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Development and testing of methyl anthranilate based formulations against rodents

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Abstract
Rodents are responsible for causing damage under agricultural and commensal situations and these can be managed using various types of rodent management strategies but each has one or more disadvantage(s). Methyl anthranilate is a GRAS listed by the U.S. Food and Drug Administration. No methyl anthranilate based formulation has been reported for rodent pest management. Our objective was to evaluate the repellent effect of an effective dose of methyl anthranilate against house rat in grain stores. Our laboratory studies showed that 2.5% methyl anthranilate treated bait is effective antifeedant repellent as well as primary repellent under bi-choice conditions so it can be effectively used under field conditions. Seven different formulations of methyl anthranilate were tested out of which six were emulsified liquid and one was microencapsulated form of methyl anthranilate. Results revealed the microencapsulated formulation of methyl anthranilate prevent rodent attack for a longer time period in grain stores.

Keywords: Rodents; repellent; methyl anthranilate; grain stores

1. Introduction
Agriculture is the backbone of the Indian economy which plays the key role in the socioeconomic development of the country. In agricultural and commensal situations rodents are the serious pests. With about 2,277 species throughout the world [1] rodents are considered as most abundant and diversified order of mammals [2]. Although there are various species of rodents, only 77 species are reported to cause damage to crops [3].
Rodents are capable to adjust themselves to various crops depending upon the food availability. They establish during the initial period and breed during growth period of crop resulting in rapid growth of their population due to increase in food availability and emigrate after crop harvest [4]. A national study conducted by the Indian Grain Storage Management and Research Institute (IGMRI) reported 4.75% rodent damage to stored grains [5]. They are not only responsible for physical damage to crops but also cause environmental and crop contamination with microbes [6] and toxigenic fungi [7] and physical contamination with their body hairs, urine and faeces. Faeces of the rodents are classified as dangerous contaminants as they contain numerous pathogens and allergens [8]. These pathogens include *Leptospira interrogans*, *Rickettsia typhi*, *Yersinia pestis* and *Streptobacillus monilliformis* [9].
Rodent can be managed using various types of rodent management strategies. Physical killing, trapping, use of biological agents and chemicals comes under reductional methods. Rodenticides are of two types: anticoagulant and acute rodenticides but excessive use of rodenticides increases environmental pollution, indirect and direct poisoning leads to the death of non-target organisms [10].
Repellents are being used to manipulate animal behaviour. These can be considered as a communication device that sends signal from which message is extracted by the animal. Repellents act by exciting the primary or secondary defense mechanisms causing food rejection. Till date, works on rodents repellency is mainly based on chemical compounds like thiram (tetra methyl thiuram disulphide), ziram (zinc dimethyl dithiocarbamate) etc. which could not be used in fields due to their toxicity [11]. There is an increasing interest in developing plant based repellents for reducing rodent damage due to their effectiveness and low impact on non-target organisms [12]. Plant based essential oils are the volatile lipophilic compounds. For the applications of these essential oils, there is need to develop formulations using chemical constituents that prevent them from degradation, evaporation and also allow sustained release at the same time [13].

Recently, potential of 5% cinnamic aldehyde as secondary repellent against house rat has been reported [12].

Methyl anthranilate (MA) is an anthranilic acid ester [14]. It is a GRAS (generally recognized as safe) listed compound which is used as a food flavoring and fragrance additive. It is a natural element of concord grapes and other materials of plants [15]. It rapidly decomposes into non-toxic components leaving nil or negligible residues on plant products. If ingested it is metabolized rapidly and its byproducts are excreted out. MA based formulations have been found to be efficient bird aversion agents repelling birds from crops fields. Different MA 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) against birds are commercially available. A synergistic, environmentally safe repellent liquid formulation containing pongamia oil, citronella oil, methyl anthranilate and kerosene oil against rodents has been also reported [16]. However, MA alone was also found effective as repellent against *Mus musculus* [17]. Therefore present systematic investigation was conducted to study its effectiveness against house rat *R. rattus* which is a dominant rodent species under commensal situations in Punjab.

2. Materials and methods

The present study was carried out at Animal House Laboratory and Rodent Research Laboratory, Department of Zoology, Punjab Agricultural University (PAU), Ludhiana located at an intersection of 30˚55' N parallel of latitude and 75˚54' E line of longitude. Commercially available MA was used in present study.

2.1 Collection and maintenance of animals

For present study, the house rat, *R. rattus* of both sexes were trapped with the help or single catch and multicatch rat traps from store houses, grocery shops and poultry farms in and around Ludhiana. In Laboratory, rats were acclimatized individually in cages of size 36 x 23 x 23cm for 15 - 20 days before the commencement of experiment with food and water provided *ad libitum*. Food provided was prepared by mixing cracked wheat, powdered sugar and groundnut oil (WSO bait) in ratio 96: 2: 2. The metallic/plastic trays were kept under each cage for collection and disposal of urine and faeces for experimentation. After acclimatization healthy rats were weighted and grouped for experimentation. Animal were used and maintained as per the guidelines of Institutional Animal Ethics Committee. Approval of Institutional Animal Ethics Committee Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana was obtained for the usage of animals.

2.2 Antifeedant used

MA was used as antifeedant against *R. rattus* in present studies. This chemical was purchased from Loba Chemical Private Limited, Mumbai, India.

