Pestic. Phytomed. (Belgrade), 39(3), 2024, 53–60
DOI: https://doi.org/10.2298/PIF2403053B

UDC 632.958:633.15:664.8.035:547.21:599.323.4
Original scientific paper

Improvement of rodent pest control strategy: II - Response of wild brown rats to baits containing preservative previously exposed to unfavourable environmental conditions

Tanja Blažić[1*], Rada Đurović-Pejčev[1], Tijana Đorđević[1], Ivana Jovičić[1], Irena Međo[1], Bojan Stojnić[2] and Goran Jokić[1]

[1] Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia
[2] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia
** Corresponding author: tanja.scepovic@pesting.org.rs*

Received: 15 October 2024
Accepted: 4 November 2024

SUMMARY

Control of brown rats (*Rattus norvegicus*) is an indispensable measure of protection of human population and domestic animals, aimed at preventing the spread of infectious diseases, and a measure of protection of goods and commodities that the brown rat can damage by its presence and activities. Rodent control procedures in environments with unfavourable conditions, such as high temperature and humidity, may fail due to reduced bait palatability caused by its degradation. Baits were prepared based on OEPP/EPPO recommendations, while ECHA recommendation was followed for bait exposure to unfavourable environmental conditions. Placebo baits were prepared by mixing ground maize grain and paraffin. Test baits, made by mixing the placebo bait with sorbic acid, were exposed to unfavourable environmental conditions (temperature 30-35 ^{0}C and humidity 90-95%). Brown rats previously captured from a wild population were used as the test organism. After an acclimation period, the animals were subjected to a four-day choice feeding test in which they were simultaneously offered placebo and test baits. The average bait palatability was unchanged, and the palatability of bait containing the preservative was 48.85 %. Furthermore, the preservative had no influence on bait consumption, compared to baits in the pre-test period, as there was no statistical difference in total amounts of bait consumed in the tests. The results indicate that 1% sorbic acid can be used as a potentially good additive to baits that are planned to be used in sewage systems and other areas where unfavorable environmental conditions prevail and there is a risk of rapid mold development and bait decay.

Keywords: *Rattus norvegicus*, rodent, preservatives, mold, palatability

INTRODUCTION

Rodents, especially commensal rodent species, cause enormous damage by their presence and activities. Being the hosts and vectors of a number of zoonoses, they pose a serious health threat to humans and domestic animals (Battersby et al., 2008; Kataranovski & Kataranovski, 2021; Gajdov et al., 2024). Wild-born rodents are reported to be reservoir hosts for at least 80 zoonotic diseases that pose serious threat to public health (Meerburg et al., 2009).

Rodent activities lead to enormous economic losses. They greatly increase the cost of production, storage and transport of products by their feeding, contamination and damage to facilities (Buckle, 1994; Almeida et al., 2013). Researchers have estimated the overall global economic cost of invasive rodents between 1930 and 2022 at USD 3.6 billion (USD 87.5 million annually). The costs reported for brown rat (*Rattus norvegicus*) specifically amounted to USD 156.6 million. Of that total cost, 87% were damage-related, principally impacting agriculture, and predominantly reported in Asia (60%), Europe (19%) and North America (9%). The dynamics of cost showed an exponential increase over time. The global cost was assessed to be much higher in reality, reaching more than 80-fold higher (USD 297.4 billion), using less conservative data filtering (Diagne et al., 2023).

Half of the world's population currently lives in urban environments and the trend of urbanization is accelerating. Proper management of urban areas is therefore crucial for creating desirable living conditions in those environments (Pickett et al., 2011; Luo et al., 2012), and it is particularly important because urbanization promotes the spreading and development of rodent populations. As a result, the use of rodenticides in urban environments has become a necessity and it is rather different from agricultural applications, which are limited to specific crops (Wittmer et al., 2010).

The brown rat will eat anything that is edible and it is strongly attracted to human dwellings because they offer ample sources of food in the form of garbage. Urban sewers are the perfect man-made rat habitats. Given their small fluctuation in ambient temperature that provides indoor warmth in winter and coolness in summer, and continuous influx of food through sewer pipes, the sewer network provides an environment in which rats can breed undisturbed all year round. As the sewage system protects brown rats from predators, their populations can expand to maximum numbers (Channon et al., 2006; Battersby et al. 2008).

