Indian Journal of Experimental Biology
Vol. 64, July 2026, pp.631-640
DOI: 10.56042/ijeb.v64i07.25966

Microencapsulated *citronella* oil: A novel antifeedant and repellent for managing house rats (*Rattus rattus*)

Kashish Saini & Neena Singla*

Department of Zoology, Punjab Agricultural University, Ludhiana 141004, India

Received 12 December 2025; revised 1 March 2026

This study presents an innovative, eco-friendly approach to rodent deterrence through sodium alginate-based microencapsulation of citronella oil, designed to enhance its stability, longevity, and efficacy against house rats (*Rattus rattus* Linnaeus). Microcapsules containing 3%, 4%, and 5% oil were incorporated into bait at 5%, 10%, and 20% concentrations and assessed for antifeedant and repellent activity under controlled laboratory and simulated storage conditions. The 5% oil microcapsules, delivered at 20% bait concentration, demonstrated the highest and most sustained antifeedant response, achieving 90.62% deterrence maintained across all 7 days. Repellency consistently ranged from 77.05% to 92.23%, with the 5% formulation outperforming the others throughout the exposure period. Maze assays confirmed a strong behavioural avoidance, as rats markedly reduced their presence in treated zones. Under simulated storage conditions, jute bags containing wheat grains and sprinkled with 5% oil microcapsules showed significant reductions in grain loss, spillage, and bag damage for up to 10 days. Effects peaked on day 2 and remained functionally effective thereafter. This work highlights microencapsulated citronella oil as a promising, sustainable, and non-toxic alternative to chemical rodenticides, offering an innovative strategy for protecting stored commodities and mitigating rodent-related losses.

Keywords: Behavioural avoidance, Eco-friendly rodent management, Essential oil formulation, Plant-derived bioactive compounds, Sustained release technology

During storage, food grains are highly vulnerable to damage from insects, rodents, and microorganisms, leading to substantial quantitative and qualitative losses. Recent estimates in India have revealed about 10% of total food grain production losses at post-harvest stages[1,2]. Globally[3-5], grain loss due to rodents during the post-harvest period has been estimated at 5–40%. Among rodent pests, the house rat (*Rattus rattus* Linnaeus) is one of the most notorious and widespread commensal rodents globally[6,7]. Beyond direct consumption losses, these rodents contaminate stored products through urine and faeces, posing significant zoonotic risks[8-10]. Thus, controlling rodent populations is critical to ensuring long-term food and public health security.

The use of natural compounds presents a safe, eco-friendly alternative to conventional rodent control methods[11,12]. Botanical extracts act either as primary repellents through unpleasant odours or as secondary repellents (antifeedants) that induce physiological discomfort upon ingestion, leading to learned avoidance[13]. Plant secondary metabolites (PSMs), such as essential oils; possess distinctive odours and tastes that naturally defend plants against pests. Numerous studies have demonstrated the potent repellent and antifeedant properties of PSMs against rodents[14-18].

Citronella oil, a light-yellow extract derived from the leaves and stems of the citronella plant (*Cymbopogon* spp.), is widely used in the food, flavouring, perfume, and refining industries. Though the mosquito-repellent properties of citronella oil have been well-documented[19-24] reports on its antifeedant and repellent effects against rodents are limited. Inhalation of citronella oil, particularly β-citronellol, has been shown to suppress appetite and food consumption by Sprague-Dawley rats[25]. The overwhelming scent of citronella oil has also been reported to stop feeding and avoid food sources by rats. Its strong repellent activity is attributed to volatile compounds such as citronellal, geraniol, and citronellol[26]. Citronellal, a major monoterpenic compound in citronella oil, has been studied for its central nervous system depressant and anti-nociceptive properties in mice[27]. However, due to its

*Correspondence:
Phone: +91 9357325446
E-mail: neenasingla1@gmail.com
E-mail: neenasingla@pau.edu

high volatility and sensitivity to light, oxygen, and temperature, citronella oil rapidly evaporates, limiting its long-term efficacy[28].

Microencapsulation offers a promising strategy to overcome these limitations. Microencapsulation of citronella oil has been explored for enhancing mosquito-repellent applications[29-31]. The limitations of essential oils, such as volatility, instability, and susceptibility to oxidation, are well discussed with possible solutions in Farina *et al.*[32]. Gel and alginate-based carriers are increasingly being explored for the controlled release of essential oils in pest management[33]. Such encapsulation systems protect oils from rapid volatilization and extend their bioactivity[34,35]. For instance, alginate microcapsules loaded with eucalyptus oil have shown significant repellent and antifeedant effects lasting up to 15 days under simulated storage conditions[36].

Building on this foundation, the present study formulated sodium alginate–based microcapsules loaded with different concentrations of citronella oil and systematically evaluated their antifeedant and repellent efficacy against *R. rattus*. This approach not only enhances the stability and persistence of citronella oil but also introduces a novel botanical-based strategy for rodent deterrence. By demonstrating markedly prolonged repellency compared to free oil applications, which typically remain effective for only 1–4 days[37,38] the present study provides strong evidence for the advantages of controlled-release botanical formulations. The findings offer meaningful insights for researchers and pest management professionals aiming to integrate sustainable, eco-friendly alternatives into rodent management programs across agricultural, residential, and storage environments. Ultimately, such innovations have the potential to reduce reliance on toxic rodenticides, minimize environmental contamination, and support safer, more resilient pest control strategies.

