Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 608-617

Received: 01-01-2018
Accepted: 03-02-2018

Shipra Sachdeva
Department of Zoology, Punjab Agricultural University, Ludhiana, Punjab, India

Dr. Neena Singla
Senior Zoologist (Rodents)
Department of Zoology Punjab Agricultural University, Ludhiana, Punjab, India

Correspondence
Dr. Neena Singla
Senior Zoologist (Rodents)
Department of Zoology Punjab Agricultural University, Ludhiana, Punjab, India

Antifeedant and repellent potential of alginate based microcapsules containing eucalyptus oil against house rat, *Rattus rattus*

Shipra Sachdeva and Dr. Neena Singla

Abstract
Microencapsulation of the desired oil reduces the evaporation rate due to limited permeability of thin wall material thereby increasing efficacy for a longer period. For evaluating antifeedant effect, microcapsules containing three different concentrations of the oil (3, 5 or 7%) and mixed in food at 5, 10 and 20% concentrations were exposed to house rats, *Rattus rattus*. Significant antifeedant effect of microcapsules was found at all the three concentrations of oil and that of microcapsules. Significant repellent effect of the oil was observed on all the 7 days at 5% concentration. Maze experiment revealed reduced activities of rats in zone treated with microcapsules containing 5% oil compared to untreated zone. Under simulated store conditions, microcapsules containing 5% oil showed significant repellent and antifeedant effect which persisted for all the 15 days of the experiment. Present study suggests the use of microcapsules containing 5% eucalyptus oil to reduce rodent damage in storage.

Keywords: antifeedant, eucalyptus oil, microcapsules, *Rattus rattus*, repellent

1. Introduction
Agricultural growth in India has been closely associated with the well known 'Green Revolution' which saw the development and adoption of new technologies and high yielding varieties of wheat, rice and other food crops. At present India is self-supportive in food production as a consequence of Green uprising. Rodents have gained the position as one of the most serious vertebrate pests disturbing human populations at the global level. As a result of the developments in agriculture and suitable tropical and sub-tropical climatic conditions, the rodent populations have increased due to their higher adaptability and high rate of reproduction and population growth. In addition to causing economic losses by direct consumption of food grains, rodents also destroy food grains by contaminating them with their urine, faecal droppings and hair. Up to the present time, the control of rodents has depended extensively on the usage of rodenticides [1] or localized trapping nearby the sites of importance [2, 3] which are to a certain extent labour intensive.

The house rat, *Rattus rattus* is one of the most common commensal rodent pest worldwide [4]. It often damages, contaminates and spoils packed food and non-food materials in transit and storage [5]. It is the predominant rodent pest species infesting poultry farms in India with highest annual productivity of 69.59 young/ female/ year reported for any Indian rodent species [6]. The species is also famous for its role in disseminating the bubonic plague that took millions of lives in the middle ages and reservoir of a number of parasites of zoonotic importance [7]. The economic losses and health problems associated with this pest lay emphasis on the necessity to develop techniques for its management.

Plant based non-lethal repellents are most suitable for rodent control [8]. These can be useful for the prevention of rodent damage to food grains in storage and seeds and seedlings in crop fields and nurseries. Repellents act by stimulating the primary or secondary defense mechanisms, causing the food to be rejected. Unpleasant taste and odour cues function as initial deterrents against ingestion of food that contains toxins leading to primary food aversion. Reducing the risk associated with pesticides by the use of plant essential oils as repellents or anifeedants may prove an excellent alternative [9]. Also, organic food production is growing steadily in the developing and developed world, and organic farmers will only accept natural pesticides. Plant essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes [10].

Eucalyptus oil is the generic name used for purified oil achieved from the foliage of *Eucalyptus* spp. The oil is used commercially in food, essence and perfumery, and in remedial industries [11]. It also holds insecticidal, herbicidal and fungicidal activities. Among the various components of eucalyptus oil, eucalyptol (1, 8-cineole) is the most important and, in fact, a characteristic compound of the genus *Eucalyptus*, largely responsible for a variety of its pesticidal properties [12, 13]. Eucalyptus oil has been placed under Generally Regarded as Safe category by Food and Drug Authority of USA and classified as nontoxic [14].

Relatively little work has been carried out on plant derived repellents as compared to other aspects of rodent control. Short term repellent effect (1-2 days) of eucalyptus and citronella oils against *R. rattus* has been reported earlier [15, 16]. The biological action of the oil can be wiped out by volatilization of active components via the act of oxidation, high temperatures and UV light [17]. Formulation of essential oils in hard forms (microcapsules or microspheres), semi-liquid forms (gels, liposomes etc.) or fluid forms (micelles, fluid, solutions suspensions etc.) have to be engaged for regulatory discharge of active elements and defending them from outside atmosphere [18]. Microencapsulation is the technique by which solids, fluids or even vapours containing the required characteristics may be encircled in enormously miniscule quantities in microscopic particles having thin coverings of wall material with some degree of permeability [19, 20]. The ultimate objective of microencapsulation is to decrease the degree of vaporization of the oil, increase shelf live and have controlled release of the active components for a longer period of time. Naturally occurring polymers such as alginate and chitosan are widely used in biomedical and pharmaceutical fields in various forms such as nanoparticles, microcapsules and emulsions.

The present study is the first report of its kind reporting antifeedant and repellent potential of alginate based microcapsules containing eucalyptus oil against *R. rattus* and their potential in reducing rodent damage under stored conditions.

2. Materials and Methods

The present work was carried out in the Department of Zoology, Punjab Agricultural University (PAU), Ludhiana, Punjab (India) located at an intersection of 30º55' N parallel of latitude and 75º54'E line of longitude. Commercially available eucalyptus oil was used in present studies.

2.1 Standardization of procedure for preparation of microcapsules containing eucalyptus oil

For standardization of procedure for preparation of microencapsulation containing eucalyptus oil, slight modifications were done in the emulsion extrusion technique described by Chan [21]. As per the procedure standardized, 1g sodium bicarbonate (1% w/v) and the desired concentration of eucalyptus oil were dissolved in 100 ml distilled water. The solution was stirred on a magnetic stirrer at a speed of 300 rotations per minute (rpm) for 15 minutes. Then 1g of sodium alginate powder (1% w/v) was mixed into the solution and again the solution was stirred on the magnetic stirrer at a speed of 300 rpm for 1 hour. This solution was then transferred into a specially designed encapsulator and was allowed to drop slowly in the form of small microcapsules into a tray containing 0.5% calcium chloride solution. Microcapsules (Figure 1) were allowed to harden in the calcium chloride solution for 30 minutes, after which these were sieved out, dried and stored in refrigerator for use in experimentation.

Fig 1: Microcapsules containing different concentrations of eucalyptus oil. 1a. Fresh microcapsules formed after hardening in calcium chloride solution. Ib. Air dried microcapsules used for experimentation.

2.2 Determination of loading capacity and encapsulation efficiency of microcapsules

The loading capacity and encapsulation efficiency of alginate microcapsules was measured as per the method described in Soliman *et al.* [22]. For the purpose, 0.5 g of microcapsules containing eucalyptus oil was dissolved in 5 mL of sodium citrate (0.055 M) and 5 mL of *n*-hexane solutions. The absorbance of upper layer of solution containing oil was measured spectrophotometrically at 340 nm. Microcapsules without oil were taken as controls. The quantity of eucalyptus oil loaded was calculated from the plotted standard curve of the eucalyptus oil.

