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**Behavioral Responses of *Rhynchophorus Ferrugineus* (Olivier) (Coleoptera: Curculionidae) to Ethyl Acetate as a Kairomone**

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**Abstract**

The different cultivars of date and decorative palms are attacked by a variety of insect species, most notably the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), which has become a global palm destroyer pest. Adult *R. ferrugineus* behavioral responses to different concentrations of ethyl acetate were assessed using a four-arm-choice olfactometer. A higher ethyl acetate concentration (1000 ng) resulted in higher attract, with significant differences found in response of virgin ( $F = 8.50$ ;  $P 0.003$ ) and mated female after 1 and 2 weeks of meeting ( $F = 11.73$ ;  $P 0.0007$  for 1 week and  $F = 5.75$ ;  $P 0.01$  for 2 weeks, respectively). A deeper knowledge of these attracted individuals may increase the efficacy of mass trapping in the control of adult red palm weevils.

**Keywords:** Insect behavior, freedom of choice, Olfactometer, Red palm weevil, Semiochemicals.

## INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the world's oldest fruit trees and has been strongly tied with Middle Eastern life from ancient times. These trees are vulnerable to a variety of insect infestations. The red palm weevil, *Rhynchophorus ferrugineus*, is a serious insect pest of these plants that is found across tropical and equatorial Africa. However, it was first recorded in Egypt in the Sharqiya area in 1992 (Cox, 1993). Early diagnosis of these pest-infested palms is challenging but necessary in order to take suitable steps as soon as possible (Faleiro, 2006; Dembilio and Jacas, 2011). However, in order to avoid serious environmental damage caused by excessive insecticide use, the feasibility of adopting sustainable RPW control strategies is being investigated, with special attention being paid to the possibility of developing a mass trapping method (Gomez Vives *et al.*, 2009).

Semiochemicals frequently have a synergistic effect on the behavior of phytophagous insects, with many reports focusing on aggregation pheromones and plant volatiles, primarily in Coleoptera (Landolt and Phillips 1997). Such behavioral synergy is well exemplified in Rhynchophorinae weevils, whose responses to aggregation pheromones are dramatically enhanced by perception of host plant odors, as evidenced by comparative captures in the field by odor-baited traps. (Landolt and Phillips 1997; Giblin-Davis *et al.* 1996; Wertheim *et al.* 2005). The volatile compounds emitted by host plant tissues have been studied using electrophysiological bioassays. Palm tissue compounds, including 'palm kairomone and ester' such as ethyl acetate and ethyl propionate, were found to be responsive to *R. ferrugineus* antennae (Guarino *et al.*, 2011; Vacas *et al.*, 2014). According to several studies, the majority of these compounds significantly increase the attractiveness of pheromone-baited traps (Faleiro, 2006; Al-Saoud, 2009; Guarino *et al.*, 2011). Recently, the use of ethyl acetate in Integrated Pest Management (IPM) program has starting to get attention in Egypt. Therefore, the purpose of this study is to evaluate the response of *R. ferrugineus* adult to different concentration of ethyl acetate under olfactometer bioassay. This will allow for 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 collected in the field and transported to the laboratory. The adults were reared in plastic boxes (30x20x15 cm.) with tight fitting perforated covers and reared on freshly sugarcane stem tissues as a source of food and oviposition site for adults at  $26 \pm 2$  °C,  $60 \pm 10$  % RH 12:12 L:D photoperiod. The sugarcane pieces were checked daily and replaced with fresh cuttings as needed.

