2019

Effect of Neem-based Botanicals and Abamectin 1.8% EC against *Phyllocnistis citrella*1 in *Citrus reticulata* (Rutaceae) Nursery Plantations

Muhammad Arshad

Muhammad Irfan Ullah

Muhammad Afzal

Jaime Molina-Ochoa

Omar Francisco Prado-Rebolledo

See next page for additional authors

Authors
Muhammad Arshad, Muhammad Irfan Ullah, Muhammad Afzal, Jaime Molina-Ochoa, Omar Francisco Prado-Rebolledo, and John E. Foster

Effect of Neem-based Botanicals and Abamectin 1.8% EC against *Phyllocnistis citrella*[1] in *Citrus reticulata* (Rutaceae) Nursery Plantations

Muhammad Arshad[2], Muhammad Irfan Ullah[2], Muhammad Afzal[2],
Jaime Molina-Ochoa[3,4]*, Omar Francisco Prado-Rebolledo[3], and John E. Foster[4]

Abstract. Citrus leafminer, *Phyllocnistis citrella* Stainton, is an economically important insect pest of citrus in Pakistan, Mexico, the United States and many other citrus-producing countries. Extract of *Azadirachta indica* A. Juss at 5 and 7% and its oil at 1 and 1.5% concentrations were tested in comparison to synthetic insecticide abamectin 1.8% EC against *P. citrella* in nursery plantations of *Citrus reticulata* L. Control of all larval instars of citrus leafminer was good with application of abamectin and *A. indica* oil during fall 2015 and summer 2016. Abamectin suppressed 75-90% of *P. citrella* larvae both seasons. *A. indica* oil at greater concentration (1.5%) provided the most control (65-88%) like abamectin but at less concentration (1%), control decreased slightly. Larvae of *P. citrella* were more sensitive to *A. indica* oil than to extract. Like abamectin, *A. indica* oil also better controlled citrus leafminer larvae and might be considered a promising tool for management of citrus leafminer.

Resumen. El minador foliar de los cítricos (MFC), *Phyllocnistis citrella* Stainton, es un insecto plaga económicamente importante de los cítricos de Pakistan, México, Estados Unidos de Norteamérica y de muchos países productores de cítricos. El extracto de *Azadirachta indica* A. Juss a las concentraciones de 5% y 7% y su aceite al 1% y 1.5% fueron probadas en comparación con el insecticida sintético abamectina al 1.8% CE en contra de *P. citrella* en plantaciones de vivero de mandarina, *Citrus reticulata* L. Los resultados mostraron que la tasa de control de todos los estadios larvarios del MFC fue alta con la aplicación de abamectina y el aceite de *A. indica* durante el otoño de 2015 y el verano de 2016. Sin embargo, el aceite de *A. indica* a la concentración más grande (1.5%) proporcionó el nivel de control más grande así como la abamectina, pero al disminuir su concentración (1%), la tasa de control disminuyó ligeramente. Las larvas de *P. citrella* fueron más sensibles al aceite que al extracto de *A. indica*. Además, también la abamectin y el aceite de *indica* también dieron mejor control de larvas del MFC; esto podría ser considerado como una herramienta promisoria para el manejo del MFC.

---

[1]Lepidoptera: Gracillariidae

[2]University of Sargodha, Department of Entomology, Sargodha, 40100, Pakistan; e-mail: makuaf@gmail.com, mirfanullah@uos.edu.pk, chafzal64@yahoo.com
[2]Universidad de Colima-Coordinación General de Investigación Científica-Centro Universitario de Investigación y Desarrollo Agropecuario-Facultad de Medicina Veterinaria y Zootecnia, Km. 40 autopista Colima-Manzanillo, Tecomán, Colima 28930, México; e-mail: jmolina@ucol.mx, jmolina18@hotmail.com
[3]Department of Entomology, Institute of Agricultural and Natural Resources, University of Nebraska-Lincoln, Lincoln, NE 68583-0816, USA.
*Corresponding author: jmolina@ucol.mx

Citrus growers consider the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), a destructive insect pest in citrus nurseries (Villanueva-Jiménez and Hoy 1998, Amiri-Besheli 2008, Grafton-Cardwell and Montez 2009, El-Afify et al. 2018). *P. citrella* feeds mostly on young flushes, making serpentine mines on leaves, with the impact of feeding greatest in nursery stock (Grafton-Cardwell and Montez 2009). According to Garcia-Mari et al. (2002), 45% of new leaf area can be damaged by mining by *P. citrella*. Shivankar et al. (2002) reported that *P. citrella* affected more than 80% of citrus nurseries in central India, and the percentage increased to >87% with severe infestations. Two mines per leaf are enough to reduce growth of plants younger than 5 years old (Beattie and Hardy 2004). Besides direct feeding, *P. citrella* causes indirect damage to new flushes by exacerbation of canker infection; controlling *P. citrella* is a dynamic component for management of canker disease (Peña et al. 1996, Belasque et al. 2005). High fertility rate and dispersal as well as the epidermis that protects against direct contact of the larval body with chemicals makes effective management of *P. citrella* complicated (Amiri-Besheli 2011).