2.3 Topography of microcapsules under Scanning Electron Microscope

Microcapsules loaded with different concentrations of eucalyptus oil (0, 3, 5 and 7%) were examined for topographical features using high-resolution scanning electron microscope (SEM) with appropriate magnifications and accelerating voltage available at Electron Microscopy and Nanoparticle Laboratory, PAU, Ludhiana. Fine coat-ion sputter device, comprising of rotary and basic pump unit was used to perform efficient and rapid metal coating of microcapsules. The diameters (mm) of microcapsules along the minor and major axes were also determined.

2.4 Collection and maintenance of animals for experimentation

For present studies, mature male *R. rattus* were live trapped from poultry farms at Ludhiana. In the laboratory, rats were acclimatized individually in laboratory cages of size 36 x 23 x 23 cm for 15-20 days before the commencement of the experiment. During acclimatization, the food and water were provided *ad libitum*. Food consisted of a loose mixture of cracked wheat, powdered sugar and groundnut oil (WSO) in the ratio 96:2:2. The metallic trays were kept under each cage for the collection and disposal of urine and faeces. The experimental protocol met the National guidelines on the proper care and use of animals in the laboratory research. The Institutional Animal Ethics Committee approved the experimental design.

2.5 Antifeedant effect of microcapsules

After acclimatization, healthy rats were weighed and selected for experimentation. A total of four laboratory pens (each of size 252 x 100 x 72 cm), were used for each experiment. Each pen further consisted of three chambers of equal size. One rat was released in each chamber. Each chamber in a laboratory pen, on its opposite facing sides was connected with holes (each of diameter 6 cm) to two small nest boxes (each of size

20 x 15 x 15 cm). Rat in a chamber had free access to both the nest boxes. During treatment microcapsules containing three different concentrations of eucalyptus oil (3, 5 or 7%) were mixed in WSO bait in three different concentrations (5, 10 or 20%) to test their antifeedant effect for seven consecutive days. For each concentration of oil and each concentration of microcapsule in bait, a new set of 12 rats was taken to eliminate the effect of any previous exposure. During pre-treatment and post-treatment periods, rats were fed on the plain WSO bait. Food consumption for both of these periods was recorded after every 24 hours for five consecutive days to determine the effect of treatment on rats. Treated food taken in a bowl was kept in the nest box on one side of the chamber. In other nest box of the chamber, bowl containing plain WSO bait was placed. The microcapsules mixed in WSO bait on day 1 were not changed subsequently up to the whole treatment period of seven days to record the persistence of antifeedant effect if any. Consumption of treated and untreated baits was recorded daily after every 24 hours to calculate mean daily bait consumption {g/100g body weight (bw)}. Based on mean daily bait consumption, the antifeedant index (%) was calculated as per the formula given below:

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

2.6 Repellent effect of microcapsules

For testing the repellent effect, two laboratory cages each of size 36 x 23 x 23 cm were joined to each other so that the rat had access to both the cages. Bowls containing plain WSO bait were kept in both the cages. The microcapsules containing the desired concentration of eucalyptus oil (3, 5 or 7%) were kept in a separate bowl in one of the cage only. This was taken as the treated side and the other side as untreated. Microcapsules kept on day 1 were not changed subsequently up to the whole treatment period of seven days to record the persistence of repellent effect if any. Consumption of WSO bait from both treated and untreated sides was recorded daily after every 24 hours to calculate mean daily bait consumption (g/100g bw). Based on mean daily bait consumption, per cent repellency was calculated as per the formula given below:

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

Where,

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

BT is the mean daily bait consumption from treated side

2.7 Rat behaviour in response to microcapsules exposed in Maze experiment

In maze experiment, the behaviour of rats (n = 4) was recorded in response to their exposure to microcapsules containing 5% eucalyptus oil. I-Maze consisting of two arms (each of length 47 cm and height 18.5cm) on opposite sides connected with a central hub was used for this purpose (Figure 2). During treatment, in one arm of I-Maze (called treated zone), microcapsules containing 5% eucalyptus oil were placed, whereas no capsules were placed in other arm as well as in the central hub (called untreated zones). One rat was released at a time in the maze in the central hub initially. Observations were recorded through camera operated rodent behaviour test system having Ethovision Pro software of Netherlands. For each rat, experiment was continued for 5 days of a week. For first two days of the week, the behavior of rat was recorded in I-maze for 2 hours without placing the microcapsules in any arm. It was done in order to reduce the fear and anxiety in rats when exposed to new environment followed by exposure to microcapsules in one of the arms on third day. Again behaviour of the rat was recorded for 2 hours in a day from third to fifth day to record the effect of treatment. Microcapsules kept on day 3 were not changed subsequently up to the whole treatment period of five days to record the persistence of repellent effect if any. Each day, the movement of rat in the I-maze was recorded in the form of tracks and the quantitative data on in zone frequency, in zone total duration of time spent (s), in zone latency of first occurrence, total distance moved (cm), velocity (cm/s) of movement and total duration (s) of movement in both treated and untreated zones was recorded.

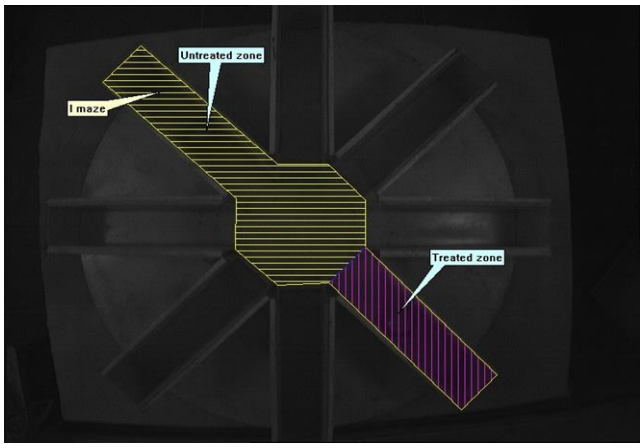

Fig 2: Maze used for evaluation of repellent effect of microcapsules containing eucalyptus oil in bi-choice experiment.

2.8 Potential of microcapsules in reducing rodent damage under simulated store conditions

A total of four laboratory pens (one for each rat) at a time were used. Each pen consisted of three chambers of equal size. All the three chambers were interconnected with each other through small holes (each of diameter 6 cm). Each chamber in a laboratory pen, on its opposite facing sides was connected with holes (each of diameter 6 cm) to two small nest boxes (each of size 20×15×15 cm). One rat was released in each pen. Rat released in each pen had free access to all the three chambers and nest boxes attached to these. Two chambers at extreme ends of a laboratory pen were provided with 11 bags of wheat grains kept in a tray in two layers (first layer has 6 bags and second layer has 5 bags) so as to look like the simulated store condition. Each bag was filled with 500 g of wheat grains. Bags kept in one chamber (treated side) were sprinkled with microcapsules containing 5% eucalyptus oil, whereas bags of other side remained untreated. Microcapsules kept on day 1 were not changed subsequently up to the whole treatment period of 15 days to record the persistence of repellent or antifeedant effect if any. Data on consumption of wheat grains (g/100 g bw), spillage of grains and number of cuts on bags was recorded daily for 15 days.

