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# BIO ASSAY OF FOOD VOLATILES ON SORGHUM AND PULSE FEEDING POPULATION OF *SITOPHILUS ORYZAE* L

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**ABSTRACT**
The experiment was conducted to assess the bioassay of five available food volatiles *viz.,* decane, tricosane, octacosane, docosane and penatacosane at Entomology laboratory, Department of Agricultural Entomology, TNAU during the year of 2014-2015. The volatile preference for sorghum and pulse breeding population was assessed separately using six arm and Y tube olfactometer. 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%), octocosane (13.33%) and docosane (2.67%) at 10 Minutes After Release. The higher number of attraction was recorded in decane (37.33%) followed by tricosane (32.67%) and octocosane (21.33%) at 20 MAR. 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%).

## 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, cosmopolitan in nature and is known to infest sound cereal seeds (Hill, 1990) and causes severe loss in rice, maize, barley and wheat (Bhatia *et al.,* 1975; Neupane, 1995). Though the storage grain loss is caused by insect pests, pathogens and rodents it is generally believed that half of the storage loss is usually caused by insects (FAO, 1968). Considering the loss caused by storage insect pests, effective methods of control are of paramount importance. 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 (Latha and Naganagoud, 2015).

Reports about its occurrence on legumes are scanty. Pemberton *et al* (1981) studied its breeding behaviour on carob, *Ceratonia siliqua* (L.), a tree legume native to the Mediterranean region. Coombs *et al.* (1977) reported the successful development by Trinidad strain of *S.oryzae* on yellow split pea. In India, the pest was recorded for the first time to feed on red gram at Coimbatore. We collected a population of rice weevil feeding on split red gram dhal was sent to IARI, identified as *Sitophilus oryzae* by V.V.Ramamoorthy, (2011, Personal communication). The study was taken to analyse the preference of food volatiles collected from sorghum and pulse breeding population through Air Entrainment Technique. In the present investigation, the feeding preference of sorghum and pulse bred population was identified based on the results of GCMS using five available synthetic food standards. The experiment was conducted using standards *viz.,*decane, tricosane, octocosane, docosane, pentacosane with untreated check using six arm and Ytube olfactometer.

## MATERIALS AND METHODS

The experiment was conducted by using the available technical standards of food volatiles such as decane, tricosane, octacosane, docosane and penatacosane 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 $\mu$l 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 experiment was conducted using five available food volatiles standards *viz.*,decane, tricosane, octocosane, 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%), octocosane (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 octocosane (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). 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. 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).

Germinara *et al.* (2008) 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

**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* (Pulse 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 | $\chi^2$ Value |
|---|---|---|---|---|---|---|
| 1. | Decane Vs Control | Decane | 0.9 | 0.3 | 0.011 | 0.915$^{NS}$ |
| | | Control | 0.1 | 0.3 | | |
| 2. | Tricosane Vs Control | Tricosane | 0.9 | 0.3 | 0.011 | 0.915$^{NS}$ |
| | | Control | 0.1 | 0.3 | | |
| 3. | Octocosane Vs Control | Octacosane | 0.7 | 0.4 | 0.206 | 0.573* |
| | | Control | 0.3 | 0.4 | | |
| 4. | Docosane Vs Control | Docosane | 0.5 | 0.5 | 1.000 | 0.317* |
| | | Control | 0.5 | 0.5 | | |
| 5. | Pentacosane Vs 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* (Pulse population) using Y tube olfactometer.**

| S.No | Treatments | | Mean | SD | P Value | $\chi^2$ Value |
|---|---|---|---|---|---|---|
| 1. | Decane Vs Control | Decane | 0.9 | 0.3 | | |
| | | Control | 0.1 | 0.3 | 0.011 | 0.915$^{NS}$ |
| 2. | Tricosane Vs Control | Tricosane | 0.7 | 0.4 | | |
| | | Control | 0.3 | 0.4 | 0.206 | 0.650* |
| 3. | Octocosane Vs Control | Octacosane | 0.8 | 0.4 | | |
| | | Control | 0.2 | 0.4 | 0.058 | 0.810* |
| 4. | Docosane Vs Control | Docosane | 0.7 | 0.4 | | |
| | | Control | 0.3 | 0.4 | 0.206 | 0.650* |
| 5. | Pentacosane Vs Control | Pentacosane | 0.2 | 0.4 | | |
| | | Control | 0.8 | 0.4 | 0.058 | 0.810* |

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

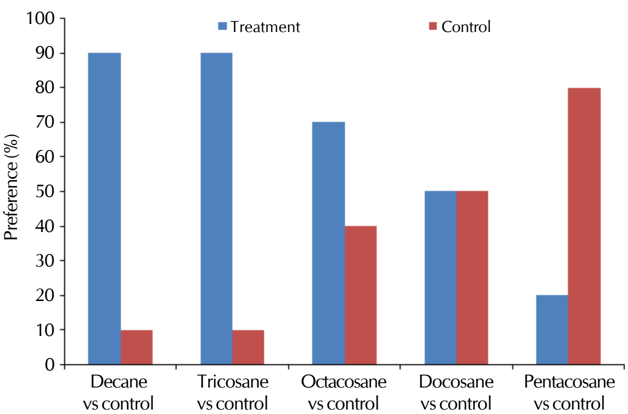


**Figure 1: Bio assay of food volatiles (Sorghum population) by using Y tube olfactometer**

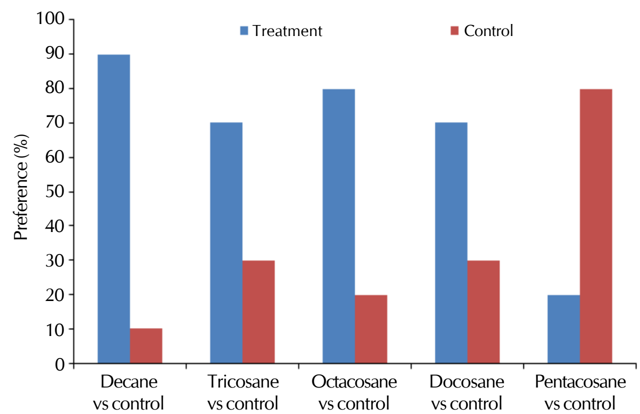


**Figure 2: Bio assay of food volatiles (Pulse population) by using Y tube olfactometer**

as repellents. However, in the case of 1- pentanol, (E,E)- 2,4-heptadienal, and phenylacetaldehyde, 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 (Maga, 1978) and, consequently, may act as kairomones. Pierce *et al.* (1990) 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), whereas phenylacetaldehyde was an attractant for several lepidopteran species including *Ephestia cautella* (Walker) and *Plodia interpunctella* (Hubner) (El-Sayed, 2006 and Olsson *et al.,* 2005). Piggot *et al.* (1991) 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 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, octocosane, 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 %), octocosane (70.0 %) and docosane (50.0 %) respectively, whereas there was no preference for pentacosane. The calculated $\chi^2$ values were represented in (Table 3).

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 $\chi^2$ value were represented in (Table 4).

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). 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).

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