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Original scientific paper

Improvement of rodent pest control strategy: I - Selection of an adequate preservative for bait base longevity under unfavourable environmental conditions

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SUMMARY

Preservation of the appearance, structure and most importantly the attractiveness of baits targeting harmful rodents over extended periods of their exposure is one of the main tasks in the process of bait formulation. The impact of several preservatives, different methods of homogenization of main bait components, and base-paraffin ratios on the extension of longevity of bait base was examined under controlled laboratory conditions. Baits based on ground maize grain were exposed to unfavourable environmental conditions, such as high temperature (30 °C-35 °C) and air humidity (90%-95%). The exposure period was 9 days, and potential mold development was monitored over the same period of time. Sodium benzoate was found deficient in providing a satisfactory effect on bait longevity (<5 days), in contrast to potassium sorbate (>7 days) and sorbic acid, which provided the longest extension in bait sustainability (>9 days). Preservative application can significantly extend bait longevity under unfavourable environmental conditions. Extended bait functionality is important for rodent pest control procedures in habitats where unfavourable environmental conditions prevail, such as sewers and water supply grids (collectors and pumping stations), public areas, housing facilities (boiler rooms, moist sellers, etc.).

Keywords: baits, preservatives, mold, longevity, palatability

INTRODUCTION

The brown rat (*Rattus norvegicus*) is one of the most important harmful rodent pests that cause significant economic losses in the process of production, processing and storage of plant and animal products. Brown rats pose health risks for humans, as well as domestic and wild animals, being the hosts and vectors of many infectious diseases (Battersby et al., 2008; Kataranovski & Kataranovski, 2021; Jokić et al., 2022). Their exceptional adaptability to extremely variable types of habitats (rural, suburban and urban), and not being exacting in nutritional requirements, enables them to survive in habitats with unfavourable environmental conditions, such as sewers and water supply grids and other moist areas (Lund, 1994).

Considering the damage that they cause, and a need to protect public health, as well as the health of domestic and wild animals, it is necessary to undertake regular monitoring and control of brown rats. Rodenticide applications are the most frequent measure, as well as the most effective way of controlling harmful rodents (Bentley, 1972; McGee et al., 2020). In order for a rodenticide to acheive good efficacy in rodent control, it needs to demonstrate adequate toxicity, as well as satisfactory palatability. It means that the structure, quality and appearance of bait need to stay unchanged throughout the exposure period in order to ensure satisfactory intake of the toxic active ingredient in bait (Buckle, 1994).

Rodent control in habitats characterized by high humidity and temperature (sewer pipes, moist sellers, canals) is often hindered to a significant degree by reduced bait palatability, which results in lower bait efficacy. Under conditions of high humidity and temperature, baits undergo degradation, which allows microorganisms to thrive and spoil the structure and quality of bait. Such baits loose their attractiveness, which is based on good organoleptic properties. The consequence of reduced bait attractiveness is a decrease in its consumption, even its complete cessation, which results in a failed procedure of rodent pest control (Blažić et al., 2017).

Good palatability, and widespread availability, make cereals one of the most frequent base materials for rodenticide baits. A good-quality cereal is sufficiently attractive to rodents to produce excellent results when used with an effective rodenticide. However, increased temperature and moisture turn cereals into excellent bases for microorganism development. They further produce toxins that affect in a negative way the appearance, taste and odour of baits, which decreases their practical value (Buckle, 1994).

Substances that may significantly extend the freshness and persistence of products are known as preservatives. Sorbic acid and its salts (sorbates) are listed as additives in legislative provisions of the Republic of Serbia. Sorbic acid (E200), potassium sorbate (E202) and sodium benzoate (E211) have broad applications in food processing and pharmaceutical industries as preservatives (Lazarević et al., 2012; Shahmohammadi et al., 2016). The use of these substances is allowed, and their non-toxicity is of great importance from an ecotoxicological point of view. After breakdown of any product containing them, they would no longer be expected to pose a danger to non-target animals, considering the results obtained in some earlier studies where sorbic acid, applied at a concentration of 5%, did not have any harmful effect on laboratory rats (Demaree et al., 1955).

