

Identification of the biological active compounds of two natural extracts for the control of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (Coleoptera - curculionidae)

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ABSTRACT

Two natural insecticides, rotenone and limonene caused antifeedant and growth inhibition of the red palm weevil *Rhynchophorus ferrugineus* larvae and adults. Rotenone was extracted from the root of *Lanchoarpus sp.* or *Derris sp.* and limonene extracted from citrus fruit oil. The concentrations of 1.5, 3, 6, 9, 12 and 15% of both insecticides were used for treatment of the sugar cane pieces. Adult stage was more tolerant than the larvae. Also, the rotenone insecticide was more effective than limonene insecticide. Moreover increasing of rotenone and limonene concentrations were reduced the food consumption and increased the larval and adult mortality. The chemical analysis was suggested the structure of rotenone is flavonoid compound and its chemical formula is $C_{23}H_{22}O_6$. While the chemical nature of limonene is alkaloid with the chemical formula of $C_{10}H_{16}O$.

Keywords: The red palm weevil, natural insecticides, rotenone and limonene

INTRODUCTION

There are problems of pesticide resistance and negative effects on non-target organisms including man and the environment (Rembold, 1984 and Dorow and Rembold, 1993). The pool of plants possessing insecticidal substances is enormous (Jacobson, 1989). The toxic action of rotenoids against variety of insect species was reported against *Tenebrio molitor* when applied topically (Negherbon, 1959); the mustard web worm, *Crocidolomia binotalis*; and the diamond-back moth, *Plutella interpunctella* and *Idiocerus sp.* (Puttarudriah and Bhatia, 1955); and *Spodoptera litura*, Krishnamurthy, 1982). On the other hand, rotenone possessed antifeedant effect to many insects, such as larvae of *Spodoptera litura* (Srimmannarayan and Rao, 1985). In addition the isolated rotenone from Leguminous plants, had antifeedant effect against different insects (Jacobson and Crosby, 1971).

Limonene reported to be insecticidal against many insects as the pulse beetle, *Callosobruchus phaseoli* (SU, 1976); and the rice weevil, *S. oryzae* and the red flour beetle, *Tribolium castaneum* (Don Pedro 1985; El-Sayed and Abdel-Rahik 1986, and Prakash *et al.*, 1987). However, limonene showed juvenile-hormone mimic activity against 5th instars larvae of the red cotton bug, *Dysdercus cingulatus* (Kareem 1984). Limonene had antifeedant activity against different insects, pulse beetle *C. maculatus* (Don Pedro, 1985); and *Leptinotarsa decemlineata* (Prakash *et al.* (1990c). On the other hand, the limonene caused inhibition of oviposition against different insects, El-Sayed and Abdel-Rahik (1986) reported limonene oil to show inhibition

oviposition of *C. maculatus*. however complete inhibition of oviposition occurred to the cotton leaf hopper, *Amrasca devastans* (Saxena and Basit, 1982 and Su and Horvat (1988). Although the limonene was act as repellent, the fruit juice of sweet orange had attractant activity when tested against the leaf cutting ants (Jacobson, 1958).

The red palm weevil *Rhynchophorus ferrugineus* came to attention because it was recently introduced during the last decade in the Near East region. The European and Mediterranean plant protection Organization (EPPO, 2002) reported that *R. ferrugineus* found in Jordan and Israel (1999), in Egypt (Cox, 1993) In Asia, mid 1980 (El-Ezaby, 1997). *R. ferrugineus* attacks many plant tree species (*Areca catechu*, *Arenga pinnata*, *Borassus flabelifer*, and *Caryota maxima* (Abbas *et al.*, 2001). Severely damage attacked palm trees show a total loss of the palms and rotting of the trunk, which lead to the death of the tree (Anonymous 2000).

The aim of this work was to study the effects of the rotenone and limonene biopesticides on the growth inhibitory, antifeedant, mortality of the red palm weevil *Rhynchophorus ferrugineus*.

The spectrophotometer analysis using UV, IR, Mass spectrum and the element analysis of the two botanical insecticides were studied.

