

Evaluation of Attractiveness and Volatile Profiling of Food Baits for Monitoring of Stored Product Pests in Paddy

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Abstract: Food baits are one of the strategies for monitoring and mass trapping of the stored product insects. Based on this principle, a study was conducted at central farm storage godown, Agricultural College and Research Institute, Madurai during March-April, 2021 by placing wheat flour, sorghum flour, pearl millet flour, rice flour, cracked corn, crushed groundnut, rice bran + rice flour as luring materials. Observations on trapped adult insects were taken on 25 days after placement of bait traps. Amongst the baits tested, wheat flour, cracked sorghum, pearl millet flour were the most attractive for stored grain pests of paddy. Wheat flour attracted 21.01% of Angoumois grain moth, *Sitotroga cerealella* and 13.38% of rice weevil, *Sitophilus oryzae*. Cracked sorghum registered attraction of 10.93% lesser grain borer, *Rhyzopertha dominica* and 13.10% of red flour beetle, *Tribolium* spp. Pearl millet flour attracted 11.65% of saw-toothed grain beetle, *Oryzaephilus surinamensis*. These effective baits were also test verified through four-arm olfactometer and found the highest orientation in the arm containing wheat flour by attracting *S. oryzae*, *Tribolium* spp. and *R. dominica* of 43.19, 39.61 and 37.41% respectively which possessed nonane, undecane, 3-octen-1-ol, butanal and pentanal volatile compounds. Therefore, the wheat flour may be exploited as an effective bait material for monitoring and mass trapping of *S. cerealella*, *R. dominica*, *Tribolium* spp. and *O. surinamensis* in storage godowns of paddy.

Keywords: Bait traps, Olfactometer, Rice godown, Stored product insects, Wheat flour, Volatile compounds

Rice is one of the most important food crops for more than half of the world's population. Losses in storage due to insects greatly influence food availability. Most commonly the insects, birds, mites, fungi, rodents and moisture are the major problems in storage godowns causing damage to rice. Stored product insects particularly adult beetles, due to their harbourage seeking behaviour, seek refuge in cracks and crevices of warehouse and storage godown. Lesser grain borer, Rhyzopertha dominica (Fabricius) (Bostrichidae; Coleoptera), rice weevil, Sitophilus oryzae (L.) (Curclionidae; Coleoptera), red flour beetle, Tribolium spp. (Tenebrionidae; Coleoptera), and Angoumois grain moth, Sitotroga cerealella (Olivier) (Gelechidae; Lepidoptera) are the most destructive insects which are found in stored cereals, flour mills, and storage godowns/warehouses (Kim et al 2010, Ahmed and Raza 2010, Duehl et al 2011, Ahmad et al 2013). Food attractant and repellent are used to manage several stored product insects (Mohan and Fields 2002). The food bait material used for detecting stored product insects may be a liquid or solid. Volatile chemical olfactory cues play an important role as attractants and diversity of substances such as kairomone can be exploited for the management of stored product insects (Mahroof and Phillips 2007). The granary weevil, Sitophilus granarius is the most widely studied storage insect species concerning its response to kairomone, with its reaction to crushed seed or whole seed (Rietdorf and Steidle 2002). Pheromones are normally species specific and a lot of insect species feed on one food stuff due to the volatiles from this food attract more than one species (Collins et al 2007). Combining food volatiles and pheromone odour can increase the pitfall traps efficiency against *Sitophilus* spp. (Likhayo and Hodges 2000, Wakefield et al 2005). Accordingly, the present study is aimed to exploit easily available, cheap, effective and attractive bait sources for the management of major pests in stored paddy.

MATERIAL AND METHODS

Trapping efficiency of different food baits: The test was conducted at Central Farm storage godown (14.5 x 6.5 x 3.5 m), Agricultural college and Research Institute, Madurai. The crushed grains and flours of wheat, sorghum, maize, groundnut, rice, rice bran and pearl millet were taken and filled in the polythene receptacles of probe trap-like structures, which contain rustproof silver metal hollow cylinders (23 x 5 cm) with 280 evenly spaced 4 mm holes. One end of the trap was closed by a removable cap. The bait traps were inserted in the interspaces between the bags arranged in a stack and the trap was filled with 50 g of bait material. The insects were crawled by orientation through the 4 mm hole and dropped into the bottom part of the receptacle.

The trap catches were recorded for 25 days. The number of insects caught in control (without bait material) was compared with the number of insects caught in the other treatments. The attraction index was calculated by using the formula (Smith et al 1993).

