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Insecticidal activity of essential oils isolated from Rue (*Ruta graveolens* L.) and Galbanum (*Ferula gummosa* Bioss.) on *Callosobruchus maculatus* (F.)

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Abstract: In recent years, essential oils of medicinal plants have received much attention as pest control chemical agents because of their insecticidal, repellent and antifeedant properties. In this research, the fumigant activity of essential oils of *Ruta graveolens* L. and *Ferula gummosa* Boiss. was tested against adults of *Callosobruchus maculatus* (F.). The essential oils were obtained from dry leaves of Rue and gum of Galbanum plant and subjected to hydrodistillation using a modified Clevenger-type apparatus. The experiments were conducted at 27 ± 1°C, 65 ± 5% RH and in dark conditions. Fumigant toxicity of essential oils was tested against 1-7 days old adults of *C. maculatus*. Mortality of adults was tested at different concentrations ranging from 7.1 to 57.1µl/l air. Results showed that LC_{50} values of Rue and Galbanum to be 14.7 and 29.41µl/l air, respectively for 24 hours fumigation. This finding show that essential oil of *R. graveolens* may be much more effective than *F. gummosa*. Therefore, these essential oils could have potential for controlling of *C. maculatus* in organic food management.

Key words: *Ruta graveolens*; *Ferula gummosa*; *Callosobruchus maculatus*; *b*otanical insecticides; fumigant toxicity

Introduction

The tremendous increase in crop yields associated with the 'green' revolution has been possible in part by the discovery and utilization of chemicals for pest control. The widespread use of synthetic insecticides has led to many negative consequences (i.e. insecticide resistance, toxicity to mammals and other non-target animals, residue problems, environmental pollution) resulting in increasing attention being given to natural products (Copping and Menn, 2000; Isman, 2006). Aromatic plants are among the most efficient insecticides of botanical origin and essential oils often constitute the bioactive fraction of plant extracts (Shaaya et al., 1991, 1997; Regnault- Roger, 1997). In stored-product insect pest control, essential oils may have numerous types of effects (Papachristos and Stamopoulos, 2002): they may have a fumigant activity (Risha et al.,1990; Rice and Coats, 1994; Regnault-Roger and Hamraoui, 1995; Shaaya et al., 1997), they may penetrate inside the insect body as contact insecticides (Saxena et al., 1992; Weaver et al., 1994; Schmidt and Streloke, 1994), they may act as repellents (Saim and Meloan, 1986; Ndungu et al., 1995), they may act as anti-feedants (Huang et al., 1997) or may affect some biological parameters such as growth rate, life span and reproduction (Saxena et al., 1992; Pascual-Villalobos, 1996). In industrialized countries, essential oils could be useful alternatives to synthetic insecticides in organic food production, while in developing countries, they can be a means of low cost protection (Isman, 2006).

The objective of this study was to investigate the fumigant toxicity of essential oils extracted from two plants of the Iran flora, *Ruta graveolens* belonging to Rutaceae and *Ferula gummosa* from Apiaceae, which were tested on the common pest of stored products, *Callosobruchus maculatus* (Coleoptera: Bruchidae).

Material and methods

Insect culture and rearing conditions

The cowpea beetle; *C. maculatus*, had been cultured on mung bean grains in plastic cylinder (15cm diameter × 30cm height). The container was covered with fabric net (40 mesh). Adult insects, 1–7 days old were used for experiments. The cultures were put in the dark in a growth chamber under conditions of 27 ± 1°C and relative humidity (RH) of 65 ± 5%. All experiments were carried out under the above rearing conditions.

Essential oils

Aerial parts of *R. graveolens* were collected at flowering stage in July, 2008 from Rudsar mountainside, Guilan/North province of Iran, and shade dried at room temperature (23-25°C) for 6 days until crisp. The dried material was stored at -24°C until needed and then hydrodistilled to extract its essential oil. The essential oil of Rue was extracted from the dry aerial parts of plant using a Clevenger-type apparatus where the plant material is subjected to hydrodistillation. Conditions of extraction of the essential oil were: 50g of air-dried sample; 1:12 plant material/water volume ratio, 4h distillation. Hydrodistillation furnished 2% (dry weight basis) essential oil. The oil of Galbanum extracted from the gum of plant that was purchased from ZARDBAND Co., in Tehran/Iran. The essential oils were dried over anhydrous sodium sulphate and were stored in a refrigerator at +4°C.

