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Efficiency of *Chrysoperla carnea* and *Trichogramma chilonis* Against Infestation of Citrus Leafminer (*Phyllocnistis citrella* Stainton)

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Abstract: Citrus leafminer, *Phyllocnistis citrella* Stainton (Gracillariidae: Lepidoptera), is a major and potential pest of citrus fruits which causes extensive damage to young leaf flush by making shallow tunnels. Its young larvae after emergence from eggs feed in tunnels and later on curling of leaves occur. This study evaluated the efficiency of two bio-control agents i.e. *Chrysoperla carnea* (5, 10 and 15 larvae per plant along with control) and *Trichogramma chilonis* (1, 2 and 3 egg cards along with control and each card contained 200 eggs) on population infestation of citrus leafminer during the spring sprouting season in 2014. Experiment was conducted according to randomized complete block design with three replications. Population infestation of citrus leafminer was recorded 24 h pre- and 24, 48 and 72 h post-treatment of *Chrysoperla carnea* larvae and at 24 h pre- and 3, 6 and 9 days post-treatment of *Trichogramma chilonis* egg cards. Results reflected that *Chrysoperla carnea* and *Trichogramma chilonis* significantly reduced the population infestation of citrus leafminer by 14 and 17%, respectively.

Key words: Bio-control agents · Kinnow · Population infestation · Rutaceae

INTRODUCTION

Citrus fruits belong to family Rutaceae. These fruits, particularly mandarins, oranges and grape fruit, are important fruit crops in the world. These are the most numerous grown fruits in Pakistan. Today, Pakistan is among the top ten citrus growing countries in the world. Within Rutaceae family, Kinnow mandarins are the most widely planted citrus cultivars. It is a very famous citrus plant known for its delicious juicy fruit. Environmental and soil conditions are ideal for kinnow mandarins in Punjab. Citrus is the vital fruit crop in Pakistan both area and production wise [1].

About 95 % of the citrus area, in Pakistan, is located in the Punjab province, because of favorable temperature and environmental conditions [2]. The area under cultivation of citrus fruits is about 199.9 thousand hectares and fruit production is 2.132 million metric ton [3]. That is why it is the biggest producer of Kinnow in the world. Out of total area under citrus cultivation, about 60% is found under Kinnow production [4].

However, citrus crop in Punjab (Pakistan) is attacked by many diseases and insect pests which are deleterious for citrus industry. Among insect pests, citrus leafminer is the most important one. The citrus leafminer, *Phyllocnistis citrella*, is originated from Eastern and Southern parts of Asia and after 1993, it was rapidly spread to all citrus growing areas of America and also in the Mediterranean basin [5].

Adult female of leafminer lays eggs underside the leaves of citrus plants. The eggs hatch out within 2 to 10 days. The larvae hatch, enter in the leaves of citrus plants and start feeding. The young larvae make mines under the surface of leaves but in the case of heavy infestation mines are made on the both surfaces of leaves. Generally one leaf has one mine, but in the case of heavy infestation two to three mines could be found per leaf. *Phyllocnistis citrella* not only causes direct injure to the new sprouts of leaves, but also brutally infect the twigs and fruits [6,7] and it is also a vector of citrus canker outbreak which is cause by *Xanthomonas axonopodis* pv. citri and *Xanthomonas axonopodis* pv. [8].

Chrysoperla carnea Stephens (Neuroptera:

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Chrysopidae) is predominantly an important and widely distributed species of green lacewing in Pakistan [9] and other parts of the world [10]. Larvae are voracious predators of cotton aphid, *Aphis gossypii* (Glov.); eggs and young larvae of tobacco budworm, *Heliothis virescens* (F.); corn earworm, *Helicoverpa zea* (Boddie); pink bollworm, *Pectinophora gossypiella* (Saund.) and Colorado potato beetle, *Leptinotarsa decemlineata* (Say) [10-12]. *Chrysoperla carnea* occurs naturally in a wide range of agro-ecosystems including citrus orchards and is commercially available in Europe and North America [13]. It has primarily been used through augmentative release to control various aphid species in greenhouses and outdoor crops [14].