Materials and Methods

Fabrication of microcapsules

For the present study, pure citronella oil was freshly extracted from citronella grass (*Cymbopogon winterianus*) available at Punjab Agricultural University, Ludhiana (India) Campus, using a Soxhlet apparatus. Sodium alginate-based microcapsules incorporating 3%, 4%, and 5% concentrations of citronella oil (Fig. 1a & b) were prepared with slight modifications to the method of Chan[39]. The oil loading capacity and encapsulation efficiency of the alginate-based microcapsules are influenced by the concentration of sodium alginate, calcium chloride, and the cross-linking time. In this study, we used 1% sodium alginate, 0.5% calcium chloride, and 20 minutes of cross-linking time. Sodium bicarbonate (1 g, 1% w/v) and the required concentration of citronella oil were dissolved in 100 mL of distilled water and stirred at 300 rpm for 15 minutes using a magnetic stirrer. Sodium alginate (1 g, 1% w/v) was then added, and stirring was continued at 300 rpm for 1 hour. The resulting solution was transferred to a specially designed encapsulator and extruded dropwise into a 0.5% calcium chloride solution to form microcapsules. The microcapsules were allowed to harden for 30 minutes, then collected by sieving and dried. All the microcapsules were prepared in a single batch and stored carefully under refrigerated conditions, and used for experimentation within one month. The loading capacity and encapsulation efficiency of the microcapsules were determined following the methodology of Parris *et al.*[40].

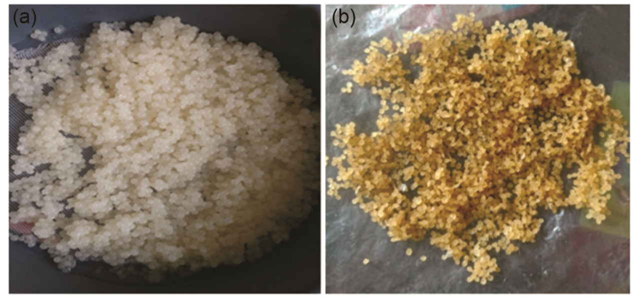

Fig. 1 — Typical appearance of microcapsules, (a) Fresh microcapsules formed after hardening in calcium chloride solution, and (b) Air dried microcapsules ready for experimentation.

Acclimatization of test animals

Adult male house rats (*R. rattus*) having scrotal testes and body weight 140-160 g, were collected from poultry farms in Ludhiana, housed individually in cages and allowed an acclimatization period of 15–20 days, with food and water provided *ad libitum*. The diet comprised a loose mixture of cracked wheat, powdered sugar, and groundnut oil (WSO bait) in a 96:2:2 ratio. Following acclimatization, healthy rats were weighed and grouped (n = 6 in each group) for experimentation. Rats were used only once per trial and were not reused in subsequent experiments. All animal-related procedures were carried out in compliance with the guidelines and approval of the Institutional Animal Ethics Committee (IAEC), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, as per the protocol approved (GADVASU/2019/IAEC/51).

Evaluation of the antifeedant effect of microcapsules

Two large pens (each measuring 2.52×1.00×0.72 m and physically separated into three equal chambers using galvanized metal sheets) were utilized for these experiments. Each chamber was connected to two small boxes (measuring 0.20×0.15×0.15 m each) positioned on opposite outer sides, accessible through small openings (of 3 inches diameter) from inside. One rat was released into each chamber, six rats in total across the two pens, allowing free access to both the connected boxes. To evaluate the antifeedant effect, microcapsules containing 3%, 4%, or 5% citronella oil were incorporated into WSO bait, each at concentrations of 5%, 10%, and 20%. Separate trials were conducted for each oil and microcapsule concentration, using different groups of rats randomly. In each trial, bait (20 g) mixed with microcapsules was placed in a bowl inside the small box on one side of the chamber (designated as the treated side) and chosen randomly, while plain WSO bait (20 g) was placed in the box on the opposite side (untreated side). The position of treated and untreated bowls was changed daily to eliminate any positional bias. The experiment was monitored over 7 days, with bait consumption (g/100g body weight (bwt)) recorded every 24 hours and fresh bait replenished daily. After each trial, a resting period of 7 days was given during which the chambers were cleaned and disinfected properly to remove traces of previous odour. The antifeedant index (%) was calculated as per the formula described below:

$$\text{Antifeedant index (\%)} = \frac{\text{Consumption of untreated bait} - \text{Consumption of treated bait}}{\text{Consumption of untreated bait} + \text{Consumption of treated bait}} \times 100$$

Evaluation of the repellent effect of microcapsules in laboratory cages

Two laboratory cages (each measuring 0.23×0.14×0.14 m) were interconnected to create a single experimental unit. One rat was released into each unit, allowing free movement between the two connected cages. A total of six such units (six rats in total) were used for each experiment. In both cages of each unit, plain WSO bait (20 g) was placed in bowls. Additionally, a separate bowl containing microcapsules (10 g) loaded with 3%, 4%, or 5% citronella oil was placed in one of the cages (designated as the treated side) chosen randomly, while the opposite cage served as the untreated side. Microcapsules kept on treated side were not part of the bait matrix. Empty microcapsules without any oil were not kept on the untreated side, the untreated bait served as the practical control. In each experimental unit, the position of the bowl containing microcapsules was changed daily to eliminate any positional bias. Separate trials were conducted for each oil concentration randomly, using different groups of rats. The effect of the treatment was monitored over 7 days. Bait consumption (g/100g body weight) was recorded every 24 hours, and bait was replenished daily. After each trial, a resting period of 7 days was given during which the cages were cleaned and disinfected properly to remove traces of previous odour. Per cent repellency was calculated as per the formula described below:

$$\text{Per cent repellency} = \frac{\text{BUT - BT}}{\text{BUT}} \times 100$$

Where, BUT is the mean daily bait consumption from the untreated side, and
BT is the mean daily bait consumption from the treated side

