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**SHORT COMMUNICATION**

# STUDIES ON REPELLENT AND FEEDING BEHAVIOUR OF HOUSE RAT, *Rattus rattus*, IN RESPONSE TO CINNAMAMIDE

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## ABSTRACT

**Rodenticides are the preferred option to prevent rodent attacks to crop produce. However, secondary poisoning and development of shyness and resistance among rodents after exposure to toxic chemicals has increased the search for plant based effective, safe and ecofriendly control methods. In present study, repellent and feeding behaviour of house rat in response to 0.4% cinnamamide based formulation was studied using automated Ethovision video tracking system and feed scale consumption monitor. Results revealed absence of primary repellent effect of cinnamamide formulation but existence of its secondary repellent effect against house rat. Repellent effect of cinnamamide increased with time during treatment period. Moreover, during treatment period, rats handled treated bait less frequently as compared to the untreated bait because feeding bouts events and feeding bout time also decreased during treatment period.**

**Key words:** Cinnamamide; Ethovision video tracking system, feeding behaviour; *Rattus rattus,* feed scale consumption monitor, repellent

## INTRODUCTION

Among the vertebrate pests, rodents are most destructive to agricultural produce. They cause damage to the standing crops due to their burrowing, cutting and hoarding activities as well as to stored food, poultry farms and other commodities (Meerburg and Kijlstra, 2008; Chattopadhyay *et al*., 2010). Rodents are also responsible for transmitting various types of dreaded diseases (Sullivan, 2007). The regulation of rodent population constitutes a difficult and largely unsolved contemporary problem because of its prolific reproductive potential and immense adaptive capabilities (Gillespie and Myers, 2004). Several approaches being used to control rodent infestation are environmental, cultural, biological, mechanical and chemical methods. Chemical control by rodenticides is most common method available to control rodent pests both under agricultural and commensal situations (Mutz and Sinclair, 2004). However, the overuse of rodenticides in agriculture fields has polluted not only agro-ecosystem but also water and other natural resources. This alarming situation demands alternative safe approaches to control pests without affecting non-target organisms and environment. There is increasing interest in developing biopesticides to control pests due to their effectiveness at low concentration and low impact on non-target organisms (Packiam *et al*., 2012).

Many plant extracts have repellent properties against rodents. Methanol extracts of *Piper nigrum*, *Aucklandia lappa*, *Cinnamomum cassia*, *Illicium verum*, *Rheum coreanum* and *Pinus densiflora* have potent antagonizing activity against mice (Yun *et al*., 1998). Many *Cinnamomum* species are rich in essential oil and tannins. The main components of essential oil obtained from the bark of *Cinnamomum cassia* are cinnamic aldehyde, cinnamic acid, cinnamyl alcohol and coumarin (He *et al*., 2005). Cinnamamide is a synthetic compound derived from cinnamic acid. Cinnamic aldehyde (5%) acts as antifeedant against house rat (*Rattus rattus*) and spray of its formulation containing sodium bicarbonate as emulsifier can prevent rodent damage under simulated storage conditions for upto 15 days (Babbar *et al.*, 2015). In order to evaluate the efficacy of rat repellents, a number of laboratory test methods such as food acceptance test, barrier test and graded strip test are available. Presently, mazes are used to determine whether different treatments or conditions affect learning and memory in rats. Rats are particularly gifted at running mazes. Their maze-running ability comes from their evolutionary history. Rats are small burrowing rodents that have spent millenia digging and find their way around underground tunnels. In present study, an attempt was made to assess the natural behaviour of house rats in maze or in chambers in response to cinnamamide, a natural plant-based repellent using automated Ethovision video tracking system (EVTS) and feed scale consumption monitor (FSCM). The EVTS is used for the analysis of locomotor activity or anxiety while FSCM to study the feeding behaviour of rats in response to cinnamamide.

## MATERIALS AND METHODS

The present study was carried out at Rodent Research Laboratory, Department of Zoology, PAU, Ludhiana (India) located at an intersection of 30º55’ N parallel of latitude and 75º54’E line of longitude. The approval of Institutional Animal Ethics Committee, GADVASU, Ludhiana was obtained (VMC/12/3901-35 dated 6.8.12) for the use of animals in present study.

