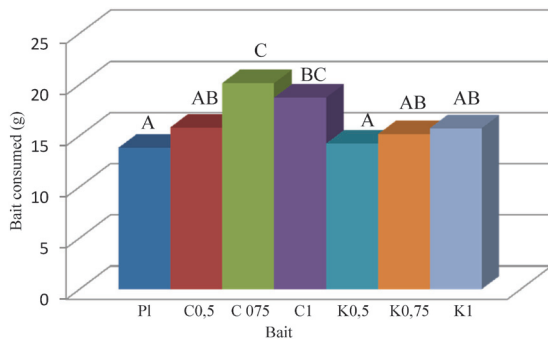


Figure 6. Average consumption of placebo bait and baits with different contents of cinnamon and clover essential oils by house mice 7 (seven) days after the beginning of experiment in the mill storage

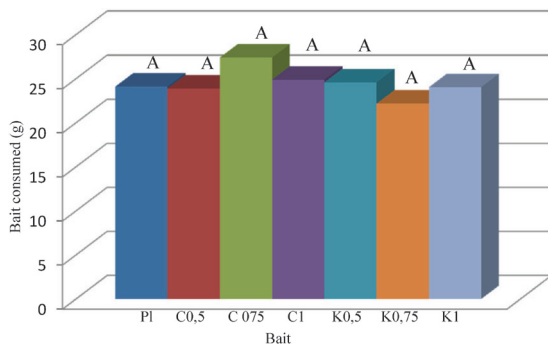


Figure 7. Average consumption of placebo bait and baits with different contents of cinnamon and clover essential oils by house mice 14 (fourteen) days after the beginning of experiment in the mill storage

Daily consumption of placebo baits in the facility for storing seeds and seedling material, assessed over five days, is presented in Table 2. Mouse presence was

assessed to be 59 animals at the beginning, and 61 animals at the end of the experiment.

Table 2. Daily consumption of placebo baits in the seed and seedling storage (facility II)

Baiting duration	Placebo bait consumption per day (g)				
	1 st day	2 nd day	3 rd day	4 th day	5 th day
Beginning of experiment	324.4	334.7	374.5	361.1	389.7
End of experiment	355.6	394.2	367.7	354.4	370.4

Figures 8-12 show the average consumption of placebo baits and baits supplemented with different concentrations of cinnamon and clover essential oils by house mice in the storage for seeds and seedlings.

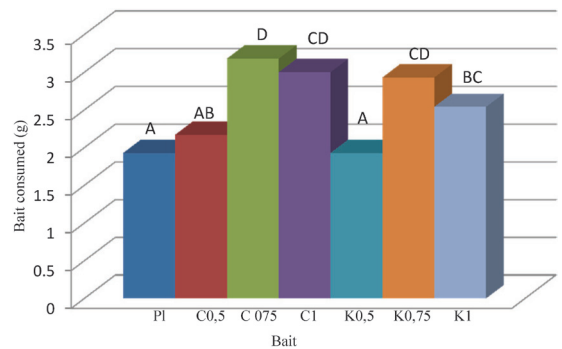


Figure 8. Average consumption of placebo baits and baits supplemented with different concentrations of cinnamon and clover essential oils by house mice on the 1st (first) day of experiment in the seed and seedling storage

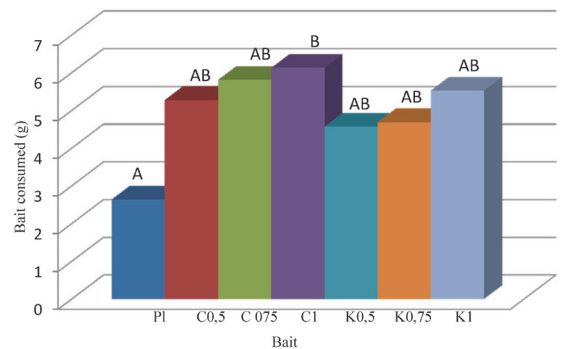


Figure 9. Average consumption of placebo baits and baits supplemented with different concentrations of cinnamon and clover essential oils by house mice after 2 (two) days of experiment in the seed and seedling storage

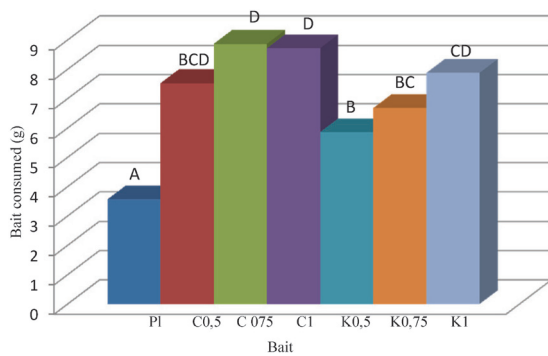


Figure 10. Average consumption of placebo baits and baits supplemented with different concentrations of cinnamon and clover essential oils by house mice after 3 (three) days of experiment in the seed and seedling storage

