USING SOME PLANT ESSENTIAL OILS AS NATURAL FUMIGANTS AGAINST ADULTS OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE)

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ABSTRACT**:** Present study was aimed to investigate fumigant toxicity of essential oils extracted from *Lippia citrodora* Kunth., *Rosmarinus officinalis* L., *Mentha piperita* L. and *Juniperus Sabina* L. against adults of cowpea weevil, *Callosobruchus maculatus* (Col.: Bruchidae). Essential oils were extracted from aerial parts of plants via hydrodistillation. Adults (1-7 days old) of insects were exposed to a series of concentrations of essential oils for 24 h and then the numbers of dead and alive insects were counted. Results showed that essential oil of *M. piperita* (LC_{50} 7.86 µl/L air) was significantly more toxic than *L. citrodora* (LC_{50} 187.51 µl/L air), *J. Sabina* (LC_{50} 134.35 µl/L air) and *R. officinalis* (LC_{50} 46.81 µl/L air). Results of this investigation suggested that essential oils of these plants could be used as safe fumigants for controlling of *C. maculatus* in small scales.

KEY WORDS: *Lippia citrodora*, *Rosmarinus officinalis*, *Mentha piperita*, *Juniperus sabina*; *Callosobruchus maculatus*, natural fumigants, essential oils.

The major storage pest of cowpea is *Callosobruchus maculates* (F.) (Col.: Bruchidae) (Epidi et al., 2008). It is an important insect pest of many grains such as cowpea, chickpea, lentil and soybean (Mahfuz & Khalequzzaman, 2007). Stored products pests control mainly depends on fumigation by methyl bromide or phosphine (Butler & Rodriguez, 1996). Due to ozone-depleting properties, application of methyl bromide is being restricted (MBTOC, 1998). Insect resistance to phosphine has been reported from many countries and using of this fumigant may become limited (Daghlish & Collins, 1999). Low toxicity to mammals, biodegradability and high volatility of essential oils posses these compounds as alternatives to conventional fumigants (Shaaya et al., 1997; Li & Zou, 2001).

This study was designed to evaluate the fumigant toxicity of essential oils of four plants, *Lippia citrodora* (Verbenaceae), *Rosmarinus officinalis* (Lamiaceae), *Mentha piperita* (Lamiaceae) and *Juniperus sabina* (Pinaceae) against adults of *C. maculatus*. *L. citrodora*, is native to South America and contain a strong lemon-scented volatile oil. *R. officinalis* is native to Mediterranean and cultivated throughout the world. *M. piperita* is used to flavour non-alcoholic beverages, ice-creams and candies and finally *J. sabina* is bitter and has a disagreeable smell and volatile oil. All of these plants have medicinal values (Prajapati et al., 2004).

MATERIALS AND METHODS

Aerial parts of *Lippia citrodora*, *Rosmarinus officinalis*, *Mentha piperita* and *Juniperus sabina* were collected from the garden of medicinal plants of Shahed

University in july, 2009 and then shade dried at room temperature. Dried materials were subjected to hydrodistillation using Clevenger type apparatus. Extracted essential oils were dried via anhydrous sodium sulphate and stored at 4°C in darkness. In all tests pure essential oils were employed.

The cowpea weevil, *C. maculatus* was reared on mung bean grains in the growth chamber under conditions of 27 ± 1°C and 65 ± 5% R.H and darkness. Adults of insects (1-7 days old) were used for experiments.

The fumigant toxicity of essential oils has been tested in glass vials (70 ml) each of them containing 10 adults of insects. Filter paper disks (Whatman No. 1) were cut into 2cm diameters and were attached to undersurface of screw caps of glass vials. Filter papers were impregnated with series of concentrations of each essential oil. Then the caps were tightly screwed on glass vials. Concentrations used for essential oils of *L. citrodora* were 114.24, 142.8, 171.36, 256.04 and 285.80 µl/L air, and for *R. officinalis* oil were 21.42, 42.84, 71.40, 100 and 128.52 µl/L air. Likewise, concentrations used for *M. piperita* were 4.28, 5.71, 7.14, 10, 14.28, 17.14 and 21.42 µl/L air and finally for *J. sabina* were 128.57, 157.14, 200, 228.57 and 271.43 µl/L air. Four replicates were run for each concentration and control. After 3, 6, 9, 12 and 24h from the beginning of exposure, numbers of dead and alive insects were counted. There was no mortality in control.

The 50% and 90% lethal concentrations (LC_{50} and LC_{90}) values were assessed by Probit analysis (Finney, 1971).

