Research Paper

Behavior Responses and Histological Changes in Sense Organ of House Rats Exposed to Methyl Anthranilate: A Known Repellent

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Rodents are the serious pests in both agricultural and commensal situations and are managed with rodenticides. However, the effectiveness of rodenticides in reducing the rodent population is questioned. Many plant-based repellents were developed for reducing rodent damage. Methyl anthranilate is a known bird repellent. Repellent behavior of house rat (*Rattus rattus*) was studied using 2.5% methyl anthranilate (MA) in Feed Scale Consumption Monitor (FSCM) and I-maze under Ethovision Video Tracking System. Behavior studies revealed the existence of the olfactory repellent effect of MA against house rat (*R. rattus*). Histomorphological studies of rats exposed to methyl anthranilate (2.5%) revealed sloughing of filiform and fungiform papillae, degeneration of keratin layer, mild hyperkeratosis and dysplasia, exophytic hyperplasia of the epithelial layer of the tongue. Change in shape of maxilloturbinates and nasoturbinates, loss of cilia, degeneration of goblet cells and decrease in thickness of the epithelial layer of the nasal septum was also recorded. Behavior and histomorphological studies therefore confirmed the existence of both olfactory and gustatory repellent effects of MA against house rat. MA can be used in the future as a repellent in rodent management programs.

Keywords: Methyl anthranilate; Repellent; House Rats, Tongue; Nasal Tissue

Introduction

Rodents are serious pests in both agricultural and commensal situations. They cause negative impacts on the well-being of people as competitors for food such as cereals and other staple food (Singleton *et al.*, 2010) or also act as carriers of various human diseases (Meerburg *et al.*, 2009). There is a need to control these pests effectively to overcome the problems caused by rodents (Chopra *et al.*, 2008). Different approaches can be used to control rodents like mechanical, biological and chemical methods. However, these methods are either too costly to use or are not ecofriendly. These problems can be tackled by the use of ecologically-based rodent management technology (Singleton *et al.*, 1999) that uses a toolbox of techniques that are ecologically, socially, and economically appropriate.

One of the promising tools to manage rodent problems is the application of repellents. These are tools developed by humans to keep the pests away from crop fields and food stores. Repellents can be visual, gustatory, olfactory, acoustic, chemicals, or mixture of these characteristics (Mason *et al.*, 1989). These repellents can cause sensory irritation (primary repellency) or/and gastrointestinal malaise (secondary repellency) (Norman *et al.*, 1992; El Hani and Conover, 1997) and can be highly effective against rodents with little impact on non-target organisms (Packiam *et al.*, 2012; Babbar *et al.*, 2015).

Many eco-friendly, easily available, economically viable plant-based repellents are found to be effective under laboratory conditions against rodents (Singla and Kaur, 2014; Singla *et al.*, 2014; Babbar *et al.*, 2015; Bala and Babbar, 2019). These include cinnamic

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aldehyde, eucalyptus oil, citronella oil, methylanthranilate etc., which were able to repel rats under the laboratory and simulated storage conditions.

Methyl anthranilate (MA) is a monocyclic aromatic amine. It is a natural compound mainly obtained from Concord grapes (*Vitisla brusca* L.) (Sun *et al.*, 2011), is GRAS-listed (Generally Recognized As Safe), and commonly used as a flavoring agent in different food items. MA acts as an active component in commercial products used to prevent loss of crops by birds (Mason and Clark, 1996). There are various commercially available methyl anthranilate based formulations (ReJeX-iT AG-145, ReJeX-iT AG-36, ReJeX-iT MA, ReJeX-iT TP-40, ReJeX-iT AP-50, Bird Shield Bird Repellent Concentrate) used to repel birds from crop fields (Vogt, 1992; Curtis *et al.*, 1994; Belant *et al.*, 1995; Cummings *et al.*, 1995; Lewis, 1996).

A previous preliminary study conducted in our laboratory based on bait consumption data revealed the primary repellent effect of MA (2.5%). Results also revealed the efficacy of MA (2.5%) based microencapsulated formulation in preventing rodent damage under simulated storage conditions (Bala and Babbar, 2019). However, behavioral responses and site of action of MA in exposed rats are not known and need to be studied further to increase its repellent efficacy in synergism with other compounds.