Broad-spectrum insecticides like pyrethroids, organophosphates, and neonicotinoids are used to suppress *P. citrella* (Mafi and Ohbayashi 2006, Powell et al. 2007). Abamectin with nematicidal, acaricidal, and insecticidal activity (Lasota and Dybas 1991) is considered the chemical most effective against the pest. It rapidly breaks down by exposure to sunlight or when present as a thin film (Clark et al. 1995) and easily reached *P. citrella* larvae in mines (Damavandian and Moosavi 2014) because its reservoirs remain within the mesophyll layer of the leaf, mostly when applied with oil (Lasota and Dybas 1991).

Insecticide is expensive, effective for short periods, and needs to be applied repeatedly (Se´tamou et al. 2010). Continual use can change the ecological equilibrium between natural enemies and pests and speed selection for resistance to insecticides by *P. citrella* (Tan and Huang 1996). Target pests might resurge (Dutcher 2007, Guedes and Cutler 2014, Guedes et al. 2016) and secondary pests like Tetranychidae, scales, caterpillars, and leafminer might outbreak (Saito 2004), that require additional applications of insecticide (Croft 1990).

Biopesticides are considered safer and effective techniques for controlling major insect pests. Among botanicals, neem tree, *Azadirachta indica* A. Juss (Family: Meliaceae) also known as Indian lilac (Schmutterer 1990) is the most promising (Nboyine et al. 2013). It suppresses the feeding sensation of insects, even at concentrations of <1 parts per million (Isman et al. 1991) and contains more than 200 allelochemicals in different plant parts, providing significant insecticidal properties (Koul et al. 2004). Neem seeds contain 40% oil of azadirachtin as the major active ingredient, which is the main constituent for insecticidal activity (Isman et al. 1991). The leaves of the plant are repellent against stored grain insect pests (Koul et al. 1990). All parts of the plant are intrinsically reported an internal chemical defense repelling insect pests, and used in effective pest control (Chaudhary et al. 2017).

Considering drawbacks of application of synthetic insecticides, suitability of alternate and safe naturally occurring plant-based chemicals against *P. citrella* need to be studied and confirmed under field conditions. The main objective of the study was to evaluate the efficacy of neem-based botanicals compared with a field dose of abamectin against *P. citrella* in nursery plantations.

Materials and Methods

The experiment was done at nursery plantations of *Citrus reticulata* in the research area of College of Agriculture, University of Sargodha, during fall 2015 and summer 2016. Coordinates of the nursery are 32°07'53.0"N 72°41'02.5"E. One- to 2-year-old plants of 'Kinnow' mandarins having dense foliage were used. The nursery was closely planted with an inter-row distance of 12.7 cm and plant-to-plant distance of 15.2 cm. The plants were grown in six consecutive rows of 80-90 plants. The plants were inspected carefully, and stunted and unhealthy plants were excluded from experimental units.

The essential oil of *A. indica* and synthetic insecticide abamectinTM® 1.8% EC were purchased from the local market (Musaji Adam & Sons, Liaison Office) of Sargodha. For preparation of *A. indica* aqueous extract, the leaves were collected from a natural area near the university, cleaned, and washed with tap water. After grinding the leaves, the powder was soaked in distilled water and filtered through cloth (Rashid and Ahmad 2013) as well as through Whatman No. 1 filter paper (Naniwadekar and Jadhav 2012). After filtration, the solvent (water) was evaporated by a rotary vacuum evaporator (Laborota 4001, Heidolph, USA) and the extract was stored in a refrigerator for further use.

Treatments (1) *A. indica* oil 1%, (2) *A. indica* oil 1.5%, (3) *A. indica* extract 5%, (4) *A. indica* extract 7%, (5) abamectin 1.8% EC at field-recommended doses and (6) check were tested against *P. citrella.* The experiment was replicated three times, and five plants were selected for each replication of a single treatment ensuring no repetition of the same treatment in a row. Five plants were treated with tap water as a check. To avoid spray drifting, selected plants were surrounded by nontreated border plants. A handheld sprayer was used to apply each chemical as a foliar spray. During fall 2015, the first application was made on 19 and second on 27 September. Abundance of *P. citrella* was comparatively low in April 2016, so only one application was made.

The first sample was collected just before the first application, and then data were recorded on the 3rd and 7th day after the first and second application. At each sampling, three shoots were selected randomly from each tree, and five young leaves were selected randomly from each shoot. The selected leaves were collected and labeled separately in plastic bags and transferred in ice boxes to the laboratory of entomology. To record live larvae, *P. citrella* larvae were classified into two categories: small and large larvae based on the length and color as described by Kerns et al. (2001).

Data were analyzed by one-way ANOVA, with treatments as the main factor, and the variable measured per replication of each treatment was the average number of live *P. citrella* larvae. Data normality was assessed by transforming data through $\sqrt{X+1}$ transformation, whereas x denoted the live number of *P. citrella* larvae. Non-transformed means were presented in the tables and figures. Means were separated by Tukey HSD (P = 0.05) pairwise comparison test using SPSS 20.0 statistical software.

