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EVALUATION OF METHYLANTHRANILATE BASED REPELLENT FORMULATIONS AND MODE OF DELIVERY TO PREVENT RODENT DAMAGE UNDER STORAGE CONDITIONS

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ABSTRACT

Rodents are the serious pests in both agricultural and commensal situations. Our previous surveys in grain stores revealed that there is a need to manage rodents in August and December. Five methyl anthranilate (2.5%) based formulations (F1-F5) were prepared using different components like emulsifiers, photostabilizers, stickers, botanicals (like pongomia oil, citronella oil, eucalyptus oil), cinnamic aldehyde, anthraquinone and kerosene oil ,etc. in varying proportions and applied on wheat bags under simulated storage conditions for testing their efficacy in managing rodent pests in storage. The results indicated that the formulation (F2) prepared by mixing methyl anthranilate (2.5%) with pangomia oil (5.0%), citronella oil (0.5%), kerosene oil (17.5%) and emulsifier Tween 80 (4.5%) was more effective in comparison to other formulations. Further when this formulation was delivered through wood waste medium, rodent damage was prevented for 15-35 days under simulated storage situations and in grain stores. This formulation can be integrated with other methods of rodent management.

Keywords: Delivery Systems, Formulations, Grain Stores, Repellent, Rodents

Agriculture is an important and indispensable sector for the sustenance and growth of the Indian economy (Ravallion *et al.,* 2007). Rodents are serious pests in both agricultural and commensal situations. They cause negative impacts on the well-being of people as competitors for food (Singleton *et al.,* 2010) and also as carriers of various human diseases (Singla *et al.,* 2008 a,b; Meerburg *et al.,* 2009; Himsworth *et al.,* 2013). Previous studies revealed 10-15% damage in grain stores is due to rodents (Kambarajan *et al.,* 2004). The post-harvest losses in India due to pests amount to 12 to 16 million MT of food grains each year (Meerburg and Kijlstra, 2008; Singh, 2010; Oerke, 2006). They not only consume stored food items but also contaminate it by urination and defecation, thus making it unfit for human consumption (Parkash and Ghosh, 1992; Drummond, 2001; Brown *et al.,* 2007). So rodent infests stored agricultural produce both in terms of quantity and quality, and due to this, the damaged food grains get less market value. Storage losses by rodents in India alone cost at least $5 billion annually (Cao *et al.,* 2002).

Several measures are currently being used to prevent rodent damage in stores (Spragins, 2006). Presently, there is no single method that can control the rodent population below the economic threshold level. Haines (2000) reported that integrated pest management technology is not yet developed for storage conditions. Therefore, there is a need to develop an integrated rodent control program adopting proper methods at appropriate timings to manage rodents under storage conditions. Survey of grain stores revealed that after stacking rabi produce in April, rodent damage increases with an increase in storage period. Stacks of wheat bags are fumigated with 56% aluminium phosphide thrice in a year (First fumigation-end of June to the first week of July, second fumigation- October-November and third fumigation- February) for managing storage insects, which reduced rodent infestation also for 30-40 days after each fumigation (Pandey, 2017). Surveys revealed that as rodent infestation increases in August and December both in outdoor grain stores and in indoor grain stores (not rodent proofed), there is a need to manage them during these months.

Repellents are used to keep the rodents away from crops and stored areas through cues like visual, gustatory, olfactory, acoustic, chemicals, or some mixture of these (Mason *et al.,* 1989). These are highly effective against rodents with little impact on non-target organisms (Packiam *et al.,* 2012; Babbar *et al.,* 2015). Various rat repellents are available commercially as spray or adhesives, which are effective only in small enclosures. However, for large godowns and commensal situations, effective and economical repellent along with a delivery system is required, which can be integrated with other methods for sustainable rodent management in storage.

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MATERIALS AND METHODS

The present study was carried out in breeding cages at Animal House and Experimental Rattery, Department of Zoology, Punjab Agricultural University (PAU), Ludhiana, Punjab, India (30º55' N; 75º54'E) and wheat grain store (not rodent proofed) at village Sherpur, Jagraon in district Ludhiana. Animals were used and maintained with the approval of Institutional Animal Ethics Committee, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Preparation of methyl anthranilate based repellent formulations

