Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1903- 1914
Received 05 July 2021; Accepted 01 October 2021.

Eco-friendly control of Citrus Leaf miner, *Phyllocnistis Citrella* Stainton (Lepidoptera: Gracillariidae) using the biological agents and pesticide oil: towards an integrative approach.

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Abstract

The citrus leaf miner (CLM), *Phyllocnistis citrella* Stainton, (Lepidoptera: Gracillariidae) is an important pest of citrus that attacks the new flushes of citrus. In this study, biological agents like *Chrysoperla carnea* and *Trichogramma chilonis* were used along with pesticide oil (Diver® 95%) to control this pest by using exclusive method. Analysis revealed that maximum mortality was observed in 9 day by *C. carnea* larvae and *T. chilonis* adults. Results showed that accumulative mortality maximum in 9 day were 18.75±0.85 as compared to 3, 6 day, and control with mean mortality 7.25±0.85, 8.5±1.25, and 0.25±0.25 respectively. In addition, maximum mortality was observed in 9 day when combined 5 Larvae+5ml/L was used with mean mortality 18.00±0.70 as compared to 3, 6 day, and control with mean mortality 6.5±0.64, 7.25±0.47 and 0.75±0.25 respectively. While in case of combine action of *T. chilonis* and pesticide oil, maximum mortality 15.5±0.86 was observed in 9 day when combined 5 adults+5ml/L was used as compared to 3, 6 day, and control with mean mortality 4±0.40, 5±0.91, and 0.25±0.25 respectively.

Key words: *P. citrella*, *C. carnea*, *T. chilonis*, Pesticide oil, Citrus plant, Biological control

Introduction

The extensive used of insecticides that have long been applied as the main solution for the management of crop pests is not without risk; environment pollution, insecticide resistance, resurgence, and chronic effects on beneficial biodiversity Casida (2009). Without impressive crop management, losses in agricultural production vary from 10 to 50 % due to insect pests, weeds, and diseases have been estimated Beddington (2010).

The citrus leaf miner (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is an devastating pest of citrus which causes direct infection to the leaves of new shoots, branches and fruits (Prates *et al*. 1996) along correlation with citrus canker has been observed (Gottwald *et al*. 1997; Chagas *et al*. 2001). This pest was foremost reported in Sao Paulo state, collected in the plant numbers which are damaged with the same pest (Prates *et al*. 1996), as result the variations were happened in the technique for removing of citrus canker (Gimenes-Fernandes *et al*. 2000; Chagas *et al*. 2001). Integrated pest management (IPM) approaches comprising on several control tactics. Therefore, it is essential to understand the possible drawbacks of agro-chemicals along augmentative and conservation biological control (Matsuda *et al*. 2001).

Different egg parasitoids are successful biological agents of important agro-farms, forestry insects and many other agriculture crop pests. Amongst these, the genus *Trichogramma* Westwood is broadly used in different insect pests control programs Li-Ying (1994). For example, *Cirrospilus quadristriatus* (Hymenoptera: Eulophidae) is a solitary ectoparasitic wasp specie, parasitizing second and third instar larvae and pre-pupa stage of the citrus leaf miner (Ding & Huang., 1989). It is responsible about 37% to 84% parasitization of the leafminer's larval instars (Argov & Rossler., 1996). Furthermore, many preliminary researches showed that *Trichogramma* has effective suitability against different host species as well as various characteristics like as chorion thickness (Pak *et al*. 1990), nutritional values (Barrett & Schmidt., 1991), age Garcia (2000).

The green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) is naturally occurring, polyphagous insect predator Duelli (2001). Its voracious feeding capacity, including almost all soft-bodied arthropods, its wide distribution as well as its extensive range of prey, make this natural promising candidate for pest management strategies (Tauber *et al*. 2000). Additionally, A lacewing larva, *C. boninensis* could consume 49 *P. citrella* larvae in its life time (Yingfang *et al*. 2010).

Furthermore, Petroleum-derived spray oils contain emulsifiers that allow the oil molecules to form semi-stable emulsions when mixed with water, and the types and concentrations used influence pesticide efficacies (Ebeling 1936; Campbell 1972). Generally, it is believed that increasing the concentration of emulsifier reduces the pesticidal efficacy of oil, because less oil is deposited on sprayed surfaces Campbell (1972). Horticultural mineral oils (HMO, contemporary PDSO that may also be called narrow-range petroleum spray oils) suppress oviposition of citrus leaf miner, *P. citrella* (Beattie *et al*. 1995; Rae *et al*. 1996). No research work was found on biological control of CLM in Pakistan. In the present study, biological control (*Chrysoperla*, *Trichogramma*.) and pesticide oil, was used for integrated pest management of *P. citrella* through potential biological agents as well as pesticide oil.

Materials and Methods

Plants and Insects

Citrus seedlings were planted as nursery in the field area of Department of Entomology, Bahauddin Zakariya University, Multan Pakistan, using square system as described by (Richardson *et al*., 2011). All the cultural practices were carried out for the management of these plants and mean diurnal temperature cycle of 35°C-23°C in the summer and 32°C-20°C in the winter. *Chrysoperla carnea* larvae and *Trichogramma chilonis* adults were taken from NIAB (Nuclear Institute for Agriculture and Biology) Faisalabad. The pesticide oil (Diver oil® 97%, E.C El-Helb pesticides and Chemical Co. Egypt) was purchased from local market.

