



Research Article

Insecticidal Suppression of Citrus Leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and Asian Citrus Psyllid, *Diaphorina citri* (Homoptera: Liviidae) in Citrus Orchards

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Authors' Contributions

MIU and ABMR presented the study concep. MA and MIU collected the data. MA planned methodology, computation and performed formal analysis. MA and NA wrote the manuscript.

Keywords

Cranionometry, Evolution, Habitat fragmentation, Morphometry

Abstract | Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Homoptera: Liviidae) and citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) are imperative insect pests of citrus orchards due to their relation with bacterial diseases. The objective of this study was to evaluate the effectiveness of soil-applied insecticides against these insect pests. The results of present study showed that soil application of imidacloprid gave a significant control compared to other insecticides. The lowest population of both ACP adults (1.80/plant) and nymphs (10.4/plant) was observed after exposure to imidacloprid than the rest of the chemicals. Similarly, CLM larval population was also observed lower (1.13/plant) when imidacloprid was applied. The least affected chemical was spirotetramat, as the population of both pests was recorded higher on citrus plants after application of this insecticide compared to others. Our findings indicate that imidacloprid can be considered the best insecticide for managing CLM and ACP population in an integrated approach.

Novelty Statement | The present study will be helpful in the selection of effective insecticide for the management of two major insect pests of citrus orchards.

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Introduction

In the fruit business, citrus is the leading fruit and plays a vital role in the economy of Pakistan. Sargodha is an agricultural region of Pakistan, having an average production of approximately 23% of Pakistan's aggregate citrus (Tahir *et al.*, 2015). Unfortunately, the citrus yield is challenged by a number of insect pests. One of the major insect pests of citrus is citrus leafminer (CLM) *Phyllocnistis citrella*,

Stainton (Lepidoptera: Gracillariidae). Larvae of CLM make serpentine mines on the surface of leaf, causing leaf curl, leaf deformation, necrosis, and finally, drop of leaves. CLM mostly prefers the young leaves and infestation remains higher in nursery plantations (Mustafa *et al.*, 2014). However, it also damages the young flushes of mature trees (Urbaneja *et al.*, 2003). The intensity of citrus canker also increases with a higher infestation of CLM. The plants remain vulnerable for a long duration due to infection of this disease (Achor *et al.*, 1996).

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The second most destructive and significant insect

pest of citrus is Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Homoptera: Liviidae). Notable losses to citrus orchards have been reported due to their role in carrying the particular phloem-limited pathogen of Huanglongbing (HLB) disease (Yang *et al.*, 2006; Hall *et al.*, 2011). Adults and nymphs of ACP can transmit the bacteria belonging to the genus *Candidatus Liberibacter* that cause HLB disease (Pelz-Stelinski *et al.*, 2010). There is no cure for this HLB disease; thus, the management of HLB has been done mainly by spraying synthetic insecticides against its vector (Boina and Bloomquist, 2015). To control the population of ACP and CLM in citrus orchards, farmers mostly rely on synthetic insecticides. Soil application of insecticides has been recommended previously to control the citrus insect pests (Rogers *et al.*, 2008) as it is incredibly effective than foliar spray. The soil application provides prolonged-lasting insecticide activity to control pests for a long duration compared to foliar-applied insecticides (Bindra *et al.*, 1968).

Insecticides are currently a critical constituent of ACP and CLM management. Some systemic neonicotinoid insecticides, thiamethoxam, imidacloprid, and a new insecticide cyantraniliprole are permitted in Florida citrus, but rate restrictions limit their soil applications in young trees (Qureshi *et al.*, 2011).

Keeping in view the great economic importance of citrus canker and HLB disease, CLM and ACP are needed to be controlled by suitable measures (Rogers and Sawyer, 2007). In this study, we evaluated four different soil-applied insecticides against ACP and CLM populations in citrus orchards.

Materials and Methods

The control of ACP and CLM by four different soil-applied insecticides was assessed in a field trial conducted at Chack#100 N13, of District Sargodha, Punjab, Pakistan. Four different insecticides; imidacloprid (12.5ml/L), chlorpyrifos (2.5ml/L) spirotetramat (1.25ml/L and spinetoram (0.4ml/L) were tested. Water was applied in the bases of control plants.

