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Evaluation of Synthetic Insecticides and Essential Oils for the Management of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)

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Authors' Contribution
MA and MIU conceived, designed and performed the experiments. YI, SK and ZH interpreted the data and wrote the paper. MA, JMO and JEF reviewed and edited the manuscript.

Key words
Botanicals, Citrus, Bioassay, Insecticides, *P. citrella.*

ABSTRACT

Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a destructive insect pest of citrus nurseries as well as orchards in Pakistan. The aim of this study was to assess the effectiveness of some synthetic insecticides and essential oils against citrus leafminer larvae. Two laboratory bioassays, leaf dip bioassay (LDB) and topical bioassay (TB) were developed to check the efficacy of tested chemicals and essential oils. As seen from results, abamectin showed significant mortality (63.5%) of CLM larvae when topical bioassay was performed. Similarly, the percent mortality of CLM larvae was 53.8% after application of abamectin, when leaf dip bioassay technique was used. However, among tested botanicals, *Azadirachta indica* oil showed better results with percent CLM mortality of 35.6%, through topical bioassay and 31.8% through leaf dip bioassay. In the case of *A. indica*, the LC_{50} value was also observed lower (1.88±0.37, 1.73±0.29) in LDB and TB, respectively, as compared to other botanicals. So, our study suggested that abamectin gave better management of citrus leafminer larvae. However, higher concentrations of *A. indica* oil can be used to control CLM larvae.

INTRODUCTION

Citrus production plays a significant role in the economy of Pakistan. Pakistan ranks at 10th in the citrus production worldwide (Ullah *et al*., 2016). Unfortunately, it is threatened by a number of insect pests. Among all insect pests, the citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a major one that has deleterious effects on citrus and related species worldwide (Elekcioğlu, 2017). It was first originated from Eastern and Southern parts of Asia and become the most threatening issue to all citrus growing areas of America and also in the Mediterranean basin after 1993 (Beattie, 1993).

At early stages, the mines can be found only on the lower surface of the leaves, but both sides of the leaves might be infested with a severe infestation. Mostly, a single larva generates only a single mine over leaf but in severe infestation; it can generate about 2 to 3 mines (Badawy, 1967; Heppner, 1993; Garcia-Marí *et al*., 1997). CLM larvae mostly damage the newly emerged leaves due to which plant growth and development affected along with less fruit quality (Elekcioglu and Uygun, 2013). Citrus canker disease has also been examined on leaves infested by CLM larvae (Mustafa *et al*., 2013). The economic impact of direct damage is particularly greater at nursery level (Grafton-Cardwell and Montez, 2009). Therefore, it is critical to protecting young seedling or top grafting trees from CLM damage.

Repeated applications of insecticide are required for better control of CLM due to its many generations per year (Yumruktepe *et al*., 1996) and higher costs involved for

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multiple applications. Recently the increasing trend of using biopesticide is also very effective to control major insect pests and alternative to conventional insecticides (Broderick *et al*., 2000; Lacey *et al*., 2001).

Mineral oil can be used as a surfactant and reduce surface tension and can be very helpful to manage CLM by minimizing the protection of epidermal layer (Dias *et al*., 2005). Using of mineral oils are the most popular alternative for the management of citrus leafminer population and are recommended for the use in home gardens, nurseries, and orchards (Khalid *et al*., 2012). The foliar application of oils proved as a repellent for a female to egg laying (Beattie *et al*., 1995; Liu *et al*., 2001). The oils are also proved to be safer for beneficial fauna as compared to insecticidal spray. Thus, they may incorporate well with biological control agents used for pest control (Cranshaw and Baxendale, 2005).

Amiri-Besheli (2011) specified the use of biopesticides like tondexir extracts from hot pepper dispirited CLM adults from egg laying on leaves and posed a lower risk to humans and the environment than other pesticides. Botanicals are comparatively harmless against the non-target organisms as compared to insecticides (Isman, 2006). The aim of present study was to check the comparative efficacy of different essential oils and synthetic insecticides against citrus leafminer larvae.

MATERIALS AND METHODS

The experiments were conducted in the laboratory of Department of Entomology, University of Sargodha, Pakistan. Four synthetic insecticides, (Spinosad@ 0.75ml/liter, Abamectin@ 0.4ml/liter, Deltamethrin@ 0.6ml/liter and Imidacloprid@ 0.3gm/liter) along with control (water) were selected to assess their effectiveness against CLM larvae. Similarly, different essential oils (*Eucalyptus camaldulensis*, *Citrus sinensis* and *Azadirachta indica*) at 0.5%, 1% and 1.5% concentrations were tested against CLM larvae. The essential oils were purchased from the local market (Musaji Adam and Sons, Liaison office, Sargodha, Pakistan).

