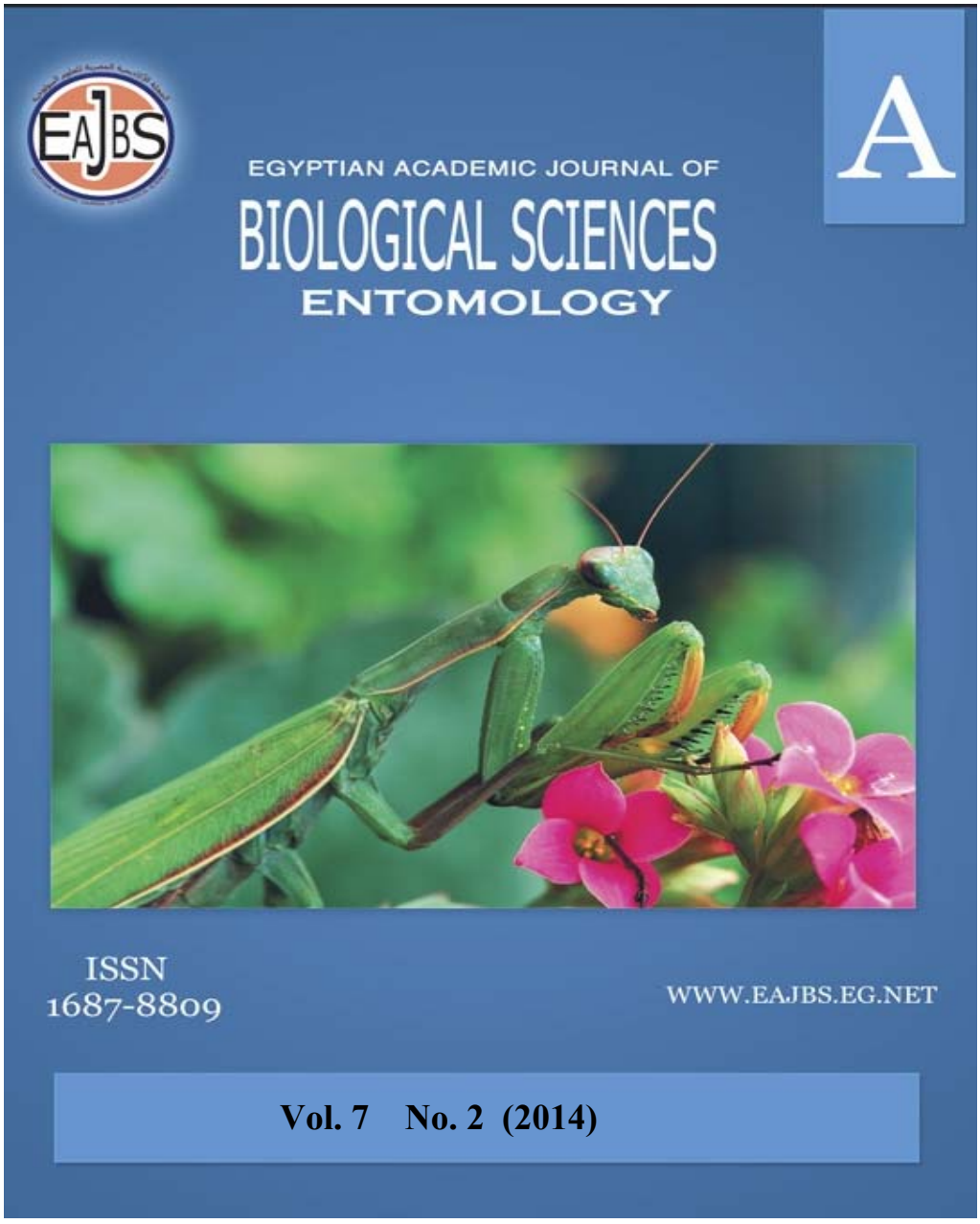

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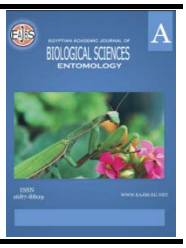

Effect of different larval instars of *Phyllocnistis citrella* Stainton on some biological aspects of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) under laboratory conditions

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ABSTRACT

Predatory potential of *Chrysoperla carnea* (Stephens) was studied on different larval instars of *Phyllocnistis citrella* (i.e. second, third and mixture of all larval instars) under laboratory conditions (26± 2ºC, 65±5% R.H, 16:8 L: D photo period).

