Journal of Sohag Agriscience (JSAS) 2024, 9(2): 246-252


**ISSN 2357-0725**
**https://jsasj.journals.ekb.eg**
**JSAS 2024; 9(2): 246-252**

**Received: 17-11-2024**
**Accepted: 09-01-2025**

**Hany A. Fouad**
**Ragaa Elnahas**
Plant Protection Department
Faculty of Agriculture
Sohag University
Sohag
Egypt

**Eman F. M. Tolba**
Plant Protection Department
Faculty of Agriculture
New Valley University
New Valley
Egypt

**Corresponding author:**
**Ragaa Elnahas**
alnahasragaa677@gmail.com

# Behavioral Responses of *Rhynchophorus Ferrugineus* (Olivier) (Coleoptera: Curculionidae) Adult To Its Aggregation Pheromone under Laboratory Conditions.

**Hany A. Fouad, Ragaa Elnahas and Eman F. M. Tolba**

## Abstract

The different cultivars of date and decorative palms are infested by a range of insect species, most notably the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), which has become a worldwide pest, devastating palm trees. Managing RPW infestations presents significant challenges due to their social behavior and the absence of identifiable early indicators. The behavioral response of *R. ferrugineus* adult to the concentration of aggregation pheromone at (1000) ng/µl hexane was assessed using a Y-shaped olfactometer. The result indicated that virgin females responded to the aggregation pheromone at 1000 ng more than virgin males and mated males and females. According to our results, additional electrophysiological and field research are required to reveal communication behavior and responses to semiochemicals, for a better understanding of the observed differences in male and female responses of RPW. A deeper knowledge of these attracted individuals may increase the effectiveness of mass trapping in the control of red palm weevil adults.

**Keywords**: Insect behavior, Y-shaped olfactometer, red palm weevil, aggregation pheromone.

# INTRODUCTION

The date palm (*Phoenix dactylifera* L.), a vital fruit-bearing crop in the Palmaceae family, is widely cultivated across arid regions globally, including Egypt. Unfortunately, various ornamental palm (*Phoenix canariensis*) and cultivars of date palm (*Phoenix dactylifera* L.) are vulnerable to infestation by multiple insect species, particularly the red palm weevil *R. ferrugineus* (RPW), which has emerged as a worldwide pest devastating palm species (Habib et al., 2017; Milosavljevic et al., 2019). Early diagnosis of these pest-infested palms is challenging; however, necessary to take suitable steps as soon as possible (Faleiro, 2006; Dembilio and Jacas, 2011). In order to avoid serious environmental damage caused by excessive insecticide use, the feasibility of adopting sustainable practices is crucial. Particular emphasis is placed on the potential for establishing a mass trapping strategy (Gomez Vives et al., 2009). The management of RPW presents considerable difficulties. Moreover, the repeated application of insecticides in fruit crops can adversely affect beneficial arthropods (Solomon et al., 2001). Among semi chemicals, pheromones are typically species-specific and environmentally benign. This characteristic makes pheromones particularly valuable in integrated pest management, serving both as indicators of pest presence and as behavioral modifiers that disrupt the spread of target pests. Controlling pest populations can be achieved through various attractant-based methods, including attract-and-infect approaches, push-pull strategies, attract-and-kill, mass trapping, and mating disruption (Soroker et al., 2015). Aggregation pheromone traps serve as the primary component in red palm weevil control programs across most of its global distribution areas (Faleiro, 2004, Abdallah and Al-Khatri, 2005, Al-Saoud, 2008, and Al-Saoud *et al.*, 2010). Recently, the use of aggregation pheromone in Integrated Pest Management (IPM) programs has started to gain attention in Egypt. Therefore, this research aima to assess the response of *R. ferrugineus* adults to aggregation pheromones using an olfactometer bioassay. This will enable the development of more effective management and control strategies that will ultimately lead to the eradication of the pest.

# MATERIALS AND METHODS

## 1. Insects

Adult weevils for the olfactometer tests were gathered from the field and transported to the laboratory. The adults were reared in plastic boxes (30x20x15 cm.) with securely fitted, perforated lids. They were provided with fresh sugarcane stem tissue both as a food source and as a site for egg-laying, at a controlled temperature of 26 ± 2 ºC, 60± 10 % RH and a 12:12 L:D photoperiod. The sugarcane segments were inspected daily and substituted with fresh ones as needed.

