



Integrated Management of Citrus Leafminer in the Nursery

Sumaiya Akter ¹, Mohammad Mahir Uddin ¹, Md. Shamsul Alam ², Naznin Nahar ¹, *✉

¹ Department of Entomology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

² Horticulture Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202, Bangladesh

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Correspondence

Naznin Nahar

✉: naznin.nahar@bau.edu.bd



ABSTRACT

Citrus is one of the most important fruit crops of Bangladesh in terms of acreage, production and economic value to growers. However, citrus production is severely compromised by citrus leafminer (CLM) affecting both nursery and orchard. Citrus growers conventionally spray different chemicals to control this insect which negatively impact their environment and health. Therefore, alternative management options to conventional practice was required. To provide effective integrated pest management (IPM) option(s) to manage CLM in the nursery, studies were conducted in a commercial horticultural nursery. First, seasonal infestation of this insect was monitored under grower's management practice. Second, an experimentation was conducted in randomized complete block design with seven treatments each replicated thrice: (i) Sanitation (removal of infested leaves weekly) (ii) Imidacloprid (0.5 ml/L of water) + Sanitation (iii) Detergent mix water (28 gm/L of water) + Sanitation (iv) Spinosad (0.5 ml/L of water) + Sanitation (v) Cypermethrin (1 ml/L of water) + Sanitation (vi) Grower's conventional practice (Chlorpyrifos @1 ml/L of water) and (vii) untreated control. Seasonal infestation of CLM remained over 30% of the three months (June, July and August) monitored. All the implemented treatments reduced percent leaf infestation, number of mines and larvae per twig compared to untreated control. Sanitation alone substantially reduced leaf infestation to 21.50%, number of mines per twig to 13.33 and number of larvae per twig to 5.11. Combining spraying (Imidacloprid, Cypermethrin, Detergent mix water) with sanitation practice could not provide further control. However, combining a biorational insecticide Spinosad provided a better control and further reduced the leaf infestation, number of mines and larvae per twig. Number of a generalist predator, ladybird beetle was reduced in all treatment plots, however, sanitation alone or in combination with Spinosad spraying, both practice, harmed it less. Citrus growers can be recommended to implement only sanitation or in combination with Spinosad spraying.

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1. Introduction

Citrus is a worldwide cultivated major group of fruit crops ranking third position among various fruits grown throughout the world (FAO, 2020). Citrus species are indigenous to South-East Asia, the Malayan Archipelago as well as other tropical and subtropical regions (Gmitter et al., 2007). Citrus production in Bangladesh substantially increased over the years due to its high profitability and consumers demand. Indeed, citrus fruit production increased from 18,712 tons in 1972 to 164,008 tons in 2021, expanding at an average yearly rate of 5.5% (Knoema, 2021). Especially after the COVID pandemic, demand of different citrus markedly increased in Bangladesh (Shakil, 2021).

Citrus production is greatly constrained by the infestation of various insect pests (Nawaz et al., 2019). Among these pests citrus leafminer (CLM) *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) causes a significant loss of citrus fruits (Nawaz et al., 2021). In Bangladesh CLM is

one of the most important constraints of citrus production causing 80% of infestation (Rahman et al., 2005). However, more significant damage by this pest occurs in the nursery as larvae prefer new flushes (Ullah et al., 2019). Leafminers infest nursery throughout the year but infestation becomes severe during rainy season and autumn on emergence of new flushes (Rahman et al., 2005). Adult leafminers lay their eggs on young leaves; after hatching immature larvae enter inside the leaf burrowing between epidermises. While moving and feeding on the epidermis, larvae leave several serpentine mines throughout the leaves. As a result, leaves become twisted, curled, drop out from plants and infested saplings shows reduced growth (Beattie et al., 2004). Planting such saplings decrease canopy development and thus fruit production. CLM also acts as a vector of citrus canker disease (Khair, 2004).

To manage CLM citrus growers conventionally use variety of chemical insecticides both in orchard and nurseries (Hasan et al., 2021). However, chemical control of CLM is

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difficult as larvae stay in between epidermal layers and pupae stay inside the rolled leaf. Thereby it compelled growers to increase the frequency of insecticide application. As a consequence, abundance of natural enemies decreases, environmental and farmers' health hazard occur (Rani et al., 2021). In this regard, Integrated pest management (IPM) might play a crucial role to manage this pest while minimizing environmental hazards.

