



Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) Control Potential of Essential Oil Isolated from Iranian *Cymbopogon citratus* Stapf

Asgar Ebadollahi^{1,*}, Jafar Geranmayeh², and Morteza Kamrani¹

¹Moghan College of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil 56971-94781, Iran

²Department of Plant Protection, Ardabil Branch, Ministry of Agriculture-Jihad, Ardabil 56169-14543, Iran

Abstract – Colorado potato beetle is a most destructive insect pest of potato throughout the world. Although utilization of chemical insecticides is a main method for management of this pest, their negative side-effects such as threat to humans and the environmental pollution prompted researchers to search for natural alternatives. Recently plant essential oils with low or without side-effects against non-targeted organisms and with high availability were considered as safe bio-pesticides. In the present study, toxicity of essential oil of Iranian lemongrass, *Cymbopogon citratus* Stapf, was evaluated against 3th instar larvae and adults of Colorado potato beetle by a leaf dipping method. Results displayed essential oil had notable toxicity against both larvae and adults after 24 and 48 h exposure times. Probit analysis revealed LC₅₀ values (lethal concentration to kill 50% of population) with 95% confidence limits were 10.32 (9.17 – 11.72) and 7.76 (6.80 – 8.74) µl/ml for larvae and 6.27 (4.82 – 8.15) and 4.35 (3.24 – 5.62) µl/ml for adults after 24 and 48 h, respectively. Based on regression analysis, a positive correlation between log concentration of essential oil and insect mortality was achieved. Results indicated *C. citratus* essential oil can be candidate as a natural alternative to the harmful chemical insecticides in the management of Colorado potato beetle.

Keywords – *Cymbopogon citratus*, Essential oil, *Leptinotarsa decemlineata*, Toxicity

Introduction

After China and India, Iran is a third chief producer of potato (*Solanum tuberosum*) in Asia so that its cultivation in Iran is about 146,000 ha with an annually production of 4,300,000.¹ The Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), is a key pest of potato throughout the world. It has been considered as most destructive pest in Ardabil and Hamadan provinces at north-west of Iran.² Its adults and larvae feed on the leaves and stems of potato plants. Further, the adults considered as a vector of plant diseases.³ Numerous chemical insecticides have been used to the management of *L. decemlineata*. Along with the negative side-effects of chemical pesticides such as environmental contamination and threat to human health, there are several studies indicating the resistance of *L. decemlineata* to the most classes of insecticides such as organochlorine, organophosphorus, carbamate, and pyrethroid ones.⁴⁻⁷ So, there

is an urgent need to introduce an eco-friendly and effective method to control this pest. Plants have undergone a co-evolution with their natural enemies including insects, in which plant secondary metabolites are developed against them. Essential oils as demotic secondary metabolites evinced a major role in the plant-enemies interactions. Plant essential oils have a complex mixture of aromatic and aliphatic components which affected by several endogenous and exogenous factors such as environmental conditions, plant genetic and extraction method.⁸ These materials have been characterized with low or non-toxicity to fish, birds and mammals. They are easily biodegrade in the environment because of their volatile quiddity. Further, pest resistance to the essential oils is lower than synthetic chemicals because of their multiple modes of action and several bio-effects.^{9,10} Consequently, essential oils considered as potential bio-agents to the insect pest management.^{11,12} Although lemongrass [*Cymbopogon citratus* (DC) Stapf (Poaceae)] is native of Southeast Asia, it is usually used by folk medicine in the numerous countries.¹³ Several biological effects of lemongrass such as anti-amoebic, antifungal and antidiarrheal activities have been documented.¹⁴⁻¹⁶

*Author for correspondence

Asgar Ebadollahi, Moghan College of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil 56971-94781, Iran

Tel: +98-919-243-6834; E-mail: ebadollahi@uma.ac.ir

Terpenic compounds such as citral and its isomers have high amount in the *C. citratus* essential oil and it was found that the biological effects of this oil have been related to these components.^{8,9,17} The aim of the present study is to evaluate the toxicity of *C. citratus* essential oil against the damaging stages of *L. decemlineata*; larvae and adults. Findings may be useful to recognize a bio-rational and safe agent for management of *L. decemlineata*.

Experimental

Plant materials and essential oil extraction – The leaves of *C. citratus* were collected at flowering stage from Tehran, Iran and dried in the shade. *C. citratus* was identified through its morphological structures by using the Flora Iranica.¹⁸ Extraction of essential oil was carried out using a Clevenger apparatus by hydro-distillation method via 50 g dried leaves and 500 ml distilled water within 3 h. Obtained essential oil was dried by anhydrous sodium sulfate and stored in refrigerator at 4 °C.

Tested insect – The first generation adults of *L. decemlineata* were collected from potato fields in Anzab region, Ardabil province, Iran and reared on potato, *Solanum tuberosum*, cv. Agria, under 26–27 °C, 60–65% RH and 16: 8 h (L: D) photoperiod conditions. Adults and 3th instar larvae obtained from a colony were considered for insecticidal bioassays.