## 2. Solution Preparation

One µL of synthetic aggregation pheromone of *R. ferrugineus* adult [(4*RS*,5*RS*)-4-methylnonan-5-ol (ferrugineol) and (4*RS*,5*RS*)-4-methylnonan-5-one (ferrugineone)], at a 9:1 ratio (Santa Cruz Biotecnology Inc., Texas, USA) was mixed with 999 µL of hexane 97% (Aldrich/Sigma Chemical Co., USA) to prepare a stock solution 1 µg $\mu L^{-1}$.

## 3. Olfactory Bioassays

Assays were performed with a Y-shaped olfactometer (leg length 12 cm, arms 10.5 cm long, spaced at an angle of 77 degrees, inner diameter 2.5 cm, outer diameter 12 mm). Weevils were transferred into an environmental room (28 ± 2 °C) an hour before each bioassay at the beginning of the photophase when the weevils were at peak activity (Avand-Faghih, 2004). The *R. ferrugineus* adults were found to be very sensitive to light, therefore, experiments were performed to determine the best protocol for this factor. Air enters the system (flow of 3 ml/min) through an activated charcoal filter. The weevils were collected in plastic vials of 250 ml capacity and left to starve overnight in the testing room. Individual responses were tested at concentrations of 1000 ng of aggregation pheromone in 1 µl of hexane 99% (Aldrich/Sigma Chemical Co., USA) applied to

1 $cm^2$ pieces of filter paper. Hexane on filter paper served as a control to assess the sensitivity of individuals with varying statuses (virgin/mated) and sexes (male/female). In one arm of the olfactory meter, the pheromone was applied, and in the other arm, hexan was supplied. This sequence was randomly exchanged at a later time. Females and males of the red palm weevil (one at a time separately) were released into the central arm hole of the Y-tube and exposed to the two arms. One replicate was defined as the total count of individual visits to the arms recorded over eight observations. Thirty-two to 48 individuals have been tested for each sex and mating status in four replicates. Each red palm weevil was given 5 minutes in a Y tube for selection. Only insects that entered one of the test arms and stayed there for at least 30 seconds in the tube were counted. Individuals who did not decide within 5 minutes were recorded as 'not responded'. The side on which the treatment was administered was switched to avoid positional bias. Following each trial, the adult *R. ferrugineus* was taken out, and the Y-tube was cleaned with water and then dried using filter and tissue paper, then put in the oven at 250 degrees for 20hours. The side on which the treatment was given was alternated.

**4. Data analysis**

Data from Y-shaped olfactometer was subjected to binomial analysis model to determine the effective of pheromone to attractive the *R. ferrugineus* adult. All statistics were performed using the statistical software R version 3.6.2 (R Core Team 2016) using RStudio version 1.2.5033 (RStudio Team 2016).

# RESULTS AND DISCUSSION

Results of Y-olfactometer bioassay testing synthetic aggregation pheromone at concentration of 1000ng /µl hexane to establish whether they are attracting adults of *R. ferrugineus* or not were showed in (Table 1; figures 1, 2 and 3). Virgin females of *R. ferrugineus* (78%, $P = 0.002$) chose the treated arm of the Y-tube olfactometer than hexane. No significant differences were found between pheromone and hexane in Y-olfactometer bioassays of virgin males and mated females and males. Results showed that gender and mating condition had a notable impact on response levels. The findings indicated that response levels were significantly influenced by gender and mating state.

Table 1. Percentage of response ± SE of virgin and mated male and female of *Rhynchophorus ferrugineus* to 1000 ng of the aggregation pheromone, tested in a Y-shaped olfactometer (single individual experiment)

| Weevil type | No. | No. of non-response | % response | | *P* value |
|---|---|---|---|---|---|
| | | | Pheromone | Hexane | |
| Virgin males | 34 | 2 | 47 | 53 | 0.86 |
| Virgin females | 32 | 0 | 78 | 22 | 0.002 |
| Mated males (after one week of mated) | 38 | 5 | 41 | 59 | 0.38 |
| Mated females (after one week of mated) | 44 | 12 | 66 | 34 | 0.11 |
| Mated males (after four weeks of mated) | 38 | 5 | 62 | 38 | 0.22 |
| Mated females (after four weeks of mated) | 48 | 16 | 53 | 47 | 0.86 |