Sewer networks often provide shelter for rat colonies, and infestation spreads from there into other parts of urban environments. Brown rats are protected inside sewers from rodent control procedures that are only undertaken on the surface, which is why they later, especially when they overpopulate, leave sewer networks and come out onto the surface (Channon et al., 2000). Sewer systems should therefore be considered in conjuction with surface control efforts (Twigg, 1975). As rapid urbanization is synchronized with more intense development and transmission of infectious zoonoses, especially because rats living in sewers come into contact with infective waste material and cause damage to pipelines, rat control in sewer networks has become a necessity (Hrgović et al., 1991; Akhtar et al., 2023). Therefore, the best results can only be achieved by simultaneous treatments of surface and underground habitats of rodents (Channon et al., 2000; Fozzard et al., 2014).

The conditions existing in sewer networks stimulate the growth and development of rodent populations while creating difficulties for successful application of rodenticides. High temperature and high humidity stimulate the development of microorganisms on baits, causing their degradation. As cereals, the standard bait carriers, are prone to mold development, predominantly by the genera Penicillium, Aspregilus and Fusarium, mico- and aflatoxins are produced easily on baits (Wagacha & Muthomi, 2008). As a result of altered structure, taste and odour, such baits become less attractive to rodents (Blažić et al., 2017; Jokić et al., 2024). Reduced palatability, resulting from deteriorating organoleptic properties of baits, may then lower their efficacy.

By applying substances that extend the longevity of baits, rodent control practice may gain in efficacy, most particularly in environments that offer conditions for quick bait deterioration. Sorbic acid [E 200] has ample uses as a preservative in food processing and cosmetic industries. The mechanism of its activity has not been fully clarified but it is assumed to penetrate the membrane of microorganisms and inhibit enzymes involved in metabolism (Stopforth et al., 2005).

Sorbic acid, which is widely used to inhibit the growth of microorganisms, has also shown effectiveness in rodenticide baits. Bait longevity extends considerably when sorbic acid is added (Jokić et al., 2024; Blažić et al. 2024). The purpose of the present study was to investigate the palatability of baits containing sorbic acid as a preservative for brown rats.

MATERIALS AND METHODS

Animals

Brown rats were captured on a pheasant farm in Belgrade suburbs. Mature and healthy animals, with body weight ranging from 100-400 g, were transported to the laboratory. The animals were treated with an insecticide to control ectoparasites. All animals were under observation during a three-week acclimation period. They were kept individually in standard cages (400 × 300 × 300 mm) under laboratory conditions of 20±2 °C ambient temperature, relative humidity varying from 40-70% and a 12:12 h light/dark cycle. Ventilation was provided at 15 min intervals for complete air refreshment. Standard laboratory diet was offered to all animals. It was commercially available and manufactured by Versele Laga nv, Belgium. Water was available *ad libitum*.

Bait preparation

Bait was prepared using a modified method of EPPO (2004). Ground maize grain was used as the carrier for bait preparation. Control bait consisted of a carrier and paraffin mixture. The bait components were mixed on a rotary mixer for an hour at 20 rpm. The carrier:paraffin ratio was 60:40 %. Cold homogenization was chosen for bait preparation, so that the basic components were added without increasing temperature in the mixture, i.e. paraffin was added into mixture without melting, so that it remained in solid state.

A preservative was also added into the test bait besides the carrier and paraffin. Sorbic acid was chosen as the preservative and used at a concentration of 1 %. The components were mixed on a rotary mixer following the same procedure that was used for control baits, and bait components were gradually added. After mixing the carrier and preservative, paraffin was added to the mixture. Baits containing the preservative were then exposed to unfavourable environmental conditions according to ECHA (2023) recommendations. The baits were exposed to high temperature (30 °C -35 °C) and humidity (90%-95%) inside an incubator for a period of five days. They were then placed in the incubator in succession to make sure that all baits have been offered to rodents after being previously exposed to unfavourable environmental conditions for five days.

Pre-test period

After acclimation, and before the trial began, the animals were divided into two groups (5 females + 5 males) and exposed to a four-day pre-test period. Standard laboratory diet was offered to all animals. Laboratory conditions were the same as during the acclimation period.

The rats were fed for four consecutive days and bait consumption was recorded. Spilled food was collected daily under each cage. Water was available *ad libitum*.