Experiment #1: Predation of *P. citrella* by *C. carnea*

In this experiment, I have measured the effect of *Chrysoperla carnea* on the mortality of *Phyllocnistis citrella* inserted in citrus nursery. Firstly, I observed the experiment on daily bases by using a hand lens to determine peak period of *P. citrella* egg hatch. Newly hatched first instar larvae (one day old) were identified by marking the adjacent leaf surface with dark ink. Only one first instar larva was kept per leaf, the remaining larvae were removed by hand. *C. carnea* cards consisting 5, 10, and 15 larvae were released on different selected plants which were covered with a net cages (35-15 cm diameter, white color made from screen netting) to hinder the entrance of any other irrelevant entity. For each treatment, plants were tagged as 1, 2 and 3 respectively and 4th plant was control. The predation data of *Chrysoperla carnea* was recorded after 3, 6, and 9 day.

Experiment #2: Parasitism of *P. citrella* by *T. chilonis*

In this experiment, *T. chilonis* adults were released on selected plants which were covered with net cages as described above experiment. Plants were tagged as 1, 2 and 3 respectively and 4th plant was control.

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Parasitization of the parasitoid was recorded after 3, 6 and 9 day. The numbers of dead larvae likely causes of the death were also noted.

Experiment #3: Combined effect of *C. carnea* and *T. chilonis* on *P. citrella*

In this experiment, *C. carnea* and *T. chilonis* both were used in combination on selected plants and treatments were (5 *T. chilonis* adults+ 5 *C. carnea* larvae, 10 *T. chilonis* adults+10 *C. carnea* larvae, and 15 *T. chilonis* adults+15 *C. carnea* larvae, and control) applied. Biological agents were resealed as same in above experiment 1. Their controlling potential was recorded after 3, 6, and 9 day.

Experiment #4: Combined effect of *C. carnea* and pesticide oil on *P. citrella*

In this experiment, combined effect of different doses of Diver (1ml/L, 3ml/L, and 5ml/L) were sprayed in the combination along 5 larvae of *C. carnea* on plants leaving the fourth one as control. Selected plants and treatments were same as described in above experiments. The data was collected after 3 days, 6 days and 9 days to check their controlling potential.

Experiment #5: Combined effect of *T. chilonis* and pesticide oil on *P. citrella*

In this study, different doses of Diver (1ml/L, 3m/L, and 5ml/L) were sprayed in the combination along with 5 adults of *T. chilonis* on plants leaving the fourth one as control. Selected plants and treatments were same as described in above experiments. The data was collected after 3 days, 6 days and 9 days to check their controlling potential.

Statistical Analysis

Mean and percentage larval mortality was recorded for each treatment and used for statistical analysis. Data obtained were first normalized by using the arsine square-root transformation (px + 0.5) and then analyzed with one-way analysis of variance (ANOVA) followed by the Tukey–Kramer honestly significant difference (HSD) test to determine significant treatments effects ($P < 0.05$, JMP Version 7.01, SAS 2007).

Results

Experiment #1: Predation of *P. citrella* by *C. carnea*

The mean mortality of *P. citrella* caused by *C. carnea* is shown in Table 1. The number of CLM preyed by *C. carnea* differed significantly between treatments (5 larvae, 10 larvae, 15 larvae, and control) ($F= 17.9$; $DF=2$; $X^2= 1.75$; $P=0.003$). Maximum mortality was observed in 9 day when 15 *C. carnea* larvae were released with mean mortality 15±1.68 as compared to 3, 6 day and control with mean mortality 11±0.91, 13.5±1.84, and 0.25±0.25 respectively. Similar trend was observed when 10 *C. carnea* larvae were released with mean mortality 11.5±1.44 in 9 day as compared to 3 and 6 day. Minimum mortality 5.75±0.85 was observed in 3 day when 5 larvae were released against CLM on citrus plant. Whereas, no significant difference was recorded between same treatments among the 3, 6, and 9 day were released respectively.

There was a significance difference in percentage mortality of CLM when different ratios (5, 10, and 15) of *C. carnea* were released in Fig. 1. Maximum percentage mortality (75±1.68%) was observed in 9 day at 15 larvae as compared to 3, 6 day, (5 larvae; 28.75±0.85%, 23.75±0.85%) and control (1.25±0.25%) was recorded minimum percentage mortality respectively.

Table 1. Mean (±SE) mortality of *P. citrella* caused by *C. carnea* after 3, 6, and 9 day respectively.

Treatments	Time		
	3 Day	6 Day	9 Day

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5 larvae	5.75±0.85c	4.75±0.85c	6.00±1.08c
10 larvae	9.00±0.81b	8.25±1.65b	11.5±1.44ab
15 larvae	11.00±0.91ab	13.5±1.84a	15±1.68a
Control	0.25±0.25d	0.25±0.25d	0.25±0.25d
F value		17.9	
P value		0.003	
HSD		3.2678	

Different letters within columns denotes significant differences between treatments. One-way ANOVA ($P \leq 0.05$).