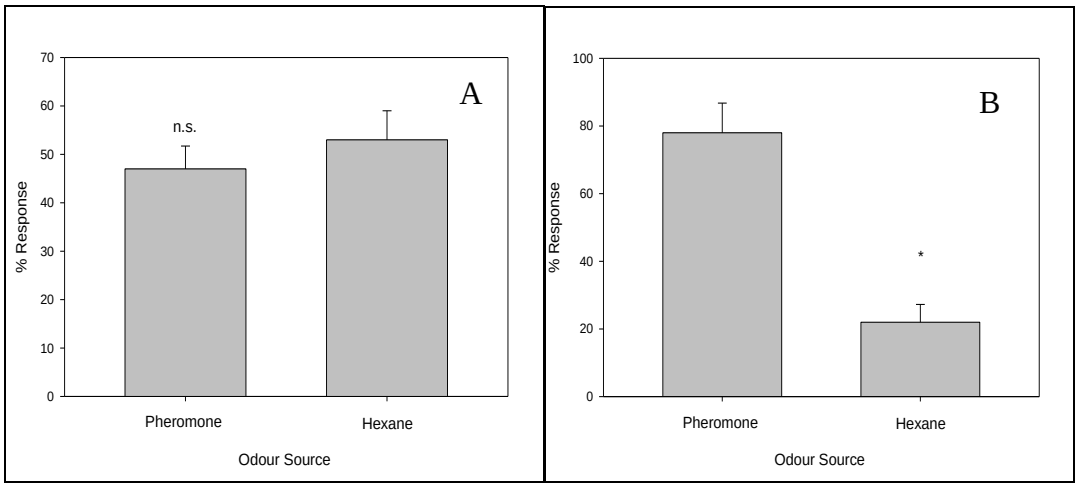


Figure 1. Percentage of response ± SE of virgin males (A) and females (B) of *Rhynchophorus ferrugineus* to 1000 ng of the aggregation pheromone, tested in a Y-shaped olfactometer (single individual experiment).
n.s. - There was no significant difference, while (*) indicated a significant difference at the 0.05

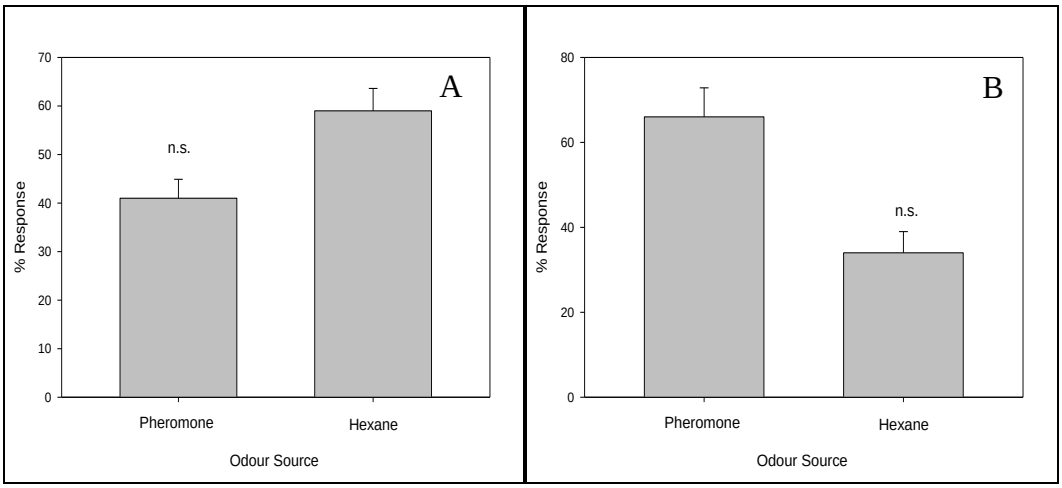


Figure 2. Percentage of response ± SE of mated males (A) and females (B) of *Rhynchophorus ferrugineus* (one week after mated) to 1000 ng of the aggregation pheromone, tested in a Y-shaped olfactometer (single individual experiment).
n.s. - no significant difference at 0.05.