Bioassay – Leaf-dipping bioassay was used to evaluate the toxicity of *C. citratus* essential oil against synchronized 3th instar larvae and adults of *L. decemlineata*. Five concentrations of essential oil ranged from 5.00 to 17.00 µl/ml for larvae and 2.00 to 19.00 µl/ml for adults were organized using Tween-80 (0.02 %) and distilled water. Leaf discs with 3 cm diameter were cut from fresh leaves of potato and were immersed in prepared concentrations for 20 seconds. After drying at room temperature for 10 minutes, the discs were placed in the Petri dishes and 5 larvae and adults separately were transferred to them. The control groups were kept under the same conditions on leaf disks without any essential oil's concentrations. Mortality counted after 24 and 48 exposure times. The insects were touched with the tip of a fine hair brush. If there were not any movement, they were considered as dead.

Statistical analysis – Experiments were organized in a completely randomized design and the data were analyzed using the SPSS 16 software. Differences between means were determined using Tukey's test at $P = 0.05$. Program also calculates the LC_{50} values and confidence limits with Probit analysis. Regression analysis was done to denote

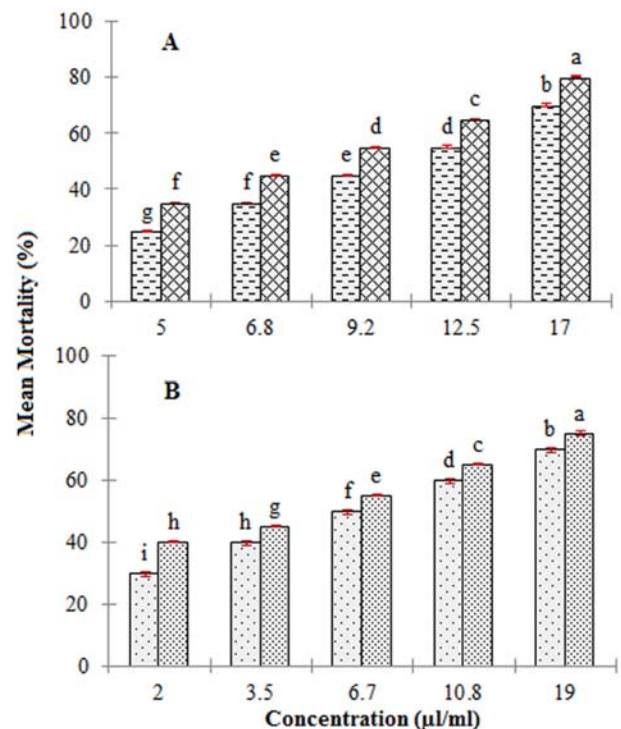


Fig. 1. Toxicity of essential oil of *Cymbopogon citratus* against the 3th instar larvae (A) and adults (B) of *Leptinotarsa decemlineata* after 24 and 48 h exposure times. According to Tukey's test, means with the dissimilar letters are significantly different at $P = 0.05$. There was not any mortality in the control groups.

mortality in contradiction of essential oil concentrations.

Result and Discussion

Results indicated that the essential oil of *C. citratus* showed significant toxicity against both larvae and adults of *L. decemlineata* after 24 and 48 h exposure times while there was not any mortality in the control groups. Comparison of mean mortality by Tukey's test revealed there are statistically significant differences between mortality of larvae and adults of insect against essential oil concentrations and exposure times (Fig. 1).

The results of Probit analysis are shown in Table 1; The LC_{50} values with 95% confidence limits were 10.32 (9.17–11.72) and 7.76 (6.80–8.74) µl/ml for larvae after 24 and 48 h exposure time, respectively. These values were, respectively, calculated as 6.27 (4.82–8.15) and 4.35 (3.24–5.62) µl/ml for adults. The LC_{50} values for both stages decreased with increasing of exposure time and there are statistically significant differences between these values after 24 and 48 h (Table 1).

Further, based on regression analysis and according to R^2 values, a positive correlation between logarithm of

Table 1. Probit analysis of the obtained data from toxicity *Cymbopogon citratus* essential oil against 3th instar larvae and adults of *Leptinotarsa decemlineata*

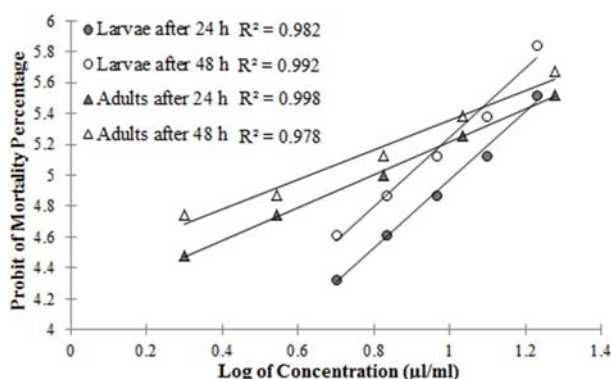
Insect stage	Time (h)	LC ₅₀ with 95% confidence Limits (µl/ml)	Slope SE	χ^2 (df = 3)	ρ values ^a	Relative median potency ^b
Larvae	24	10.32 (9.17 – 11.72)	2.19 ± 0.32	0.38	0.94	-
	48	7.76 (6.80 – 8.74)	2.21 ± 0.32	0.87	0.83	0.75 (0.62 – 0.89)
Adults	24	6.27 (4.82 – 8.15)	1.06 ± 0.17	0.09	0.99	-
	48	4.35 (3.24 – 5.62)	0.96 ± 0.17	0.75	0.86	0.69 (0.46 – 0.99)