2.3 Determination of effective concentration of MA

Six different concentrations of MA (2, 2.5, 3, 4, 6, and 8%) were tested. Treated baits containing different concentrations of chemical were prepared by dissolving the required chemical in known volume of methanol and then mixing in known quantity of plain WSO bait. Rats of treated and untreated groups (n=6 for treated group and n=1 for untreated group; 3 males and 3 females in each group) were fed on plain WSO-mix bait for three consecutive days. After this pre-treatment period, rats were fed on WSO bait mixed with different concentrations of MA (2, 2.5, 3, 4, 6, and 8%) in bi-choice feeding tests for five consecutive days. After five days of treatment period, rats were again fed on plain WSO bait to record the post-treatment bait consumption. Daily bait consumption of both treated and untreated baits was calculated by the following formula:

$$\text{Daily bait consumption (g/100 g body weight)} = \frac{\text{Daily consumption of bait by rat}}{\text{Weight of rat (g)}} \times 100$$

Antifeedant index (AFI) both in no-choice and bi-choice tests were calculated by following formula:

In Bi-choice test:

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

In No-choice test:

$$\text{AFI (\%)} = \frac{\text{Consumption of pre-treated bait} - \text{Consumption of treated bait}}{\text{Consumption of pre-treated bait}} \times 100$$

2.4 Primary/secondary repellent effect of effective concentration of MA

Experiments were also conducted in laboratory cages to record the hourly consumption of groups of rats treated with MA (2.5% and 8%) in bi-choice feeding test and using 2.5% MA in no-choice feeding test up to 6 hours to confirm the existence primary repellent effect of MA against house rats. For this acclimatized rats were weighed and grouped. Under bi-choice feeding test, rats of treated groups (n=10, 5 males and 5 females for each concentration) were fed on a known weight of 2.5 and 8% MA treated bait, simultaneously rats of untreated group (n=10, 5 males and 5 females) were also fed on plain WSO-mix bait for six consecutive hours. Rats of treated group (n=10, 5 male, 5 females) were also fed on known weight of 2.5% MA treated bait under no-choice conditions for six consecutive hours for observing the hourly consumption of treated bait. After every hour, the consumption of WSO bait treated with effective dose and plain WSO bait was recorded by quickly weighing the bowls.

Hourly bait consumption of both treated and untreated baits was calculated by the following formula:

$$\text{Hourly bait consumption (g/100 g body weight)} = \frac{\text{Hourly consumption of bait by rat}}{\text{Weight of rat (g)}} \times 100$$

2.5 Preparation of MA formulations

Effective concentration of MA (2.5%) was used to prepare different formulations of MA by using different chemicals in different proportion (Table1).

Formulation 1 (F1) was prepared by micro-encapsulation of emulsified 2.5% MA in 1% sodium alginate shell using emulsion extrusion technique. Detailed method of preparation of microcapsules is given in Table1. This formulation was prepared by mixing 2.5% methyl anthranilate, 1% Tween 80 as emulsifier and 1% sodium alginate solution. The solution thus prepared was then poured drop by drop using syringe in a trough containing 1% calcium chloride resulting in encapsulation of 2.5% MA with sodium alginate coat. Capsules so prepared were kept in calcium chloride solution for about 25 minutes for the hardening of the capsules, after

which these were sieved out (Fig1), dried and stored in refrigerator for use in experimentation.

Fig 1: Microcapsules of 2.5% methyl anthranilate

Formulation 2 (F2) contained 2.5% methyl anthranilate, 1% Tween 80 as an emulsifier, 2% tert-butyl hydroquinone as photostabilizer and 0.1% Xanthan gum as sticker. This formulation was prepared by mixing 2.5 ml meyhl anthranilate in 1 ml Tween 80. To this solution, tert-butyl hydroquinone solution prepared by mixing 2 gm of tert-butyl hydroquinone in 5 ml methanol was added along with xanthan gum solution, which was prepared by mixing 0.1 gm of xanthan gum in 20 ml boiling water. The volume of the solution then made up to 100 ml by adding water into it.

Formulation 3 (F3) contained 2.5% methyl anthranilate, 1% Tween 80, 2% pongamia oil, 2% tert-butyl hydroquinone and 0.1% Xanthan gum. This formulation was prepared by mixing 2.5 ml methyl anthranilate and 2 ml pongamia oil in 1 ml Tween 80. To this solution, tert-butyl hydroquinone solution prepared by mixing 2 gm of tert-butyl hydroquinone in 5 ml methanol was added along with xanthan gum solution which was prepared by mixing 0.1 gm of xanthan gum in 20 ml boiling water. The volume of the solution then made up to 100 ml by adding water into it.

Formulation 4 (F4) contained 2.5% methyl anthranilate, 1% Tween 80, 5% pongamia oil, 0.5% citronella oil and 17.5% kerosene oil in water. This solution was prepared by mixing 2.5 ml methyl anthranilate, 5 ml pongamia oil, 0.5 ml citronella oil and 17.5 ml of kerosene oil and 1 ml Tween 80. The volume of the solution then made up to 100 ml by adding water into it.

Formulation 5 (F5) contained 2.5% methyl anthranilate, 1% Tween 80 as an emulsifier and 0.1% Xanthan gum as sticker. This formulation was prepared by mixing 2.5 ml meyhl anthranilate in 1 ml Tween 80. To this solution, xanthan gum solution was added, which was prepared by mixing 0.1 gm of xanthan gum in 20 ml boiling water. The volume of the solution then made up to 100 ml by adding water into it.

Formulation 6 (F6) contained 2.5% methyl anthranilate, 1% Tween 80, 2% pongamia oil and 0.1% Xanthan gum. This formulation was prepared by mixing 2.5 ml methyl anthranilate and 2 ml pongamia oil in 1 ml Tween 80. To this solution xanthan gum solution was added which was prepared by mixing 0.1 gm of xanthan gum in 20 ml boiling water. The volume of the solution then made up to 100 ml by adding water into it.

Formulation 7 (F7) contained 2.5% methyl anthranilate, 1% Tween 80, 5% pongamia oil, 0.5% citronella oil, 17.5% kerosene oil and 0.1% xanthan gum in water. This solution was prepared by mixing 2.5 ml methyl anthranilate, 5 ml pongamia oil, 0.5 ml citronella oil, 17.5 ml of kerosene oil and 1 ml Tween 80. To this solution xanthan gum solution was added which was prepared by mixing 0.1 gm of xanthan gum in 20 ml boiling water. The volume of the solution then made up to 100 ml by adding water into it.

Table 1: Composition of different formulations of 2.5% MA.

