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Original Article

TRACE DETERMINATION OF RED PALM WEEVIL, *Rhynchophorus ferrugineus*, PHEROMONE AT TRAPING LOCATIONS UNDER EGYPTIAN CLIMATE

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Abstract

A simple and reliable methodology was developed for the determination of the release rate and profile of red palm weevil (RPW) pheromone from its commercial lures, in order to evaluate their longetivity. Analysis were performed on a narrow bore (0.25 µm) GC-column [30 m x 0.25 mm ID coated with DB-5ms (Phenyl Arylene polymer virtually equivalent to a (5%-Phenyl)-methylpolysiloxane] utilizing flame ionization detection (FID, temp.: 250 ^{o}C), with sample-splitting injection (50%, temp.: 230 ^{o}C). Pure N_2 was used as carrier gas at a flow rate of 1 mL min^{-1}. Initial column temperature was kept at 40 ^{o}C for 1 min., and then programmed at a rate of 15 ^{o}C min. up to 120 ^{o}C (2 min.), 20 ^{o}C min $^{-1}$ to 250 ^{o}C (4 min.), with a total analysis time of ~ 17.5 min. per run. Moreover, residues have been detected in the surrounding environment, including the plants in the nearby area of the trapping.

Key words: Pheromone, 4-methyl-5-nonanol, 4-methyl-5-nonanone, Red palm weevil (RPW, Rhynchophorus ferrugineus), GC analysis.

Introduction

Red palm weevil (*RPW*), *Rhynchophorus ferrugineus* [(Oliv.), *Coleoptera*: *Curculionidae*] is the most economically harmful pest of date palm throughout the Middle East (Al-Elimi et al., 2000; Ferry et al., 2002), where date palm, plays a principal role in the farming production and human diet (Murphy. and Brisco, 1999). One of the most practical methods to control adult weevils in date and coconut plantations is monitoring and mass trapping with lures baited with aggregation pheromones (Kaakeh, 2000; Kaakeh et al., 2001; Abozuhairah et al., 1996; FAO, 1999; El-Ezaby et al., 1998). 4-Methyl-5-nonanol is a male-produced aggregation pheromone of the Asian palm weevil, *Rhynchophorus bilineatus* [(Montr.), *Coleoptera: Curculionidae*] (Perez et al., 1996; Hallett et al., 1993a,b). Only the (4*S*, 5*S*)-isomer is also an aggregation pheromone of two other Asian palm weevils; *R. ferrugineus* (Oliv.) and *R. vulneratus* (Panz.) (Öehlschlager et al.,1995). Stereoisomeric mixture of 4-Methyl-5-nonanol (pheromone) and 4-Methyl-5-nonanone (synergist) is used frequently for monitoring and mass trapping of the relevant insect (*RPW*) (Hallett et al., 1999;

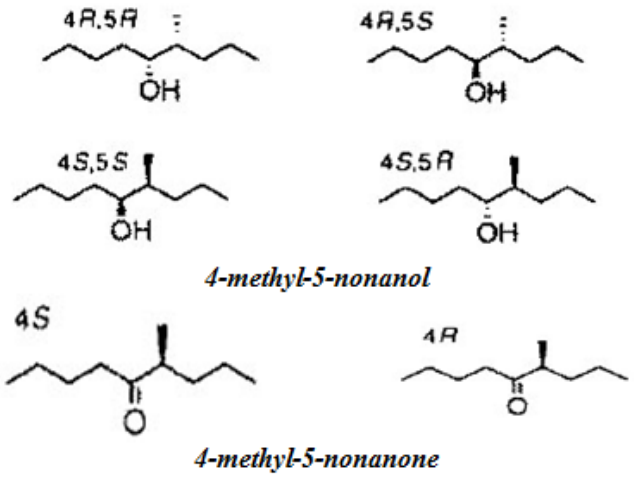

Figure 1

Vidyasagar et al., 2000a,b). It is reported that the most active ratio of pheromone: synergist is 9: 1 (Hallett et al., 1993b; Abraham et al., 1998) (Structures in Figure 1). Gas chromatographic (GC) analysis was performed for quantification of 4-Methyl-5-nonanol pheromone using their trifluoroacetyl derivatives using an internal standard (Zada et al., 2002).In this paper, a simple, rapid and reliable GC method was developed for the accurate determination of the compositional substances in their admixtures without derivatization.

