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**Muhammad Faisal Shareef**  
Department of Entomology,  
University College of  
Agriculture, University of  
Sargodha, Punjab, Pakistan.

**Abu Bakar Muhammad Raza**  
Department of Entomology,  
University College of  
Agriculture, University of  
Sargodha, Punjab, Pakistan.

**Muhammad Zeeshan Majeed**  
Department of Entomology,  
University College of  
Agriculture, University of  
Sargodha, Punjab, Pakistan.

**Kanwer Shahzad Ahmed**  
Department of Entomology,  
University College of  
Agriculture, University of  
Sargodha, Punjab, Pakistan.

**Waqas Raza**  
Department of Plant Pathology,  
University College of  
Agriculture, University of  
Sargodha, Punjab, Pakistan.

**Hafiz Faqir Hussain**  
Department of Entomology,  
University of Agriculture,  
Faisalabad, Punjab, Pakistan.

#### Correspondence

**Kanwer Shahzad Ahmed**  
Department of Entomology,  
University College of  
Agriculture, University of  
Sargodha, Punjab, Pakistan.

## Effect of botanicals on the infestation of citrus leaf miner, *Phyllocnistis citrella* stainton

**Muhammad Faisal Shareef, Abu Bakar Muhammad Raza, Muhammad Zeeshan Majeed, Kanwer Shahzad Ahmed, Waqas Raza and Hafiz Faqir Hussain**

#### Abstract

Citrus leafminer, *Phyllocnistis citrella* Stainton (Gracillariidae: Lepidoptera), is a serious and primary pest of citrus and causes extensive damage to new leaf flush in citrus nurseries and orchards. Control of this pest mostly relies on synthetic insecticides and there are no known alternate effective control measures. In this study, effect of different plant extracts was evaluated on the population infestation of citrus leafminer. In this regard, four plants extracts were used viz; neem (*Azadirachta indica* A. Juss), *datura* (*Datura stramonium* L.) leaf extracts and lime (*Citrus aurantiifolia* Swingle), kurtuma (*Citrullus colocynthis* L.) peel extracts with 10, 20 and 30 percent concentrations against citrus leafminer infestation on kinnow nursery seedlings. Spray of plant extracts were repeated three times with the interval of 15 days on selected plants. Experiment was laid out in randomized complete block design (RCBD) with three replications. Percent population infestation of citrus leafminer was recorded before the application and after 24, 48, and 72 hrs of spray. Results revealed that peak infestation of leafminer appeared in end August to early October, and moreover, foliar application of 30% neem and kurtuma leaf extracts gave up to 12% reduction in the population infestation of citrus leafminer at 72 hrs post application. Therefore, neem extract is recommended to manage and control citrus leafminer infestation on citrus nursery seedlings.

**Keywords:** Botanicals, *Phyllocnistis citrella*, nursery, young sprouts, plant extracts, neem, kurtuma, citrus leafminer

#### 1. Introduction

Citrus fruits, particularly mandarins, sweet oranges and grapefruits, are important crops in the world. It ranks first in area wise and production wise among the entire fruits tree in world. Citrus is the vital fruit crop in Pakistan both in the area wise and production wise. The area under cultivation of citrus fruit is (199.9 thousand hectares) and fruit production is 2.132 million metric ton (MMT) [1]. Today, Pakistan is among the top ten citrus growing countries of the world. Within Rutaceae family of citrus genus, kinnow (Blanco) is the largest planted species. About 94 percent of the citrus area in Pakistan is located in the Punjab province [2]. Hence, it is the biggest producer of kinnow in the world.

However, Pakistan is far behind regarding per unit area production of citrus as compared to other citrus production countries. One of the reasons for this lower production is the attack of citrus crop by many diseases and insect pests that are a severe threat to citrus industry. One of the major insect pests of citrus is citrus leafminer (CLM).