### 2. Bioassays with olfactometer

Adult *R. ferrugineus* behavioral responses to different concentrations of ethyl acetate were assessed using a four arm-choice olfactometer (Model QT-WII01) custom-made by Channel Tech Co., Ltd., Beijing, China. At the start of the photo phase, weevils were transferred into the testing room ( $26 \pm 2$  °C) an hour before each bioassay. The four-arm olfactometer was linked to a pump, which maintained constant pure air in the four arms while vacuuming out the odors emitted by the treatments via central suction. The four arms connected the four airflow meters to ensure that the control and odorant airstreams were equal in each arm. Individual responses were tested at concentrations of 250, 500, and 1000 ng of ethyl acetate (>99% purity, BDH. Chemicals limited poole, England) in 1  $\mu$ l of hexane 97% (Aldrich/Sigma Chemical Co., USA) applied to 1 cm<sup>2</sup> pieces of filter paper to determine the sensitivity of different status of the individuals' virgin/mated and male/female. Each concentrate was randomly placed in one of three chambers (three active chambers), while the fourth chamber was left empty (inactive chamber). As a control, hexane on filter paper was used. A single adult weevil was placed on the surface of the central arena. The assay was terminated when the individual made a choice (entered the jar for more than 1 minute) or after 5 minutes if no choice was made. Each adult was used only once. At least thirty-two to 40 individuals have been used in each experiment in four replicates. After each test, adult of *R. ferrugineus* was removed and the chambers were rinsed with water and dried with filter paper, then put it in the oven at 250 degrees for 20 hours.

### 3. Data analysis

Data were analyzed to determine differences in the effect of different concentrations of ethyl acetate on the behavior of *R. ferrugineus* adult. The Shapiro-Wilk test was used to check normality and homogeneity of variances. So, the Kruskal-Wallis's test, as an alternative to analysis of variance, was used with significance level = 0.05 to analyze the response by the weevil to choose one arm of olfactometer using (R Development Core Team 2014).