### *Collection and maintenance of animals*

The house rats of both sexes were trapped with the help of single- and multi-catch rat traps from storehouses and poultry farms in and around Ludhiana (India). The rats were acclimatized individually in cages of 36×23×23 cm size each for 15-20 days in laboratory. Food and water were provided *ad-libitum*. Food consisted of a mixture of cracked wheat, powdered sugar and groundnut oil (WSO) in 96:2:2 ratio. The metallic trays were kept under each cage for the collection and disposal of urine and faeces. After acclimatization, healthy mature rats of both sexes were weighed. The animals were maintained as per the guidelines of Institutional Animal Ethics Committee.

### *Rodent behaviour in maze kept under camera connected to ETVS in response to cinnamamide*

To study the behaviour of house rats {n = 3 of each sex} in response to cinnamamide under natural conditions, a formulation of 0.4% cinnamamide was prepared following the procedure used for the preparation of formulation III of 5% cinnamic aldehyde (Babbar *et al*., 2015). For this, 0.4% cinnamamide (dissolved in methanol) was mixed with 1% sodium bicarbonate (used as emulsifier). I-maze consisting of two arms (length 47 cm; height 8.5 cm) on opposite sides and one central hub was kept under a camera connected to computer (Fig. 1) installed with Ethovision software version 3.0 (Noldus Information Technology, Netherland). This software is an integrated system for automatic detection, recording and analysis of movement and behaviour of an animal. During treatment period, the formulation of 0.4% cinnamamide was sprayed on bags (containing 500 g wheat grains), kept at the extreme end of one arm of I-maze (called treated zone) whereas the bags kept at extreme end of second arm (called untreated zones) were not sprayed. Only one rat was released at a time in I-maze. For the first 3 days, the behaviour of a rat released in I-maze was recorded automatically for 2 h without spraying on bags kept in any of the arm. It was done to reduce the fear developed in rats on exposure to new environment. On $4^{th}$ day, the bag, kept in one of the arms, was treated with 0.4% cinnamamide and behaviour of rat recorded again for 2 h in a day from $4^{th}$ to $6^{th}$ day. The same experiment was repeated for other 5 rats also. Each day, the movement

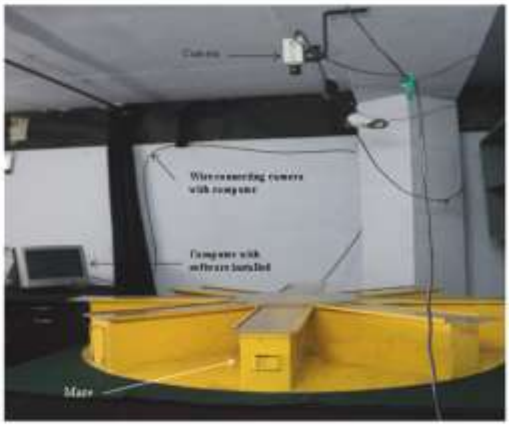


**Fig. 1: Camera operated Ethovision video tracking system with maze**

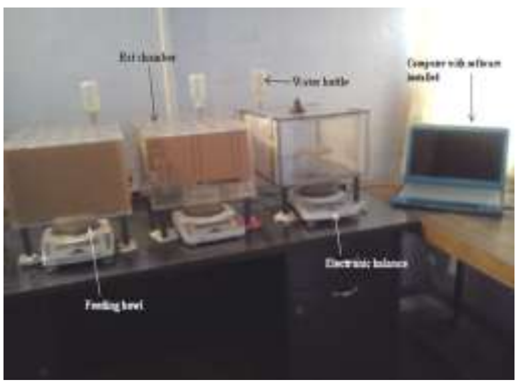


**Fig. 2: Feed scale consumption monitor (FSCM)**

of rat in I-maze was recorded as movement tracks (Fig. 3) and data was recorded in terms of zone frequency (number of visits to zone$h^{-1}$), total distance moved (cm), frequency of rearing (number of times rat's body is vertically erected $h^{-1}$) and frequency of mobility $h^{-1}$ in both treated and untreated zones. Rat can either be mobile or immobile. Mobility of rats can be affected under stressful conditions in response to cinnamamide.