Similarly, tiny insect parasitic wasps in the genus *Trichogramma* are very effective egg parasitoids being used against lepidopterous pests all over the world [15, 16]. Approximately, 18 different species of this genus are applied worldwide on an area of about 15 million ha per annum to control pests of cotton, sugarcane, rice, corn, soybean, sugar-beet, vegetables, fruits and forests [17, 18].

Biological control is considered the most economical and environmentally sound long-term solution for managing *Phyllocnistis citrella* [19]. Amalin *et al*. [20] also reported that lacewings, ants, hunting spiders and host feeding ecto-parasitoids as predators of *Phyllocnistis citrella*.

The main objective of this study was to evaluate the in-situ efficacy of different bio-control agents on the population dynamics of citrus leaf miner, which was eco-friendly and long lasting control measure.

MATERIALS AND METHODS

This study was designed at the citrus (Kinnow) nursery of University College of Agriculture, University of Sargodha during spring sprouting season from February to April 2014. Leaves on one branch were counted and it was multiplied by total number of branches on a plant to calculate total number of leaves per plant. For calculation of percent infestation (damage) of citrus leafminer, total number of damaged leaves were counted and divided with total number of leaves on a seedling or citrus plant and then multiplied by hundred as described in the formula below [21];

Infested leaves (%) = damaged leaves / total leaves x 100

Experimental Layout: Cards of *Chrysoperla carnea* and *Trichogramma chilonis* were acquired from Ayub Agriculture Research Institute (AARI) Faisalabad, Punjab, Pakistan. Larvae of *Chrysoperla carnea* were released on 12 selected plants under randomized complete block design (RCBD) with three replications. These plants were covered by muslin net to obstruct the entry of any other insect or invertebrate. Three plants were treated with 5, 10 and 15 larvae, respectively and one plant was considered as control in each block. *Trichogramma chilonis* egg cards were installed on 12 selected plants under randomized complete block design (RCBD). In the case of *Trichogramma chilonis* egg cards, three plants were treated through one, two and three cards (200 eggs per card) respectively and one plant treated as control in each block.

The data regarding percent leafminer population infestation was recorded on the whole plant 24 h pre-and 24, 48 and 72 h post-treatment of *Chrysoperla carnea* larvae and at 24 h pre- and 3, 6 and 9 days post-treatment of *Trichogramma chilonis* egg cards. Statistical extrapolation of date was done by M.Stat-C 8.1 versions.

RESULTS

Infestation Dynamics of Citrus Leafminer: On nursery plants, percent population infestation was recorded with the intervals of 10 days during this study. Maximum infestation was recorded at April 10, which was 38.1%. Population infestation gradually increases during the whole period of study with its peak during end March to end April as shown in Figure 1.

Effect of *Chrysoperla Carnea* on the Population Infestation of Citrus Leafminer

Pre-Application Leafminer Infestation (%): As demonstrated in Figure 2, percent population infestation of citrus leafminer recorded before the release of *Chrysoperla carnea* larvae was maximum (24.8%) in treatment T1 (5 larvae per plant), while it was minimum in T0 (control treatment) with an average of 13.7% infestation of leafminer which was not significantly different from T3 (15 larvae of *Chrysoperla carnea* per plant) bearing 14.35% infestation. Plants in treatment T2 (10 larvae per plant) showed a population infestation of 20.16% (Figure 2).

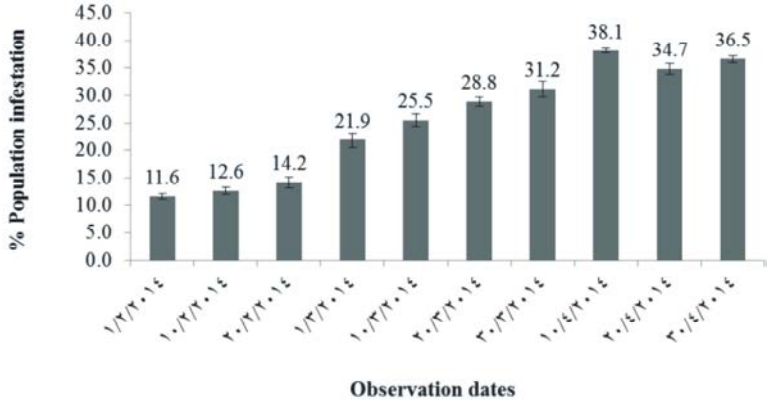

Fig. 1: Percent population infestation of citrus leafminer during spring sprouting of 2014