Results

During fall 2015, initial abundance of small and large larval instars of *P. citrella* differed significantly ($P < 0.05$) at the first date of sampling just before applying the first application of insecticide (Tables 1-2). Similarly, a significant difference (P <

Table 1. Average Number (±SEM) of Live Small Larvae of *Phyllocnistis citrella* after Applications of Insecticides in Nursery Plantations during Fall 2015

	1st spray			2nd spray	
Treatment	Before	3 DAT[a]	7 DAT	3 DAT	7 DAT
Abamectin	0.551 ± 0.016a	0.284 ± 0.012c	0.124 ± 0.008d	0.093 ± 0.011d	0.080 ± 0.011d
A. indica extract 5%	0.484 ± 0.022ab	0.422 ± 0.023b	0.364 ± 0.019b	0.280 ± 0.013b	0.201 ± 0.009b
A. indica extract 7%	0.533 ± 0.023ab	0.431 ± 0.021b	0.288 ± 0.015c	0.201 ± 0.013c	0.133 ± 0.011c
A. indica oil 1%	0.493 ± 0.024ab	0.275 ± 0.015c	0.168 ± 0.011d	0.128 ± 0.014d	0.107 ± 0.008cd
A. indica oil 1.5%	0.475 ± 0.023ab	0.262 ± 0.013c	0.151 ± 0.012d	0.124 ± 0.011d	0.080 ± 0.009d
Check	0.444 ± 0.022b	0.533 ± 0.027a	0.595 ± 0.022a	0.627 ± 0.018a	0.671 ± 0.014a
F value	3.17	31.24	132.56	216.8	447.5
P value	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001

Means followed by the same letter in a column are not significantly different ($P > 0.05$), $P < 0.05$ = significant, $P < 0.001$ very significant

[a]DAT = day after treatment

Table 2. Average Number (±SEM) of Live Large Larvae of *Phyllocnistis citrella* after Applications of Insecticides in Nursery Plantations during Fall 2015

	1st spray			2nd spray	
Treatment	Before	3 DAT[a]	7 DAT	3 DAT	7 DAT
Abamectin	0.440 ± 0.016a	0.275 ± 0.011c	0.182 ± 0.010d	0.102 ± 0.008d	0.044 ± 0.008d
A. indica extract 5%	0.502 ± 0.017ab	0.444 ± 0.016b	0.387 ± 0.013b	0.315 ± 0.013b	0.244 ± 0.011b
A. indica extract 7%	0.467 ± 0.017ab	0.386 ± 0.014b	0.333 ± 0.021bc	0.253 ± 0.014c	0.182 ± 0.014c
A. indica oil 1%	0.524 ± 0.024a	0.413 ± 0.022b	0.316 ± 0.012c	0.226 ± 0.012c	0.137 ± 0.012c
A. indica oil 1.5%	0.507 ± 0.016ab	0.311 ± 0.010c	0.201 ± 0.014d	0.115 ± 0.010d	0.066 ± 0.006d
Check	0.498 ± 0.021ab	0.528 ± 0.020a	0.564 ± 0.021a	0.604 ± 0.018a	0.680 ± 0.022a
F value	2.56	30.53	78.68	188.6	318.1
P value	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001

Means followed by the same letter in a column are not significantly different ($P > 0.05$), $P < 0.05$ = significant, $P < 0.001$ = very significant

[a]DAT = day after treatment

0.001) of small and large larvae was found at all sampling dates after the first and second applications. The initial average number of live larvae before the first application of insecticide was comparable among treatments, from 0.444-0.551 small and 0.440-0.524 large larvae per leaf during fall 2015. Abamectin resulted in significantly fewer small larvae (0.124) per leaf compared to the check (0.595 larva per leaf) after the 7th day of first application. The second most effective chemical was *A. indica* oil that at 1.5 and 1% concentrations resulted in significantly fewer small larvae (0.151 and 0.168) per leaf, respectively, at the 7th day after first application. Similarly, at the 7th day after second application, abamectin and *A. indica* oil at 1.5% concentration resulted in significantly fewer small larvae (0.08 per leaf) compared to the check (0.671 larva per leaf).

Similar findings were observed for large larvae during fall 2015 in which abamectin and *A. indica* oil at 1.5% concentration resulted in significantly fewer large larvae per leaf (0.182 and 0.201, respectively) compared to the check (0.564 larva per leaf) at the 7th day after first application. The average number of large larvae at the 7th day after second application was only 0.044 and 0.066 per leaf by application of abamectin and *A. indica* oil at 1.5% concentration, respectively (Table 2). The check reached 0.68 larva per leaf.

During summer 2016, no significant difference was observed in the initial number of small (F = 2.05, df = 5, P > 0.05) and large (F = 1.96, df = 5, P > 0.05) larvae just before application. However, significant difference was observed in small larvae the 3rd (F = 45.66, df = 5, P < 0.001) and 7th days (F = 108.6, df = 5, P < 0.001) after insecticide application. Similarly, for large larvae, a significant difference in the number of live larvae was found the 3rd (F = 27.1, df = 5, P < 0.001), and 7th (F = 116.6, df = 5, P < 0.001) days after application. During summer 2016, average abundance ranged from 0.346-0.426 small larvae (Fig. 1) and 0.315-0.373 large larvae per leaf (Fig. 2).