Fumigant toxicity

In order to determine the fumigant toxicity of Rue and Galbanum essential oils, filter paper disks (Whatman No. 1, 2cm diameter) were treated with essential oils at concentrations ranging from 36-500µl/l in air. The treated filter papers were then attached to the underside the screw lids of plastic vials (70ml volume); each of which contained severally 10 adults (1-7 days old) of insects. Lids were screwed firmly on the vials. Laboratory film (Parafilm) strip was swathe around the lids as an impervious blanket. To impede the contact toxicity a fabric net (40 mesh) was placed under the lids between the impregnated papers and insects. Mortality was calculated after 2, 4, 6, 8, 10, 12 and 24h from the beginning of exposure. While the insects' legs and antenna has not any movement when goaded with a fine brush, were considered dead. No mortality was observed in control vials.

Sake to assess LC_{50} and LC_{95} values, the filter paper disks were impregnated with series of concentrations. Ten adult insects (1-7 days old) were put into 70ml plastic vials, which were detailed in above experiment. Concentrations of essential oils tested were 0, 7.1, 14.3, 21.4, 28.6, 35.7, 42.9, 50 and 57.1µl/l in air. Control vials were kept under the same conditions without any essential oil. Five replicates were set up for each concentration and control. 24h after initial exposure to essential oils the number of dead and live insects in each vial was counted. There was no mortality in untreated controls. The mortality was calculated as described in the first experiment above. Treatment vials were monitored 48h after recording the data and no influenced insects recovered. The 50% and 95% lethal doses were computed by Probit analysis (Finney, 1971).

Results and discussion

Referring to the graph in Fig. 1 it can be observed that Rue oil (LC_{50} 14.7µl/l in air) much more toxic than Galbanum oil (LC_{50} 29.2µl/l in air) against *C. maculatus*. The lowest concentration (36µl/l in air) obtained 50% mortality of the insect with Rue oil and 15% with the Galbanum oil. At concentration 186µl/l in air the essential oils of Rue and Galbanum after 12h exposure caused 77.5% and 62.5% mortality, respectively. Both of oils killed entire the insects after 24 h in this concentration. At concentration of 300µl/l in air achieved 87.5% and 82.5% mortality after 12h by Rue and Galbanum oil, respectively. At highest concentration (500µl/l in air), Rue oil caused 67.5%, 95% and 100% mortality after 2, 4 and 6h exposure times, respectively, and Galbanum oil obtained 47.5%, 80% and 100% mortality after the same exposure interval.