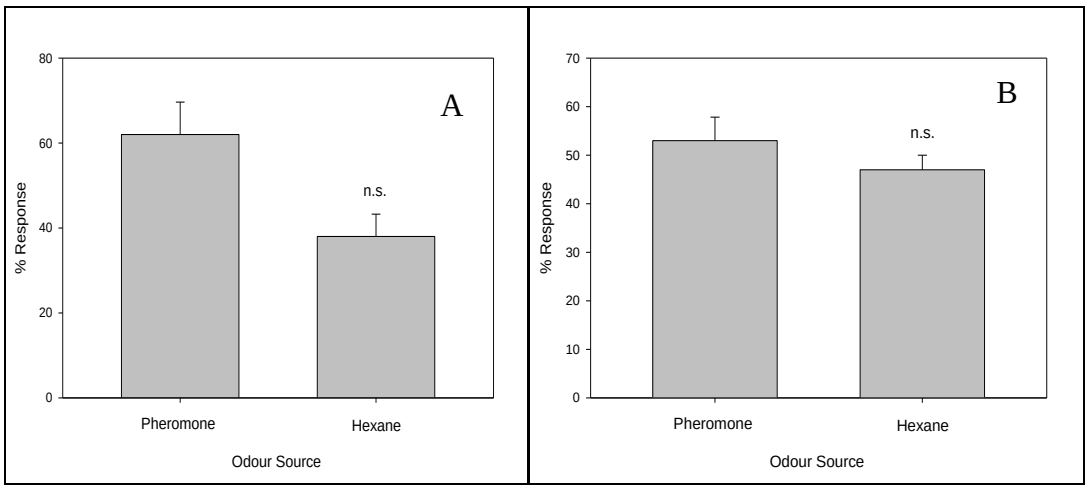


Figure 3. Percentage of response ± SE of mated males (A) and females (B) of *Rhynchophorus ferrugineus* (four weeks after mated) to 1000 ng of the aggregation pheromone, tested in Y-shaped olfactometer (single individual experiment).
n.s. - no significant difference at 0.05.

Weevils have been found to respond differently to aggregation pheromones depending on their sex and mating status (Graaf et al., 2005; Poorjavad et al., 2009). Poorjavad et al. (2009) reported that the RPW adult responded to 1000 times more hexane than to 1, 10, and 100 ng/µl. They also found that both sexes' aggregation pheromone reaction rises with mating. According to Avand-Faghih (1998) and Soroker et al. (2005), females' higher activity levels could be the cause of the bias they exhibit in field trap captures. These results therefore suggest that females may be more sensitive to the aggregation pheromone than males. Another experiment showed a sex ratio of 1:2.2, with female *R. ferrugineus* weevils constantly predominating in trap captures throughout the research (Al Saoud, 2013). The fact that female RPW have more basioconic sensillae on their antennae than males (Avand-Faghih, 2004) and that the weevil *R. palmarum* basioconic sensillae have been shown to be sensitive to the aggregation pheromone (Said et al., 2003) support this possibility. In comparison to the control group, Chakravarthy et al. (2014) demonstrated that the aggregation pheromone of the red palm weevil attracted a much greater number of weevils—13.4 females and 7.6 males.

# CONCLUSION

Sex and mating status had significant effect on the level of responses. Consequently, these findings indicate that females might exhibit greater sensitivity to the aggregation pheromone compared to males may be attributed to their elevated activity levels. Electrophysiological and field research are required to reveal communication behavior and responses to semiochemicals. And adeeper knowledge of these attracted individuals may increase the efficacy of mass trapping in the control of red palm weevil adults.

# REFERENCES

Abdall , F.F. and Al-Khatri , S.A. (2005) The effect of pheromone, kairomone and food bait on attracting males and females of red palm weevil. *Egyptian Journal of Agricultural Research* 83, 169–177

Al-Saoud , A. H. (2008). The Role of Red Palm Weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) Aggregation Pheromone Traps Sets on The Number of Catch Weevils. In *First International Conference of Agricultural Sciences "Strengthening the Role of Scientific Research to Support Plans of Sustainable*

*Agricultural Development" Organized by the Faculty of Agriculture, Aleppo University& the Faculty of Agriculture and Forest, Al-Mosul University* (pp. 13-15).

Al-Saoud, A. H. (2013). Effect of ethyl acetate and trap colour on weevil captures in red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) pheromone traps. *International Journal of Tropical Insect Science*, 33:202-206.

Al-Saoud, A.H., Al-Deeb, M.A. and Murchie, A.K. (2010). Effect of Color on the Trapping Effectiveness of Red Palm Weevil Pheromone Traps. *Journal of Entomology*, 7 (1): 54-59.