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was originated from Eastern and Southern parts of Asia. After 1993, it was rapidly spread to all citrus growing areas of America and also in the Mediterranean basin. Citrus leafminers are one of the major pests that have very deleterious effects on Citrus and related species worldwide [3]. According to Ujiye [4], citrus leafminer is a destructive pest of citrus crops. Its adults are silvery white tiny moths which lay eggs in dawn or dusk time on young foliage of citrus plants. Larvae upon hatching enter among the epidermis and start feeding within leaf lamina without any delay. At early stages, insect mines under the surface of leaves, but in the case of heavy infestation mines are made on both surfaces of leaves. Generally one larva makes one mine but in the case of heavy infestation two to three mines may be formed per leaf by the same larva. The larvae of Citrus leaf miner have four instars and development of the larvae takes 5 to 20 days. Pupation of *P. citrella* occurs in a particular type of pupal cell which is formed by larva at the leaf edge within the mine under a simple leaf curve.

Total life cycle of the pest is about 3 weeks in summer and may prolong to 2 months in winter season.

Leaf miner's infestation has been a constant threat to citrus industry all over the world. Particularly young citrus nurseries and spring sprouting are highly prone to be attacked by citrus leafminers. A wide range of insecticides and botanical pesticides are being evaluated against these deleterious insect pests. For instance, Bhatia and Joshi [5] evaluated dimethoate, monocrotophos, phosphamidon, fenvalerate, parathion-methyl, quinalphos, cypermethrin, (all at 0.05%) and deltamethrin (at 0.0017%) for the management of *P. citrella* on kinnow mandarin nursery in Rajasthan, India. They found deltamethrin, fenvalerate, monocrotophos and quinaphos as the most effective ones against citrus leafminers. Similarly, Johi *et al.* [6] evaluated the toxicity of neem, mahua and pongamia oil (2 and 4%) and seed extract (2%) of neem and pongamia against citrus leafminer on citrus line in Karnataka, India. All the treatments reduced the population of *P. citrella*, but neem seed extract was most effective. Neem oil (1%) against *P. citrella* has been reported effective against citrus leafminers by Singh and Azam [7] and Katole *et al.* [8] on citrus mandarin. Borad *et al.* [9] organized a study during 1997-99 on eight different types of botanical insecticides viz; mint (*Metha piperita*), tulsi (*Ocimum sanctum* L.), arduisi (*Adhatoda vasica* L.), karen (*Nerium indicum* M), naffatia (*I. fistulosa* Mart.), neem (*A. indica*), ami (*Clerodendrum multiflorum* Bur.) and bougainvillea (*Bougainvillea* spp.) against different insect pests of kagzi lime (*Citrus aurantiifolia* S.). Spray of neem (*A. indica*) and (*I. fistulosa*) leaves extract of 10% suspension solution successfully managed the population of citrus leafminers and citrus psylla (*Diaphorina citri*).

Nevertheless, it is difficult to control citrus leafminer infestation due to its obscured nature of feeding within tunnels. Consequently, pesticides are often overdosed or applied more frequently with poor or unsatisfied control of pests and pronounced phytotoxic effects. Therefore, keeping in view this context, present study was aimed to evaluate the effect of different plant extracts against citrus leafminer infestation.

## 2. Materials and methods

This study was designed at citrus (kinnow mandarin) nursery of University College of Agriculture, University of Sargodha (32° 7' 48" N and 72° 41' 8" E) from August to October 2013. Plants were collected from the surroundings of farm area of the University of Sargodha and were brought to Entomology laboratory of the University College of Agriculture for extraction. Leaf extracts of neem (*Azadirachta indica* A. Juss) and datura (*Datura stramonium* L.) and peel extracts of lime (*Citrus aurantiifolia* Swingle) and kortuma (*Citrullus colocynthis* L) were prepared using ethyl alcohol as extraction solvent using an orbital shaker set at 150 rpm at ambient room

temperature for three days and then was filter through Whatman No.1 filter paper. Starting material taken for each extraction was 250 g powdered plant material in 250 ml ethyl alcohol with 1:1 ratio. Three concentrations i.e. 10, 20 and 30% were used with three replications for each treatment. The extracts were stored at 4 °C in refrigerator in dark colored airtight vials until downstream application in experiments.