RESULTS

The results of LC_{50} values of various essential oils are shown in Table 1. Results of this research showed that percentage of insect mortality was increased with the concentration and the time of exposure. In the other hand, toxicity depends on concentration, type of essential oil and exposure time. Figs. 1-4 show that in all cases mortality was increased with increasing the concentration and exposure time.

Essential oils of *L. citrodora* at lowest concentration (114.24 µl/L air) caused 20% mortality in insect population after 24 h compared to 85% mortality at the highest concentration (285.8 µL/l air) (Fig. 1). Lowest concentration of essential oil of *R. officinalis* (21.42 µl/L air) achieved 14% mortality after 24 h exposure and at the highest concentration (128.52 µl/L air) caused 88% mortality after the same time (Fig. 2). Lowest concentration of essential oil of *M. piperita* (4.28 µl/L air) killed 5% of insects after 3 h exposure and 7.5%, 20% and 37.5% after 9, 12 and 24 h exposure, respectively (Fig. 3). Exposing *C. maculatus* adults to 128.57 µl/L air of *J. sabina* essential oil for 3h caused no mortality and after 6, 12 and 24h exposure caused 2%, 6% and 14% mortality in insect population. Likewise, with increasing the concentration to 271.43 µl/L air, also mortality was increased to 28% after 6 h exposure, 84% and 96% mortality after 12 and 24 h exposure to essential oil of *J. sabina*, respectively (Fig. 4).

Comparison of the estimated LC_{50} values of essential oils indicated that *M. piperita* essential oils is significantly more toxic to *C. maculatus* than other essential oils which were reported in this study. Furthermore, adults of *C. maculatus* were highly more tolerant to essential oil of *L. citrodora* than essential oils of *M. piperita*, *R. officinalis* and *J. sabina* (Table 1).

DISCUSSION

Employing the synthetic insecticides remains undesirable effects such as mammalian toxicity, food chain disruption and increases resistance in insect population (Regnault-Roger, 1997). Natural compounds from plant origins could be efficient alternatives for conventional fumigants because of their low toxicity to mammals, fast degradability properties and regional availability (Rajendran & Sriranjini, 2008). Essential oils of *M. piperita* and *R. officinalis*, displayed considerable fumigant toxicity on adults of *C. maculatus*. In previous investigations demonstrated that essential oils of *Artemisia unnua* L. (LC_{50} 14.1 µl/L air) and *Eucalyptus camodulensis* Deh. (LC_{50} 17.77 µl/L air) have toxic effect on adults of *C. maculatus* (Hosseinpour et al., 2009; Abbasipour et al., 2009). Comparing the estimated LC_{50} values of present investigation with LC_{50} values of mentioned essential oils indicated that essential oils of *M. piperita* is more toxic to *C. maculatus* than essential oils of *A. annua* and *E. camodulensis*. Results of this investigation clearly illustrated that insects varied in their susceptibility to various essential oils that probably referring to insecticidal ability of their active constituents. Abbasipour et al (2009) reported that essential oils of *Rosemarinus officinalis* possess toxic effect in gaseous phase against adults of *Tribolium castaneum* (LC_{50} 103.28 µl/L air). The noticeable signification between the LC_{50} values of *T. castaneum* and *C. maculatus* can be of their differences in morphology, physiology, sensitivity and other factors.

Results of this study suggested that essential oils of *L. citrodora*, *R. officinalis*, *M. piperita* and *J. sabina* plants are good choice for controlling this pest. Also, we need some additional studies for formulating and improving methods of application of them.

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Table 1. Fumigant toxicity of *L. citrodora*, *R. officinalis*, *M. piperita* and *J. sabina* essential oils against *C. maculatus*.

Essential oils	n[a]	LC_{50}[b] (μl/L air)	LC_{90}[b] (μl/L air)	Slope±SE	Df	Chi square (χ^2)
Lippia citrodora	200	187.51 (171.33-206.05)	338.65 (290.74-433.71)	4.99±0.68	3	3.96
Rosmarinus officinalis	200	46.81 (39.27-54.22)	135.76 (110-186.07)	2.77±0.34	3	1.34
Mentha piperita	280	7.86 (5.90-9.66)	37.76 (25.02-90.39)	1.88±0.36	5	1.28
Juniperus sabina	200	134.35 (113.86-149.35)	258.51 (228-320.17)	4.50±0.73	3	3.82

n[a]: number of insects
[b] 95% lower and upper fiducially limits are shown in parenthesis.

