

## Using of *Chrysoperla carnea* (Stephens) Larvae as a Biological Control Agent against *Phyllocnistis citrella* Stainton.

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### ABSTRACT

Experiments were carried out in the orchards farm of the Fac. of Agriculture at Moshtohor, Benha University, Egypt on navel orange (*Citrus sinenses*) seedlings grafted on *Citrus volckamerianat* to study the effect of releasing 2<sup>nd</sup> instar larvae of the aphid lion (*Chrysoperla carnea*) on citrus leaf miner (*Phyllocnistis citrella*) infestation rate in nursery of citrus plants. The efficiency of *Ch. carnea* as a bio-agent against the citrus leaf-miner was estimated. A net wooden cage was divided into 12 cages that measured 75×75×250 cm. each, and covered tightly with screened fine nylon from all sides. Three treatments were examined compared with control (Tr.1: one & Tr.2: two and Tr.3: four 2<sup>nd</sup> instar larvae of *Ch. carnea*/ seedling). Obtained data clarified that releasing *Ch. carnea* on citrus seedlings caused considerable reduction in both the whole mean number of infested leaves and whole mean percentage of infestation by *P. citrella*. Treatment 2 was the best in reducing the seasonal mean infestation rate by *P. citrella* in citrus nursery by 43.70% while, the treatment 3 gave the best results in decreasing the numbers of *P. citrella* mines, larvae, pupae and infested leaves by 50.86, 78.37, 72.47 and 52.68 %, respectively. The mentioned treatments caused reductions in the whole mean number of mines, larvae and pupae of *P. citrellato* be 7.14, 1.14 and 0.68/seedling, respectively.

**Keywords:** Biological control, *Chrysoperla carnea*, *Phyllocnistis citrella*

### INTRODUCTION

The citrus leaf miner (CLM), *Phyllocnistis citrella* (Lepidoptera:Gracillariidae) is considered as an Asian pest species on citrus plants (Heppner, 1993). Within few years, this pest has moved into citrus growing regions of the world with incredible widespread in many countries (Beattie, 1993). This pest was recorded as outbreak population in the most citrus orchards of Egypt in 1994 (Tawfik *et al.*, 1996). The citrus leaf-miner causes wide damage in citrus orchards such as; leaf curling and the presence of serpentine mines that are distinctly seen on the lower leaf surfaces and also on the upper leaf surfaces when heavy infestations occurs. Citrus leaf-miner may cause a great damage to trees and the leaves become severely destroyed. It is therefore necessary to control this serious pest (Garrido, 1994). Many control measures should be done for the control of this pest. Chemical control is the common method for controlling this pest but the efficacies of different insecticides are expected not to be high because larvae are protected in their mines and pupae in their pupal chambers. In addition, the repeated use of pesticides for control of this pest is very harmful because of its residues in trees and fruits and also its harmful effect on natural enemies and causing distance in the whole ecosystem. Shareef *et al.* (2016) reported that generalist predators in their feeding can reduce pest populations effectively (Wise, 1993 and Romeldi *et al.*, 2012). Several predators of *P. citrella* have been reported. Among these *Chrysoperla spp.* are the major contributors for *P. citrella* predation (Chen *et al.*, 1989). The present study was carried out to control the citrus leaf miner by using nonchemical safe effective methods. Releasing of *Chrysoperla carnea* larvae on *P. citrellato* infested citrus plants was evaluated as a biological control tool.

### MATERIALS AND METHODS

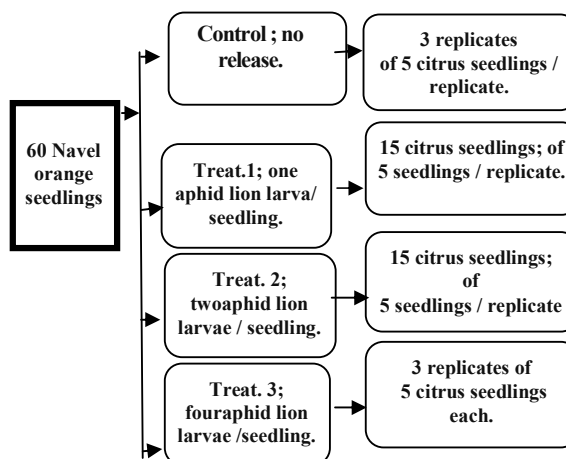
Experiments were conducted at the citrus orchards of the Fac. of Agriculture, Moshtohor, Benha University, Egypt on seedlings of some citrus varieties. The study dealt with releasing the second instar larvae

of *Chrysoperla carnea* for the biological control of the citrus leaf miner (CLM) *Phyllocnistis citrella* Stainton.