Formulations	MA	Emulsifier (Tween 80)	Photo Stabilizer (Tert butyl hydroxyquinon)	Sticker (Xanthan gum)	Pongamia Oil	citronella oil	Kerosene oil	Micro encapsulation
F1 (Capsules)	2.5%	1%						√
F2	2.5%	1%	2%	0.1%				
F3	2.5%	1%	2%	0.1%	2%			
F4	2.5%	1%			5%	0.5%	17.5%	
F5	2.5%	1%		0.1%				
F6	2.5%	1%		0.1%	2%			
F7	2.5%	1%		0.1%	5%	0.5%	17.5%	

-MA indicate Methyl Anthranilate

-√ indicate Microencapsulation has performed

2.6 Testing of formulations in grain stores:

Wheat grain store (indoor but not rodent proofed) at the Director Seed farm and experimental rattery, Punjab Agricultural University (PAU), Ludhiana and at village Sherpur, Jagraon in district Ludhiana were selected to study the effect of different formulations of 2.5% MA on rodent population.

At PAU Seed farm, five stacks of 20 small wheat bags with each bag weighing 200gm were installed in 5 stores (Fig 2). Four different formulations of 2.5% MA i.e F1, F2, F3 and F4 were sprinkled/sprayed on four stacks installed in four different stores whereas the fifth stack was kept as untreated control. 250 ml of each formulation was sprayed on stack no. 1, 2 and 3 while 5 gms of microcapsules were sprinkled on stack no. 4. After application of formulations, all the stacks were covered with tarpaulin. Rodent population in each store was estimated by bait census method. This method gives evidence for the resident population of stacks as well as rodents coming from surrounding areas. For estimating rodent population by this method, plain 2% WSO (Cracked wheat: Sugar: Oil; 96:2:2) bait, prepared one day before the application was kept under stacks @ 50gm/ points. After 48 hours, left over bait was collected and weighed to calculate percent bait consumption. This rodent census method is an indirect method of estimation of rodent population and only provides an index of population size rather than their absolute number. Rodent population/activity was estimated using this method at different time intervals throughout the observation period till the appearance of signs of rat infestation again after treatment. Rodent species visiting stacks was confirmed from pellets present on bags. Rodent damage was recorded in terms of number and size of cuts (cm^2) on bags of small stacks.

Formulations tested at the PAU Seed farm were also tested in indoor grain store at village Sherpur, Jagraon in district Ludhiana. Five godowns with severe rodent infestation were selected in this store. 8-12 large stacks of 50kg gunny bags filled with wheat were stored in each godown. Each stack consists of 2650 - 2800 gunny bags covering 60Sq. metre area. In each godown, three small stacks of 3 wheat bags each with each bag weighing 50 kg were installed (Fig 2).

Fig 2: 2a. Small stacks treated and covered with tarpaulin in PAU Seed farm. 2b. Small and large stacks in a godown at village Sherpur

Small stacks installed in godown number 1,2,3 & 4 were treated with four different formulations i.e F1, F2, F3 and F4 respectively while small stacks of godown number. 5 were not treated. 1000 ml of each formulation i.e. F2, F3 and F4 was sprayed on three small stacks of godown number 2, 3 and 4 respectively and 10 gm of F1 microcapsules was sprinkled on three small stacks of godown number 1 whereas stacks of godown number 5 were kept as untreated control. Rodent population was recorded at intervals before and after treatment by bait census method as given above till the appearance of signs of rat infestation again after treatment. Plain bait was kept under each of the small stack as well as under large stacks @10 gm/point and left over bait was collected after 7 days to record the consumption. Effect of treatments was recorded for 5 weeks. Rodent species visiting stacks was confirmed from pellets present on bags. Rodent damage was recorded in terms of number and size of cuts (cm^2) on bags of small stacks.

At experimental rattery, Department of Zoology, PAU, Ludhiana, two chambers were selected and in each chamber, six stacks of 6 small wheat bags with each bag weighing 400gm were installed. Five different formulations of 2.5% MA i.e F1, F4, F5,F6 and F7 were sprinkled/ sprayed on five different stacks installed in each chamber where as sixth stack was kept untreated control. 250 ml of each formulation was sprayed on stack no. 1, 2, 3 and 4 while 5gm of capsules were sprinkled on stack no. 5. After application of formulations, all the stacks were properly covered with tarpaulin. One house rat of equal weight and same sex were released in each room. Plain 2% WSO bait (Cracked wheat: Sugar: Oil; 96:2:2), prepared one day before the application was kept under stacks @ 10gm/ points. After 48 hours, left over bait was collected and weighed to calculate percent bait consumption. Rodent activity was estimated using this method at different time intervals throughout the observation period till the appearance of signs of rat infestation again after treatment. Damage caused by rat was recorded in terms of number and size of cuts (cm^2) on bags of small stacks.

2.7 Statistical analyses

Values were determined as mean ± SE. Significance of difference were determined at 5% level of significance. Significance of difference between treated and untreated bait, among concentrations, sex, formulations and different durations after treatment was determined by ANOVA using fCRD. The statistical software used was SAS version 9.3

3. Results

3.1 Determination of the effective concentration of MA as an antifeedant against *R. rattus* in laboratory cages

Rats were fed on bait treated with MA (2.0, 2.5, 3.0, 4.0, 6.0 & 8.0%) in bi-choice feeding tests for five consecutive days (Table 2).

Table 2: Antifeedant index and average mean daily consumption of plain bait and of bait treated with different concentrations of MA.