Materials and Methods

Animals

Animals were maintained and used by following the guidelines of the Institutional Animal Ethics Committee Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The present study was conducted at Animal House Laboratory and Rodent Research Laboratory, Department of Zoology, Punjab Agricultural University (PAU), Ludhiana (30°55' N parallel of latitude and 75°54' E line of longitude). For the present study, the house rat, *R. rattus* of both sexes, were trapped with the help of single catch and multi-catch rat traps from storehouses, poultry farms and grocery shops in and around Ludhiana. In the laboratory, rats were acclimatized individually for 15-20 days in the cages of size 36 x 23 x 23 cm. Before the commencement of the experiment, rats were given *ad libitum* access to food and water. Food provided

was prepared by mixing cracked wheat, powdered sugar and groundnut oil (WSO bait) in the ratio of 96: 2: 2. The metallic/plastic trays were kept below each cage for collection and disposal of urine, faeces and spilled grains. For experimentation, acclimatized healthy rats were weighed and grouped.

Repellents Used

Methyl anthranilate (MA) was purchased from Loba Chemical Private Limited, Mumbai, India.

Experimental Design for Behavior Study Using Ethovision Video Tracking System

Changes in the behavior of male and female house rats (n=3 for each sex) in response to MA (2.5%) were recorded using Ethovision video tracking system (EVTS). An I-Maze consisting of two arms (each with length 47.0 cm and height 18.5 cm) on opposite sides and one central hub was used for this purpose. The extreme end of one arm referred to as zone 1 was treated with MA (2.5%) while the central hub (zone 2) and second arm (zone 3) were not treated. The maze was kept under the camera to record the movement of rats, and this camera was connected to the computer installed with the software (Ethovision Video Tracking System) (Kaur and Babbar, 2017). During the treatment period, MA (2.5%)-based formulation (F1) prepared by mixing MA and tween 80 in the equal ratio was sprayed on the bag (containing wheat grains) placed at the extreme end of the zone 1. Walls of the extreme end of zone 1 were also sprayed with MA (2.5%)-based formulation. However, zone 2 and bag kept at the extreme end of zone 3 as well as its side walls were not treated with the formulation. For three days, the behavior of the rat was recorded in I-Maze for two hours in a day without the MA application. This was done in order to reduce the fear, *i.e.*, neophobia developed in rats on exposure to a new environment followed by the spray on the bag kept in zone 1 on the fourth day. Again, the behavior of the rat was recorded for two hours a day from the first day of treatment until the rats started inflicting damage on the treated bag. Each day, the movement of the rat was recorded in the form of visual movement tracks in the I-Maze, and the observations *viz.*, zone frequency, frequency of rearing, total distance moved, and mobility in both untreated and treated zones were recorded.

Experimental Design for Behavior Study Using Feed Scale Consumption Monitor

Alteration in feeding behavior of rats (n=4 of each sex) in response to MA (2.5%) mixed bait in comparison to plain bait was studied using Feed Scale Consumption Monitor (FSCM). This system comprised of four rat chambers, and each chamber was kept on an electronic balance provided with a food bowl. The whole apparatus was connected to a computer having the software installed for recording the feeding behavior of these rats (Kaur and Babbar, 2017). For this purpose, rats were released in chambers of FSCM, and their feeding behavior was recorded for six hours in a day for five days using plain WSO bait in order to reduce the anxiety and fear developed in rats on exposure to a new environment. It was then followed by exposure of all the four rats of each sex to WSO bait mixed with MA (2.5%). Again the feeding behavior of rats in response to MA (2.5%) mixed bait was recorded for six hours in a day for 30 consecutive days. Treated bait was prepared by mixing the known volume of MA in a known volume of methanol, which was then mixed in a known quantity of WSO plain bait to prepare MA (2.5%) bait. Each day quantitative data on feeding bout events/h, feeding bout time (s)/h, and feed consumed (g)/h was recorded to determine the changes in feed behavior of rats exposed to MA (2.5%) in comparison to plain bait.