### 2.1 Leafminer infestation determination

For assessing *P. citrella* infestation (damage), total number of damaged leaves were counted and divided with total number of leaves on a citrus seedling and multiplied by hundred. In this way percent damage was calculated. In the same way, leaves on one branch were counted and it was multiplied by total number of branches on the plant to calculate total number of leaves on the seedling and percent damaged leaves as follows [10];

$$\text{Infested leaves (\%)} = \frac{\text{damaged leaves}}{\text{total leaves}} \times 100$$

### 2.2 Field application of plant extracts

Plant extracts were applied through hand sprayer as foliar spray after making three concentrations i.e. 10, 20 and 30% in tap water. Only water was sprayed in control plots. Experiment was designed under randomized complete block design (RCBD) with three replications for each treatment with the intervals of 15 days in moon soon sprouting season.

### 2.3 Observations and statistical analysis

The data of percent population infestation of citrus leafminer was recorded through the percent infested leaves of citrus on the whole plant before the application of spray and 24, 48 and 72 hrs post spray. Statistical software M.Stat-C V 8.1 was used for data exploitation. One way analysis of variance (ANOVA) was used to determine statistical significance of treatments followed by Fischer's least significance difference (LSD) test to differentiate among treatment means. Values at 95% confidence interval were considered statistically significant.

## 3. Results

This study encompassed in-situ evaluation of different plant-derived chemicals against leafminer infestation on citrus nursery seedlings. The experiment was laid out under RCBD in citrus nursery. The leafminer percent population infestation was recorded before and 24, 48 and 72 hrs post treatment.

On nursery plants, percent population infestation was recorded with the intervals of 10 days during this study. Maximum infestation was recorded in the end of August and during September. Maximum leafminer percent infestation was recorded in the end of August which was 44.5%, and minimum was in end of October i.e. 28.5% as shown in Figure 1.

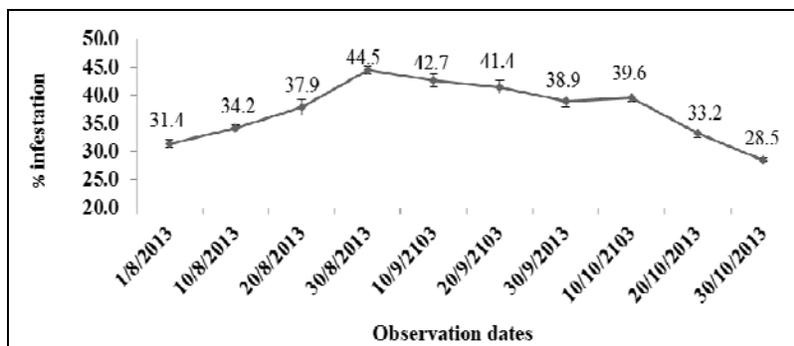


Fig 1: Percent population infestation of citrus leafminer on kinnow mandarin seedlings during monsoon sprouting season

Regarding effect of plant extracts on leafminer infestation, it was found that before the application of 1<sup>st</sup> spray the percent population infestation was about 46.14% in T0 plots which showed the highest population infestation, which was not significantly differs from all other treatments because other plants also contain similar population infestation. The plants contain T9 (42.98%), T3 (42.8%) and T12 (41.95%) infestation were closely similar to each other. The plant T2 (40.1%) and T8 (40.06%) were also similar to each other. T4 (31.87) gave minimum infestation as shown in Figure 2.