***Feeding behaviour of house rats using feed scale consumption monitor (FSCM)***

Feeding behaviour of rats (n = 3 of each sex) was recorded using FSCM [version 1.3.4; Columbus Instruments Inc.] (Fig. 2) in response to the formulation of 0.4% cinnamamide prepared as above. Hardware of this system consists of 3 rat chambers, each kept on an electronic balance provided with food bowl and connected to a computer having software for recording feeding behaviour of rats in terms of number of feeding bouts, feeding bout length (sec.) and feed consumed (g). For this, overnight hungry rats were released in chambers of FSCM and their feeding behaviour recorded using plain WSO bait for 2 h in a day for 6 consecutive days. It was done to reduce the fear and anxiety in rats on exposure to new environment. This was followed by exposing the rats to WSO bait mixed with 0.4% cinnamamide and the feeding behaviour was again noted in terms of the number of feeding bouts, feeding bout length and feed consumed in response to cinnamamide mixed bait for 2 h in a day for 6 days.

The values were calculated as mean ± SE. The significance of differences between various treatments was determined at $P \leq 0.05$ level. Significance of differences among various parameters was determined by using factorial completely randomized design. Various factors taken for comparison in different experiments were sexes (male and female), treatments (control and treated) and days of treatment. Analysis was done using general linear model (GLM) in SAS version 9.3. All pair-wise treatment comparisons were made using Tukey's HSD test (David, 2010).

## RESULTS AND DISCUSSION

***Behavior of house rat in response to 0.4% cinnamamide under bi-choice conditions in I-maze***

Rat behaviour in response to 0.4% cinnamamide under bi-choice condition in I-maze kept under EVTS revealed insignificant difference between sexes, hence the data of both sex was pooled. There was also insignificant difference between treated and untreated zones with respect to in-zone frequency, total distance moved and frequencies of rearing and mobility; but the values were low in treated zone indicating reduced activity in treated zone (Table 1). Rats showed movement in both treated and untreated zones from the very beginning of their exposure to cinnamamide indicating absence of primary repellent effect or olfactory repellency in response to cinnamamide (Fig. 3). Babbar *et al.* (2015) reported the absence of primary repellent effect of 5% cinnamic aldehyde against rats. The rats showed movement in both the zones but the total distance moved was less (30.3%) in treated zone during treatment period (Table 1). The in-zone frequency in both zones during pre- and post-treatment periods was insignificantly more than observed during treatment period. The total distance moved and frequencies of both rearing and mobility were insignificantly less during pre-treatment period than during treatment period indicating disruptive behaviour of rats exposed to 0.4% cinnamamide even in untreated zone of I-maze. Frequencies of rearing and mobility were more (49.0 and 63.5%) during pre-treatment period in treated zone but after cinnamamide treatment both were reduced by 31.6 and 29.5%, respectively, in treated zone. In treated zone, the total distance moved by rats and the frequencies of both rearing and mobility decreased by 9.7, 48.8 and 9.8%, respectively, from day 1 to day 3 of treatment period but there was insignificant difference among the days of treatment showing increase in repellency toward cinnamamide with time. There was absence of primary repellent effect to 0.4% cinnamamde but repellent effect increased with time, possibly due to secondary repellent effect of cinnamamide against rats (Table 1). Repellency is mediated either through primary defense mechanism (sensory irritation by contact or olfactory avoidance) [Mason *et al.*, 1991] or through secondary defense mechanism causing development of conditioned taste aversion (CTA) in response to post-ingestional malaise (Johnson *et al.*, 1982). Repellents inducing CTA are more effective as they promote long term avoidance responses in animals. After feeding bait from treated bag, rats might have developed gastrointestinal malaise resulting in reduction of their activity (mobility and rearing) with time in treated zone. Kaland-akanond-Thongsong *et al*. (2010) reported decrease in the number of visits of rats in response to repellents to treated core as compared to untreated core of circular open field. In present study, the use of automated ETVS allowed undisturbed recording of behavioural activities of house rat.

***Feeding behaviour of house rat to 0.4% cinnamamide in FSCM in no-choice feeding test***

There was an insignificant difference in the number of feeding bouts, feeding bout length and feed consumed between both the sexes but a significant ($p \leq 0.05$) reduction in the values of all the three