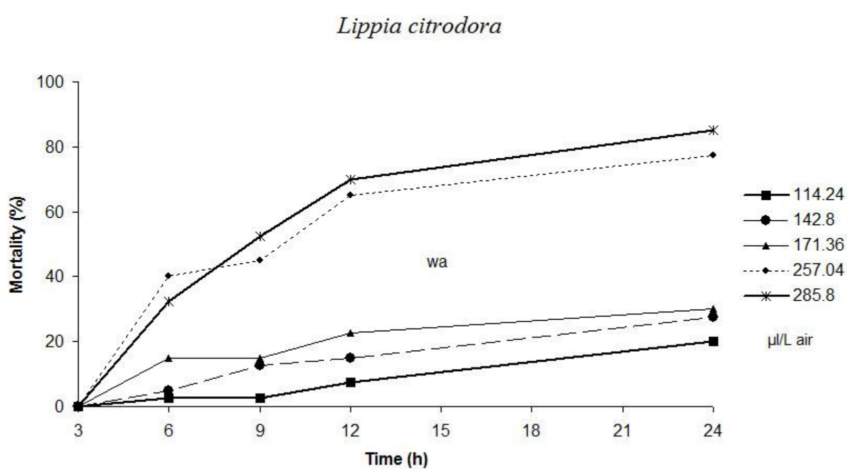

Figure 1. Mortality of adults of *C. maculatus* exposed to *L. citrodora* essential oil in different concentrations and times.

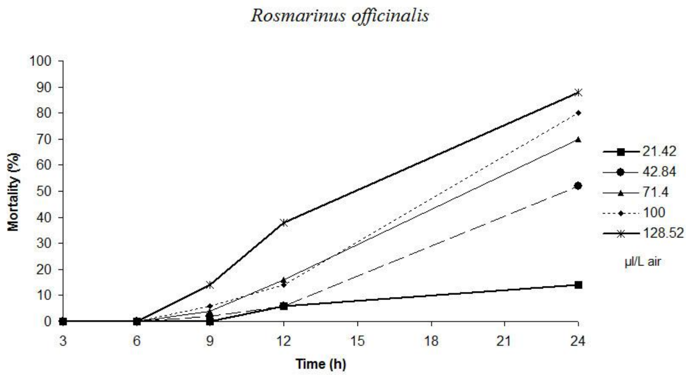

Figure 2. Mortality of adults of *C. maculatus* exposed to *R. officinalis* essential oil in different concentrations and times.

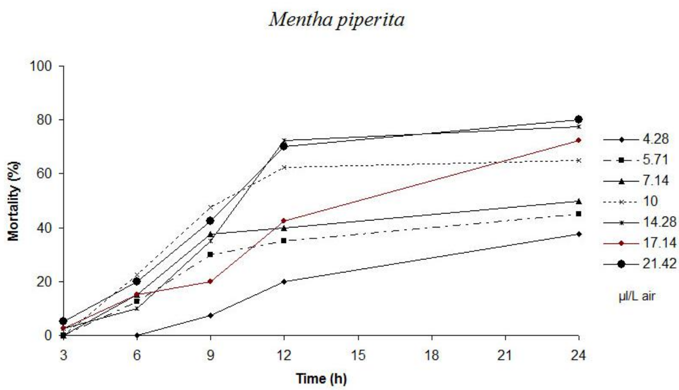

Figure 3. Mortality of adults of *C. maculatus* exposed to *M. piperita* essential oil in different concentrations and times.

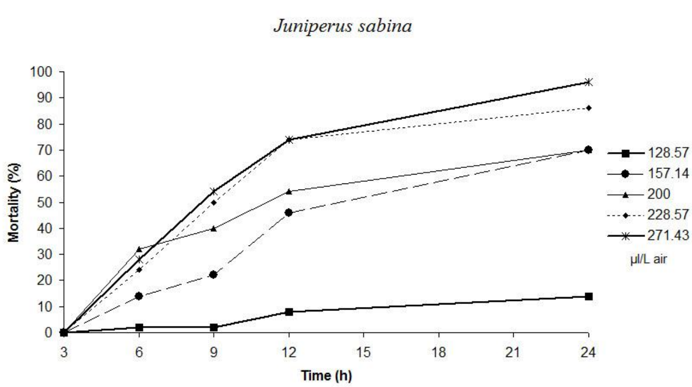

Figure 4. Mortality of adults of *C. maculatus* exposed to *J. Sabina* essential oil in different concentrations and times.