Tissue Processing and Photography

Paraffin blocks of nasal tissue and tongue of rats exposed to MA (2.5%) mixed bait for 30 days were processed for histomorphological studies. The nasal tissue of rat was divided into four different segments, and the only second segment was taken for further

histomorphological processing as this segment permitted view of all epithelial cell types.

Sections of nasal tissue and tongue were obtained at 5µm thickness with a rotary microtome, stained with haematoxylin and eosin, dehydrated in ascending ethanol series, and then cleared in xylene and mounted in DPX (Humason, 1966). The tissue sections were evaluated for histological changes under a compound light microscope. Photomicrographs were captured using a digital camera. For the evaluation of the slides, different layers and cells of the tongue and nasal tissue were identified (Hebel and Stromberg, 1986; Harkeema, 1990; Thind *et al.*, 2017) under a microscope connected to a computer installed with Magvision software.

Statistical Analyses

Values were calculated as mean $\pm$ SE. The significance of difference was determined at a 5% level of significance. Significance of difference among different parameters recorded using EVTS and FSCM was determined by applying ANOVA using statistical software SPSS 16.0.

RESULTS

Study of Repellent Effect Using Ethovision Video Tracking System

Study of the behavior of both sexes of *R. rattus* in untreated I-maze using EVTS revealed that both male and female rats were very active during the pre-treatment period. Their mobility in terms of in zone frequency, total distance moved, frequency of rearing, and frequency of mobility were very high (Table 1; Fig. 1A). However, during the treatment period, the decline in the mobility of all rats in both treated and

Table 1: Effect of 2.5% methyl anthranilate (2.5%) on the behavior of house rat

Gender	Zone	Treatment period	In zone frequency	Total distance moved (cm)	Frequency of rearing	Frequency of mobility
Female	Zone 1-Treated arm	PT	17.00 $\pm$ 2.04 ^a	874.66 $\pm$ 100.93 ^a	109.00 $\pm$ 6.00 ^a	102.32 $\pm$ 19.27 ^b
		T	2.67 $\pm$ 0.70 ^{ab}	206.49 $\pm$ 83.18 ^a	21.21 $\pm$ 10.47 ^a	47.46 $\pm$ 15.33 ^a
	Zone 2 -Untreated central hub	PT	18.00 $\pm$ 1.57 ^a	2014.01 $\pm$ 727.41 ^a	183.02 $\pm$ 53.13	616.18 $\pm$ 148.61 ^a
		T	1.17 $\pm$ 0.17 ^b	907.35 $\pm$ 141.91 ^a	74.08 $\pm$ 6.62 ^a	163.62 $\pm$ 28.84 ^a
	Zone 3- Untreated arm	PT	14.31 $\pm$ 0.49 ^a	1432.24 $\pm$ 110.39 ^a	99.04 $\pm$ 17.38 ^a	597.80 $\pm$ 133.74 ^a
		T	2.84 $\pm$ 0.66 ^{ab}	101.97 $\pm$ 26.35 ^a	38.82 $\pm$ 33.48 ^a	82.87 $\pm$ 73.61 ^a

Values are mean $\pm$ SE; PT- Pre-treatment period; T- Treatment period

Fig. 1: Activity of rat (*R. rattus*) in untreated, central and zone treated with MA (2.5%)-based formulation (A) during the pre-treatment period; (B-D) during the treatment period

untreated zones (Zones 1) was recorded (Table 1; Figs. 1B-D). Similar behavior responses of all the rats in treated I-maze revealed that these effects could be repeatable under field conditions. Although F1 was sprayed on the extreme end of the treated arm, the smell of F1 dispersed both in the untreated central hub as well as in untreated arm resulting in a reduction in movement of rats even in untreated zones.

For the first three to four days after treatment, rats only moved in untreated sides (zones 2 and 3), and their mobility in untreated zones also reduced to a great extent (Fig. 1B). They entered treated zone 1 on the fourth to fifth day after treatment but not up to the extreme end of the treated arm. Their movements in terms of in zone frequency, total distance moved, frequency of rearing, and frequency of mobility increased gradually from 4-6 days after treatment (Fig. 1C). From 6-12 days, their movement in the treated

zone increased but remained less than in untreated zones. After that, they started moving towards treated bag kept at the extreme end of treated zone 1 and started inflicting cuts on treated bags from twelfth to the fourteenth day of the treatment period (Fig. 1D). A comparison of different parameters of mobility in different zones, however, revealed the non-significant difference between pre-treatment and treatment periods (Table 1).

