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Full Length Research Paper

Effect of diethyl maleate on toxicity of linalool against two stored product insects in laboratory condition

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Essential oil extracted from plants has been widely investigated for pest control properties, with some proving to be toxic in insect pests. In this study, effect of the synergist diethyl maleate (DEM) on toxicity of one monoterpenoids, linalool was studied against two most common stored-product insects such as *Callosobruchus maculatus* and *Rhyzopertha dominica*. Diethyl maleate was combined in mass ratios (1:8 and 1:4) with acetone used and applied on *C. maculatus* and *R. dominica* adult. Five concentrations of linalool were tested with four replications at 24 and 48 h with 30 adult insect in each replication. After 24 h of exposure, the LC_{50} values were estimated to be 23.61 and 31.01 µl/l air, and after 48 h, they were 15.07 and 21.84 for each insect, respectively. A combination of inalool with the synergist after 24 h of exposure, the LC_{50} values was estimated to be 11.93 and 13.07 µl/l air and after 48 h, they were 7.38 and 7.93, respectively for each insect. The synergist is able to block the specific system of enzymes involved in selection of tolerance in susceptible generations. Diethyl maleate is an inhibitor of glutathione S-transferase (GST) activity. These results show that diethyl maleate decrease doses of linalool.

Key words: Diethyl maleate, linalool, LC_{50}, synergism, *Rhyzopertha dominica, Callosobruchus maculatus.*

INTRODUCTION

Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses and insect contamination in food commodities is an important quality control problem of concern for food industries (Rajendran and Sriranjini, 2008). Currently, phosphine (from metal phosphide preparations, cylinderized formulations and on-site generators) and methyl bromide (available in cylinders and metal cans) are the two common fumigants used for stored-product protection world over. Insect resistance to phosphine is a global issue now and control failures have been reported in field situations in some countries (Taylor, 1989; Collins et al., 2002). Methyl bromide, a broad-spectrum fumigant, has been declared an ozone-depleting substance and therefore, is being phased out completely (Rajendran and Sriranjini, 2008).

During the past few decades, application of synthetic pesticides to control agricultural pests has been a standard practice. However, with growing evidence that many conventional pesticides can adversely affect the environment, requirements for safer means of pest management have become crucial. Therefore, the use of safe, low toxicity botanical pesticides is now emerging as one of the prime means to protect crops, their products and the environment from pesticide pollution, which is a global problem (Rozman et al., 2007).

The toxic and repellant properties of monoterpenoids have been studied by several authors (Obeng-Ofori et al., 1998; Ngoh et al., 1998; Pascual-Villalobos et al., 2003; Garia et al., 2005). In recent study, one of the monoterpenoids such as linalool was shown to be possible alternatives to synthetic insecticides against stored-product pests such as *Callosobruchus maculatus* and *Rhyzopertha dominica.*

Resistance to organophosphates, pyrethroids or

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Abbreviation: DEM, Diethyl maleate; PBO, TPP, etc

Table 1. LC_{50} and LC_{95} values of linalool without synergist to *C. maculatus* and *R. dominica* at 24 h.

Insect	LC_{50} (µl/l air)	Slope (± SE)	Intercept (±SE)	LC_{95} (µl/l air)	χ^2
C. maculatus	23.61 (22-25)	5.24 (±0.44)	-2.20 (±0.62)	48.59 (42-57)	0.79
R. dominica	31.01 (29-32)	6.06 (±0.56)	-4.03 (±0.83)	57 .09 (51-67)	1.58

Table 2. LC_{50} and LC_{95} values of linalool without synergist DEM to *C. maculatus* and *R. dominica* at 48 h

Insect	LC_{50} (µl/l air)	Slope (± SE)	Intercept (±SE)	LC_{95} (µl/l air)	χ^2
C. maculatus	15.07 (14-15)	5.47 (±0.47)	-1.44 (±0.58)	30.12 (27-34)	1.56
R. dominica	21.84 (20-22)	6.11 (±0.59)	-3.18 (±0.78)	40.58 (36-47)	2.35

phosphine is common in many stored-product pests (Nayak et al., 2003). According to Miller (1988), the tolerance mechanisms of insecticides can be classified into four categories: behavioral change, resistance to penetration, insensitivity of sit of action and metabolic resistance (Lopez et al., 2010). The study of tolerance mechanisms can show the possibility of insects developing tolerance to a pesticide and helps in the development of strategies to avert this (Jensen, 2000).

In this study, we selected one extracted essential oil, linalool and one synergist diethyl maleate (DEM) to enhance the activity of linalool.

MATERIALS AND METHODS

Insect cultures

All the test insects were gotten from laboratory colonies maintained in the Entomology Laboratory of the Department of Entomology, Islamic Azad University, Tabriz Branch, Iran. *R. dominica* (F.) was reared in one liter glass container containing whole wheat grains and *C. maculatus* (F.) was reared on a diet of bean. The cultures were maintained in continuous darkness at 29 ± 1°C and 60 ± 5% relative humidity (RH). The insects used in these experiments were 1 to 7 days old adults.

Contact toxicity tests of synergist

In the first bioassay, diethyl maleate was combined in mass ratios (1:8 and 1:4) with acetone used and applied on *C. maculatus* and *R. dominica* adult. The filter paper (9 cm of diameter) received 1 ml of this solution, and was placed on Petri dish (9 cm of diameter). Acetone was evaporated by placing dishes under the laboratory fume hood for 20 min. Insects were exposed to treated dishes for 1 h and then placed in the glass bottles (2 cm high) above.