IPM is a pest management approach that focuses on the long-term prevention or suppression of pest issues using a combination of physical, cultural, mechanical, and biological measures to keep pest population below economic injury level (Nahar, 2020). Sanitation is considered as the most important cornerstone of any good IPM program. It involves removal and destruction of sources of pest infestation, diseases and weeds from the field. Removing infested plant parts greatly reduces infestation in various crops (Morrison et al., 2019). IPM also advocates use of biorational insecticides when needed. It is claimed that these insecticides are less harmful to natural enemies and environment (Haddi et al., 2020). Spinosad is a well-known biorational originated from a bacterium *Saccharopolyspora spinosa* and used in control of different insects including CLM (Guojun et al., 2016). Detergent mix water is also considered as selective insecticide because of its minimal adverse effects on non-target organisms and safety to natural enemies (Cranshaw, 1996). Combining biorational insecticides with sanitation practice might provide better control compared to growers' conventional practice of spraying.

The main aim of this study was to find out best IPM option(s) to manage citrus leafminer in the nursery, and to assess the effect of implemented practices on the abundance of a generalist predator, ladybird beetle. Moreover, seasonal infestation of CLM in the nursery under grower's conventional practice was also monitored.

2. Materials and Methods

2.1. Experimental site

The experiment was conducted in a commercial nursery "Adhunik Nursery and Horticulture Centre", Gouripur, Mymensingh (24°41'22.0" N latitude and 90°28'13.2" E longitude) located under Old Brahmaputra Flood Plain. Grower has divided the nursery into different zones according to fruit saplings grown there. Present study was conducted in the 'lemon zone' of the nursery where local variety of lemon saplings were maintained by grower following standard horticultural practices. Saplings were eight months old during the study. Present study was divided into two experiments: first, determination of seasonal infestation of citrus leaf miner; second, determination of best management option(s) to tackle citrus leaf miner in the nursery.

2.2. Determination of seasonal infestation of citrus leafminer in the nursery

Seasonal infestation of CLM was recorded from saplings under grower's conventional practice of pest management (weekly spraying of chemicals by grower). First, five random spots were selected from the lemon nursery and then three random plants were chosen per spot for the

observation of infestation. Infestation of CLM was monitored by counting number of infested leaves and healthy leaves per plant at 10 days interval for three months (June, July and August) with the availability of new leaf flush. Later, percent leaf infestation was calculated.

2.3. Determination of best management option(s) for citrus leafminer in the nursery

A part of the nursery was sacrificed by the grower for this experiment and it was assured that only researcher's management practice will be maintained throughout the experimental period. Thus, the experiment was laid out in randomized complete block design with seven treatments each replicated thrice. Selected experiment field was divided into three equal blocks each consisting of seven plots. Each plot was 2m x 2m in size consisting six saplings. Two adjacent unit plot and blocks were separated by 1m buffer zone to avoid influence of one treatment to others. Implemented treatments were: **(i)** Sanitation only (weekly removal of infested leaves) **(ii)** Spraying Imidacloprid (Imidagold 20SL @ 0.5 ml/L of water) + Sanitation **(iii)** Spraying Detergent (Wheel washing powder) mix water @ 28 gm/L of water + Sanitation **(iv)** Spraying Spinosad (Libsen 45SC @ 0.5 ml/L of water) + Sanitation **(v)** Spraying Cypermethrin (Limper 10EC @ 1 ml/L of water) + Sanitation **(vi)** Grower's conventional control (spraying Chlorpyrifos: Ashaban 48EC @ 1 ml/L of water), and **(vii)** Untreated control.

Sanitation practice plots were carefully monitored and all infested leaves from saplings were removed, collected in a bag and buried under soil. A leaf was considered infested if there was any larva, pupa and mine present on the leaves. In total, five sprays were given. Spraying was done while monitoring the level of infestation, thereby, first three sprays were given at 7 days interval whereas last two sprays were adjusted at 15 days interval.

2.3.1. Determining percent leaf infestation and counting number of mines and larvae per twig

Number of infested and healthy leaves per plant per plot was counted weekly. Afterwards, per cent leaf infestation over the replicates of each treatment was calculated. Number of mines (tunnel like appearance) were counted visually from a randomly selected twig of each plant per plot. Later, mean number of mines per twig was calculated over the replicates. Moreover, larvae present inside the mine were observed with hand lens and counted. Later, mean number of larvae per twig was calculated over the replicates.

2.3.2. Monitoring of a generalist predator, ladybird beetle per plot

Number of ladybird beetle per plot was monitored by installing a yellow sticky trap in each plot. The traps were installed with bamboo stick roughly 10 cm above the plant canopy. The sticky traps were collected from the field after 15 days of installation, carefully wrapped with polythene paper and brought to the laboratory for counting the ladybird beetles.

2.4. Statistical analysis

The data were analyzed by R version 3.6.3. Normality and homogeneity of the data were tested by Shapiro-Wilkinson

and Levene's test, respectively. Then, a standard analysis of variance was performed. Means of different variables were separated according to Tukey's HSD test.

