

Toxic Effects of Eight Plant Essential Oils Against Adults of Colorado Potato Beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)

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ABSTRACT

Toxicity of the essential oils extracted from *Artemisia dracuncululus* L., *Crambe orientalis* L., *Hypericum perforatum* L., *Hypericum scabrum* L., *Rosmarinus officinalis* L., *Salvia multicaulis* Vahl, *S. sclarea* L. and *Tanacetum agrophyllum* (L.) were tested against adults of the Colorado potato beetle (CPB), (*Leptinotarsa decemlineata* Say) under laboratory conditions. Doses of 10, 15 and 20 µl were applied and mortality of adults was determined at 24, 48, 72 and 96 h. The toxic effect was influenced by the doses and the exposure time of the 8 plant essential oils. All the tested essential oils had toxic effects against the adults of CPB. Essential oils of *R. officinalis* and *T. agrophyllum* were the most effective ones within the first 24 hrs at their lowest dose applications. In the 10 and 20 µl doses, after 96 hrs of treatments, 88.8 and 100% mortalities were achieved by *C. orientalis* and *R. officinalis*, respectively. The results indicated that both essential oils had a potential in controlling CPB.

Key words: *Leptinotarsa decemlineata*, Potato, Essential oil, Toxicity.

INTRODUCTION

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say, (Coleoptera: Chrysomelidae) is native to Mexico. It is a polyphagous species found on a wide range of host plants; potatoes, egg plants, pepper and also some tomato species in the world (Hare, 1990 and Capinera, 2001). Potato is the most preferred hosts for the pest, but it may feed and reproduce on a number of other plants in the Solanaceae family. Adults and larvae feed on the foliage of the host plants but larvae are the most damaging life stage that cause economic injury if their populations are moderate or above (Ferro *et al.*, 1983 and Mailloux *et al.*, 1991). CPB is economically important pests defoliating potatoes and reducing drastically tuber yields. It is also a vector of bacterial ring rot which can be of economic importance if climatic conditions are appropriate to the disease (Gökçe *et al.*, 2007).

Synthetic insecticides and fumigants are commonly used for the control of the pests. However, there is a considerable problem in the use of these chemicals due to their residual toxicity in the post-harvest products and occurrence of insecticide resistant (Gelman *et al.*, 2001). Synthetic pesticides also cause environmental pollution owing to their slow biodegradation in the environment (Barnard *et al.*, 1997). Thus, there is an increasing interest in research concerns with the development of new

alternative pesticides, such as toxic natural products including plant essential oils, extracts and secondary metabolites for pest control in agricultural production (Scott *et al.*, 2004 and Laborda *et al.*, 2013). Plant essential oils are a mixture of many flavours and fragrances grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) that provide characteristic odours. Insecticidal properties of numerous essential oils and some monoterpenes have been extensively studied against various insect species (Lee *et al.*, 2003; Kordali *et al.*, 2006 and Laborda *et al.*, 2013).

Aim of the present study was to assess the toxicity of 8 essential oils compared to izoldesis (deltamethrin), against adults of CPB.

MATERIALS AND METHODS

Biological material

Adults of CPB were collected from potato fields of the eastern Anatolia (Erzurum) and reared in laboratory at 25±1°C, 64±5 RH in the Department of Plant Protection at Ataturk University, Turkey The adults obtained from laboratory cultures were stored in separate insect cages including appropriate fresh potato leaves. All tests were carried out under the same laboratory conditions.