Fumigant toxicity

The fumigation toxicity of the linalool was tested following the method of Wang et al. (2001) with some modification. For the second bioassay, wide mouth bottles of 310 ml capacity with lids were used as exposure chamber. Filter papers of 3 cm diameter were treated with five different essential oil concentrations to give a range of 20 to 80% mortality. The range of concentrations was chosen on the basis of a number of preliminary trials. The filter paper was attached to the undersurface of the screw cap of bottles. Thirty insects were hung at the center of the glass bottle (2 cm high) above. The bottles were then closed tightly with a lid. Each treatment with respective control was replicated four times. Mortality was checked after 24 and 48 h. The insects were considered to be dead as no leg or antennal movements were observed.

Data analysis

Mortality data were analyzed with SPSS software (SPSS Inc, 1993). Probit analysis was used to determinate LC_{50} and LC_{95} values. The values significance of χ^2 was estimated according to Robertson and Preisler (1992). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test to estimate statistical differences between means at $\alpha = 0.05$.

RESULTS

Fumigant toxicity

Linalool showed variable toxicity to adults of test insects. *C. maculatus* showed high susceptibility to linalool than *R. dominica,* after 24 and 48 h (Tables 1 and 2). The 24-h LC_{50} values against the beetles were 23.61 and 31.01 µl/l air and the 48-h LC_{50} values were 15.07 and 21.84 µl/l air for *C. maculatus* and *R. dominica*, respectively (Table 1 and 2). In general, mortality increased as the doses of linalool and exposure period increased. On the other hand, the LC_{50} decreased with the duration of exposure to the linalool (Table 1 and 2).

According to the results of ANOVA, the effect of doses and exposure time interactions of the linalool on beetles were significant at $P< 0.01$. The results show that there were positive and linear significant relationships between percent mortality of *C. maculatus* and *R. dominica* and duration of exposure to the linalool vapors within all concentration levels. This indicates that higher dosage is more efficient in management of pests.

Effect of synergist

The results from the experiments with DEM indicate

Table 3. LC_{50} and LC_{95} values of linalool with synergist DEM to *C. maculatus* and *R. dominica* at 24 h.

Insect	LC_{50} (µl/l air)	Slope (± SE)	Intercept (±SE)	LC_{95} (µl/l air)	χ^2
C. maculatus	11.93 (10-13)	2.14 (±0.23)	2.31 (±0.26)	69.61 (49-116)	2.07
R. dominica	13.07 (11-14)	3.54 (±0.29)	2.95 (±0.33)	38.08 (31-48)	1.13

Table 4. LC_{50} and LC_{95} values of linalool with synergist DEM to *C. maculatus* and *R. dominica* at 48 h.

Insect	LC_{50} (µl/l air)	Slope (± SE)	Intercept (±SE)	LC_{95} (µl/l air)	χ^2
C. maculatus	7.38 (6-8)	1.94 (±0.21)	4.68 (±0.26)	51.97 (36-88)	1.16
R. dominica	7.93 (7-8)	2.81 (±0.23)	3.53 (±0.22)	30.44 (24-40)	1.32

differences between the two insect species used (Tables 3 and 4). With synergist after 24 h of exposure, the LC_{50} values were estimated to be 11.93 and 13.07 µl/l air and after 48 h, they were 7.38 and 7.93 µl/l air for *C. maculatus* and *R. dominica*, respectively (Tables 3 and 4). It was found that synergist decreased doses of the linalool to control *C. maculatus* and *R. dominica.*

DISCUSSION

In general, aromatic plants contain oils only in concentrations of 1 to 3% w/w (Cakir, 1992). Certain compounds in the oils exhibit much stronger activity than others. Plant varieties that produce these compounds in larger quantities should be sought, or synthetic production methods should be explored as an option to gain enough material for full-scale use (Rozman et al., 2006). Linalool is a major component of essential oil of this annual mint, representing 60 to 90% of the total volatile collected (Ntezurubanza, 1987). Linalool (3,7-dimethyl-1,6-octadien-3-ol) is a common component of floral scents and is an olfactory cue in the seeking of host plants by numerous phytophagous invertebrates (Ryan and Byrne, 1988), and has been suggested as alternative to conventional insecticides in controlling all life stages of the cat flea, *Ctenocephalides felis* (Bouche), (Hink et al., 1988). The essential oil of *Ocimum canum* contains up to 90% linalool. Lee et al. (2001) showed that menthone, limonene and linalool isolated from *Mentha arvensis* L. had toxic activity against rice weevil.

Analysis of the toxicity data in the present study shows that the linalool exhibited a variable toxic action against the adult of the two beetles. Rozman et al. (2008) has reported fumigant toxicity of the compounds such as linalool, against *Tribolium castaneum, R. dominica* and *Sitophilus oryzae.* For *R. dominica*, linalool was highly effective and produced 100% mortality in the same conditions. With *T. castaneum* after 7 days exposure, linalool produced 70% mortality. The observed difference between our results and those of Rozman et al. (2008) against *R. dominica,* seems to be reasonable because of different species and size of insects.

Insects use enzymes not only to maintain their homeostasis, but also to protect themselves against xenobiotics. Preliminary evidence of their involvement in insecticide resistance can be obtained by using synergists (Brindley and Selim, 1984; Scott, 1990; Bernard and Philogene, 1993). The synergists DEM, PBO and TPP are inhibitors of the main detoxification enzymes commonly involved in insecticide resistance (Raffa and Priester, 1985; Bernard and Philogene, 1993; Scott, 1999). The use of insecticide synergists for providing preliminary evidence on the resistance mechanism has seldom been fully explored in stored-grain insect pests (Guedes and Zhu, 1998). Decreased linalool resistance was achieved in this study by the synergist DEM, suggesting that it is able to enhance enzymatic activity and decrease mechanism of resistance.

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