Study of Feeding Behavior of House Rats Using Feed Scale Consumption Monitor

There was a non-significant difference in the number of feeding bouts/hour, feed consumed/hour, and antifeedant index among treatment days. However, a significant difference was recorded among days for feeding bout length (s)/hour. There was, however, an overall decrease in the number of feeding bouts, feeding bout length and feed consumed (g/hour) during the treatment period as compared to during the pre-treatment period, although the difference was non-significant for these parameters between pre-treatment and treatment period. Overall antifeedant index for MA (2.5%) treated bait was 55.80 ± 12.79 and percent acceptance of treated bait over plain bait was only 26.82 percent indicating less preference of rats towards treated bait compared to untreated bait even under no-choice conditions (Table 2). These results indicated that although rats avoided treated bait during the exposure period of 30 days under no-choice conditions as compared to plain bait during the pre-treatment period. However, the repellent effect towards treated bait was more for the first two days (might be due to olfactory repellent effect), but after that, the consumption of treated bait increased. It might be due to the absence of alternative lure bait under no-choice condition, which forced the rat to feed on treated bait.

Effect of MA (2.5%) on the Tongue of House Rat

Histomorphological study of the tongue of rats fed on 2.5MA (2.5%) treated bait revealed the disorganization of the epithelial layer of tongue and degeneration of both filiform and fungiform papillae in comparison to untreated rats indicating sloughing of papillae of treated rats (Figs. 2A, B). The presence of the keratin layer and stratified squamous epithelium helps in the prevention of damage caused by the

Table 2: Effect of methyl anthranilate (2.5%) treated bait on the feeding behavior of house rat, *Rattus rattus*

Days	Pre-treatment period			Treatment period			Antifeedant Index (%)	Percent acceptance of treated bait over plain bait
	No. of feeding bouts/h	Feeding bout length (s)/h	Feed consumed (g)/hour	No. of feeding bouts/h	Feeding bout length (s)/ h	Feed consumed (g)/hour		
1-5	6.89±2.08 ^a	112.60±22.69 ^{ab}	0.80±0.13 ^a	4.07±0.96 ^a	48.02±9.69 ^{ab}	0.22±0.03 ^a	64.82±12.11	33.72%
6-10				2.66±0.61 ^a	53.36±14.00 ^{ab}	0.25±0.06 ^a	51.49±29.62	47.62%
11-15				2.86±0.42 ^a	39.19±8.64 ^b	0.33±0.06 ^a	36.62±4.10	13.12%
16-20				1.29±0.45 ^a	20.64±5.04 ^b	0.14±0.02 ^a	67.43±13.40	32.20%
21-25				3.45±0.76 ^a	69.96±15.19 ^{ab}	1.25±0.38 ^b	44.04±2.26	5.95%
26-30				3.15±0.31 ^a	71.80±10.62 ^{ab}	0.20±0.02 ^a	70.44±10.19	28.33%
		Mean±SE		2.91±0.12 ^a	50.49±2.97 ^{ab}	0.39±0.04 ^a	55.80±12.79	26.82%

Values are Mean ± SE; Different superscripts show a significant difference in different parameters during the pre-treatment and treatment period.

Similar or no superscript show non-significant difference along with the columns

ingestion of harmful food. However, in treated rats, there was sloughing and separation of the keratin layer present above the stratified squamous epithelium of rat tongue (Figs. 2C, D). On the anterior side of the tongue also, the keratin layer got separated from the underlying epithelial layer in treated rats. Histoarchitecture of the epithelial layer of the tongue of the untreated rat was normal (Fig. 3A), but mild hyperkeratosis and dysplasia occurred in the epithelial layer of the treated rat (Fig. 3B). Histological studies also revealed that there was the formation of severe exophytic hyperplasia in the epithelium of the tongue tip of the treated rat (Fig. 3C).