Test period

After the pre-test period, all animals were included in a choice feeding test for four consecutive days. The choice test was conducted according to relevant OEPP/EPPO (2004) methodology. The animals were simultaneously offered placebo and test baits in two symmetrically arranged feeding bowls at the front of each cage. To avoid possible accustomed feeding in the same position, bowl positions were alternated daily. Consumed baits in both bowls were recordered daily. Baits were replaced by fresh ones each day. Standard diet for laboratory rodents was offered after the trial was over. Water was available *ad libitum*. The weight of each animal was recorded before and after trial.

Data analysis

Mean bait consumption (g/g body weight) was calculated for each individual. Mean consumption was calculated as the average daily consumption over the period (g), divided by starting weight (g).

Statistical analysis was conducted using a one-way ANOVA and Tukey's HSD test to analyse the influence of sex on bait consumption and palatability. A two-way ANOVA was used to analyse the influence of sex and bait, and their interactions on consumption during pre-test and test periods.

In all analyses, P-values below 0.05 were considered statistically significant.

Ethics statement

The experiment was conducted in accordance with ethical principles and was approved by the Ministry of Agriculture, Forestry and Water Management (Republic of Serbia) - Veterinary Directorate (No. 323-07-04155/2023-05/3 16.05.2023).

RESULTS

Brown rats had uniform consumption during pre-test and test periods (Figures 1 and 2). The amount of consumed conditioning bait was not statistically different from the amount of placebo and test baits during the test. The main effects of sex and baits, and interactions of effects were insignificant (Table 1).

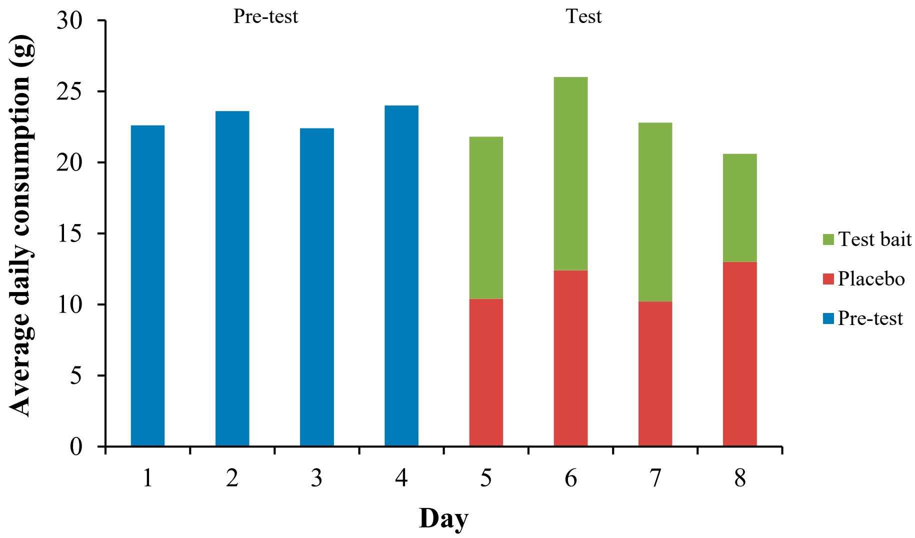

Figure 1. Average daily bait consumption (g) of brown rat females during pre-test period (conditioning) and test period (test bait with 1% sorbic acid and placebo bait)