#### 1. Effect of releasing the aphid lion larvae on citrus leaf miner infestation rate in the citrus nursery as a biological control method.

60 Navel orange (*C. sinenses*) seedlings grafted on *Citrus volckameriana* were used for studying the effect of releasing 2<sup>nd</sup> instar larvae of the aphid lion (*Chrysoperla carnea*) on citrus leaf miner infestation in citrus plants nursery. These citrus seedlings were put in net wooden cage divided into 12 cages with dimensions 75×75×250 cm. each. Each cage was tightly covered with finely screened nylon from all directions. In each cage, 5 citrus seedlings were placed as a replicate. 2<sup>nd</sup> instar larvae of *Chrysoperla carnea* were obtained from the aphid lion production unit at the Fac. of Agric., Cairo Univ. and released on the examined citrus seedlings at three different rates under randomized complete block design (RCBD) with three replications /treatment (Shareef *et al.*, 2016). The number of infested and healthy leaves, mines, larvae and pupae of *P. citrella* were counted before releasing 2<sup>nd</sup> instar larvae of *Ch. carnea* and weekly counted after that during successive 11 weeks.

Seedlings were divided into 4 groups as shown in the following diagram according to that described by Smith and Hoy (1995) and Maf1 and Ohbayashi (2010):



The number of infested and healthy leaves, mines, larvae and pupae of *P. citrella* were counted to calculate the infestation rate with citrus leaf miner. The infestation percentage was calculated according to the following formula:

$$\text{Infestation rate (\%)} = \frac{\text{No. of infested leaves / sample}}{\text{Total No. of leaves / sample}} \times 100$$

**Experimental Design and Analysis:**

All the data of experiments were analyzed in a randomized complete block design (RCBD) due to those obtained on the ANOVA tables by MSTAT-C version 1.41 according to Snedecor & Cochran (1980). Also, significance between means was determined by Duncan's multiple range tests at 0.05 probability level according to Steel & Torrie (1980).



Unit of experimental cage . Releasing 2nd instar larvae 5 citrus plants represented of (*Chrysoperla carnea*) on one replicate. nursery

**RESULTS AND DISCUSSION**

**1- Effect of releasing *Chrysoperla carnea* larvae on citrus leaf-miner infestation in citrus nursery plants during 2016.**

Data presented in Table (1) indicate that the percentage of infestation by citrus leaf-miner, *P. citrella* decreased by releasing the aphid lion 2<sup>nd</sup> instar larvae. Data in this table proved that releasing two 2<sup>nd</sup>-instar *Ch. carnea* larvae per citrus seedling gave the highest reduction in the infestation rate with citrus leaf-miner by -43.70% followed by releasing four 2<sup>nd</sup>-instar

larvae (-33.73%), while there was an increase in the infestation rate in the control treatment by +14.30%. These data were in harmony with Shareef *et al* (2016) who reported that using *Ch. carnea* reduced the population infestation of citrus leaf miner by 14%. In similar studies, Ahmed *et al*, (2013) observed slight reductions in citrus leaf miner infestation rates with time in *Ch. carnea* treatment, but the pest's population had non-significant differences in terms of increasing the releasing rate and number of days after treatment. Parasitoid alone and in combination with others was found less effective in comparison with *Ch. carnea* on *P. citrella* populations (Amalin *et al*, 2002).

**2- Effect of releasing *Chrysoperla carnea* larvae on reduction percentage of mines, larvae and pupae of the citrus leaf miner on citrus plants in nursery during 2016.**

As shown in Table (2) the highest reduction in mines caused by *P. citrella* (-50.86%), was recorded in the treatment 3, in which four 2<sup>nd</sup> larvae of *Ch. carnea* were released on each citrus seedling, while the lowest reduction rate in mines number (-37.70) was in treatment 1 (one *Ch. carnea* 2<sup>nd</sup> instar larvae / seedling). On contrary, in control treatment there were no difference in larval counts from the beginning to the end of the experiment in the citrus leaf-miner mines rates.