MATERIAL AND METHODS

1- Insect colony

The different developmental stages of *Rhynchophorus ferrugineus* was obtained from the infected palm trees at Wady-Aldawaser region in Saudi Arabia. The colony were fed on sugar –cane pieces and the females laid eggs between the sugar cane tissues till hatching. Rearing colony was kept under $32\pm 3^{\circ}\text{C}$ and $50\%\pm 5$ R.H. according to the method of Nassar and Abdullah (2001) with some modification.

2- Botanical insecticides

Two Botanical insecticides (Rotenone and limonene) were obtained from Sigma Chemicals Company. Rotenone is the extract of *Lanochocrapus* species or *Derris* species root. Limonine is the extract of citrus fruit peels. Different concentrations (1.5, 3, 6, 9, 12, and 15%) of rotenone and limonene insecticide were prepared using water as solvent.

3- Antifeedant of larvae

In the present investigation, feeding inhibition or repellency was demonstrated by treating sugar-cane pieces with the different concentrations of rotenone and limonene. The different concentrations (2, 4, 6, 8, 10, and 12%) of the rotenone and limonene insecticides were topically treated with 1ml of each concentration on the sides of sugar cane pieces (5 pieces measured $15\times 3\times 3\text{cm}$ /replicate) using 1ml glass pipette. The treated sugar-cane pieces were air-dried then introduce to the starved 6th days old larvae. Sugar-cane pieces without any treatment were introduced to the 6th days old larvae as control. The treatment was replicated three times (10 starved larvae /replicate) for each concentration. The experiments were performed according to the method adopted by Morallo-Rejesus (1980).

4-Toxicity study and biostatistics

Larvae (8-days old) and newly emerged adults repeated three times (10 larvae or adults/replicate), were topically received 4μ of rotenone and

limonene concentrations (1.5, 3, 6, 9, 12, and 15%) using micropipette (1-10 μ). The treated larvae and adults supported with small sugar-cane pieces in plastic cups for feed. Control insects were given sugar cane without any treatment. Percent of larval and adult mortality was recorded after 24 hours post treatment and the corrected mortality adjusted using Abbot formula (1925). The recorded data was tabulated depending on *T*-distribution and was estimated according to Finney (1971).

5- Spectrum nature of rotenone and limonene.

Rotenone and limonene were subjected to spectrophotometer analysis by using ultra violet (UV), infrared (IR) and mass (Ms) spectrophotometers at the Chemistry Department, Faculty of Science, King Khalid University.

RESULTS AND DISCUSSION

Antifeedant activity

Repellants, on the other hand, drive the insects away after exposure the sugar-cane pieces to the rotenone and limonene insecticides without necessarily feeding. The behavior of the larvae was observed immediately after exposure for 6hr but the consumption of sugar-cane pieces measured after 24hr. Data recorded in Table (1) revealed that, the larval consumption of treated sugar cane with rotenone was 22.3, 13.5, 8.7 and 5.7cm² at the concentration of 1.5, 3, 6 and 9%, respectively. Whereas at the higher concentration, 12 and 15% of rotenone, no food was recorded by larvae. In the case of treated sugar cane with limonene by concentration of 1.5, 3, 6, 9 and 12% the areas of food consumption were 27.6, 17.2, 10.4, 8.7 and 5.7 cm², respectively. While at the concentration of 15% only the limonene insecticide caused no consumption food by larvae compared to 45.4 \pm 3.12cm² of control (Table 1). The rotenone and limonene treated sugarcane were ingested by larvae of *R. ferrugineus* to an appreciable degree and this feeding was less than the feeding in control sugarcane. It is quite clear from these results that rotenone considered as antifeedant, more than limonene and this indicates that the both of natural insecticides contained volatile chemicals, which deter insects. The reduced feeding could be either due to direct toxic action of both insecticides on larvae but the food intake is reduced until the insects die. The present results clearly confirmed with those obtained by El-Sayed and Abdel- Rahik (1986) who reported that the odor of the citrus oils prevents the beetles of *Callosobruchus maculatus* from wandering away from the cowpea seeds. Don Pedro (1985) conducted an intensive study into the growth inhibitory and antifeedant effects of limonene to *C. maculatus* when admixed with stored cowpea at 4% w/w. Its peel powder also reduced the grain damage and weight loss due to *Sytophilus oryzae* when admix with wheat grains (Rout, 1986). Both rotenone and limonene proved repellent to larvae of *R. ferrugineus* and a significantly ($P < 0.05$) less feeding was observed on the treated sugar-cane pieces. Components of rotenone and limonene may be responsible for antifeedant and repellent activity against larvae of *R. ferrugineus*. Similar to this result, the active component of rotenone insecticide shows antifeedant activity to the 4th instar larvae of *Spodoptera litura* (Srimannarayan and Rao, 1985). Rotenone was also isolated from various species belonging to family Leguminosae, had antifeedant effect