Initial attempts to delay rodenticide degradation under conditions of high humidity were made in the 1950s. Paraffin was then used for the first time as an additive to rodenticides intended for controlling brown rats in sewers because it was noticed that it extends bait longevity. Paraffin was found to have a number of advantages which ensure its wide applicability. Primarily, it significantly delays bait degradation (Marsh & Plesse, 1960). Considering the firmness that baits are given by its application, they have become safer due to a level of selectivity as some species are repelled by the need to bite and chew in order to get to the tastier but toxic part of the bait. Baits also became more tolerant to insect infestation, which resulted in lower bait dissipation. However, paraffin deficits include higher production cost, as well as melting and change of structure at high temperatures (Marsh, 2012). Another important shortcoming is its effect on rodenticide persistence in natural environments. Paraffin slows down rodenticide degradation, and thereby extends its availability to nontarget species, which is a serious issue from the ecotoxicological aspect (Lund & Lodal, 1990). Depending on the type of formulation and intended purpose of bait, some 40-50 % of paraffin in baits is considered sufficient to ensure bait persistence over longer periods of exposure in rodent habitats characterized by unfavourable environmental conditions (Marsh, 2012). However, paraffin has a negative impact on bait palatability, so that bait becomes less acceptable to rodents as the content of paraffin increases.

Baits must remain palatable and attractive to rodents for an eradication effort to be effective. Extended periods of bait persistence would significantly improve rodent control effectiveness. Short longivity periods of rodenticide carriers of up to 5 days under unfavourable environmental conditions are insufficient for successfull rodent control, which had been confirmed in our earlier preliminary research (Blažić et al., 2017). Efforts were therefore directed towards finding a procedure that would delay mold development on bait carriers, and extend the longevity and attractiveness of bait by adding preservatives. Research tasks focused on choosing the proper type and concentration of preservative (sorbic acid, potassium sorbat and sodium benzoate), content of paraffin in bait, and method of mixing the components, in order to achieve a more persistent and effective bait for rodent control.

MATERIALS AND METHODS

Bait preparation

Test baits

Three types of preservatives were used to prepare the test bait: sodium benzoate (E211), potassium sorbate (E202) and sorbic acid (E200). The effectiveness of the chosen preservatives was determined for 4 contents in bait: 0.3%, 0.5%, 1% and 2%.

The effect of four different contents of paraffin on bait persistence was also tested in combinations with different types and contents of preservatives: 75%:25%,70%:30%, 65%:35% and 60%:40%, respectively.

The longevity of two types of bait were tested: bait without paraffin (base+preservative) and bait containing paraffin (base+preservative+paraffin). Different contents of preservatives and paraffin were tested for each type of bait. Bait longevity was tested for baits without paraffin: base+E211, base+E202, base+E200; and baits with paraffin: base+E202+paraffin (25%-40%) and base+E200+paraffin (25%-40%). Sodium benzoate was excluded from the second trial stage (with paraffin) because of unsatisfactory results in the first stage.

Bait homogenization

Bait preparation was based on maize grain ground to a fineness sufficient to facilitate the required homogenization of bait components (Table 1). Cold homogenization was chosen as a method of bait preparation in which the main components are added without raising temperature in the mixture. The bait components were mixed on a rotary mixer for an hour at a speed of 20 rounds per minute.

Preparation of paraffin-free baits

Baits were made by mixing the product base with preservatives. Control baits consisted of the base without added preservative. They were exposed to the same conditions as test baits in order to determine the period of bait persistence without any added preservative.

Preparation of baits with paraffin

Besides determining the effects of preservatives in baits containing only the base and preservative, the impact of paraffin on bait persistence was also tested.