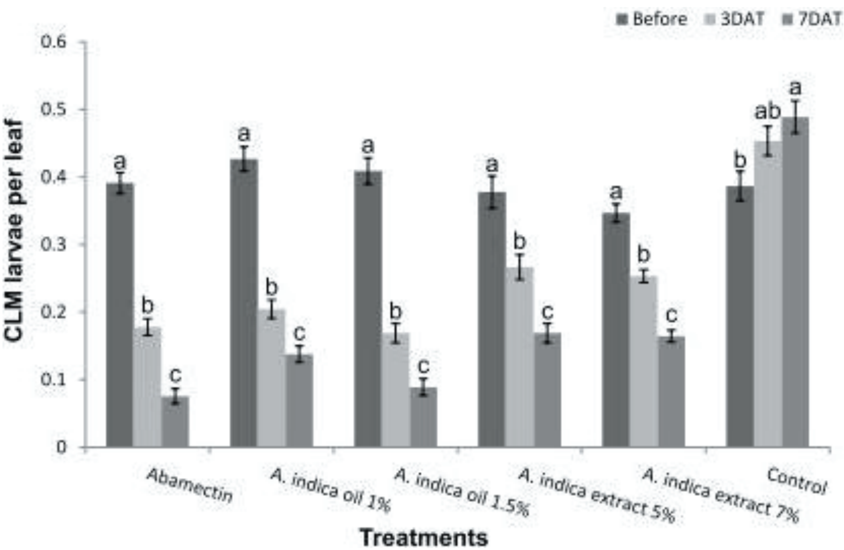

Fig. 1. Average number (±SEM) of live *Phyllocnistis citrella* larvae (small) after different time intervals during summer 2016. Means sharing the same letter are not significantly different (P > 0.05). DAT = day after treatment.

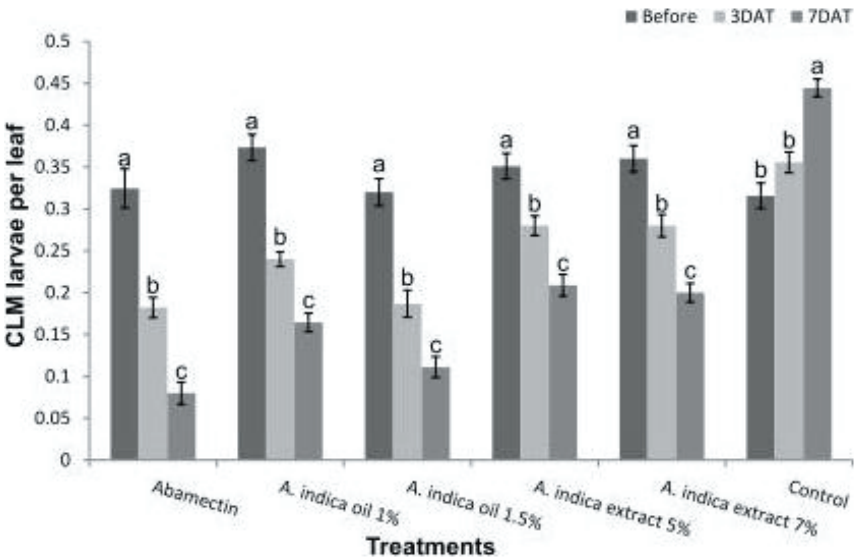

Fig. 2. Average number (±SEM) of live *Phyllocnistis citrella* larvae (large instars) after different time intervals during summer 2016. Means sharing similar letters are not significantly different ($P > 0.05$). DAT = day after treatment.

Abamectin and *A. indica* oil at 1.5% concentration resulted in significantly fewer small larvae per leaf (0.075 and 0.088, respectively) at the 7th day of exposure compared to the check (0.488) (Fig. 1). At the 7th day after application, similar control of large larvae was by application of abamectin and *A. indica* oil (Fig. 2).

The number of *P. citrella* larvae with *A. indica* extract was comparatively low as with abamectin and *A. indica* oil during both seasons. But, compared with the check, the *P. citrella* population remained low.

Discussion

Phyllocnistis citrella is a major insect pest of all citrus cultivars especially in nursery plantations in Pakistan. The pest causes severe damage to young flushes, so there is dire need to protect new flushes of citrus. Our results showed that abamectin reduced abundance of small and large larvae of *P. citrella*. At the last day of sampling both seasons, abamectin had controlled 75-90% of *P. citrella* larvae.

Abamectin belongs to the avermectin group, is comprised of two homologous compounds (80% of avermectin B1a and 20% of avermectin B1b) (Pozo et al. 2003), and has high molecular weight, so it reduces penetration capacity of the insect cuticle (Stock and Holloway 1993). It also can be absorbed easily and translocated into leaf tissues, leaving minimal residue on the surface (Morse et al. 1987) and is safer for natural enemies (Bacci et al. 2007). Efficacy of abamectin against *P. citrella* larvae was confirmed by Rao et al. (2002) and Abou-Fakhr Hammad and Antar (2003). According to Patil (2013), 0.0007% of abamectin 1.9% EC controlled the pest.