After 24 hrs of 1<sup>st</sup> spray, means of each treatment was statistically similar to each other, but after 48 and 72 hrs, there was an apparent reduction in the infestation of citrus leafminer (Figure 3). Neem extract was most effective, although kortuma and datura were also effective. After 48 hrs of spray, 30% neem extract gave the highest reduction (7.63%) in the population infestation of citrus leafminer, while 20% neem extract also reduced the population infestation up to 4.82%. Lime extract showed no reduction in the population infestation of citrus leafminer (Figure 3).

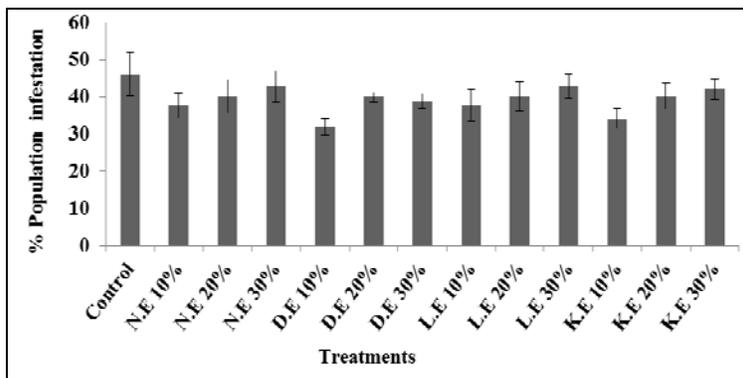


Fig 2: Percent population infestation of citrus leafminer on kinnow mandarin seedlings before 1<sup>st</sup> spray of plant extracts

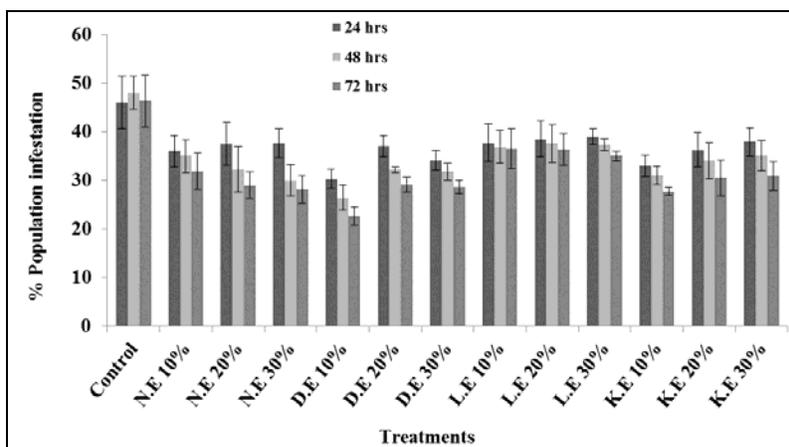


Fig 3: Percent population infestation of citrus leafminer on kinnow mandarin seedlings at 24, 48 and 72 hrs after 1<sup>st</sup> spray of plant extract

Percent population infestation before the application of 2<sup>nd</sup> spray showed that plot T0 contained (43.1%) as the highest population infestation before treatment application and it was significantly different from T10 (27.7%) which showed

minimum population infestation. The infestation in other treatment plots were the intermediate between these two values (Figure 4).

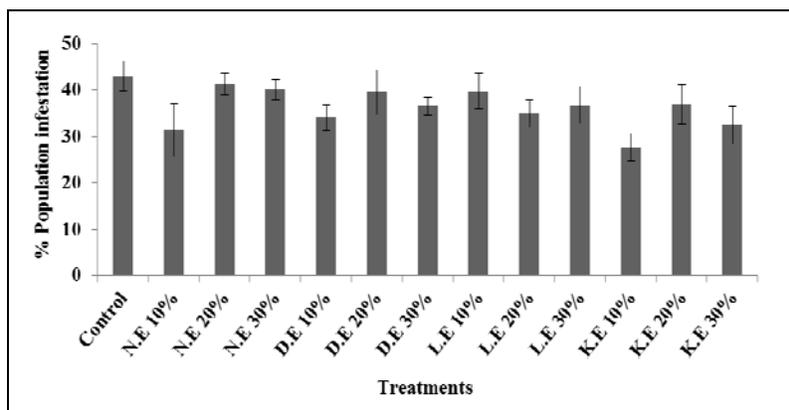


Fig 4: Percent population infestation of citrus leafminer on kinnow mandarin seedlings before 2<sup>nd</sup> spray of plant extracts