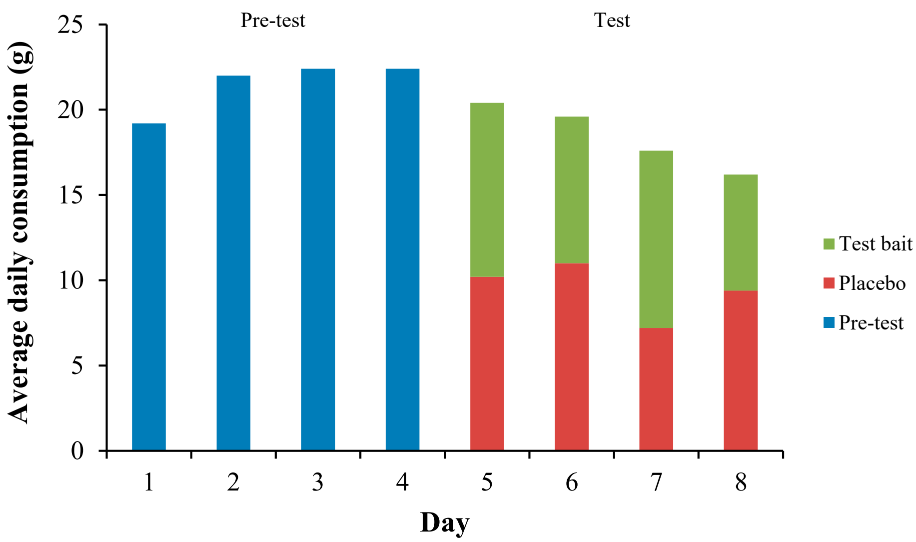

Figure 2. Average daily bait consumption (g) of brown rat males during pre-test period (conditioning) and test period (test bait with 1% sorbic acid and placebo bait)

Table 1. Two-way ANOVA for bait consumption by brown rats over pre-test and test periods

Effect	Degree of freedom	F	p
Bait	1	0.73	0.40
Sex	1	2.28	0.15
Bait*Sex	1	0.46	0.51
Error	16		

The palatability of preservative-supplemented baits ranged from 33% to 59%. It exceeded 50% in six animals (Table 2). Bait palatability was not significantly affected by sex ($F_{1,8}$= 0.06; p= 0.81).

Table 2. Palatability of test baits containing preservative (sorbic acid 1%) by wild-born brown rats

N_0	Initial weight (g)	Final weight (g)	Baits	I day (g)	II day (g)	III day (g)	IV day (g)	Σ (g)	Palatability (%)
Females									
1	287	274	Test bait	14	14	15	8	51	51.00
			Placebo bait	12	17	7	13	49	
2	326	322	Test bait	13	18	17	11	59	48.76
			Placebo bait	17	14	15	16	62	
3	210	208	Test bait	16	12	12	8	48	53.33
			Placebo bait	5	12	11	14	42	
4	149	144	Test bait	4	16	13	5	38	56.72
			Placebo bait	9	5	6	9	29	
5	192	183	Test bait	10	8	6	6	30	38.46
			Placebo bait	9	14	12	13	48	
Males									
6	126	116	Test bait	3	5	4	6	18	33.33
			Placebo bait	13	10	8	5	36	
7	178	177	Test bait	14	12	8	7	41	58.57
			Placebo bait	5	6	6	12	29	
8	255	249	Test bait	11	8	16	8	43	52.44
			Placebo bait	10	14	6	9	39	
9	213	210	Test bait	7	14	10	9	40	50.63
			Placebo bait	13	8	9	9	39	
10	229	221	Test bait	16	4	14	4	38	45.24
			Placebo bait	10	17	7	12	46	

The amount of consumed test and placebo baits over the test period was not statistically different between females ($F_{1,8}$= 0.03; p= 0.87) and males ($F_{1,8}$= 0.42; p= 0.53). Nominally, female mean consumption of test bait was higher (Ms_{test}= 0.21 g/g bw; $Ms_{placebo}$= 0.20 g/g bw) than male mean consumption (Ms_{test}= 0.18 g/g bw; $Ms_{placebo}$= 0.21 g/g bw).

DISCUSSION

The results indicate that 1% sorbic acid preservative had no negative impact on bait palatability for brown rats. In some previous studies rats had been found to be sensitive to changes in bait taste and odor caused by mold development. In our earlier research (Blažić et al., 2017), rats significantly reduced bait consumption as mold started to develop on bait and the process progressed. During a 6-day test period, animals were offered bait which had been previously exposed to unfavourable environmental conditions for periods progressively extended by 24 h on each consecutive day of the test. Five days after the animals were given bait that had spent 120 h under unfavourable conditions, bait palatability was reduced to merely 12.38%, while consumption completely ceased on the sixth day after bait had spent 144 h in incubator, and the trial was terminated. The situation was similar in the same study with house mouse (*Mus musculus*), which indicates rodent sensitivity to changing organoleptic properties of bait. No change was observed in the study regarding bait consumption during a pre-test period, as opposed to the test period. Additionally, there was no change in the consumption of placebo and test baits over the test period, which shows that the preservative did not alter the flavor of baits or had a repellent effect on brown rats. This is especially important from the aspect of rodent control practice. These results are consistent with the fact that sorbic acid has neutral taste and odor, which is a general characteristic of sorbate salts too, so its addition would not affect the organoleptic properties of product (Bajčić et al., 2021).