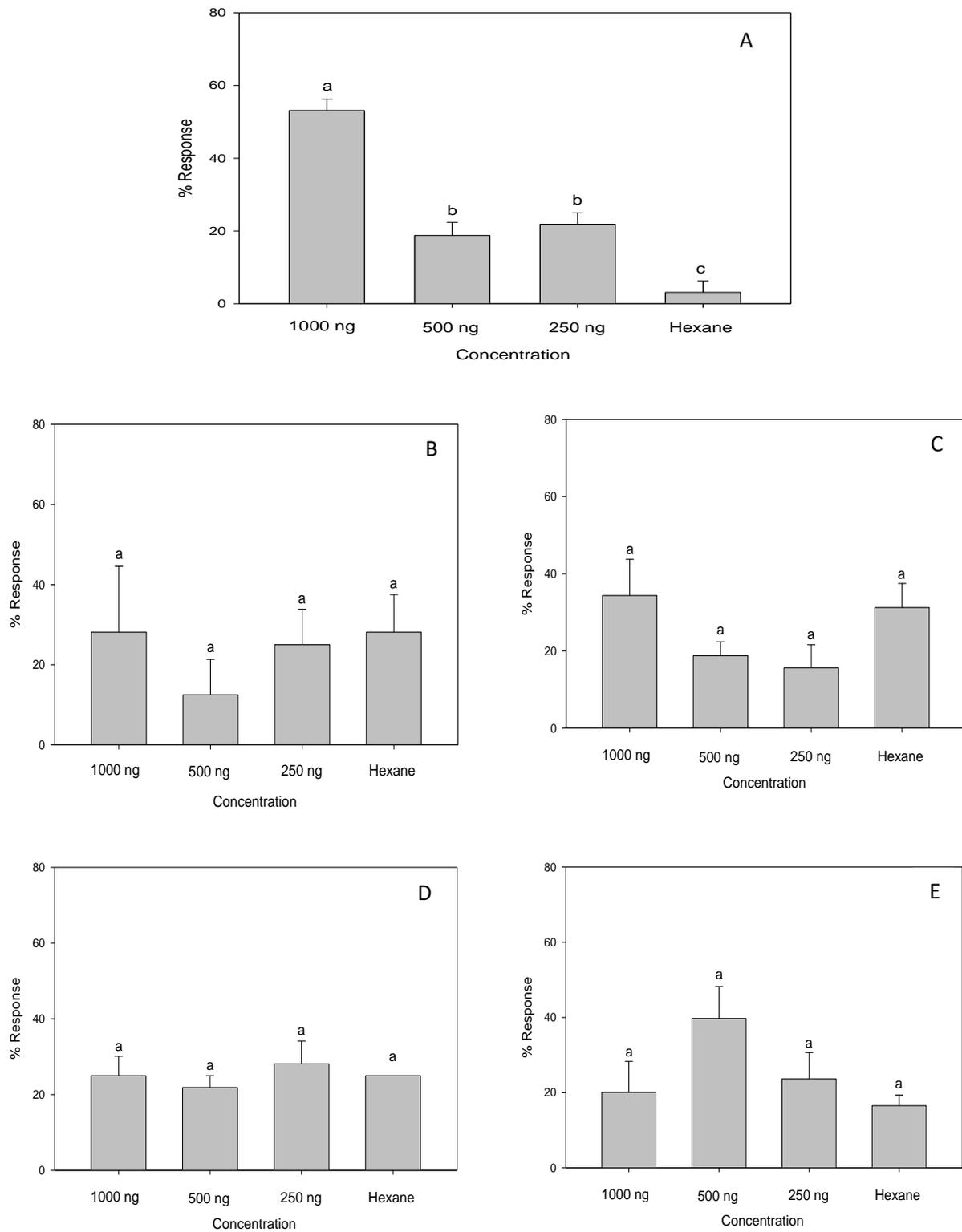
## RESULTS AND DISCUSSIONS

Response of *R. ferrugineus* adult in olfactometer to three ethyl acetate emission concentrations were subsequently evaluated (Table 1; Figures 1 and 2). In all experiments, the emission concentrations of 500 and 250 ng did not significantly differ from the control in terms of the number of adults attracted. However, a higher ethyl acetate concentration (1000 ng) resulted in higher attract, with significant differences found in respond of virgin (F = 8.50; P 0.003) and mated female after 1 and 2 weeks of meeting (F = 11.73; P 0.0007 and F = 5.75; P 0.01 for 1 and 2 weeks, respectively). According to these findings, the percentage of females who responded to 1000 ng of ethyl acetate was consistently higher than the percentage of males who responded in all treatments. The mated male's behavior, on the other hand, differed slightly: the three ethyl acetate concentrations achieved the same efficacy as the control. However, in most treatments, some individuals were present in the arena but did not make a decision (Table 1; Figures 1 and 2). In **Table 1. shows the percentage of response  $\pm$  SE of virgin and mated male and female *Rhynchophorus ferrugineus* to different concentrations of ethyl acetate in a 4-arm olfactometer (single individual experiment).**