2.9 Statistical analysis

Values were determined as mean ± SE. The data on bait consumption, spillage of grains and number of cuts on bags from treated and untreated sides at different concentrations of eucalyptus oil and microcapsules under both laboratory and simulated store conditions was collected using factorial

experiments in completely randomized design. Analysis was done using general linear model in SPSS 20.0 software. All pairwise treatment comparisons were made using Tukeys' test at the 5% level of significance.

3. Results and Discussion

3.1 Loading capacity and encapsulation efficiency of microcapsules

Loading capacity of microcapsules was a minimum at 3% (86%) and maximum (100%) at 5% concentration of eucalyptus oil. The loading capacity of microcapsules containing 7% eucalyptus oil was 96% which may be due to leakage of excess oil as observed when the microcapsules were kept in calcium chloride solution for hardening. The encapsulation efficiency of microcapsules was 14.33, 10 and 6.86% at 3, 5 and 7% concentrations, respectively being maximum at 3% concentration. The oil loading capacity and encapsulation efficiency of these alginate based microcapsules depended upon the concentration of sodium alginate and calcium chloride as well as upon the cross linking time. In present study, we used 1% sodium alginate, 0.5% calcium chloride and 30 minutes cross linking time. Soliman *et al.* [22] also reported oil loading capacity and encapsulation efficiency of alginate based microcapsules to be 50-90% and 10-25%, respectively at cross linking time of 25-30 minutes. They found 2% (w/v) sodium alginate, 0.5% (w/v) calcium chloride and 20 minutes cross-linking time to be the best to achieve maximum loading capacity of 90-94% and maximum encapsulation efficiency of 22.5- 23.5% of different oils.

3.2 Topography of microcapsules under Scanning Electron Microscope

Topography of microcapsules not loaded and loaded with different concentrations of eucalyptus oil (3, 5 and 7%) using a high-resolution Scanning Electron Microscope (SEM) revealed three-dimensional, porous, sponge like structure (Figures 3 and 4). The micrograph of microcapsules without oil revealed smooth textural characteristics. However, oil loaded microcapsules exhibited crimpy surface with the appearance of noticeable lumps. These lumps may be due to deposition of oil onto the outer or inner surface of the external layer of microcapsules. Deposition of oil in the form of bulging at the surface was also seen on the surface of microcapsules containing 7% eucalyptus oil. Bursted oil bulgings were also seen on the surface of these microcapsules. Soliman *et al.* [22] also observed similar textural characteristics in SEM micrographs of plain and oil loaded microspheres. The average diameter of microcapsules without any oil along the minor and major axes was found to be 1.27±0.05 and 1.51±0.03 mm, respectively. The average diameters of microcapsules containing 3, 5 and 7% eucalyptus oil along the minor and major axes were found to be 1.25±0.07 and 1.38±0.09, 1.06±0.02 and 1.13±0.03, and 1.43±0.11 and 1.62±0.14, respectively. No significant differences were found in the diameters of microcapsules without oil and those containing 3, 5 and 7% eucalyptus oil.

3.3 Antifeedant effect of microcapsules

3.3.1 Effect of different concentrations of microcapsules containing 3% oil

Results (Table 1) revealed a significant ($P \leq 0.05$) difference in mean daily consumption between treated and untreated baits at all the three concentrations (5, 10 and 20%) of microcapsules on all the 7 days of treatment. At 5 and 20% concentrations of microcapsules, the consumption of treated bait on day 1 was non-significantly less than that of untreated bait thus indicating that rats may have started avoiding the bait containing microcapsules after first ingesting it on day 1. The overall antifeedant index (Table 4) was found to vary from 72.3 to 77.8% at all the three concentrations of microcapsules containing 3% oil. No significant difference was observed in the mean daily consumption of bait between pre-treatment (9.7 to 13.1 g/100g bw) and post-treatment (10.1 to 12.9 g/100g bw) periods indicating no toxic effect of microcapsules containing eucalyptus oil.

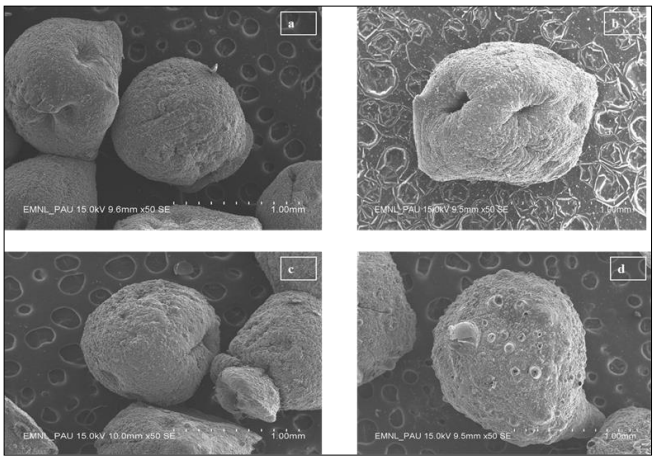

Fig 3: Scanning Electron Microphotographs of microcapsules loaded and unloaded with eucalyptus oil. 3a. microcapsules without oil. 3b. microcapsules containing 3% eucalyptus oil. 3c. microcapsules containing 5% eucalyptus oil. 3d. microcapsules containing 7% eucalyptus oil at 15.0kV 9.6mm×50SE.

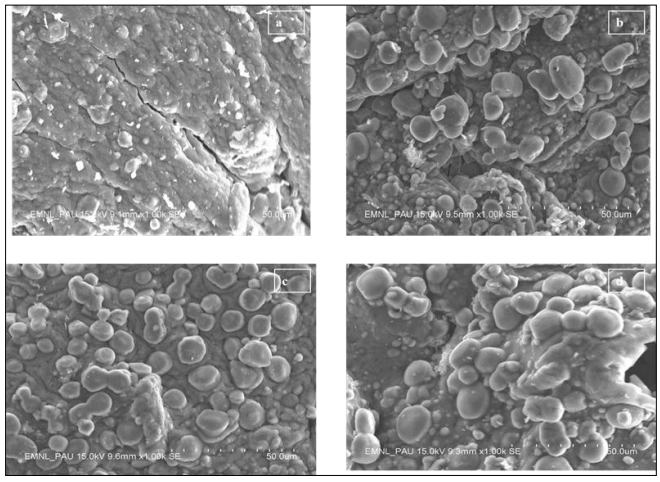

Fig 4: Scanning Electron Microphotographs of microcapsules loaded and unloaded with eucalyptus oil. 4a. microcapsules without oil. 4b. microcapsules containing 3% eucalyptus oil. 4c. microcapsules containing 5% eucalyptus oil. 4d. microcapsules containing 7% eucalyptus oil at 15.0kV 9.6mm×1.00K SE.