Evaluation of the repellent effect of microcapsules in a Maze experiment

For this experiment, an I-Maze setup with two arms connected to a central hub was utilized. Microcapsules containing 5% citronella oil were randomly placed in one arm (designated as the treated zone), while the other arm remained empty (untreated zone). Each rat (a total of four male rats) was initially released into the central hub. Rat activity was continuously monitored using a camera-operated rodent behaviour test system (Ethovision, Netherlands) for two hours each day. During the first two days, each rat was acclimatized to the I-Maze for two hours daily.In next 3 days, rats were exposed to the treated arm containing the microcapsules (10 g) in a bowl. The position of the bowl containing microcapsules was reversed daily to eliminate any positional bias. After each trial with a rat, a resting period of 7 days was given, during which the arms of the maze were cleaned and disinfected properly to remove traces of previous odour. Data collection focused on several behavioural parameters: in-zone frequency, total duration spent in each zone (seconds), latency to first entry, total distance travelled (m), movement velocity (m/s), and total movement duration (seconds) in both zones.

Evaluation of repellent/antifeedant effect under simulated store conditions

Four large pens (each measuring 2.52×1.00×0.72 m) were utilized, with the three chambers (measuring 0.84×1.00×0.72 m each) of each pen interconnected via a small hole of 3 inches diameter to allow a rat free access to all chambers. In two chambers located at opposite ends, metallic trays containing small bags of wheat grains arranged in two layers were placed. In one of these chambers, the bags were sprinkled with microcapsules containing 5% citronella oil (designated as the treated side) randomly, while the opposite chamber remained untreated. The position of the tray containing bags sprinkled with microcapsules was changed daily to eliminate any positional bias. The treatment effect was monitored over 15 days. Per cent repellency was calculated according to the formula described above. Spillage and consumption of grains (g/100g body weight), along with the number of bag cuts, were recorded every 24 hours.

Statistical analyses

Values are presented as mean ± SE. Data were collected through factorial experiments arranged in a completely randomized design and analyzed using the General Linear Model procedure in SPSS 20.0 software. Pairwise treatment comparisons were conducted using Tukey's test at a 5% level of significance.

Results

Loading capacity and encapsulation efficiency of microcapsules

The oil loading capacity of microcapsules increased dramatically with higher oil concentrations, measuring 50% for 3% oil, 80% for 4%, and peaking at 94% for 5% citronella oil. However, encapsulation efficiency did not follow the same trend, with values of 8.3±0.1%, 10.0±0.1%, and 9.4±0.1%, respectively, suggesting optimal encapsulation performance at the 4% oil level. Scanning electron microscopy revealed smooth textural characteristics for microcapsules without oil, while oil-loaded microcapsules exhibited crimped surfaces with lumps due to oil deposition.

Antifeedant effect of microcapsules

The antifeedant efficacy of citronella oil microcapsules varied with both oil and bait concentrations and exposure time. For 3% citronella oil microcapsules, strong antifeedant activity was observed during the first four to five days, peaking on day 2, followed by a marked decline. Average antifeedant indices ranged from 61.7% to 78.7%

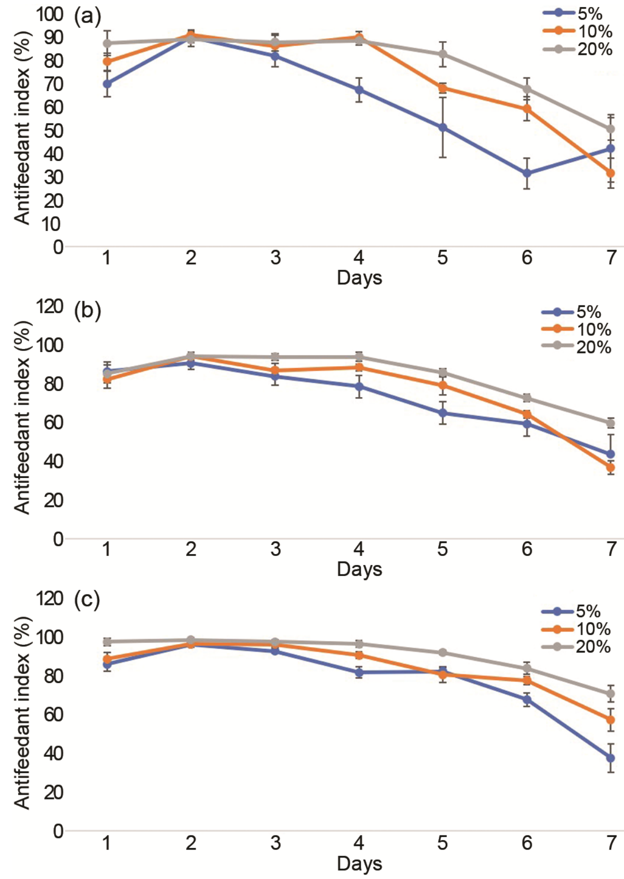

Fig. 2 — Comparison of the antifeedant effect of microcapsules mixed in bait at different concentrations along different days of exposure against *Rattus rattus* exposed in laboratory pens, (a) Microcapsules containing 3% citronella oil, (b) Microcapsules containing 4% citronella oil, and (c) Microcapsules containing 5% citronella oil. Values represent Mean ± SE (n = 6).

across bait concentrations (Fig. 2a; Table 1S). A similar trend was recorded for 4% citronella oil microcapsules, with higher initial activity than the 3% formulation and a gradual reduction after day 2. The average antifeedant index ranged from 72.3% to 83.4% (Fig. 2b; Table 2S). Microcapsules containing 5% citronella oil showed the strongest and most sustained effect. Although activity also peaked on day 2 and declined over time, higher bait concentrations maintained comparatively greater efficacy. The average antifeedant index ranged from 77.5% to 90.6% (Fig. 2c; Table 3S). Large variations in standard errors for most of the observations were due to both individual animals within a group that did not consume as much food as others, and daily variation within an individual across the 7 days.

