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Volatiles responses of adult *Sitophilus oryzae* L. feeding on sorghum and split pulses

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Abstract

Volatiles responses of *S. oryzae* to odours/volatiles from sorghum, red gram, chick pea, black gram, green gram, fried gram and lentil were compared in olfactometer bioassay. Results indicated that sorghum populations were more attracted towards sorghum followed by split pulses. Whereas pulses populations were more attracted towards red gram followed by green gram, chick pea, black gram, lentil and fried gram. The experiment was conducted using five available food volatiles standards viz., decane, tricosane, octacosane, docosane, pentacosane with untreated check using six arm olfactometer. The experiment was conducted separately for sorghum and pulse population of *S. oryzae*. The results showed significant variation in orientation behavior of *S. oryzae* towards food volatiles of *S. oryzae*. In sorghum population, maximum number of orientation was recorded in decane followed by tricosane, octacosane and docosane. In case of pulse population, maximum number of insects orientated towards in decane followed by octacosane, tricosane and docosane. Maximum number of pulse population oriented towards in decane and octacosane when compared to sorghum population. Y tube olfactometer was used to identify the choice and no choice tests of sorghum and pulse population. All the volatile compounds attracted significantly more weevils than the control treatment. The active weevils of sorghum population were strongly attracted to decane, tricosane, octacosane and docosane respectively, whereas there was no preference for pentacosane. In case of pulse population the active weevils were strongly attracted to decane, octacosane, tricosane and docosane respectively, whereas there was no preference for pentacosane. The data show that rice weevil adults have the ability to respond behaviorally to a wide range of cereal volatiles and that responses may change as a function of concentration. The results suggest that host finding behavior of weevils will depend on the balance of positive and negative volatile stimuli from grain as the relative concentrations of volatiles may change during storage.

Keywords: Behavioural response, *Sitophilus oryzae* L., sorghum and split pulses

Introduction

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the most destructive pest of stored cereals worldwide. It is classed as a primary pest, one which can easily infest sound cereal seeds (Hill, 1990) ^[12]. It is the most cosmopolitan in nature, and causes severe losses in rice, maize, barley, wheat, and other crops (Neupane, 1995) ^[21]. Storage grain losses of major cereal crops can be attributed primarily to attack by insect pests, pathogens, and rodents. Currently, the primary means of rice weevil control in warm climates is the use of fumigants and residual chemical insecticides (Farukiet al., 2005 and Moharramipour, 2007) ^[9]. Synthetic chemical pesticides are usually applied by the farmers to reduce losses during storage (Adaneet al., 1996) ^[1]. In recent years, however, the overreliance and the use of chemical insecticides in crop pest control programs (around the world) has resulted in environmental damage, pest resurgence, pest resistance to insecticides, and lethal effects on non-target organisms. Furthermore, because of cost, these pesticides are becoming increasingly inaccessible to farmers, particularly in developing countries. This fact, combined with the consumer's demand for residue-free food, prompted researchers to evaluate other alternative reduced risk control methods for stored-grain protection. These methods include, among others, the application of chemical ecology in the control of stored-grain insects. Semiochemical-based pest management systems in stored-products are being necessitated by the withdrawal of approval for use of many synthetic insecticides and the fumigant methyl bromide. Control often depends on a sound knowledge of the ecology and on the effects of a multitude of environmental factors on the life history of a pest.

It is a primary pest of stored rice, sorghum, cumbu and maize and reports about its occurrence on legumes is scanty. Pemberton *et al.*, (1981) ^[24] studied its breeding behaviour on carob, *Ceratonia siliqua*(L.), a tree legume native to the Mediterranean region. Coombs *et al.*, (1977) ^[4] reported the successful development by Trinidad strain of *S.oryzae* on yellow split pea. Info chemical cues are generally considered to play a pivotal role in the location, evaluation and utilization of hosts by herbivorous insects (Francis *et al.*, 2005) ^[11]. Numerous species of insects have been shown to be attracted to (single or blends of) volatiles of their host plants (Visser, 1986) ^[30]. The response of maize weevil to olfactory stimuli has been demonstrated earlier and food-related odours may contain potential attractants (Honda, and Oshawa, 1990. Ben, and Xuan, 1992) ^[2, 14]. However, it is not clear whether *S. zeamais* is capable of using olfactory cues to discriminate between volatiles from suitable and unsuitable plant species during the host-finding process.