3.3.2 Effect of different concentrations of microcapsules containing 5% oil

Results (Table 2) revealed a significant ($P \leq 0.05$) difference in mean daily consumption between treated and untreated baits at all the three concentrations of microcapsules on all the 7 days of treatment. The overall antifeedant index (Table 4) was found to vary from 65.5 to 76.7% at all the three concentrations of microcapsules containing 5% oil. No significant difference was observed in mean daily consumption of bait between pre-treatment (9.2 to 10.9 g/100g bw) and post-treatment (9.0 to 9.5 g/100g bw) periods indicating no toxic effect of microcapsules containing

eucalyptus oil.

3.3.3 Effect of different concentrations of microcapsules containing 7% oil

Results (Table 3) revealed a significant ($P \leq 0.05$) difference in mean daily consumption between treated and untreated baits at all the three concentrations of microcapsules on all the 7 days of treatment. The overall antifeedant index (Table 4) was found to vary from 69.6 to 78.8% at all the three concentrations of microcapsules containing 5% oil. No significant difference was observed in the mean daily consumption of bait between pre-treatment (10.3 to 13.0 g/100g bw) and post-treatment (9.1 to 12.9 g/100g bw) periods indicating no toxic effect of microcapsules containing eucalyptus oil.

Overall, no significant difference was found in antifeedant index among the three concentrations (5, 10 and 20%) of microcapsules (Figure 5) as well as among the microcapsules containing three concentrations (3, 5 and 7%) of eucalyptus oil (Figure 6).

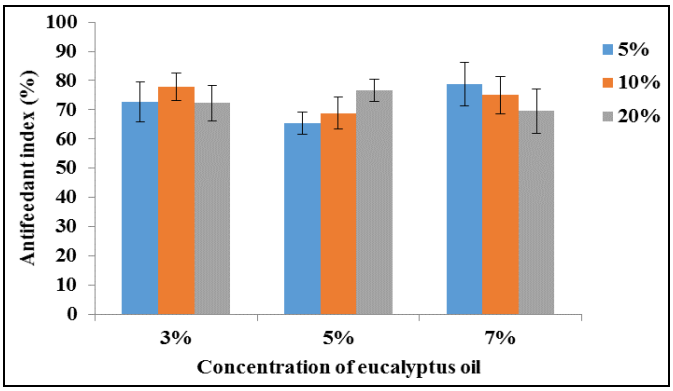

Fig 5: Comparison of antifeedant index of three different concentrations of microcapsules against *R. rattus*. Bars with no superscript indicate no significant difference at $P \leq 0.05$.

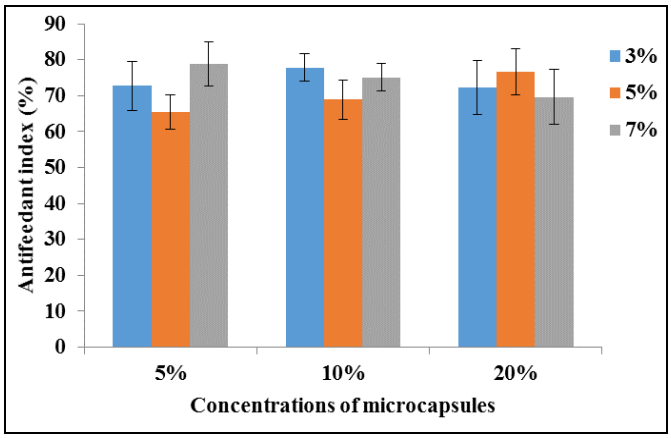

Fig 6: Comparison of antifeedant index of microcapsules containing 3, 5 and 7% eucalyptus oil against *R. rattus*. Bars with no superscript indicate no significant difference at $P \leq 0.05$.

3.4 Repellent effect of microcapsules

Results (Table 5) revealed no significant difference in mean daily consumption of plain bait between treated and untreated sides at 3 and 7% concentrations of eucalyptus oil indicating no significant repellent effect. At 5% concentration of the oil, a significant ($P \leq 0.05$) difference in mean daily consumption of plain bait was observed between treated and untreated sides indicating a significant repellent effect. Repellency (Table 6) with microcapsules containing 3% eucalyptus oil was observed only on days 1 (48.2%) and 6 (60.6%) of exposure indicating ineffective concentration or low concentration of eucalyptus oil in microcapsules. Per cent repellency with microcapsules containing 5% eucalyptus oil was maximum on day 1 (96.1%) of exposure which decreased slowly to 70.2% on 7 days of exposure thus indicating persistence of repellent effect on all the 7 days. There was no repellency observed with microcapsules containing 7% eucalyptus oil on day 1 of exposure. Reduced repellency was however, observed from days 2 to 6 (40.1-59.0%) which may be due to the spillage of oil from the microcapsules as also indicated in the SEM images of the microcapsules. Also there was no significant difference in bait consumption between pre-treatment (9.4 to 11.5 g/100g bw) and post-treatment (10.2 to 11.9 g/100g bw) periods at all the three concentrations of oil indicating no adverse effect of exposure to microcapsules containing eucalyptus oil.

Overall, microcapsules containing all the three concentrations were found to have antifeedant effect whereas, microcapsules containing only 5% eucalyptus oil were found to have repellent effect on all the 7 days of treatment. The microcapsules containing 5% eucalyptus oil were therefore evaluated for affecting behaviour of rats in Maze experiment and their potential in reducing rodent damage under simulated store conditions.

3.5 Rat Behaviour in response to microcapsules exposed in Maze experiment

Recording of behaviour of male *R. rattus* in response to microcapsules containing 5% eucalyptus oil exposed in bi-choice test in I-Maze under rodent behaviour test system revealed (Table 7) no significant difference in in zone frequency, latency of first occurrence (s), total distance moved (cm) and velocity (cm/s) of movement between untreated and treated zones, however, a significant ($P \leq 0.05$) difference was observed in in zone total duration of time spent (s) and total duration of mobility (s) between untreated and treated zones. There was also no significant difference in different activities in treated zone among the 3 days of treatment. In zone total duration of time spent by rats was significantly ($P \leq 0.05$) less in treated zone that that in untreated zone on first 2 days of treatment, however there was no significant difference between treated and untreated zones on day 3 of treatment. This indicated that the effect of microcapsules remained for first 2 days. The total duration of mobility was found to significantly ($P \leq 0.05$) less in treated zone on day 1 of treatment than that in untreated zone, however, there was no significant difference in total duration of mobility between treated and untreated zones on days 2-3 of treatment thus indicating quick dissipation of repellent effect or the spread of odour of eucalyptus oil in both the arms of the Maze due to volatile nature of eucalyptus oil and smaller area of I-Maze. The animal tracking data in two zones of I-Maze revealed comparatively less animal tracks in treated zone (Figure 7). Similar experiment to study repellent efficacy of chilli, wintergreen oil, bergamot oil, peppermint oil and geranium oil was conducted by Kalandakanond-Thongsong *et al.* [23] in the circular open field against adult male Wistar rats. Rats spent less time in the inner zone of the circular field exposed to repellents compared to the control indicating that the rats were most likely trying to avoid the closed contact to these substances.