Method and Materials

Chemicals

4-Methyl-5-nonanol pheromone purity > 98 % / 4-Methyl-5-nonanone Synergist purity > 99 % were supplied by ABCR GmbH & Co. KG.; D-76187 Karlsruhe–Germany.

Gas Chromatography

Analysis were performed on GC-17A Gas Chromatograph, Shimadzu, connected to CBM-102 communication Bus-Module, Shimadzu, Japan; equipped with technically advanced GC-column [30 m x 0.25 mm ID; 0.25 μm), coated with DB-5ms (phenyl arylene polymer virtually equivalent to a (5%phenyl)-methylpolysiloxane] for optimum performance, flame ionization detector , and a split/splitless injector. Nitrogen was used as the carrier gas with a flow rate 1 ml /min. The analysis was performed in the split mode 50: 50. The column was kept at 40 ^{o}C for 1 min. and then programmed at a rate of 15 ^{o}C /min to 120 ^{o}C (2 *min*.), 20 ^{o}C /min to 250 ^{o}C (*4min*). The injector and detector temp were maintained at 230 ^{o}C and 250 ^{o}C respectively.

Stability Cabinet

MMM Medcenter cooling incubator, series 4 KBK 4200, MMM Medcenter Einrichtungen GmbH&Co KG electrically heated unit (cabinet) was fitted with electronic temperature regulator (0 ^{o} C to 80 ^{o} C), whereby the range of the humidity sensor can also be regulated. An air-circulating motor cycles the air with the ultrasonic humidifying principle to reach the required temperature and relative humidity (for studying the rates of release).

Procedure of Analyses

Solutions of 4-methyl-5-nonanol pheromone and 4-methyl 5-nonanone synergist were prepared in diethyl ether at concentration of (6.32 mg/ ml) and (0.69 mg/ ml) respectively. Representative aliquots of each solution equivalent to 1580 – 15800 μg of pheromone and 550 – 1390 μg of synergist were transferred accurately from their stock solutions into series of 10–ml calibrated measuring flasks, and complete to volume with diethyl ether. One µl of each prepared solution was injected separately into the gas chromatograph under the specified chromatographic conditions. The calibration curve representing the relationship between the relative peak areas (using external standard technique) *versus* the corresponding concentration were constructed and the regression equations were computed. Figures 2 & 3 represent the calibration curves for both pheromone and synergist, respectively.