Methyl anthranilate (MA) based five formulations with repellent properties were prepared in laboratory by mixing different chemicals/ oils in varying proportions on a magnetic stirrer along with Tween 80 (4.5%) as an emulsifier (Table 1).The formulation F1 was the basic formulation with only MA (2.5%) and Tween 80 (2.5%) as emulsifier. However, the formulation F2 contained MA (2.5%) + Tween 80(4.5%) and three oils like pongomia oil (5%), citronella oil (0.5%) and kerosene oil (17.5%). Likewise, F3 was prepared by mixing cinnamic aldehyde (5.0%), anthraquinone (0.5%) and eucalyptus oil (5.0%) along with MA (2.5%) + Tween 80(4.5%). The composition of formulations F4 and F 5 was similar to the F2 formulation, except that F4 contained xanthan gum solution (0.1%) as sticker while F5 had an additional component of tert-butyl hydroquinone solution (2%) as a photo stabilizer along with xanthan gum solution. Xanthan gum solution (0.1%) used in F4 and F5 was prepared by mixing 0.1g of xanthan gum in 20 ml boiling water. Similarly tert-butyl hydroquinone solution (1%) used in F5 was prepared by mixing 2g of tert-butyl hydroquinone in 5 ml methanol. The volume of all the formulations was made to 100ml by adding water. Final Solution so prepared was again mixed on a magnetic stirrer for half an hour.

Evaluation of persistence of formulations F1 to F3 in animal house

Three house rats, *Rattus rattus* of each sex (n=6) were used to test formulations F1 to F3 under simulated storage conditions in breeding cages at Animal house of Department of Zoology, PAU, Ludhiana. Six breeding cages (for three rats of each sex) (72×36×37cm) were used to test the effectiveness and persistence of each formulation. One wheat bag (weighing 500g) was kept at the extreme ends of each breeding cage. Bag at one end was treated with formulation, whereas the bag of the second end remained untreated (Plate 1). Formulations F1, F2, and F3 were used as a spray. Rodent damage was recorded after every three days till the appearance of damage. Rodent damage was recorded in terms of the number of cuts, size of cuts (cm), the quantity of spilled grains (g) and percent UV fluorescence of spilled wheat grains and wheat grains filled in both treated and untreated bags for each formulation..

Rat urine fluoresces under UV light. UV fluorescence of wheat grains was recorded in the UV chamber to determine the contamination caused by rat urine to the grains (Babbar *et al.,* 2015). Consumption of wheat grains (g/100g body weight) from both treated and untreated bags was recorded after the termination of each experiment.

Evaluation of formulations (F2, F4 and F5) and a delivery system in experimental rattery

At PAU experimental rattery, experiment was performed to test different formulations (F2, F4, and F5) and a delivery system. Out of three formulations tested in laboratory cages in previous experiment, F2 was most effective. Therefore, F1 and F3 were not tested in experimental rattery. Experimetal rattery was constructed in field area and was receiving sunlight and rain, therefore F2 was further stabilized by using stickers and photo stabilizer to prevent runoff by rain

Table 1. Composition of methyl anthranilate based repellent formulations

Formulation	Methyl anthranilate MA (%)	Emulsifier (Tween 80) (%)	Photo Stabilizer (Tert-butyl hydro-quinone) (%)	Sticker (Xanthum gum) (%)	Ponga-mia oil (%)	Citronella oil (%)	Kero-sene oil (%)	Cinnamic aldehyde (%)	Anthra-quinone (%)	Eucaly-ptus oil (%)
F1	2.5	2.5	-	-	-	-	-	-	-	-
F2	2.5	4.5	-	-	5.0	0.5	17.5	-	-	-
F3	2.5	4.5	-	-	-	-	-	5.0	0.5	5.0
F4	2.5	4.5	-	0.1	5.0	0.5	17.5	-	-	-
F5	2.5	4.5	2	0.1	5.0	0.5	17.5	-	-	-

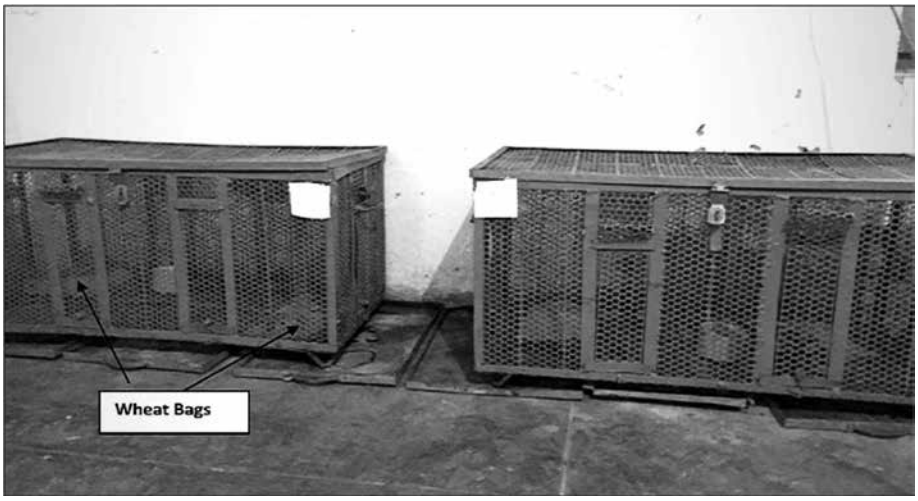

Plate 1. Breeding cages in Animal House with wheat bags kept at extreme ends for testing

and photo degradation. As mentioned earlier 1% xanthan gum (sticker) and 2% tert-butyl hydroquinone (photo stabilizer) added in F2 for preparing formulation F4 and F5 was to increase the efficacy of the repellent further.