Simultaneous mixing of bait components

Simultaneous mixing implies that all components (base+preservative+paraffin) are added and homogenized at the same time. The content of the tested base and paraffin was 75%:25%. The test results showed that there was no need for testing the other three proportions of base and paraffin (70%:30%, 65%:35% and 60%:40%).

Gradual mixing of bait components

Gradual mixing implies that bait components were mixed by gradually adding and mixing them. The base and preservatives were the first to undergo homogenization. After their homogenization on a rotary mixer, paraffin was added and the process of homogenization proceeded. All four proportions of base and paraffin were examined (75%:25%,70%:30%, 65%:35% and 60%:40%).

Experimental design

Dishes were filled with 100 g of bait preparation each and put in a climate chamber. Each type of bait was exposed in 4 replicates. Following the ECHA (2023) methodology, chamber conditions were set to simulate unfavourable environmental conditions of high temperature and humidity. Ambient temperature was 30-35 °C, while air humidity ranged from 90% to 95%. Mold development was inspected daily, ending with day 9. The dishes were removed from the chamber after first symptoms of mold have appeared. After day 9, baits were checked once more two days later (i.e. on day 11). All baits were removed from the climate chamber after 11 days.

Table 1. Grinding fineness of maize grain (MS-mean value of different fractions of ground maize grain, SE-standard error)

Sieve opening (mm)	Replicates					MS (%)	SE
	I (%)	II (%)	III (%)	IV (%)	V (%)		
2	1.86	2.81	1.66	1.2	1.69	1.87	0.23
1.25	22.2	24.16	25.69	20.21	22.22	19.28	4.04
0.8	25.85	25.31	23.27	26.94	23.6	20.96	4.46
0.5	17.33	13.81	15.11	26.94	23.75	16.24	4.13
≤ 0.5	32.74	33.9	34.26	24.74	28.75	30.88	1.82

Data analysis

Statistical analysis was conducted using ANOVA and Tukey's HSD test to examine the influence of preservative content and base-paraffin ratio on bait longevity. For all groups in which bait longevity exceeded the test period of 9 days, an 11-day period was used for the analysis. P-values below 0.05 were considered as statistically significant in all analyses.

RESULTS

Longevity of paraffin-free baits

Mold development was detected on baits containing low concentrations of sodium benzoate (0.3% - 0.5%) between the 3rd and 5th day. Bait longevity was 4-6 days when higher contents of that preservative were applied (Table 2).

Baits containing low concentrations of potassium sorbate had 5-6 days longevity, while higher contents allowed either 7-9 days logevity period or there were no mold symptoms at all on the 9th day.

The lowest concentration of sorbic acid was found to allow mold development from 6 to ≥9 days of the test. No mold development was found in dishes with the three highest concentrations of the preservative until the end of testing (>9).

Table 2. The effects of preservatives on bait longevity (paraffin-free)

Preservatives	Contents (%)	Symptoms appearance (days)
Sodium benzoate	0.3	3.75
	0.5	4.75
	1	5.5
	2	5.25
Potassium sorbate	0.3	5.25
	0.5	5.25
	1	7.75
	2	>9
Sorbic acid	0.3	7.0
	0.5	>9
	1	>9
	2	>9

Control baits developed symptoms of mold after three days (Figure 1).

a)

b)

Figure 1. Development of microorganisms on bait base (ground maize grain): a) one and three days, b) four and six days after baits were exposed to unfavourable environmental conditions (temperature 30-35 °C and air humidity 90-95%).

Longevity of baits containing paraffin

Simultaneous mixing of components

When bait components were simultaneously mixed, the longevity of those containing potassium sorbate did not exceed 5 days in the climate chamber (Table 3). Initial symptoms of mold were detected as early as on the 3rd day of the test. In dishes containing sorbic acid, symptoms were found to develop after 4-5 days.

Table 3. The effects of preservatives on bait longevity after simultaneous mixing of components

Preservatives	Contents (%)	Symptoms appearance (days)
Potassium sorbate	1	4
	2	4.5
Sorbic acid	0.5	4.5
	1	4.75

Mold symptoms in control dishes developed after three days.