3. Results and discussion

3.1. Seasonal infestation of citrus leafminer in the nursery

CLM infestation was monitored for three months period (June-August) in a citrus nursery under grower's conventional management practice (spraying only) to understand the seasonal incidence of the infestation. At the beginning of the monitoring in June, leaf infestation by CLM was 32.48% (Figure 1). Afterwards, the infestation slightly decreased over time and again reached the peak on July. Infestation remained over 30% from June to August. This level of infestation severity might be influenced by rain (average 383 mm) and availability of new flush during rainy season. Leafminer larvae prefer to feed on young flushes, thereby, infestation severity becomes high in rainy season with the availability new flush compared to autumn and winter (Nawaz et al., 2019). Influence of rain and new flush emergence of plants on the severity of leafminer infestation has been reported earlier (Chhetry et al., 2012; Prabhudev et al., 2021).

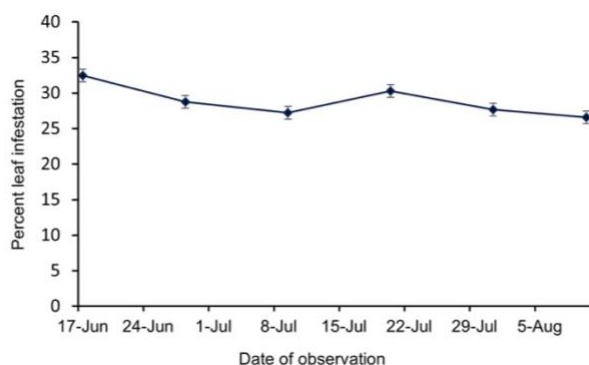


Figure 1. Seasonal infestation of citrus leafminer in the nursery under grower's management. Error bar represents standard errors of the mean.

Interestingly, over three months period leaf infestation caused by CLM was statistically similar ($P>0.05$) of the six observations time. This signifies grower's management practice of spraying could not reduce the infestation in the nursery. Repeated calendar spraying might influence resistant development by the insect against insecticides (Qureshi et al., 2011). Inefficacy of pesticides has been reported earlier in other crops in Bangladesh (Nahar et al., 2020). This type of futile spraying not only increased the cost of production but also hampers the environment including natural enemies (Nahar, 2020), thereby, growers should be encouraged to try out alternatives to chemical spraying.

3.2. Effect of various management options on citrus leaf infestation, mines per twig and larvae per twig

All the implemented practices reduced leafminer infestation substantially ($P<0.05$) compared to untreated

control (Table 1). The highest percentage of leaf infestation (42.09%) was observed in untreated control plots whereas in treated plots infestation ranged from 15.04-25.19%. Sanitation practice alone can reduce the infestation by 21 percentage points compared to untreated control. Sanitation was effective as infested leaves were removed with eggs, larvae or pupae attached with them which reduced the further spread of the infestation. Role of sanitation in reducing leaf infestation in citrus and other crops has been reported earlier (Rathee et al., 2018). One of the big advantages of sanitation practice is that it is safe for environment and natural enemies. However, there remains an obscurity that this practice might be laborious and thus increase cost of production. We argue that this concern is more relevant for managing orchards but for nursery it might be less expensive. In this aspect, a further separate research is required.

Combining either Cypermethrin or Imidacloprid spraying with sanitation could not reduce the leaf infestation over sole sanitation. Combining spraying of detergent mix water with sanitation practice also could not provide any significant control over sanitation. However, combining a biorational insecticide Spinosad substantially reduced (6 percentage points more reduction) the infestation over sanitation and more 27 percentage points over untreated control (Table 1). Efficacy of Spinosad to reduce leaf infestation by CLM has been reported in few other studies (Mane et al. 2016; Nandi et al., 2021). Sanitation alone or with Spinosad spraying both provided better control than grower's conventional spraying (Chlorpyrifos). Although Chlorpyrifos spraying provided some sort of control, however, application of this insecticide should be carefully considered as it is less degradable and highly toxic to environment (Raj and Kumar, 2022).

All the implemented practices also reduced number of mines as well as larvae per twig substantially ($P<0.05$) compared to untreated control (Table 1). The highest number of mines and larvae per twig (28.06 and 11.78 respectively) were observed in untreated control plots whereas in treated plots number of mines per twig ranged from 7.17-19.06 and larvae per twig from 2.44 to 7.50 (Table 1). Sanitation alone can reduce the number of mines per twig by 52% and number of larvae per twig by 56% compared to untreated control. Adding either Cypermethrin or Imidacloprid spraying with sanitation could not further reduce the number of mines and larvae per twig over sole sanitation. Adding spraying of detergent mix water with sanitation practice also could not provide any significant control over sanitation. However, adding a biorational insecticide Spinosad substantially reduced number of mines per twig (46% more reduction) and number of larvae per twig (52% more reduction) over sole sanitation. Sole spraying of Chlorpyrifos also reduced the number of mines and larvae per twig as this is a broad-spectrum and highly toxic insecticide. However, this spraying should be discouraged as it has negative impact on environments and non-target organisms (Walton and Pringle, 2001). Sanitation alone or in combination with Spinosad both practices provided better control than sole Chlorpyrifos application. Practice of sanitation is environmentally benign and Spinosad is claimed to be safer for environment and natural enemies (Kollman, 2002; Ghosh et al, 2010), therefore, both the practices could be safely recommended to growers to follow in the

