

Possibility Using Camphene as Biorational Insecticide against the Red Palm Weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)

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Abstract: The Red palm weevil, *Rhynchophorus ferrugineus* (RPW) (Coleoptera: Curculionidae) is the most important pest for the palm trees in all of the Arab Gulf area. The possible use of the terpene (Camphene) as toxic product alternative to chemical insecticide through the IPM program for controlling RPW and decreasing generating resistance phenomenon has been carried out, where there is a need for different compounds having different modes of action. Camphene is bicyclic monoterpene. It volatilizes readily at room temperature and has a pungent smell, is used in the preparation of fragrances and as a food additive for flavoring. Camphene is attractive for both male and female adults of the red palm weevil. Effect of camphene as fumigant or contact poison against different stages of *Rhynchophorus ferrugineus* (egg, 4th instar larvae and adults) was determined, it cleared a toxic effect for all stages of the insect, especially for eggs and larvae as contact or fumigant poisons. LC₅₀ as contact poison was 249.2, 610.1 and 777.3 µl./100 ml., while LC₅₀ as fumigant poison was 34, 47.9 and 99.9 µl./l. air on egg, 4th larval stage and adult respectively. Median lethal concentration (LC₅₀ concentration) of camphene induced some biological changes on treated eggs and 4th instar larvae.

Keywords: Red palm weevil, *Rhynchophorus ferrugineus*, camphene, terpene, toxicity, biological activity

1. Introduction

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier.) (Coleoptera:Curculionidae) is a major pest of various palms in the Middle East, South and South East Asia, North Africa and Southern Europe (Nirula,1956). Females lay eggs in damaged or wounded plants. Upon hatching, the larvae burrow into the fresh tissue and migrate to the bud region and heart of the crown where they feed for two to four months and eventually kill the host plant (Abraham,1971). The RPW has a wide geographical and host range. It was reported to infest 40 palm species worldwide (Vidyasagar *et al.*, 2000). Infestation with RPW starts with gravid females attracted to palm volatiles for laying eggs which hatch into damage inflicting larvae. Infestation date palms exhibit several symptoms depending on the stage of attack, e.g. oozing of brownish fluid mixed with palm tissue excreted by feeding larvae that has a typical fermented odor, tunneling of palm tissue by larval, presence of adults and pupae at the base of fronds, drying of infested offshoots, fallen pupae around an infested palm, drying of outer leaves niches and topping of trunk in case of sever and extensive tissue damage (Vidyasagar *et al.*, 2000). Management of agricultural pests over the past half century has been largely depending on the use of synthetic pesticides for field and post-harvest protection of crops (Ferry *et al.*, 2004). Several reviews on the control strategies including integrated pest management for RPW are available (Vidyasagar & Subahanran, 2000). But the development of insect resistance to these products, the high operational cost and environmental pollution has created the need for developing of alternative approach to control many insect pests. In this sense, the usage of essential oils is an alternative to control many field and household insect

pests (Sarawae *et al.*, 2005; 2012; 2013). The essential oils are natural, volatile and complex compounds, and their characteristic odors are formed by secondary metabolites in plants. Many plant essential oils and their constituents from terpenes show a broad spectrum of activity against pest insects ranging from insecticidal, repellents, attractants, oviposition, deterrents, growth regulators and anti-vector activity (Bakkali *et al.*, 2008). Plant essential oils and monoterpenes are shown as botanical pesticides. One of the most important features of botanical pesticides is to perform different effects on some insects. Most essential oil chemicals are relatively non-toxic to mammals and fish in toxicological tests, and introduce the criteria of reduced pesticides risk. Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential oil based pesticides owing to the complex mixture of constituents that characterize many of these oils. Monoterpenes, the chemical constituents of essential oils found in plants, are known biologically active compounds. Essential oils and their constituents demonstrate fumigant and topical toxicity as well as antifeedant and repellent effects (Shaya *et al.*, 1997; Sharaby & EL- Dosary 2014; Sharaby & EL-Nugibal, 2015). Camphene is bicyclic monoterpene .It volatilizes readily at room temperature and has a pungent smell, is a minor constituent of many essential oils such as turpentine, cypress oil, camphor oil, nerol, ginger oil, and valerian. It is produced industrially by catalytic isomerization of more common α -pinene. Camphene is used in the preparation of fragrances and as a food additive for flavoring. Its mid-19th century use as a fuel for lamps was limited by its explosiveness. Sharaby & EL-Dosary, (2014) recorded that Camphene was attracted for

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both sex of the RPW. The aim of the current research is to estimate the toxic effect of Camphene as contact or as fumigant toxicant on different stages of the red palm weevil, *Rhynchophorus ferrugineus*, and investigate the effect of LC₅₀ of Camphene on some biological parameters to find their mode of action and its possible use as alternative natural material to chemical pesticide in the IPM program for controlling the insect. Studies have not yet been conducted to evaluate the insecticidal activities of monoterpene Camphene towards the RPW.

2. Materials & Methods

1- Insect culture and rearing conditions.