The results of efficacy of abamectin against *P. citrella* indicated it could be part of integrated pest management in citrus. However effective, use of abamectin is limited in other citrus-growing countries. Three applications per year are recommended in Florida (Knapp 1996) because of resistance. According to Metcalf and Luckmann (1994), abamectin ranked very dangerous and should be used in case of severe infestation by insects. Similarly, Damavandian and Moosavi (2014), reported that abamectin, dursban, and confidor might enhance infestation by citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae). The insecticides are registered and have been used against *P. citrella* for many years in Iran.

To reduce the application rate and numbers, minimize the cost of insecticides, and overcome the resistance problem, insecticides can be used in combination with botanicals for better management of *P. citrella*. Successful control of *P. citrella* using abamectin with petroleum oil was reported by Raga et al. (2001) and its better control depends on timing of application and amount of oil used (Liu et al. 2001). Wang et al. (2005) concluded that application of abamectin in combination with oil provided synergistic effect to manage *P. citrella*.

Botanicals or naturally occurring chemicals are considered safe, cheaper, and easily available to control many insect pests (Pandao et al. 1992, Raveendran et al. 1998, Ukey et al. 1999, Charleston et al. 2005, Kraiss and Cullen 2008, Italo et al. 2009, Kumral et al. 2010, Ali et al. 2011) and can replace synthetic insecticides. In our study, *A. indica* oil at 1.5% concentration suppressed 65-88% of *P. citrella* larvae both seasons. Azadirachtin-based biopesticides with a different mode of action have well-known effect on many insect pests and were reported by Kraiss and Cullen (2008), Italo et al. (2009), Kumral et al. (2010), and Ali et al. (2011). It can delay reproduction in insects with growth-deterring properties and cause morphological deformations such as molt blocking and partial ecdysis during the pupal stage that inhibit adult formation (Boulahbel et al. 2015, Chaudhary et al. 2017). As insect growth regulator, neem oil also was reported by Kraiss and Cullen (2008) against soybean aphid, *Aphis glycines* Matsumura. Azadirachtin compound is similar in structure to insect hormone “ecdysone” that plays an important role in metamorphosis in insects. It displays antifeedancy through stimulation of deterrent cells and blocks feeding stimulation in insects through firing receptor cells known as “sugar” (Mordue Luntz et al. 1998). Injection of azadirachtin induces physiological impairment in the midgut of insects that leads to reduction in digestive efficiency (Chaudhary et al. 2017).

Consequently, need for synthetic insecticides can be modified easily by incorporating neem oil for eco-friendly management of insects (Adnan et al. 2014). In addition, abamectin and neem oil have a characteristic translaminar action and rapid penetration into leaf tissues that kills *P. citrella* larvae (Mujica et al. 2000). Overall, our findings are in accordance to Abou-Fakhr Hammad and Antar (2003) who reported that azadirachtin, abamectin, and mineral oil had significant effect on all larval instars of *P. citrella* at field conditions in novel orange plantations.

However, extracts of *A. indica* were least effective against all *P. citrella* larval instars during both study seasons when compared to *A. indica* oil and abamectin insecticide, but abundance of *P. citrella* was less than the check. Foliar application of *A. indica* extract also reduced abundance of *P. citrella* because of direct toxicity or repellent action. However, the amount of toxicity was much less than synthetic insecticide or *A. indica* oil and might be due to less concentration of extracts reaching mines. According to Shareef et al. (2016), neem extract at 30% concentration was effective against *P. citrella*. Thus, neem extract if applied at higher concentration at regular intervals could be effective in managing *P. citrella*. Another possibility why neem extract is less toxic against *P. citrella* might be less knockdown action.

According to McKenna et al. (2013), plant extracts need more time to expose their insecticidal efficacy compared to synthetic insecticides having quick knockdown action. However, efficiency might be enhanced by combining mineral oils that help the chemicals penetrate easily into leaf tissues (Bográn et al. 2006).

Our results showed that abamectin and *A. indica* oil had significant effect on all *P. citrella* larval instars in nursery plantations of *C. reticulata*. Unfortunately, we did not study the effects of the insecticides on biological control agents of *P. citrella* because to build an IPM program, the basic component is to check the compatibility of emerging insecticides with natural enemies. However, earlier studies confirmed that abamectin and neem-based insecticides with low acute toxicity to parasitoids were effective in managing *P. citrella* in laboratory and field experiments (Villanueva-Jiménez and Hoy 1998, Villanueva-Jiménez et al. 2000, de Morais et al. 2016). But, the impact of natural insecticides on biological control agents in ecological conditions of Pakistan needs to be studied.