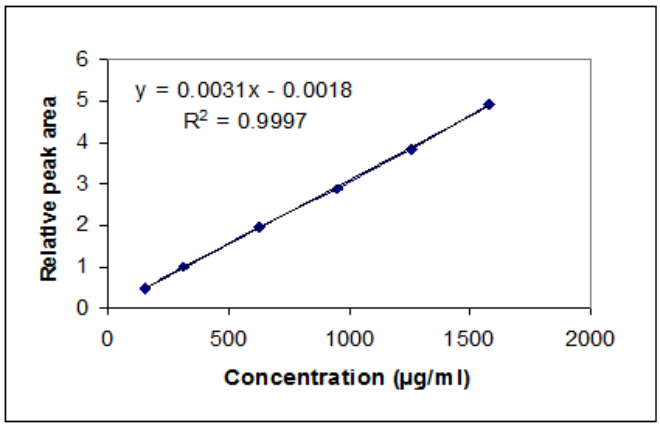

Figure 2

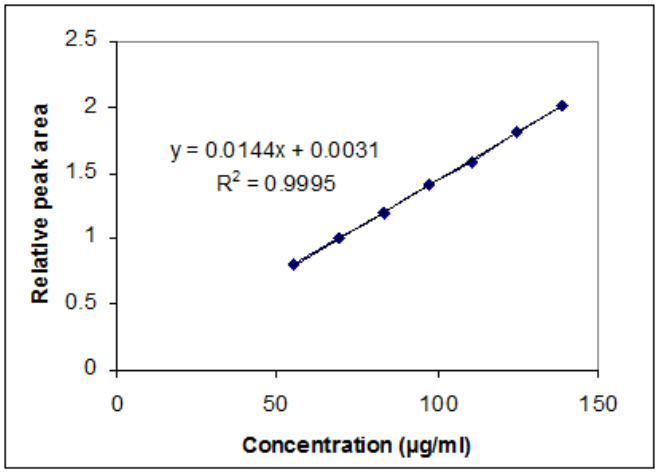

Figure 3

Application of the Method with Red Palm Weevil Pheromone Lures and Monitoring Release Rate (mg/day) Under Different Climatic Conditions Simulating the Average Conditions in Arab Republic of Egypt (ARE) [lab study]

P028 Ferrolure^{+} [Batch No. 11.158.F40.0] were supplied from ChemTica (Costa Rica) They contained 700 mg of 4-methyl-5-nonanol pheromone (9 parts) and 4-methyl-5-nonanone synergist (1 part), in addition to colorant and stabilizer, enclosed in a special polymer bubble slow-release device. The pheromone formulation is registered in Saudi Arabia for the Welfare of the administration of Agricultural Services of Guidance, Ministry of Agriculture & Water. The purity of the combined components in the mixture was claimed to be only $\geq$ 95%. The preparation was kept cooled (~ 5 °C) in its metallic sealed packages till the investigations (May-July). Two different Egyptian climate conditions were used in study; delta summer climate [medium, (Average)], temp. (30 ^{o}C) – RH (65 %) and desert summer

