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Application of Organic Fertilizers Affect the Citrus Leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) Infestation and Citrus Canker Disease in Nursery Plantations

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ABSTRACT: Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is one of the most important insect pests of Pakistan's citrus nursery stock and caused extensive damage to young flushes. The organic compost is a widespread technique used to manage insect pests and plant diseases. Different composts (biofert, tara root and vermicompost) at 0.5 and 0.25 kg/plant concentration in comparison to NPK fertilizer at 0.4 and 0.2 g/plant were evaluated for CLM infestation and the associated citrus canker disease in nursery plantations of *Citrus reticulata* Blanco. Application of biofert at 0.5 kg/plant reduced the CLM infestation up to 54.5% during Fall-2016 and 39.1% during Summer-2017 in comparison to control treatment. The CLM larval density was also found lower by the application of biofert followed by vermicompost during both seasons. Both concentrations of biofert followed by vermicompost at 0.5 kg/plant resulted in remarkable protection against citrus canker disease in both flushes. The incidence of canker associated with CLM infested leaves was also studied and found lower by the application of biofert and vermicompost compared with control treatment. Conclusively, the soil amendment using biofert and vermicompost affects the CLM population and canker infection in nursery plantations. These organic fertilizers can be used in future citrus IPM programs as a tool to suppress the CLM population and citrus canker disease.

KEYWORDS: *Citrus reticulata*, compost, integrated pest management, *Phyllocnistis citrella*, *Xanthomonas axonopodis*

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Introduction

Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton, (Lepidoptera: Gracillariidae) is one of the most important insect pests of citrus, and mostly attacks on young flushes.¹ It causes direct and indirect damage to citrus crops and is widely distributed in all citrus producing areas of Pakistan.^{2,3} Larvae generate serpentine mines on newly emerged leaves due to which curling, necrosis, and drop of leaves occur.⁴ In citrus nurseries, CLM reduces the normal growth of plants and decreases the canopy development for fruit production.⁵ CLM also exacerbates the citrus canker disease by providing an entry hole for the bacterium, *Xanthomonas axonopodis* pv *citri*.⁶ Canker-infected trees show lesions on the leaves, stem, and fruits, and in severe infestation, defoliation, twig dieback, and fruit drop occur.⁷ To prevent the disease, most of the citrus-growing areas restrain the citrus import from those countries or areas that are well known to have endemic canker.⁸ Previously, it has been reported that CLM may provide the access to the bacteria for infection on the leaf surface and the incidence may increase with the increase in CLM damage.^{6,9} Although it may

enhance the disease severity on citrus leaves, it is not an efficient vector of this disease.¹⁰

Various groups of synthetic insecticides, horticultural mineral oils, and botanicals are being used to manage the CLM population.^{3,4,11} But the effective control of CLM is very difficult because its larva remains under the epidermis layer of the leaves that act as a protective layer. Similarly, the pupae also remain in the rolled margin of leaves.⁴ Furthermore, the excessive use of chemicals is also harmful to the natural enemies and the development of resistance in insects is another concern.¹² For high-yielding production, nutritional management is also an important factor that may affect the response of insect pests and disease due to change in the microclimate of the plants. Organic material increases the plant vigor and also enhances the ability of plants to tolerate against insect attack.¹³

Use of compost such as cattle manure and vermicompost is considered an important component of the bio-organic concept of orange cultivation.¹⁴ Compost increases the soil biodiversity that is very important to maintain soil health.¹⁵ Meyer¹⁶ reported that nutrient availability in the soil not only affects



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the amount of damage caused by insect herbivores but also render the plant diseases. The inclusion of organic materials as a fertilizer could be the alternative to synthetic insecticides for the management of insect pests and diseases.¹⁷ In comparison to synthetic, the organic fertilizers may be more effective to induce plant growth and to increase the tolerance level of plants against insect pests. Current effort to optimize the inputs of organic fertilizer in the citrus nursery plantations and the aspect on plant response to biotic stress like CLM damage and the citrus canker infection could be an important factor in the integrated management program for citrus crops. Our main objective of this study was to evaluate the response of nursery plantation of Kinnow, *Citrus reticulata* Blanco to CLM damage and canker infection after application of organic fertilizer or composts during two seasons.