Seven small stacks of 2 bags (each bag weighing 2kg) were installed. Out of the seven small stacks, one was kept as untreated stack, on three small stacks, F2, F4 and F5 were sprayed directly on bags while on remaining three small stacks, wood waste treated with F2, F4 and F5 (F2+WW, F4+WW and F5+WW) was kept. After the application of formulations, all the stacks were covered with tarpaulin. One rat (weighing more than 150g) was released in the rattery chamber for each replication (n=3). Damage caused by rats was recorded after every 5 days as given above.

Evaluation of effective formulation(F2) with different delivery systems in the grain store

Effective formulation F2 determined from the previous experiments conducted in breeding cages and rattery along with wood waste and two more delivery systems (Wheat straw and maize cobs) were tested in grain store at village Sherpur, Jagraon in district Ludhiana. Three godowns with severe rodent infestation were selected in this store. 7-10 large stacks of 50 kg gunny bags filled with wheat were stored in each godown. Each stack consists of 2650 - 2800 gunny bags covering the 60 sq m area. As it was not possible to record rodent damage from these large stacks, therefore small stacks were constructed in each selected godown. Four small stacks with 2 wheat bags each weighing 50 kg were installed in each godown for this study. Three small stacks installed in godowns were treated with F2 applied in three different modes of applications/delivery system i.e. wheat straw (WS), maize cobs (MC), and wood waste (WW) while one small stack in godowns was kept untreated (Plate 2). Formulation F2 was sprayed on each mode of application/delivery system and kept on small stacks installed in godowns. Rodent population was recorded before treatment by the bait census method. For this, plain bait was kept under each small stack and large stack @50 g/point and leftover bait was collected after five days to record the consumption. Consumption was recorded to confirm the presence of rodents in each godown. The effect of treatments was recorded after every 7 days for 35 days. Rodent species visiting stacks were also confirmed from faecal pellets present on bags. Rodent damage was recorded in terms of number and size of cuts (cm^2) on bags of small stacks.

Statistical analyses

Values were calculated as mean ± SE. Data collected for the dependent variables like number of cuts, size of cuts, consumption, spillage and contamination of grains using factorial completely randomized design was subjected to analysis of variance using PROC GLM procedure of statistical software SAS 9.3. Tukey's multiple comparison method was applied to compare the significant difference among different formulations for different parameters at $p \leq 0.05$.

RESULTS AND DISCUSSION

Evaluation of the efficacy of formulations F1 – F3 as repellent in Animal House

Repellent efficacy of three formulations (F1-F3) tested in breeding cages under bi-choice simulated storage conditions against both male and female house rats (n=3 for each sex) revealed that the odour on stacks treated with formulation F1, F2, and F3 retained for 12, 21, and 15 days respectively. All three formulations were prepared using different essential

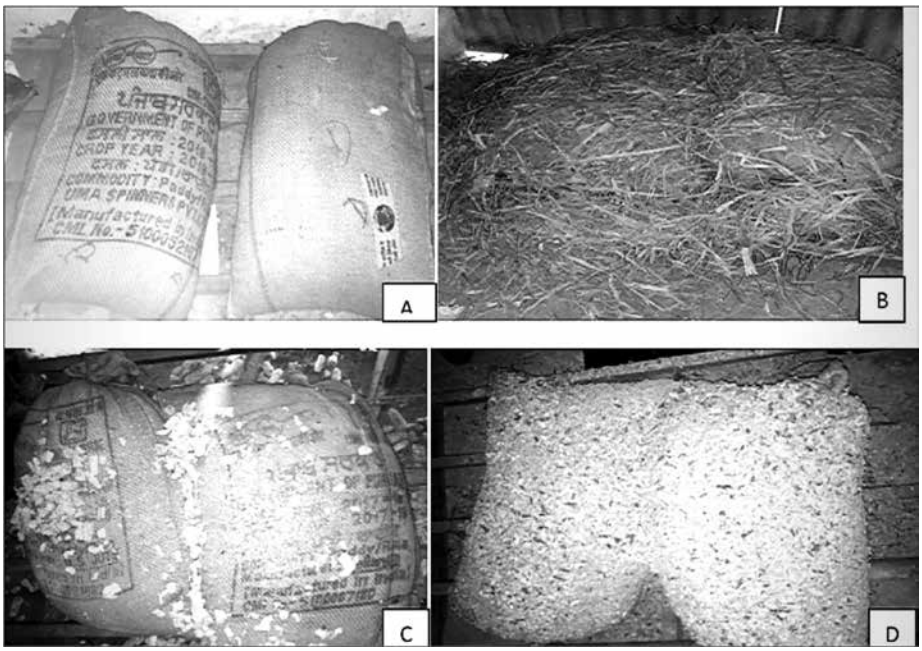

Plate 2. Bags treated with F2 using different modes of applications: A-Control B-Formulation F2+Wheat straw (WS), C-Formulation F2+Maize cobs (MC0< D-Formulation F2+WW (Wood waste)