nursery. Apart from environmental benefit, Spinosad have special mode of action over other insecticides i.e. translaminar access (Eger et al., 1998). As CLM larvae remain under leaf cuticle, thereby, it is better to choose

Spinosad other than any other insecticides to manage CLM.

Table 1. Effect of various management options on percent leaf infestation, number of mines and larvae per twig in the nursery

Treatments	Percent leaf infestation	Number of mines per twig	Number of larvae per twig
Sanitation	21.50 ± 1.71 bc	13.33 ± 1.03 c	5.11 ± 0.25 c
Imidacloprid + Sanitation	24.22 ± 1.65 bc	16.06 ± 0.56 bc	5.56 ± 0.25 bc
Detergent mix water + Sanitation	20.15 ± 1.53 bc	11.33 ± 0.86 cd	4.28 ± 0.21 cd
Spinosad + Sanitation	15.04 ± 1.38 c	7.17 ± 0.75 d	2.44 ± 0.17 d
Cypermethrin + Sanitation	22.33 ± 0.94 bc	16.11 ± 0.83 bc	6.11 ± 0.26 bc
Growers' practice (Chlorpyrifos)	25.19 ± 1.42 b	19.06 ± 0.52 b	7.50 ± 0.34 b
Untreated Control	42.09 ± 2.25 a	28.06 ± 2.28 a	11.78 ± 0.71 a

Values within a column followed by the same letter did not differ significantly ($P>0.05$) according to Tukey's HSD test. Values presented here are the mean ± standard error of the mean.

3.3. Effect of various management options on a generalist predator, Ladybird beetle

Abundance of ladybird beetles was highest (28 beetles per plot) in untreated control plots (Figure 2). All the implemented practices substantially ($P<0.05$) reduced the number of ladybird beetles. However, the least reduction occurred in the plots treated with detergent mix water along with sanitation (12.33 beetles per plot) and Spinosad along with sanitation (11 beetles per plot). In contrast, highest reduction occurred in the plots under grower's conventional (Chlorpyrifos) spraying plots; only 6.67 ladybirds per plot was observed. Chlorpyrifos is a broad-spectrum insecticide and highly toxic to non-target organisms (Sud et al., 2020). Adverse effects of Chlorpyrifos on different natural enemies including ladybirds have been well-documented (Walton and Pringle, 2001; Cloyd, 2012) whereas many studies have reported Spinosad and insecticidal soap as comparatively safer to natural enemies (Williams et al., 2003; Jalali et al., 2009; Jansen et al, 2010).

4. Conclusion

The present study tested several integrated pest management options for CLM in the nursery. Sanitation alone or in combination with biorational insecticide Spinosad spraying appeared to be the best two management options in terms of reducing leaf infestation, number of mines as well as larvae per twig. Grower's conventional spraying (Chlorpyrifos) provided sort of control, however, it drastically reduced number of ladybird beetles compared to all other tested IPM options. To understand the profitability of the IPM options further study on economic analysis is required in future.

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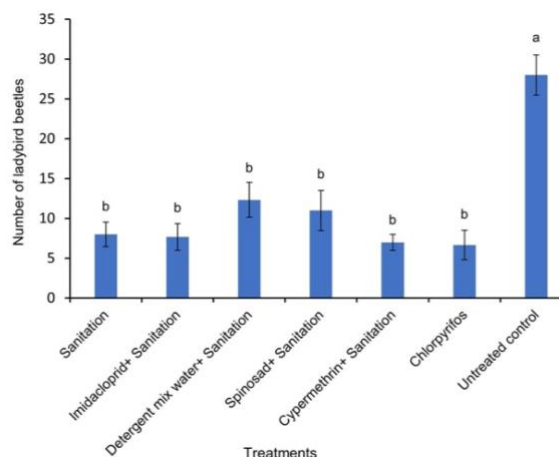


Figure 2. Effect of various management options on number of ladybird beetles. Bars with different letters differ significantly ($p<0.05$) according to Tukey's HSD test. Error bar represents standard errors of the mean.

Conflict of interest

There was no conflict with any financial institution or funding agency that affect the results or the interpretation of the manuscript.

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