The RPW culture was maintained in laboratory from larvae, pupae and adults collected from infested palm trees in Al-Kharj region of Saudi Arabia. The insect culture was established under laboratory condition at 25±2C° temperature and 30% RH, and L: D10:14h. Sugarcane stem split longitudinally in 10cm, pieces to provide food source for (larvae and adult weevils) as well as oviposition substrate. From the laboratory bred pupae, the requisite number of emerging adults was collected. The adults after emergence from the pupae were sexed and maintained in separate 1 L jars with sugarcane pieces as food. Regular addition of field-collected adults to avoid inbreeding of the insects.

2- Camphene: Obtained as a pure compound from Sigma Co. (St. Lous. Mo., U.S.A).

3- Fumigant toxicity

Different concentrations of essential oil were prepared by dilution of oil µl./100ml. with distilled water, 0.1% Triton X-100 was added as emulsifier for different stages (eggs, 4th instar larvae and adult stage). They were applied on filter-paper (Whatman No.1) strip measuring 5 cm that attached to the lower side of the jars lid. Five normal and healthy adults 4days old were placed in empty 1l. capacity glass Jar then sealed with air-tight glass lid. Thus, there was no direct contact between the oil and the insect. For the control jars, filter papers were treated with emulsifier and water only without the oil. Mortality was determined after 24 h from comment of the exposure time. Each test was replicated five times for each concentration or each tested stage. Signs of egg mortality indicated as no hatching, no motion of larvae and no leg or antennal movement observed for adult mortality.

4- Contact toxicity

Different tested stages (eggs-, 4th larval instar and 4days old adult) were sprayed by the different concentrations of the oil emulsion using small plastic hand sprayer under open air and

left to dry from moisture, then placed inside glass jar for mortality observation. An aqueous solution with the same amount of water and emulsifier used as in the treatment served as control. Each test repeated five time, 100 egg and 25 larvae or adult were used.

5- Biological activity of Camphene on egg and 4th larval instar.

Concentration of the Camphene (LC₅₀) by fumigation or contact on egg stage and 4th larval instars that determined from the previous test was prepared as emulsion in distilled water with two drops of Triton x100 (as emulsifier). Camphene emulsion was applied by fumigation or contact treatment as mentioned before, and then the hatched larvae from the treated eggs, and also the still living treated 4th instar larvae were introduced on the normal Sugarcane stem split as a food source for follow-up growth and development. Different biological aspects were recorded as follows, larval duration, pupal duration, percentage pupation, and percentage of adult emergence adult longevity percentage of larval and adult deformation, percentage of deposited eggs / female, and percentage of egg hatching. Foregoing biological aspects was evaluated by daily observations.

6- Statistical analysis.

Data were corrected using Abbot's formula (Abbot, 1925) for the control mortality, then subjected to Probit analysis to estimate LC₅₀ values according to (Finny ,1971). The percentage of mortality was analysed using analysis of variance (ANOVA) test. Significant differences for means were compared using Duncan's multiple range test (Duncan, 1955) using computer software (SPSS, 1999). Significance between samples were indicated by probability values at P< 0.05%.

3. Results & Discussion

Table 1 show a positive relation between Camphene concentration and percentage of mortality for all the tested stages of the RPW. Camphene has both contact and vapor phase activity, it was more effective by fumigation than as contact poison for all stages, LC₅₀ was 34.1, 46.9 and 99.9 µl oil/l. air by fumigation while it was 1249.2, 610.1 and 777.3 µl. oil /100 ml. water as a contact on egg, 4th instar larvae and adult stage respectively. Egg was the most sensitive stage by fumigation followed by larvae then adults. Data cleared that Camphene have different effects on the insect , it block the air pore (egg micropyle in insect egg envelops or chorion) through which air oxygen enters, and cause egg asphyxiation.

Table 1: Toxicity of Camphene on different stages of *Rhynchophorus ferrugineus* by fumigation or contact treatments

Tested Stage	Fumigation µL/L					Contact µL/100ml				
	LC ₅₀	LC ₉₅	Slope±SE	LM	UM	LC ₅₀	LC ₉₅	Slope±SE	LM	UM
Egg	34.1	296.9	1.8±0.3	28.4	45.1	1249.2	3075.4	1.5±0.4	199.1	377.5
4 th larval instar	46.9	100.9	5.1±0.5	36.6	63.7	610.1	2689.4	2.6±0.4	519.6	799.0
Adult	99.9	8113.9	1.8±0.3	839.3	1236.9	777.3	1785.9	4.6±0.7	---	---

Table 2 recorded very low number of egg hatching 26.7% after exposed to Camphene vapor, some of the hatched larvae still alive for 90 day then all died without reached the pupal

stage in comparing with 27 day larval duration for control untreated test. LC₅₀ of Camphene as contact treatment on eggs, also shows some biological aspects abnormalities

where 13.3% of egg hatching was obtained, 6% of the hatched larvae reached to the pupal stage after 99.5 day. 30% of larval abnormalities was observed comparing with 10% for the control. All obtained pupae were small in size comparing with the control ones. All pupae died without reached the adult stage.