oils/compounds. The odour of oils is mainly due to volatile organic compounds, which dissipate with time resulting in the reduction in the intensity of their odour with an increase in the treatment period (Singla and Kaur, 2014). Moreover, essential oils also degrade when exposed to various environmental factors (Avery, 1992). However, the odour of formulation F2 was retained for maximum time (21 days) that might be due to the synergistic effect of different essential oils and compounds added in this formulation (Bai, 2008).

Results also revealed a significant reduction ($p \leq 0.05$) in the number and size of cuts on treated bags as compared to untreated bags with all the three formulations tested. It might be due to the repellent effect of compounds/oils used in these formulations. Formulation F1 prevented rodent damage up to 3 days, F2 up to 15 days, and F3 up to 6 days. However, rodent damage was recorded on all the untreated bags during the first observation after three days of treatment onwards. A total number of cuts and cut sizes were maximum on bags treated with formulation F1 and F3 with complete damage to bags after 15 and 18 days, respectively and minimum on bags treated with formulation F2 even after 21 days of treatment (Table 2).The odour of formulation F2 was also retained for a maximum period, thus prevented rodent damage for longer duration as compared to F1 and F3. Damage to untreated bags was also less in laboratory pens, where on one side bags were treated with F2. This might be due to percolation of smell from treated to untreated chamber.

Earlier studies conducted in our laboratory had revealed both primary (olfactory and gustatory) and secondary (causing gastrointestinal distress) repellent effects of methyl anthranilate (2.5%), which has been used in all the formulations i.e., F1 to F3 in the present study (Bala, 2018; Bala and Babbar, 2019; Kaur, 2019). Reduction in the odour of a formulation on treated bags resulted in a reduction in repellent efficacy, indicating the olfactory repellent effect is playing a major role in preventing damage. Bai (2008) also reported that rodent repellent formulation containing pongamia oil, citronella oil, methyl anthranilate, and kerosene oil provides repellency against rats and mice for 7-15 days.

Percent consumption and spillage due to rodent damage from untreated bags and bags treated with different formulations were significantly ($p \leq 0.05$) different. However, extent of wastage (both consumption and spillage) was at par among different treatments. Percent consumption and spillage were minimum from bags treated with formulation F2 even after 21 days of treatment followed by F1 and F3 and was maximum from untreated bags (Table 3). Data indicated that percent consumption from F2 treated bags was significantly less compared to F1 and F3 (Fig. 1). There was a significant ($p \leq 0.05$) difference in percent contamination to wheat grains collected from untreated and treated bags. However, there were non-significant ($p \leq 0.05$) differences in contamination on wheat grains collected from bags treated with F1 and F3 but significant difference between F1 and F2 as well as between F2 and F3 indicating significantly reduced contamination

Table 2. Efficacy of three repellent formulations (F1, F2 and F3) on rodent damage to grain stacks undercaged conditions

Formulation	Treatments (n=6; 3 for each sex)	Number of cuts							Total number of cuts	Total cut size (cm^2)
		After 3 days	From 4-6 days	From 7-9 days	From 10-12 days	From 13-15 days	From 16-18 days	From 19-21 days		
F1	UT	1.00± 0.00	0.33± 0.19	0.67± 0.19	0.33± 0.19	Complete damage	-	-	2.33± 0.38^{bc} up to 12 days	2.30± 0.47^{abc}
	T	0.00± 0.00	0.33± 0.19	0.50± 0.20	0.50± 0.20	Complete damage	-	-	1.33± 0.19^{ab} up to 12 days	0.97± 0.19^{ab}
F2	UT	0.67± 0.19	1.00± 0.00	0.33± 0.19	0.67± 0.19	0.16±0.15	0.50± 0.20	0.50± 0.20	3.83± 0.28^{d} up to 21 days	3.79± 0.45^{c}
	T	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00±0.00	0.16± 0.15	1.00± 0.00	1.16± 0.15^{a} upto 21 days	0.55± 0.26^{a}
F3	UT	0.67± 0.19	1.00± 0.00	0.33± 0.19	0.16± 0.15	0.50±0.20	Complete damage	-	2.67± 0.19^{c} up to 15 days	2.67± 0.58^{bc}
	T	0.00± 0.00	0.00± 0.00	0.33± 0.19	0.67± 0.19	0.50±0.20	Complete damage	-	1.50± 0.20^{ab} up to 15 days	1.20± 0.32^{ab}

Values are mean±SE; UT- Untreated; T- Treated
a,b,c shows significant difference among different treatments along the columns.

of wheat grains collected from bags treated with F2 (Fig. 2). Maximum percent contamination (68.22±3.77) was recorded on wheat grains collected from untreated bags and minimum (12.33±2.60) on the grains collected from bags treated with formulation F2 (Table 3).