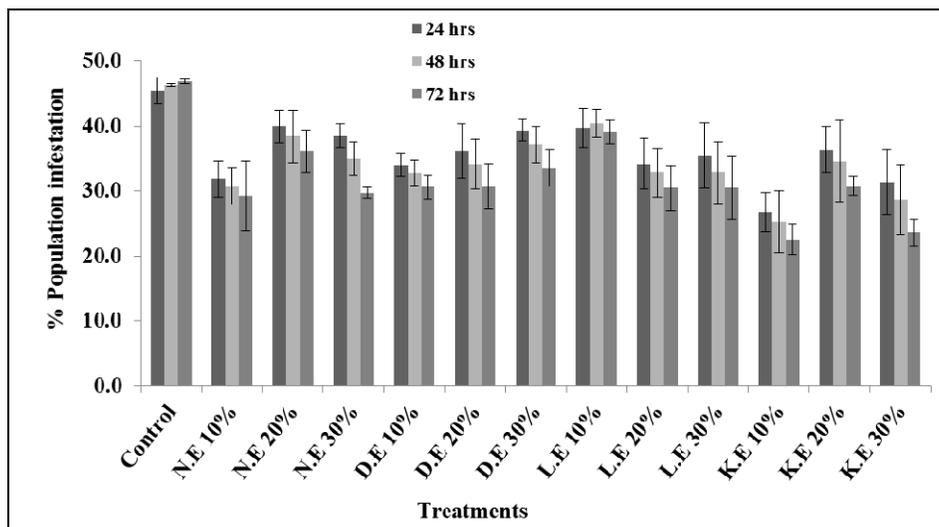


Fig 5: Percent population infestation of citrus leafminer on kinnow mandarin seedlings at 24, 48 and 72 hrs after 2<sup>nd</sup> spray of plant extracts

Twenty-four hours after 2<sup>nd</sup> spray, data showed that neem extract with 30% concentration gave the maximum reduction in infestation of (i.e. 1.6%) and treatment T2 (20% neem extract) gave 1.3% reduction in the infestation but other treatments did not show any reduction (Figure 5). After 48 hrs, treatment T3 (30% neem) gave maximum reduction (3.6%) in the population of citrus leafminer, while treatment T12 (30% kurtuma extract) showed infestation of about 28.7% and 30% lime extract (T9) gave 32.9% with a reduction in leafminer population infestation of about 2.7 and 2.6%, respectively. After 72 hrs, data showed that T3 (29.73%) gave maximum reduction (5.27%) in the population infestation of citrus leafminer population, while T12 (23.63%) showed 5.1% reduction in the population. T6 (33.5%) and T8 (30.5%) also showed good reduction of (i.e. 3.6% and 2.4%) in leafminer population infestation (Figure 5).

Before the application of 3<sup>rd</sup> spray of plant extracts, population infestation of citrus leafminer was highest in T1 (42.03%) and minimum in T11 (22.15%), as shown in Figure 6. Plants in treatment T3 and T2 had 36.6 and 33.17% which were less than T1 infestation. T6 (32.58%), T10 (31.3%), T7 (31.1%) and T0 (30.3) showed relatively similar population infestation