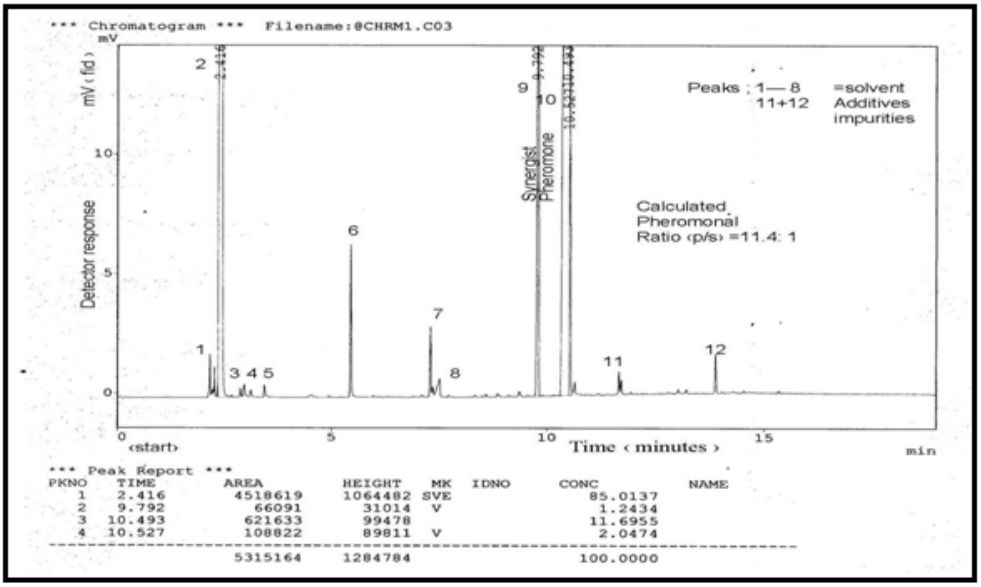

Figure 4

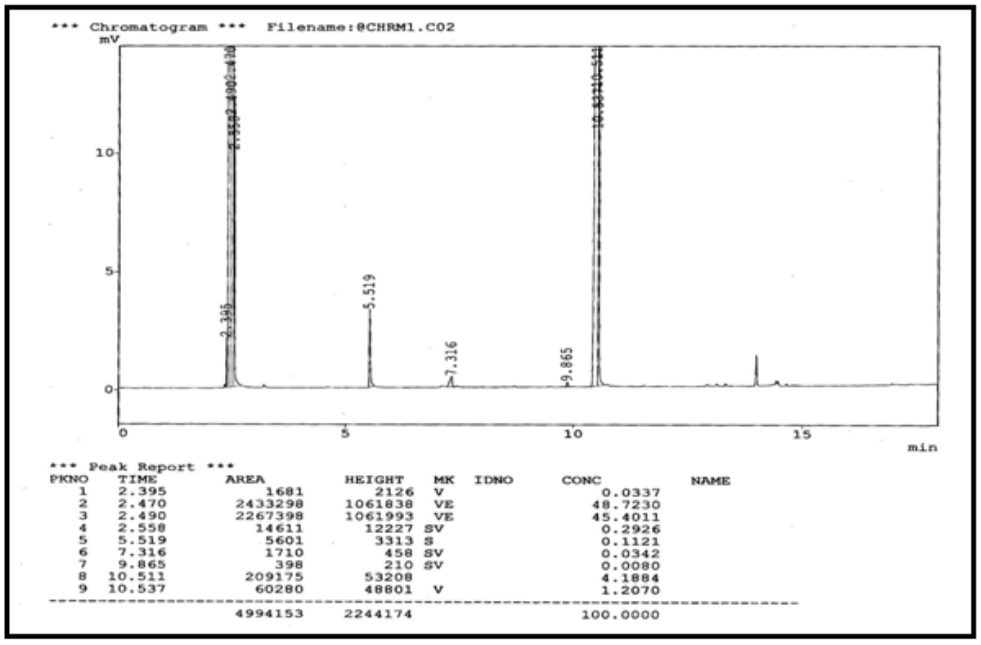

Figure 5

climate [medium, (Average)], temp. (34 ^{o}C) – RH (50 %). Both conditions were controlled using a stability cabinet in which the lures were placed. At the 5 th, 10 th, 20 th, 30 th, 45 th, 60 th d, three lures were taken randomly from the cabinet for analysis. The content of each lure was transferred quantitatively into 10–mL calibrated volumetric flask. The volume was completed with the rinse of the lure bubble using diethyl ether. For GC analysis, sample was diluted by a factor of 100. Then, an aliquot of one *µl* was chromatographed under the specified chromatographic conditions immediately or the extract kept at 5 ^{o}C until analysis.

Determination of the Traces of Pheromone on Surrounding Plants [field study]

Leaves of *Solanum nigrum* (eggplant) were chosen and collected from the surroundings of palm trees as an example of plants cultivated in the nearby area of the *RPW* pheromone trap. The lures were placed in weevil bucket traps in date and *Solanum nigrum (*eggplant) plantation in a garden at El-

