

Antifeedant activity of three essential oils against the red palm weevil, *Rhynchophorus ferrugineus*

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Abstract

Three essential oils (EOs) from flowers and leaves of crofton weed, *Eupatorium adenophorum* Spreng. and aerial parts of Indian wormwood, *Artemisia nilagirica* (C.B. Clarke) Pamp., were evaluated for antifeedant activity against adults of red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier, a major pest of coconut palm, date palm, and Canary Island date palm. The specific bioassay method for evaluating the antifeedant activity of these EOs was used against *R. ferrugineus* adults and feeding-mark responses were analyzed at every 24 h interval for a period of 96 h. EOs from *E. adenophorum* flowers and *A. nilagirica* aerial parts, showed significant antifeedant activity at 96 h against RPW adults at 1000 ppm, as evident from 60.13 ± 11.31 and 60.13 ± 7.94 feeding prick-marks, respectively, as compared to 113.75 ± 13.55 and 104.81 ± 11.23 feeding prick-marks in their respective controls. However, the EO from *E. adenophorum* leaves was not found effective as antifeedant. GC-MS studies revealed differences in chemical composition, showing 40.87% oxygenated sesquiterpenes and 64.25% sesquiterpene hydrocarbons as the major constituents in the EOs of *E. adenophorum* flowers and leaves, respectively, whereas major class of compounds in EO from aerial parts of *A. nilagirica* comprised 37.02% sesquiterpenes and 32.92% monoterpene hydrocarbons. EOs from *E. adenophorum* flowers and aerial parts of *A. nilagirica*, have potential as environment-friendly alternative method for management of the RPW.

Key words: *Rhynchophorus ferrugineus*, antifeedant, essential oil, *Eupatorium adenophorum*, *Artemisia nilagirica*, Coleoptera, Curculionidae.

Introduction

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera Dryophthoridae), is an invasive pest of several palms, including coconut palm (*Cocos nucifera* L.), date palm (*Phoenix dactylifera* L.), and Canary Islands date palm (*Phoenix canariensis* hort. ex Chabaud). A native of South and South-East Asia (Nirula, 1956a,b), it has spread to Middle-East region in late eighties (Abraham *et al.*, 1998), and subsequently has been reported from several European countries including Spain (Barranco *et al.*, 1996a,b) and Italy (Sacchetti *et al.*, 2005; 2006). More recently, RPW was reported from Caribbean Islands (Roda *et al.*, 2011) and USA (http://cizr.ucr.edu/red_palm_weevil.html). Several reviews on the control strategies including Integrated Pest Management (IPM) for RPW are available (Vidyasagar and Subaharan, 2000; Faleiro, 2006; Abbas, 2010). Various methods of control are used against RPW in South-Asia (Abraham *et al.*, 2002), Middle-East (Abdallah and Al-Khatri, 2000; Vidyasagar *et al.*, 2000a; Faleiro, 2006) and Europe (Nardi *et al.*, 2011; Jacas *et al.*, 2011; Massa *et al.*, 2011; Tapia *et al.*, 2011; Triggiani and Tarasco, 2011). One of the important components of IPM strategies for *R. ferrugineus* is mass pheromone trapping system using synthetic aggregation pheromone, food baits and with or without kairomones (Oehlschlager, 1998; Vidyasagar *et al.*, 2000b). For controlling *R. ferrugineus*, synthetic pesticides are used in preventive sprays and curative injections (Abraham and Vidyasagar, 1993; Abozuhairah *et al.*, 1996; Abraham *et al.*, 1998; Vidyasagar *et al.*, 2000a). Since its report in Canary Island date palm in Spain, Italy and other European countries, imidacloprid is recommended for the control of this alien pest (Barranco *et al.*, 1998; Her-