Effect of MA (2.5%) on Nasal Tissue of House Rat

Histoarchitecture studies of untreated rats revealed that the nasal cavity was surrounded by surface epithelium. Nasoturbinate and maxilloturbinate were present in the nasal cavity of the rat. The nasoturbinate extended from the dorsal wall and projects ventrally to half the depth of the nasal cavity. This turbinate turned laterally and dorsally, producing a hook in the cross-section of turbinate. The maxilloturbinate was attached to the lateral wall and projected dorsally into the nasal cavity (Fig. 4A). Distal nasal septum incompletely separated the two nasal passages, also known as “septal window,” present just rostral to the nasopharynx. There was un-coiling of nasoturbinate, change in the shape of maxilloturbinate (Fig. 4B), loss of cilia, degeneration of goblet cells resulting in

Fig. 2: Section of the tongue of *Rattus rattus* (A& B) Comparison of the epithelium (E), Fungiform Papillae (Fu P), Filiform Papillae (Fi P) and TB (Taste Buds) of the tongue of untreated and treated rat; (C& D) Separation and thinning of Keratinized layer(KL) on epithelial layer of the tongue of treated rat in comparison to untreated rat. Epithelium (EP), Lamina propria (LP) and Muscle fibers (M) (400X)

vacuolization of the epithelial layer of the nasal septum and degeneration of lamina propria in nasal tissue of treated rats (Figs. 4C, D). Respiratory epithelial layer and lamina propria cover the nasal septum on both sides in the untreated rat (Fig. 5A). However, there was intermixing of both respiratory epithelial layer and lamina propria and sloughing of lamina propria and the degeneration of blood vessels present in lamina propria just beneath the respiratory epithelium of treated rat (Fig. 5B).

DISCUSSION

A previous preliminary study conducted in our laboratory revealed the efficacy of MA (2.5%)-based formulation as a repellent in preventing rodent damage in grain stores (Bala and Babbar, 2019). The present study was conducted to determine the mode of action of MA (2.5%) as a repellent and to study alteration in the behavior of rats in response to MA (2.5%) treated baits under laboratory conditions to further increase the repellent efficacy of formulation by mixing other repellents with a different mode of action. It is the first study of its kind comparing behavior responses with histomorphological changes in sensory organs. If the repellents were able to repel the rats, then the number of visits and the time the rats spent in the treated zone would decrease, and the time the rats spent away from the treated zone would be increased. Reduction in the mobility of rats in I- maze observed during the present investigation after treatment indicated its repellent effect. However, reduction in the smell of F1 in I-maze with time resulted in increased mobility of rats in treated I-maze, revealed that repellent effect of MA (2.5%) might be due to its effect on nasal sensory responses. Quick dissipation of repellent effect or the spread of odor of MA in both the arms of the maze as well as the reduction in the smell with time might be due to the volatile nature of MA. Previous studies indicated the repellent property of MA against birds (Mason *et al.*, 1996). However, this is the first study indicating the repellent efficacy of MA against rodents. The previous study conducted using eucalyptus oil as a repellent against *R. rattus* also reported low persistence of repellent effect due to the volatile nature of oil (Singla *et al.*, 2014). Essential oils generally act as a repellent in the vapor phase (Gnankin'e and Bassol'e, 2017; Islam *et al.*, 2017) and are effective only for a short period. The drawback of the short protection time

could be improved through formulation technology so that active components could be retained on the surface for longer periods or by mixing several repellents with a different mode of action to synergies their repellent action. Previous studies reported that combinations of plant secondary metabolites (PSM) could increase repellent efficacy, but higher concentrations did not. Neither pure compounds nor mammalian scents were as effective as repellents as essential oils or the combination of several PSM with different characteristics. Testing of 17 different plant products singly or in combination with each other in T-maze revealed that the combination of essential oils was more effective than a single compound. A combination of black pepper oil and carrot seed oil (BP+CS) proved as an ecologically best deterrent than a single compound (Schlötterburg *et al.*, 2018).