Table 2. Mean (±SE) mortality of *P. citrella* caused by *T. chilonis* after 3, 6, and 9 day respectively

Treatments	Time		
	3 Day	6 Day	9 Day
5 Adults	5.25±1.19c	6.5±1.32c	6.00±1.82c
10 Adults	10.75±1.65b	9.5±0.64b	10.25±1.10b
15 Adults	12.00±1.23a	11.5±1.32a	12.5±1.64a
Control	0.00±0.00d	0.25±0.25d	0.25±0.25d
F value		95.0	
P value		0.000	
HSD		1.36	

Different letters within columns denotes significant differences between treatments. One-way ANOVA ($P \leq 0.05$).

Table 3. Mean (±SE) mortality of *P. citrella* caused by combined effect of *C. carnea* and *T. chilonis* after 3, 6, and 9 day respectively

Treatments	Time		
	3 Day	6 Day	9 Day
5 Adults+5 Larvae	7.25±0.85c	8.5±1.25c	9.5±1.32c
10Adults+10 Larvae	13±0.91b	14.5±1.55b	16.75±1.97ab
15 Adults+15Larvae	18.25±0.85a	18±0.81a	18.75±0.85a
Control	0.25±0.25d	0.25±0.25d	0.25±0.25d
F value		45.6	
P value		0.0002	
HSD		3.22	

Different letters within columns denotes significant differences between treatments

Experiment #2: Parasitism of *P. citrella* by *T. chilonis*

The mean mortality of *P. citrella* parasitoid by *T. chilonis* is shown in Table 2. The number of CLM dead by *T. chilonis* adults differed significantly between treatments (F= 95; DF=2; X^2= 0.12; P=0.0001). Maximum mortality was observed in 9 day when 15 *T. chilonis* adults were released with mean mortality 12.5±1.64 as compared to 3, 6 day, and control with mean mortality 5.25±1.19, 6.5±1.32, and 0.25±0.25

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respectively. Similar, minimum mortality was observed in 3 day when 5 larvae were released against CLM on citrus plant. However, no significant difference was recorded within same treatments in 3, 6, and 9 day respectively.

In case of percentage mortality of CLM, there was a significance difference in all treatments when *T. chilonis* adults were released, Fig. 2. Maximum percentage mortality (62.5±1.64%) was observed in 9 day at 15 *T. chilonis* adults as compared to 3, 6 day, (5 *T. chilonis* adults; 27.5±1.19%, 32.75±1.32%) and in control minimum percentage of mortality (1.25±0.25%) was recorded respectively.

Experiment #3: Combined effect of *C. carnea* and *T. chilonis* on *P. citrella*

The mean mortality of *P. citrella* caused by *C. carnea* larvae and *T. chilonis* adults is shown in Table 3. The analysis data of CLM dead by *C. carnea* larvae and *T. chilonis* adults was differed significantly among treatments (5 adults+5 larvae, 10 adults+10 larvae, 15 adults+15 larvae, and control) (F= 45.6; DF=2; X^2= 3.17; P=0.0002). Maximum mortality was observed in 9 day, when 15 *T. chilonis* adults+15 *C. carnea* larvae were released with mean mortality 18.75±0.85 as compared to 3. 6 day, and control with mean mortality 7.25±0.85, 8.5±1.25, and 0.25±0.25 respectively. Similar, minimum mortality was observed in 3 day when 5 adults+5 larvae were released against CLM on citrus plant. However, no significant difference was recorded within same treatments in 3, 6, and 9 day respectively.

Similar trend was observed in percentage mortality of CLM in all treatments when different ratios were released showed significance difference in Fig. 3. Maximum percentage mortality (93.75±0.85%) was observed in 9 day at 15 *T. chilonis* adults+15 *C. carnea* larvae as compared to 3, 6 day, (5 *T. chilonis* adults+5 *C. carnea* larvae; 36.25±0.85%, 42.5±1.25%) and control (1.25±0.25%) was recorded with minimum percentage mortality respectively.

Experiment #4: Combined effect of *C. carnea* and pesticide oil on *P. citrella*

The accumulative mortality of *P. citrella* caused by *C. carnea* larvae and pesticide oil is shown in Table 4. The statistical analysis data was differed significantly among all treatments (5 Larvae+1ml/L, 5 Larvae+3ml/L, 5 Larvae+5ml/L, and control) (F= 24.2; DF=2; X^2= 1.53; P=0.0013). Maximum mortality was observed in 9 day when combined 5 Larvae+5ml/L was used with mean mortality 18.00±0.70 as compared to 3, 6 day, and control with mean mortality 6.5±0.64, 7.25±0.47, and 0.75±0.25 respectively. However, minimum mortality was observed in 3 day when 5 larvae+1ml/L were used against CLM on citrus plant. However, no significant difference was observed within same treatments in 3, 6, and 9 day respectively.

Similar trend was observed in percentage mortality of CLM in all treatments when different ratios were used in 3, 6, and 9 day showed significance difference in Fig. 4. Maximum percentage mortality (90.00±0.28%) was observed in 9 day at 5 Larvae+5ml/L as compared to 3 day (5 *C. carnea* larvae+1ml/L; 32.5±0.64%) and control (3.25±0.25%) was recorded with minimum percentage of mortality respectively.