nandez-Marante *et al.*, 2003; Dembilio *et al.*, 2010). However, inconsistent control of pest and high frequency of insecticide applications were reported from Spain (Ferry and Gomez, 2002), which may also contribute to environmental pollution. Recent studies revealed pesticide residues exceeding permissible limits in date fruits probably due to large-scale and indiscriminate use of pesticides for the control of RPW, thus contaminating date fruits and also environment with toxic substances (Kamel *et al.*, 2007; El-Saeid and Al-Dosari, 2010). Therefore it is desirable to reduce the number of synthetic pesticide sprays by alternate sprays of potent environment-friendly natural products. This will not only reduce the pesticide load in the date orchards but also help in insect resistance management. Bioactive natural products from plants are known to be both biorational and environment-friendly (Isman, 2006; Dayan *et al.*, 2009). The natural products, rotenone and limonene, tested for antifeedant effect on RPW larvae and toxicity on RPW larvae and adults were shown to be efficacious at very high doses (Abdullah, 2009). Amongst the variety of natural products available, essential oils (EOs) are being investigated since a decade to provide environmentally-safe alternatives to pesticides (Isman, 1999, 2000). They can be obtained from various plant parts such as flowers, leaves, stem, fruits, seeds, roots, etc. EOs are volatile and lipid-soluble natural mixtures of terpenes, terpenoids, polyphenols, fatty acid esters, etc. They have been reported to possess several types of bioactivities including antibacterial, antiviral, antifungal, antifeedant, insecticidal and medicinal (analgesic, sedative, anti-inflammatory, spasmolytic, locally anesthetic) (Isman, 2002; Bakkali *et al.*, 2008; Adorjan and Buchbauer, 2010). Antifeedant activity of EOs can be useful in preventing new infestations of

RPW. Though it has been investigated against a few stored-grain coleopteran pests including curculionidae weevils (Huang *et al.*, 2000; Perez *et al.*, 2010), there appears to be no attempt to find potential essential oils with antifeedant activity against the global pest, *R. ferrugineus*. Hence, we evaluated the antifeedant activity of three EOs extracted from two plants of Asteraceae family, namely, crofton weed, *Eupatorium adenophorum* Spreng. (flowers and leaves) and Indian wormwood, *Artemisia nilagirica* (C.B. Clarke) Pamp. (aerial parts) against *R. ferrugineus* adults. As there is no standard bioassay method for testing the antifeedant activity against the RPW adults, we also developed a specific method for evaluating the EOs.

Materials and methods

Insect culture and rearing conditions

The *R. ferrugineus* culture was maintained in the laboratory from the pupae collected from the infested palm trees in Al Kharj region of Saudi Arabia. This insect culture was established in our laboratory in the year 2009 and continued till date with regular addition of field-collected adults to avoid inbreeding of the insects. The insects were reared at 25 ± 2 °C temperature and 30% RH, and L:D cycle of 10:14 h. Sugarcane stem pieces were procured from the local market, cleaned with mild detergent under tap water, split longitudinally in 10cm bits and were used for feeding the insects. The adults after emergence were sexed and kept in separate containers with sugarcane pieces. From the laboratory-bred pupae, the requisite number of emerging adults were collected, sexed and separately kept in cylindrical wide-mouth bottles with screw-cap (length, 11 cm and diameter, 9 cm) provided with sugarcane bits.

Essential oils

Essential oil samples extracted from *E. adenophorum* flowers (EEOF) and leaves (EEOL), and *A. nilagirica* leaves (AEOL) were obtained from Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi, India. Both the plant materials were collected from Himalayan region, near Palampur, India.