Treatments were applied to 4 years old Kinnow plants (*Citrus reticulata* Blanco). Tree spacing was 3.5m (11.4ft) within rows and 5.5m (18.04ft) between rows. The average temperature and humidity was 29±2 °C and 76±10% respectively in selected locality during study period. Treatments were allocated to a plot consisting of 25 trees (5 rows wide x 5 trees in length) in a randomized complete block design (RCBD) and replicated five times. Treatments were applied as soil drench using an applicator to deliver spray solution at the tree base (Rogers and Sawyer, 2007). Five trees were selected for each treatment,

and three shoots were chosen randomly from three sides of each tree for ACP nymph counting. Shoots were collected and examined under a stereomicroscope microscope to count the number of psyllid nymphs. For adult psyllid, three-minute visual counts were made for each shoot. For CLM, three shoots were selected randomly, and numbers of live larvae were recorded per shoot of each tree. Data were recorded in the morning time (9:00-11:00 am) at weekly intervals for two months; 1st week of September to the last week of October.

Data were analyzed by one-way ANOVA using Minitab 16.1 software, and means were compared by LSD test when significant F values ($P \leq 0.05$) were found. Log (x+1) data were transformed before statistical analysis (Rogers and Sawyer, 2007), and untransformed means are shown in Tables 1, 2, 3.

Results

The results revealed that adult ACP counts were significantly lower in all treatments than control plots except in spinetoram on the 12th and 26th of October. Among all treatments, imidacloprid gave significant control of ACP adults having the lowest number compared to control plots. On the last day of the experiment, the mean number of ACP adults was only 1.80±0.34 and 5.73±0.54 in control plots after applying imidacloprid. The second most effective insecticide was spirotetramat, having 2.47±0.13 number of ACP adults on 26th October. The less efficient insecticide was spinetoram, in which the mean number of ACP adults was higher (5.47±0.84) compared to other treatments (Table 1).

Similar results were observed for ACP nymphs, in which all treatments significantly reduced the number of ACP nymphs compared to control plots except spinetoram. There was no significant difference ($P > 0.05$) in the mean number of ACP nymphs in spinetoram and control plots on the 1st and 14th of September. Imidacloprid has significantly reduced the ACP nymphs among all sampling dates compared to other treatments. On 26th October, psyllid nymphs were 10.4±0.38 numbers after applying imidacloprid compared to control plots (31.67±0.98 numbers). A higher number of ACP nymphs (20.07±0.54) was found after the application of spinetoram compared to other treatments on the last day of sampling (Table 2).

In CLM, all the treatments significantly ($P < 0.05$) reduced the population compared to control during all sampling dates. Imidacloprid was also proved best against CLM. The lowest numbers of mines were observed after the application of imidacloprid than other insecticides. After applying imidacloprid, the mean number of mines was only 1.13±0.08 on the last day of observation

Table 1: Number (mean±SE) of Asian citrus psyllid adults after application of insecticides.