In case of percentage mortality of CLM, when values were taken in 3, 6, and 9 day showed significance difference in all treatments in Fig. 5. Maximum percentage mortality (77.50±0.86%) was observed in 9 day at 5 adults+5ml/L as compared to 3 day, (5 adults+1ml/L; 20±0.40%) and control (1.25±0.25%) was recorded with minimum percentage of mortality respectively.

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Received 05 July 2021; Accepted 01 October 2021.

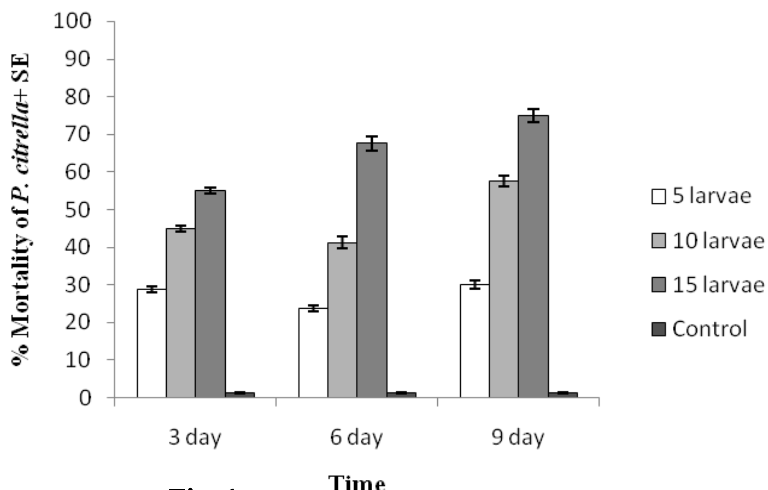


Fig. 1

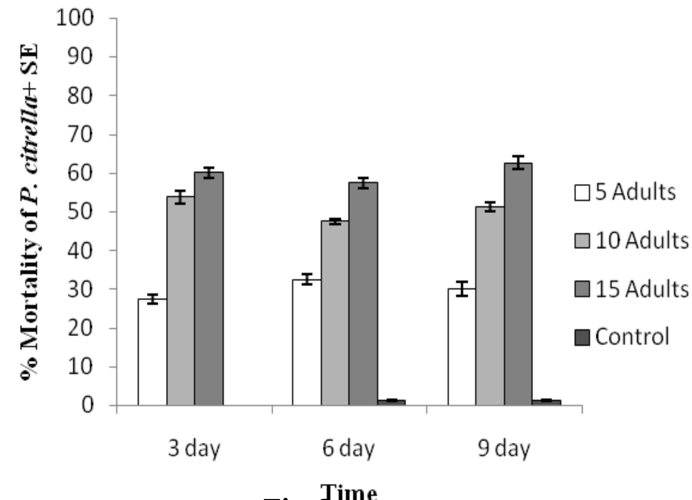
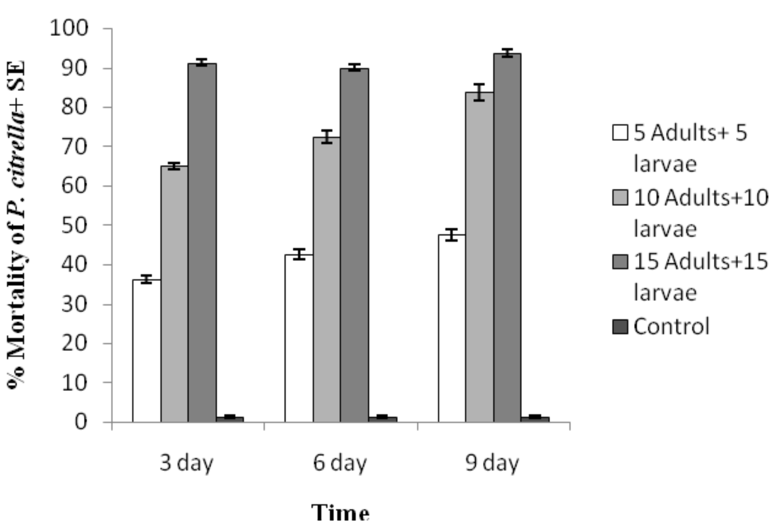
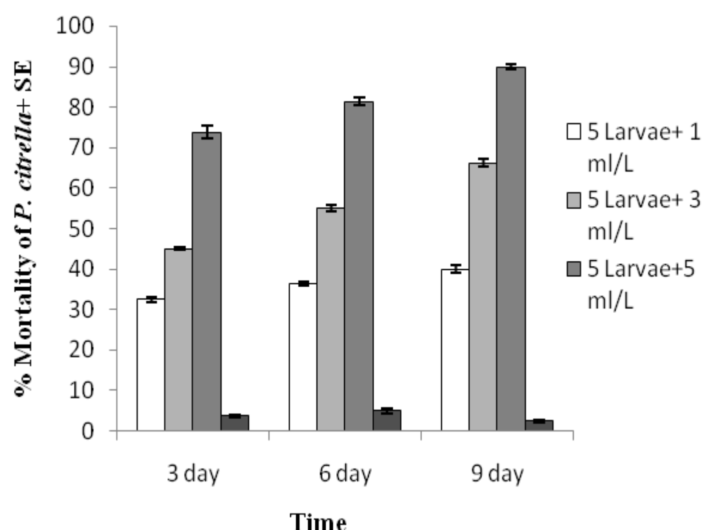


Fig. 2

Figure 1. Percentage (±SE) mortality of *P. citrella* caused by *C. carnea* after 3, 6, and 9 day. **Figure 2**. Percentage (±SE) mortality of *P. citrella* caused by *T. chilonis* after 3, 6, and 9 day.





Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1903- 1914
Received 05 July 2021; Accepted 01 October 2021.

Fig. 3 **Fig. 4**

Figure 3. Percentage (±SE) mortality of *P. citrella* caused by *C. carnea* and *T. chilonis* after 3, 6, and 9 day. **Figure 4**. Percentage (±SE) mortality of *P. citrella* caused by *C. carnea* and pesticide oil after 3, 6, and 9 day.

Discussion

Different experiments were performed by using two bio-agents along pesticide oil against *P. citrella* in citrus nursery under natural conditions. The mean mortality and percentage mortality were described here. First experiment was concerned to evaluate predation of *C. carnea* against *P. citrella*. In this experiment, results showed that maximum mortality was observed in 9 day when 15 larvae were released against *P. citrella* as compared to 3, 6 day , and control (Table 1). The author observed that maximum mortality of *P. citrella* in 9 day due to maximum exposure time, and maximum numbers of predators which were allowed for predation. Consequently, the predation of *P. citrella* by *C. carnea* recorded in this study at different ratio is coincided with the previous experiment on functional response of *C. carnea* towards *N. ribisnigri* (Shrestha 2011), in which predation of *N. ribisnigri* was reported at the highest supplied density. In addition, our results showed that maximum percentage mortality was observed at 15 larvae in 3rd week of release for *P.citrella* (Fig. 1). Hence, findings of this experiment are also agreed with the previous works on predation rates of green peach aphids, *Myzus persicae* (Scopes 1969), cabbage aphids, *Brevicoryne brassicae* (Huang & Enkegaard 2010), and mealy plum aphids, *Hyalopterus pruni* (Atlihan *et al*., 2004), have been reported for 3rd instar *C. carnea* at different environmental conditions. While, preliminary research by (Browning & Pena 1995); (Amalin *et al*., 1996, 2002) found that green lacewing larvae *C. rufilabris* (Burmeister), and mirid bugs, causing approximately 34 to 39% of the mortality, are predators of CLM larvae in citrus groves in south Florida.

Table 4. Mean (±SE) mortality of *P. citrella* caused by combined effect of *C. carnea* and pesticide oil after 3, 6, and 9 days respectively.

Treatments	Time		
	3 Day	6 Day	9 Day
5 Larvae+1ml/L	6.50±0.64c	7.25±0.47c	8.00±1.08c
5 Larvae+3ml/L	9.00±0.40b	11.00±0.70b	13.25±0.85ab
5 Larvae+5ml/L	14.75±1.65ab	16.25±0.94a	18;00±0.70a
Control	0.75±0.25d	1±0.57d	0.50±0.28d
F value		24.2	
P value		0.001	

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1903- 1914
Received 05 July 2021; Accepted 01 October 2021.

HSD	4.02

Different letters within columns denotes significant differences between treatments

Table 5. Mean (±SE) mortality of *P. citrella* caused by combined effect of *T. chilonis* and pesticide oil after 3, 6, and 9 days respectively.

Treatments	**Time**		
	3 Day	6 Day	9 Day
5 Adults+1ml/L	4.00±0.40b	5.00±0.91b	7.50±0.64ab
5 Adults+ 3 ml/L	6.50±0.95ab	10.75±1.70a	11.50±0.64a
5 Adults+5 ml/L	9.75±0.85ab	12.00±0.91a	15.50±0.86a
Control	0.00±0.00c	0.50±0.28bc	0.25±0.25bc
F value		5.75	
P value		0.040	
HSD		6.29	

Different letters within columns denotes significant differences between treatments

In second experiment, I observed the parasitism of *P. citrella* by *T. chilonis* released at different levels. In this experiment, results reflected that maximum parasitism of *P. citrella* was reported at 15 *T. chilonis* adults in 9 day than the other weeks and control (Table 2). Similarly, in case of percentage mortality, maximum mortality was observed in 3rd week at 15 adults compared to other (Fig. 2). The results were agreed with the findings of (Perales-Gutierrez *et al*., 1996) recorded the relative dominance of *Z. multilineatum* as a parasitoid of *P. citrella* in the Mexican state of Colima. In addition, (Bautista-Martinez *et al*., 1998) observed *Z. multinleatum* as a parasitoid of *P. citrella* in the state of Veracruz. Similarly, (Legaspi *et al*., 1999) conducted a surveys in south Texas in 1995 and 1996 designates the native parasitoid complex attacking *P. citrella*. Furthermore, *T. chilonis* parasitoid of *P. citrella* is usually polyphagous that parasitoid other lepidopteran leaf miners (Pena *et al*., 1996). Furthermore, previous work by (Hoy *et al*., 1995); (Hoy & Nguyen 1997); (Pomerinke & Stansly 1998); (Villanueva-Jimenez *et* al., 2000) indicated that up to 99% of the *P. citrella* parasitized by *Ageniaspis citricola* (Hymenoptera: Encyrtidae).