Bioassay for antifeedant activity

The bioassays were conducted with EEOL, EEOF and AEOL at concentrations of 1000 and 500 ppm. The EOs were dissolved in minimum quantity of acetone and then volume was made up with distilled water. To clear the turbidity of the solution, 0.1% Triton X-100 emulsifier was used before making up the volume. An aqueous solution with the same amount of solvent and emulsifier as used in the treatment served as control. A bioassay method was developed specifically for the RPW adults to evaluate the antifeedant activity on the basis of feeding prick-marks. In this method, bioassay was done using fresh and cleaned 10 cm long pieces of sugarcane stem and each piece was split into two equal longitudinal halves. Each half (exposed area ~ 32.5 cm²) was treated by dipping in the 10ml working solution of EO

formulation for ten seconds and then air-dried, whereas control included only solvent, emulsifier and water. One treated sugarcane piece was placed in a rectangular plastic box of $18 \times 12 \times 6.5$ cm (L×W×H) dimensions. The 24-hours old, 6 h-starved male and female adults were obtained from our culture and one pair (male and female) was released in each box. Each treatment was replicated ten times in a randomized block design. Observations on feeding were taken after 24, 48, 72 and 96 hours. All feeding marks were analyzed by counting with a square window of 2×2 cm² in the location of maximum and minimum density of pricks on sugarcane piece and then averaging them. Several other window counts, including square windows of 1×1 cm² and rectangular windows did not provide the accurate account of the feeding marks. The holes without the eggs were counted and the ones drilled by females for depositing the eggs were ignored. The following formula was worked-out for calculating total number of pricks on sugarcane piece (~ 32.5 cm² area) of each replicate:

$$\text{Total number of prick-marks} = \left[\frac{(P_1 + P_2)}{2} * Z \right] / 4$$

P_1 = Maximum number of pricks in 2×2 cm² window area
 P_2 = Minimum number of pricks in 2×2 cm² window area
 Z = Area (~ 32.5 cm² for sugarcane piece in each replicate)

After the completion of the experiment, i.e. 96 h, the insects from all the treatments were fed with untreated sugarcane bits for 7 more days to monitor them for symptoms of inactivity or mortality.

Chemical analyses of essential oils

Chemical analyses of EOs were performed using gas chromatography (GC) and gas chromatography – mass spectrometry (GC-MS). GC analyses for EEOF, EEOL and AEOL were done with Agilent 7890A instrument equipped with DB-5 column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) and FID. Diluted samples (1/200, w/w, in dichloromethane) of $1.0 \mu\text{l}$ each were injected in the split-less mode. For EEOF and EEOL, the oven temperature was raised from 60 °C to 180 °C at 4 °C/min, held isothermal for 5 min and finally ramped to 220 °C at 10 °C/min. For AEOL analysis, the temperature was ramped from 60 °C (2 min) to 200 °C at 3 °C/min. Injector and detector temperatures were held at 230 °C and 250 °C, respectively, while carrier (He) and make-up (N₂) gases were maintained at the flow rates of 1ml/min and 25 ml/min, respectively. The relative percentage of the EO constituents was calculated by FID peak area normalization without using correction factors.

GC-MS analyses were performed with DB-5 MS column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) and Agilent 5975C mass analyzer with ionization potential of 70eV, transfer line temperature at 280 °C and scan range of m/z 40-450. The identification of EO constituents was done by one or more methods including matching their mass spectra with NIST and Wiley computer libraries, with the reported literature (Jennings and Shibamoto, 1980; Adams, 2007), with spectra from authentic samples (bisabolol, camphor and caryophyllene from Fluka Inc.), and by comparing their retention times with pure authentic samples and their linear retention indices relative to the series of n-hydrocarbons (C₅ to C₁₆).

Statistical analysis

Statistical analysis was performed using SPSS® 15.0 for Windows® software. Data were analyzed by using one-way ANOVA to examine the effects of the three tested EOs on feeding activity of *R. ferrugineus* adults and the means were separated using Tukey's test. Statistical significances between the samples were indicated by probability values of $P < 0.05$.