The present investigation was carried out to compare host preference of population collected from red gram dhal to other split pulses and the normal population that occurs on sorghum.

Materials and Methods

Extraction, identification and bioassay of food volatiles

The food volatiles were collected from both uninfested and infested grains of sorghum and split pulses viz., redgram, chick pea, black gram, green gram, fried gram and lentil.

Extraction of food volatiles by using Air Entrainment Technique (AET)

The experiment was carried out to collect the volatiles of both uninfested and infested grains and to identify the volatile compounds. The experimental set up is having the glass container with firmly tightened lid with two holes for inlet and outlet tube. Approximately 10 g of healthy and infested food grains were enclosed in the separate containers. The air filter with charcoal was inserted into one hole of the lid. The aquarium pump was used to release pressure on one end of the filter. The volatile collection system consists of a glass chamber (25.7 cm long and 7.6 cm ID) constructed of pyrex glass with an inlet and a ground-glass joint outlet. A glass cartridge containing 50 mg of 'Porapak Q' adsorbent was connected to the collection chamber at one end and another glass cartridge containing activated charcoal (50 mg) was connected to the other end. This unit was connected to an aquarium pump by a Teflon tube. Air was pushed through the charcoal cartridge to purify the air passing inside the chamber containing sample. The air-flow was adjusted to 600 ml./min. by a flow meter (Syntech, Hilversum, The Netherlands). The experiment was run for 24 hrs and volatiles collected on the 'Porapak Q' were eluted using 2 ml of HPLC grade hexane (Merck) solution for four times and collected in an air tight glass tubes. The entire set up was washed thoroughly with hexane solution and the unit was oven dried between experiments to make odour-free. The same process was repeated for the eluting solvent dichloro methane. The volatile compounds collected in hexane and methylene chloride were then identified by Gas Chromatograph Mass Spectrometer (GCMS).

Identification of food volatiles by using Gas Chromatography - Mass

Spectrometry (GC-MS)

Chromatographic separation was carried out with GCMS-QP (Shimadzu, 2010) plus equipped with a capillary column RXI-IMS, 30mX 0.25 mm, 0.25 mm ID. Helium (99.999% pure) was used as a carrier gas with a flow rate of 0.98 ml/min. The GC-MS

oven temperature was programmed as follows:

- i. 60 °C (hold 2 min.) at the rate of 5 °C/min.
- ii. Increased to 280 °C (hold 0min.) at the rate of 10 °C/min.
- iii. Increased to 250 °C (hold 10min.)

1	Mode	:	Split (1:5)
2	Injector Temperature	:	250°C
3	Ion source temperature	:	220°C
4	Interface temperature	:	250°C
5	Solvent out time	:	4 min.
6	Mass range	:	20- 400 m/z

Injection was performed in split less mode (1:5) and the volume was 1 µL. The mass spectra of compounds in samples were obtained by ionization voltage at 70 eV and the detector was operated in scan mode from 500 amu. The chemical constituents were identified by matching mass spectra with spectra of reference compounds in mass library of National Institute of Standards and Technology (NIST) Version 2.0. The relative amounts of individual components were expressed as percent peak areas relative to total peak area.

Bio assay of food volatiles on sorghum and pulse feeding population of *S.oryzae* by using 6 arm and Y tube olfactometer

The experiment was conducted by using the available technical standards of food volatiles such as decane, tricosane, octacosane, docosane and pentacosane purchased from Sigma Aldrich Company. The 1000 ppm (0.01g in 10 ml of HPLC grade hexane) of each volatile was used for both experiments. For both experiments 10 µl of volatile compound applied in the what man no. 4 filter paper pieces were used before release of insects. The size of the filter paper pieces was 4 x 1.5 cm. In 6 arm olfactometer 50 test insects were used to determine the preference of food volatiles. Observation was made on number of insects settled on each arm at 0, 5, 10, 15, 20, 25 and 30 MAR.

In Y tube olfactometer, individual adult female was released one at a time within the first cm of the base tube of the olfactometer, using a small size aspirator. Activated by the odour-loaded airflow and additionally motivated by the light, insects move upward. Incidentally an insect make no choice between control and treated filter paper within 3 min. this was scored as no-choice and the insects were discarded. Each treatment was replicated ten times using fresh Insects. After having tested 10 insects, the entire set-up was cleaned with ethanol for further use.