Plant materials and isolation of the essential oils

Artemisia dracuncululus L., *Crambe orientalis* L., *Hypericum perforatum* L., *Hypericum scabrum* L., *Rosmarinus officinalis* L., *Salvia multicaulis* Vahl, *S. sclarea* L. and *Tanacetum agrophyllum* (L.) were collected from different localities of Turkey. Voucher specimens have been deposited in the herbarium of Ataturk University, Faculty of Agriculture, the Department of Plant Protection, Erzurum, Turkey. Aerial parts of the plants were dried in shade and ground in a grinder. The dried plant samples (500g) were subjected to hydrodistillation for 4h using a Clevenger-type apparatus. The oils were dried over anhydrous Na₂SO₄ and stored under N₂ in a sealed vial until required, and then stored at 4°C until used for GC analyses and toxicity bioassays. The oil yields of *A. dracuncululus*, *C. orientalis*, *H. perforatum*, *H. scabrum*, *R. officinalis*, *S. multicaulis*, *S. sclarea* and *T. agrophyllum* were 1.00, 0.1, 0.24, 0.19, 1.46, 0.80, 1.35, and 9.8% (w/w, dry weight basis), respectively.

Bioassays

Glass Petri dishes (9 cm wide x 1.5 cm deep, corresponding to 120 ml volume) were used as exposure chambers to test the toxicities of the essential oils against the adults of CPB. To determine the contact toxicity effects of the oils, they were dissolved in DMSO–water solution (10%, v/v). The final concentrations of the treatments were 10, 15 and 20 µL/petri. A filter paper was placed on bottom of each of Petri dishes (9 cm×1.5 cm deep) and 15 adults of CPB were placed on this filter paper, containing the appropriate amounts of potato leaves. Thus, there was direct contact between the oils and the adults. The emulsions were sprayed to Petri dish (9 cm diameter) placed on the bottom two layers of filter paper (1 ml/ dish). Afterwards, 15 adults of the adults were placed on the filter paper. 10, 15 and 20 µL/petri of the essential oils were sprayed to adults of CPB by using a spray equipment. The Petri dishes were covered with a lid and transferred into incubator, and then kept under standard conditions of 25±1°C, 64±5 RH and 16:8 (light: dark) photoperiod for 4 days. The treatments were arranged in a completely randomized design with three replications including controls. İzoldesis (Deltamethrin 2.5 g/l) was used as positive control in the same conditions above mentioned. 10, 15 and 20 µl of positive control reactive were applied, corresponding to 0.025 mg, 0.0375 mg and 0.05 mg/petri dishes respectively. After exposure, mortality of adults was counted at 24, 48, 72 and 96 h. Each experiment was replicated three times.

Data analysis

The results of mean mortality were subjected to one-way variance analyses (ANOVA), using SPSS 17.0 software package. Mortality was expressed as

mean (percentage) ± standard error. Differences between means were tested through Duncan test and values with $p < 0.05$ were considered significantly different. LD₂₅, LD₅₀ and LD₉₀ values at 96 h were calculated with regression analysis by probit using SPSS. Probit analysis of dose-mortality data was conducted to estimate the LD₂₅, LD₅₀ and LD₉₀ values and associated 95 % confidence limits for each treatment.

RESULTS AND DISCUSSION

Toxicity of the 8 essential oils obtained from *A. dracuncululus*, *C. orientalis*, *H. perforatum*, *H. scabrum*, *R. officinalis*, *S. multicaulis*, *S. sclarea* and *T. agrophyllum* were determined against adults of CPB. The 10, 15 and 20 µL\petri concentrations were applied and their toxicities were compared with toxicity of izoldesis (deltamethrin) a commercial insecticidal at the same (10, 15 and 20µL\petri) concentrations (Table 1). The results showed that the essential oils exhibited various toxicities against the adults depending on exposure time and treatment concentrations of the oil. In general, the mortality increased with increasing doses and exposure times. Among the tested 8 plant essential oils, (100%) mortality of CPB adults was achieved at the 20 µL\petri concentration of *C. orientalis* and *R. officinalis* after 96 h. On the contrary, the least mortality (40%) was determined at all doses by *S. multicaulis* oil after 96 h. Mortality rates in the alldoses of the essential oils of *A. dracuncululus*, *C. orientalis*, *H. perforatum*, *H. scabrum*, *R. officinalis*, *S. multicaulis*, *S. sclarea* and *T. agrophyllum* after 24, 48, 72 and 96 hrs against the adults of CPB were presented in table (1) and fig. (1). In addition, the highest mortality rates after 24 h of treatment with the 10, 15 and 20 µl doses of *R. officinalis* oil were determined as 26.6, 28.80 and 35.5% for the adults of CPB, respectively. In contrast, the lowest mortality rates (2.22, 4.44 and 8.88%, respectively) were found after 24h of treatment with the 10, 15 and 20 µL\petri doses for the essential oil of *H. perforatum* (Table 1 and Fig. 1).