In conclusion, abamectin and *A. indica* oil had an adverse effect on *P. citrella*, and there was no difference in their abilities to suppress all larval instars. For eco-friendly management of *P. citrella*, *A. indica* oil should be incorporated into an integrated management program that will be cost-effective, lessen resistance to insecticide, and be safe for the environment by minimizing the number of insecticide applications. Further research is needed to determine factors responsible for larval mortality and whether the biopesticide can be used in future citrus IPM programs as a tool for effective management of *P. citrella*.

Acknowledgment

The authors gratefully acknowledge the authorities of Institute of Food Science and Nutrition, University of Sargodha for laboratory facilities, and the Universidad de Colima for supporting the publication.

References Cited

Abou-Fakhr Hammad, E. M., and N. M. Antar. 2003. Efficacy of selected insecticides on *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in navel orange (*Citrus sinensis* L.). Tests Agrochem. Cult. 24: 6-7.

Adnan, S. M., M. M. Uddin, M. J. Alam, M. S. Islam, M. A. Kashem, M. Y. Rafii, and M. A. Latif. 2014. Management of mango hopper, *Idioscopus clypealis*, using chemical insecticides and neem oil. Sci. World J. 2014: 1-5.

Ali, H., S. Ahmad, G. Hassan, A. Amin, and M. Naeem. 2011. Efficacy of different botanicals against red pumpkin beetle (*Aulacophora foveicollis*) in bitter gourd (*Momordica charantia* L.). Pak. J. Weed Sci. Res.17: 65-71.

Amiri-Besheli, B. 2008. Efficacy of *Bacillus thuringiensis*, mineral oil, insecticidal emulsion and insecticidal gel against *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). Plant Prot. Sci. 44: 68-73.

Amiri-Besheli, B. 2011. The effect of some botanical pesticides against citrus leafminer (CLM) and two spotted mite (TSM). *In* M. Stoytcheva [ed.], Pesticides in the Modern World - Pesticide Use and Management. InTech.

Bacci, L., A. L. B. Crespo, T. L. Galvan, E. J. G. Pereira, M. C. Picanco, G. A. Silva, and M. Chediak. 2007. Toxicity of insecticides to the sweet-potato whitefly (Hemiptera: Aleyrodidae) and its natural enemies. Pest Manage. Sci. 63: 699-706.

Beattie, A., and S. Hardy. 2004. Citrus Leafminer. Agfacts H2.AE.4, 4th ed. NSW Department of Primary Industries, New South Wales, Australia. https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/137634/citrus-leafminer.pdf

Belasque, J., A. L. Parra-Pedrazzoli, J. Rodrigues-Neto, P. T. Yamamoto, M. C. M. Chagas, J. R. P. Parra, B. T. Vinyard, and J. S. Hartung. 2005. Adult citrus leafminers (*Phyllocnistis citrella*) are not efficient vectors for *Xanthomonas axonopodis* pv. *citri*. Plant Dis. 89: 590-594.

Bográn, C. E., S. Ludwig, and B. Metz. 2006. Using oils as pesticides. Texas A&M University System; AgriLife Extension. E-419 11/06. http://insects.tamu.edu/extension/publications/epubs/e-419.cfm

Boulahbel, B., M. Aribi, S. Kilani-Morakchi, and N. Soltani. 2015. Activity of neem oil in *Drosophila melanogaster*: toxicity and delayed effect on the progeny. J. Entomol. Zool. Stud. 3: 306-310.

Charleston, D. S., R. Kfir, L. E. M. Vet, and M. Dicke. 2005. Behavior response of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) to extract derived from *Melia azedarach* and *Azadirachta indica*. Bull. Entomol. Res. 95: 457-465.

Chaudhary, S., R. K. Kanwar, A. Sehgal, D. M. Cahill, C. J. Barrow, R. Sehgal, and J. R. Kanwar. 2017. Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. Front. Plant Sci. 8: 610.

Clark, J. M., J. G. Scott, F. Campos, and J. R. Bloomquist. 1995. Resistance to avermectins: extent, mechanisms, and management implications. Annu. Rev. Entomol. 40: 1-30.

Croft, B. A. 1990. Arthropod Biological Control Agents and Pesticides. Wiley, New York.

Damavandian, M. R., and S. F. K. Moosavi. 2014. Comparison of mineral spray oil, confidor, dursban, and abamectin used for the control of *Phyllocnistis citrella* (Lepidoptera: Gracillaridae), and an evaluation of the activity of this pest in citrus orchards in northern Iran. J. Plant Prot. Res. 54: 156-163.

deMorais, M. R., O. Z. Zanardi, G. R. Rugno, and P. T. Yamamoto. 2016. Impact of five insecticides used to control citrus pests on the parasitoid *Ageniaspis citricola* Longvinovskaya (Hymenoptera: Encyrtidae). Ecotoxicology 25: 1011-1020.

Dutcher, J. D. 2007. A review of resurgence and replacement causing pest outbreaks in IPM, pp. 27-43. *In* A. Ciancio and K. G. Mukerji [eds.], General Concepts in Integrated Pest and Disease Management. Springer, Amsterdam, Netherlands.