Sex	Treat-Ments	Body weights (g)	Consumption (g/100gm bw) of bait				Antifeednt Index (%)
			Pretreatment Period	Treatment period		Post Treatment Period	
				Plain	Treated		
Male[A]	2.0	156.66±8.34	6.72±0.15	7.81±0.49	1.99±0.44	6.97±0.34	60.73±5.38
	2.5	161±10.2	8.04±0.30	6.62±0.94	1.49±0.15	8.01±0.25	71.67±1.69
	3.0	161.66±6.54	5.81±0.74	5.86±0.39	2.10±1.14	7.42±0.08	57.37±8.74
	4.0	157.45±1.36	8.45±0.25	8.54±0.47	0.48±0.17	6.04±0.46	90.05±3.15
	6.0	160.66±3.03	8.13±0.15	11.11±0.12	0.36±0.11	6.24±0.36	93.69±1.82

	8.0	159±10.21	8.04±0.30	9.03±0.97	0.90±0.20	7.92±0.32	84.15±2.10
	Control	155.36±4.25	7.21±0.47	9.11±0.12		7.45±.89	
Female[B]	2.0	152.31±7.98	6.36±0.50	9.88±0.24	2.21±0.53	6.27±0.80	65.37±7.01
	2.5	152.33±7.40	7.67±0.33	6.17±0.26	1.46±0.19	6.47±0.29	75.97±2.61
	3.0	145.16±6.61	6.55±0.61	7.23±0.11	0.59±0.20	7.03±0.53	82.32±5.80
	4.0	151±4.78	8.42±0.74	9.67±0.43	1.93±0.35	6.04±0.25	66.77±1.67
	6.0	150±6.44	7.3±0.43	8.96±1.19	2.55±0.02	5.21±0.54	64.29±1.11
	8.0	152.33±7.40	7.67±0.33	10.35±0.52	0.81±0.21	5.68±0.94	85.95±3.29
	Control	149.25±6.12	8.44±1.47	8.45±0.11		6.24±0.47	
M±SE			7.48±0.21[a]	8.48±0.40*	1.40±0.20**	6.64±0.21[b]	

-Mean values with [*,**] along the row indicate significant difference in overall average consumption of plain and treated bait by male and female rats at $P \leq 0.05$
-Mean values with [a,b] along the row indicate significant difference in overall average food consumption between pre- and post- treatment period at $P \leq 0.05$
-Mean values with [A,B] in a column indicate significant difference in overall consumption of treated bait between male and female rats at $P \leq 0.05$

Results (Tables 2) revealed a significant difference in overall mean daily food consumption (g/100g bw) between pre and post- treatment period (Fig 3), however this difference in overall mean daily food consumption (g/100g bw) was non-significant between male and female rats. Average consumption (g/100g bw) of treated bait was significantly ($P \leq 0.05$) low as compared to untreated bait in case of each concentration. There was also a significant difference in overall consumption of treated bait between male and female rats. However there was a non-significant difference in average mean daily consumption of treated bait from day 1 to day 5 of treatment period as well as among different treatment groups. Therefore antifeedant or secondary repellent effect of MA was evident on the first day of feeding and consumption of treated bait remained depressed till day 5 of the treatment period. Rats might have consumed the treated bait during the first few hours of day 1 of the treatment period, which caused the gastrointestinal malaise in rats resulting in the development of antifeedant or secondary repellent effect among rats against different concentrations of MA. However MA was earlier reported as a nociceptive primary bird repellent [18]. It was reported to act as a repellent by irritating pain receptors associated with taste and smell. It was also found effective as a repellent against *Mus musculus* [17].

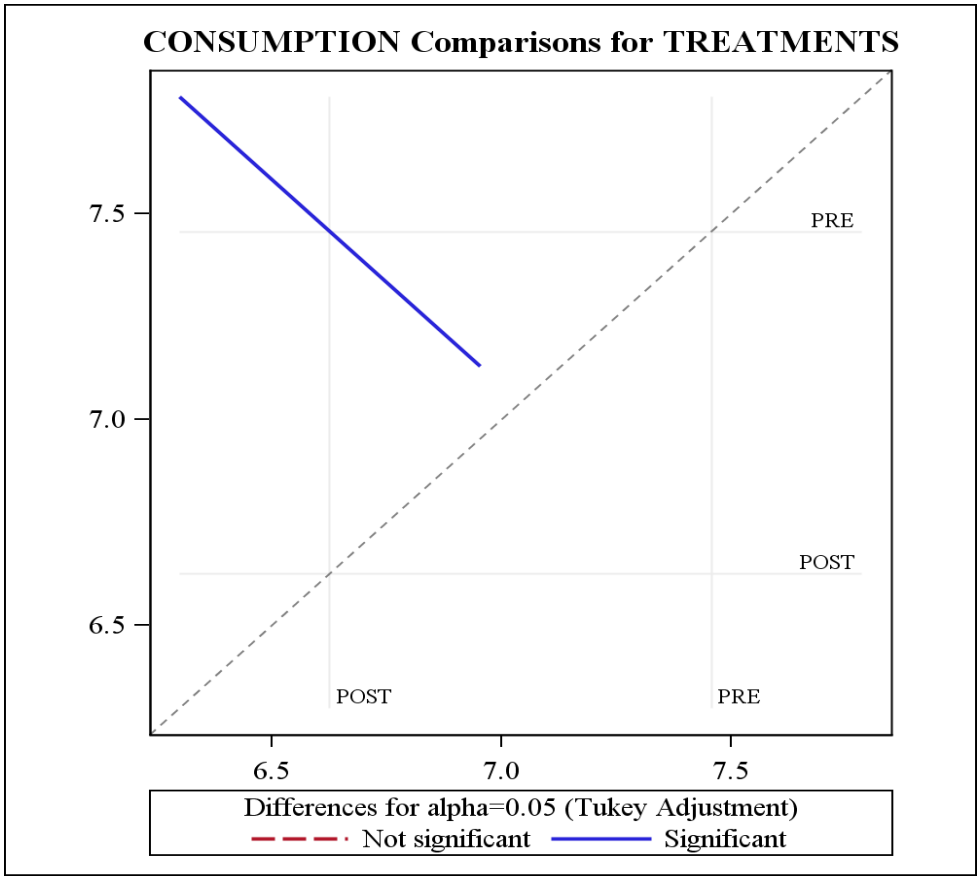

Fig 3: Shows significant difference in consumption of plain bait between pre and post- treatment period

3.2 To determine the existence of primary repellent effect of 2.5 and 8% MA under no-choice and bi-choice conditions against *R. rattus*

As there was non-significant difference in consumption of treated bait from day 1 to day 5 with reduced consumption of treated bait during the treatment period in above mentioned experiment, existence of a primary repellent effect of MA could not be established. Moreover there was non-significant difference in consumption of treated bait and antifeedant index among different treatment groups, therefore only two concentrations of MA were selected to study the existence of a primary repellent effect of MA under bi-choice conditions.