Although the mortality rate of (60%) after 48 h of treatment with the minimum dose (10 µL\petri) of *R. officinalis* oil was determined against adults of CPB, the lowest mortality rate at the same exposure time and dose of *S. multicaulis* oil was (11.1%) (Table 1 and Fig. 1). Furthermore, the mortality rate of (75.50%) occurred at the treatment after 48 h with 15 µl dose of essential oil of *R. officinalis*. The lowest mortality rate (17.70%) was found after 48 h in the 15 µL\petri dose of *H. scabrum* oil (Table 1 and Fig. 1). Although the least mortality rate (24.40%) was recorded after 48 h of treatment with the 20 µL\petri in dose of essential oil of *H. perforatum*, the highest

Table (1): Toxicity of essential oils of 8 plant essential oils on adults of *L. decemlineata* after 24, 48, 72 and 96 hrs

Essential oil	Dose (µl/l)	Mortality(%)			
		Exposure time (h)			
		24	48	72	96
<i>Artemisia dracunculus</i>	10	4.44 ± 4.44 ^{ab}	24.4 ± 4.44 ^{cdefg}	40.0 ± 3.84 ^{cd}	68.8 ± 2.22 ^e
	15	13.3 ± 3.84 ^{bcd}	31.1 ± 5.87 ^{efgh}	68.8 ± 2.22 ^h	86.6 ± 3.84 ^{ijk}
	20	26.6 ± 3.84 ^{gh}	46.6 ± 3.84 ^{jk}	80.0 ± 3.84 ⁱ	97.7 ± 2.22 ^{lm}
<i>Crambe orientalis</i>	10	8.88 ± 2.22 ^{abcd}	28.8 ± 2.22 ^{efgh}	84.4 ± 2.22 ^{ij}	93.3 ± 3.84 ^{klm}
	15	8.88 ± 2.22 ^{abcd}	31.1 ± 2.22 ^{efgh}	88.8 ± 2.22 ^{jk}	97.7 ± 2.22 ^{lm}
	20	15.5 ± 2.22 ^{cdef}	37.7 ± 2.22 ^{hij}	93.3 ± 0.0 ^{kl}	100 ± 0.0 ^m
<i>Hypericum perforatum</i>	10	2.22 ± 2.22 ^a	15.5 ± 2.22 ^{bc}	24.4 ± 2.22 ^b	48.8 ± 4.44 ^{cd}
	15	4.44 ± 2.22 ^{ab}	20.0 ± 3.84 ^{bcd}	40.0 ± 3.84 ^{cd}	73.3 ± 3.84 ^{ef}