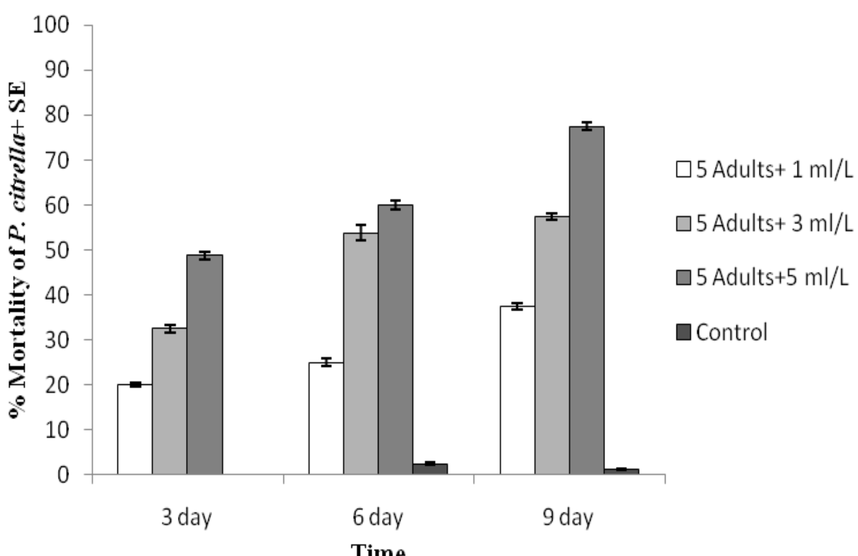


Figure 5. Percentage (±SE) mortality of *P.citrella*caused by *T. chilonis* and pesticide oil after 3, 6, and 9 day.

In third experiment, we noticed that combination of *C. carnea* and *T. chilonis* showed more effective against the *P. citrella*. In this experiment, maximum mean mortality was recorded when 15 *C. carnea* larvae+15 *T. chilonis* adults were released toward *P. citrella* at 9 day than 3, 6 day, and control (Table 3). In this study, maximum percentage mortality was observed in 9 day at 15 *C. carnea* larvae+15 *T. chilonis* adults released against *P. citrella* (Fig.3). In addition, similar findings were also reported by (Nasreen *et al*., 2000; 2004) in which combined effect of *C. carnea* and *T. chilonis* on *Helicoverpa armigera* eggs. Furthermore, (Abera-Kalibata *et al*., 2007) have observed combined effect of two ant species against banana weevil *Cosmopolites sordidus* (Germar). While, preliminary research by (Browning & Pena 1995); (Amalin *et al*., 1996, 2002) found that green lacewing larvae *Chrysoperla rufilabris* (Burmeister), and mirid bugs, causing approximately 34 to 39% of the mortality, are predators of CLM larvae in citrus groves in South Florida, U.S.A. Our experiment therefore suggests that both the predators and parasitoid will have greater impact on *P. citrella* at earlier stages than later. The larger prey could usually fragileness the predator and parasitoid species and overcome the predator, resulting in a reduced predator and parasitoid population. For instance, consumption rate has been shown to decline with increased prey size in coccinellid beetles and anthocorid bugs (Dixon & Russel 1972).

In fourth experiment, we assessed the combined effect of *C. carnea* and pesticide oil toward *P. citrella* at different levels. In the present study, results indicated that maximum mean and percentage mortality was recorded at 5 larvae+5ml/L against *P. citrella* in 9 day compared to other days and control (Table 4 & Fig. 4). Our results coincided with (Nasreen *et al*., 2000; 2004) work in which combined action of *C. carnea* and pesticide on *H. armigera* eggs observed. Furthermore, many researchers work showed that petroleum oil, due to its fewer effects on natural enemies and short residual activity, and predators were useful for the control of CLM (Beattie *et al*. 1995b; Knapp *et al*. 1995; Rae *et al*. 1996). We may conclude that the combined effect of bio-agents and pesticide applications suppressed CLM densities below the economic threshold.

In fifth experiment has been recorded that combined action of *T. chilonis* adults and pesticide oil showed affected control on CLM larvae. In the present study, results indicated the maximum mean and percentage mortality were observed at treatment 5 adults+5ml/L toward *P. citrella* in 9 day (Table 5 & Fig. 5). Findings of this study are correlated with previous experiment reported by (Nasreen *et al*., 2000; 2004); Beattie *et al*., 1995b); (Knapp *et al*., 1995); (Rae *et al*., 1996). In addition, effect of pesticide oil on natural enemies has not significant (Beattie & Smith 1993; Erkilic & Uygun 1997) and thus valuable for IPM programs (Lowery & Isman 1995).

In conclusion, the author recommended that biological control agents and selective pesticide oil are useful for the population depression of *P. citrella* in citrus groves. For this purpose, different trials in laboratory as well as field are required in citrus orchard for known suitability in different environmental conditions, compatibility to different insecticides, and effectiveness of these bio-agents. Such research may be helpful to developing an IPM program for managing citrus leaf miners in Pakistan as a means of reducing the spread of citrus canker, because increased use of these biological control agents and safe pesticide to control this disease vector could have environment friendly and less costs, as well as benefits.