Avand-Faghih A., 2004.- Identification et application agronomique de synergistes végétaux de la phéromone du charançon *Rhynchophorus ferrugineus* (Olivier) 1790.- *These pour obtenir le titre de docteur de l'INA-PG*, Institut National Agronomique Paris-Grignon et Institut National de la Recherche Agronomique, France. 95 pp.

Avand-Faghih A.,( 1998). Research on the Control of red palm weevil, Rhynchophorus ferrugineus Oliv. (Col.: Curculionidae) with synthetic attractants in Sistan & Blouchestan province (Iran). *A thesis submitted in partial fulfilment for the degree of M.Sc.* in Agricutural Entomology, Department of Plant Protection, College of Agriculture, University of Tehran, (pp. 162)

Dembilio O. and Jacas, J.A. (2011). Basic bio-ecological parameters of the invasive red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in *Phoenix canariensis* under Mediterranean climate. *Bulletin of Entomological Research*, 101: 153-163

Faleiro J. R. (2006). A review of the issues and management of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. *International Journal of Tropical Insect Science*, 26: 135-150.

Faleiro, J. R. (2004). Pheromone based strategy for the management of red palm weevil in date palm and coconut agro-ecosystems: Implications, protocols and impact. In *Proceedings of The Date Paper presented at Date Palm Regional Workshop on Ecosystem based IPM for Date Palm in the Gulf Countries UAE University, Al-Ain/UAE* (pp. 28-30).

Gomez-Vives S., Ferry M., Barbado J., Hernandez F., Montero Aplicacio N. (2009). Aplicaciónde una estrategia de control integrado del picudo rojo de las palmeras *(Rhynchophorus ferrugineus). Phytoma Espana*. 206: 1–6.

Graaf, J., Govender, P., Schoeman, A. S., and Viljoen, A. (2005).Efficacy of pheromone seasonal trapping of the banana weevil, *Cosmopolites sordidus* in South Africa.- *International Journal of Pest Management*, 51 (3): 209-218.

Habib, D.M., Mouna, N., and Wiem, H. (2017). Red Palm Weevil *Rhynchophorus ferrugineus* chemical treatments applied on ornamental palms in Tunisia: results of extensive experiments. *International Journal of Agriculture Innovation and Research*, 5(6):1062-1068.

Karthikeyan, R., Suresh Kumar, K., Singaravadivel, K., and Alagusundaram, K. (2014). Volatile elements of coconut toddy (*Cocos nucifera*) by gas chromatography–mass spectrometry. *Journal of Chromatography & Separation Techniques*, *5*(213):10-4172.

Milosavljević, I., El-Shafie, H.A., Faleiro, J.R., Hoddle, C.D., Lewis, M. And Hoddle, M.S., (2019). Palm ageddon: the wasting of ornamental palms by invasive palm weevils, *Rhynchophorus spp*. *Journal of Pest Science*,92(1):143-156.

Poorjavad, N., Goldansaz, S. H., and Avand-Faghih, A. (2009). Response of the red palm weevil *Rhynchophorus ferrugineus* to its aggregation pheromone under laboratory conditions. *Bulletin of Insectology*, 62(2): 257-260.

R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna,Austria.http:// www.R-project.org.

Said, I., Tauban, D., Renou, M., Mori, K., and Rochat, D. (2003). Structure and function of the antennal sensilla of the palm weevil

*Rhynchophorus palmarum* (Coleoptera, Curculionidae). *Journal of Insect Physiology*, 49(9): 857-872.

Solomon, M. G., Jay, C. N., Innocenzi, P. J., Fitzgerald, J. D., Crook, D., Crook, A. M., ... and Cross, J. V. (2001). Natural enemies and biocontrol of pests of strawberry in northern and central Europe. *Biocontrol Science and Technology*, 11(2), 165-216.

Soroker, V., Blumberg, D., Haberman, A., Hamburger-Rishard, M., Reneh, S., Talebaev, S., Anshelevich, L., Harari, A. R. (2005). Current status of red palm weevil infestation in date palm plantations in Israel. *Phytoparasitica*, 33(1), 97- 106.

Soroker, V., Harari, A., and Faleiro, J. R. (2015). The role of semiochemicals in date pest management. *Sustainable pest management in date palm: Current status and emerging challenges*, *21*:315-346.