Overall, antifeedant activity increased with both citronella oil concentration (3% < 4% < 5%) and bait concentration (5% < 10% < 20%). The highest and most persistent efficacy was achieved with 5% citronella oil microcapsules applied at 20% bait concentration (Fig. 3).

Repellent effect of microcapsules in laboratory cages

The repellent efficacy of citronella oil microcapsules formulated at 3%, 4%, and 5% concentrations was strikingly evident throughout the 7-day evaluation period (Table 1). At 4% and 5% citronella oil, a consistently and significantly lower average daily consumption of plain WSO bait was recorded on the treated side compared to the untreated side across all days, resulting in high repellency levels of 87.3% and 92.2%, respectively. At 3% oil concentration, although repellency was substantial during the first five days, ranging from 47.7% to 94.4%, a reversal occurred after day 5, with bait consumption increasing on the treated side, indicating a drop in repellent effectiveness.

Overall, the average repellency ranged from 77.0% to 92.9%, peaking at the 5% oil concentration (Fig. 4), clearly highlighting a dose-dependent response. Due to these promising results, the microcapsules were further assessed for their repellent potential in the Maze experiment and under simulated storage conditions to validate their practical application and sustained efficacy.

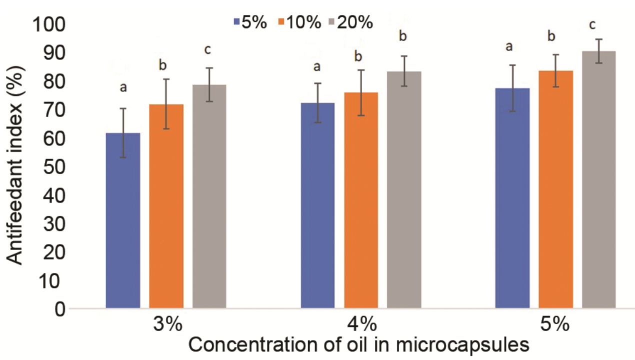

Fig. 3 — Comparison of the antifeedant index of microcapsules containing different concentrations of citronella oil and mixed in different concentrations in bait against *Rattus rattus* exposed in laboratory pens. Bars with different superscripts (a-c) indicate significant differences at $P \leq 0.05$. Values represent Mean ± SE (n = 6).

Repellent effect of microcapsules in a Maze experiment

Movement tracking analysis (Table 2) clearly demonstrated behavioural aversion of male *R. rattus* to the treated zone in the I-maze bi-choice test using microcapsules containing 5% citronella oil. Although no significant differences were observed in latency to first entry or velocity of movement between treated and untreated zones, spatial tracking data revealed significantly fewer movement tracks in the treated zone, confirming strong repellency. Significant reductions were recorded in key behavioural parameters, including in-zone frequency, total time spent (s), total distance moved (cm), and total mobility duration (s) between the treated and untreated zones.

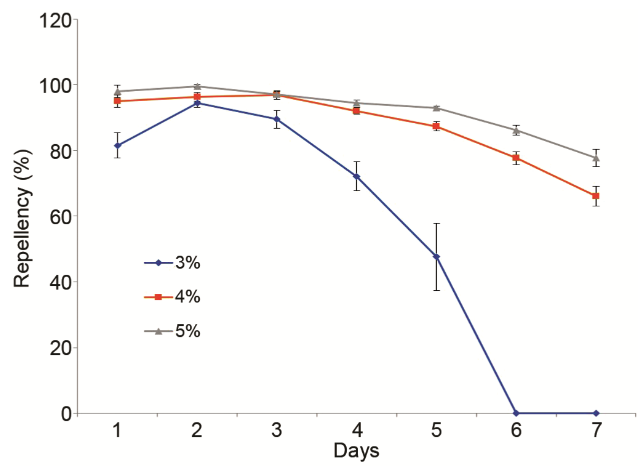

Fig. 4 — Comparison of the repellent effect of microcapsules containing different concentrations of citronella oil against *Rattus rattus* exposed in laboratory cages. Values represent Mean ± SE (n = 6).

Table 1 — Repellent effect of microcapsules containing different concentrations of citronella oil on *Rattus rattus* (n = 6 each)

Days of treatment	Microcapsules containing 3% oil: Mean daily consumption of plain bait (g/100g bwt), Treated side	Untreated side	Repellency (%)	Microcapsules containing 4% oil: Mean daily consumption of plain bait (g/100g bwt), Treated side	Untreated side	Repellency (%)	Microcapsules containing 5% oil: Mean daily consumption of plain bait (g/100g bwt), Treated side	Untreated side	Repellency (%)
1	1.7±0.3	9.9±0.7*	81.5±3.8^{ab}	0.6±0.2	11.1±0.4*	94.9±1.9^{a}	0.2±0.2	11.3±0.4*	97.9±1.9^{ab}
2	0.5±0.1	9.6±0.9*	94.4±1.3^{ab}	0.4±0.1	10.8±0.4*	96.3±1.3^{a}	0.1±0.1	11.3±0.4*	99.5±0.5^{a}
3	0.9±0.2	8.5±0.8*	89.5±2.7^{ab}	0.3±0.1	10.2±0.4*	96.8±1.2^{a}	0.3±0.1	11.0±0.5*	97.1±1.1^{ab}
4	1.8±0.3	6.7±0.5*	72.1±4.4^{ab}	0.8±0.1	9.6±0.5*	92.0±1.1^{ab}	0.6±0.1	10.5±0.5*	94.4±1.0^{ab}
5	2.2±0.3	4.0±0.4*	47.7±10.2^{b}	1.1±0.1	8.7±0.4*	87.3±1.4^{b}	0.7±0.1	9.9±0.4*	92.9±0.6^{b}
6	2.7±0.3	1.8±0.3	-	1.5±0.1	6.9±0.3*	77.6±2.0^{c}	1.2±0.1	8.7±0.5*	86.1±1.5^{c}
7	2.6±0.3	0.6±0.2	-	1.7±0.2	5.0±0.2*	66.1±3.0^{d}	1.5±0.1	6.9±0.4*	77.7±2.5^{d}
Average	1.8±0.3	5.9±1.5*	77.0±7.5	0.9±0.2	8.9±0.9*	87.3±4.7	0.7±0.2	9.9±0.7*	92.2±3.2