3.6 Potential of microcapsules in reducing rodent damage under simulated store conditions

Results (Table 8) revealed a significant difference in mean

daily consumption of wheat grains between treated and untreated sides from day 2 to 15. No consumption of grains was found on either side on day 1. Average mean daily consumption in 15 days from treated side was significantly ($P \leq 0.05$) low (3.1 g/100g bw) than that observed on untreated side (9.7 g/100g bw). Mean daily consumption of grains on treated side increased slowly up to day 15 of treatment indicating that repellent effect of microcapsules dissipated slowly day by day. Highest per cent repellency (Table 8) was found on day 3 (92.0%) of treatment which decreased slowly and reached 30.7% by day 15. The average repellency observed in 15 days was 64.7%.

No spillage of wheat grains was found on either side on day 1 (Table 9, Figure 8a-b). Spillage of grains started on both treated and untreated sides from day 2 onwards but was comparatively more on untreated side as compared to treated side (Figure 8c-d). Average mean daily spillage of grains in 15 days from treated side was significantly ($P \leq 0.05$) low (4.7 g/100g bw) than that observed on untreated side (9.0 g/100g bw). No cuts to bags were found on either side on day 1 (Table 9). Average mean daily cuts on bags in 15 days from treated side were significantly ($P \leq 0.05$) lower (1.3) than that observed on untreated side (2.3). Number of cuts to bags on treated side were low initially but increased slowly up to day 15 of treatment again indicating that repellent effect of microcapsules dissipated slowly day by day. The size of cuts of the bags was small on treated side as compared to untreated side.

Essential oils of eucalyptus seem principally potent as repellent against mosquitoes and other arthropods [24-28]. Since a wide spectrum of biological activities is possessed by eucalyptus oil and are regarded as safer compounds, there have been efforts to commercialize and market the insecticides/repellent products containing eucalyptus oil as such or based upon them [29].

A number of plant products as antifeedanting agents have been tested against rodents in bi-choice tests [30-35]. Singla *et al*. [16] studied repellent effect of eucalyptus oil at 5, 10 and 20% concentrations against *R. rattus* by applying the oil as spray in bi-choice feeding test. Results revealed the higher repellent effect of the oil when applied daily as compared to when applied once in a week indicating low persistence of repellent effect which may be due to the volatile nature of oil. So it was suggested to prepare different formulations to increase the persistence of the effect of the oil. Singla and Kaur [36] conducted study in order to increase the efficacy of eucalyptus oil by controlling the release of oil through encapsulated wax blocks and the 5% concentration of the oil was found to be most effective as repellent. Singla *et al*. [37] also tested the repellent potential of encapsulated wax blocks containing 5% concentrations of eucalyptus oil under storage conditions and found them effective for 3-4 days in reducing rodent damage.

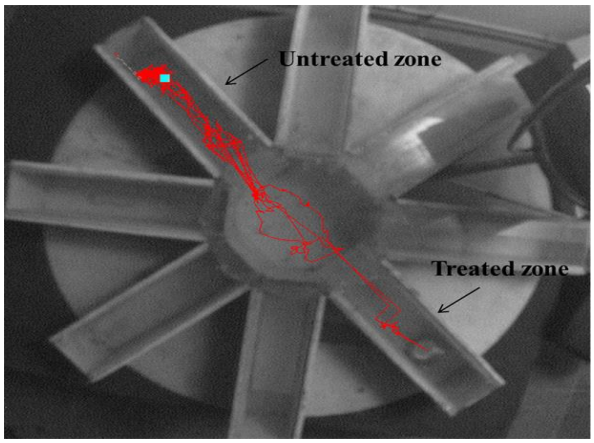

Fig 7: Maze showing movement tracks of rat in treated and untreated zones.

Fig 8: Wheat grain bags kept on treated and untreated sides of the laboratory pen. 12a. No rodent damage to untreated bags on day 1. 12b. No rodent damage to treated bags on day 1. 12c. Rodent damage to untreated bags after day 1. 12d. Comparatively less rodent damage to treated bags after day 1.

Table 1: Antifeedant effect of different concentrations of microcapsules containing 3% eucalyptus oil against *Rattus rattus.*

Days of treatment	Mean daily bait consumption (g/100g bw)					
	5%		10%		20%	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
1	4.5±1.0	629±1.2	0.3±0.1	9.0±0.6*	3.9±1.3	7.2±1.2
2	2.4±1.8	7.7±0.9*	0.7±0.1	10.1±0.9*	1.0±0.4	7.5±0.6*
3	2.5±1.4	7.2±1.3*	1.1±0.2	10.0±0.8*	1.9±0.8	5.1±1.0*
4	1.1±0.5	7.7±0.6*	1.1±0.2	10.1±0.6*	0.7±0.6	7.1±0.8*
5	0.7±0.2	8.6±0.5*	1.7±0.2	9.2±0.6*	0.2±0.1	7.6±0.6*
6	0.7±0.3	8.2±0.7*	1.7±0.5	9.5±1.0*	0.8±0.4	7.9±0.9*
7	0.9±0.4	9.0±0.9*	1.9±0.3	9.3±1.2*	1.1±0.4	7.4±0.7*
Average	1.8±0.6	7.8±0.4*	1.2±0.2	9.6±0.2*	1.4±0.5	7.1±0.4*

-Mean values with * indicate significant difference between consumption of treated and untreated baits at $P \leq 0.05$

Table 2: Antifeedant effect of different concentrations of microcapsules containing 5% eucalyptus oil against *Rattus rattus.*

Days of treatment	Mean daily bait consumption (g/100g bw)					
	5%		10%		20%	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
1	1.4±0.5	5.4±0.7*	0.4±0.3	8.2±0.8*	0.4±0.2	9.3±1.8*
2	0.4±0.4	7.3±1.3*	2.7±0.8	6.6±1.2*	0.4±0.1	9.4±1.4*

3	1.7±0.3	8.4±0.9*	0.6±0.3	5.4±1.0*	0.5±0.2	8.8±1.3*
4	2.1±0.4	8.1±0.8*	1.3±0.4	4.8±1.3*	0.6±0.2	8.4±1.6*
5	1.8±0.3	7.7±0.7*	0.9±0.5	5.9±1.0*	1.5±0.3	7.3±1.6*
6	1.9±0.2	7.4±1.0*	1.2±0.2	6.3±1.0*	2.1±0.2	7.7±1.0*
7	1.7±0.4	6.4±0.8*	0.9±0.5	7.7±0.3*	2.2±0.2	7.9±1.0*
Average	1.6±0.2	7.2±0.4*	1.2±0.3	6.4±0.5*	1.1±0.3	8.4±0.3*

-Mean values with * indicate significant difference between consumption of treated and untreated baits at $P \leq 0.05$

Table 3: Antifeedant effect of different concentrations of microcapsules containing 7% eucalyptus oil against *Rattus rattus.*

Days of treatment	Mean daily bait consumption (g/100g bw)					
	5%		10%		20%	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
1	2.24±0.45	6.45±0.63*	1.38±0.32	7.49±0.35*	3.21±0.93	11.48±1.41*
2	1.52±0.89	6.25±0.64*	0.74±0.25	8.43±0.37*	4.05±0.73	7.97±2.05
3	0.62±0.42	6.54±0.70*	0.72±0.27	7.88±0.31*	1.39±0.66	9.69±2.07*
4	0.17±0.17	4.58±0.43*	0.66±0.35	7.89±0.75*	1.90±0.70	11.79±2.25*
5	0.54±0.13	4.55±0.47*	1.23±0.45	7.92±0.74*	0.39±0.18	7.30±0.65*
6	0.18±0.18	4.43±0.33*	1.82±0.25	7.10±0.56*	1.94±1.24	8.47±1.18*
7	0.29±0.13	3.78±0.32*	1.24±0.34	7.70±0.87*	0.79±0.62	5.51±1.26*
Average	0.79±0.32	5.23±0.47*	1.11±0.17	7.77±0.17*	1.95±0.53	8.89±0.92*

-Mean values with * indicate significant difference between consumption of treated and untreated baits at $P \leq 0.05$

Table 4: Antifeedant index of different concentrations of microcapsules containing 3, 5 and 7% eucalyptus oil.