before the application of 3<sup>rd</sup> spray (Figure 6). After 24 hrs of botanicals spray, there was a reduction in the population infestation of leafminer (Figure 7). T3 (34.19%) gave maximum reduction of (2.41%) in the population of citrus leafminer, while T10 (29.5%), T12 (21.9%), T2 (32.1%) and T6 (31.5%) also showed reduction by about 1.8, 1.3, 1.07 and 1.08%, respectively, which all significantly differed from the T3 infestation. After 48 hrs, T3 (30% neem extract) gave maximum reduction of 3.29% in the population infestation of citrus leafminer, while T2 (20% neem extract) also significantly reduced the population by 2.5% but less than T3. T7, T6 and T5 also showed population reduction by 1.67, 1.54 and 1.52% but statistically these were all similar to each other. After 72 hrs of spray, reduction in the infestation of citrus leafminer was maximum (4.3%) in T3 (26.56%), while T12 (30% kurtuma extract) and T2 gave showed reduction in the population of citrus leafminer (i.e. 3.63 and 3.3%). T5, T10, T11 and T1 showed relatively similar results and reduction in population was about 1.92, 1.84, 1.56 and 1.53%, respectively. T0 (29.46%) did not show any reduction rather a population increase of 1.0% which highly significantly differ from all others treatments.

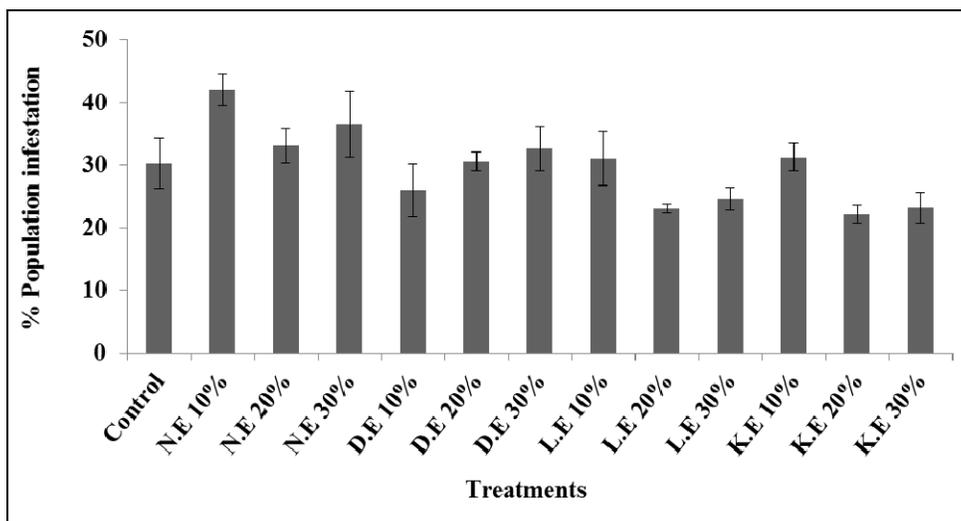


Fig 6: Percent population infestation of citrus leafminer on kinnow mandarin seedlings before 3<sup>rd</sup> spray of plant extracts