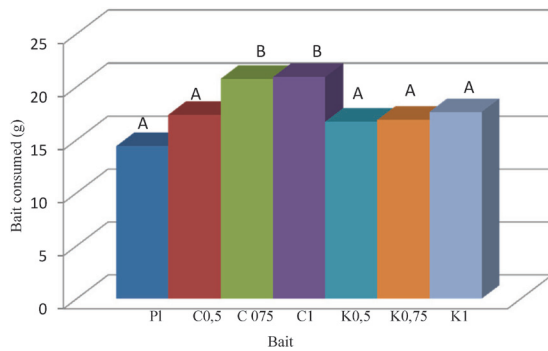


Figure 11. Average consumption of placebo baits and baits supplemented with different concentrations of cinnamon and clover essential oils by house mice after 7 (seven) days of experiment in the seed and seedling storage

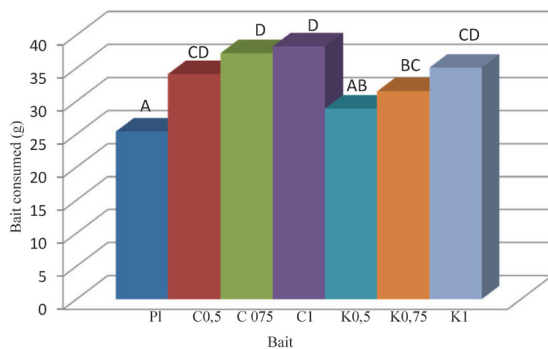


Figure 12. Average consumption of placebo baits and baits supplemented with different concentrations of cinnamon and clover essential oils by house mice after 14 (fourteen) days of experiment in the seed and seedling storage

The average consumption of placebo baits and baits supplemented with different concentrations of cinnamon and clover essential oils by house mice in the mill storage (facility I), and seed and seedling storage (facility II) showed significant statistical differences after 1, 2, 3 and 7 days. No significant difference was detected in daily bait consumption in the mill storage after 14 days ($F_{6,21}=0.688$; $P=0.6611$). In the seed and seedling storage, bait consumption data for the same time period also revealed significant differences (Table 3).

On the first day of baiting in facility I, the highest daily consumption was noted for baits containing 0.75 and 1 % cinnamon essential oil, and for the highest concentration of clover essential oil. In facility II, the highest level of initial attractiveness was demonstrated by baits with 0.75 % cinnamon oil, and the average daily consumption was 3.2 g.

On the second day of baiting in facility I, the highest attractiveness was shown by baits with 0.75 and 1 % concentration of cinnamon oil, and the average daily consumption was 6.8-7.3 g/day. Excepting placebo baits, which achieved the lowest attractiveness for house mice, there were no significant statistical differences among any of the other baits. In facility II, the highest daily consumption was found for baits containing 1 % cinnamon oil concentration and for the lowest concentration of clover oil.

Comparing data for facility I on the 3rd day of experiment with the other test baits, cinnamon oil concentrations of 0.75 and 1 % showed the best attractiveness for house mice. The average daily consumption of those baits was steadily at around 9.3 g/day. Daily consumption of all other baits, including placebo bait, was not significantly different. In facility II, baits with 0.75 and 1 % cinnamon oil concentrations, as well as baits with the highest clover oil concentration, showed attractiveness for the house mouse. Daily consumption of those baits showed no mutual difference on average in statistical terms.

Seven days after the experiment started in facility I, the highest level of attractiveness for the house mouse was demonstrated by cinnamon oil bait with 0.75 % concentration as its average daily consumption was 20.1 g. The average daily consumption of baits with the lowest concentration of clover oil and placebo baits was at the same level and showed no statistical difference over the given period. In facility II, the average daily consumption of baits containing the two top concentrations of cinnamon oil was 20.8 g on average and higher than consumption of all other baits.

At the end of the experiment (14 days), the average daily bait consumption in facility I ranged from 22.2 g (clover oil concentration 0.75 %) to 27.4 g (cinnamon oil concentration 0.75 %). In facility II, baits with all three concentrations of cinnamon oil and the highest concentration of clover oil showed the highest attractiveness.

Table 3. Effects of different concentrations of cinnamon and clover essential oils on bait consumption by house mice in the mill, and in the facility for storing seeds and seedling material (one-way ANOVA)

Assessment intervals	Facility I*		Facility II†	
	$F_{6,21}$	P	F	P
1 st day	2.786	0.0372	9.678	0.0001
2 nd day	3.019	0.0275	6.643	0.0001
3 rd day	2.972	0.0290	9.307	0.0001
7 th day	3.514	0.0141	3.917	0.008
14 th day	0.688	0.6611	11.728	0.0001

* Facility I = mill storage; † Facility II = seed and seedling storage

DISCUSSION

The approximate counts of house mice in the facilities where the attractiveness of baits with different contents of cinnamon and clover essential oils were evaluated indicate stable populations which were not exposed to any other harmful activity.