Attraction Index =
$$\frac{T-C}{N} \times 100$$

Where, N- Number of insects attracted in treatment, C-Number of insects trapped in the control, N- Total number of individuals

Olfactometer bioassay: To study the chemoreception and attraction an experiment was conducted using specially designed four-star shaped olfactometer made of plastic (25 x 25 x 11.5 cm), with a 2.5 cm diameter opening in four corners. Each corner had a small tube, through which air could go from the arena along the central tube. The corner tubes were linked to Tygon tubing with four different gas washing bottles, all filled with a 10 g of olfactory stimulus (food attractants). This set-up provided the insects to recognize the olfactory cues of the food attractants. Air was blown to each of the four arms by the pump. The odour residue in the arena of the tubes was cleaned with 70% ethanol (Arnold et al 2012). The test adults viz., R. dominica, Tribolium spp. and S. oryzae were starved for 24 h in Petri plates before the commencement of olfactory bioassay. Twenty unsexed adults were released in the centre of the olfactometer (7 mm hole) and it was covered with cloth to minimise the phototactic response of insects. After 10 minutes, the location of the insects was observed (Ukeh et al 2010). Three different best odour/volatile sources were used to observe the response of R. dominica, Tribolium spp., S. oryzae. Each treatment was replicated 10 times.

Volatile Profiling of Food Baits in GC-MS/MS

Sample preparation: Spectroscopic analysis of food baits was done with Gas chromatograph-mass spectrometer (GC-MS/MS) to find out the chemical cues. For this purpose, a fresh sample of the selected food bait was dried and ground into powder. Samples (10 g each) were extracted with 30 ml of methanol in an ultrasonic bath for 30 min and filtered through a 0.45 μ m polyvinylidene fluoride syringe filter for GC-MS/MS analysis (Kim et al 2020)

GC- MS/MS analysis: The methanol extract was characterized using GC-MS/MS (GC 2010 plus, GCMS – TQ 8040 SHIMADZU), in Central Instrumental Laboratory, Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai. The compounds were separated on the capillary column (Rxi° - 5 Sil MS). The carrier gas was helium (purity percentage > 99.99%) with a column flow rate of 1ml/min and the injection was done in split less mode. The oven temperature was scheduled at 110°C,

which was constantly increased to 150°C at a rate of 10°C/min and held for 5 min, then increased to 200°C at a rate of 10 °C/min. After holding 20 min again, it was finally increased to 240°C at a rate of 10°C /min and held for 5 min. The MS was operated in electron ionization (EI) mode at 70 eV, ion source temperature 200°C, interface temperature was 230°C and scan range was 45–600 m/z. The solvent cut time was 3 min. Each sample was allowed to run for 30 minutes. The spectrum of the unknown volatile compounds was identified by the NIST 17 (National Institute of Standards and Technology) MS library database. The obtained compounds were tabulated along with the per cent area of the peaks and retention time (RT).

Statistical analysis: The attraction index and the difference in the behavioural response/orientation of the beetles were analysed using Completely Randomized Design by using Duncan's Multiple Range Test (DMRT) with IBM SPSS 22.0 software.

RESULTS AND DISCUSSION

The attraction of S. cerealella R. dominica, Tribolium spp., S. oryzae, O. surinamensis adults varied with food attractants (Table 1). Among the food bait attractants tested, wheat flour (21.01%) was the most attractive to S. cerealella followed by pearl millet flour, rice bran and the least attraction was noticed in crushed groundnut (4.62%). For R. dominica, the cracked sorghum was highly attractive (10.93%) followed by pearl millet flour, cracked corn and the least attraction with rice flour (4.04%). Cracked sorghum (13.10%) was highly attractive to Tribolium spp. followed by sorghum flour, wheat flour and the least attraction was in cracked corn (3.35%). The attraction of S. oryzae to wheat flour and sorghum flour was high (13.27 and 11.67% respectively) followed by cracked sorghum, pearl millet flour and crushed groundnut. Pearl millet flour was highly attractive for O. surinamensis (11.65%) followed by rice bran (7%), sorghum flour, cracked sorghum. wheat flour and the least attraction with cracked corn (2.10%). Wheat flour attracted more S. oryzae (13.38%) compared to other food attractants. Earlier studies indicated that larger grain borer, Prostephanus truncatus and R. dominica had a positive attraction towards cereal host odour (Bashir et al 2001, Edde and Phillips 2006). The cracked wheat alone attracted Sitophilus zeamais six times more than S. oryzae (Likhayo and Hodges 2000). The maximum attraction of R. dominica was due to some odour/volatile produced by cracked sorghum (10.93%) and S. cerealella to wheat flour (21.01%) and O. surinamensis to pearl millet flour (11.65%). Ahmad et al (2013) who reported that R. dominica had been attracted more to wheat flour. The comparative efficacy of different food baits to various storage

insect pests revealed that the wheat flour was more attractive to *S. cerealella, S. oryzae and O. surinamensis* sorghum flour/ cracked sorghum to *R. dominica, S. oryzae, Tribolium* spp. and *O. surinamensis* and the pearl millet flour to *S. cerealella, R. dominica, S. oryzae* and *O. surinamensis* (Fig. 1). The total attraction index of wheat flour > sorghum flour > cracked sorghum > pearl millet flour was found to be 56.60, 45.73, 45.23 and 43.71% respectively (Table 1). Proving that wheat, sorghum and pearl millet flours were effective attractants to different stored grain pests of paddy.