Table 2 —Movements and activities of male *Rattus rattus* in response to microcapsules containing 5% citronella oil kept in a bi-choice test under Maze experiment (n = 4)

Parameters	Treatment days	Treated zone	Untreated zone
In-zone frequency	Day 1	9.7±6.8	68.5±42.5
	Day 2	1.5±0.3	33.5±13.0*
	Day 3	4.2±2.9	65.5±42.0
	Average	5.2±2.1	55.7±9.7*
In-zone total duration (s)	Day 1	907.9±889.3	2692.0±889.3
	Day 2	22.6±9.6	4477.2±903.3*
	Day 3	14.0±5.4	3585.8±5.4*
	Average	314.9±256.8	3585.0±446.3*
In-zone latency of first occurrence (s)	Day 1	769.2±728.9	268.4±262.5
	Day 2	442.0±302.6	67.8±49.3
	Day 3	14.2±13.3	4.4±19.0
	Average	408.5±189.3	113.6±68.9
Total distance moved (m)	Day 1	52.3±50.2	182.9±70.3
	Day 2	1.1±0.7	173.8±104.0
	Day 3	3.2±2.1	225.7±44.5*
	Average	18.9±14.5	194.2±13.8*
Velocity (m/s) of movement	Day 1	0.1±0.0	0.7±0.5
	Day 2	0.1±0.0	0.2±0.0
	Day 3	0.2±0.1	0.2±0.0
	Average	0.1±0.0	0.4±0.1
Total duration of mobility (s)	Day 1	228.8±218.5	872.5±338.5
	Day 2	6.0±3.1	1766.1±1322.5
	Day 3	6.6±2.2	1152.1±334.2*
	Average	80.5±64.2	1263.6±228.5*

Mean values are Mean ± SE, Mean values with * indicate a significant difference in rat activity between treated and untreated zones at $P \leq 0.05$ The daily assessments were of 2 hours duration only. Large variations in standard errors on most of the mean values were due to the small sample size (only 4 individuals) for this I-maze experiment.

Across the 3-day observation period, no overall differences in rat activity were detected within the treated zone. In-zone frequency was lower in the treated zone on day 2. The total time spent, total distance moved, and total mobility duration in the treated zone were significantly reduced on days 2 and 3, though not on day 1. The daily assessments were of 2 hours duration only, and the large variations in standard errors on most of the mean values were due to the small sample size (only 4 individuals) for this I-maze experiment. The consistent reduction in movement tracks and activity metrics over time highlights the progressive and sustained repellency effect of 5% citronella oil microcapsules. Movement tracking further confirmed this pattern, with noticeably fewer tracks recorded in the treated zone, reinforcing the strong repellent effect over time.

Repellent effect of microcapsules under simulated store conditions

Results (Table 3) showed significantly lower mean daily consumption of wheat grains (g/100 g bwt) from the treated side compared to the untreated side for up to 12 days. After day 12, consumption from the treated side increased, becoming significantly higher on days 14 and 15, indicating that the effect of microcapsules containing 5% citronella oil persisted for approximately 12 days. The overall mean daily consumption was significantly lower on the treated side (2.8±1.4 g/100 g bwt) than on the untreated side (7.5±1.3 g/100 g bwt). The average repellency was 75.6%, peaking at 100% on day 2 and gradually declining to 0% by day 13. Grain spillage from bags was also significantly lower on the treated side for up to 10 days, with non-significant differences observed on days 11 and 12. By day 15, spillage was significantly higher on the treated side. Similarly, the number of bag cuts was significantly reduced up to day 10, after which differences diminished and later reversed (days 13–15). The size of cuts varied from 20 to 50 mm. Over the 15-day period, the treated side recorded significantly lower mean grain spillage (3.0±1.2 g) and fewer bag cuts (1.8±0.7), with smaller cut sizes compared to the untreated side.

Table 3 — Efficacy of microcapsules containing 5% citronella oil in reducing damage caused by *Rattus rattus* under simulated store conditions (n =4)