Results

Antifeedant activity of essential oils

Amongst the three evaluated EOs, the interaction between concentration and time was found significant in cases of EEOF (72 h and 96 h) and AEOL (24 h, 48 h, and 96 h) only, whereas, EEOL was found not effective in preventing the normal feeding marks (table 1). At higher concentration of 1000 ppm, the EEOF significantly affected the normal feeding of the *R. ferrugineus* adults showing 54.84 ± 11.07 , and 60.13 ± 11.31 mean prick-marks as against 102.78 ± 10.75 , 113.75 ± 13.55 mean prick-marks in control after 72 h and 96 h, respectively. However, after 24 h and 48 h, EEOF treatment did not affect the feeding significantly. The AEOL showed significant antifeedant activity against *R. ferrugineus* adults at 1000 ppm with 18.96 ± 5.07 , 38.82 ± 6.24 , and 60.13 ± 7.94 mean prick-marks as against 48.34 ± 10.82 , 78.41 ± 9.82 , and 104.81 ± 11.23 mean

prick-marks in control after 24 h, 48 h and 96 h, respectively, however in the observation taken after 72 h, AEOL treatment was found to not affect the feeding significantly. At 500 ppm, all the three evaluated EOs did not significantly affect the feeding of *R. ferrugineus* adults. No inactivity or mortality of the test insects was observed in any of the treatments during the experiments till 96 h and subsequent 7 days of monitoring period.

Chemical composition of essential oils

Results of chemical composition analysis by GC and GC-MS of EEOF revealed that out of total 89.66% identified components more than 84% content comprised oxygenated sesquiterpenes, sesquiterpene hydrocarbons and oxygenated monoterpenes (table 2). Six major components of EEOF were found to be amorphene derivatives, amorph-4,7(11)-diene-8-one (7.33%) and amorph-4,7-dien-11-ol (3.79%), sesquiterpenes, curcumene (4.24%), β -bisabolene (3.56%) and caryophyllene (3.26%), and oxygenated sesquiterpene, bisabolol (3.49%) (table 3). EEOL composition was found to be dominated by sesquiterpenes and their oxygenated derivatives comprising over 87% of total 92.71% identified components. Five out of six major components of EEOL, viz. β -farnesene (4.98%), curcumene (4.58%), β -sesquiphellandrene (3.80%), α -bergamotene (2.86%) and caryophyllene (2.79%) were sesquiterpene hydrocarbons, whereas the sixth component, amorph-4,7(11)-dione-8-one (4.15%) is an oxygenated derivative of

Table 1. Mean number of feeding-marks of *R. ferrugineus* on sugarcane pieces (area $\sim 32.5 \text{ cm}^2$) after treatment with essential oils of EEOF, EEOL and AEOL at two concentrations and control at 24 h, 48 h, 72 h and 96 h. Data was analyzed with one-way ANOVA and means were separated with Tukey's test.

Essential oil	Concentration (ppm)	Feeding - marks \pm S E			
		24 h	48 h	72 h	96 h
EEOF	control	44.28 ± 8.29 a	74.75 ± 14.38 a	102.78 ± 10.75 b	113.75 ± 13.55 b
	500	27.53 ± 6.8 a	41.98 ± 9.59 a	78.54 ± 11.33 ab	86.67 ± 13.30 ab
	1000	23.56 ± 2.83 a	38.2 ± 8.9 a	54.84 ± 11.07 a	60.13 ± 11.31 a
		$F = 3.048; P = 0.065$	$F = 3.205; P = 0.057$	$F = 4.870; P = 0.016$	$F = 4.582; P = 0.020$
EEOL	control	7.6 ± 2.81 a	26.91 ± 11.20 a	45.70 ± 16.41 a	53.82 ± 19.31 a
	500	17.27 ± 6.55 a	31.48 ± 8.04 a	42.66 ± 8.45 a	59.92 ± 10.12 a
	1000	12.6 ± 4.13 a	28.84 ± 6.31 a	40.62 ± 7.82 a	58.91 ± 11.51 a
		$F = 0.978; P = 0.391$	$F = 0.068; P = 0.934$	$F = 0.054; P = 0.948$	$F = 0.052; P = 0.950$
AEOL	control	48.34 ± 10.82 b	78.41 ± 9.82 b	97.5 ± 12.57 a	104.81 ± 11.23 b
	500	31.15 ± 6.42 ab	62.3 ± 10.49 ab	91.63 ± 17.62 a	105.17 ± 19.56 b
	1000	18.96 ± 5.07 a	38.82 ± 6.24 a	56.88 ± 7.7 a	60.13 ± 7.94 a
		$F = 3.381; P = 0.050$	$F = 4.807; P = 0.017$	$F = 2.728; P = 0.085$	$F = 3.846; P = 0.034$