Thus, the experiments under simulated storage conditions in breeding cages revealed F2 to be the most effective formulation in preventing rodent damage up to15 days. It might be due to the synergistic effect of different repellents like methyl anthranilate, pongamia oil, and citronella oil using Tween 20 as an emulsifier, which reduced the volatility of oils and thus retained the odour of F2 formulation on treated bags and thus prevented the rodent damage for a longer duration. An earlier study also reported that a combination of essential oils was more effective as repellent than a single compound (Noosidum *et al.,* 2014). Many methods have been described for the improvement of repellent efficacies of essential oils. The most general method to increase the effectiveness of a repellent is to combine several essential oils from different plants, leading to a synergistic effect (Harris, 2002). A combination of black pepper oil and carrot seed oil was also reported as an ecologically best deterrent against

Table 3. Efficacy of three repellent formulations (F1, F2 and F3) on rodent damage to grain stacks under caged conditions

Treatment	Consumption of grains (%)	Spillage of grains (%)	Wastage due to rodent damage (Consumption + spillage) (%)	Contamination of grains due to rat urine (%)
Control	54.17±1.19^{a}	40.75±2.68^{a}	94.92±3.08^{a}	68.22±3.77^{a}
F1 (After 12 days)	39.08±0.64^{b}	25.88±2.96^{ab}	64.96±2.61^{a}	39.33±2.45^{b}
F2 (After 21 days)	27.76±2.88^{c}	24.98±2.3^{b}	52.75±4.71^{a}	12.33±2.60^{c}
F3 (After 15 days)	36.75±1.11^{b}	29.01±6.67^{ab}	65.76±7.06^{a}	30.67±3.08^{b}

Values are Mean ± SE
^{a,b,c} shows significant difference among different treatments along the columns.

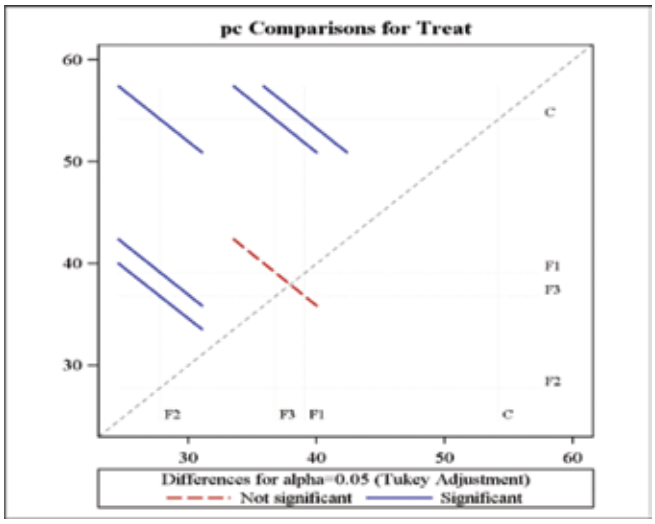

Fig. 1. Comparison for percent consumption (pc) of grains among treatments

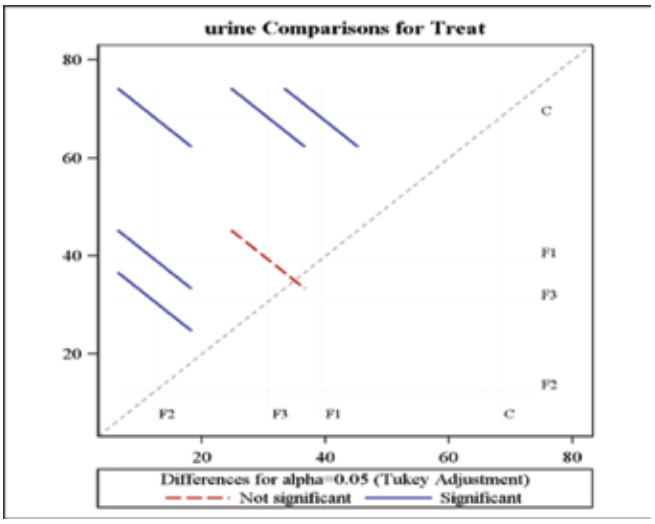

Fig. 2. Comparison for percent contamination(urine) of grains among treatments

common voles, *Microtis arvalis* than a single compound (Schlötelburg *et al.,* 2018).