Materials and Methods

Citrus plants

One-year-old plants of *C. reticulata* were collected from nursery plantations at College of Agriculture, University of Sargodha and transplanted into plastic pots (9 × 5 in). The plants were pruned off for young flushes and maintained with adequate water requirements throughout the experimental period. Twenty to twenty-five days after cutting, the plants were left in the field for natural infestation of CLM. All the plantings were susceptible to CLM and infestation was moderate to severe across the selected place. The plants were kept free from synthetic insecticides during the whole experimental period.

Preparation of vermicompost

Vermicompost was prepared using pit below method suggested by Arancon et al.¹⁸ Earthworms, *Pheretima posthuma*, were collected from the moist soil at water channel nearby University. First, the layer of 2 kg organic waste (dry banana leaves 250 g + cow dung 2 kg) was spread on the polythene sheet. The size of vermibed was 2 m × 1 m × 10 cm. Finally, the 50 g earthworms were released on the bedding material. To avoid the ants and termites, 20 g neem, *Azadirachta indica* A. Juss. leaves were also added and covered with straw to protect them from sunlight. The water was sprinkled regularly to keep the heap moist. The material was left for 20 to 25 days under the soil and then earthworms were separated from the vermicompost.

Soil amendment

The response of citrus plants was evaluated to CLM and canker infection after application of organic fertilizers: vermicompost, biofert, and tara root feed compost in comparison to synthetic fertilizer, NPK. Two concentrations of organic fertilizers (0.5 and 0.25 kg/plant) and two of NPK fertilizer (0.4 and 0.2 g/plant) were tested. The biofert and tara root

composts were purchased from the local market (Adil Sons Market Sargodha). No application of fertilizer was done for the control plants. First application of fertilizer was done during August 2016 by mixing in the soil before transplanting of plants into plastic pots. Second application was done during mid-January 2017 and was applied as a top dressing in the pots. Five plants were selected for each treatment, considering each plant as 1 replication. The infected or damaged leaves were removed before experimentation. Plants were treated with only given treatments while no other application of pesticides or chemical fertilizer was used. The experiment was performed under completely randomized design with five replications.

Percent CLM infestation

Number of CLM infested and total leaves per plant were recorded at weekly interval during August 2016 to May 2017. Percent infestation of CLM was recorded by the formula suggested by Mustafa et al¹⁹:

Percent

$$\text{CLM Infestation} = \frac{\text{Infested leaves per plant}}{\text{Total number of leaves per plant}} \times 100$$

CLM larval density

Number of mines per leaf per plant was recorded to determine the CLM density. The mines containing larvae at any stage, pupae, and empty mines were considered for larval density. The CLM larval density was calculated using the formula²⁰:

CLM

$$\text{larval density} = \frac{\text{Number of mined leaves per plant}}{\text{Total number of leaves per plant}} \times 100$$

Citrus canker-CLM injury

The plants were also evaluated for the presence of citrus canker disease symptoms. In addition, numbers of canker lesions associated with CLM injury or mined-leaves and numbers not associated with CLM injury were counted. The disease incidence per plant was calculated using the formula²¹:

Disease

$$\text{incidence} = \frac{\text{Number of infected leaves per plant}}{\text{Total number of leaves per plant}} \times 100$$

Percentage of citrus canker-infected leaves associated with CLM injury (CC-CLM) was calculated using the formula⁹:

$$\text{Percent CC-CLM leaves} = \frac{\text{Number of infected leaves with CLM damage}}{\text{Total number of CLM damaged leaves}} \times 100$$

while CC-CLM shows the citrus canker-infected leaves associated with CLM damage.