$P < 0.05$ is significant; Values followed by dissimilar alphabets in superscript depict significant differences.

Table 2. Chemical composition of EEOF, EEOL and AEOL by GC and GC-MS analyses.

Chemical class of constituents	Percentage in essential oil*		
	EEOF	EEOL	AEOL
monoterpene hydrocarbons	02.46	00.64	32.92
oxygenated monoterpenes	09.41	02.10	05.67
sesquiterpene hydrocarbons	34.05	64.25	37.02
oxygenated sesquiterpenes	40.87	23.10	13.14
diterpenes and others	02.87	02.62	05.98

* Cumulative content in percentage of each class of constituents.

Table 3. Major constituents of EEOF, EEOL and AEOL by GC and GC-MS analyses.

Major Constituents (%)		
EEOF	EEOL	AEOL
amorph-4,7(11)-dione-8-one (7.33)	β -farnesene (4.98)	camphor (26.50)
curcumene (4.24)	curcumene (4.58)	β -bisabolene (7.57)
amorph-4,7-dien-11-ol (3.79)	amorph-4,7(11)-diene-8-one (4.15)	β -farnesene (6.17)
β -bisabolene (3.56)	β -sequiphellandrene (3.80)	caryophyllene oxide (5.01)
bisabolol (3.49)	α -bergamotene (2.86)	(+)-epi-bicyclosesquiphellandrene (4.55)
caryophyllene (3.26)	caryophyllene (2.79)	α -gurjunene (4.06)

amorphene. In case of AEOL, composition analysis showed higher relative abundance of monoterpene and sesquiterpene hydrocarbons followed by oxygenated sesquiterpenes, with all the three totalling to more than 83% of 94.73% total identified components (table 2). Camphor (26.50%) was found to be the main constituent of AEOL followed by β -bisabolene (7.57%), β -farnesene (6.17%), caryophyllene oxide (5.01%), (+)-epi-bicyclosesquiphellandrene (4.55%) and α -gurjunene (4.06%) (table 3).

Discussion

There is a renewed interest amongst scientists to study the bioactivity of plant EOs against phytophagous arthropod pests (Bakkali *et al.*, 2008; Adorjan and Buchbauer, 2010). The antifeedant and repellent effects of various plant EOs have been reported on weevils *Sitophilus granarius* (L.) and *Sitophilus zeamais* Motschulsky (Coleoptera Dryophthoridae), both widespread stored grain pests (Conti *et al.*, 2010, 2011; Mossi *et al.*, 2011).

In our studies, the EEOF showed significant antifeedant activity after 72 h and 96 h, causing 53.35 and 52.86 per cent reduction in feeding, respectively, at 1000 ppm. Ding *et al.* (1999) referred to their preliminary studies and reported antifeedant activity of petroleum ether extracts from flowers of *E. adenophorum*, though they omitted the name of test insect.

The AEOL was also found to exhibit potent antifeedant activity against RPW at 1000 ppm, with 18.96 ± 5.07 prick-marks in the first 24 h as against 48.34 ± 10.82 in the control which in effect amounts to about 60% reduced feeding. There was an increase in the total prick marks after 96 h, being 60.13 ± 7.94 in case of AEOL as compared to 104.81 ± 11.23 in control, the antifeedant activity of AEOL remained significant throughout the period of observation except at 72 h (table 1). Since we have found significant differences in other observational time-points, we assume non-significance at 72 h time-point as a natural biological difference.