Gradual mixing of bait components

The longevity of baits containing sorbic acid was longer than 9 days, i.e. no symptoms of microorganism development appeared on those baits until the end of the observed test period, regardless of paraffin content. Potassium sorbate showed a similar effect, considering that microorganisms developed after 8 days (Table 4).

A statistically significant influence of preservative concentration on bait longevity was found ($F_{3,60}$=53.97; P=0.00). The longest longevity period was found in test baits that contained sorbic acid, while baits with potassium sorbate achieved longer longevity periods only at higher concentrations. Regarding potassium sorbate bait (2%), paraffin was found to influence bait longevity ($F_{3,12}$=97.00; P=0.00). The longevity period was longer for baits with higher paraffin contents (65-35% and 60-40%). For baits with a low content of potassium sorbate (1%), no statistically significant effect of paraffin on bait longevity was detected ($F_{3,12}$=2.86; P=0.08).

Table 4. The effects of types and contents of preservatives on the longevity of baits with different contents of paraffin

Base-paraffin ratio (%)	Preservatives	Contents (%)	Symptoms appearance (days)
75:25	Potassium sorbate	1	8.25
		2	9
	Sorbic acid	0.5	>9
		1	>9
70:30	Potassium sorbate	1	8
		2	8.75
	Sorbic acid	0.5	>9
		1	>9
65:35	Potassium sorbate	1	8.75
		2	>9
	Sorbic acid	0.5	>9
		1	>9
60:40	Potassium sorbate	1	9
		2	>9
	Sorbic acid	0.5	>9
		1	>9

DISCUSSION

The results of this research indicate that the use of preservatives can significantly delay the development of microorganisms on bait base and prolong its freshness and persistence. Our earlier preliminary research had indicated that the first visible symptoms of microorganism development on baits made from broken wheat grain occurred after three days, and after that an accelerated decrease in bait consumption took place before it fully stopped by the sixth test day (Blažić et al. 2017). Ground maize grain as bait base was exposed to elevated temperatures and humidity, and the first visible symptoms of mold developed after three days of the test, which is consistent with previous results of our research, in which ground wheat grain was used as bait base. When preservatives were added, the longevity of baits whose carrier was ground maize grain was extended to more than 5 days for potassium sorbate and sorbic acid. On baits that contained potassium sorbate (2%) or sorbic acid (≥0.5%), microorganisms were unable to develop until the end of the observed period (>9 days).

Demaree et al. (1955) found that 5% sorbic acid had no harmful effect on rats in feeding tests lasting 90 days. Concentration increase to 8% resulted in some liver problems in the observed rats. Low doses of sorbic acid preservative which were used in our research are not expected to have any negative impact on other non-target species that may come into contact with bait through secondary poisoning. The tested content of 0.5% sorbic acid is ten times lower than the content tested in toxicity tests on laboratory rats over the period of 90 days.

Our results indicate that the method of mixing bait components significantly affected the effectiveness of preservatives and bait longevity. When all bait components (base+preservative+paraffin) were mixed at the same time, bait longevity was shorter than 5 days. It is assumed that simultaneous mixing does not achieve good homogenization, and the preservative does not have a good dispersion within a mixture. By mixing baits gradually, i.e. by initially mixing the base and preservative, and then adding paraffin, an optimal homogenization of the mixture and dispersion of preservative are achieved. Baits obtained in this way showed much better longevity (>9 days), compared to those with the same preservative content but mixed simultaneously (<5 days).

Howald et al. (2004) and Alifano et al. (2010) reported rapid degradation of rodenticides in their studies due to bait exposure to tropical environments. As a result, the first signs of rodenticide degradation were noted after merely two days of exposure. During the five-day test period, degradation was observed in most of the exposed baits. These results are consistent with data in our present study, where baits without preservatives were completely covered with mold after 5 days of the test.