	20	8.88 ± 2.22 ^{abcd}	24.4 ± 4.44 ^{cdefg}	46.6 ± 3.84 ^{de}	82.2 ± 5.87 ^{hij}
<i>Hypericum scabrum</i>	10	2.22 ± 2.22 ^a	15.5 ± 2.22 ^{bc}	37.7 ± 4.44 ^c	46.6 ± 3.84 ^{bc}
	15	6.66 ± 3.84 ^{abc}	17.7 ± 5.87 ^{bcd}	40.0 ± 7.69 ^{cd}	55.5 ± 3.87 ^d
	20	13.3 ± 3.84 ^{bcd}	33.3 ± 3.84 ^{gh}	66.6 ± 3.84 ^{gh}	80.0 ± 3.84 ^{gh}
<i>Rosmarinus officinalis</i>	10	26.6 ± 3.84 ^{gh}	60.0 ± 6.66 ^l	82.2 ± 2.22 ^{ij}	88.8 ± 2.22 ^{kl}
	15	28.8 ± 4.44 ^{hi}	75.5 ± 4.44 ^m	88.8 ± 2.22 ^{kl}	95.5 ± 2.22 ^{lm}
	20	35.5 ± 5.87 ^l	88.8 ± 2.22 ⁿ	97.7 ± 2.22 ^l	100 ± 0.0 ^m
<i>Salvia multicaulis</i>	10	2.22 ± 2.22 ^a	11.1 ± 2.22 ^{ab}	22.2 ± 2.22 ^b	40.0 ± 3.84 ^b
	15	11.1 ± 2.22 ^{abcde}	24.4 ± 2.22 ^{cdefg}	35.5 ± 2.22 ^c	55.5 ± 2.22 ^d
	20	28.8 ± 4.44 ^{hi}	55.5 ± 5.87 ^{kl}	82.2 ± 2.22 ^{ij}	93.3 ± 3.84 ^{klm}
<i>Salvia sclarea</i>	10	6.66 ± 3.84 ^{abc}	26.6 ± 3.84 ^{defg}	51.1 ± 2.22 ^e	77.7 ± 2.22 ^{efg}
	15	15.5 ± 2.22 ^{cdef}	22.2 ± 2.22 ^{cdef}	53.3 ± 3.84 ^{ef}	75.5 ± 3.84 ^{efg}
	20	24.4 ± 2.22 ^{efgh}	44.4 ± 2.22 ^j	64.4 ± 2.22 ^{gh}	88.8 ± 2.22 ^{kl}
<i>Tanacetum agrophyllum</i>	10	17.7 ± 2.22 ^{defg}	31.1 ± 2.22 ^{efgh}	60.0 ± 3.84 ^{fg}	80.0 ± 3.84 ^{gh}
	15	20.0 ± 3.84 ^{efgh}	42.2 ± 4.44 ^{ij}	66.6 ± 3.84 ^{gh}	84.4 ± 2.22 ^{hij}
	20	24.4 ± 2.22 ^{efgh}	46.6 ± 3.84 ^{jk}	77.7 ± 5.87 ^l	93.3 ± 3.84 ^{klm}
Pozitif Control (Izoldesis)	10	77.7 ± 2.22 ^j	100 ± 0.0 ^o	100 ± 0.0 ^l	100 ± 0.0 ^m
	15	88.8 ± 2.22 ^k	100 ± 0.0 ^o	100 ± 0.0 ^l	100 ± 0.0 ^m
	20	95.5 ± 2.22 ^k	100 ± 0.0 ^o	100 ± 0.0 ^l	100 ± 0.0 ^m
Control (DMSO)	-	2.22 ± 1.85 ^a	4.44 ± 1.86 ^a	6.66 ± 0.00 ^a	8.44 ± 1.76 ^a