Days of treatment	Antifeedant index (%)								
	3% oil			5% oil			7% oil		
	5%	10%	20%	5%	10%	20%	5%	10%	20%
1	**32.4±8.8^{a}**	**93.2±3.1^{a}**	**35.2±15.6^{a}**	**61.8±10.7^{b}**	**89.0±7.0^{a}**	**92.3±3.7^{a}**	**48.2±10.9^{b}**	**69.4±7.3^{a}**	**57.6±10.0^{ab}**
2	74.8±14.8^{b}	86.2±2.0^{ab}	76.6±10.0^{b}	93.3±6.7^{a}	54.3±6.9^{a}	91.0±3.1^{a}	66.3±15.8^{ab}	84.0±5.3^{a}	31.1±12.5^{b}
3	76.6±9.5^{b}	79.2±3.4^{abc}	57.9±15.3^{b}	66.0±6.2^{b}	77.5±12.8^{a}	90.8±3.0^{a}	84.8±9.4^{ab}	83.4±6.0^{a}	78.4±10.5^{a}
4	75.7±9.4^{b}	81.2±3.8^{abc}	84.5±12.7^{b}	59.4±7.4^{b}	48.7±15.6^{a}	86.9±4.9^{a}	93.3±6.7^{a}	85.7±7.2^{a}	73.1±11.2^{ab}
5	85.7±5.1^{b}	69.3±2.2^{bc}	94.9±3.2^{b}	61.1±6.4^{b}	67.6±16.7^{a}	63.5±6.1^{b}	80.6±4.3^{ab}	73.9±9.7^{a}	91.3±4.1^{a}
6	82.4±6.0^{b}	70.5±7.9^{bc}	80.5±8.0^{b}	58.6±4.1^{b}	64.6±9.9^{a}	56.5±3.2^{b}	92.6±7.4^{a}	58.2±6.6^{a}	70.2±16.6^{ab}
7	81.2±8.8^{b}	64.7±6.6^{c}	76.4±8.1^{b}	58.2±8.1^{b}	80.5±4.5^{a}	56.2±2.6^{b}	85.5±7.0^{ab}	70.7±7.6^{a}	85.6±10.9^{a}
Average	72.7±7.4	77.8±4.2	72.3±8.1	65.5±5.1	68.9±5.9	76.7±7.0	78.8±6.6	75.1±4.1	69.6±8.2

-Mean values with different superscripts (a-c) in a column indicate significant difference among different days of application at $P \leq 0.05$

Table 5: Repellent effect of microcapsules containing different concentrations of eucalyptus oil against *Rattus rattus.*

Days of treatment	Mean daily bait consumption (g/100g bw)					
	3%		5%		7%	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
1	4.95±1.82^{a}	6.42±1.79	0.39±0.21^{a}	9.20±2.79*	7.21±3.76^{a}	6.53±1.61
2	7.89±1.54^{a}	4.06±0.97	0.76±0.43^{ab}	9.25±2.79*	5.07±1.34^{a}	6.61±1.88
3	5.44±1.23^{a}	4.20±0.85	1.52±0.43^{bc}	8.92±2.79*	6.21±1.88^{a}	6.27±1.34
4	4.85±0.90^{a}	4.09±0.80	1.82±0.64^{c}	8.14±3.22*	4.12±2.15^{a}	4.48±1.88
5	8.06±1.91^{a}	5.80±1.51	2.40±0.86^{c}	8.16±3.01*	3.82±2.95^{a}	5.49±0.54
6	4.04±1.32^{a}	4.79±1.29	1.99±1.07^{c}	7.63±2.36*	3.76±2.15^{a}	3.90±1.61
7	5.95±0.63^{a}	4.66±1.87	2.10±1.07^{c}	7.34±2.79*	5.34±1.07^{a}	4.64±3.49
Average	5.88±0.63	4.86±0.37	1.57±0.30	8.38±0.31*	5.08±0.53	5.42±0.45

-Mean values with * indicate significant difference between consumption of plain bait from treated and untreated sides at $P \leq 0.05$
-Mean values with different superscripts (a-c) in a column indicate significant difference among different days of application at $P \leq 0.05$

Table 6: Per cent repellency with microcapsules containing different concentrations of eucalyptus oil against *Rattus rattus.*

Days of treatment	Per cent Repellency		
	3%	5%	7%
1	48.23±17.65^{a}	96.13±37.68^{a}	-
2	-	91.46±34.54^{ab}	42.15±17.57^{a}
3	-	82.24±34.54^{abc}	40.06±10.05^{a}
4	-	76.68±32.66^{bc}	44.80±26.00^{a}
5	-	69.57±29.16^{c}	55.00±17.08^{a}
6	60.61±19.79^{a}	71.40±22.27^{c}	59.02±5.89^{a}
7	-	70.21±25.12^{c}	-
Average	54.42±4.38	79.67±4.36	48.21±3.33

-Mean values with different superscripts (a-c) in a column indicate significant difference among different days of application at $P \leq 0.05$

Table 7: Behaviour of *Rattus rattus* in response to microcapsules containing 5% eucalyptus oil exposed in bi-choice test in I-Maze.

Parameters	Treatment days	Treated zone	Untreated zone
In zone frequency	Day 1	26.75±24.75(49.51)[a]	50.5±31.79(63.58)
	Day 2	217±166.87(333.73) [a]	228.5±165.37(330.74)
	Day 3	432±426.68(853.35) [a]	438.5±426.51(853.01)
	Average	225.25±101.38(202.75)	239.17±97.11(194.22)
In zone total duration (s)	Day 1	95.3±44.44(88.89) [a]	4379.55±908.15(1816.31)*
	Day 2	569.5±497.15(994.30) [a]	3798.85±1206.21(2412.41)*
	Day 3	663.15±646.97(1293.93)[a]	3793.4±1236.50(2473.00)
	Average	442.65±152.22(304.44)	3990.6±168.43(336.85)*
In zone latency of first occurrence (s)	Day 1	47.05±18.17(36.35) [a]	34.8±18.34(36.67)
	Day 2	2.1±1.84(3.69) [a]	18.3±11.69(23.39)
	Day 3	49.25±24.05(48.09) [a]	20.45±7.29(14.58)
	Average	32.8±13.30(26.61)	24.52±4.49(8.97)
Total distance moved (cm)	Day 1	1008.48±847.19(1694.38) [a]	44630.49±27293.71(54587.41)
	Day 2	18346.22±17034.31(34068.62)[a]	38951.15±11166.06(22332.11)
	Day 3	44092.04±43688.23(87376.45)[a]	71997.73±34443.72(68887.43)
	Average	21148.91±10839.05(21678.09)	51859.79±8834.821(17669.64)
Velocity (cm/s) of movement	Day 1	10.67±4.21(8.42)[a]	23.87±3.09(6.18)
	Day 2	25.52±5.90(11.80)[a]	61.9±48.45(96.89)
	Day 3	32.84±13.67(27.34)[a]	63.08±42.83(85.66)
	Average	23.01±5.65(11.29)	49.61±11.15(22.31)
Total duration of mobility (s)	Day 1	90.7±40.97(81.94)[a]	4272.5±888.30(1776.60)*
	Day 2	538.35±466.83(933.65)[a]	3552.05±1290.62(2581.24)
	Day 3	453.15±436.97(873.95)[a]	3752.35±1226.08(2452.17)
	Average	360.73±118.85(237.70)	3858.97±185.94(371.87)*