(Jacobson and Crosby, 1971). Nomilin and obacumone of citrus oils were more active growth inhibitors even than the azadirachtin at 10ppm concentration (Klocke and Kubo, 1982). El-Sayed and Abdel-Rahik (1986) reported the citrus oil to show inhibition of growth and oviposition of *C. maculates* on the treated cowpea seeds using 0.73 to 1.0% formulations. El-Sayed *et al.* (1991) found its oil treatment with stored at 0.25% w/w to significantly reduce the populations of wheat weevil, *Sitophilus granarius* and to cause 44% mortality 16 days after treatment. Furthermore the effect of limonene was inhibited and prevented oviposition of the cotton leaf hopper, *Amrasca devastans* (Saxena and Basit 1982).

Table (1): Antifeedant effects of rotenone and limonene insecticides on *R. ferrugineus* larvae.

Concentration %	Peaces (cm ²) eat/larvae	
	Rotenone	Limonene
1.5	*22.3±1.5	*27.6±3.6
3	*13.5±0.7	*17.2±2.5
6	*8.7±2.6	*10.4±2.1
9	*5.7±1.2	*6.8±1.4
12	*0.0	*2.3±0.3
15	*0.0	*0.0
Control	45.4±3.12	45.4±3.12

* a significance (P<0.05).

Lethality effect of rotenone and limonene:

Botanical insecticides of rotenone and limonene resulted in a considerable mortality compared to control. The highest mortality rates attained 92.3% and 81.2% in treated larvae with the higher concentration (15%) of rotenone and limonene, respectively (Table 2). Whereas the highest concentration of rotenone and limonene caused 79.2 and 71.1% mortality of adult stage respectively compared to 0% of control group. The larval and adult mortalities were increased with an increasing in the concentration of both insecticides. The lower larval mortality was 11.3 and 8.7% while adult mortality was 6.2 and 4.2% after treatment of both stages with the lower concentration (1.5%) of rotenone and limonene, respectively. Mortality percent are probably the result of the toxic power of these two compounds. Furthermore, decreased locomotion, trembling of appendages were noted in the treated adults which demonstrating the effect of rotenone and limonene on the center of locomotion in the nervous system as neurotoxin insecticides. These symptoms were accompanied by the production of liquid feces, thus indicating a disruption of water balance. This phenomenon is often noted in insects treated with neurotoxic insecticides (Proux *et al.*, 1993). Similar to this result, the rotenone acted as a contact insecticide and also as a stomach poison of slow action, against the rice weevil, *Sitophilus oryzae* seed powder of *Derris elliptica* (Nagai, 1902).

On the other hand ,Fabaceae plants (contained rotenone) was found to protect stored wheat when admixed with the grains (Krishnamurthy and Rao, 1982). Puttarudriah and Bhatia (1955) and Fukami *et al.* (1959) reported the root powder of *Derris* to be toxic to the pulse beetle, *Calosobruchus sp.* Similarly, toxic effect of rotenone was also reported against *Tenebrio molitor* when applied topically (Negherbon, 1959). Data recorded in Table (2) indicated that the larvae and adults of *Rhynchophorus ferrugineus* were more susceptible to rotenone than the limonene insecticide. This may be due to the different of chemical nature between rotenone and limonene. Limon oil was reported to be toxic to the pulse beetles, *Callosobruchus chinensis* and *C. maculates* (SU,