All storages were isolated units in which no rodenticides had been used for at least six months prior to the experiment.

Buckle and Smith (1994) reported that cinnamon essential oil may be used as an attractant for rodents, but neither a formulation nor a rate were suggested. In our earlier studies, the predominant constituent of cinnamon essential oil with 96.7 % content was cinnamaldehyde (IUPAC: (E)-3-phenyl-2-propenal), known synonymously also as: cinnamic aldehyde, 3-phenyl acrolein, 3-phenyl propenal, benziliden acetaldehyde, cinamal, 3-phenyl acrilaldehyde, and under its CAS identifier 104-55-2. Eugenyl acetate (2.2 %) and eugenol (0.5 %) were found as minor ingredients. Eugenol is known to have been used for trapping rodents (Safety Data Sheet, 2016). Eugenol (CAS: 97-53-0),

an organic compound with the chemical formula $C_{10}H_{12}O_2$, is the main component of clover essential oil (75 %), while eugenyl acetate content is lower, 4.4 %.

Data analysis showed that all test concentrations of cinnamon and clover oils increased bait acceptability as early as 24 h after the experiment started. Regarding the 2-7 days period of baiting, cinnamon essential oil was found to have positive effect on bait acceptability, especially its 0.75 and 1 % concentrations. The highest concentration of clover oil was also found to influence bait consumption by house mice over the period, only less significantly than the cinnamon oil concentrations mentioned. After seven days, the average consumption of baits containing cinnamon oil concentrations 0.75 % and 1 % was up to 46 %, i.e. 35.5 % higher than the consumption of placebo baits. This level of contribution of clover and cinnamon essential oils to bait attractiveness is consistent with laboratory data (Jokić et al. 2013).

No significant difference was observed between the effects of the test concentrations of essential oils and placebo baits in the mill storage at the end of the experiment, i.e. after 14 days of assessment. Over the interval between the last two assessments, average bait consumption was approximate in the test facility, ranging from 7 to 11 g. Based on the results, as well as the fact that essential oils are highly volatile, the test baits were assumed to have lost their attractiveness to the house mouse over the trial period. Furthermore, dust may also have spoiled the baits and decreased their attractiveness. As manufacturers recommend that baits for rodent control be placed for periods of 7-10 days, the formulated products at concentrations tested in this study may be expected to achieve full utilization.

Significant statistical differences in bait consumption were revealed in the seed and seedling storage in all assessment intervals. The average consumption of baits containing 0.75 % and 1 % essential oil of cinnamon was 47 % and 51 % higher, respectively, compared to placebo baits.

Analysing the results, baits containing 0.75 and 1 % cinnamon essential oil were found to possess feeding attractiveness for the house mouse. As the data showed steady average daily consumption of baits with those two concentrations of cinnamon, the lower concentration of 0.75 % was chosen for further optimization tests for bait formulation improvement.

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Uticaj etarskih ulja na ishranu jedinki domaćeg miša: I - cimet i karanfilić ispitivani u skladištima

REZIME

Utvrđivan je uticaj etarskih ulja cimeta (*Cinnamomi zeylanicum*) i karanfilića (*Eugenia caryophyllata*) na prihvatljivost mamaka na bazi žita za domaćeg miša u skladišnim uslovima. U ciju optimizacije sadržaja etarskog ulja, ispitivan je efekat tri različite koncentracije, 0,5, 0,75 i 1 %. Eksperimenti su izvedeni u skladišnim objektima mlina i objektima za skladištenje semenskog i sadnog materijala.

Atraktantno delovanje ispitivanih koncentracija etarskih ulja cimeta i karanfilića u odnosu na placebo mamac bilo je statistički značajno od početka pa do sedmog dana eksperimenta.

Sedmog dana, u objektima mlina, prosečna konzumacija mamaca sa sadržajem etarskog ulja cimeta u koncentracijama od 0.75 % i 1 % bila je do 46 %, odnosno 35.5 % viša u odnosu na placebo mamac. Četrnaestog dana nije zabeležena statistički značajna razlika u konzumaciji ponuđenih mamaca u objektima mlina. U skladišnim objektima semenskog i sadnog materijala, zabeležena je statistički značajna razlika u konzumaciji ispitivanih mamaca. Prosečna konzumacija mamaca sa sadržajem od 0.75 % i 1 % etarskog ulja cimeta, četrnaestog dana od početka eksperimenta bila je za 47 %, odnosno 51 % viša u odnosu na placebo mamac. U odnosu na druge ispitivane mamce, mamci sa sadržajem etarskog ulja cimeta u koncentracijama od 0.75 % i 1 % ispoljili su atraktantnije delovanje u odnosu na druge mamce i međusobno se nisu statistički značajno razlikovali.

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