Results and Discussion

The volatile alkanes such as pentane, heptadecane, heneicosane, tetracosane, hexatriacontane, tetratetracontane and nonacosane were present in both uninfested and infested grains of sorghum. The compounds present only in infested grains were eicosane, tricosane, hexacosane, hexasiloxane and tetracontane. The alcohol compound nonanal was present only in uninfested sorghum. In case of aromatic group, naphthalene was present only in infested sorghum grains. The other volatile compounds such as hexane-2 nitro, 2 pentanone, 3- ethyl-3-methyl, butoxy butanone were present in both uninfested and infested grains. The terpene compound longifolene was present in both uninfested and infested grains. The ketone compound, pentanone was present in both uninfested and infested grain of sorghum.

In case of pulses, the most abundant volatile alkanes such as decane, pentane, nonadecane, tetracosane, hexatriacontane, tricosane, octacosane nonacosane and eicosane were present in both uninfested and infested grains. The alcohol compound such as nonanal was present in both infested and uninfested pulses, whereas octanol was present only in uninfested and infested lentil.

In case of alkene group, pentene which is most abundant in infested pulses. The other volatile compound such as hexane 2 one, hexane 2 nitro and butoxy butanone present in redgram, whereas hexane- 2 one, hexanoic acid, hexane 2, 3, 5 trimethyl, butoxy butanone and carboxylic acid were present in chick pea. The terpene compound, longifolene was present in redgram and lentil. The alkane compound such as caffeine was present only in fried gram. The ketone compound pentanone was present in both uninfested and infested grain of pulses.

The present findings are in accordance with Maga *et al.* (1978) [18] who identified the cereal food volatiles such as pentanol, propanol, methanol, hexanol, 2 hexanone, 2 octanone and methyl butanol. Nguyen, (2006) [22] reported that 1-hexanol, nonanal, 2-nonenal and bromochloro-methane were present in wheat and maize. Hougnet *et al.*, (1971) [15] identified the following compounds in maize grain such as methanol, ethanol, acetaldehyde, propanal, acetone, 2-methyl propanal, butanal, 2-butanone, 3-methyl butanal or 2-methyl butanal, pentanal, hexanal and heptanal. McWilliams and Mackey (1969) [19] also identified the compounds in wheat grain such as acetaldehyde, 2-methylpropanal, butanal, hexanal, octanal, crotonaldehyde and 3-methyl-2-butanone. Sides *et al.* (2001) [29] reported that hexanal, octanal, nonanal and decanal were identified in oat samples. Zhou *et al.* (1999) [31] identified the volatile components in oats such as hexanal, pentanol, octanal, nonanal, 3-methyl-1-butanol and decanal. In present investigation sorghum and split pulses released a range of different volatiles and some major and minor components were common in uninfested and infested grains.

In present study five available food volatile standards were used to test the behavioural response of *S.oryzae* such as decane, octacosane, tricosane, docosane and pentacosane by using six arm and Y tube olfactometer. Sorghum and pulse breeding populations was oriented towards the four compounds such as decane, octacosane, tricosane and docosane when compared to pentacosane (Fig. 1 and 2).

The experiment was conducted using five available food volatiles standards viz., decane, tricosane, octacosane, docosane, pentacosane with untreated check using six arm olfactometer. The experiment was conducted separately for sorghum and pulse population of *S.oryzae*. The results showed significant variation in orientation behavior of *S.oryzae* towards food volatiles of *S.oryzae*. In sorghum population, maximum number of orientation was recorded in decane (24.67%) followed by tricosane (17.33%), octacosane (13.33%) and docosane (2.67%) at 10 MAR. At 20 MAR, higher number of attraction was recorded in decane (37.33%) followed by tricosane (32.67%) and octacosane (21.33%). The same trend was observed at 30 MAR (Table 1).

In case of pulse population, maximum number of insects orientated towards in decane (17.33%) octacosane (14.00%), tricosane (8.67%) and docosane (4.67 %) at 10 MAR. At 20 MAR, higher number of attraction was recorded in decane (30.67%) followed by octacosane (28.67%), tricosane (19.33%) and docosane (16.00%). Similar results were observed at 30 MAR. Maximum number of pulse population oriented towards in decane and octacosane when compared to sorghum population (Table 2).