The impact of paraffin content on bait longevity was not clearly determined because it was not possible to distinguish between the observed groups of baits. All groups containing sorbic acid, unlike those with potassium sorbate, had the same values of bait longevity, and therefore showed no variance, which is why it was not possible to apply statistical analysis. However, the results show that the longevity periods of baits from all observed base-preservative ratio groups exceeded the observed test period. Such results may indicate that only the type and content of preservative had effect on bait longevity, rather than the base-preservative ratio. Future research is planned to test an extended period of bait exposure, which is expected to further clarify the impact of preservatives, as well as the base-preservative ratio, on bait longevity periods.

CONCLUSION

The use of preservatives can significantly improve bait quality during periods of exposure to unfavorable environmental conditions. By maintaining good quality and attractiveness, baits remain palatable to rodents for longer periods of time, which is vital for the process of rodent pest control. The results indicate that sorbic acid application would achieve the best results in practice, considering its positive impact through extending bait longevity under unfavourable environmental conditions. The same applies to potassium sorbate applied at the concentration of 2%. The results indicate no practical significance of sodium benzoate applications. The focus of future research will be on bait palatability.

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REFERENCES

Alifano, A., Wegmann A., Pott M., & Oberg, E. (2010). Palmyra Atoll restoration rainforest project: rat genetic sampling, detection method testing, and canopy bait testing, https://www.researchgate.net/publication/255482802_APPENDIX_G_PALMYRA_ATOLL_RAINFOREST_RESTORATION_PROJECT_RAT_GENETIC_SAMPLING_DETECTION_METHOD_TESTING_AND_CANOPY_BAIT_TESTING

Battersby, S., Hirschhorn, R.B, & Amman, B.R. (2008). Commensal rodents. In: X. Bonnefoy, H. Kampen, & K. Sweeney, *Public health significance of urban pests* (pp 387-419). Copenhagen: WHO Regional Office for Europe. https://iris.who.int/bitstream/handle/10665/107363/E91435.pdf;jsessionid=A4803AAA2CA6759371D8D33A9F3289F2?sequence=1

Bentley, E.W. (1972). A review of anticoagulant rodenticides in current use. *Bulletin of World Health Organization*, *47*, 275-280. https://iris.who.int/bitstream/handle/10665/263422/PMC2480728.pdf?sequence=1&isAllowed=y

Blažić, T., Jokić, G., Vukša, M., & Đedović, S. (2017). Acceptability of wheat baits pre-treated with high temperature and humidity to commensal rodent species. In Z. Borowski, W. Olech and A. Suchecka (eds.), *Abstracts of 11th European Vertebrate Pest Management Conference* (p 14). Warsaw: European Bison Friends Society.

Buckle, A.P. (1994). Rodent control methods: chemical. In Buckle A.P. & Smith R.H. (eds.), *Rodent pests and their control* (pp 127-160). Oxon, UK: CAB International.

Demaree, G.E., Sjogren, D.W., McCashland, B.W., & Cosgrove, F.P. (1955). Preliminary studies on the effect of feeding sorbic acid upon the growth, reproduction, and cellular metabolism of albino rats. *Journal of American Pharmaceutical Association (Scientific Ed.), 44*(10), 619-621. https://doi.org/10.1002/jps.3030441013

ECHA (2023). *Guidance on the biocidal products regulation*, Volume II: Efficacy, Parts B and C: Assessment and evaluation (Parts B+C). Version 6.

Howald, G., Samaniego, A., Buckelew, S., McClelland, P., Keitt, B., Wegmann, A. ... Barclay, S. (2004). *Palmyra Atoll rat eradication assessment trip report, August 2004 (Technical report to US Fish and Wildlife Service, Honolulu, HI,* pp 61). http://www.pacificinvasives-initiative.org/rk/tools/Further_Information/2.%20Feasbility%20Study/Palmyra%20Atoll%20Rat%20Eradication%20Feasibility%20FINAL.pdf

Jokić, G., Blažić, T., Rajković, M., Radić Vukičević, O., Sibinović Zlatić, R., Dragićević, D., & Stojnić, B. (2022). Prisustvo bakterije Leptospire kod sivog pacova (*Rattus norvegicus*) poreklom iz skladišta žitarica. *Zbornik rezimea radova, XVII savetovanje o zaštiti bilja*, Zlatibor (pp 25-26). Beograd: Društvo za zaštitu bilja Srbije.