Results revealed that feeding and rearing on different larval instars of *P. citrella* affected predator biology with different degrees. It had no significant effect on incubation period of *C. carnea* eggs females, pupation period, adult longevity and pre-and post - oviposition periods. On the other hand, it had significant effect on *C. carnea* larval period and its survival, female fecundity and eggs fertility. In general, third instar larvae of *P. citrella* was the most preferred prey for *C. carnea*. The results illustrate the potential importance of prey resources (life stage) on *C. carnea* population growth and indicate that *C. carnea* has considerable potential for the biological control of *P. citrella.*

INTRODUCTION

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) originated from South East Asia and has become a global pest of citrus, in many parts of the world. (Heppner and Dixon, 1995; Pena *et al*., 1996; Legaspi *et al*., 1999; Diez *et al*., 2006). *P. citrella* attacked all varieties of citrus, other Rutaceae plants, and several ornamental species (Heppner, 1993; Legaspi *et al*., 1999). These authors reported that females lay eggs on the leaves of host tree and eclosing larvae feed on the leaf epidermis ingesting sap and causing chlorosis and curled leaves. Larvae of *P. citrella* make characteristic serpentine mines under the leaf cuticle, which may reduce photosynthesis (Cook, 1988). Feeding tunnels produced by *P. citrella* larvae on citrus leaves may facilitate infection by the citrus canker bacterium, *Xanthomonas axopodis* pv. citri (Sohi and Sandhu, 1968; Cook, 1988; Gottwald *et al*., 1997). High population densities of *P. citrella* are usually recorded in spring and summer due to greater availability of leaf flushes and new shoots, as well as higher temperatures (Pena *et al*., 1996; Legaspi *et al*., 1999; Diez *et al*., 2006).

P. citrella is an important pest in citrus nurseries and top-grafted trees (Diez *et al*., 2006), and heavy infestation causes significant impact on growth and yield (Pena

et al., 2000; Browning *et al*., 2006).

Control of *P. citrella* is typically accomplished through multiple applications of conventional insecticides, which are often ineffective because the larvae are usually concealed within the mines which protected them from insecticide sprays (Legaspi *et al*., 2001).

Biological control is generally regarded as the most economically sound and environmentally sustainable management practice for *P. citrella* (Knapp *et al*., 1995; Hoy and Nguyen, 1997). Several predatory arthropods are known to feed on *P. citrella*, including lacewing larvae, ants, and hunting spiders (Argov and Rossler, 1996, Pomerinke, 1999; Amalin *et al*., 2001a, b; Xiao *et al*., 2007), and many of these studies have identified predation as the most important natural mortality factor acting on *P. citrella* in many parts of the world (Chen *et al*., 1989; Amalin *et al*., 1996, 2002; Hoy *et al*., 2007). Green lacewing, *Chrysoperla carnea* (Stephens) generally known as aphid-lion is the most intensively studied species of Chrysopids because of its wide geographical distribution, broad habitats with high relative frequency of occurrence, good searching ability to find prey, high resistance to commonly applied insecticides, have a high reproductive rate, have a short developmental time and easy rearing in the laboratory make it a valuable biological control agent (Khan *et al*, 2013). The larvae of lacewing feed on a wide range of pest species while adults are free living and feed only on nectar, pollen and honey dew (El-Serafi *et al*., 2000).

MATERIALS AND METHODS

The experiments were conducted on the biological parameters and predatory potential of *C. carnea* feeding on different immature stages of *P. citrella* (CLM) at Plant Protection laboratories, Faculty of Agriculture, Al-Azhar university, Nasr City, Cairo under controlled conditions of 26± 2ºC , 65±5% relative humidity and 16:8 L: D photo period .

Culture maintenance of the prey (*P. citrella*)

Infested citrus leaves were collected daily from citrus orchard of faculty farm (that contained different instars of *P. citrella* larvae) and transferred to the laboratory. Leaves were cleaned from any other pests and divided into three divisions: leaves contained different instars of *P. Citrella* larvae (mixed larval instars which contain of the first, second, third and fourth instars), leaves contained only the second instar larvae and the third division contained only the third instar larvae of *P. citrella*.