Treatments	Post treatment means								
	1-Sept	7-Sept	14-Sept	21-Sept	28-Sept	5-Oct	12-Oct	19-Oct	26-Oct
Imidacloprid	2.13±0.29 ^c	1.80±0.24 ^d	2.40±0.52 ^c	2.40±0.28 ^c	2.20±0.32 ^c	2.07±0.16 ^c	2.07±0.56 ^c	1.67±0.18 ^d	1.80±0.34 ^c
Spinetoram	2.93±0.22 ^c	3.07±0.26 ^d	3.40±0.24 ^c	3.40±0.41 ^c	3.20±0.30 ^c	3.47±0.32 ^{bc}	3.00±0.39 ^c	2.45±0.17 ^d	2.47±0.13 ^{bc}
Chlorpyrifos	5.20±0.51 ^b	4.87±0.38 ^c	5.07±0.62 ^b	5.73±0.71 ^b	4.60±0.46 ^b	4.93±0.28 ^b	4.40±0.33 ^b	3.93±0.32 ^c	3.53±0.35 ^b
Spirotetramat	6.53±0.74 ^b	6.93±0.35 ^b	6.07±0.40 ^b	6.67±0.63 ^b	4.87±0.42 ^b	5.00±0.34 ^b	5.67±0.34 ^a	5.07±0.24 ^b	5.47±0.84 ^a
Control	11.5±0.86 ^a	9.53±1.07 ^a	12.3±0.61 ^a	12.5±0.30 ^a	12.0±0.53 ^a	11.4±1.25 ^a	6.33±0.58 ^a	6.60±0.47 ^a	5.73±0.54 ^a
	F=41.44	F=29.41	F=62.46	F=70.56	F=80.15	F=42.73	F=21.36	F=38.23	F=11.13
	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Means within column followed by same letters are not significantly different using LSD ($P>0.05$). Data were subjected to $\text{Log}_{10}(X+1)$ transformation for statistical analysis. Untransformed means are presented for comparison.

Table 2: Number (mean±SE) of Asian citrus psyllid nymphs after application of insecticides

Treatments	Post treatment means								
	1-Sept	7-Sept	14-Sept	21-Sept	28-Sept	5-Oct	12-Oct	19-Oct	26-Oct
Imidacloprid	11.13±0.51 ^d	11.33±0.55 ^d	11.60±0.69 ^c	12.00±0.56 ^d	12.47±0.61 ^d	12.60±0.55 ^d	12.00±0.38 ^d	11.60±0.26 ^c	10.4±0.38 ^c
Spinetoram	17.27±0.65 ^c	17.80±0.57 ^c	18.33±0.43 ^b	19.07±0.48 ^c	19.53±0.61 ^c	20.60±0.69 ^c	20.13±0.82 ^c	19.80±0.74 ^b	19.00±0.72 ^b
Chlorpyrifos	19.33±1.14 ^{bc}	19.13±1.41 ^{bc}	19.47±1.36 ^b	20.60±0.97 ^{bc}	21.20±0.73 ^{bc}	21.80±0.54 ^{bc}	21.20±0.53 ^{bc}	20.73±0.4 ^b	19.07±0.4 ^b
Spirotetramat	20.33±0.69 ^{ab}	21.07±0.68 ^b	21.60±0.56 ^{ab}	22.47±0.43 ^b	23.40±0.66 ^b	23.87±0.76 ^b	22.87±0.78 ^b	21.93±0.43 ^b	20.07±0.54 ^b
Control	23.00±1.91 ^a	24.40±1.74 ^a	24.20±2.01 ^a	25.60±1.89 ^a	31.73±1.51 ^a	34.07±2.01 ^a	33.33±1.65 ^a	32.80±1.38 ^a	31.67±0.98 ^a
	F=19.61	F=22.28	F=16.82	F=23.59	F=67.69	F=54.84	F=71.59	F=102.4	F=160.7
	P<0.0001	P<0.0001	P<0.0001						

Means within column followed by same letters are not significantly different using LSD ($P>0.05$). Data were subjected to $\text{Log}_{10}(X+1)$ transformation for statistical analysis. Untransformed means are presented for comparison.

Table 3: Number (mean±SE) of citrus leafminer larvae after application of insecticides.