El-Afify, A. H., R. M. Shreel, N. M. Ghanim, and M. A. Hendawy. 2018. Seasonal activity of the citrus leafminer, *Phyllocnistis citrella* Stantion in navel orange orchards during autumn season. IOSR J. Agric. Vet. Sci. 11: 16-21.

Garcia-Mari, F., C. Granda, S. Zaragoza, and M. Agusti. 2002. Impact of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) on leaf area development and yield of mature citrus trees in the Mediterranean area. J. Econ. Entomol. 95: 966-974.

Grafton-Cardwell, E., and G. Montez. 2009. Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). Citrus Entomology, University of California (26 April 2013). http://citrusent.uckac.edu/leafminer.htm

Guedes, R. N. C., and G. C. Cutler. 2014. Insecticide-induced hormesis and arthropod pest management. Pest Manag. Sci. 70: 690-697.

Guedes, R. N. C, G. Smagghe, J. D. Stark, and N. Desneux. 2016. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu. Rev. Entomol. 61: 43-62.

Isman, M., O. Koul, J. Arnason, J. Stewart, and G. Salloum. 1991. Developing a neem-based insecticide for Canada. Mem. Entomol. Soc. Can.123: 39-46.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51: 45-66.

Italo, C. G., H. F. Amanda, and L. R. Diego. 2009. Physical and chemical characterization of *Melia azedarach* L. fruit and leaf for use as botanical insecticide. Chilean J. Agric. Res. 69: 38-45.

Mordue Luntz, A. J., M. S. J. Simmonds, S. V. Ley, W. M. Blaney, W. Mordue, M. Nasiruddin, and A. J. Nisbet. 1998. Actions of azadirachtin, a plant allelochemical, against insects. Pest Manag. Sci. 54: 277-284.

Kerns, D., G. Wright, and J. Loghry. 2001. Citrus leafminer (*Phyllocnistis citrella*). *In:* Citrus Arthropod Pest Management in Arizona. University of Arizona, College of Agriculture, Cooperative Extension, Tucson, AZ. http://ag.arizona.edu/crop/citrus/insects/leafminer.pdf

Knapp, J. L. 1996. Florida Citrus Pest Management Guide. SP-43. Florida Cooperative Extension Service, IFAS, University of Florida, Gainesville, FL.

Koul, O., M. B. Isman, and C. Ketkar. 1990. Properties and uses of neem, *Azadirachta indica*. Can. J. Bot. 68: 1-11.

Koul, O., G. Singh, R. Singh, J. Singh, W. Daniewski, and S. Berlozecki. 2004. Bioefficacy and mode-of-action of some limonoids of salannin group from *Azadirachta indica* A. Juss and their role in a multicomponent system against lepidopteran larvae. J. Biosci. 29: 409-416.

Kraiss, H., and E. M. Cullen. 2008. Insect growth regulator effects of azadirachtin and neem oil on survivorship, development and fecundity of *Aphis glycines* (Homoptera: Aphididae) and its predator, *Harmonia axyridis* (Coleoptera: Coccinellidae). Pest Manag. Sci. 64: 660-668.

Kumral, N. A., S. Cobanoglu, and C. Yalcin. 2010. Acaricidal, repellent and oviposition deterrent activities of *Datura stramonium* L. against adult *Tetranychus urticae* (Koch). J. Pest Sci. 83: 173-180.

Lasota, J. A., and R. A. Dybas. 1991. Avermectins, a novel class of compounds: implications for use in arthropod pest control. Annu. Rev. Entomol. 36: 91-117.

Liu, Z., G. Beatie, M. Hodgkinson, and L. Jiang. 2001. Influence of petroleum derived spray oil aromaticity, equivalent n-paraffin carbon number and emulsifiers concentration on oviposition of citrus leafminer *Phyllocnistis citrella* Stainton. J. Aust. Entomol. Soc. 40: 193-196.

Mafi, S. A., and N. Ohbayashi. 2006. Toxicity of insecticides to the citrus leafminer, *Phyllocnistis citrella*, and its parasitoids, *Chrysocharis pentheus* and *Sympiesis striatipes* (Hymenoptera: Eulophidae). Appl. Entomol. Zool. 41: 33-39.

McKenna, M. M., E. M. Abou-Fakhr Hammad, and M. T. Farran. 2013. Effect of *Melia azedarach* (Sapindales: Meliaceae) fruit extracts on citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). SpringerPlus 2: 144.

Metcalf, R. L., and W. H. Luckmann. 1994. Introduction to Insect Pest Management. 3rd ed., Wiley, New York.