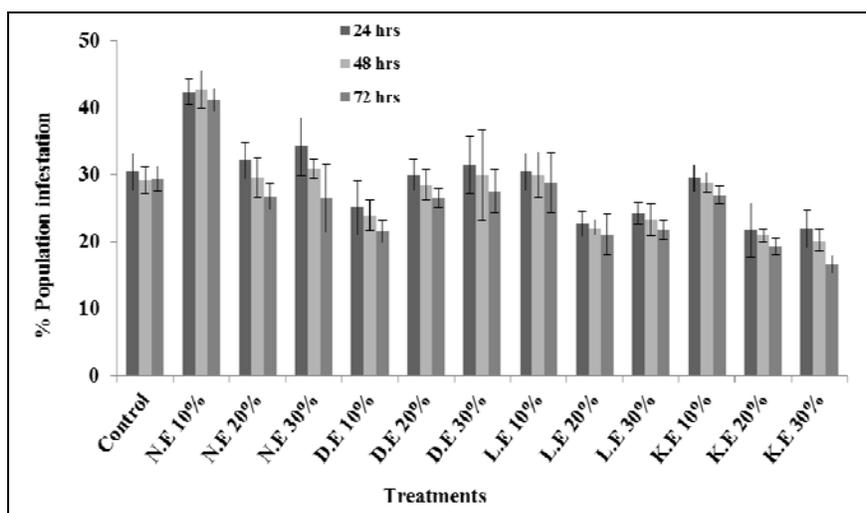


Fig 7: Percent population infestation of citrus leafminer on kinnow mandarin seedlings at 24, 48 and 72 hrs after 3<sup>rd</sup> spray of plant extracts

#### 4. Discussion

Citrus leafminer (*P. citrella*) is one of the devastating pests of citrus crops all over the world. It is particularly destructive to young seedlings in citrus nurseries and sprouting. Therefore, it is dire need to protect new shoots of young or top grafting trees from the damage caused by summer and autumn generation of citrus leafminer. The present study was carried out to evaluate the effect of botanical extracts of plants known previously and bio control agent on the population dynamics/ trend of citrus leaf miner. Four plants extracts i.e. neem, datura, lime and kurtuma were applied against citrus leafminer each with three different concentrations. Spray of plants extracts were repeated three times with the intervals of 15 days from end of August to end of September 2013.

In general, about 25- 48% population infestation of citrus leafminer was recorded in citrus nursery under study during monsoon and spring sprouting season. Density of citrus leafminer infestation reached to the maximum during the spring sprouting in the month of March and April while it reached to maximum as well during the month of August. These finding are supported by observations of Singh and Azam [7] and Legaspi *et al.* [11] worked on the seasonal occurrence and population dynamics of citrus leafminer, *P. citrella*, on different citrus cultivars and reported that population of citrus leafminer were at peak during February-April and July- October. However, Zeb *et al.* [12] assessed population dynamics of citrus leafminer in Northern areas of Pakistan and found that the pest remains active throughout the year on new plant growth and young sprouting with peak populations in second fortnight of September and to first fortnight of October.

Regarding effect of botanical extracts on citrus leafminer infestation, Results reflected that, foliar application of datura and neem leaves extracts with 30% concentration gave up to 12% reduction in the population citrus leaf miner after 72 hrs. These results are in line with those of Borad *et al.* [9], who evaluated a wide range of botanicals against different insect pests and found that that 10% leaf extract of neem (*A indica*) and morning glory (*I. fistulosa*) successfully managed the population of citrus leafminer (*P. citrella*) and citrus psylla (*Diaphorina citri*).

In this study, foliar application of neem leaf extract reduced the population of citrus leafminer by their direct toxicity and/or repellent actions and also there would antifeedant activity of kortuma causing about 6% reduction of leafminer

infestation. However, this reduction in infestation of citrus leafminer by spray of plant extracts seems much less as compared to different synthetic insecticides or biocontrol agents release as determined by Villanueva-Jimenez and Hoy [13], Legaspi *et al.* [11] and Setamou *et al.* [14]. However, if applied timely and with regular intervals during peak infestation levels of pest, plant derived chemicals such as botanical extracts could be effective to control and manage population infestation of citrus leafminer on young seedlings and in citrus nurseries. Nevertheless, neem plant extracts have usually a dual mode of action (insecticidal, replant and antifeedant) against a wide variety of phytophagous insect pests [15]. Therefore based on results of this study, application of a high concentration of neem extract is recommended for checking the infestation of citrus leafminer on citrus nursery seedlings and young sprouts.

#### 5. Conclusion

Farmers mostly rely on synthetic rather than organic insecticides to control citrus leafminer without knowing the ill effects of these chemicals. Therefore, results of present study demonstrated that if timely application of botanical insecticides with regular intervals during peak infestation levels of pest, could be effective to control and manage population infestation of citrus leafminer on young seedlings and in citrus nurseries.

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