The Y tube olfactometer was used to identify the choice and no choice tests of sorghum and pulse population for the food volatiles

viz., decane, tricosane, octacosane, docosane and pentacosane. All the volatile compounds attracted significantly more weevils than the control treatment. The active weevils of sorghum population were strongly attracted to decane (90.0 %), tricosane (90.0 %), octacosane (70.0 %) and docosane (50.0 %) respectively, whereas there was no preference for pentacosane. The calculated χ^2 values were represented in (Table 2).

In case of pulse population the active weevils were strongly attracted to decane (90.0 %), octacosane (80.0 %), tricosane (70.0 %) and docosane (70.0 %) respectively, whereas there was no preference for pentacosane. The calculated χ^2 value were represented in (Table 4).

Germinara *et al.* (2008) [10] reported that many cereal volatiles elicit a significant behavioral responses of *S. granarius* to various compounds (1-butanol, 3-methyl-1-butanol, pentanal, maltol, and vanillin) acted uniquely as attractants, while twelve [1-hexanol, butanal, hexanal, heptanal, (E)-2-hexenal, (E,E)- 2,4-nonadienal, (E,E)- 2,4-decadienal, 2,3-butanedione, 2-pentanone, 2-hexanone, 2-heptanone, and furfural) acted only as repellents. However, in the case of 1-pentanol, (E,E)- 2,4-heptadienal, and phenyl acetaldehyde, granary weevils were attracted to low concentrations but repelled by higher concentrations, thus showing the ability of the species to detect and respond differentially to variations in concentration of host volatiles. Some of the volatiles that attract *S. granarius* have been identified in the aroma of freshly broken grains of several cereals (Mega, 1978) and, consequently, may act as kairomones. Pierce *et al.* (1990) [26] reported that higher concentrations of food volatiles ((E,E)-2,4-heptadienal, vanillin, and maltol) were required to elicit a response from granary beetles, *O. surinamensis* and *O. mercator*. The number of compounds might be used as kairomones by other species: pentanal, vanillin, and maltol were attractive to *S. oryzae* (Phillips *et al.*, 1993); pentanal attracted *O. surinamensis* and *O. mercator* (Pierce *et al.*, 1990) [25, 26], whereas phenyl acetaldehyde was an attractant for several lepidopteran species including *Ephestia cautella* (Walker) and *Plodia interpunctella* (Hubner) (El-Sayed, 2006 and Olsson *et al.*, 2005) [8]. Piggott *et al.* (1991) [23] reported that optimal doses of (E,E)-2,4-nonadienal, (E)-2-hexenal, 2-pentanone, 2-heptanone, (E,E)-2,4-decadienal, and furfural were as effective as the repellent control against granary weevil.

The results of this study suggest that host finding by the rice weevil is a complex process that depends on the balance of positive and negative stimuli that change over time. Consequently, a clear understanding of the biological activity of different cereal and legume volatiles, individually and in combination, might be essential for developing semiochemical based rice weevil management strategies. Furthermore impact of volatiles on insects might have on other species that utilize the same resources to ensure that compounds to repel one pest is not an attractant for another. For example, while hexanal, heptanal, and (E,E)- 2,4-nonadienal (at low doses) repel the granary weevil, they have been reported as attractants for *O. surinamensis* and *O. mercator*, two secondary pests of stored grain and processed cereals (Pierce *et al.*, 1990) [26]. Such differential responses to the same semiochemicals that originate from a heterogeneous food substrate undoubtedly play an important role in niche partitioning within the stored grain ecosystem (Phillips *et al.*, 1993) [25].