-Mean values with * indicate significant difference between treated and untreated zones at $P \leq 0.05$
-Mean values with similar superscript (a) in a column for each parameter indicate no significant difference among different days of application at $P \leq 0.05$

Table 8: Effect of microcapsules containing 5% eucalyptus oil on consumption of wheat grains by *Rattus rattus* under simulated store conditions.

Days of treatment	Mean daily consumption of grains (g/100 g bw)		Percent repellency
	Treated side	Untreated side	
Day 1	0.00±0.00[a]	0.00±0.00	0±0[d]
Day 2	0.30±0.30[ab]	4.50±1.38*	75±20.41 [ab]
Day 3	0.66±0.39[abc]	7.39±0.82*	91.67±3.93[a]
Day 4	1.57±0.16[abcd]	7.74±0.62*	79.58±1.40[ab]
Day 5	1.11±0.38[abc]	10.10±0.74*	88.69±3.11[a]
Day 6	2.20±0.16[abcde]	11.46±0.95*	80.62±0.88[ab]
Day 7	2.09±0.28[abcde]	10.90±0.47*	80.62±2.39[ab]
Day 8	2.20±0.83[abcde]	10.68±1.13*	77.15±7.21[ab]
Day 9	3.26±0.51[cde]	11.54±0.91*	71.86±3.18 [ab]
Day 10	3.10±0.4[bcde]	12.99±0.90*	76.51±1.79 [ab]
Day 11	3.98±0.48[def]	10.79±1.81*	60.81±5.15 [abc]
Day 12	4.80±1.10[ef]	11.85±0.91*	59.20±8.33 [abc]
Day 13	5.86±0.71[f]	12.40±1.59*	51.10±6.21[abc]
Day 14	6.23±0.87[f]	11.13±1.49*	46.59±7.93[bc]
Day 15	8.65±0.87[g]	12.46±1.15*	30.69±1.25[d]
Average	3.07±1.23	9.73±1.76*	64.67±12.24

-Mean values with * indicate significant difference in consumption of wheat grains between treated and untreated sides at $P \leq 0.05$
-Mean values with different superscripts (a-g) in a column indicate significant difference among different days of application at $P \leq 0.05$

Table 9: Effect of microcapsules containing 5% eucalyptus oil on spillage of wheat grains from bags and number of cuts on bags by *Rattus rattus* under simulated store conditions.

Days of treatment	Spillage of grains from bags (g)		No. of cuts on bags	
	Treated side	Untreated side	Treated side	Untreated side
Day 1	0.00±0.00 [a]	0.00±0.00	0.00±0.00[a]	0.00±0.00
Day 2	1.00±0.71[ab]	4.00±1.35	0.75±0.25[ab]	1.50±0.29
Day 3	2.25±0.48[ab]	7.50±1.89*	0.75±0.25[ab]	1.75±0.25*
Day 4	3.00±0.91[ab]	8.50±2.25	1.00±0.00[ab]	2.00±0.41*
Day 5	3.00±0.71[ab]	8.75±1.11*	1.25±0.25[b]	2.25±0.63
Day 6	3.50±1.19[bc]	14.50±2.02*	1.50±0.29[b]	3.00±0.41*
Day 7	3.75±1.65[bc]	10.50±4.50	1.50±0.29[b]	3.00±0.41*
Day 8	5.75±1.18[bc]	10.00±4.53	1.25±0.25[b]	2.75±0.48*
Day 9	6.25±1.60[cd]	7.00±1.91	1.50±0.29[a]	2.50±0.29*
Day 10	5.25±1.70[bc]	7.50±2.60	1.75±0.25[b]	2.75±0.48
Day 11	9.00±2.04[d]	8.00±2.80	2.00±0.41[b]	2.75±0.48

Day 12	7.50±1.55^{cd}	6.00±2.04	1.50±0.29^{b}	2.50±0.29*
Day 13	7.50±1.19^{cd}	13.75±4.15	1.25±0.25^{b}	2.75±0.48*
Day 14	4.75±0.63^{bc}	11.00±2.68*	1.50±0.29^{b}	2.50±0.29*
Day 15	7.25±1.49$^{c\ d}$	17.75±2.25*	2.00±0.00^{b}	2.25±0.48
Average	4.65±1.30	8.98±2.16*	1.30±0.26	2.28±0.38*

-Mean values with * indicate significant difference in consumption of wheat grains between treated and untreated sides at $P \leq 0.05$
-Mean values with different superscripts (a-d) in a column indicate significant difference among different days of application at $P \leq 0.05$

4. Conclusions

The present study is the first of its kind evaluating repellent/antifeedant potential of alginate based microcapsules containing eucalyptus oil against rodent pests so as to have a slow release of the oil through the minute pores present in the wall material. The study revealed, a significant difference in mean daily bait consumption between treated and untreated sides at all the three concentrations of microcapsules (5, 10 and 20%) containing 3, 5 and 7% eucalyptus oil. Microcapsules containing 5% eucalyptus oil showed a maximum repellent effect which persisted for all the seven days of the experimental period. Under simulated store conditions, microcapsules containing 5% eucalyptus oil were able to reduce damage by *R. rattus* to bags containing wheat grains up to the whole experimental period of 15 days. The study suggests that the microcapsules containing 5% eucalyptus oil can be used for reducing rodent damage under stored conditions.

5. Acknowledgements

Authors are thankful to the Professor & Head, Department of Zoology, Punjab Agricultural University, Ludhiana and Indian Council of Agricultural Research, New Delhi for providing facilities and financial assistance.