1976). While the extract and powder of dried citrus peels of sweet lime inhibited the multiplication of stored product insects (Su *et al.*, 1972). On the other, hand the volatile oils of limon was inhibited the oviposition of the cotton leafhopper, *Amrasca devastans* (Saxena and Basit, 1982). Jacobson (1975) reported insecticidal activity in the oil extracted from sour orange peels against the diamond-back moth, *Plutella xylostella*. El-Sayed *et al.* (1991) reported that oil of sweet orange to show 64% mortality to wheat weevil, *Sitophilus granarius* in stored treated grains at 0.25%w/w. Also, Su and Horvat (1988) isolated and characterized four insecticide components from biologically active lemon peel extract which were moderate toxic to *Sitophilus oryzae* and *Callosobruchus maculates*. Toxicity of insects, mammals and fishes to rotenone was due to the ease of entrance to the cellular level (Fukami *et al.* 1969). On the other hand, rotenone has ability to inhibit cellular respiration in almost every living organism, including mammals, fish, amphibians, insects, and even plants (Bradbury 1986). Insects poisoned with rotenone show symptoms of intoxication: drop in oxygen consumption and respiratory depression and ataxia leading to convulsions and finally to paralysis and death (Gonzalo 2004). On the other hand, The symptoms of insects poisoned by rotenone differed from those produced by neurotoxic insecticides and were characterized by reduction of oxygen-consumption, depressed respiration and eventual paralysis (Martin, 1964). Whereas the mode of limonene action is similar to that of pyrethrum. Its effect the sensory nerve of the nervous system, but it is not a ChE inhibitor (George *et al.*, 2004).

Table (2) Effect of rotenone and limonene on the larvae and adult mortality of *R. ferrugineus*.

Concentration %	Insecticides			
	Rotenone		Limonene	
	% of larval and adult Mortalities		% of larval and adult Mortalities	
	Larva	Adult	Larva	Adult
1.5	11.3	6.2	8.7	4.2
3	23.4	18.3	14.4	9.1
6	36.5	28.1	26.5	18.6
9	58.2	47.3	45.2	36.2
12	73.2	65.1	60.3	47.3
15	92.3	79.2	81.2	71.1
Control	00.0	00.0	00.0	00.0

Spectrophotometer analysis of rotenone and limonene

a-Ultraviolet Spectrophotometer (UV)

Ultraviolet (UV) spectra of rotenone are shown in Fig. (1). The active compound showed UV maxima at 294, 337nm and low intensity region of 207.5nm at absorbance of 0.592, 0.512, and 1.188 absorbance, respectively. These absorbances indicated the presence of flavon group.

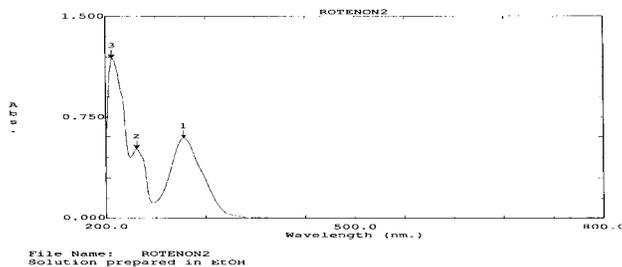


Fig. (1) : Ultraviolet(UV) spectrophotometer of Rotenone

While UV analysis of limonene (Fig.2) was contains unsaturation connected with C=O and C=N. The unsaturation may be present in the ring itself or alpha to the carbon. The active components of limonene showed UV high-resolution maxima at 273.5, 256.5, and 214.5nm with absorbance of 0.013, 0.013, and 1.907 respectively, which indicated the presence of aromatic alkaloid group.