Table 1: Bioassay of food volatiles to *S.oryzae* (Sorghum population) using 6 arm olfactometer

S.No	Treatments	Number of weevils settled (%)*					
		Minutes After Release (MAR)					
		5 MAR	10 MAR	15 MAR	20 MAR	25 MAR	30 MAR
1.	Decane	12.67 (20.85) ^b	24.67 (29.78) ^b	32.00 (34.45) ^a	37.33 (37.66) ^a	37.33 (37.66) ^a	37.33 (37.66) ^a
2.	Tricosane	8.00 (16.43) ^c	17.33 (24.60) ^c	26.67 (31.09) ^b	32.67 (34.86) ^a	32.67 (34.86) ^a	32.67 (34.86) ^a
3.	Octocosane	5.33 (13.35) ^c	13.33 (21.42) ^c	16.67 (24.09) ^c	21.33 (27.51) ^b	21.33 (27.51) ^b	21.33 (27.51) ^b
4.	Docosane	2.00 (8.13) ^d	2.67 (9.40) ^d	3.33 (10.52) ^d	2.67 (9.40) ^c	2.67 (9.40) ^c	3.33 (10.52) ^c
5.	Pentacosane	0.67 (4.68) ^e	1.33 (6.63) ^d	2.00 (8.13) ^e	1.33 (6.63) ^c	1.33 (6.63) ^c	1.33 (6.63) ^d
6.	Control	2.67 (9.40) ^d	2.67 (9.40) ^d	2.00 (8.13) ^e	2.00 (8.13) ^c	2.00 (8.13) ^c	2.00 (8.13) ^{cd}
7.	Unsettled	70.00 (56.79) ^a	38.00 (38.06) ^a	17.33 (24.60) ^c	2.67 (9.40) ^c	2.67 (9.40) ^c	2.00 (8.13) ^{cd}
	SEd	1.6108	1.7786	0.9604	1.6580	1.6580	1.5429
	CD Value (0.05)	3.4452	3.8152	2.0601	3.5564	3.5564	3.3094

*Mean of three replications. Figures in parentheses are arc sin transformed values. Mean followed by same letter (s) in a column are not significantly different by DMRT (P=0.05)

Table 2: Bioassay of food volatiles to *S.oryzae* (Pulses population) using 6 arm olfactometer

S.No	Treatments	Number of weevils settled (%)*					
		Minutes After Release (MAR)					
		5 MAR	10 MAR	15 MAR	20 MAR	25MAR	30MAR
1.	Decane	11.33 (19.67) ^b	17.33 (24.60) ^b	26.67 (31.09) ^a	30.67 (33.63) ^a	30.67 (33.63) ^a	30.67 (33.63) ^a
2.	Tricosane	6.67 (14.96) ^c	8.67 (17.12) ^c	18.67 (25.60) ^b	19.33 (26.08) ^b	19.33 (26.08) ^b	19.33 (26.08) ^b
3.	Octocosane	12.00 (20.27) ^b	14.00 (21.97) ^b	26.67 (31.09) ^a	28.67 (32.37) ^a	28.67 (32.37) ^a	28.67 (32.37) ^a
4.	Docosane	4.67 (12.48) ^{cd}	4.67 (12.48) ^d	14.67 (22.52) ^b	16.00 (23.58) ^b	16.00 (23.58) ^b	16.00 (23.58) ^b
5.	Pentacosane	0.67 (4.68) ^e	1.33 (6.63) ^e	1.33 (6.63) ^d	0.67 (4.68) ^d	0.67 (4.68) ^d	0.67 (4.68) ^d
6.	Control	2.67 (9.40) ^d	2.00 (8.13) ^e	2.00 (8.13) ^d	2.00 (8.13) ^c	2.00 (8.13) ^c	2.00 (8.13) ^c
7.	Unsettled	62.00 (51.94) ^a	52.00 (46.15) ^a	10.00 (18.43) ^c	2.67 (9.40) ^c	2.67 (9.40) ^c	2.67 (9.40) ^c
	SEd	1.7001	1.9306	1.9682	1.5002	1.5002	1.5002
	CD Value (0.05)	3.6467	4.1412	4.2218	3.2179	3.2179	3.2179

*Mean of three replications. Figures in parentheses are arc sin transformed values. Mean followed by same letter (s) in a column are not significantly different by DMRT (P=0.05)

Table 3: Bio assay of food volatiles to *S.oryzae* (Sorghum population) using Y tube olfactometer