Data also indicated that releasing four 2<sup>nd</sup> instar larvae of *Ch. carnea* on each citrus seedling gave the highest reduction rate in citrus leaf-miner larvae by -78.37%. On contrary, the lowest decreasing rate was obtained in control treatment by rate -18.33%.

As for the pupal counts, data in Table (2) revealed that the treatment 3 (four 2<sup>nd</sup> instar larvae of *Ch. carnea* per each citrus seedling) gave the highest reduction in leaf-miner pupal stage counts (-72.47%), while the lowest decreasing rate was obtained in control treatment by mean rate -20%.

The present findings are supported by those of Urbaneja *et al.* (2004) who reported that *Ch. carnea* could complete its development feeding on *P. citrella* only during the laboratory rearing. The same authors indicated that predation upon *P. citrella* had been satisfactorily correlated to flushing in Eastern Spain.

**Table 1. Effect of releasing *Chrysoperla carnea* larvae at different rates on citrus leaf miner infestation rates on citrus nursery plants during 2016.**

Parameter	Treatment	Pretreatment	Investigation period (successive weeks)											Mean
			1	2	3	4	5	6	7	8	9	10	11	
%	Control	36.79	44.88	42.17	40.83	42.62	32.09	40.50	37.94	46.26	46.80	44.47	43.98	42.05
		100%	+21.99	+14.62	+10.98	+15.85	-12.78	+10.08	+3.13	+25.74	+27.21	+20.88	+19.54	+14.30
		49.17	49.26	29.59	36.09	40.04	42.17	42.24	43.17	43.39	45.94	48.50	50.73	42.83
	T1	100%	+0.18	-39.82	-26.6	-18.57	-14.24	-14.09	-12.20	-11.76	-6.57	-1.36	+3.17	-12.90
		53.04	39.29	28.38	26.39	20.36	25.64	31.49	30.97	28.79	27.3	34.01	35.79	29.86
		100%	-25.92	-46.49	-50.25	-61.61	-51.66	-40.63	-41.61	-45.72	-48.53	-35.88	-32.52	-43.70
	T2	62.49	44.58	34.21	38.83	35.67	33.18	43.85	38.31	42.65	45.46	49.77	48.96	41.41
		100%	-28.66	-45.26	-37.86	-42.92	-46.90	-29.38	-38.69	-31.75	-27.25	-20.36	-21.65	-33.73
		T3												

T1: one *Ch. carnea* larva / seedling  
 T2: two *Ch. carnea* larvae / seedling  
 T3: four *Ch. carnea* larvae / seedling

**Table 2. Effect of releasing *Chrysoperla carnea* larvae on reduction percentage of mines, larval and pupal rates of citrus leaf miner on citrus nursery plants during 2016.**