^a Since the ρ values are greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits.

^b Relative median potency was considered for each stage separately. According to the relative median potency records, differences between LC₅₀ values of each stage are statistically significant.

Table 2. Chemical composition of *Cymbopogon citratus* essential oil from different locations

Source country	Main components
Argentina	Geranial (45.4%), neral (31.3%), neryl acetate (13.6%), caryophyllene oxide (9.7%). ²⁴
Brazil	Geranial (E-citral) (53.2%), neral (Z-citral) (36.4%), geraniol (2.6%) and geranyl acetate (1.5%). ²⁷
Cameroun	Geranial (39.3%), neral (21.9%), geraniol (15.6%) and myrcene (14.0%). ²⁸
China	Citral (45.9%) and verbenol (37.9%). ²⁹
Columbia	Geranial (34.4%), neral (28.4%), geraniol (11.5%) and β -Pinene (8.1%). ²³
Cuba	Geranial (51.1%), neral (35.2%), myrcene (6.5%) and geraniol (2.2%). ²⁷
India	α -Citral (17.9%), β -citral (29.0%), 1,8-cineole (7.5%) and caryophyllene (6.4%). ²⁶
Sri Lanka	α -Citral (46.2%), β -citral (31.6%), geraniol (3.6%) and geranyl acetate (1.3%). ³⁰

**Fig. 2.** Concentration-response lines for different concentrations of *Cymbopogon citratus* essential oil against 3th instar larvae and adults of *Leptinotarsa decemlineata*.

concentrations and mortality has been achieved for both larvae and adults (Fig. 2).

Chemical composition of *C. citratus* essential oil from different country was assessed in the recent studies (Table 2). According to the Table 2, different isomers of citral including as α -citral, β -citral, geranial and neral, geraniol, myrcene, neryl acetate, caryophyllene, caryophyllene oxide, geranyl acetate, verbenol, 1,8-cineole, and β -pinene were the main components, which all are among terpenic compounds. Toxicity of such terpenic compounds was approved against *L. decemlineata* and some of other insect pests.^{17,19}

Further, toxicity of some essential oils was evaluated against *L. decemlineata* in the recent years. For example, toxicity of *Eugenia caryophyllus* (Sprengel), *Mentha spicata* L., *Myrtus communis* L., *Ocimum basilicum* L., *Satureja khuzistanica* Jamzad and *Thymus daenensis* Celak essential oils were evaluated against the 4th instar larvae and adults of *L. decemlineata*. All essential oils were toxic against both stages in which the essential oil of *S. khuzistanica* was the most efficient.²⁰ Toxicity of four *Achillea* species including *A. biebersteinii* Afan., *A. coarctata* Poir., *A. wilhelmsii* Koch and *Achillea biserrata* Bieb. was investigated against the adults of *L. decemlineata* by Cakır et al.²¹ *L. decemlineata* adults were affected by the concentrations and exposure times of all essential oils. They concluded that observed toxicity might be caused by the occurrence of main components or by synergistic effect of them with the other compounds. In the present study, the insecticidal activity of *C. citratus* essential oil was approved against the larvae and adults of *L. decemlineata*. This effects may be related to being some terpenic compounds specially citral, geranial, neral, geraniol, myrcene, and 1,8-cineole. Furthermore, the insecticidal effects of *C. citratus* essential oil were reported against some major insect pests such as *Aedes aegypti* L., *Tribolium castaneum* Herbst, *Sitophilus oryzae* L., *Zabrotes subfasciatus* (Boheman), and *Musca domestica* L., which support the

results of present study for insecticidal potential of this oil.²²⁻²⁶ One of the most attractive features of plant essential oils is that they are low-risk materials to mammals and other non-target organisms. Essential oils have short residual half-lives. So, their consumption can reduce risks to foraging pollinators such as honeybees. Furthermore, because of being several components with multiple modes of action against pests, pest resistance to these bio-agents will be low. Based on the previous findings *C. citratus* essential oil has a great potential for management of some insect pests and according to the our results it was a sound candidate as a new bio-pesticide for *L. decemlineata* management. However, additional studies should be made for enhancement of its toxicity against *L. decemlineata* and other economical insect pest, improvement its stability in the field application and reducing its cost problem.

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