Kataranovski, D., & Kataranovski, M. (2021). *Štetni glodari: biologija, epizootiologija, ekologija i kontrola brojnosti* (pp 1-1058). Beograd: NNK Iternacional.

Lazarević, K., Stojanović, D., & Đonović, N. (2012). Primena prehrambenih aditiva i zdravlje: sorbinska kiselina i sorbati (Application of food additives and healh: sorbic acid and sorbate). *Zdravstvena zaštita*, *41*, *6*, 41-45.

Lund, M. (1994). Commensal rodents. In Buckle A.P. and Smith R.H. (eds.), *Rodent pests and their control* (pp. 23-43). Oxon, UK: CAB International.

Lund, M., & Lodal, J. (1990). Transportation of blocks by rats and mice. *Danish Pest Inify Laboratories Annual Report*.

Marsh, R.E. (2012). The history of paraffinized rodent baits. In R. M. Timm (Ed.), *Proceedings of 25th Vertebrate Pest Conference* (pp 172-185). Davis: University of California.

Marsh, R.E., & Plesse, L.F. (1960). Semipermanent anticoagulant baits. *Bulletin of California Department of Agriculture*, *49*(3),195-197.

McGee, C.F., McGilloway, D.A., & Buckle, A.P. (2020). Anticoagulant rodenticides and resistance development in rodent pest species – A comprehensive review. *Journal of Stored Product Research*, *88*, 10168. Doi: 10.1016/j.jspr.2020.101688

Shahmohammadi, M., Javadi, M., & Nassiri-Asl, M. (2016). An overview on the effects of sodium benzoate as a preservative in food products. *Biotechnology and Health Sciences*, *3*(3), e35084. doi: 10.17795/bhs-35084.

Unapređenje strategije suzbijanja štetnih glodara: I - Izbor odgovarajućeg konzervansa za održivost baze mamka u nepovoljnim uslovima sredine

REZIME

Očuvanje izgleda, strukture i ponajviše atraktivnosti mamka za ciljane štetne vrste glodara u dužem vremenskom periodu izlaganja jedan je od glavnih zadataka u procesu njihovog formulisanja. U laboratorijskim, kontrolisanim uslovima ispitivan je uticaj različitih konzervanasa na produženje perioda održivosti osnove mamka, način homogenizacije osnovnih komponenti mamka kao i odnos baze i parafina na period održivosti mamka. Mamci na bazi mlevenog zrna kukuruza izlagani su nepovoljnim uslovima sredine kao što su visoka temperatura (30° C-35° C) i vlažnost vazduha (90%-95%). Period izlaganja bio je 9 dana, koliko je trajao i period praćenja razvoja prouzrokovača plesni. Utvrđeno je da natrijum benzoat nema zadovoljavajući uticaj na održivost mamka (<5 dana), za razliku od kalijum sorbata (>7 dana) i sorbinske kiseline, koja je uticala na najdužu održivost mamka (>9 dana). Primenom konzervanasa značajno se može produžiti period održivosti mamka u nepovoljnim uslovima sredine. Produžena upotrebna vrednost mamka, značajna je u praksi u postupku suzbijanja štetnih glodara na staništima gde preovladavaju nepovoljni uslovi sredine, kao što su kanalizacioni i vodovodni sistemi (kolektori, crpne stanice), javne površine, stambeni objekti (kotlarnice, vlažni podrumi i sl).

Ključne reči: mamci, konzervansi, plesan, održivost, prihvatljivost