**Table 1: Repellent behaviour of rats to 0.4% cinnamamide under bi-choice condition in I-maze**

| Parameters | Treatment/days | | Untreated zone (n = 6) | Treated zone (n = 6) |
|---|---|---|---|---|
| In zone frequency (number of visits to zone $h^{-1}$) | Pre-treatment period | | 171.2± 102.07 | 79.8± 31.39 |
| | Treatment period | Day 1 | 53.0± 26.86 | 44.0± 28.24 |
| | | Day 2 | 20.1± 6.66 | 35.8± 20.10 |
| | | Day 3 | 13.2± 6.78 | 41.6± 0.86 |
| | Post-treatment period | | 151.0± 96.72 | 44.9± 29.16 |
| Total distance moved (cm) | Pre-treatment period | | 61174± 2300.92 | 6162.9± 2016.62 |
| | Treatment period | Day 1 | 21070.6± 11812.94 | 16216.7± 7795.22 |
| | | Day 2 | 26533.5± 5586.58 | 3141.1± 1112.27 |
| | | Day 3 | 21526.6± 7555.37 | 1571.1± 1085.44 |
| | Post-treatment period | | 93769.1± 2523.34 | 4331.9± 1796.90 |
| Frequency of rearing (events $h^{-1}$) | Pre-treatment period | | 369.5± 157.72 | 754.1± 79.29 |
| | Treatment period | Day 1 | 1009.5± 123.86 | 549.0± 203.17 |
| | | Day 2 | 1411.5± 513.94 | 367.0±158.86 |
| | | Day 3 | 1326.0± 664.91 | 268.0± 46.90 |
| | Post-treatment period | | 532.3± 212.84 | 334.9± 125.78 |
| Frequency of mobility ($h^{-1}$) | Pre-treatment period | | 599.4± 254.46 | 944.1± 256.20 |
| | Treatment period | Day 1 | 1511.8± 752.92 | 1316.3± 599.73 |
| | | Day 2 | 2234.3± 539.32 | 279.2± 102.28 |
| | | Day 3 | 2098.5± 661.81 | 128.5± 86.66 |
| | Post- treatment period | | 707.2± 263.32 | 461.4± 223.85 |

Values are mean ± SE; No superscript on values indicate insignificant difference between treated and untreated zone as well as among days

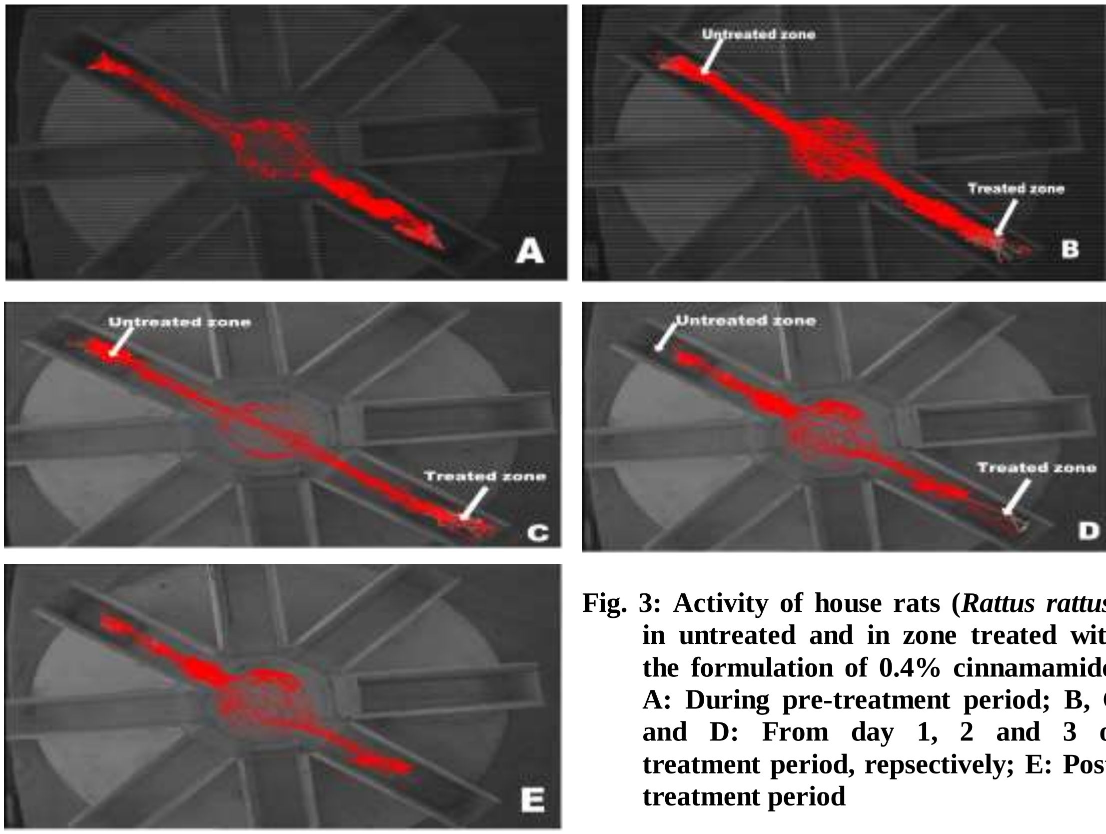


**Fig. 3: Activity of house rats (*Rattus rattus*) in untreated and in zone treated with the formulation of 0.4% cinnamamide. A: During pre-treatment period; B, C and D: From day 1, 2 and 3 of treatment period, repsectively; E: Post-treatment period**