Results (Tables 3) revealed that rats completely avoided treated baits during the first hour of their exposure to treated bait under bi-choice conditions indicating primary/olfactory repellent effect of MA. After that rats started eating treated

bait. There was non-significant difference in overall mean hourly consumption (g/100g bw) between both sexes as well as between treated and untreated baits upto 5 hours of their exposure to treated bait indicating primary repellent effect towards MA treated bait disappeared after one hour of their exposure to treated bait and rats started eating treated bait. However consumption of both treated and untreated bait remained depressed upto 5 hours and there was non-significant difference in consumption between treated and untreated bait but consumption of untreated bait increased significantly and there was significant difference in consumption between treated and untreated bait during 6th hour in both sexes indicating the development of antifeedant/secondary repellent effect against MA after the 5th hour of their exposure to treated bait (Tables 3). Secondary repellent effect might have occurred because of development of gastrointestinal malaise after eating treated bait which retained in rats throughout the treatment period of 5 days recorded in previous experiment.

Table 3: Hourly consumption of bait treated with 2.5 and 8% MA by two different groups of rats in bi-choice and 2.5% in no-choice feeding tests.

Hours of consumption	Consumption of plain bait and bait treated with 2.5% MA under bi-choice conditions				Consumption of plain bait and bait treated with 8% MA under bi-choice conditions				Consumption of plain bait and bait treated with 2.5% MA under no-choice conditions			
	Male		Female		Male		Female		Male		Female	
	Plain	Treatment	Plain	Treatment	Plain	Treatment	Plain	Treatment	Plain	Treatment	Plain	Treatment
1st	1.43±0.00	0.00±0.00	2.72±0.38	0.00±0.00	4.05±0.29	0.00±0.00	2.31±0.48	0.00±0.00	1.65±0.12	0.10±0.00[a]	0.87±0.14	0.20±0.00[1]
2nd	0.93±0.00	1.32±0.25	0.74±0.02	0.04±0.02	1.03±0.22	0.00±0.00	1.56±0.40	1.67±0.00	1.11±0.11	0.17±0.02[a]	0.65±0.07	0.18±0.00[1]
3rd	1.41±0.14	0.83±0.27	1.22±0.24	1.10±0.33	0.80±0.05	1.32±0.00	1.37±0.10	1.05±0.25	0.89±0.08	0.59±0.16[b]	1.20±0.17	0.40±0.20[2]
4th	1.92±0.48	0.88±0.25	1.39±0.40	0.54±0.00	1.95±0.78	1.91±0.57	1.58±0.28	0.63±0.02	1.99±0.30	0.82±0.15[b]	1.10±0.11	0.47±0.21[2]
5th	1.97±0.39	1.50±0.01	0.83±0.19	1.72±0.90	1.69±0.37	0.89±0.32	1.58±0.31	0.47±0.01	1.21±0.09	0.90±0.14[b]	0.88±0.10	0.50±0.21[2]
6th	8.18±0.93*	0.82±0.05**	5.80±1.02[#]	1.95±0.41[##]	3.50±0.68*	1.41±0.32**	8.52±1.3[#]	1.07±0.24[##]	1.55±0.11	1.02±0.15[b]	0.84±0.19	0.53±0.24[2]
Mean	2.64±1.020	0.89±0.19	2.11±0.72	0.89±0.31	2.17±0.49	0.92±0.81	2.82±1.04	0.81±0.21	1.4±0.15	0.63±0.14	0.92±0.07	0.38±0.05

-Mean values with *, ** along the row indicate significant difference in mean consumption of plain and treated bait in male rats under bi-choice conditions at $P \leq 0.05$
-Mean values with [#,##] along the row indicate significant difference in mean consumption of plain and treated bait in female rats under bi-choice conditions at $P \leq 0.05$
-Mean values with [a,b] in a column indicate significant difference in mean consumption of treated bait in male rats during treatment period in no-choice condition at $P \leq 0.05$
-Mean values with [1,2] in a column indicate significant difference in mean consumption of treated bait in female rats during treatment period in no-choice condition at $P \leq 0.05$

However under no-choice conditions rats consumed negligible quantity of treated bait for first two hours but after that during 3rd there was a significant increase in mean consumption of treated bait and non-significant change from 3rd hour to 6th hour in both the sexes indicating existence of a primary repellent effect for 2 hours under no-choice conditions but primary repellent effect against bait treated with MA was more evident under bi-choice conditions. There was also a non-significant difference in overall average hourly consumption (g/100g bw) between both sexes as well as between treated and untreated bait (Table 3). From the above experiment, it is concluded that baits treated with 2.5 and 8.0% MA are effective as primary repellent in house rats under bi-choice conditions and there was non-significant difference in effectiveness of 2.5 and 8% MA treated bait. Therefore 2.5% MA was used for preparation of formulations for filed applications. Both the experiments clearly indicated that MA is effective both as primary as well as secondary repellent under bi-choice conditions.

3.3 Testing the effect of different formulations of 2.5% MA on rodent damage after different periods of treatment

During present investigation, different formulations of 2.5% MA were prepared and tested in indoor wheat grain stores at director Seed farm, PAU, Ludhiana, experimental rattery, PAU, Ludhiana and one grain store at village Sherpur, Jagraon in district Ludhiana.

3.3.1 Testing the effect of different formulation of 2.5% MA at PAU Seed farm

At PAU seed farm, small stacks installed in four different stores were treated with four different formulations of 2.5% MA i.e. F1, F2, F3 and F4 while stack in fifth store was kept as untreated control stack. Rodents population and their damage in each grain store was recorded at three days interval till the appearance of signs of damage.