Bait palatability data are particularly important considering rodent pest control purposes. Reduced bait palatability in the field leads to weaker control effectiveness. Refusal of bait may lead to an erroneous conclusion that the number of rodents in the field is not high, which may consequently result in huge damage and irreparable losses. Generally, the brown rat causes enormous damage, both in terms of public health and economic losses. Potential damage exceeds many times the funds required for their effective control (Buckle, 1994; Battersby et al., 2008; Almeida et al., 2013; Kataranovski & Kataranovski, 2021; Jokić et al., 2022; Jokić et al., 2023; Diagne et al., 2023; Pustahija et al., 2024; Gajdov et al., 2024). The results indicated a good palatability of baits containing sorbic acid preservative as it exceeded 50 % in more than half of the test animals, while it ranged from 33% to 49% in all other animals. The average palatability of bait containing the preservative was 48.85 %.

Sorbic acid is considered one of the least toxic antimicrobial preservatives that are used in food processing industries (Stopforth et al., 2005). Sorbic acid has been found nontoxic to rats and mice in acute oral toxicity studies. In subchronic studies, no significant adverse effects have been observed on rats, mice or dogs when 10% sorbic acid was included in their diet. At concentrations exceeding 10%, sorbic acid was irritating (Anonimus, 1988).

CONCLUSION

The test results show that 1% sorbic acid added to bait had no negative effect on bait palatability for brown rats. Since sorbic acid postponed mold development on maize-based baits and had no effect on bait palatability, it was concluded that it is potentially a good additive for baits that are planned to be exposed to unfavourable environmental conditions, such as high temperature and high humidity. This is especially important for baits intended for applications in habitats with high temperature and high humidity, such as underground facilites or sewage systems.

ACKNOWLEDGEMENT

This study was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-66/2024- 03/200214.

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Unapređenje strategije suzbijanja štetnih glodara: II - Prihvatljivost mamaka sa konzervansom koji su prethodno izlagani nepovoljnim uslovima sredine za jedinke sivog pacova

REZIME

Suzbijanje sivog pacova (*Rattus norvegicus*) predstavlja neizostavnu meru zaštite stanovništa i domaćih životinja sa ciljem sprečavanja prenošenja i širenja zaraznih bolesti kao i meru zaštite ljudskih dobara od šteta koje sivi pacov može proizvesti svojim prisustvom i aktivnošću. Postupak kontrole brojnosti glodara u prostoru gde preovladavaju nepovoljni uslovi sredine, kao što su povišena temperatura i vlažnost, može biti neuspešno zbog smanjene prihvatljivosti mamka uzrokovane njegovom degradacijom. Priprema mamaka izvedena je u skladu sa preporukom OEPP/EPPO metode, dok je sam postupak izlaganja mamaka nepovoljnim uslovima sredine izveden u skladu sa preporukom ECHA. Placebo mamci pripremljeni su mešanjem mlevenog zrna kukuruza i parafina. Test mamci, pripremljeni mešanjem placebo mamka i sorbinske kiseline, izlagani su nepovoljnim uslovima sredine (temperatura 30-35 ^{0}C i vlažnost vazduha 95-99 %). Kao test organizam korišćene su jedinke sivog pacova, prethodno izlovljene iz divlje populacije. Nakon perioda adaptacije jedinke su uključene u pre-test u trajanju od četiri dana. Prosečna prihvatljivost ispitivanog mamka je bila ujednačena, odnosno prihvatljivost mamak sa dodatkom konzervansa je bila 48,85 %. Utvrđeno je da sorbinska kiselina nema negativnog uticaja na prihvatljivost mamka. Takođe, konzervans nije uticao ni na konzumaciju mamka u poređenju sa mamcima iz pre-test perioda s obzirom da se ukupna količina konzumiranog mamka tokom testa nije statistički razlikovala od količine konzumiranog mamka tokom pre-test perioda. Rezultati ukazuju da se sorbinska kiselina pri sadržaju od 1% može koristiti kao potencijalno dobar aditiv za pripremu mamaka koji će biti izlagani u kanalizacionim sistemima i drugim prostorima u kojima preovladaju nepovoljni uslovi sredine i u kojima postoji rizik od brzog razvoja plesni i degradacije mamka.

Ključne reči: *Rattus norvegicus*, glodari, konzervansi, plesni, prihvatljivost