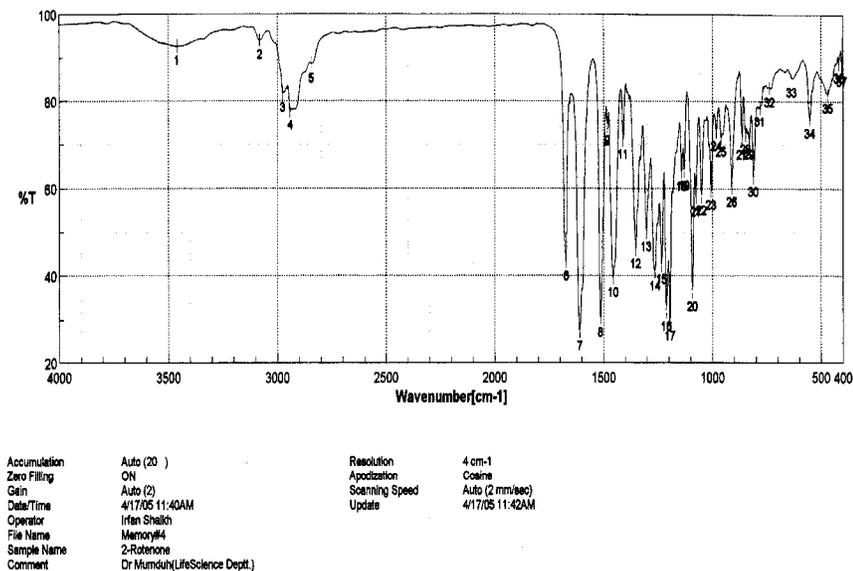


Fig. (2): Infrared (IR) spectrophotometer of Rotenone

Data obtained by UV analysis of rotenone show the presence of two bands of low intensity in the region 520-640nm. The shorter wavelength 243 nm revealed the high intense bands that indicate the unsaturation double bonds. For limonene UV and visible spectra, the bands 520-700nm may refer to transition in simple ketenes, acids, esters and amides.

b-Infrared Spectrophotometer (IR)

IR-data of the active compound of rotenone is showed in Fig. (3). Infrared analysis supported the present of many function groups at wavenumber of 3400cm⁻¹, 3100cm⁻¹, and 2816cm⁻¹, 2700cm⁻¹ attached to flavonoid. On the other hand, low absorbance wavenumber was obtained at different of transition.

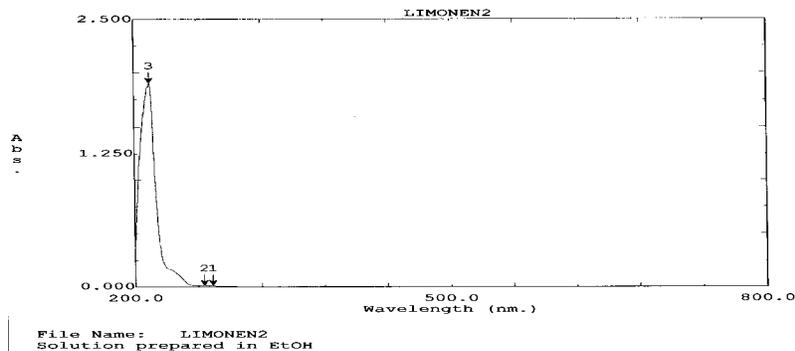


Fig. (3): Ultraviolet (UV) spectrophotometer of Limonene

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These absorbance was $1500-1700\text{cm}^{-1}$, $1000-1400\text{cm}^{-1}$, and $500-980\text{cm}^{-1}$ which suggested the presence of aliphatic chemical structure attached to the function groups of C=O, C=N, C=CL, OH and CH. These function groups which supported by IR have principal important of rotenone toxicity. IR analysis of limonene insecticide (Fig. 4) revealed the presence of different maxima with different wave number ($2340-2900\text{cm}^{-1}$, $1430-800\text{cm}^{-1}$, $800-750\text{cm}^{-1}$ and $400-600\text{cm}^{-1}$ which suggested the presence of alkaloid structure. The presence OH and CH group of limonene is supported by IR analysis at wavenumber of 3450 and 2854cm^{-1} respectively. On the other hand, the presence of chloride that indicated by IR at $800-600\text{cm}^{-1}$.