*Values followed by different letters in the same column differ significantly at $P \leq 0.05$ according to Duncan Multiple test; a Mean ± SE of three replicates, each set up with 15 adults.

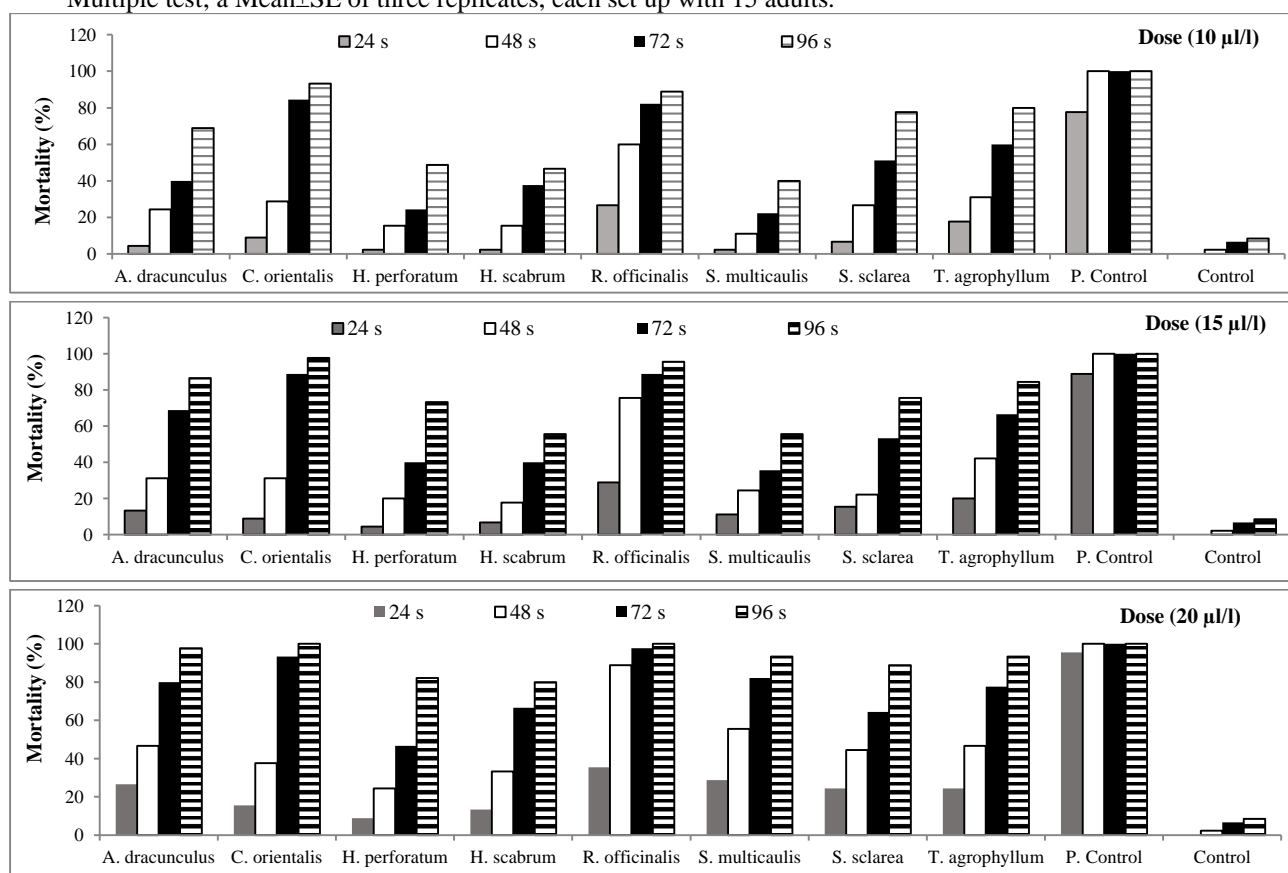


Fig. (1): Mortality rates of of *Leptinotarsa decemlineata* (Say) adults in relation to exposure different time of 8 essential oils in the 10, 15 and 20 µL/petri doses.

mortality rate (88.80%) was found by using 20 µL\petri of essential oil of *R. officinalis* (Table 1 and Fig. 1). Similarly, the least mortality rate (22.20 %) was determined after 72 hrs of treatment with the 10 µL\petri dose of essential oil of *S. multicaulis*. However, the most mortality (84.40%) after 72 hrs in the 10 µL\petri dose was found for the essential oil of *C. orientalis* (Table 1 and Fig.1). On the other hand, the highest mortality rates (88.80%) occurred at essential oils of *C. orientalis* and *R. officinalis* after 72 hrs at the 15 µl, whereas in the same time and dose *S. multicaulis* occurred the lowest one (35.50%) (Table 1 and Fig. 1).

However, the mortality rates (between 2.22 and 100%) were recorded after 96 hrs treatment with all doses for essential oils of all tested plants against adults of CPB. Although the lowest mortality rate (40%) at 10 µL\petri was found for *S. multicaulis*, the highest mortality (100%) was achieved for *C. orientalis* and *R. officinalis*. In the present study, we revealed that the essential oil from *R. officinalis* had an insecticidal effect between 26.60 and 100% (after 24 hrs in the 10 µL\petri dose and 96 hrs in the 20 µl, respectively) for adults of CPB. *S. sclarea* oil had an insecticidal effect of (6.66% (after 24 h in the 10 µL\petri dose) and 88.80% (after 96 h in the 20 µL\petri) mortality rates on adults of CPB (Table 1 and Fig. 1).