Table 2: Some biological aspects of *Rhynchophorus ferrugineus* after egg treatment with LC₅₀ of Champhene by different methods

Biological parameters	Control Champhene treatments		
	Fumigant Contact		
	mean± SD	mean± SD	mean± SD
% egg hatching	26.7	13.33	98
Larval duration	----	99.5 ± 0.44	27± 3.33
% larval deformation	----	30	10
% pupation	-----	6	88
Pupal duration	-----	-----	22± 12.04
% adult emergence	-----	-----	77

(----) all died. No results.

Table 3 recorded some biological aspects of RPW after 4th instar larvae treated with the LC₅₀ of Champhene by the two different methods, as a contact poison all the treated 4th instar larvae were died within 2.5 month without reached pupal stage, while as a fumigant poison, larval duration were significantly increased to 69.4 compared 23.5 day for the control. There were a great number of deformed larvae reached to (24%), larva lost its motion and was unable to complete the molting process during the development. Great decrease in pupation percentage reached to 16% compared with 87.33% for the control normal larvae. No emerged adults were obtained, where deformed pupae and uncompleted adult in the form of pupal adult intermediate were noticed inside the pupal cocoons, for the control 72% emerged normal adults were obtained. It can be speculated that Champhene was toxic as fumigant or contact on all stages of RPW, it is potent JH mimic affecting the hormonal balance in RPW, resulting in strong suppression of embryogenesis, metamorphosis, and adult formation (Itaya, 1987 Kehler & Peterson, 1991). Sharaby & EL-Dosary (2014) mentioned that Champhene was attractive for both sex of RPW adults, with 60% percentage on males and with 40% on females. Champhene have different effects on the insect and the most important is that they block the air holes (Spiracles) through which the insect breathes, and causes asphyxiation. AL-Sharook *et al.* (1991) recorded that the oil effects on the insect respiratory and nervous system leading to insect death, disrupt the normal hormone regulation. In some cases oils also may act as poisons, interacting with the fatty acids of the insect and interfering with normal metabolism. Some researches have demonstrated that essential oils have neurotoxic, citotoxic and mutagenic action among others in different organism, and the essential oils act as multiple levels in the insect, so the possibility of generating resistance is little probable (Bakkali *et al.*, 2008). Won-II *et al.* (2003) mentioned that insects response varied according to oil type, dose and developmental stage. Oils work primarily by covering and suffocating the pest. In some cases, they can disrupt certain membranes of exoskeleton (Cranshaw & Baxendale, 2014). Henandez-Lambrano *et al.* (2015) mentioned that the essential oils extracted from the citronella genus (*Cymbopogon*) can be used to control *Oryzaephelium*

surinamensis (L.) and maize weevil *Sitophilus zeamais* Motsch. It can also reduce the risk associated with the use of synthetic insecticides. Turpentine oil is a type of essential oil that is extracted from turpentine (Saeidnia, 2014). The main component includes α -thujene, α -pinene, camphene, β -pinene, myrcene, 3-carene, p-cymene, limonene, and β -phyllandrene among several others (Liu *et al.*, 2013). Regnault-Roger & Hamraoui, (1995) recorded that the point of entry of the toxin is one of apart from the variability of phytochemical patterns that involves several factors. Commonly terpene oil can be inhaled, ingested or skin absorbed by insects. The fumigant toxicity of essential oils and their main components, the volatile monoterpenes, has been described (Weaver *et al.*, 1991; Regnault-Roger *et al.*, 1993). The present results from this study agreed also with that recorded by Sarwar & Salman, 2015 that many plant essential oils and their constituents of monoterpenes show a broad spectrum of activity against insect pests, these natural chemicals have multiple mode of action, including antifeedant and repellent activity, moulting and respiration inhibition, growth and fecundity reduction and cuticle disruption, and can act as contact, fumigant, and oviposition inhibition toxicant. These multiple mode of action are advantageous because they delay the development of resistance among arthropod pest population.

Table 3: Some biological aspects of *Rhynchophorus ferrugineus* after 4th larval instar treatment with LC₅₀ of Champhene by different methods.

Biological parameters	Control Champhene treatments		
	Fumigant Contact		
	mean± SD	mean± SD	mean± SD
Larval duration	69.4± 0.54	----	23.5± 7.9
% larval deformation	24	----	12
% pupation	16	-----	87.33
Pupal duration	-----	-----	22± 12.04
% adult emergence	----	----	72%

(-----) all died

4. Conclusion

From all these results, it could be deduced that terpene Champhene present a widespread range of activities on *R.ferrugineus* as contact or fumigant poison, it works on the endocrine system and therefore on the enzyme system of adults. Champhene could be used for environmentally safer pest management option. It is active against all stages of the insect as contact and as fumigant poison. Possibility of generating resistance is little probable, and can be effectively used as part of current alternative integrated pest management strategies of *R.ferrugineus* aiming at decreasing the use of classical insecticides, ecochemical control based on plant- insect interaction relationships is one of the most promising methods.

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