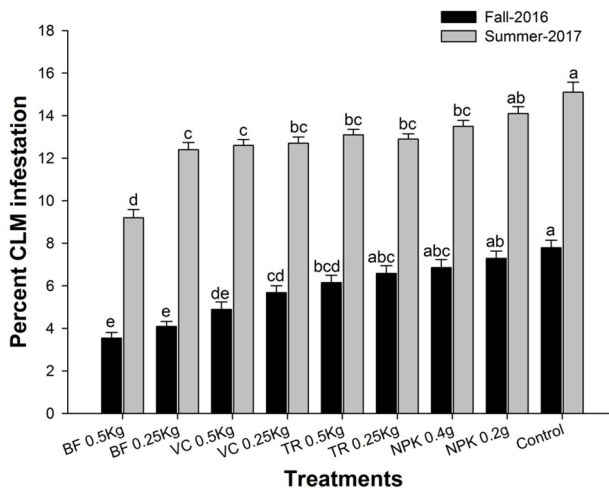


Figure 1. Effect of synthetic and organic fertilizer on percent infestation (means \pm SE) of *Phyllocnistis citrella* per plant during two flushes; means sharing similar letters across treatments for each season are not significantly different at $P > .05$; percent infestation was significantly ($P < .05$) different for each treatment across two seasons. BF indicates biofert; CLM, citrus leafminer; NPK, synthetic fertilizer; TR, tara root; VC, vermicompost. Small letters in superscripts indicate significant differences in different treatment groups during two flush seasons.

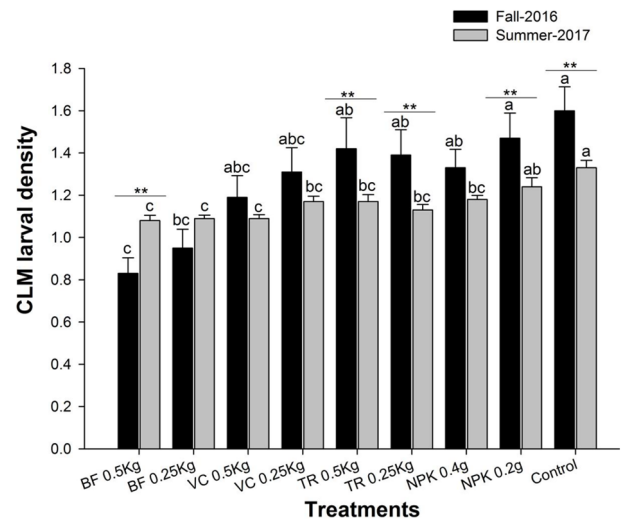


Figure 2. Effect of synthetic and organic fertilizer on larval density (means \pm SE) of *P. citrella* per plant during two flushes; means sharing similar letters across treatments for each season are not significantly different at $P > .05$. BF indicates biofert; CLM, citrus leafminer; NPK, synthetic fertilizer; TR, tara root; VC, vermicompost. The symbol "*" shows the significance ($P < .05$) for treatment across two season.

Data Analysis

Data for CLM infestation and larval density and canker incidence were pooled from August to November considering the Fall-2016 flush and from February to May considering the Summer-2017 flush season. One-way analysis of variance (ANOVA) under completely randomized design was performed to determine the significance of treatments for two seasons. Means were separated using the Tukey honestly significant difference (HSD) all pairwise comparison test at 5% probability level. All the analyses were performed using Minitab 16.1 software.

Results

The results showed that treatments had significant impact on percent infestation of CLM during Fall-2016 ($F = 20.3$, $P < .05$) and Summer-2017 ($F = 24.5$, $P < .05$) flush. Across the season for each treatment, there was also a significant ($P < .05$) difference in CLM infestation. CLM activity remained higher during Summer flush than Fall. Percent CLM infestation was found higher: 7.79% during Fall and 15.1% for Summer flush in untreated or control plants. Biofert had a significant negative impact on the CLM activity during both flushes. The infestation was lower: 3.54% during Fall and 9.2% during Summer flush with the application of biofert compost at 0.5 kg/plant compared with other treatment. Following trend of CLM infestation among the treatments was found: control > NPK > tara root > vermicompost > biofert (Figure 1).