Parameter	Treatment	Pre-treatment	Reduction rates during investigation period (successive weeks)										Mean		
			1	2	3	4	5	6	7	8	9	10		11	
Mines	Control	10.13	8.73	8.27	8.53	9.60	10.27	10.07	12.13	14.20	9.73	10.47	9.40	10.13	
		100%	-13.82	-18.23	-15.79	-5.23	+1.38	-0.59	+19.74	+40.18	-3.95	+3.36	-7.21	0%	
	T1	12.20	6.60	9.33	6.13	7.73	7.73	6.60	7.87	7.07	8.87	7.47	8.20	7.6	
		100%	-45.90	-23.52	-49.75	-36.64	-36.46	-45.90	-35.49	-42.05	-27.30	-38.77	-32.79	-37.70	
	T2	11.33	7.07	6.53	6.53	6.00	6.07	7.00	6.73	7.80	7.40	7.00	7.13	6.84	
		100%	-37.60	-42.37	-42.37	-47.04	-46.43	-38.22	-40.60	-31.16	-34.69	-38.22	-37.07	-39.63	
	T3	14.53	6.07	6.93	6.87	7.07	5.87	7.73	7.00	8.13	7.47	7.80	7.60	7.14	
		100%	-58.22	-52.31	-52.72	-51.34	-59.60	-46.8	-51.82	-44.05	-48.60	-46.32	-47.70	-50.86	
	Larvae	Control	1.8	2.07	3.4	2.4	1	1.07	1	0.87	0.73	1	1.2	1.4	1.47
			100%	+15	+88.89	+33.33	+44.44	-40.56	-44.44	-51.67	-59.44	-44.44	-33.33	-22.22	-18.33
		T1	2.13	2.6	3.07	2.8	0.6	0.6	0	0.41	0.73	0.33	0.13	1.13	1.13
			100%	+22.07	+44.13	+31.46	-71.83	-71.83	-100	-80.75	-65.73	-84.51	-93.90	-46.95	-46.95
T2		2.93	3.2	3.33	2.67	0.6	0.87	0	0.6	1.13	0.67	0.67	0.73	1.13	
		100%	+9.22	+13.65	-8.87	-79.52	-70.30	-100	-79.52	-61.43	-77.13	-77.13	-75.09	-61.43	
T3		5.27	2.93	2.67	2.6	0.73	0.4	0	0.4	0.8	0.4	0.8	0.8	1.14	
		100%	-44.40	-49.34	-50.66	-86.15	-92.41	-100	-92.41	-84.82	-92.41	-84.82	-84.82	-78.37	
Pupae		Control	1.4	1.33	2	1.33	0.4	0.53	1.1	1.13	1.4	0.9	1.03	1.22	1.12
			100%	-5	+42.86	-5	-71.43	-62.14	-21.43	-19.29	0	-35.71	-26.43	-12.86	-20
		T1	1.8	1.53	2	1.67	0.27	0.27	0	0	0.47	0.2	0.13	0.07	0.6
			100%	-15	+11.11	-7.22	-85	-85	-100	-100	-73.89	-88.89	-92.78	-96.11	-66.67
	T2	2.13	1.93	2.07	1.33	0.2	0.4	0	0.47	0.73	0.47	0.47	0.47	0.78	
		100%	-9.39	-2.82	-37.56	-90.61	-81.22	-100	-77.93	-65.73	-77.93	-77.93	-77.93	-63.38	
	T3	2.47	1.67	1.53	1.47	0.27	0.13	0	0.27	0.53	0.27	0.67	0.67	0.68	
		100%	-32.39	-38.06	-40.49	-89.07	-94.74	-100	-89.07	-78.54	-89.07	-72.87	-72.87	-72.47	

**ACKNOWLEDGEMENT**

Thanks for all staff members of Plant Protection Department, Faculty of Agriculture at Moshtohor, Benha University for their help and encouragement.

**REFERENCES**

Ahmed, S., M.M. Shakir and A.Younis, 2013. Integrated management of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) through natural enemies, mineral oil and insecticide in a citrus nursery of Faisalabad, Punjab, Pakistan. *Thai J. of Agri. Sci.*, 46(3): 135-140.

Amalin, D.M., J.E. Peña, R.E. Duncan, H.W. Browning and R. Mcsorley, 2002. Natural mortality factors acting on citrus leaf miner, *Phyllocnistis citrella*, in lime orchards in South Florida. *Biocontrol*, 4: 327- 347.

Beattie, G.A., 1993. Integrated control of the citrus (Hymenoptera: Trichogrammatidae) at low leaf miner. N. S. W. Agriculture, Rydalmere, N. S. W., temperatures. *Pakistan Journal of Zoology*, 42: 63-67. Australia CAB, IIBC.

Chen, R.T., Y.H. Chen and M.D. Huang, 1989. Biology of green lacewing, *Chrysopa boninensis* and its predation efficiency to citrus leafminer, *Phyllocnistis citrella*, pp. 96-105. In *Studies on the Integrated Management of Citrus Insect Pests*, Beijing, China Academic Book and Periodical Press.

Garrido, A., 1994. *Phyllocnistis citrella* Stainton, biological aspect and natural enemies found in Spain. In: *Proceedings of the Meeting of the IOBC/WPRS Working Group on Integrated Control in Citrus FruitsCrops*, Antibes, 27-28 Oct. 1994, IOBC/WPRS *Bullet.* 18(5): 1-14.

Heppner, J. B., 1993. Citrus leaf miner, *Phyllocnistis citrella*, in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). *Trap. Lepid.* (Gainesville), 4:49-64.

Mafi1, Sh. and N. Ohbayashi, 2010. Biology of *Chrysocharis pentheus*, an endoparasitoid wasp of the citrus leaf miner *Phyllocnistis citrella* Stainton. *J. Agr. Sci. Tech.*, 12: 145-154.