The success of a repellent is also dependent on population size, alternative food availability and weather conditions (Mason, 1997). Therefore it is essential to study the behavioral responses of pests and properties of repellent used for the management of a pest before field application. Under field conditions, a repellent based on olfaction could be more appropriate. Odorous compounds could change movement patterns to regulate the intake of PSM treated food (Iason and Villalba, 2006) and to reduce the activity of animals (Fischer *et al.*, 2013). Behavior studies using EVTS also revealed a reduction in activity patterns in treated I-maze indicating its olfactory repellent effect. Similar experiment to study repellent efficacy of chili, wintergreen oil, bergamot oil, peppermint oil and geranium oil was conducted in the circular open field against adult male Wistar rats. Rats spent less time in the repellent treated zone compared to the control indicating that the rats avoided closed contact with treated substances (Kalandakanond-Thongsong *et al.*, 2010). The repellent effect of cat urine and faeces was also studied in Y-maze. Rats showed movement in both treated and untreated zones of the maze but distance moved and rat activities were less in the treated zone as compared to untreated zone (Bramley and Waas, 2001; Babbar *et al.*, 2015; Sachdeva and Singla, 2018).

The study of feeding behavior using FSCM revealed that rats avoided MA (2.5%) treated bait even under no-choice conditions and had not developed

Fig. 3: Section of the tongue of treated and untreated rats (A) Epithelial layer (EP), Lamina propria (LP) and Filiform Papillae (Fi P) of an untreated rat; (B) Mild dysplasia and hyperkeratosis of the epithelial layer of the tongue of a treated rat; (C) exophytic hyperplasia in the epithelial layer of tongue tip of treated rat (400X)

habituation against treated bait throughout the treatment period of 30 days. The number of feeding bouts, bout length and food consumption remained less throughout the treatment period as compared to during the pre-treatment period, indicating that not only the consumption of treated bait was reduced, but rats also handled the treated bait less frequently. However, previous studies reported that repellents were more effective in the presence of alternate plain bait or under bi-choice (Bala and Babbar, 2019). Several plant products as antifeedant agents had been tested against rodents in bi-choice tests (Schnutterer and Aschr, 1987; Jermy, 1990; Trigg, 1996; Singla and Parshad, 2007; Babbar *et al.*, 2015). A study on different plant secondary metabolites (PSMs) like methyl nonyl ketone with the addition of methyl anthranilate (MNK+MA) against voles and methyl

Fig. 4: Section of the nasal cavity of *Rattus rattus*. (A & B) Comparison of Nasoturbinates (NT), Maxilloturbinates (MT) and Septum (S) of nasal tissue of untreated and treated rat; (C & D) More vacuolization in the epithelium of nasal septum (ES) of treated rat compared to untreated rat (E). Goblet Cells (GC), Basal Cells (BC), Basal Lamina (BL) (400X)

nonyl ketone (MNK) against mice revealed that the consumption of treated bait increased after four days of the treatment. It might be due to the degradation of repellents with time due to various environmental factors (Baumler *et al.*, 1990; Hansen *et al.*, 2016). Reduction in repellent efficacy of MA (2.5%) was also recorded after two days of treatment during the present investigation. It might be to the reason that rats were provided treated bait under no-choice conditions. However, the consumption of treated bait remained low throughout the experimental period of 30 days as compared to plain bait. Previous studies conducted in our laboratory also revealed that rats do not develop habituation against cinnamic aldehyde (5%) treated bait (Kaur and Babbar, 2018). Herbivores change their behaviors in response to toxic

Fig. 5: Respiratory epithelial (RE) layer and lamina propria (LP) covering nasal septum (A) Untreated (B) treated rat. Septum (S), Blood vessel (BV) (100X)

plant secondary metabolites (Dearing *et al.*, 2005). They can connect information from the gastrointestinal tract like pain and nausea with a specific taste or smell through neurophysiological feedback (Provenza *et al.*, 1992; Welzl *et al.*, 2001). Therefore, they learn from negative post ingestive effects and reduce food uptake and develop a conditioned food aversion (Bryant *et al.*, 1991; Torres-Contreras and Bozinovic, 1997; Torregrossa and Dearing, 2009). Previous studies conducted in our laboratory revealed gastrointestinal distress in rats exposed to MA (2.5%) treated bait under no-choice conditions (Bala, 2018). However, no report is yet available regarding its effect on sensory organs of taste and smell.