Similar results were found for the CLM larval density per plant with the application of synthetic and organic fertilizers. A significant difference was found in larval density after application of fertilizer during Fall ($F = 5.06$, $P < .05$) and Summer

($F = 8.02$, $P < .05$) flush. However, across the season, a significant ($P < .05$) difference of larval density was found in biofert at 0.5 kg, both concentrations of tara root compost, NPK 0.2 g/plant and control treatment. CLM density was found higher: 1.6% during Fall and 1.33% during Summer on the control plants. The lowest activity of CLM larvae was found 0.83% to 1.09% in biofert treatment during both flushes (Figure 2).

The fertilizers had also significant ($P < .05$) effect on the citrus canker incidence during both flushes. The disease incidence was found higher: 2.03% in Fall and 3.28% in Summer flush on the control plants. Lowest disease incidence was found: 0.30% to 0.82% in biofert and 0.50% to 1.28% in vermicompost treatment during both flushes (Table 1).

Citrus canker on the leaves associated with CLM mines was also significantly ($P < .05$) different among the treatments. In control treatment, 22.7% to 23.4% CLM damaged leaves also showed the symptoms of citrus canker and the percentage was higher than other treatments. However, 3.8% to 7.5% CLM damaged leaves showed the disease symptoms after the application of biofert compost (Table 1).

Discussion

CLM is a major insect pest of the citrus nursery as well as mature orchards and affects the citrus production. The CLM activity remained lower on the plants receiving the organic fertilizer, biofert, and vermicompost compared with synthetic fertilizer. Reduction of insect pests after application of organic fertilizer has been reported earlier.^{22,23} The inorganic N fertilization may reduce plant resistance to insects, by enhancing the dietary quality that helps the plant to produce more succulent and fresh leaves.^{24,25} CLM female prefers the young leaves for egg laying and thus, the nitrogen fertilizer could be helpful to increase its

Table 1. Incidence of citrus canker disease per plant after application of synthetic and organic fertilizers during two flush seasons.

TREATMENTS	DISEASE INCIDENCE		CC-CLM	
	FALL-2016	SUMMER-2017	FALL-2016	SUMMER-2017
Biofert: 0.5 kg	0.30 ± 0.151 ^c	0.38 ± 0.138 ^e	3.80 ± 1.041 ^d	5.39 ± 1.047 ^d
Biofert: 0.25 kg	0.44 ± 0.172 ^{bc}	0.82 ± 0.103 ^{de}	4.31 ± 1.053 ^{cd}	7.46 ± 1.083 ^{cd}
Vermicompost: 0.5 kg	0.50 ± 0.160 ^{bc}	1.15 ± 0.201 ^{cde}	5.20 ± 1.225 ^{cd}	9.53 ± 1.304 ^{bcd}
Vermicompost: 0.25 kg	0.77 ± 0.332 ^{bc}	1.28 ± 0.205 ^{cd}	8.41 ± 2.536 ^{bcd}	11.1 ± 1.934 ^{bcd}
Tara root: 0.5 kg	0.87 ± 0.241 ^{bc}	1.63 ± 0.184 ^{bc}	11.9 ± 2.374 ^{bcd}	12.7 ± 2.489 ^{bcd}
Tara root: 0.25 kg	0.87 ± 0.263 ^{bc}	1.81 ± 0.382 ^{bc}	10.8 ± 2.482 ^{bcd}	13.9 ± 2.482 ^{bc}
NPK: 0.4 kg	1.01 ± 0.206 ^b	2.09 ± 0.174 ^b	12.7 ± 2.483 ^{bcd}	15.8 ± 1.937 ^b
NPK: 0.2 kg	1.10 ± 0.274 ^b	2.21 ± 0.163 ^b	13.9 ± 2.732 ^b	16.1 ± 2.492 ^b
Control	2.03 ± 0.327 ^a	3.28 ± 0.284 ^a	23.4 ± 2.143 ^a	22.8 ± 2.034 ^a
F-value	4.16	9.18	4.02	4.94
P-value	<.05	<.05	<.05	<.05
Df _{8, 179}				