parameters during treatment period as compared to pre-treatment period. The number of feeding bouts and feeding bout length reduced significantly by 47.4 and 26.0%, respectively, during treatment period as compared to pre-treatment period on day 1 of treatment but the values of these parameters decreased to nil after day 4 of treatment period in case of male rats and by 36.9, 43.8 and 9.6% after day 2 of treatment period in female rats showing severity of gastrointestinal malaise developed in rats exposed to 0.4% cinnamamide treated bait (Table 2). The study revealed that consumption of treated bait declined completely after day 4 in male rats and remained depressed after day 2 of treatment in female rats. The feeding response of rats to 0.4% cinnamamide treated bait is again indicative of the development of conditioned taste aversion. The rats not only reduced

**Table 2: Antifeedant behaviour of rats (*Rattusrattus*) to 0.4% cinnamamide in FSCM**

| Gender | Days | Pre-treatment period* | | | Treatment period** | | |
|---|---|---|---|---|---|---|---|
| | | Feeding bout events | Feeding bout length (sec.) | Feed consumed (g) | Feeding bout events | Feeding bout length (sec.) | Feed consumed (g) |
| Male* | Day 1 | 6.3±2.12 | 327.0±134.77 | 0.72±0.30 | 3.00± 1.41 | 85.0±49.03 | 0.74± 0.37 |
| | Day 2 | 17.7±11.57 | 543.0±354.61 | 1.07±0.69 | 1.00± 0.82 | 5.0±4.08 | 0.01± 0.00 |
| | Day 3 | 10.3±7.22 | 580.0±371.34 | 1.33±0.62 | 1.00±0.47 | 5.0±2.05 | 0.02±0.01 |
| | Day 4 | 8.3±3.21 | 405.3±108.96 | 1.67±0.54 | 0.66±0.27 | 4.3±1.78 | 0.05±0.02 |
| | Day 5 | 8.7±2.33 | 455.3±160.75 | 1.70±0.42 | 0 | 0 | 0 |
| | Day 6 | 21.3±4.45 | 537.7±169.51 | 2.07±0.38 | 0 | 0 | 0 |
| Female* | Day 1 | 4.0±0.47 | 456.7±50.40 | 0.64±0.11 | 3.00±2.44 | 89.7±73.21 | 0.66±0.53 |
| | Day 2 | 8.0±2.49 | 483.0±65.64 | 3.33±0.82 | 1.33±0.72 | 23.0±13.14 | 0.17±0.07 |
| | Day 3 | 10.7±1.78 | 557.7±189.44 | 2.45±0.60 | 0 | 0 | 0 |
| | Day 4 | 11.7±2.68 | 665.7±95.15 | 2.28±0.85 | 2.66±2.17 | 104.0±84.92 | 0.04±0.02 |
| | Day 5 | 11.7±1.65 | 522.0±186.23 | 2.86±1.30 | 0.33±0.27 | 8.0±6.53 | 0.13±0.10 |
| | Day 6 | 8.7±3.34 | 430.7±229.35 | 3.91±2.27 | 0.33±0.27 | 4.7±3.81 | 0.02±0.01 |

*, **In overall, significant difference between pre-treatment and treatment period at 5% level of significance.

the consumption of treated bait but also showed decline in the mean number of feeding bouts and mean length of feeding bout during treatment period. The rats also avoided to touch the treated bait even if they were not provided with alternative bait during treatment period and kept hungry before their exposure to treated bait. This behavior may be a displacement activity as a result of irritation or inflammation induced by treated bait (Krebs and Davies, 1987). In conclusion, the study showed that 0.4% cinnamamide is not a primary repellent but an effective secondary repellent against both sexes of rats. The rats were provided natural conditions and kept undisturbed during recording of their behaviour using automated EVTS and FSCM. The secondary repellent effect of 0.4% cinnamamide could be utilized under field conditions.

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