Days of treatment	Mean daily consumption of grains (g/100 g bwt)		Spillage of grains from bags (g)		Number of cuts on bags		Percent repellency
	Treated side	Untreated side	Treated side	Untreated side	Treated side	Untreated side	
Day 1	0.2±0.2	11.0±0.5*	0.2±0.2	10.7±0.5*	0.2±0.2	6.2±0.2*	98.6±1.4^{a}
Day 2	0.0±0.0	10.3±0.8*	0.0±0.0	9.2±0.5*	0.0±0.0	5.5±0.5*	100.0±0.0^{a}
Day 3	0.9±0.2	9.5±0.7*	1.4±0.2	9.2±0.6*	1.0±0.0	5.2±0.2*	90.2±2.0^{a}
Day 4	0.5±0.1	9.2±0.8*	1.4±0.2	7.5±1.0*	1.0±0.0	4.5±0.3*	94.0±0.8^{a}
Day 5	0.8±0.2	9.7±0.9*	1.1±0.1	9.2±1.1*	1.0±0.0	5.7±0.5*	91.6±2.4^{a}
Day 6	1.1±0.2	9.4±0.7*	2.4±0.2	7.9±1.0*	1.0±0.0	6.0±0.4*	88.1±2.5^{a}
Day 7	1.2±0.1	8.9±0.7*	2.0±0.4	6.2±0.6*	1.0±0.0	4.7±0.6*	86.8±1.2^{a}
Day 8	1.5±0.1	8.6±0.8*	1.7±0.3	7.1±0.8*	1.2±0.2	4.5±0.3*	81.5±2.1^{ab}
Day 9	2.2±0.2	7.3±0.7*	1.9±0.1	6.1±0.7*	1.5±0.3	3.5±0.5*	69.3±4.5^{ab}
Day 10	3.1±0.3	6.7±0.6*	3.5±0.3	5.8±0.5*	2.0±0.0	3.0±0.4*	52.1±8.7^{b}
Day 11	3.8±0.6	5.9±0.7	4.0±0.6	4.9±0.4	2.2±0.5	2.7±0.5	30.8±16.5^{b}
Day 12	4.8±0.6	5.6±0.3	4.5±0.6	4.9±0.6	2.7±0.2	2.2±0.2	24.4±9.2^{b}
Day 13	6.2±0.6	4.6±0.2	5.5±1.2	4.9±0.3	3.2±0.2	2.0±0.0	-
Day 14	7.5±0.9	3.9±0.6	6.0±0.4	3.5±1.0	4.2±0.5	2.0±0.6	-
Day 15	8.3±0.8	1.9±0.7	8.7±1.3	1.4±0.5	4.2±0.5	1.0±0.4	-
Average	2.8±1.4	7.5±1.3*	3.0±1.2	6.6±1.2*	1.8±0.7	3.9±0.8*	75.6±13.0

Mean values are Mean ± SE, Mean values with * indicate significant difference in respective parameters between treated and untreated sides at $P \leq 0.05$, Mean values with different superscripts (a-b) indicate a significant difference among days at $P \leq 0.05$

Overall, microcapsules containing 5% citronella oil effectively reduced feeding damage, grain spillage, and bag cutting by *R. rattus* for 10–12 days, after which reapplication is necessary to maintain protection.

Discussion

Despite the promising potential of plant essential oils, significant challenges remain, such as their volatility, poor water solubility, and susceptibility to oxidation due to various physical factors[35]. In a previous study[38] we investigated the repellent effect of citronella oil against *Rattus rattus* at 5, 10, and 20% concentrations applied weekly as a paint. However, the repellent effect was only observed on the first day of treatment. We also examined the persistence of the repellent effect of 5% citronella oil encapsulated in wax blocks under stored conditions, and found that the effect lasted for only 3-4 days[37].

In the present study, we encapsulated different concentrations of citronella oil into sodium alginate-based microcapsules to achieve a slow, sustained release through the microcapsules' minute pores. The evaluation of the antifeedant and repellent effects against *R. rattus* revealed that microcapsules containing 5% citronella oil provided the maximum and most sustained effects, lasting throughout the entire 7-day experimental period. Under simulated indoor storage conditions, the application of microcapsules containing 5% citronella oil on wheat grain bags successfully reduced damage caused by *R. rattus* for up to 12 days. Thereafter, repellency gradually decreased, suggesting a time-dependent reduction in volatile release and/or sensory adaptation. The sustained but declining effect is consistent with the known volatility-driven mode of action of essential oils, where behavioural avoidance is closely linked to vapour concentration[41,42]. A similar study using alginate microcapsules containing varying concentrations of eucalyptus oil[36] also demonstrated significant repellent and antifeedant effects with microcapsules containing 5% oil. This improved persistence aligns with findings in polymer encapsulation systems[43].

In the current study, a marked repellent effect of 5% citronella oil microcapsules was also evident in the I-maze experiment. This aligns with a similar experiment by Kalandakanond-Thongsong *et al.*[44], who studied the repellent effects of various oils, including chilli, wintergreen, bergamot, peppermint, and geranium, on adult male Wistar rats. Their results showed that rats spent significantly less time in the inner zone of the field exposed to repellents, suggesting avoidance behaviour due to the overwhelming scent.

In the present study, the maximum antifeedant effect of different concentrations of citronella oil and microcapsules was observed on day 2 of exposure, indicating learned aversion, a behavioural phenomenon in which rodents develop a strong avoidance of a particular food source after experiencing an unpleasant consequence, such as nausea or discomfort, following consumption[15]. This form of conditioned learning occurs when rodents associate the taste or smell of a bait with negative experiences, leading to a reduction in their feeding behaviour toward that bait.

From a health and safety perspective, citronella oil is widely regarded as having comparatively low mammalian toxicity and is commonly used in household products, topical repellents, and food-related applications[45,46]. When microencapsulated, controlled release further reduces direct exposure and enhances stability, improving user safety and environmental compatibility. Nevertheless, large-scale application requires careful evaluation of inhalation exposure, non-target effects, and occupational safety under field conditions.

The development and duration of aversion observed in this study suggest that citronella primarily functions as a spatial and contact repellent rather than a toxicant. The strong initial avoidance response, followed by gradual recovery of activity after 10–12 days, indicates that repellency is concentration-dependent and reversible. There is limited evidence that rodents develop true physiological resistance to plant essential oils, however, behavioural habituation or reduced sensitivity over time may occur if exposure levels decline below the repellent threshold[41]. In the present findings, decreased feeding and bag damage after day 12 likely reflect diminished volatile emission rather than recalcitrance or learned tolerance. This suggests that efficacy can be maintained through timely reapplication before repellency falls below an effective level.