Evaluation of effective and stable formulations with different delivery systems in rattery

Based on the efficacy of formulation F2 in the previous experiments, the trials with this formulation were conducted in rattery under natural conditions for further investigation along with other two formulations (F4 and F5), which were prepared from F2 only by adding 0.1% Xanthum gum as stickers (in F4) and by adding both 0.1% sticker and 2.0% photo stabilizer (in F5 only) to prevent degradation due to environmental factors like sunlight and rainfall. Two modes of delivery of treatments i.e. direct spray on bags and spray on wood waste (WW) were employed.

Results revealed that the odour of formulation F2 was retained for a longer period when sprayed on wood waste. Maximum smell intensity was recorded on bags treated with F2+WW and minimum on bags treated with F4 and F5. These results indicated that the addition of stickers and photo stabilizers had no effect on increasing the stability of repellent formulation. However, spraying of F2 on wood waste retained the repellent odour on treated bags for a longer duration. It is the first study in which the efficacy of repellents against rodents was enhanced by mixing different repellents in F2. The efficacy of formulation (F2) was further enhanced by using wood waste as carrier, which retained the odour of oils for longer duration and thus increased its efficacy against rodents.

The total number of cuts by rats reduced significantly ($p \leq 0.05$) on treated bags as compared to untreated bags (Table 4). However, all the treatments were significantly

Table 4. Efficacy of repellent Formulation F2, F4 and F5 applied as direct spray on bags and through use of Wood waste as delivery systems on extent of rodent damage in experimental rattery

Treatment (n=3)	Number of cuts						Total number of cuts	Total cut size (cm^2)
	From 1-5 days	From 6-10 days	From 11-15 days	From 16-20 days	From 21-25 days	From 26-30days		
Control	1.00±0.00	1.00±0.47	1.00±0.47	1.33±0.27	0.00±0.00	1.00±0.47	5.33±1.68^{b}	5.97±3.10^{b}
F2	0.00±0.00	0.00±0.00	0.00±0.00	0.67±0.27	0.33±0.27	0.00±0.00	1.00±0.54^{a}	0.43±0.26^{a}
F2+WW	0.00±0.00	0.00±0.00	0.00±0.00	0.33±0.27	0.00±0.00	0.66±0.27	1.00±0.54^{a}	0.20±0.09^{a}
F4	0.00±0.00	0.00±0.00	1.00±0.00	1.00±0.00	0.33±0.27	0.00±0.00	2.33±0.27^{a}	2.68±1.02^{a}
F4+WW	0.00±0.00	0.00±0.00	0.67±0.27	0.33±0.27	0.67±0.27	0.00±0.00	1.67±0.81^{a}	1.79±0.95^{a}
F5	0.00±0.00	0.00±0.00	1.00±0.00	0.67±0.27	0.00±0.00	0.67±0.27	2.34±0.54^{a}	2.94±1.90^{a}
F5+WW	0.00±0.00	0.00±0.00	0.67±0.27	0.33±0.27	0.00±0.00	0.00±0.00	1.00±0.54^{a}	0.63±0.47^{a}

Values are Mean±SE; WW-Wood waste

^{a,b,c} shows significant difference among different treatments along the columns.

Table 5. Efficacy of repellent formulation F2, F4 and F5 applied as direct spray on bags and through use of Wood waste as delivery systems on consumption, spillages and contamination of stored wheat grains in experimental rattery

Treatment (n=3)	Consumption of wheat grains (%)	Spillage of wheat grains (%)	Wastage due to rodent damage (Consumption + spillage) (%)	Contamination of grains due to rat urine (%)
Control	8.00±1.08[a]	7.58±1.18[a]	15.58±2.08[a]	87±2.12[a]
F2	4.03±0.14[b]	4.02±0.20[b]	8.05±0.31[b]	32±1.41[b]
F2+WW	0.75±0.11[c]	0.40±0.08[c]	1.16±0.20[c]	08±5.65[c]
F4	5.01±0.33[b]	4.90±0.64[ab]	9.91±0.98[b]	40±4.41[b]
F4+WW	4.45±0.14[b]	4.45±0.40[ab]	8.89±0.55[b]	31±0.70[b]
F5	4.61±0.25[b]	3.98±0.26[b]	8.59±0.19[b]	40±1.41[b]
F5+WW	4.05±0.07[b]	3.68±0.36[b]	7.74±0.44[b]	36±2.82[b]

Values are Mean ± SE; WW-Wood waste
[a,b,c] shows significant difference among different treatments along the columns.