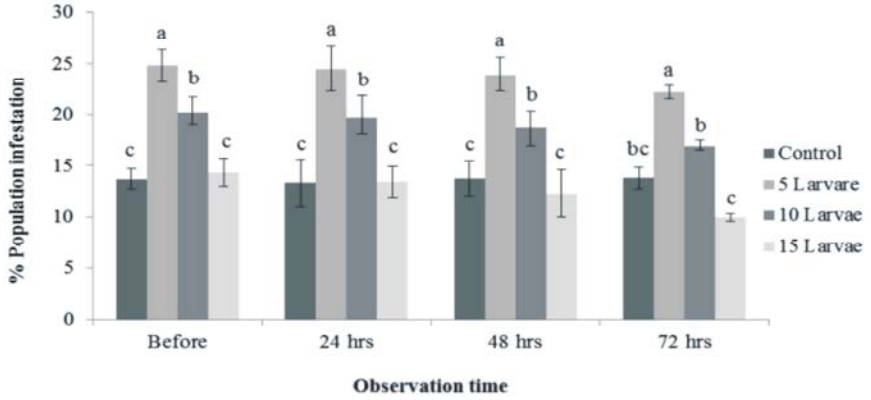

Fig. 2: Population infestation of citrus leafminer before and after 24, 48 and 72 h of the application of *Chrysoperla carnea* larvae

Post-Application Leafminer Infestation (%): After 24 h of *Chrysoperla carnea* introduction on plants, reduction in the leafminer population infestation was found maximum (1.0%) in T3, reduced from 14.35% to 13.45%. In case of T2 (19.7%) and T0 (13.3%), this infestation reduction was about 0.5% and 0.4% respectively. Minimum reduction of leafminer population infestation was observed in T1 (24.5%) that was only 0.3%. After 48 h, observation for reduction in the population infestation showed that treatment T3 (12.26%) has maximum reduction of 1.19% in the population of citrus leafminer, T2 (18.67%) showed 1.03% and T1 (23.93%) showed 0.57% reduction. T0 (13.74%) showed no reduction rather the leafminer population increased by 0.44%. After 72 h, reduction in the population infestation showed that T3 (9.93%) had maximum reduction of 2.33% in the population of citrus leafminer, T2 (16.80%) showed (1.87%) reduction and T1 (22.21%) show (1.72%) reduction. Control treatment, T0 (13.79%), showed not any reduction but population increase (0.05) (Figure 2).

Effect of *Trichogramma chilonis* on the Population Infestation of Citrus Leafminer

Pre-Application Leafminer Infestation (%): Percent population infestation of citrus leafminer recorded before the application of *Trichogramma chilonis* egg cards (Figure 3) showed that, treatment T1 plants contained maximum population of citrus leafminer i.e. 19.21%, treatment T0 also had maximum population infestation (17.28%) but less than T1. Treatment T2 plants had 16.21% population and treatment T3 have minimum population of citrus leafminer (15.65%) before application of *Trichogramma chilonis* egg cards.

Post-Application Leafminer Infestation (%): After three days of installation of *Trichogramma chilonis* egg cards, reduction in the population infestation showed that T3 (3 cards per plot) (14.13%) had maximum reduction of 1.52% in the population of citrus leafminer infestation, while T2 (2 cards per plot) showed 14.93% infestation with an average reduction of 1.28% reduction and T1 (18.8%) showed 0.41% reduction. T0 (18.65%) had minimum