For practical implementation, farmers and storage managers should adopt a rotational or scheduled reapplication strategy, ideally at 7–10 day intervals under comparable environmental conditions, to sustain deterrence. Environmental factors such as temperature, ventilation, and humidity may accelerate volatilization and shorten effective duration. Therefore, field-based calibration of reapplication intervals is recommended. Integrating citronella microcapsules within broader integrated pest management frameworks, combining sanitation, proofing, and habitat modification, would likely maximize long-term effectiveness while minimizing reliance on toxic rodenticides.

Conclusions

Overall, microencapsulated 5% citronella oil represents a promising and safer tool for rodent management in enclosed storage conditions by reducing feeding damage without introducing persistent toxicants into the environment. However, further studies addressing cost-effectiveness, shelf-life, large-scale field performance under varying environmental conditions, optimal dosing schedules, long-term behavioural responses and safety for humans and non-target organisms are essential before large-scale field deployment.

Acknowledgement

The authors are thankful to the Indian Council of Agricultural Research, New Delhi, for providing financial assistance to conduct this study under the All India Network Project on Vertebrate Pest Management.

Conflict of interest

The authors declare no competing interests.

References

1 Madhukar PM, Pandey LM & Dixit US. *Post-harvest grain storage: Methods, factors, and eco-friendly solutions. Food Ctrl*, 174 (2025) 111236.

2 Shukla N, Tripathi AK, Sharma K & Tiwari A. Addressing food security threats: Rodent management in grain storage facilities. *Int J Res Agron*, 8 (2025) 40.

3 Mdangi M, Mulungu LS, Massawe AW, Eiseb SJ, Tutjavi V, Kirsten F, Mahlaba TAM, Malebane P, von Maltitz EF, Monadjem A, Dlamini N, Makundi RH & Belmain SR. Assessment of rodent damage to stored maize *(Zea mays* L.) on smallholder farms in Tanzania. *Int J Pest Manag,* 59 (2013) 55.

4 Belmain SR, Htwe NM, Kamal NQ & Singleton GR. Estimating rodent losses to stored rice as a means to assess efficacy of rodent management. *Wildl Res*, 42 (2015) 132.

5 Htwe NM, Singleton GR & Maw PP. Post-harvest impacts of rodents in Myanmar; how much rice do they eat and damage? *Pest Manag Sci,* 73 (2017) 318.

6 Shiels AB, Pitt WC, Sugihara RT & Witmer GW. Biology and impacts of Pacific island invasive species. 11. *Rattus rattus*, the black rat (Rodentia: Muridae). *Pac Sci*, 68 (2014) 145.

7 Ebani VV. Commensal rodents: Still a current threat. *Pathogens*, 11 (2022) 1483.

8 Singla LD, Singla N, Parshad VR, Juyal PD & Sood NK. Rodents as reservoirs of parasites in India. *Integr Zool*, 3 (2008) 21.