Results (Table 4) revealed that smell intensity of different formulations was not affected for six days of treatment. After 9 days of treatment, intensity of smell was reduced for all the formulations and smell intensity was least with formulations F3 followed by F2, F1 and F4. After 12 days of treatment intensity of smell was maximum with formulation F4 followed by F1-F3 indicating that intensity of smell was retained for maximum period of time with formulation F4. Presence of rat pellets on bags and consumption of plain WSO bait kept below stacks revealed the presence of rats in all the grain stores throughout the observation period but cuts were not observed on the bags upto 9 days of treatment from all the four stacks treated with different formulations (Table 4). However cuts were seen on untreated bags even after 3 days of treatment and number and area of cuts on untreated bags increased with time. After 12 days of treatment, number of cuts and area of cuts were found to be maximum on untreated stack followed by stacks treated with F3, F2, F4 and F1. Although the number of cuts were same (one) on all bags treated with different formulations but area of bags damaged was maximum on stack treated with F3 formulation (Table 4). These results clearly indicate that damage on bags increased by a reduction in intensity of smell of different formulations. 2.5% methyl anthranilate is volatile and its smell dissipates with time although its microencapsulation and/or addition of photostabilizers and stickers slow down the release/ degradation of methyl anthranilate. Proper covering of bags after application of different formulations can further increases their effectiveness. In PAU Seed farm, small stacks after treatment were partially covered with tarpaulin (Fig.2) results fast dissipation of smell and therefore protection against rodent damage for short period. Therefore proper covering of bags after treatment can further increase the effectiveness of different formulations.

3.3.2 Testing the effect of different formulation of 2.5% MA at village Sherpur, Jagraon, Ludhiana

All the formulations tested in PAU grains stores were also tested in grain stores at village Sherpur. All small stacks after treatment with different formulation were properly covered

with tarpaulin. Estimation of rodent population from all the selected godowns by recording plain bait consumption as well as presence of faecal pellets revealed the presence of rodents in all the selected godowns. Therefore, out of five godowns selected, small stacks in four godowns were treated with four different formulations.

3.3.2.1 Effect of formulations on presence of rodents adjudged from consumption of plain WSO bait and faecal pellets

Results (Table 5) revealed that consumption of 2% WSO bait kept under untreated large stacks LS1, LS2, LS3, LS4 was significantly more than from the small stacks treated with the formulations F1, F2, F3 and F4. Consumption of bait from untreated small stacks (C) was also significantly more as compared to the small stacks treated with FI, F2, F3 and F4. Therefore from this data, it is concluded that visits of rats was more near untreated small and large stacks as compared to treated small stacks. Consumption of bait kept under small stacks treated with all the four formulations was nil upto 14 days indicating rats had not visited treated stacks for upto 14 days. After 21 days of treatment small stacks treated with F4 showed least consumption of 2% WSO bait followed by F1, F3, F2, but overall there was non-significant difference among all the small stacks treated with different formulations. After 28 days of treatment, again the stacks treated with F4 show least consumption of bait followed by F1, F2 and F3. There was increase in the consumption of bait kept under small stacks with time.

Table 4: Effect of different formulations of 2.5% MA on rodent population and damage at PAU Seed farm.

Days of treatment	Treatment	Smell intensity	Rat pellets	Consumption of WSO (%)	Area of cuts on bags (cm^2)	No. of cuts
After 3 days	F1	+++++	Rr	38	0	Nil
	F2	+++++	Mm	34	0	Nil
	F3	+++++	Rr,Mm	42	0	Nil
	F4	+++++	Rr,Mm	48	0	Nil
	Untreated	---------	Rr	42	2.5	1
After 6 days	F1	+++++	Mm	50	0	Nil
	F2	++++	Mm	38	0	Nil
	F3	+++	Rr,Mm	52	0	Nil
	F4	+++++	Rr,Mm	30	0	Nil
	Untreated	----------	Rr,Mm	36	2.7	1
After 9 days	F1	++++	Rr,Mm	36	0	Nil
	F2	+++	Rr,Mm	22	0	Nil
	F3	++	Rr,Mm	40	0	Nil
	F4	++++	Rr,Mm	42	0	Nil
	Untreated	----------	Rr	28	3	1
After 12 days	F1	++	Rr,Mm	36	0.5	1
	F2	++	Rr	16	0.84	1
	F3	++	Rr	34	1.5	2
	F4	+++	Rr	38	0.65	1
	Untreated	----------	Rr	22	14.5	2
Total number of cuts and area of cuts for 12 days	F1				0.5	1
	F2				0.84	1
	F3				1.5	2
	F4				0.65	1
	Untreated				22.7	5

-Increase in the number of + signs indicate increase in the intensity of smell
-F1-F4 indicate 2.5% methyl anthranilate formulations; Mm*: Mus musculus;* Rr*: Rattus rattus*

Table 5: Percent consumption of 2% WSO bait kept under large and small stacks in the indoor store at village Sherpur, Jagraon.

Stacks	Treatments	Consumption of 2% WSO bait				
		After 7 days	After 14 days	After 21 days	After 28 days	After 35 days
SS1	F1	0.00±0.00^{a}	0.00±0.00^{a}	1.33±0.27^{a}	3.33±0.54^{a}	4.66±0.54^{a}
LS1	Untreated	70.00±8.47^{bc}	78.75±3.72^{bc}	82.5±2.93^{c}	77.5±2.33^{bc}	60.0±3.95^{b}
SS2	F2	0.00±0.00^{a}	0.00±0.00^{a}	2.50±0.95^{a}	4.33±0.27^{a}	5.33±0.54^{a}
LS2	Untreated	100.00±0.00^{c}	92.5±3.85^{c}	75.00±4.67^{bc}	87.75±3.75^{c}	87.5±3.42^{c}
SS3	F3	0.00±0.00^{a}	0.00±0.00^{a}	2.33±0.72^{a}	4.66±0.54^{a}	5.44± 0.12^{a}
LS3	Untreated	68.75±2.75^{b}	70.00±2.50^{bc}	60.00±9.55^{b}	62.50±6.31^{b}	61.25±5.21^{b}
SS4	F4	0.00±0.00^{a}	0.00±0.00^{a}	0.66±0.54^{a}	1.33±0.27^{a}	1.47±0.12^{a}
LS4	Untreated	67.5±4.23^{b}	67.75±4.56^{b}	62.5±4.23^{b}	57.77±4.20^{b}	60.11±2.22^{b}
SS5	Untreated(C)	60.00±4.71^{b}	63.33±2.72^{b}	53.33±2.72^{b}	73.33±5.44^{bc}	70.00±4.71^{bc}
LS5	Untreated	70.00±3.06^{bc}	68.75±4.82^{b}	76.66±7.20^{bc}	75.0±0.58^{bc}	71.42±0.19^{bc}