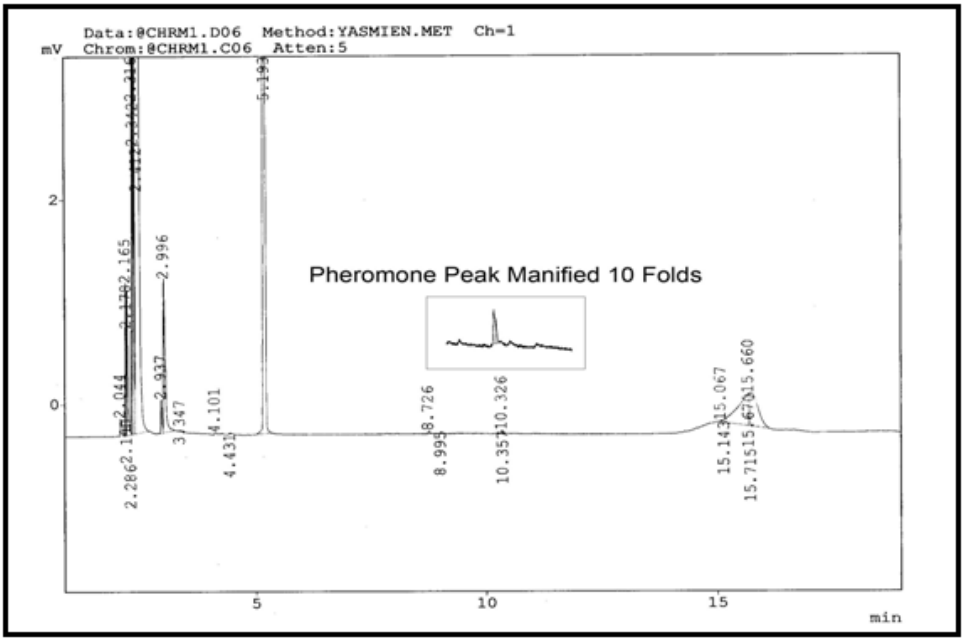

Figure 6

Sherouk Suburb, East-Cairo, Egypt under the actual experimental and Egyptian summer field conditions (July 2008) 22 $^{o}C_{(min.)}$ - 34 $^{o}C_{(max.)}$ and relative humidity (RH) 58 %. The trap also contained a mixture plant material (sugarcane and dates) and a bottle of ethyl acetate (kairomone) in the same trap, each one consists of plastic bucket modified by cutting holes and entry slots in the sides (25 *mm* diameter) within 3 *cm* of the trap top (Öehlschlager et al., 1992). Lure devices were hung directly under the cup of cover of the trap together with kairomone (ethyl acetate). The bottom of the bucket is filled with water to maintain high humidity level and to drown the weevils. The trap was put in the farm, placed on the ground and buried to the entry holes for monitoring traces of pheromone and synergist by analyzing samples of fresh leaves of *Solanum nigrum* (eggplant). Every 2 days, ten grams of fresh leaves of eggplant *(Solanum nigrum*) of either control or test samples were weighed, cut into small pieces and immersed in diethyl ether for 5 min. Then, the extract was decanted and the extraction was repeated three times (3x100 ml), to assure the complete extraction. The extract was concentrated using rotary evaporator, dissolved in diethyl ether, and then transferred quantitatively into 10-ml calibrated volumetric flask. One *µl* of the final extract was injected into GL-Chromatograph.

Results and Discussion

Quality Assurance of the Most Commercially Applied Pheromone Preparation

Triple injections of each working solution of the components and the preparation were made onto the fused-silica GC Column. The contents were 706 $\pm$ 3 *mg/lure* (most slightly above the limit, 700 *mg/lure*). Figure 4 demonstrates a typical GChromatogram indicating the resolution of the components of newly-opened (zero-time) originally sealed lure. The relative composition of pheromone: synergist was found to be 11.3 ($\pm$0.4): 1. This ratio was considered the basis to calculate the uniformity in the quality assurance of the preparation. The synergist resolved as a single signal (retention time t_R = 9.79 + 0.02 *min*.), while the pheromone indicated more than one peak, up to 4 peaks, with retention times of 10.29 – 10.43 *min*. This can be explained by the various steric configuration of the secondary alcoholic – OH group in the pheromone 4-Methyl-5-nonanol molecule as shown in Figure 1.

Release Rate (mg/day) Under Different Climatic Conditions Simulating the Average Conditions in Arab Republic of Egypt (ARE)

A study has been performed by measuring the rate of release (*mg /day*) under the predominating Egyptian delta and desert climate conditions in agreement with the reported *RPW* higher capture rates during April- June in Egypt (El-Garhy, 1996). This is probably due to increase in temperature and emergence of broods whose development is slowed by the cooler winter months. Table 1 demonstrates the rate of release and the composition percentage of the studied commercial preparation. The relative content uniformity percentage (RCU%) of the pheromone and synergist under the two studied conditions (delta and desert) shows that synergist is more volatile as measured by its lower boiling point 71.5 ^{o}C (boiling point of pheromone = 70 ^{o}C @ 24 mm Hg). The higher temperature with lower relative humidity allows more release of both components, but with relatively

Table 1: The component (%) and relative composition of pheromone (p) and synergist (s) under different simulated climatic conditions