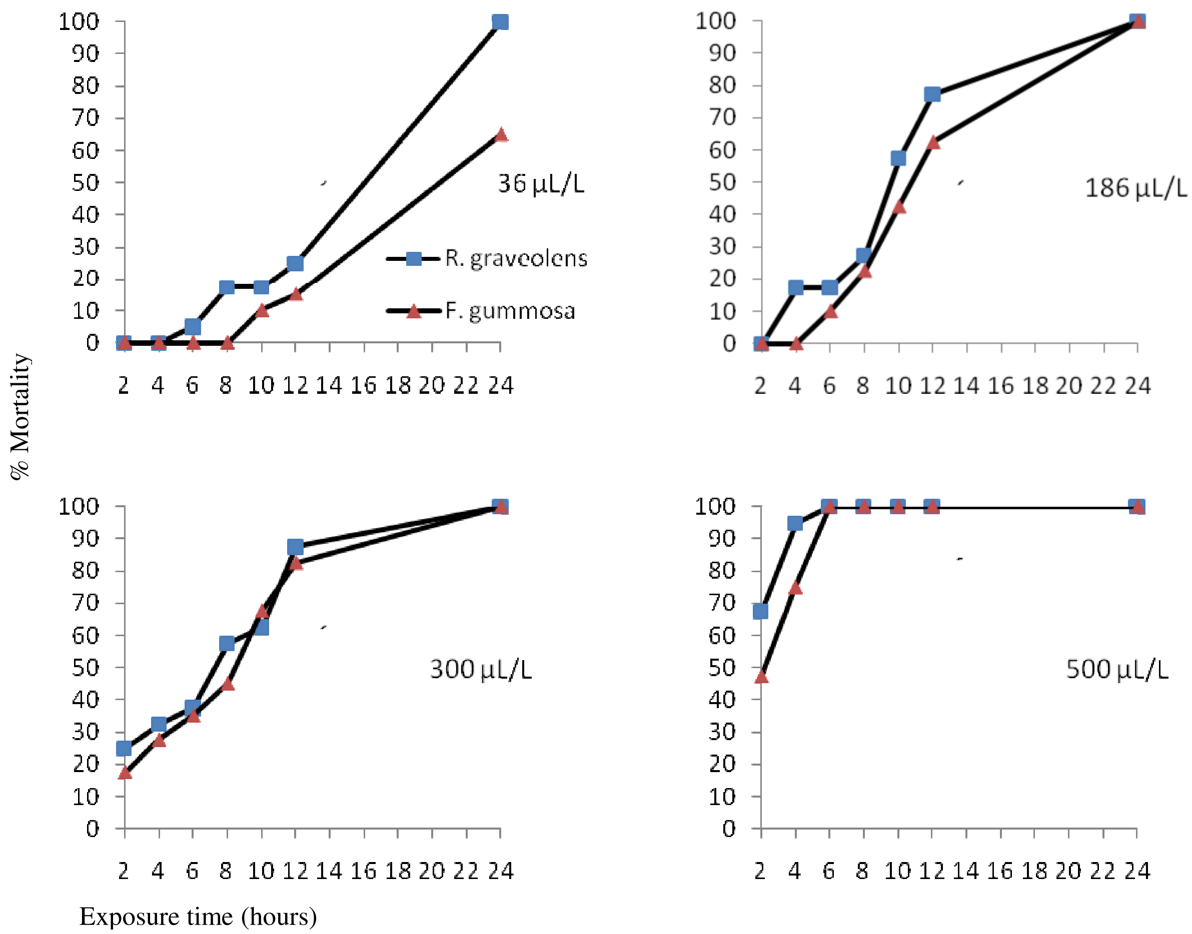

Figure 1. Percentage mortality of *Callosobruchus maculatus* exposed for various periods of time to essential oils from *Ruta graveolens* and *Ferula gummosa* impregnated on filter paper discs and held at 27°C and 65% r.h.

The 50% and 95% lethal concentration were computed by Probit analysis (Finney, 1971), and LC_{95} values for Rue and Galbanum essential oils were 49.9 and 115.5µl/l in air, respectively (Table 1).

Table 1. Fumigant toxicity of *R. graveolens* and *F. gummosa* essential oils against *Callosobruchus maculatus*

Essential oils	LC50[a,b]	LC95[a,b]	Slope±SE	Degrees of freedom	Chi square (χ^2)
R. graveolens	14.7 (12.3-17)	49.9 (60.8-80.3)	2.67±0.27	6	2.7
F. gummosa	29.2 (25.9-32.7)	115.5 (89.6-167.8)	2.75±0.28	6	9.9

[a] Units LC_{50} and LC_{95} µl/l in air, applied for 24 h at 27°C and 65% r.h.
[b] 95% lower and upper fiducial limits are shown in parenthesis.

Essential oils of *R. graveolens* and *F. gummosa* displayed good fumigant toxicity against cow pea beetle, *C. maculatus.* The insect respond differently to Rue and Galbanum essential oil and much more susceptible to first than another oil. Insecticidal activity varied with type and concentrations of the essential oils and exposure times. No studies had been reported previously toward the fumigant toxicity of *R. graveolens* and *F. gummosa* on insect pests. Tendency toward using plant origin materials as safe alternatives for hazardous chemical fumigants (i.e. methyl bromide) increased. Many other plant essential oils show potential as natural insecticides particularly in enclosed places. For example, fumigant toxicity of *Artemisia sieberi, Salvia officinalis* and *Zataria multiflora* on *C. maculatus* had been reported (Negahban et al., 2007; Rastegar et al., 2008). Insecticidal activity of *Artemisia annua* and *sambucus ebulus* against *Tribolium castaneum* had been demonstrated (Jalali et al*.,* 2002). In present study efficacy of *R. graveolens* and *F. gummosa* essential oils for managing *C. mmaculatus* was demonstrated. Future researches must be focus on delineate the chemical constituents of these essential oils, residues on target commodity and the effects of any residues on product acceptability.

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