- LS: Large stacks; SS: Small stacks
-F1-F4: 2.5% methyl anthranilate formulations
-Mean values with different superscripts (a-c) indicate significant difference among treatments and weeks at $P \leq 0.05$

3.3.2.2 Intensity of smell of formulations after different periods of treatment

Intensity of smell on stacks treated with four different formulations F1, F2, F3, F4 was strong upto 14 days of treatment (Table 6). After 21 days of treatment, intensity of smell was maximum on the stacks treated with formulations F1 and F4 followed by the formulations F2 & F3. However after 28 days of treatment, intensity of smell of F1 and F4 was more followed by F2 and F3. After 35 days of treatment, intensity of smell was negligible on the treated stacks. From the above results, it is concluded that smell of all formulations on stacks completely covered with tarpaulin was strong for a period of 21 days as compared to 9 days only when stacks treated with different formulations were covered partially

with tarpaulin. Effectiveness of microencapsulated formulation F1 as rodent repellent persist for longer period of time as compared to other formulations tested and prevented rodent attack on bags for longer time period.

Table 6: Effect of different formulations of 2.5% MA at village Sherpur, Jagraon.

Treatments	After 7 days	After 14 days	After 21 days	After 28 days	After 35 days
	Smell intensity				
F1	++++++	++++++	+++++	++++	++
F2	++++++	++++++	++++	+++	+
F3	++++++	++++++	++++	++	+
F4	++++++	++++++	+++++	++++	++
Untreated(C)	-	-	-	-	-
	Number of cuts on stacks of bags				
F1	0±0[a]	0±0[a]	0±0[a]	0±0[a]	2.33±0.27[c]
F2	0±0[a]	0±0[a]	0±0[a]	0.33±0.27[a]	2.33±0.27[c]
F3	0±0[a]	0±0[a]	0±0[a]	0.66±0.27[a]	0.66±0.27[a]
F4	0±0[a]	0±0[a]	0±0[a]	0.33±0.27[a]	0.33±0.27[a]
Untreated(C)	1±0.47[b]	1.33±0.54[b]	1±0.47[b]	1.33±0.54[b]	1.33±0.54[b]
	Area of bags damaged by rats				
F1	0±0[1]	0±0[1]	0±0[1]	0±0[1]	1±0.23[1]
F2	0±0[1]	0±0[1]	0±0[1]	0.16±0.13[1]	0.66±0.13[1]
F3	0±00[1]	0±0[1]	0±0[1]	0.50±0.40[1]	0.50±0.40[1]
F4	0±0[1]	0±0[1]	0±0[1]	0.16±0.13[1]	0.16±0.13[1]
Untreated(C)	6± 2.82[2]	5.66±3.47[2]	6.83±3.43[2]	3.33±1.08[2]	4.66±2.17[2]

-Increase in the number of (+) signs indicate increase in the intensity of smell
-F1-F4: 2.5% methyl anthranilate formulations
-Mean values with different superscripts (a-c) indicate significant difference in number of cuts among different treatments and weeks at $P \leq 0.05$
-Mean values with [1,2] indicate significant difference in area of bags damaged by rats among different treatments and weeks at $P \leq 0.05$

3.3.2.3 Effect of formulations on number of cuts on bags

No cut was observed on bags treated with F2, F3 and F4 for upto 21 days and upto 28 days on bags treated with F1 (Table 6). Maximum number of cuts was recorded on bags treated with F3 after 28 days of treatment. Cuts were observed on the stacks treated with F1 after 35 days of treatment. Number of cuts was significantly less on treated stacks than from untreated stacks throughout the observation period.

3.3.2.4 Effect of formulations on area of bags damaged by rats

No damage was observed on the bags treated with formulations F1, F2, F3, F4 upto 21 days of treatment (Table 6). After 28 days of treatment, area of bags damaged by rats treated with F1 was nil (Fig 4) whereas cuts were observed on the stacks treated with F2, F3, F4 Area of bags damaged by the rats in untreated (C) stacks was significantly more than from treated stacks.

Fig 4: 4a. Rodent damage on untreated bag. 4b. No rodent damage on bag treated with F1 after 28 days of treatment

3.3.3 Testing the effect of different formulations of 2.5% MA at experimental rattery, Department of Zoology, PAU, Ludhiana

At experimental rattery, five small stacks installed in each of the two ventilated open chambers were treated with five different formulations of 2.5% MA i.e. F1, F4, F5, F6 and F7 while sixth stack in each chamber was kept as untreated control stack. Damage by house rat released in each room was recorded at three days interval till the appearance of signs of damage.

3.3.3.1 Intensity of smell of formulations after different periods of treatment

Results (Table 7) revealed that the smell intensity of different formulations was not affected for six days after their application on stacks except small reduction in intensity of smell for formulation F5 and F6. However after 9 days of treatment, intensity of smell was reduced for all the formulations. After 12 days of treatment intensity of smell was maximum with formulation F1, F4 and F7 of room number 2 followed by F5 and F6 indicating that intensity of smell was retained for maximum period of time with formulation F1 and F4

3.3.3.2 Effect of formulations on number of cuts on bags

No cut was observed on the stacks treated with F1, F4 and F7 for upto 9 days of treatment (Table 7). Cuts were observed on the stacks treated with F1 and F4 after 12 days of treatment. Number of cuts was significantly less on treated stacks than from untreated stacks

Table 7: Effect of different formulations of 2.5% MA at experimental rattery, Department of Zoology, PAU.