Time (days)	*Simulated Climate (1) *x*			*Simulated Climate (2) +o*		
	P	***S***	***P / S***	***P***	***S***	***P / S***
0 (start)	105.2	10	10.5	103.2	10	10.3
5	105.1	9.3	11.3	101.1	9.1	11.0
10	104.8	9.0	11.6	100.1	8.5	11.9
20	103.8	8.7	11.9	98.8	8.1	12.2
30	103.5	8.6	12.0	96.7	7.3	13.2
45	102.1	8.3	12.3	92.1	6.6	14.0
60	102.0	8.0	12.8	88.8	6.3	14.1

(1) *: Delta condition [medium, (Average)], temp. (30 °C) – RH (65 %)
(2) +: Desert condition [medium, (Average)], temp. (34 °C) – RH (50 %)
P: Pheromone (4-methyl-5-nonanol)
S: Synergist (4-methyl-5-nonanone)
x: Release rate = 5 – 5½ mg.day^{-1}
o: Release rate = 10 – 11 mg.day^{-1}

higher P/S -ratio than at lower temperatures and higher humidity. Figure 5 shows the GC-chromatographic separation of both pheromone and synergist in P028 preparation (700 *mg/lure*) after two months controlled release which has been reported that the most active ratio of pheromone to synergist (9:1) tends to vary very much. This result was assessed by biological activity study of the *RPW* pheromone trap developed by (Hallett et al., 1993b) and (Abraham et al., 1998).They reported that bucket traps baited with a mixture of 4-methyl-5-nonanol (90%) with its related compound 4-methyl-5-nonanone (10%) captured 65% more *RPW* than traps baited with 4-Methyl-5-nonanol alone in Saudi Arabia. As a final conclusion, this study showed that:

- The active ratio of pheromone/synergist (9:1), which is in general essential for significant catch and satisfactory activity for *RPW* pheromone trap, is dependent on the release rate of the pheromone relative to the synergist in their admixtures, is principally depending on the climate (temperature & rel. humidity).
- The effective overall period of time for applying the catching traps containing the bio-controlling mixture of pheromone and synergist must be less than two months for delta conditions and 30 days only for desert summer conditions.

Trace Determination of RPW Pheromone at Trapping Locations under Egyptian Climate

Upon using the bucket trap inside the date palm field, contamination of the surrounding plants with pheromone and its synergist occurred. In the present study, leaves of *Solanum nigrum* (eggplant as an example of plants cultivated in the same nearby area field of *RPW* pheromone trap) were chosen from the surroundings of date palm trees and collected every two days then analyzed as detailed by the proposed GC–method. Traces of 4-methyl-5-nonanol were found on the leaf samples indicating environmental pollution. Monitoring these traces indicates that the proposed pheromone is declining rapidly in the summer because of the high temperature (32 ± 5 °C) during the experiment (July & August 2008) compared to winter (18 ±5 °C) in view of low boiling point and rapid evaporation of the pheromone. Moreover, it was found that following an oral administration in human, saturated aliphatic acyclic secondary alcohols and ketones were metabolized and converted to neurotoxic and hepatotoxic substances (WHO, 1999). Additionally, long chain alcohols in the range (C_6-C_{11}) are considered as irritant (Veenstra et al., 2009). So the determination of their traces on the surrounding plants was essential. Figure 6 shows contamination of the surrounding plants in the vicinity of the trap magnified by ten folds.

Conclusion

The analytical quality assurance of the pheromone preparation is of great importance to control the process of pests' aggregation at maximum efficiency. The start field or laboratorial studies as well as the follow-up screening of the aggregation procedure depend principally on the available analytical data. The additives may increase the product efficiency, so the propellant controls the rate of release of the pheromone and synergist; while the stabilizer may keep the natural properties of the components in the desired conditions. Derivatization of both of the pheromone and / or the synergist was a step more. Then, the direct GC-analysis was quite significant with high accuracy & repeatability and time-saving. The proposed chromatographic optimized method is quite applicable for rapid and accurate analysis of major components of the most commonly admixed, pheromone and synergist for *RPW* biological control.

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