6. References

1. Buckle AP, Eason ET. Control methods: Chemical. In: Buckle AP, Smith RH (eds.). Rodent Pests and their Control. 2nd edn. Cab International, UK, 2015, 123-154.
2. Parshad VR, Kaur M, Singla N. Species-specific differences in live and kill trapping of rodents in fodder berseem (*Trifolium alexandrium*) and maize (*Zea mays*) fields. Indian Journal of Ecology. 2006; 33(1):40-44.
3. Weihong J, Veitch CR, Craig JL. An evaluation of the efficiency of rodent trapping methods: The effect of trap arrangement, cover type and bait. New Zealand Journal of Ecology. 2009; 23(1):45-51.
4. Shiels AB, Pitt WC, Sugihara RT, Witmer GW. Biology and impacts of pacific island invasive species 11. *Rattus rattus*, The black rat (Rodentia: Muridae). Pacific Science. 2014; 68(2):145-184.
5. Parshad VR. Rodent control in India. Integrated Pest Management Review. 1999; 4:97-126.
6. Sridhara S, Krishnamurty TR. Population dynamics of *Rattus rattus* in poultry and implications for control. In: Borrecco JE, Marsh RE (eds.). Proceedings of 15th Vertebrate Pest Conference, University of California, Davis, USA, 1992, 224-228.
7. Singla LD, Singla N, Parshad VR, Juyal PD, Sood NK. Rodents as reservoir of parasites in India. Integrative Zoology. 2008; 3:21-26.
8. Nolte DL, Mason JR, Clark L. Avoidance of bird repellents by mice (*Mus musculus*). Journal of Chemical Ecology. 1993; 19:427-432.
9. Varona S, Martin A, Cocero MJ. Formulation of a natural biocide based on lavandin essential oil by emulsification using modified starches. Chemical Engineering Processing: Process Intensification. 2009; 48(6):1121-1188.
10. Koul O, Walia S. Dhaliwal GS. Essential oils as green pesticides: Potential and constraints. Biopesticides International. 2008; 4(1):63-84.
11. Batish DR, Singh HP, Kohli RK. Eucalyptus essential oil as a natural pesticide. Forest Ecology and Management. 2008; 256:2166-2174.
12. Duke JA. Dr. Duke's phytochemical and ethnobotanical databases. http://www.arsgrin.gov/duke, 2004.
13. Leicach SR, Yaber Grass MA, Chludil HD, Garau AM, Guarnaschelli AB, Fernandez PC. Chemical defenses in *Eucalyptus* species: a sustainable strategy based on antique knowledge to diminish agrochemical dependency In: Oteng-Amoako, A.A., (ed.), New Advances and Contributions to Forestry Research. http://www.intechopen.com/books/new-advances-and-contributions-to-forestry-research, 2012.
14. USEPA (United States Environment Protection Agency). RED Facts. Flower and Vegetable Oils. http://www.epa.gov/oppsrrd1/REDs/ factsheets/4097fact.pdf, 1993.
15. Singla N, Kaur R. Potential of citronella oil as rodent repellent measured as aversion to food**.** Applied Biological Research. 2014; 16(2):191-198.
16. Singla N**,** Thind RK, Mahal AK. Potential of eucalyptus oil as repellent against house rat, *Rattus rattus.* The Scientific World Journal, http://dx.doi.org/10.1155/2014/249284, 2014.
17. 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. Journal of Food Science. 2008; 73(4):41-47.
18. Roy A, Singh SK, Bajpa J, Bajpai AK. Controlled pesticide release from biodegradable polymers. Central European Journal of Chemistry. 2014; 12(4):453-469.
19. Yoshizawa H. Trends in microencapsulation research. KONA Powder and Particle Journal. 2004; 22:22-31.
20. Bakry AM, Abbas S, Ali B, Majeed H, Abouelwafa MY, Mousa A, *et al*. Microencapsulation of oils: A comprehensive review of benefits, techniques, and applications. Comprehensive Reviews in Food Science and Food Safety, doi: 10.1111/1541-4337.12179, 2015.
21. Chan ES. Preparation of Ca-alginate beads containing high oil content: Influence of process variables on encapsulation efficiency and bead properties. Carbohydrate Polymers. 2011; 84(4):1267-1275.
22. Soliman EA, El-Moghazy AY, Mohy El-Din MS, Massoud MA. Microencapsulation of essential oils within alginate: Formulation and in vitro evaluation of antifungal activity. Journal of Encapsulation and Adsorption Sciences. 2013; 3:48-55.
23. Kalandakanond-Thongsong S, Daendee S, Thongsong B, Chavananikul V. The efficacy of pure natural repellents on rat responses using circular open field. The Thai

Journal of Veterinary Medicine. 2010; 40(4):411-418.
24. Choi WS, Park BS, Ku SK, Lee SE. Repellent activities of essential oils and monoterpenes against *Culex pipiens pallens*. Journal of American Mosquito Control Association. 2002; 18(4):348-351.
25. Brooker MIH, Kleinig DA. Field Guide to Eucalyptus. 3rd edn. Bloomings, Melbourne, South-eastern Australia. 2006; 1:353.
26. Jaenson TGT, Garboul S, Palsson K. Repellency of oils of lemon, eucalyptus, geranium, and lavender and the mosquito repellent MyggA natural to *Ixodes ricinus* (Acari: Ixodidae) in the laboratory and field. Journal of Medical Entomology. 2006; 43:731-736.
27. Koschier EL, Sedy KA. Effects of plant volatiles on the feeding and oviposition of *Thrips tabaci*. In: Marullo R, Mound L (eds.). Thrips and Tospoviruses. CSIRO, Australia, 2001, 185-187.
28. Patyal SK, Kumar J. Olfacto-gustatory repellency of some essential oils to the Indian honey bee, *Apis cerana indica* Fabr. Indian Bee Journal. 1989; 51:5-8.
29. Lin TS. Effects of essential oil from the leaves of seven *Eucalyptus* on the control of termite. Forest Products and Industries. 1998; 17:751-760.
30. Trigg JK. Evaluation of a eucalyptus-based repellent against *Anopheles* spp. in Tanzania. Journal of American Mosquito Control Association. 1996; 12(2):243-246.
31. Wager-Page, SS, Mason JR, Aronov E, Epple G. The role of sensory cues and feeding context in the mediation of pine needle oils repellency in prairie voles. National Wildlife Research Center Repellents Conference 1995. Paper 38. http:// digitalcommons.unl.edu/ nwrcrepellants/38, 1997.
32. Singla N, Parshad VR. Antifeeding effects of a neem (*Azadirachta indica*) based formulation botanical bird repellent against the house rat (*Rattus rattus).* Indian Journal of Agricultural Sciences. 2007; 77(8):502-504.
33. Babbar BK, Kaur J, Singla N, Mahal AK. Effectiveness and persistence of cinnamic aldehyde as an antifeedant on rats under storage conditions. Crop Protection. 2015; 67:235-242.
34. Jermy T. Prospects of antifeedant approach to pest control- A critical review. Journal of Chemical Ecology. 1990; 16:3151-1366.
35. Schnutterer H, Aschr KRS. Natural pesticides from the neem tree (*Azadirachta indica A juss*) and other tropical plants. Proceedings of 3rd International Neem Conference, July 10-15, 1986, Nairobi, Kenya, 1987, 703.
36. Singla N, Kaur R. Increasing efficacy of eucalyptus oil as repellent against *Rattus rattus* by controlled release through encapsulated wax blocks. Proceedings of National Academy of Sciences (Biological Sciences), DOI 10.1007/s40011-016-0723-1, 2016.
37. Singla N, Kaur R, Babbar BK, Mahal AK. Repellent potential of eucalyptus and citronella oils against rodent pests infesting stored grain houses. Crop Improvement special issue, 2012, 1037-1038.