Olfactometer bioassay: Olfactometer bioassay revealed the significant differences in the orientation behaviour of *R. dominica, Tribolium* spp. and *S. oryzae* towards food bait attractants in a four-armed olfactometer. Among the three food baits tested, the highest attraction (43.19%) of *S. oryzae*

was in the arm containing wheat flour followed by *Tribolium* spp. and *R. dominica*. Sorghum flour attracted *S. oryzae*, *Tribolium* spp. and *R. dominica*. The least attraction was in pearl millet flour with 21.83, 18.07, 17.62% of *S. oryzae*, *Tribolium* spp., *R. dominica*, respectively (Fig. 2).

The orientation response of *R. dominica* to wheat volatiles in two-arm olfactometer bioassay was proved by Dowdy et al (1993). However, in our study a four-arm olfactometer bioassay, multi choice test a greater number of *S. oryzae* (43.19%), *R. dominica* (37.41%) and *Tribolium* spp. (39.61%) was attracted to the test arm containing wheat flour. *R. dominica* spent more time in the olfactometer arm containing de-oiled groundnut odour/volatile compared to the arm contained maize or wheat odour/volatile (Bashir 2000). In the present study also *R. dominica, Tribolium* spp. and



🛛 Sitotroga cerealella 🔍 Rhyzopertha dominica 🔍 Tribolium spp. 🖿 Sitophilus oryzae 🖉 Oryzaephilus surinamensis

Fig. 1. Response of stored product insects to various food attractants in bait traps

Attractants	Relative attraction index (%)					Total attraction index (%)
	Sitotroga cerealella	Rhyzopertha dominica	<i>Tribolium</i> spp	Sitophilus oryzae	Oryzaephilus surinamensis	_
Crushed groundnut	4.62 ^g	9.31 ^b	8.07 ^d	2.59 ^{de}	2.99 ^{cd}	27.58
Wheat flour	21.01ª	7.42°	8.93°	13.27ª	5.97 ^b	56.60
Cracked corn	7.09 ^f	8.48°	3.35 ^h	5.51 ^{bcd}	2.10 ^{de}	26.53
Sorghum flour	7.50°	7.75⁴	11.94 [⊳]	11.67ª	6.86 ^b	45.73
Rice flour	7.44 ^e	4.04 ^g	5.21 ^r	7.13 ^{bc}	1.79 ^{de}	25.61
Pearl millet flour	9.27 ^b	9.34⁵	5.36 ^f	8.09 ^b	11.65°	43.71
Rice bran	8.35°	6.92 ^f	7.66 ^e	7.12 ^{bc}	7.16 [♭]	37.22
Rice bran + Rice flour	7.97 ^d	6.66 ^f	4.39 [°]	4.21 ^{cd}	3.45°	26.68
Cracked sorghum	7.12 ^f	10.93ª	13.10ª	8.10 ^b	5.98 ^b	45.23
Control	0.00 ^h	0.00 ^h	0.00'	0.00 ^e	0.00 ^e	0.00
SEd	0.1177	0.1504	0.1542	1.4152	0.6018	-

 Table 1. Comparative response of stored product insects of paddy to various food bait attractants

Mean followed by the same letter (s) in a column are not significantly different by DMRT (P=0.05)

S. oryzae spent more time in the arm containing wheat flour. Vijay et al (2020) reported that the highest orientation (50.5%) of *S. oryzae* females and males were recorded towards sorghum in 20 minutes after release. In present study the orientation of *S. oryzae* towards sorghum flour (30.12%) was within 10 minutes after release. In the current study, the baits were arranged in the order of the number of visits or entries towards the wheat flour (37.41%), sorghum flour (28.75%) and pearl millet flour (17.62%), which are comparable with earlier works. Behavioural response of *R. dominica* to host plant (maize grain and winter wheat grains) bioassay indicated that both male and female *R. dominica* attracted to maize and wheat grain than the control (Ukeh and Umoetok 2007).

GC- MS/MS: The separation of the volatile compound was obtained using the $R_x i - 5$ Sil MS capillary column. There were over 89, 85 and 80 peaks on the chromatogram of wheat, sorghum and pearl millet respectively (Fig. 3). The major attractive volatile compounds present in the three flours analyzed were hexanol, butyric acid, propionic acid, nonane, undecane, 3-octen-1-ol, hexanal, nonanal, octanal, butanal and hexanoic acid (Table 2).