Predator, Lacewings (*C. carnea*)

Eggs were purchased from biological control laboratory, Faculty of Agriculture, Cairo University. Hatched larvae first, second and third instars of *C. carnea* were placed singly in Petri dish (9 x 2 cm) with the help of camel hair brush. In the bottom of Petri dish, a folded filter paper was placed to create shelters for larva. Known number of second, third and mixed of instars of *P. citrella* was introduced into each Petri dish for predator larval feeding. Consumption of each instar of *P. citrella* was recorded daily till completion of each larval instar of *C. carnea*. The experiment was replicated 30 times for each larval instar of *C. carnea*. After pupation, *C. carnea* male and female adults were placed in individual glass jars (12x 28 cm) with napiliras strips for egg laying. Number of eggs laid in each jar was recorded daily and transformed to separate petri dishes for fecundity and fertility percent(percent hatching).They were fed on artificial diet containing yeast + sugar + distilled water in ratio of 4:7:10.(Hagen *et al*., 1976).

All biological parameters including egg incubation, larval and pupal period (days), total food consumption, pupal and adult survival, longevity of male and female (days) pre, post and oviposition periods (days) and fecundity per female with percent fertility were recorded daily.

The period of time from egg laying to hatching was considered incubation period, from hatching till spinning of cocoon was designated the larval period and from cocoon formation and coming out from pupal case as pupal period. The time after emergence of adults and start of Oviposition was considered as pre-Ovipositional period, the period of egg-laying was considered oviposition and post oviposition period of female was recorded as period between the days of female ceased egg laying to the day of death.

Data collected on fecundity, fertility, incubation, larval instars, pupal period and other various aspects of predator biology were subjected to statistical analysis (one way analysis of variance ANOVA), (Cohrt Software 2004).

RESULTS AND DISCUSSION

Incubation period

The results listed in (Table 1) showed that the incubation period of eggs of *C. carnea* feeding on different larval instars of *P. citrella* was not significant. It was 3.40±0.89, 3.60±0.55 and 3.00±1.00 days on mixed larval instars; second and third instars of *P. citrella*, respectively. The minimum incubation period of 3.00 days was recorded for eggs laid by females emerged from larvae fed on third instar of prey. Sattar *et al*. (2011) reported that the incubation period of *C. carnea* was 2.25, 2.28, 2.36, 3.85, 2.25 and 2.80 days on *A. gossypii*, *P. solenopsis*, *S. cerealella*, *H. armigera*, *P. gossypiella* and mixed host diet, respectively.

Table 1: Mean developmental time and % survival of pre-imaginal developmental stages of *C. carnea* reared on different larval instars of *P. citrella.*

Prey instar	Developmental period of *C. carnea* (days).								
	Incubation period	1st larval instar	2nd larval instar	3rd larval instar	Total larval period	Larva survival %	Pupal period	Pupal Survival %	Total immature stages
mixed	3.40 ±0.89	6.42 ±1.38	5.58 ±0.67	7.92 ±1.50	19.91 ±1.73	70 %	9.33 ±1.08	78 %	29.00 ±2.45
2nd	3.60 ±0.55	6.00 ±1.04	4.67 ±0.89	5.58 ±1.24	16.25 ±1.54	66 %	9.08 ±0.49	62 %	25.58 ±1.73
3rd	3.00 ±1.00	4.83 ±0.83	4.17 ±0.83	4.00 ±0.74	13.00 ±1.54	80 %	9.08 ±1.16	92 %	22.08 ±2.02
LSD 5%	1.15	0.92	0.67	0.99	1.33	_____	0.79	_____	1.73

Larval and pupal period

The results indicated that larval developmental period of *C. carnea* feeding on different larval instars of *P. citrella* was significantly different. Duration of first larval instar of *C. carnea* was 6.42±1.38, 6.00 ±1.04 and 4.83±0.83 days, while for second instar was 5.58±0.67, 4.67±0.89 and 4.17±0.83 days and for the third instar was 7.92±1.50, 5.58±1.24 and 4.00±0.74 days, when they fed on mixed larval instars; second and third instars of *P. citrella*, respectively. The complete larval developmental period of the predator was 19.91±1.73, 16.25±1.54 and 13.00 ±1.54 days on mixed larval instars; second and third instars of *P. citrella*, respectively. The results indicated that feeding on the third instar larvae of the prey, the larval development of