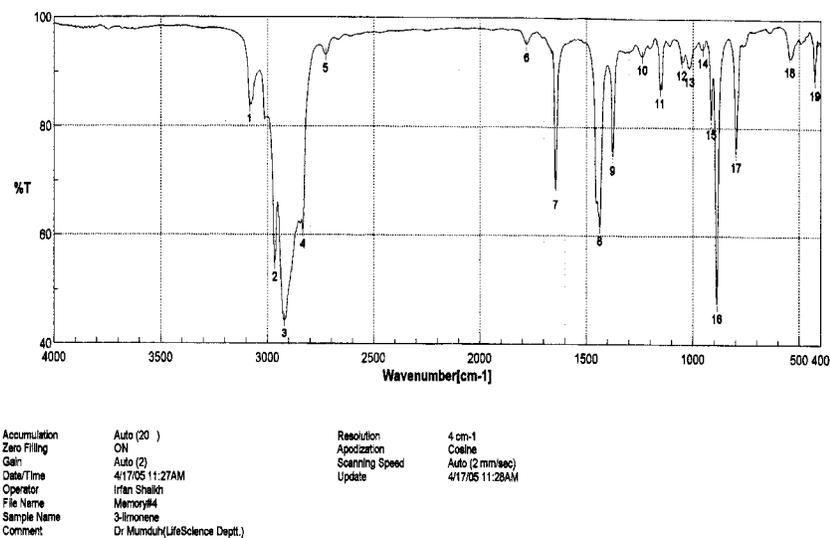


Fig. (4): Infrared (IR) spectrophotometer of Limonene

c-Mass Spectroscopy (Ms)

Mass spectrophotometer analysis, suggested by Gonzalo (2004), the molecular weight of rotenone was 394.4MW. This was revealed that rotenone is flavonoid in nature and it has some other stretching. On the other hand, EPA (2005) that the molecular weight of limonene was 187.6MW, which supported to the presence of alkaloid structure. The present results revealed that the active compound with molecular weight 394.4MW and proposed molecular formula is $\text{C}_{23}\text{H}_{22}\text{O}_6$ of rotenone might be the correct one. On the other hand, the molecular formula of limonene was $\text{C}_{10}\text{H}_{16}\text{O}_4$, however this results confirmed with the finding of George and David, (2004). Similar results are indicated by WHO (1992); Dave Kain (2000) and Gonzalo, (2004). From the previous investigation of element analysis, UV, MS, and IR spectrum the chemical nature was supported the presence of falvonoid in rotenone and alkaloids for limonene.

These positive finding could have the way for the development of a safe, potent, and cheaper plant protection component, which could be used either alone or in combination with other methods in the future to make crucifer production non-polluting, non-hazardous and at the same time profitable, especially in the developing countries.

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ARABIC SUMMARY

تعريف المركبات النشطة بيولوجياً لأثنين من المستخلصات الطبيعية لمكافحة سوسة النخيل الحمراء (ريتكوفرس - فيروجينيس) غمدية الأجنحة - كيركيلونيدى

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أدى تأثير أثنين من المبيدات الطبيعية ، الروتينون، والليمونين على يرقات وبالغات سوسة النخيل الحمراء الى فقد القدرة على الغذاء وتوقف النمو لليرقات وبالغات. ومبيد الروتينون هو مستخلص جذور نبات لانكوكاريس أو الديريس، ومبيد اليمونين هو مستخلص من زيوت ثمار الموالح وقد استخدمت التركيزات 1.5 ، 3 ، 6 ، 9 ، 12 ، 15% لكلا المبيدين فى معالجة قطع قصب السكر التى تتغذى عليها البالغات واليرقات. وكانت اليرقات أكثر تأثراً بالمبيدين عن البالغات ، كما أن مبيد الروتينون كان له تأثير أكثر من اليمونين . وقد لوحظ أيضا أن زيادة التركيزات للروتينون واليمونين أدى الى زيادة نسبة الوفيات لاطوار سوسة النخيل الحمراء وكذلك تقليل كمية الغذاء المستهلك.

وبدراسة التحليل الكيميائى عن طريق استخدام اجهزة الأشعة فوق البنفسجية والأشعة تحت الحمراء وتحليل العناصر فقد وجد أن تركيب مبيد الروتينون من مجموعة الفلافونويد المعادلة الكيميائية له هي $C_{23}H_{22}O_6$. بينما كان تركيب مبيد اليمونين من مجموعة القلويدات ومعادلتة الكيميائية هي $C_{10}H_{13}O_4$.