Development of abnormalities in histoarchitecture of the tongue of rats exposed to MA (2.5%) treated bait might be the reason for the reduction in consumption of MA (2.5%) treated bait as recorded in feeding behavior study using FSCM. These results confirmed the gustatory repellent effect of treated bait. Earlier studies also reported disorganization and sloughing of filiform papillae and thinning of fungiform papillae in response to the ingestion of chili powder (2%) (Thind *et al.*, 2017). Mild hyperkeratosis and dysplasia in the epithelial layer were also reported in rats treated with broccoli ethanolic extract (Mohammadi *et al.*, 2013). In the present study, rats started avoiding the treated bait after experiencing post-ingestion deleterious changes

on the tongue as observed during the present investigation and due to gastrointestinal distress (Bala, 2018).

Disorganization of olfactory and respiratory epithelium in rats exposed to MA (2.5%) for 30 days revealed that rats might also have avoided treated bait or bags treated with MA (2.5%) due to its deleterious effect on nasal tissue confirming the olfactory repellent effect of methyl anthranilate. A study on the exposure of esbiothrin (a mosquito repellent) in rats shows there was a loss of cilia and epithelium of nasal tissue of rat (Ünal *et al.*, 2009). Previous studies revealed that respiratory epithelium was the most susceptible area to injury caused by inhaled irritants (Harkema, 1990). There was disorganization of respiratory epithelium and loss of goblet cells or cilia due to the inhalation of toxic substances (Renne *et al.*, 2007; Renne *et al.*, 2009).

Compounds with a characteristic repellent odor may be useful for prevention against rodent damage because these odors disseminate through the environment. Such sensory repellents are highly volatile and affect the mucous membranes of the eyes, nose, mouth, and throat of animals before feeding resulting in avoidance of treated area (Mason *et al.*, 1996). Taste-based plant-derived repellents generally include a bitter or hot-tasting ingredient that affects the same membranes, but they act after ingesting. However, the present study indicated that MA has both olfactory and gustatory repellent effect. Previous studies also revealed that an odor alone could not substantially change the spatial activity of common voles, possibly because movements are essential for finding food or mates (Iason and Villalba, 2006). A combination of an odor with a stimulus detected by another sensory system may increase repellency (Mason and Reidinger, 1983; Beauchamp, 1995; Clapperton *et al.*, 2012). Several modes of action determine the effectiveness of PSMs in repelling herbivores. Pre-ingestive effects cause a deterrent impact on animals before ingestion of food. These effects are mainly due to the odor of volatile compounds. Deterrent effects caused by taste or gastrointestinal distress regarded as post-ingestive effects (Wiggins *et al.*, 2003; Marsh *et al.*, 2006).

CONCLUSIONS

Study of behavior using FSCM and EVTS and histomorphology study of nasal tissue and tongue of rats exposed to MA (2.5%) revealed that rats avoided treated bait during the exposure period of 30 days under no-choice conditions as compared to plain bait during pre-treatment period. However, the repellent effect towards treated bait was more for the first two days. Changes in the behavior of male and female house rats using the Ethovision video tracking system revealed that there was a reduction in the mobility of rats during the treatment period as compared to the untreated period. Rats had not entered into the treated zone for the first few days after treatment. The histomorphological study indicated the deleterious effect on both tongue and nasal tissue. All these results confirmed both the olfactory and gustatory repellent effect of methyl anthranilate. However, more studies need to be done to increase its efficacy in the real environment in combination with other repellents.

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Author Contributions

All authors were involved in data collection. Ravneet Kaur prepared the data for statistical analysis and wrote the manuscript, B K Babbar designed the study and wrote the manuscript, Bindu Bala and Devendra Pathak contributed in writing discussion.

Conflict of Interest Disclosure

The authors declare no conflict of interest.

Data Availability

Ethical Approval: Animals were used and maintained by following the guidelines of the Institutional Animal Ethics Committee. Approval of Institutional Animal Ethics Committee Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, was attained for the usage of animals.

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