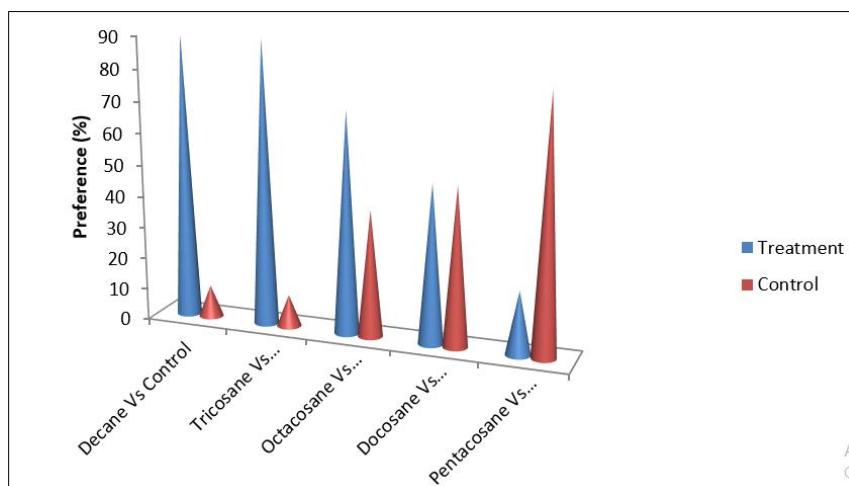
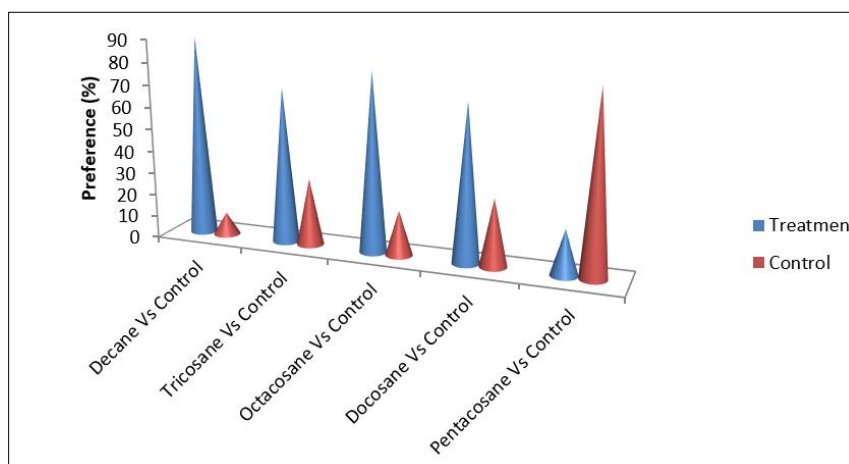
S.No	Treatments		Mean	SD	P Value	χ^2 Value
1.	DecaneVs Control	Decane	0.9	0.3	0.011	0.915 ^{NS}
		Control	0.1	0.3		
2.	TricosaneVs Control	Tricosane	0.9	0.3	0.011	0.915 ^{NS}
		Control	0.1	0.3		
3.	OctocosaneVs Control	Octacosane	0.7	0.4	0.206	0.573*
		Control	0.3	0.4		
4.	DocosaneVs Control	Docosane	0.5	0.5	1.000	0.317*
		Control	0.5	0.5		
5.	PentacosaneVs Control	Pentacosane	0.2	0.4	0.058	0.810*
		Control	0.8	0.4		

Mean of ten replications. * indicate significant differences from even distribution at $P < 0.05$ and $P < 0.01$. NS- Non Significant

Table 4: Bio assay of food volatiles to *S.oryzae* (Pulses population) using Y tube olfactometer

S.No	Treatments		Mean	SD	P Value	χ^2 Value
1.	DecaneVs Control	Decane	0.9	0.3	0.011	0.915 ^{NS}
		Control	0.1	0.3		
2.	TricosaneVs Control	Tricosane	0.7	0.4	0.206	0.650*
		Control	0.3	0.4		
3.	OctacosaneVs Control	Octacosane	0.8	0.4	0.058	0.810*
		Control	0.2	0.4		
4.	DocosaneVs Control	Docosane	0.7	0.4	0.206	0.650*
		Control	0.3	0.4		
5.	PentacosaneVs Control	Pentacosane	0.2	0.4	0.058	0.810*
		Control	0.8	0.4		

Mean of ten replications. *indicate significant differences from even distribution at $P < 0.05$ and $P < 0.01$. NS- Non Significant

**Fig 1:** Bio assay of food volatiles (Sorghum population) by using Y tube olfactometer**Fig 2:** Bio assay of food volatiles (Pulses population) by using Y tube olfactometer

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