It is not unusual to find significant differences in the bioactivities of EOs derived from different parts of the

same plant. The EO from leaves of Brazilian pepper tree, *Schinus molle* L. showed repellent action whereas, the EO from the fruit was attractive to rice weevil, *Sitophilus oryzae* (L.) (Benzi *et al.*, 2009). Different bioactivities result from different composition of essential oils and therefore different parts of the same plant may exhibit major differences in their compositions. Analysis of EO composition of the medicinal plant *Curcuma longa* L. revealed α -phellandrene as the main constituent of leaves while being totally absent in the flowers (Leela *et al.*, 2002). In the light of above facts and significant differences found in the antifeedant activity of EEOF and EEOL, chemical composition analyses was attempted to know the differences in their content profiles.

Results of GC and GC-MS analyses of EEOF and EEOL showed quite different content profiles, wherein EEOF comprised relatively higher percentage of monoterpene hydrocarbons and oxygenated derivatives of monoterpenes and sesquiterpenes compared to EEOL. The latter showed a reduction in variety and content of monoterpenes as well as oxygenated derivatives of monoterpenes and sesquiterpenes (table 2). Two major constituents of EEOF, viz., amorph-4,7-dien-11-ol (3.79%) and β -bisabolene (3.56%) were found to be absent in EEOL. Notably, β -Bisabolene is reportedly a potent non-phytotoxic antifeedant against *Leptinotarsa decemlineata* (Say) (Gonzalez-Coloma *et al.*, 1995) and *Myzus persicae* Sulzer (Gutierrez *et al.*, 1997).

Examination of AEOL chemical composition revealed very high (26.50%) camphor content though 32.92% monoterpene and 37.02% sesquiterpene hydrocarbon contents were comparable. Camphor is a known insect antifeedant and repellent as was reported by Miles *et al.* (1985) against boll weevil. The antifeedant activity of AEOL may originate from its main constituent camphor but minor constituents are known to modulate the bioactivities of major constituents with synergism or antagonism. Previous studies suggest synergistic role of other constituents in the EOs with high camphor content (Gonzalez-Coloma *et al.*, 2006; Nerio *et al.*, 2010). β -bisabolene, second major constituent of AEOL, is also known to possess antifeedant activity (Gonzalez-Coloma *et al.*, 1995; Gutierrez *et al.*, 1997) as stated above in case of EEOF.

The respective differences in effectiveness of EEOF and AEOL seem to originate from their different chemical compositions. It is to be noted that the composition of EEOF is biased towards sesquiterpene hydrocarbons and their oxygenated derivatives, whereas, in AEOL, representation of both monoterpene and sesquiterpene hydrocarbons is more than their oxygenated derivatives. The reason for gradual reduction in antifeedant activity of AEOL over the period of observation could be high volatility of its major constituent camphor. In case of EEOF, antifeedant activity became significant only after 72 h.

Conclusions

Among the three essential oils tested, the one extracted from flowers of *E. adenophorum* showed significant antifeedant activity against *R. ferrugineus* adults in the latter half of the observation period whereas the one obtained from aerial parts of *A. nilagirica* exhibited significant antifeedant activity throughout the observation period except after 72 h time-point. The essential oil from leaves of *E. adenophorum* lacked antifeedant activity. This shows that essential oils from different parts of the same plant differ in the biological activity as well as chemical composition as evidenced from this investigation. Analyses of chemical composition of the essential oils from flowers and leaves of *E. adenophorum* showed significant variation in their respective content profiles. This study has provided valuable information on the potential of EEOF and AEOL as antifeedants and requires further research on the feasibility of their field application as a preventive method.

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