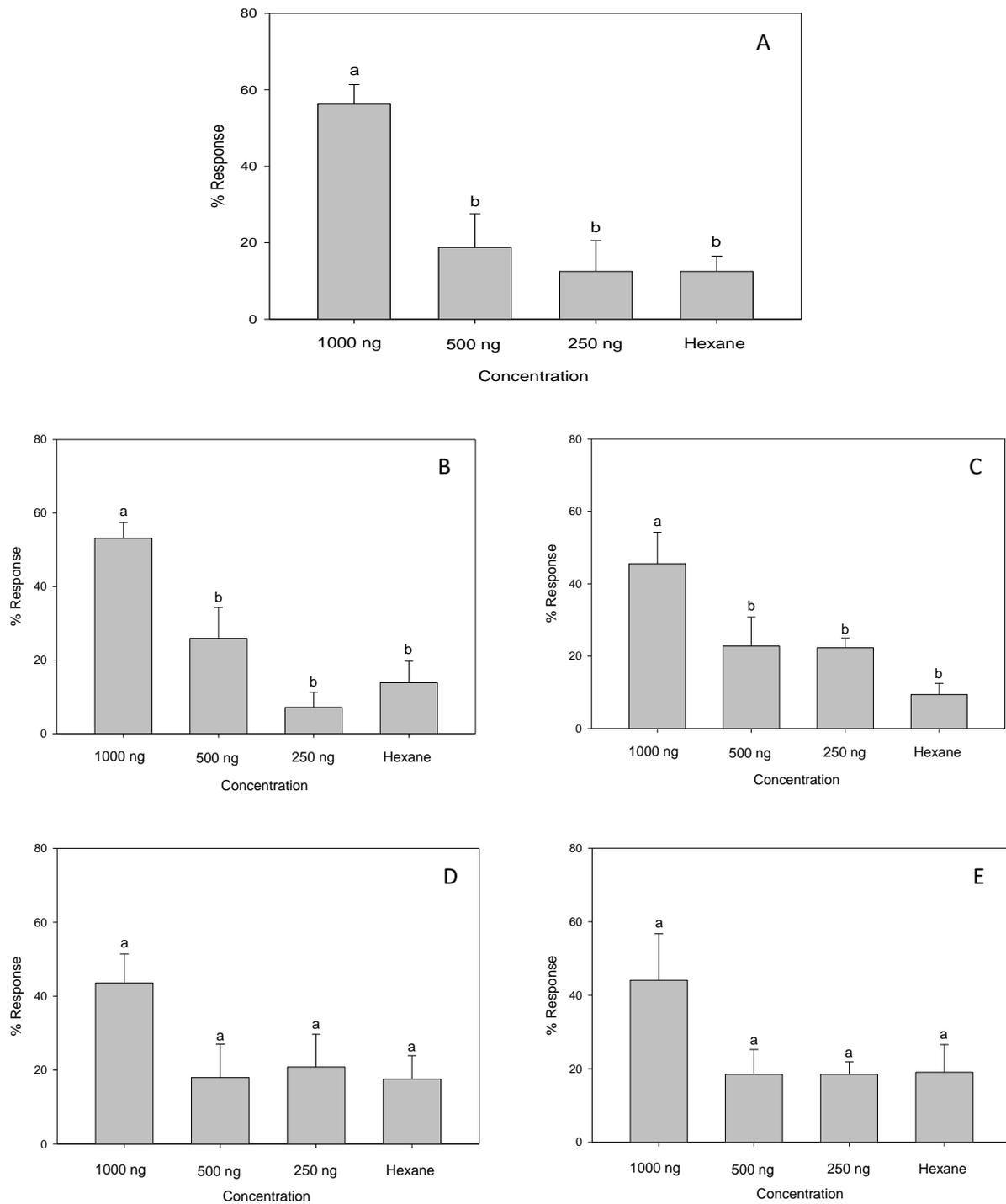
present study, it was clear that the responses of *R. ferrugineus* adults significantly differed according to the different of sex type and ethyl acetate concentration. Females of *R. ferrugineus* obviously preferred ethyl acetate compared to male. This response could be explained by females being more attracted to odor sources than males, as well as the need to provide a high quality food for egg production (Norkute *et al.*, 2020).. Rhynchophorus weevils are attracted to the fermentation volatiles of various palm tissues, palm oils, coconut, and pineapple (Jaffe *et al.* 1993, Rochat *et al.* 1991, Giblin-Davis *et al.* 1994), which include so-called "palm esters," such as ethyl acetate. For *R. ferrugineus*, the kairomone effect of a two-ester blend, ethyl acetate and ethyl propionate, was recently described (Guarino *et al.* 2011). (Venugopal and Subaharan . 2019) found that the adult of *R. ferrugineus* elicited high attractiveness response to fermented coconut sap neera compared with fresh neera. Volatile chemicals known as 'palm esters', such as ethyl acetate, ethyl propionate, ethyl butyrate, ethyl isobutyrate, and ethyl lactate, and their perception by adult weevils, have been demonstrated by electroantennogram bioassays (EAG) (Giblin-Davis *et al.* 1994; Rochat *et al.* 2000). However, another study found that ethyl acetate had no effect on attraction and that it cannot replace the synergistic effect of palm odor in attracting the red palm weevil (Vacas *et al.* 2013). Finally, understanding these interactions between RPW adult and host volatiles compounds can help to build effective RPW management techniques by focusing on *R. ferrugineus* communication and behavior.