the predator was faster (13.00± 1.54 days) and on the same time it's percent survival was much higher (80%). Thus, the larval food of the predator has significant effect on the length of its developmental period. This piece of result is in agreement with those obtained previously (Balasubramani and Swamiappan, 1994; Saminathan *et al*., 1999; Bansod and sarode, 2000; Liu andchen, 2001). For example, Liu and Chen (2001) determined the development, survival and predation of *C. carnea* on three aphid species, *A. gossypii, M. persicae* and *L. erysimi*. They found that *A. gossypii* was the better prey for *C. carnea* because the predator consumed high amounts of it with rapid development and high survival rate.

The pupal period of *C. carnea* (Table I) was significantly not affected by feeding on different larval instar of *P. citrella*. The cocoon period of *C. carnea* was 9.33±1.08, 9.08±0.49 and 9.08±1.16 days feeding on mixed larval instars; second and third instars of *P. citrella*, respectively. However, the pupal survival was much higher for those fed on the third instar larvae of the prey. Hamad *et al*., (2012) found that the pupal period was 6.92 days on eggs of moth and 6.0 days on aphids, while the emergence was 87.4% and 94.7% respectively.

Food consumption of different larval instars of *C. carnea.*

The data in the (Table 2) revealed the consumption rate of different larval instars of *C. carnea*. The third larval instar of *C. carnea* consumed an average of 66.60±5.55 of mixed larval instars of *P. citrella* during its life span followed by second instar (27.80±3.11) and first instars (23.20±4.44).Therefore, the third instar larvae of *C. carnea* consumed the largest number of prey in all life stages when compared to first and second larval instars of *C. carnea.* In other words, younger *C. carnea* larval instars were unable to attack older prey larvae.

Table 2: Food consumption of *C. carnea* larval instars on different larval instars of *P. citrella.*

Prey larval instars	Mean number consumed by *C. carnea*. larva				
	1st	2nd	3rd	total	LSD 5%
mixed	23.20±4.44	27.80±3.11	66.60±5.55	117.60±9.45	6.17
2nd	17.20±2.28	22.00±2.92	54.40±4.73	93.60±7.44	4.77
3rd	11.40±1.14	17.80±1.30	40.00±2.55	69.20±2.95	2.45
LSD 5%	4.07	3.55	6.14	9.85	___

This piece of result is in accordance with that obtained previously (Singh and Hamid, 1998, Singh and Manoj, 2000, Gautam and Tesfaye, 2002, BalaKrishnan *et al*., 2005, Huang and Enkegaard, 2010, Solangi *et al*., 2013). For example, singh and Manoj (2000) reported that the larvae of *C. carnea* consumed an average of 11.48, 79.52 and 83.00 aphids, *L. erysimi* during its first, second and third instar, respectively.

It was observed that lacewing larvae attacked randomly their prey in tender places such as abdomen or under the pronotum and could not pierce hard parts such as head or the sclerotized parts of the thorax. Same observation was reported by Sablon *et al*. (2013).

Results listed in Table (2) indicated that the consumption rate of third *C. carnea* larval instar was higher than that of first and second ones. There were many explanations for this: third instar larvae of the predator was larger in size and required higher volume of nutrients (Sattar *et al*., 2007), Cheng *et al*. (2009) observed that first

instar were less mobile than older lacewing stages, younger lacewing larval instars were unable to attack older *P. citrella* larvae probably because a young lacewing larvae was satiated without a total consumption of its prey (Sablon *et al*., 2013).

The present study allowed to quantify the predation behaviour of *C. carnea* according to prey and predator larval instar. The third instar lacewing larvae were the most voracious, as their prey consumption was higher than that of the two first instars.

Reproductive attributes of *C. carnea*

Data listed in (Table 3) revealed that, feeding of *C. carnea* larvae on different larval instars of *P. citrella* had no significant effects on its pre- and post-oviposition period. The average longest oviposition period of *C. carnea* females was 32.00 ±2.92 days when fed on third larval instar of *P. citrella*. The maximum mean adult male and female longevity was recorded for *C. carnea* feeding on third larval instar of *P. citrella* as a prey. However, there was no significant effect of feeding on different larval instars of the prey on predator adult longevity. The maximum mean fecundity per female of *C. carnea* was 14.40±1.52 eggs/ day recorded when fed as larvae on third larval instar of *P. citrella* followed by 11.00±1.58 eggs/ day on mixed larval instars, whereas, the minimum of 9.00 ±1.58 eggs/ day was recorded when fed on second instar of *P. citrella*.