References

Abera-Kalibata, A.M., Gold C.S., Van Driesche R.G & Ragama P.E. (2007). Composition, distribution, and relative abundance of ants in banana farming systems in Uganda. *Bio Control,* 40:168–178

Amalin, D.M., Pena, J.E & Mcsorley, R. (1996). Abundance of spiders in lime groves and their potential role in suppressing the citrus leafminer population, p. 72.

Amalin, D.M., Pena, J.E., Duncan, R.E., Browning, H.W & Mcsorley, R. (2002). Natural mortality factors acting on citrus leafminer, *Phyllocnistis citrella*, in lime orchards in South Florida. *Bio Control*, *47*: 327-347

Argov, Y & Rossler, Y. (1996). Introduction, release and recovery of several exotic natural enemies for biological control of the citrus leafminer, *Phyllocnistis citrella*, in Israel. *Phytoparasitica, 24*: 33-38

Atlihan, R., Kaydan, B & Ozgokce, M.S. (2004). Feeding activity and life history characteristics of generalist predator, *Chrysoperla carnea* (Neuroptera: Chrysopidae) at different prey densities. *J Pest Sci*, *77*: 17–21

Barrett, M & Schmidt, J.M. (1991). A comparison between the amino acid composition of an egg parasitoid wasp and some of its hosts. *Entomol Exp Appl*, 59:29–41

Bautista-Martinez, N., Carrillo-Sanchez, J.L., Bravo-Mojica, H & Koch, S.D. (1998). Natural parasitism of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) at Cuitlahuac, Veracruz, Mexico. *Fla Entomol*, 81:30–37.

Beattie, G.A.C & Smith, D. (1993). Citrus Leafminer. Agfacts H2.AE.4. In: 2nd Ed. NSW Agriculture, Orange, Australia.

Beattie, G.A.C., Somsook, V., Watson, D.M., Clift, A.D & Jiang, L. (1995b). Field evaluation of *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) and selected pesticides and enhancers for control of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). *J Aust Entomol Soc*, 34: 335–342

Beattie, G.A.C., Liu, Z.M., Watson, D.M., Clift, AD & Jiang, L. (1995). Evaluation of petroleum spray oils and polysaccharides for control of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). *J Aust Entomol Soci*, 34: 349-353

Beddington, J. (2010). Food security: contributions from science to a new and greener revolution. *Phil Trans R Soc*, 365:61-71

Browning, H.W & Pena, J.E. (1995). Biological control of the citrus leafminer by its native parasitoids and predators. *Citrus Industry*, 76: 46-48

Campbell, M.M. (1972). Efficiency of summer superior oils in relation to the concentration of emulsifying agent. *Exp Rec Dept Agr S Aust* , pp 36–42

Casida, J.E. (2009). Pest toxicology: the primary mechanisms of pesticide action. *Chem Res Toxico,l* 22:609–619

Chagas, M.C.M., Para, J.R.P., Namekta, T., Hartung, J.S & Yamamto, P.T. (2001). *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its relationship with the citrus canker bacterium *Xanthomonas axonopodis* pv. citri in Brazil. *Neotrop Entomol*, 30: 55-59

Ding, Y. M., Li, M & Huang, M.D. (1989). Studies on biology of two species of parasitoids *Tetrastichus phyllocnistoides* and *Cirrospilus quadristriatus* and their parasitization on the citrus leaf-miner *Phyllocnistis citrella* Stainton. In: Studies on the integrated management of citrus insect pests (ed. M.D Huang). *Beijing Academic Book and Periodical*, pp 1063-1113

Dixon, A.F.G & Russel, R.J. (1972). The effectiveness of *Anthocoris nemorum* and *A. confusus* (Heteroptera: Anthocoridae) as a predator of sycamore aphid. *Drepanosiphum platanoids*. Searching behaviour and the incidence of the predation in the field. *Entomologia Experimentalis et Applicata*, 15:35-50.

Duelli, P. (2001). Lacewings in field crops. In: McEwen P, New TR, Whittington AE, (Eds.) Lacewings in the Crop Environment. Cambridge University Press, Cambridge, pp 158–171.

Ebeling, W. (1936). Effect of oil spray on California red scale at various stages of development. *Hilgardia*, 10: 95–125

Erkilic, L.B & Uygun, N. (1997). Studies on the effects of some pesticides on white peach scale, *Pseudaulacaspis pentagona* (Targ. Tozz.) (Homoptera: Diaspididae) and its sideeffects on two common scale insect predators. *Crop Prot*, 16: 69–72

Garcia, P. (2000). Biologia de Trichogramma cordubensis Vargas & Cabello (Hym., Trichogrammatidae) numa perspectiva de controlo biológico. PhD Thesis, *Departamento de Biologia, Universidade dos Açores*, pp 1–238.