9 Singla N, Singla LD & Kaur R. Rodents as museum of helminthic parasites of public health importance in Punjab, India. *Int J Infect Dis,* 12 (2008) 381.
10 Brar SK, Singla N & Singla LD. Comparative comprehensive analysis on natural infections of *Hymenolepis diminuta* and *Hymenolepis nana* in commensal rodents. *Helminthologia*, 58 (2021) 248.
11 Nolte DL, Mason JR & Clark L. Avoidance of bird repellents by mice (*Mus musculus*). *J Chem Ecol*, 19 (1993) 427.
12 Garg N, Singla N & Mahal AK. Repulsive and attractive response of mature *Rattus rattus* towards conspecific natural urinary signals of the same and the opposite sex. *Anim Biol*, (2024).
13 Pavea R, Kovaříková K & Novák M. Botanical antifeedants: An alternative approach to pest control. *Insects,* 16 (2025) 136.
14 Babbar BK & Singla N. Evaluation of peppermint oil as repellent/antifeedant against the Indian gerbil, *Tatera indica*. *Crop Improv,* (2012) 907.
15 Fischer D, Imholt C, Pelz HJ, Wink M, Prokop A & Jacob J. The repelling effect of plant secondary metabolites on water voles, *Arvicola amphibius*. *Pest Manag Sci*, 69 (2013) 437.
16 Singla N, Thind RK & Mahal AK. Potential of eucalyptus oil as repellent against house rat, *Rattus rattus*. *Sci World J*, 2014 (2014) 7.
17 Babbar BK, Kaur J, Singla N & Mahal AK. Effectiveness and persistence of cinnamic aldehyde as an antifeedant on rats under storage conditions. *Crop Prot*, 67 (2015) 235.
18 Singla N & Kaur R. Increasing efficacy of eucalyptus oil as repellent against *Rattus rattus* by controlled release through encapsulated wax blocks. *Proc Natl Acad Sci India B Biol Sci*, 87 (2017) 1407.
19 Kim JK, Kang CS, Lee JK, Kim YR, Han HY & Yun HK. Evaluation of repellency effect of two natural aroma mosquito repellent compounds, citronella and citronellal. *Entomol Res*, 35 (2005)117.
20 Koul O, Walia S & Dhaliwal GS. Essential oils as green pesticides: Potential and constraints. *Biopestic Int*, 4 (2008) 63.
21 Sakulku U, Nuchuchua O, Uawongyart N, Puttipipatkhachorn S, Soottitantawat A & Ruktanonchai U. Characterization and mosquito repellent activity of citronella oil nanoemulsion. *Int J Pharm* 372 (2009)105.
22 Wany A, Jha S, Nigam VK & Pandey DM. Chemical analysis and therapeutic uses of citronella oil from *Cymbopogon winterianus*: A short review. *Int J Adv Res*, (2013) 504.
23 Yoon JK, Kim KC, Cho Y, Gwon YD, Cho HS, Heo Y, Park K, Lee YW, Kim M, Oh YK & Kim YB. Comparison of repellency effect of mosquito repellents for DEET, citronella, and fennel oil. *J Parasitol Res*, 2015 (2015) 6.
24 Zamora D, Klotz SA, Meister E & Schmidt JO. Repellency of the components of the essential oil, citronella, to *Triatoma rubida*, *T. protracta*, and *T. recurva* (Hemiptera: Reduviidae: Triatominae). *J Med Entomol,* 52 (2015) 719.
25 Batubara I, Suparto IH, Sa'diah S, Matsuoka R & Mitsunaga T. Effects of inhaled citronella oil and related compounds on rat body weight and brown adipose tissue sympathetic nerve. *Nutrients*, 7 (2015)1859.
26 Muller GC, Junnila A, Kravchenko VD, Revay EE, Butlers J & Schlein Y. Indoor protection against mosquito and sand fly bites: A comparison between citronella, linalool, and geraniol candles. *J Am Mosq Control Assoc*, 24 (2008) 150.
27 Melo MS, Sena LC, Barreto FJ, Bonjardim LR, Almeida JR, Lima JT, De-Sousa D & Quintans-Júnior LJ. Antinociceptive effect of citronellal in mice. *Pharm Biol*, 48 (2010) 411.
28 Ayala-Zavala JF, del Toro-Sanchez L, Alvarez-Parrilla E & Gonzalez-Aguilar GA. High relative humidity in package of fresh-cut fruits and vegetables: Advantage or disadvantage considering microbiological problems and antimicrobial delivering systems. *J Food Sci*, 73 (2008) 41.
29 Solomon B, Sahle FF, Gebre-Mariam T, Asres K & Neubert RHH. Microencapsulation of citronella oil for mosquito-repellent application: Formulation and in vitro permeation studies. *Eur J Pharm Biopharm*, 80 (2012) 61.
30 Bakry AM, Abbas S, Ali B, Abouelwafa MY, Mousa A & Lian L. Microencapsulation of oils: A comprehensive review of benefits, techniques, and applications. *Compr Rev Food Sci Food Saf*, 15 (2016) 143.
31 Ribeiroa AD, Marques J, Fortea M, Correiaa FC, Parpot P, Oliveira C, Pereira AI, Andrade L, Azenha L, Azenha C, Mendes A, Alves GM, Sousa CA & Tavares CJ. Microencapsulation of citronella oil for solar-activated controlled release as an insect repellent. *Appl Mater Today*, 5 (2016) 90.
32 Farina P & Conti B. Liabilities of essential oils as insect repellents. *Curr Opinion Environ Sci Hlth,* 40 (2024) 100564.
33 Shah JA, Vendl T, Aulicky R, Frankova M & Stejskal V. Gel Carriers for plant extracts and synthetic pesticides in rodent and arthropod pest control: An overview. *Gels,* 8 (2022) 522.
34 Zhao Y, Wang Y, Zhang Z & Li H. Advances in controllable release essential oil microcapsules and their promising applications. *Molecules*, 28 (2023) 4979.
35 Ayllón-Gutiérrez R, Díaz-Rubio L, Montaño-Soto M, Haro-Vázquez MdP & Córdova-Guerrero I. Applications of plant essential oils in pest control and their encapsulation for controlled release: A review. *Agriculture*, 14 (2024) 1766.
36 Sachdeva S & Singla N. Antifeedant and repellent potential of alginate-based microcapsules containing eucalyptus oil against house rat, *Rattus rattus*. *J Entomol Zool Stud,* 6 (2018) 608.
37 Singla N, Kaur R, Babbar BK & Mahal AK. Repellent potential of eucalyptus and citronella oils against rodent pests infesting store grain houses. *Crop Improv*, 39 (2012) 1037.
38 Singla N & Kaur R. Potential of citronella oil as rodent repellent measured as aversion to food. *Appl Biol Res*, 16 (2014) 191.
39 Chan ES. Preparation of Ca-alginate beads containing high oil content: Influence of process variables on encapsulation efficiency and bead properties. *Carbohydr Polym,* 84 (2011) 1267.
40 Parris N, Cooke PH & Hicks KB. Encapsulation of essential oils in zein nanospherical particles. *J Agric Food Chem*, 53 (2005) 4788.
41 Nerio LS, Olivero-Verbel J & Stashenko E. Repellent activity of essential oils: A review. *Bioresource Technol*, 101 (2010) 372.
42 Isman MB. Botanical insecticides in the twenty-first century—fulfilling their promise? *Ann Rev Entomol*, 65 (2020) 233.
43 López S, Tapia A, Zygadlo J, Stariolo R, Abraham GA & Cortez Tornello PR. *Zuccagnia* punctata Cav. essential oil into Poly(ε-caprolactone) matrices as a sustainable and environmentally

friendly strategy biorepellent against *Triatoma infestans* (Klug) (Hemipera, Rediviidae). *Molecules*, 26 (2021) 4056.

44 Kalandakanond-Thongsong S, Daendee S, Thongsong B & Chavananikul V. The efficacy of pure natural repellents on rat responses using circular open field. *Thai J Vet Med,* 40 (2010) 411.

45 Isman MB. Botanical insecticides, deterrents and repellents in modern agriculture and increasingly regulated world. *Ann Rev Entomol*, 51(2006) 66.

46 EPA. *Reregistration Eligibility Decision (RED): Oil of Citronella*. United States Environmental Protection Agency, Washington DC (2013).