For bioassays, the leaves with actively feeding third instar CLM larvae were collected from citrus mandarins in the field. Leaves petiole was wrapped with cotton to keep leaves moist. For leaf dip bioassay, leaves were dipped for 10 sec separately in each chemical and dipping in distilled water served as control. Then, leaves were air dried for 2 h and placed in clean Petri plates that were lined with wet filter paper. Similarly, leaves with actively feeding CLM larvae were also treated through topical application method described by Shapiro *et al*. (1998). One drop (about 4µl) of each chemical was applied on the thorax of each CLM larvae using sterilized micro-syringe. After application, leaves were transferred to clean Petri dishes. Each treatment was replicated five times in both bioassays and five leaves were treated for each replicate. Leaves were examined under a microscope to check the movement of CLM larvae. Mortality of CLM larvae was considered when it displayed a lack of external or peristaltic movement when probed.

Both studies were repeated once and for each time, data were recorded after 3, 6, 9, 12 and 24 h after application by counting dead larvae under a microscope. The percentage mortality of CLM larvae was calculated and corrected by Abbott's (1925) formula:

$$\text{Percent mortality} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) \times 100$$

Where, T is treatment and Co is control.

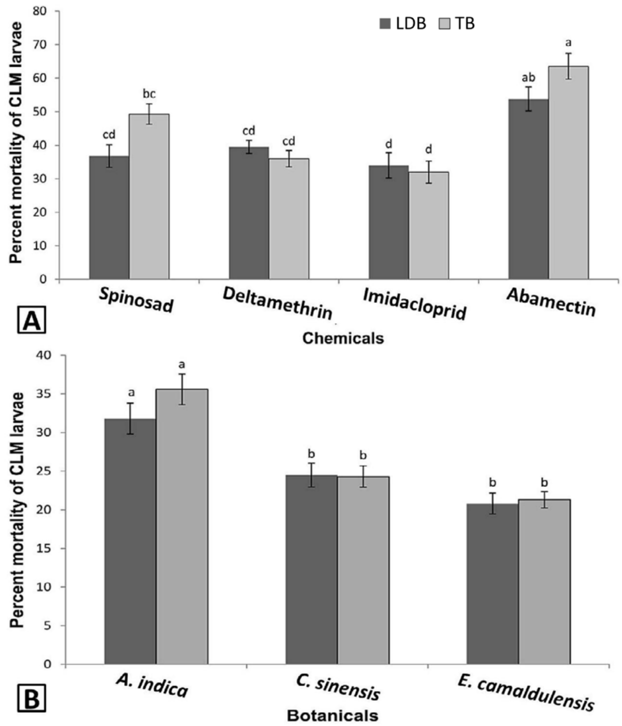

Fig. 1. Percent mortality (Means±SE) of CLM larvae after application of different synthetic insecticides (A) and botanicals (B) by using two bioassay techniques. For A, means sharing similar letters for each bioassay are not significantly different from each-others, Significance rate (F=3.11, $P<0.05$), For B, significant rate (F=1.26, $P>0.05$). LDB, leaf dip bioassay; TB, topical bioassay.

The data were analyzed by a completely randomized design using factorial arrangements of treatments, time interval and type of bioassay for insecticides and additionally concentration in essential oils.

Table I.- LC_{50} values for different essential oils against CLM larvae by using two bioassay techniques.

Essential oils	Leaf dip bioassay		Topical bioassay	
	LC_{50}±SE	95% C.I. (Lower-Upper)	LC_{50}±SE	95% C.I. (Lower-Upper)
A. indica	1.88±0.37	1.145-2.61	1.73±0.29	1.16-2.29
C. sinensis	2.54±0.75	1.06-4.01	2.54±0.67	1.23-3.85
E. camaldulensis	2.237±0.42	1.41-3.06	2.78±0.80	1.22-4.35

The lethal concentration LC_{50} of each essential oil was also calculated by probit analysis. Means comparison were tested by Tukey HSD all pairwise comparison test. All the analysis was performed using Minitab 16.1 and SPSS 20.0 software.

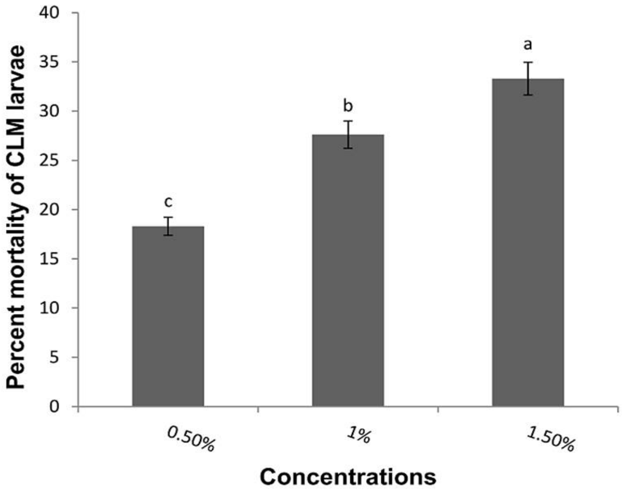

Fig. 2. Percent mortality (Means±SE) of CLM larvae after application of different concentration of botanicals, Significant effect of concentration (F=60.59, *P<0.001*) was observed. LDB, leaf dip bioassay; TB, topical bioassay.