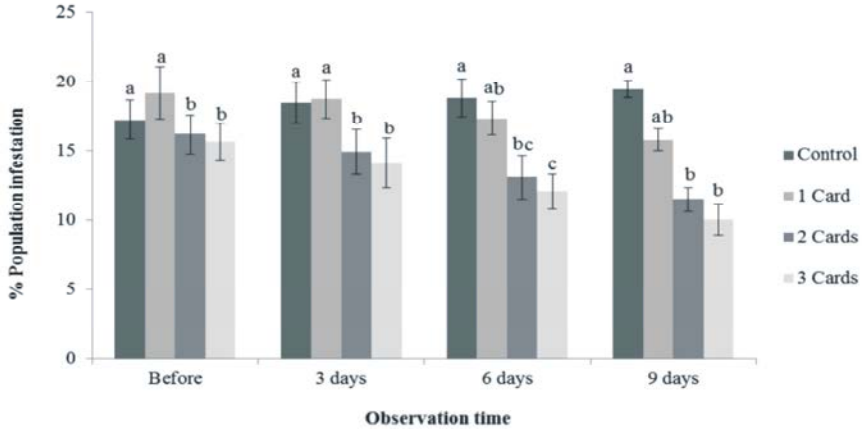

Fig. 3: Percent population infestation of citrus leafminer before and after three days, six days and nine days of application of *Trichogramma chilonis* egg cards.

infestation reduction rather there was an increase of about 1.37% observed in the population of citrus leafminer. After six days of application, maximum reduction in the population infestation was observed for T3 (i.e. 12.06%) with a reduction of about 2.24%, while treatment T2 with 13.1% infestation showed 1.83% reduction and T1 with an infestation rate of 17.37% showed about 1.43% reduction. Control treatment T0 contained 18.65% infestation with no reduction observed. Nine days post application of *Trichogramma chilonis* egg cards, reduction in the population showed that T3 (10.02%) had maximum reduction of 2.58% in the population of citrus leafminer, T2 (11.49%) showed (1.61%) reduction and T1 (15.8%) showed (1.57%) reduction. Treatment T0 (19.29%) had no reduction rather there was an increase (0.64%) in the population of citrus leafminer (Figure 3).

DISCUSSION

This study evaluated the effect of entomophagous insects, green lacewing *Chrysoperla carnea* and hymenopterous egg parasitoid *Trichogramma chilonis* against population infestation of citrus leafminer. During this study, the time of the application of bio-control agents in citrus nursery was very important because leafminers remained active throughout the year but its population was at peak during spring and during rainy seasons at appearance of young sprouting. In this study, the population of citrus leafminer reached to a maximum during the spring sprouting in the month of March and April. These finding are in line with the findings of Singh and Azam [22] observation, they conducted a field trail in Andra Pradsh, India, in 1984-1985 on the seasonal occurrence and population dynamics of *Phyllocnistis citrella* on lime and concluded that population of citrus leafminer were at peak during February-April and July- October.

During this study, the numbers of *Chrysoperla carnea* larvae were most important because the treatment which contained 15 larvae showed better results as compared to other treatments. Amalin *et al*. [20] also reported that lacewings, ants, hunting spiders and host feeding ecto-parasitoids as predators of *Phyllocnistis citrella* eggs and larvae. This study evaluated for the very first time the efficiency of *Trichogramma chilonis*, which was not used earlier for control of citrus leafminer larvae, but it was very effective against eggs of other lepidopterous insects as recorded by Ahmad *et al*. [23].

On average, there was about 14% reduction in the population infestation of citrus leafminer by introduction of *Chrysoperla carnea* larvae and about 17% reduction in case of *Trichogramma chilonis* egg cards and there was a gradual increase of leafminer infestation in the control treatments. This demonstrates the effectiveness of these bio-control agents although the reduction is not significant as tested on against other lepidopterous and sucking insect pests on other field crops [24-26].

CONCLUSION

Farmers mostly relay on pesticides to control citrus leafminer without knowing the ill effects of these chemicals. Therefore, these pests should be regularly monitored to know their abundance and peak time of activity. It will help in reducing spray application and in decision making for the management of this pest. Results of present study demonstrated that even different

bio-control agents such as *Chrysoperla carnea* and *Trichogramma chilonis* are not so much effective as synthetic insecticides against citrus leafminer infestation but the presence of these natural enemies in the field could effectively reduce the infestation propagation. Further work is needed to study the effect of environmental factors on the citrus leafminer and rearing and release of efficient bio-control agents in citrus orchards.

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