at par in recording total number of cuts. Treatment with formulations F2 and F2+WW though prevented rodent damage for up to 15 days, whereas it was only for 10 days with other treatments like, F4, F4+WW, F5, and F5+WW. Further probe into results revealed that total number of cuts was maximum on bags treated with formulation F5 (2.34**±0.54**) and minimum in case of bags treated with F2, F2+WW, F5+WW (1.00**±0.54**). Results revealed that the addition of photo stabilizer and sticker had not improved the repellent efficacy of treatments. However, the mode of application of F2 has improved its efficacy and prolonged the duration of prevention of rodent damage (Table 4).The observations on effect of these treatments on total cut size also showed similar trends where it was maximum on bags treated with formulation F5 (2.94**±1.90**) and minimum on bags treated with formulation F2+WW (0.20±0.09) after 30 days of treatment. In general, none of the treatments had any significant difference amongst each other (Table 4). Percent consumption, spillage, and wastage from untreated bags were significantly more ($p \leq 0.05$) as compared to bags treated with different formulations (Table 5).

Among the treatments, application of F2 through WW (F2+WW) was significantly superior ($p \leq 0.05$) over other treatments in reducing the extent of consumption, spillage, wastage and contamination by rodents in rattery. Other four treatments were significantly at par in all these test parameters (Table 5; Fig. 3).These results further indicated that the odour of F2 is retained for the maximum time when delivered with wood waste. It might be due to the relatively slow release of repellents from wood waste, which maintains the odour of F2 on wheat bags resulting in the prevention of rodent damage to the wheat bags treated with F2+WW for a longer duration. Bai (2008) also reported the efficacy of methyl anthranilate in preventing rodent damage when mixed with pongamia oil, citronella oil, and kerosene oil against rodents. Earlier ultrasonic repellers came on to the markets for use in premises in both India and other countries against rodents but rodents developed habituation against them (Lund, 1988). Similarly, numerous chemicals were also evaluated for their repellent action (Rana *et al.,* 1994) but in practice no single chemical is available commercially as a repellent. It might be due to low efficacy of these repellents when applied under field conditions.

Various methyl anthranilate formulations were also found effective as a repellent against birds (Curtis *et al.,* 1994, Lewis, 1996). Different formulations of 2.5%

Table 6. Evaluation of repellent formulation F2 using three delivery systems in grain stores

Treatment (n=2)	Number of cuts					Total number of cuts	Total cut size (cm^2)
	After 7 days	From 8-14 days	From 15-21 days	From 22-28 days	From 29-35 days		
Control	1.00±0.00	2.5±0.86	3.00±0.57	1.00±0.00	1.00±0.00	8.50±1.43[b]	19.25±6.63[b]
F2+WS	0.00±0.00	0.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00	2.00±0.00[a]	1.22±0.81[a]
F2+MC	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.00	1.00±0.00	2.00±0.00[a]	1.85±0.65[a]
F2+WW	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00[a]	0.00±0.00[a]

Values are Mean ± SE; WS- Wheat straw; MC- Maize cobs; WW- Wood waste
[a,b,c] shows significant difference among different treatments along with the columns.