Gimenes-Fernandes, N., Barbosa, J.C., Ayres, A.J & Massari, C.A. (2000). Plantas doentes não detectadas nas inspeções dificultam a erradicação do cancro cítrico. Summa. *Phytopathol,* 26: 320-325

Gottwald, T.R., Graham, J.H & Schubert, T.S. (1997). An epidemiological analysis of the spread of citrus canker in urban Miami, Florida, and synergistic interaction with the Asian citrus leafminer. *Fruits,* 52: 371–378

Hoy, M.A & Nguyen, R. (1997). Classical biological control of the citrus leafminer *Phyllocnistis citrella* Stainton: Theory, practice, art and science. *Trop Lepidoptera* 8: 1-19

Hoy, M.A., Nguyen, R., Hall, D., Bullock, R., Pomerinke, M., Pena, J., Browning, H & Stansly, P. (1995). Establishment of citrus leafminer parasitoid, *Ageniaspis citricola* in Florida. *Citrus Industry,* 76: 12-17

Huang, N & Enkegaard, A. (2010). Predation capacity and prey preference of *Chrysoperla carnea* on *Pieris brassicae*. *Biocontrol*, 55: 379–385

Knapp, J., Albrigo, L.G., Browning, H.W., Bullock, R.C., Heppner, J., Hall, D.G., Hoy, M.A., Nguyen, R., Pena, J.E & Stansly, P. (1995). Citrus Leafminer, *Phyllocnistis citrella* Stainton: Current Status in Florida – 1995. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, *University of Florida, Gainesville, Florida, USA.*

Legaspi, J.C & French, J.V. (1999). The Citrus Leafminer and Its Natural Enemies, Circ. B96-1. Texas A&M–Kingsville Citrus Center, *Weslaco, TX.*

Li-Ying, L. (1994). Worldwide use of *Trichogramma* for biological control on different crops: a survey biological control with egg parasitoids. In: Wajnberg E, Hassen, S, (Eds*.) CAB International, UK*, pp 37–53

Lowery, D.T & Isman, M.B. (1995) Toxicity of neem to natural enemies of aphids. *Phytoparasitica* 23: 297–306

Matsuda, K., Buckingham, S.D & Kleier, D. (2001). Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharm Sci*, 22:573–579

Nasreen, A., Ashfaq, M & Mustafa, G. (2000). Intrinsic toxicity of some insecticides to egg parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *Bull Inst Trop Agric Kyushu Univ* 23: 41-44

Nasreen, A., Mustafa, G., Ashfaq, M & Saleem, M.A. (2004). Combined Effect of *Chrysoperla carnea* Stephen (Neuroptera: Chrysopidae) and *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) on *Helicoverpa armigera* Eggs in the Presence of Insecticides. *Pak J Zool*, 36: 189-191

Pak, G.A., Van-Dalen, A., Kaashoek, N & Dijkman, H. (1990). Host egg chorion structure influencing host suitability for the egg parasitoid *Trichogramma* Westwood. *J Insect Physiol*, 36:869–875

Pena, J.E., Duncan, D & Browning, H. (1996). Seasonal abundance of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in south Florida citrus. *Environ Entomol*, 25:698–702

Perales-Gutierrez, M., Arredondo-Bernal, H.C., Garza-Gonzalez, E & Aguirre-Uribe, A. (1996). Native parasitoids of citrus leafminer, *Phyllocnistis citrella* Stainton in Colima, Mexico. *Southwest Entomol*, 21:349–350

Pomerinke, M.A & Stansly, P.A. (1998). Establishment of *Ageniaspis citricola* (Hymenoptera: Encyrtidae) for biological control of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Florida. *Florida Entomol*, 81:361-372

Prates, H.S., Nakano, O & Granena, S. (1996). Minadora das folhas de citros *Phyllocnistis citrella*, Stainton, 1856. Campinas SP. CATI. *Comunicado Técnico*, pp 129

Rae, D.J., Watson, D.M., Liang, W.G., Tan, B.L., Li, M., Huang, M.D., Ding, Y., Xiong, .J.J., Du, D.P., Tang, J & Beattie, G.A.C. (1996). Comparison of petroleum spray oils, abamectin, cartap, and methomyl for control of citrus leafminer (Lepidoptera: Gracillariidae) in southern China. *J Econ Entomol*, 89: 493–500

Richardson, M.L., Catherine, J., Westbrook, D.G., Hall., Stover, E.D & Duan, Y.P. (2011). Abundance of Citrus Leafminer Larvae on Citrus and Citrus-related Germplasm. *Hort Sci*, 46:1260–1264

SAS, Institute. (2007). SAS/STAT 9.1 User's Guide. *SAS Institute*.

Shrestha, G. (2011). Investigation of potential of the green lacewing, *Chrysoperla carnea* Stephens, (Neuroptera: Chrysopidae) in biocontrol of lettuce aphid, *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae) in field-grown lettuce. MSc Thesis, *Aarhus University, Denmark*.

Tauber, M.J., Tauber, C.A., Daane, K.M & Hagen, K.S. (2000). Commercialization of predators: recent lessons from green lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). *American Entomol*, 46:26–38

Villanueva-Jimenez, J.A., Hoy, M.A & Davies, F.S. (2000). Field evaluation of integrated pest management- compatible pesticides for the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae). *J Econ Entomol*, 93: 357-367

Yingfang, X., Henry, Y & Fadamiro. (2007). Exclusion experiments reveal relative contributions of natural enemies to mortality of citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Alabama Satsuma orchards. *Biol Contl* 54:189–196