Means sharing similar letters within columns are not significantly different at $P > .05$. CC-CLM shows the percentage of citrus canker disease incidence on leaves containing CLM mines. Small letters in superscripts indicate significant differences in different treatment groups during two flush seasons.

population. Our findings are in accordance with Rao et al²⁶ who also reported that the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), does not prefer the plants treated with organic manure and the maximum attack was noticed on the plants treated with inorganic fertilizers. Biradar et al²⁷ reported that plants showed a tolerance against the CLM after application of organic fertilizers as compared with those treated with inorganic fertilizers. Fertilizer provides the essential nutrients to the plant that are important in growth and development and help plants to resist against the diseases and pests but if applied at the right time and with balance amount. With an excessive amount of nitrogenous fertilizers, the plants show more vegetative growth with new, soft, and succulent leaves that could be helpful for insect attack. According to Schumann et al,²⁸ the amount balance of macro and micronutrients in the citrus could help in reducing the pest pressure. Brown and Tworcoski²⁹ reported that organic fertilizers affect the abundance of arthropod and are safer for natural enemies.

After biofert, vermicompost also showed good results in suppressing the population of CLM. Vermicomposting is a biotechnological process of composting, in which earthworm species are used to increase the waste conversion process and to produce a better product.³⁰ In the last decade, the use of synthetic chemicals was reduced by over 75% where vermicompost was used to manage the insect pests and diseases in global agriculture.³¹ It is due to the production of chitinase enzyme by earthworms which breaks down the chitin in the exoskeleton of insects.³² Previous studies have reported that the addition of

vermicompost to the soil reduces the insect pests such as psyllids, *Heteropsylla cubana* (Homoptera: Psyllidae),³³ ground nut leafminer, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae),³⁴ aphids jassids, and spider mites.^{18,35,36}

In our study, the organic fertilizers also showed remarkable effect on the citrus canker disease. The disease incidence was found lower on the plants received biofert and vermicompost compared with synthetic fertilizer. Our findings about the successful suppression of plant diseases using vermicompost are supported by previous studies.^{37–39} Canker disease incidence on the leaves associated with CLM injury was also determined and the results showed that 20% to 24% leaves containing mines were also canker infected in the control treatment. However, the disease incidence on CLM damaged leaves was lower on plants treated with biofert and vermicompost. The possible explanation is that the compost promotes the rapid healing of wounds created by CLM larvae, so the bacterial pathogen entry or infection reduced. During two flushes, about 39% to 55% and 16% to 37%, CLM damage was controlled using biofert and vermicompost, respectively, at 0.5 kg/plant concentration. Similarly, about 62% to 72% and 15% to 55% canker disease was suppressed using these two composts. This level of control allows citrus growers for the better management of nursery plantations and to transplant healthy and canker-free plants into the field. Organic fertilizers are the best options for reducing the CLM abundance and to get rid of canker disease in citrus nursery plantations. The compost can easily prepare and is cheaper than synthetic fertilizer. So, organic fertilizer can be

important element in an integrated pest management program for the control of CLM and canker disease and to increase the sustainability of citrus production. The use of compost in the Agricultural ecosystems should be incorporated in the sustainable management of insect pests and disease.

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Author Contributions

AHK and MA performed the experimental work and analysis in conducting this research study. MIU contributed in concept and designing of this study and also research supervision. MuR, MA, SK, NM, SA, AMK, SMAZ and MaR contributed equally in literature search, interpretation of results, manuscript drafting, improvement and critical reviewing of drafted manuscript. All authors approved and agreed for the submission of this article for publication.

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