Table 3: Effect of different reproductive attributes of *C. carnea* feeding on different larval instars *P. citrella* under laboratory conditions.

	Reproductive traits of *C. carnea*.							
Prey instars	Pre-Ovipositio n period (days)	Oviposi tion period (days)	Post ovipositi on period (days)	Fecundity (egg/female /day) ±SE	Fertility (fertile egg / day) (%)	Male longevity (days)	Female longevity (days)	Sex Ration % M:F
mixed	6.60 ±0.55	27.40 ± 2.07	7.60 ±1.14	11.00 ±1.58	81	36.00 ±2.55	41.60 ±3.13	1:1
2nd	7.20 ±0.84	27.60 ±2.70	6.60 ±1.34	9.00 ±1.58	79	34.40 ±2.30	41.40 ±2.70	1:1
3rd	6.40 ±1.14	32.00 ±2.92	6.60 ±1.40	14.40 ±1.52	86	36.80 ±0.84	45.00 ±2.55	1:1
LSD 5%	1.21	3.57	1.67	2.14	___	2.81	3.86	___

Higher fertility of eggs (% hatching) of *C. carnea* was recorded when fed on third larval instar of *P. citrella* (86 %) as larval prey followed by mixed larval instars diet (81 %). The sex ratio of *C. carnea* feeding on different instars was not affected. Sattar *et al*. (2011) reported that, the maximum fecundity were recorded when *C. carnea* was reared on *S. cerealella* eggs, while minimum fecundity were recorded for those fed on *P. gossypiella* eggs. Sarwar *et al*., (2011) recorded that, fecundity, fertility, pupation, hatchability and longevity of *C. carnea* were higher when reared on aphids followed by the pink and spotted bollworms eggs. Mannan *et al*. (1997) observed that mean fecundity of *C. carnea* was about 84.70% on *Aphis gossypii*. Jokar and Zarabi (2012).found that the fertility rate of *C. carnea* was higher when fed on aphid diet than on whitefly diet.

In conclusion, the results obtained from the present work indicated that there is a potential for the use of lacewing larvae as biological agents to control CLM immature stages but further studies should be conducted to observe if predator larvae move and find easily CLM immature stages; to estimate the predatory impact in field conditions; and to observe the lacewing choice between CLM and any alternative prey. These results should make citrus entomologists consider conservation biological

control not as a mere complement of necessary classical biological control programs, but as the cornerstone of future Integrated Pest Management in citrus systems.

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ARABIC SUMMERY

تأثير الأعمار اليرقية المختلفة لصانعات أنفاق أوراق الموالح على بعض النواحي البيولوجية لأسد المن تحت الظروف المعملية.

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تم دراسة الكفاءة الإفتراسية لأسد المن على أعمار يرقية مختلفة من صانعات أنفاق أوراق الموالح (العمر اليرقى الثانى والثالث وخليط من الأعمار اليرقية) تحت ظروف المعمل (٢٦±٢ م ˚، 65± 5 رطوبة نسبيه 16ساعه ضوء : 8 ساعة ظلام) وبينت النتائج أن تغذية وتربية أسد المن على الأعمار اليرقية المختلفة لصانعات أنفاق أوراق الموالح أثرت بيولوجيا بدرجات مختلفة. فكان له تأثير غير معنوى على فترة حضانة البيض لإناث أسد المن، فترة العذراء، طول عمرالحشرات الكاملة وفترة ما قبل و بعد وضع البيض .من ناحية أخرى، كان له تأثير معنوى على مدة الطور اليرقى لأسد المن وبقائها، كفاءة الإناث على وضع البيض وخصوبة البيض بشكل عام وكان العمر اليرقى الثالث من صانعات أنفاق الموالح الفريسة الأكثر تفضيلا لأسد المن كما أوضحت النتائج أهمية المرحلة العمرية للفريسة على النمو التعدادى لأسد المن وعلى هذا فإن أسد المن يعتبر ذوكفاءه عالية فى المكافحة البيولوجية لصانعات أنفاق أوراق الموالح.