Treatments	Post treatment means								
	1-Sept	7-Sept	14-Sept	21-Sept	28-Sept	5-Oct	12-Oct	19-Oct	26-Oct
Imidacloprid	1.00±0.15 ^c	1.07±0.12 ^d	1.20±0.08 ^d	1.40±0.12 ^d	1.60±0.12 ^d	1.60±0.12 ^d	1.47±0.13 ^d	1.33±0.11 ^d	1.13±0.08 ^d
Spinetoram	1.67±0.24 ^{bc}	1.87±0.25 ^c	2.07±0.12 ^c	2.40±0.22 ^c	2.87±0.17 ^c	2.93±0.19 ^c	2.67±0.11 ^c	2.47±0.13 ^c	2.27±0.12 ^c
Chlorpyrifos	2.20±0.22 ^b	2.53±0.13 ^b	2.67±0.21 ^b	3.20±0.25 ^b	3.60±0.16 ^b	3.73±0.22 ^b	3.47±0.13 ^b	3.33±0.15 ^b	3.07±0.26 ^b
Spirotetramat	2.27±0.36 ^b	2.73±0.25 ^b	2.93±0.19 ^b	3.33±0.18 ^b	3.73±0.16 ^b	3.93±0.16 ^b	3.60±0.19 ^b	3.33±0.18 ^b	3.07±0.29 ^b
Control	3.13±0.17 ^a	3.40±0.19 ^a	3.60±0.12 ^a	4.07±0.19 ^a	4.67±0.34 ^a	4.93±0.31 ^a	4.80±0.25 ^a	4.53±0.13 ^a	4.07±0.19 ^a
	F=10.05	F=17.21	F=30.09	F=25.29	F=28.52	F=32.67	F=42.09	F=61.85	F=27.35
	P=0.0003	P<0.0001							

Means within column followed by same letters are not significantly different using LSD ($P>0.05$). Data were subjected to $\text{Log}_{10}(X+1)$ transformation for statistical analysis. Untransformed means are presented for comparison.

compared to control plots (4.07±0.19). Spirotetramat was the second most effective insecticide for controlling CLM, and the least effective insecticides were chlorpyrifos and spinetoram (Table 3).

Discussion

This study determines the potential for long-term insect control in the citrus orchard with soil application of insecticides. The results demonstrate that all soil-applied insecticides do not perform equally in terms of CLM and ACP control. Imidacloprid provided the best protection against tested insects due to the rapid

effect as for the other treatments. Significant control was observed with imidacloprid providing quick initial control of ACP and CLM population during September and October (Rogers *et al.*, 2008) when the population of these insects remains higher in the Sargodha region. The efficiency of imidacloprid may be due to the movement of insecticide from the roots to the upper portion of the citrus plants or low absolute toxicity (Timmeren *et al.*, 2007, 2012). Imidacloprid proved the best insecticide as compared to others, and results are also confirmed by the findings of Shivankar *et al.* (2000), Qasim and Hussain (2015), and Sétamou *et al.* (2010). However, the efficacy of imidacloprid followed by spirotetramet varied with

spinetoram providing no significant control for the ACP and CLM population. Ichinose *et al.* (2012) and Tiwari and Stelinski (2013) confirmed the effectiveness of imidacloprid with the rapid killing of psyllid adults and nymphs. However, Stansly and Rogers (2006) recommend imidacloprid as a foliar application in the mature tree for long-term management of the ACP population when the population reached its peak position.

The study was conducted during September and October because CLM and ACP populations remain higher during these months in the Sargodha region. CLM's highest peak was observed by Pandey and Pandey (1964) and Batra and Sandhu (1981). They concluded that CLM infestation started and peaked during September due to the availability of new flushes and a decrease in temperature, and an increase in humidity level. On the other hand, ACP was also observed at a peak during September by Vetter *et al.* (2013) and Tahir *et al.* (2015). According to Qureshi *et al.* (2014), soil-applied systemic insecticides can be integrated to reduce the ACP population, but the additional spray could be based on pest scouting. However, there is a need to determine the non-target effect of these insecticides on beneficial insects. Insecticide application manages ACP effectively, resulting in Huanglongbing (HLB) disease reduction (Gatineau *et al.*, 2010; Ichinose *et al.*, 2010). According to Boina and Bloomquist (2015), imidacloprid significantly reduces the spread and incidence of HLB. According to Boina *et al.* (2009), imidacloprid has a more prolonged residual activity with ACP suppression at the nursery level. Similarly, Setamou *et al.* (2010) also confirmed that soil-applied imidacloprid gave long-term suppression of ACP and CLM population.

In this study, soil application of imidacloprid provided the most significant reduction of CLM and ACP population. Foliar application of insecticides should be used with soil-applied imidacloprid to aid in preventing resistance development. Further, there is a need to determine the compatibility of insecticides with other control measures like biological control for integrated management of citrus insect pests.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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