Morse, J. G., T. S. Bellows Jr., L. K. Gaston, and Y. Iwata. 1987. Residual toxicity of acaricides to three beneficial species on California citrus. J. Econ. Entomol. 80: 953-960.
Mujica, N., M. Pravatiner, and F. Cisneros. 2000. Effectiveness of abamectin and plant-oil mixtures on eggs and larvae of the leafminer fly, *Liriomyza huidobrensis* Blanchard. CIP Program Report 1999-2000: 161-166.
Naniwadekar, M. Y., and A. S. Jadhav. 2012. Process development of pesticide production from *Azadirachta indica* A. Juss. Int. J. Agric. Innov. Res.1: 65-70.
Nboyine, J. A., M. Abudulai, and D. Y. Opera-Atakora. 2013. Field efficacy of neem (*Azadirachta indica* A. Juss) based biopesticides for the management of insect pest of cotton in Northern Ghana. JEBAS 1: 321-327.
Pandao, S. K., K. R. Mahajan, S. K. Aherkar, A. Muqueen, and H. S. Thakare. 1992. Efficacy of some herbal products and insecticides against tur pod fly *Melanagromyza obtuse* Mall on Rabi Pigeon pea (*Cajanus cajan* L.) var. C-11. Pestology 15: 8-10.
Patil, S. K. 2013. Evaluation of insecticides against citrus leafminer, *Phyllocnistis citrella* Stainton in acid lime. Pest Manag. Hort. Ecosys. 19: 237-239.
Peña, J. E., R. Duncan, and H. Browning. 1996. Seasonal abundance of the citrus leafminer and its parasitoids in South Florida citrus, pp. 92. *In* M. A. Hoy [ed.], International Meeting: Managing the Citrus Leafminer, 22-25 April 1996, Orlando, Florida. University of Florida, Gainesville, FL.
Powell, C. A., M. S. Burton, R. Pelosi, M. A. Ritenour, and R. C. Bullock. 2007. Seasonal abundance and insecticidal control of citrus leafminer in a citrus orchard. HortScience 42: 1636-1638.
Pozo, O. J., J. M. Marin, J. V. Sancho, and F. Hernández. 2003. Determination of abamectin and azadirachtin residues in orange samples by liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A 992: 133-140.
Raga, A., M. E. Satol, M. F. Souza, and R. C. Siloto. 2001. Comparison of spray insecticides against citrus leafminer. Arq. Inst. Biol. 68: 77-82.
Rao, A. R., K. R. Reddy, G. S. Reddy, K. Gopal, and M. Madhavi. 2002. Evaluation of insecticides against citrus leafminer on acid lime. Indian J. Citric. 1: 79-82.
Rashid, M., and A. Ahmad. 2013. The effect of neem / *Azadirachta indica*) leaves extract on the ecdysis and mortality of immature stages of common house mosquito *Clex pipiens fatigans.* Biologia 59: 213-219.
Raveendran, N., V. Selvanaryanan, S. Ganesan, and K. Raman. 1998. Bioefficacy of certain botanical formulations against the groundnut leaf miner, *Aproaerema modicella* (Gelechiidae: Lepidoptera). Pestology 22: 26-28.
Saito, T. 2004. Insecticide susceptibility of the leafminer, *Chromatomyia hoticola* (Goureau) (Diptera: Agromyzidae). Appl. Entomol. Zool. 39: 203-208.
Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35: 271-297.
Se´tamou, M., D. Rodriguez, R. Saldana, G. Schwarzlose, D. Palrang, and S. D. Nelson. 2010. Efficacy and uptake of soil-applied imidacloprid in the control of Asian citrus psyllid and a citrus leafminer, two foliar-feeding citrus pests. J. Econ. Entomol. 103: 1711-1719.
Shareef, M. F., A. B. M. Raza, M. Z. Majeed, K. S. Ahmed, W. Raza, and H. F. Hussain. 2016. Effect of botanicals on the infestation of citrus leaf miner, *Phyllocnistis citrella* stainton. J. Entomol. Zool. Stud. 4: 1335-1340.

Shivankar, V. J., C. N. Shyam Singh, and V. N. Dhengle. 2002. Field appraisal of imidacloprid against citrus leaf miner, *Phyllocnistis citrella* Stainton. Indian J. Citric. 1: 167-170.
Stock, D., and P. J. Holloway. 1993. Possible mechanisms for surfactant-induced foliar uptake of agrochemicals. Pest. Sci. 38: 165-177.
Tan, B., and M. Huang. 1996. Managing the citrus leafminer in China, pp. 49-52. *In* M. A. Hoy [ed.], Managing the Citrus Leafminer. Gainesville, FL.
Ukey, S. P., S. V. Saroda, N. R. Naitam, and M. J. Patil. 1999. Evaluation of plant products in comparison with conventional insecticides against the pests of cowpea. Pestology 23: 23-25.
Villanueva-Jiménez, J. A., and M. A. Hoy. 1998. Toxicity of pesticides to the citrus leafminer and its parasitoid *Ageniaspis citricola* evaluated to assess their suitability for an IPM program in citrus nurseries. BioControl 43: 357-388.
Villanueva-Jiménez, J. A., M. A. Hoy, and F. S. Davies. 2000. Field evaluation of integrated pest management-compatible pesticides for the citrus leafminer *Phyllocnistis citrella* and its parasitoid *Ageniaspis citrocola*. J. Econ. Entomol. 93: 357-367.
Wang, Q., J. Cheng, Z. M. Liu, S. G. Wu, X. P. Zhao, and C. X. Wu. 2005. Influence of insecticides on toxicity and cuticular penetration of abamectin in *Helicoverpa armigera*. Insect Sci. 12: 109-119.