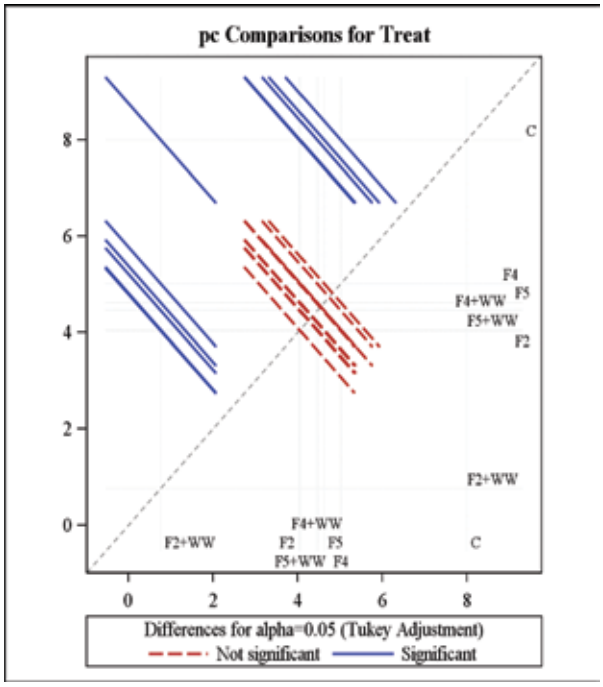

Fig. 3. Comparison for percent consumption (pc) of grains among treatments

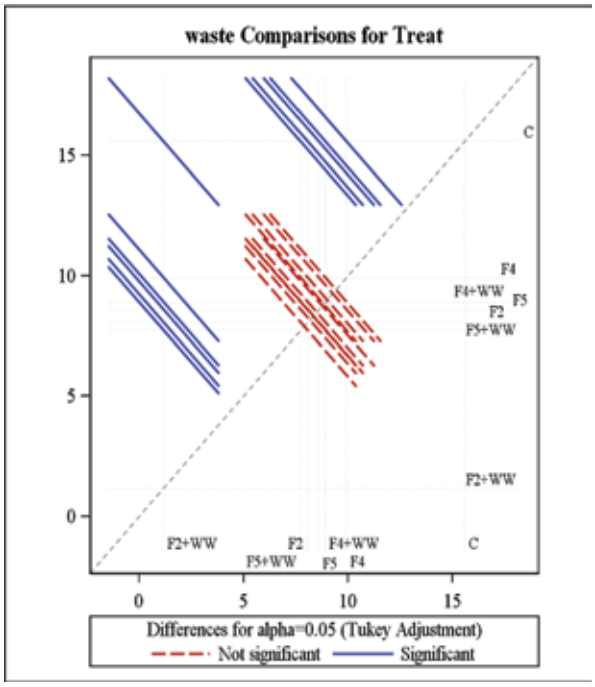

Fig. 4. Comparison for percent contamination (urine)of grains among treatments

MA were also reported to prevent rodent damage from treated stacks for 9-28 days under both indoor and outdoor conditions (Bala and Babbar, 2019). All these tests were performed by applying formulation directly on the treated area resulting in prevention from damage for a shorter duration. During the present investigation, it was observed that the application of formulation/ repellent on an adsorbent (WW) could enhance the persistence of repellent.

Evaluation of effective formulation F2 with different delivery systems in grain stores

Under this experiment, the most effective repellent formulation (F2) was evaluated under grain stores at village Sherpur, Jagraon, in district Ludhiana by spraying on three different delivery systems i.e., Wheat straw (WS); Maize cobs (MC) and Wood waste (WW). Results revealed that the odour of formulation F2 was retained for a longer duration on bags treated with F2+WW and F2+MC (up to 35 days). The presence of faecal pellets on untreated stacks during the first visit after treatment indicated rodent infestation; however, faecal pellets were not seen on the bags treated with F2+WS, F2+MC and F2+WW up to 21, 28 and 35 days, respectively indicating rats avoided treated bags which was more persistent with F2+WW treatment.

Likewise total number of cuts reduced significantly ($p \leq 0.05$) on treated bags as compared to untreated bags, however no significant difference was noticed among treatments. However, the formulations F2+WS and F2+MC prevented rodent damage for up to 20 and 27 days, respectively. However, no cuts were recorded on bags treated with formulation F2+WW even after 35 days of treatment, indicating wood waste is more effective than other modes of application in preventing rodent damage. Among treatments, the maximum total cut size was recorded on bags treated with formulation F2+MC (1.85±0.65). Cuts were not seen on bags treated with F2+WW, even up to 35 days of treatment. From these results, presented in Table 6, it may be inferred that wood waste was the best mode of application and prevented rodent damage for a long duration.

Earlier reports of Bala and Babbar (2019) also indicated that 2.5% MA-based formulations applied on stacks prevented rodent damage for up to 28 days. However, during the present investigation, no damage was recorded on stacks even after 35 days of treatment when F2 applied on wood waste was kept on stacks. The cost of F2 formulation (the minimum effective concentration) per sqm area comes out to be Rs 20 (the US $ 0.26), which may be considered to be cost-effective considering the extent of loss caused by *R. rattus* in storage through direct damage and contamination of food. The cost of one stack of wheat bags is Rs 20 lakh, and rodents are responsible for causing damage to the lower two layers of stacks, which costs about 2 lakh. The cost-effectiveness of a pest management tool in grain stores depends upon the value of crop stored, level of damage without treatment, and percent damage reduced after treatment. Hansen

et al. (2016) reported that plant secondary metabolites can repel several rodent pest species but lack of transition of laboratory studies to the field is the likely reason why only few repellents are registered for rodent management. As F2+WW prevented rodent damage for long duration even in open area, its utilization in field conditions along with other rodent pest management methods can be a breakthrough in eco-friendly rodent pest management programme.

From the above results, it is concluded that methyl anthranilate based formulation F2 prevented rodent damage for upto 15 days under simulated storage conditions in breeding cages, while F1 and F3 prevented damage for 3 and 6 days, respectively. Addition of photostabilizer and sticker in F2 could not increase the duration for prevention of rodent damage. However, adsorption of F2 on wood waste slowed down the release of repellents and considerably increased the duration of prevention of rodent damage. F2+WW prevented rodent damage for 15-35 days under simulated storage conditions and in grain stores.

Authors' contribution

Conceptualization of research work and designing of experiments (BK); Execution of field/lab experiments and data collection (RK, BK); Analysis of data and interpretation (AK, RK); Preparation of manuscript (BK, RK)

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