The food bait attraction to S. cerealella and S. oryzae in wheat flour may be due to the presence of volatile compounds like nonane, undecane, butanol and 3-octen-1-ol which were found in the present analytical study. E-2nonenal and 4-ethylacetophenone compounds induced positive response from O. surinamensis, Sitophilus granarius and Cryptolestes ferrugineus in accordance with earlier findings (Collins et al 2007, Dooley et al 2018). Hexanoic acid, 2-phenylethanol and E-3-octen-2-one had elicited a response for O. surinamensis and S. granarius (Collins et al 2007). The present investigation corroborates with other studies where the chemical compounds like hexanol and butyric acid were found to be the attractive volatile compounds present in sorghum, which resulted the orientation of R. dominica and Tribolium spp. (Giliomee et al 2007, Collins et al 2007).

Hexanal, nonanal, undecane, octanal, butanal and hexanoic acid were identified as attractive compounds (Giliomee et al 2007, Collins et al 2007, Dooley et al 2018) which are responsible for greater catch of *O. surinamensis*. Different doses of benzaldehyde and aliphatic aldehyde have been tested for *O. surinamensis* and *O. Mercator* observed positive response of both species. The present investigation corroborates with other studies that hexanol and butyric acid were the attractive volatile compounds present in sorghum flour which attracted *R. dominica* and *Tribolium* spp. . Balakrishnan et al (2017) . reported a related findings that undecane, octanal, 1-hexen-3-ol, 2-heptanone, ethyl

hexanoate and hexanoic acid had elicited the strongest electroantennographic (EAG) responses to *Tribolium castaneum*. Adults of *Callosobruchus sinensis* showed preferential behaviour towards benzaldehyde and 2-hexanal (Wang et al 2020) while *Callosobruchus maculatus* was attracted by 3-octanol, linalool oxide, 3-octanone, nonanal and 1-octanol (Adhikary et al 2015). The results of present study is also comparable with the reports of Germinara et al (2008) that *Sitophilus oryzae* and *S. granarius* showed repellent effects towards propionic acid. Appalasamy et al (2021) reported that octadecanoic acid, pentadecanoic acid and cis- Vaccenic acid acted as repellent and insecticidal activity against termites, *Macrotermes carbonarius* and cockroaches, *Periplaneta americana*, which are in line with

Table 2. Volatile profiles of food bait attractants

Volatile compounds	Peak area (%)				
	Wheat	Sorghum	Pearl millet		
3-Hexanol	-	0.09±0.0008	-		
1-Butanol	0.25±0.004	-	-		
3-Octen-1-ol	0.24±0.006				
7-Octen-4-one	-	0.12±0.0003	0.26±0.002		
1-Octanal	-	-	0.78±0.005		
Pentanal	0.32±0.0005	-	-		
Hexanal	-	-	0.58±0.014		
Butanal	-	-	0.24±0.001		
Nonanal	-	-	1.55±0.008		
Undecane	1.73±0.029	-	0.73±0.009		
Nonane	0.3±0.005	-	-		
Propionic acid	-	0.12±0.001	0.23±0.002		
Butyric acid	-	0.10±0.001	-		
Hexanoic acid	-	-	0.57±0.001		
n-Hexadecanoic acid	12.65±0.022	15.42±0.181	20.36±0.512		
cis-Vaccenic acid	-	-	-		



Fig. 2. Behavioural/Orientation response of *Rhyzopertha dominica, Tribolium* spp. and *Sitophilus oryzae* to various food attractants



 1. Propionic acid
 2. n- Hexadecanoic acid
 3. Nonanal
 4. Nonane

 5. Hexanal
 6. 1-octanal
 7. Butanal
 8. 7-octen-4-one
 9. Hexanoic acid

Fig. 3. GC MS/MS volatile profile of wheat, sorghum and pearl millet

our investigation. In study, the cis-Vaccenic acid present in rice bran (59.06%), so attraction per cent was very low towards this bait. The n-Hexadecanoic acid was present in a lower amount in wheat (12.65%), had exhibited very high attraction per cent in wheat compared to others (Table 1 and 2).

CONCLUSION

This study emphasized the attraction efficiency of different food baits to various storage insect pests that can be used to develop a multi-species lure for the control of stored grain pests. An effective food bait lures can be formulated using multiple millet-based flours like wheat flour, sorghum flour/cracked sorghum and pearl millet flour and that can be further exploited for monitoring and mass trapping of storage insect pests in rice godowns.

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Received 28 September, 2021; Accepted 19 January, 2022

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