Romeldi, F., M.I. Schneider and A.E. Ronco, 2012. Short and long-term effects of endosulfan, cypermethrin, spinosad, and methoxyfenozide on adults of *Chrysoperla externa* (Neuroptera: Chrysopidae). *J. Econ. Entomol.* 105: 1982-1987.

Shareef, M.F., A. M. Raza., K. S. Ahmed, M. A. Ali and M.Z. Majeed, 2016. Efficiency of *Chrysoperla carnea* and *Trichogramma chilonis* against infestation of citrus leaf miner (*Phyllocnistis citrella* Stainton). *Academic J. of Ento.*, 9 (1): 14-19.

Smith, J. M. and M. A. Hoy, 1995. Rearing methods for *Ageniaspis citricola* (Hymenoptera: Eulophidae) released in a classical biological control program for citrus leaf miner *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). *Florida Entomol.*, 78: 600-608.

Snedecor G.W. and W. G. Cochran, 1980. *Statistical Methods*. The Iowa state Univ., press Amer., USA, 7th.

Steel R.G. and J. H. Torrie, 1980. *Principles and procedures of statistics A biometrical approach*. 2nd ed., Mc Graw Hill book company, New York, USA.

- Tawfik, M.F.S., M.S.I. El-Dakroury, I.A. Afifi, A.M. Ibrahim and F.M. Eid, 1996. Parasitic species secured from larvae and pupae of the citrus leaf miner, *Phyllocnistis citrella* Stainton (Gracillariidae: Lepidoptera) in Egypt. *Egy. J. Bio. P. Cont.*, 6(1):111.
- Urbaneja, A., A. Muñoz, A. Garrido, and J. A. Jacas, 2004. Which role do lacewings and ants play as predators of the citrus leaf miner in Spain. *Spanish J. of Agric. Res.*, 2 (3), 377-384.
- Wise, D.H., 1993. *Spiders in Ecological Webs*. Cambridge University Press, Cambridge; New York.

استخدام يرقات أسد المن كعامل من عوامل مكافحة البيولوجية لصناعة أنفاق أوراق الموالح.  
حسين سالم إبراهيم المسلاتي<sup>١</sup>، عادل عبد الحميد حافظ<sup>٢</sup>، فوزي فائق شلبي<sup>٢</sup> والحسيني السيد نوار<sup>٢</sup>  
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أجريت التجارب في مزرعة البساتين بكلية الزراعة بمشهر، جامعة بنها، مصر على شتلات البرتقال أبو سره والمطعمة على أصل فولكا ماريانا لدراسة تأثير إطلاق العمر الثاني ليرقات أسد المن (*Chrysoperla carnea*) على نسبة الإصابة بصناعة أنفاق أوراق الموالح بنباتات الموالح في المشتل. تم تقييم كفاءة استخدام أسد المن كعامل بيولوجي لمكافحة صناعة أنفاق أوراق الموالح. تم استخدام قفس خشب مسلك مقسم إلى ١٢ قفص وكل قفص أبعاده ٧٥ × ٧٥ × ٢٥٠ سم ومغطى بإحكام بقماش نايلون ناعم من كل اتجاه. تم اختبار ٣ معاملات بالإضافة للكنترول (Tr.1) يرقة واحدة - Tr.2 - يرقتان و Tr.3 أربع يرقات من العمر الثاني ليرقات أسد المن لكل شتلة). أوضحت النتائج أن إطلاق يرقات أسد المن على شتلات الموالح تسببت في انخفاض كبير لكل من المتوسط العام لعدد الأوراق المصابة والمتوسط العام لنسبة الإصابة بصناعة أنفاق أوراق الموالح. كانت المعاملة الثانية Tr.2 هي الأفضل في خفض المتوسط الموسمي لنسبة الإصابة بصناعة أنفاق أوراق الموالح في المشتل بمعدل ٤٣.٧٠% بينما كانت المعاملة الثالثة Tr.3 هي الأفضل في تقليل أعداد الأنفاق واليرقات والعداري والأوراق المصابة بمعدلات ٥٠.٨٦، ٧٨.٣٧، ٧٢.٤٧ و ٥٢.٦٨% بالترتيب. تسببت المعاملة المذكورة في خفض المتوسط العام لعدد الأنفاق واليرقات والعداري لصناعة أنفاق أوراق الموالح لتكون ٧.١٤، ١.١٤ و ٠.٦٨ لكل شتلة بالترتيب.