Treatments	After 3 days	After 6 days	After 9 days	After 12 days
	Smell intensity			
F1	+++++	+++++	++++	+++
F4	+++++	+++++	++++	+++
F5	+++++	++++	+++	++
F6	+++++	++++	++	+
F7	+++++	+++++	++++	++
C	---------	---------	---------	---------
	Number of cuts on stacks of bags			
F1	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	1 ± 0^{b}
F4	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0.5 ± 0.35^{a}
F5	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0.5 ± 0.35^{a}
F6	0 ± 0^{a}	0 ± 0^{a}	1.5 ± 0.35^{b}	1 ± 0^{b}
F7	0 ± 0^{a}	0 ± 0^{a}	1.5 ± 0.35^{b}	1 ± 0^{b}
C	1.5 ± 0.35^{b}	1.5 ± 0.35^{b}	1 ± 0^{b}	1.5 ± 0.35^{b}
	Area of bags damaged by rats			
F1	0 ± 0^{A}	0 ± 0^{A}	0 ± 0^{A}	1.75 ± 0.53^{B}
F4	0 ± 0^{A}	0 ± 0^{A}	0 ± 0^{A}	0.5 ± 0.35^{A}
F5	0 ± 0^{A}	0 ± 0^{A}	0 ± 0^{A}	0.5 ± 0.35^{A}
F6	0 ± 0^{A}	0 ± 0^{A}	0.75 ± 0.53^{B}	2 ± 0.35^{C}
F7	0 ± 0^{A}	0 ± 0^{A}	0.75 ± 0.53^{B}	1.5 ± 0.35^{B}
C	1 ± 0.35^{B}	1.75 ± 0.17^{B}	0.75 ± 0.53^{B}	1.25 ± 0.53^{B}
	Percent consumption of 2% WSO bait kept under stacks			
F1	0 ± 0^{x}	0.5 ± 0.35^{x}	2 ± 0^{y}	3.5 ± 0.53^{x}
F4	0 ± 0^{x}	0 ± 0^{x}	1 ± 0^{x}	1.5 ± 0.22^{x}
F5	0 ± 0^{x}	0 ± 0^{x}	1.5 ± 0.14^{x}	1.5 ± 0.22^{x}
F6	0 ± 0^{x}	2 ± 0^{y}	3.5 ± 0.35^{y}	4.5 ± 0.25^{z}
F7	0 ± 0^{x}	2.5 ± 0.35^{y}	3.5 ± 0.35^{y}	6 ± 0.70^{z}
C	3.5 ± 0.35^{y}	2.45 ± 0.21^{y}	4.5 ± 0.35^{z}	4.5 ± 0.35^{z}

-Increase in the number of (+) signs indicate increase in the intensity of smell

-F1, F4, F5, F6, F7 indicate 2.5% methyl anthranilate formulation.

-Mean values with ^{a,b} indicate significant difference among different treatments and days at $P \leq 0.05$

-Mean values with different superscripts (A-C) indicate significant difference among different treatments and days at $P \leq 0.05$

Mean values with different superscripts (x-z) indicate significant difference among different treatments and days at $P \leq 0.05$

3.3.3.3 Effect of formulations on area of bags damaged by rats

No damage was observed on the bags treated with formulations for 6 days of treatment (Table 7). After 9 days of treatment, area of the bags treated with formulations F1 and F4 was nil, whereas cuts were observed on the bags treated with F5 and F6. Cuts were observed on the bags treated with F1 and F4 after 12 days of treatment.

4. Discussion

Observations recorded from field application of formulations clearly indicated that all the formulations prevented rodent damage from treated stacks for nine days at PAU Seed farm (indoor) when treated stacks were partially covered with tarpaulin resulting in fast dissipation of smell of repellent from stacks. However when treated stacks were properly covered with tarpaulin in indoor grain store at village Sherpur, dissipation of smell reduced resulting in prevention of rodent damage upto 21 days with all the formulations tested. However in experimental rattery at PAU, where small stacks were installed in ventilated chamber under outdoor condition, treatment with MA formulations prevent rodent attack for 9 days only. Primary repellent effect of MA through olfaction seems to be playing role as all these formulations were either sprayed or sprinkled as microcapsules on bags. Damage on the bags increased with reduction in intensity of smell. It is also reported that birds avoided treated fruits (7-11 days) until the fragrance of MA was dissipated [19]. Results from all the field experiments also revealed that F1 formulation prevented rodent damage for longer duration. It might be due to the reason that microencapsulation of MA slowed down the release of MA resulting in strong smell of MA for longer duration and thus prevention of rodent damage for long time. Rodents are responsible for causing 10-15% damage in grain stores [20]. Cost of one stack of wheat bags is Rs 20 lakh and rodents are responsible for causing damage to lower two layers of stacks, which costs about 2 lakh. Cost of one time treatment of one stack with MA capsules is Rs 500, which can protect a stack from rodent attack for one month. Cost effectiveness of MA formulation in grain stores depends upon the value of crop stored, level of damage without MA application and percent damage reduced after MA application. So effectiveness of MA increased with increase in crop value and anticipated damage due to rodents and other animals like birds, insect pests etc. as MA also prevent damage due to birds and insects. The effectiveness of MA formulations can be further enhanced in the presence of alternative food resources, if grains to be protected from rodent damage are treated with MA. It is already reported as GRAS (generally recognized as safe) listed compound and is being used as a food flavoring and fragrance additive. It is a natural element of concord grapes and other materials of plants [15]. It rapidly decomposes into non-toxic components leaving nil or negligible residues on plant products. If ingested it metabolized rapidly and by products are excreted out. Therefore food products to be protected from rodent damage can be treated with microencapsulated MA before packing.

5. Conclusion

The present study is the first of its kind evaluating the repellent effect of an effective dose of methyl anthranilate against house rat in grain stores as no MA based formulation has been reported for rodent pest management till now. Our laboratory studies showed that 2.5% MA treated bait is effective antifeedant repellent as well as primary repellent under bi-choice conditions and microencapsulation of 2.5% MA prevented rodent attack upto 28 days if treated grain bags are properly covered under indoor conditions.

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