RESULTS

The results showed that abamectin gave the best control of citrus leafminer larvae with percent mortality of 53.8% through leaf dip bioassay and 63.5% by topical bioassay. Abamectin showed 15.8% more performance in TB as compared to LDB. The second most effective chemical was spinosad who gave 49.3% mortality of CLM larvae through topical application. The least effected chemical was imidacloprid which showed only 34% and 32% mortality of CLM larvae in leaf dip bioassay and topical bioassay, respectively (Fig. 1A).

Among tested botanicals, *A. indica* showed better response against CLM larvae with percent mortality of 31.8% in LDB and 35.6% in TB (Fig. 1B). The percent mortality of CLM larvae was greater (33.3%) by using higher concentration (1.5%) of oils (Fig. 2).

Furthermore, LC_{50} values were also confirmed the higher toxicity of *A. indica* against CLM larvae having a minimum value 1.88%±0.373 by using LDB technique. Similarly, when we used topical bioassay, the LC_{50} value for *A. indica* was also found lower (1.73%±0.289) as compared to other tested botanicals (Table I).

DISCUSSION

CLM is a very important insect pest of the citrus crop all over the world and is very destructive to young seedling at nursery level. Different commercial insecticides and plant essential oils were tested for the management of CLM in this study. Two different bioassays were performed to demonstrate their utility. Leaf dip bioassay was tested to check the ability of tested chemicals for the penetration into leaf, and topical bioassay was tested to check the direct activities of chemicals against CLM larvae. As seen from the results, abamectin at field recommended dose show better response against CLM larvae. Abamectin is a product of natural fermentation of soil bacteria *Streptomyces avermitilis* and being used to control citrus insect pests (Khalil, 2013). The results were in accordance to Patil (2013) who sprayed acid lime leaves with different insecticides and proved abamectin as best chemical against citrus leafminer compared to spinosad, diafenthiuron and triazophos. Spinosad was the second most effective chemical against CLM larvae in our study. Spinosad directly affected the insect nervous system through ingestion (Salgado, 1998). It has the less toxic effect on the non-target organism and has the low persistence to the environment and is classified by the U.S. Environmental Protection Agency (Thompson *et al*., 2000).

Insecticide application may be quite useful at the time of new flushes when the leaves are most susceptible to CLM damage. In our study, both synthetic and botanicals gave better response against third instar CLM larvae through topical bioassay compared to leaf dip bioassay, but there was no significant difference between these two bioassays. Mafi and Ohbayashi (2006) found 3 to 44% mortality of citrus leafminer eggs due to insecticides exposure by using dip method, but the mortality of 1st instar CLM larvae was almost over 90%.

Furthermore, it is essential to be aware of the harmful effect of insecticides on beneficial fauna, so the biorational insecticides including botanicals can offer safe products for natural enemies (Grafton-Cardwell and Gu, 2003; Villanueva-Jiménez *et al*., 2000). Therefore, to overcome the CLM infestation, it is very important to select chemicals having less toxicity to natural enemies.

Among tested essential oils, *A. indica* showed significant mortality of CLM larvae as compared to others. It may be due to rapid penetration and translaminar activity of neem oil through leaf tissues (Mujica *et al*., 2000). Our findings were supported by IIHR (2006) who reported that neem oil showed 65.9% mortality of citrus leafminer larvae.

However, follow-up sprays of neem formulations can be used under severe infestation and as prophylactic sprays during young flushes (Verghese *et al*., 2004). Our results were also supported by Howard (1993) who concluded that abamectin and azadiractin both as a biorational insecticide are potentially beneficial for controlling CLM larvae.

The plant essential oils can be used as an alternate of synthetic chemicals against citrus leafminer. Similarly, in growing season of citrus plants, the frequent applications of oils may be an option to reduce insecticide usage and leafminer population (McCoy, 1985; Stansly *et al*, 2008).

Higher concentration of essential oils should be assessed to find out a dose-dependent effect against CLM larvae. It might be possible, *A. indica* oil at higher concentration enhance the penetrability into leaf mines. So, our study suggests that abamectin and neem oil poses interesting perspectives for leafminer management program. For further understanding, it is important to explore the insecticides in combination with botanicals to ensure that they perform better in the field.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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