Among all the essential oils, the high insecticidal effects were determined for the essential oils of *C. orientalis*, *R. officinalis* and *T. agrophyllum* with (80–100%). The essential oil of *H. scabrum* showed low toxic effect with low mortality rates in all doses (Table 1 and Fig. 1).

In previous studies, it was stated that the essential oil obtained from *A. dracunculus* had a weak antifungal activity (Kordali *et al.*, 2005). In addition, Manzoomi *et al.* (2010) found fumigant toxicity of essential oil from *A. dracunculus* on the adults of *Callosobruchus maculatus* and its results indicated that the mortality of adults increased with increasing of concentration and exposure time. Cheah *et al.* (2013) reported larvicidal, oviposition, and ovidal effects of *Artemisia annua* (Asterales: Asteraceae) against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus* (Diptera: Culicidae). Similarly, it was stated larvicidal and ovidal toxicities of essential oil from *A. dracunculus* on first instar larvae and eggs of *Plodia interpunctella* Hübner (Rafiei-Karahroodi *et al.*, 2011). In an earlier study, a test of the toxicity of *Hypericum scabrum* oil against *Sitophilus granarius* adults and *Ephestia kuehniella* larvae indicated that 10 µL\petri of *H. scabrum* oil showed 73.0 and 72.0% mortality, respectively (Yıldırım *et al.*, 2005) compared with

(2.22% and 80.0% on adults) of CPB (after 24 hrs in the 10 µL\petri dose and 96 hrs in the 20 µL\petri, respectively). Previous studies stated also that the extract from *R. officinalis* had an insecticidal effect between (85.9 and 89.9%) mortality rates, respectively on adults of CPB at field and laboratory conditions (Kara *et al.*, 2014). Likewise, it was found that the essential oil from *R. officinalis* had a fumigant toxicity and repellent effects against *Tribolium confusum* (Saeidi and Moharramipour, 2013). The essential oil of *S. multicaulis* had an insecticidal effects against granary weevil, *S. granarius* (Yıldırım *et al.*, 2011), while the essential oil of *S. sclarea* had no toxic effect to adults of granary weevil, *S. Granarius* (Yıldırım *et al.*, 2011).

The essential oil of *T. argyrophyllum* had a toxic effect of (17.70% (after 24 hrs in the 10 µL\petri) to 93.30% (96 hrs in the 20 µl) with mortality rates for adults of CPB in all exposure times and treatments with all doses (Table 1 and Fig. 1). However, the mortality rates of izoldesis using as positive control were established after 24 hrs in the 10, 15 and 20 µL\petri doses as 77.70, 88.88 and 95.50% for adults of CPB, respectively. Furthermore, the mortality rates after 48, 72 and 96 hrs of treatment with all doses (10, 15 and 20 µL\petri) of izoldesis were found as 100 % for adults of CPB. No mortality for adults of CPB (except 2.22% 24 h; 4.44% 48 h; 6.66% 72 h; and 8.44% 96 h) in the control (Table 1 and Fig. 1). It was indicated that the essential oil isolated from *T. argyrophyllum* had an insecticidal effect on adults of *S. granarius* (Kordali *et al.*, 2012).

In conclusion, development of natural or biological insecticides may help to decrease depending on synthetic chemicals. In this respect, natural insecticides may also be effective, selective, easily bio-degradable and relatively low pollution for environment. In the present study, the essential oils obtained from 8 plants were found to be more toxic against adults of CPB, but the essential oils of *H. scabrum* and *S. multicaulis* had less effect. In many cases, their toxicities were also identical with the toxicity of commercial insecticides, widely used as insect reagent to protect the potato against adults of CPB. Therefore, in the light of the present results, it can be suggested that these plant essential oils can be used as new insecticidal reagents against adults of CPB. However, further studies need to be conducted to evaluate the cost and safety of these reagents, particularly under field conditions.

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