type of weevil	Number of non-responders	% response				P value
		1000 ng	500 ng	250 ng	Hexane	
Virgin males	2	53.12 $\pm$ 3.12	18.75 $\pm$ 3.61	21.87 $\pm$ 3.12	3.12 $\pm$ 3.12	<.0001
Virgin female	2	56.25 $\pm$ 6.25	18.75 $\pm$ 8.06	12.50 $\pm$ 8.83	12.50 $\pm$ 5.10	0.003
Mated males (after 1)	2	28.12 $\pm$ 16.43	18.76 $\pm$ 8.84	25.00 $\pm$ 8.84	28.12 $\pm$ 9.37	0.7
mated female	4	53.12 $\pm$ 4.27	25.89 $\pm$ 8.42	7.14 $\pm$ 4.12	13.83 $\pm$ 5.84	0.0007
Mated male (after 2)	2	34.37 $\pm$ 9.37	18.75 $\pm$ 3.61	15.62 $\pm$ 5.98	31.25 $\pm$ 6.25	0.2
Mated female (after 2)	3	45.53 $\pm$ 8.70	22.76 $\pm$ 8.03	22.32 $\pm$ 2.67	9.37 $\pm$ 3.12	0.01
Mated males (after 4)	2	25.00 $\pm$ 5.10	21.87 $\pm$ 3.12	28.12 $\pm$ 5.98	25.00 $\pm$ 0	0.8
Mated female (after 4)	5	43.61 $\pm$ 7.79	18.01 $\pm$ 9.01	20.83 $\pm$ 8.83	17.55 $\pm$ 6.36	0.1
Mated males (after 6)	4	20.08 $\pm$ 8.23	39.73 $\pm$ 8.51	23.66 $\pm$ 6.96	16.51 $\pm$ 2.85	0.1
Mated female (after 6)	5	44.04 $\pm$ 12.65	18.45 $\pm$ 6.76	18.45 $\pm$ 3.42	19.04 $\pm$ 7.52	0.1

The Kruskal-Wallis's test shows that means followed by the same letter in each row do not differ (p0.05).



**Figure 1: Response percentage  $\pm$ SE of virgin and mated male *Rhynchophorus ferrugineus* to different concentrations of ethyl acetate in a 4-arm olfactometer (single individual experiment). A: virgin male. B, C, D and E: mated males after 1, 2, 4 and 6 weeks of mating, respectively.**



**Figure 2: Percentage of response  $\pm$  SE of *Rhynchophorus ferrugineus* virgin and mated females to different concentrations of ethyl acetate in a 4-arm olfactometer (single individual experiment).A: virgin female. B, C, D and E: mated females after 1, 2, 4 and 6 weeks of mating, respectively.**

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## الملخص العربي

الاستجابات السلوكية للحشرات البالغة من سوسة النخيل الحمراء *Rhynchophorus Ferrugineus* (Olivier) (Coleoptera: Curculionidae) لخلات الإيثيل باعتباره (كيرمون).

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تمثل شجرة نخيل التمر *Phoenix dactylifera* L. قيمة كبيرة وأهمية بالغة من الناحية الاجتماعية والغذائية والاقتصادية. لكن من المؤسف أن أصناف النخيل المختلفة تتعرض لهجوم أنواع مختلفة من الحشرات وخاصة سوسة النخيل الحمراء *Rhynchophorus Ferrugineus* (RPW) (Coleoptera: Curculionidae) التي أصبحت آفة عالمية مدمرة للنخيل. تم تقييم الاستجابات السلوكية للبالغين *R. Ferrugineus* لتركيزات مختلفة من خلات الإيثيل باستخدام مقياس الشم بأربعة أذرع. أدى التركيز العالي لخلات الإيثيل (1000 نانوغرام) إلى جذب أعلى، حيث كانت هناك اختلافات كبيرة في استجابة الإناث العذراء ( $F = 8.50$ ;  $P 0.003$ ) والإناث المتزاوجة بعد أسبوع وأسبوعين من التزاوج بين 1000 نانوغرام و500 و250 نانوغرام من خلات الإيثيل ( $F = 11.73$ ;  $P 0.0007$  و  $F = 5.75$ ;  $P 0.01$  وأسبوعين، على التوالي). إن المعرفة الأعمق بهؤلاء الأفراد المنجذبين قد تزيد من فعالية الاصطياد الجماعي في مكافحة سوسة النخيل الحمراء البالغة.