

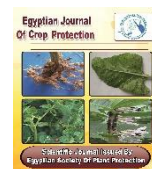


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Assessment of Two Biological Control Agents and Insecticide on *Spodoptera littoralis* (Boisd.) Under Laboratory Conditions

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ABSTRACT

Two natural insect enemies, *Chrysoperla carnea* (Stephens) and *Trichogramma evanescens* (Westwood) and one insecticide, Profenofos, were assessed against the cotton leafworm, *Spodoptera littoralis* (Boisd.) under laboratory conditions. The first, second and third larval instars of *C. carnea* consumed 35.53 ± 7.82 , 35.82 ± 3.21 and 701.75 ± 135.6 eggs of *S. littoralis*, respectively. The second and third instar larvae consumed 166 ± 39.1 and 729.91 ± 120.50 larvae of *S. littoralis*. The feeding capacity of second larval instar of *C. carnea* on eggs and larvae of *S. littoralis* differed significantly. *T. evanescens* parasitism was 71.05%, also the longevity of females and females percentage were 3.19 days and 39.00%, respectively. Oleander leaves, *Nerium oleander* infested with *S. littoralis* eggs were treated by dipping in 5 concentrations of profenofos, and LC_{50} , LC_{90} were 39.19 and 639.681 ppm after 4 days from treatment. Moreover, The results showed that eggs mortality percentage were 43.19, 67.19, 75.20, 85.20 and 85.61% as a result of profenofos application with different concentrations 37.5, 75, 150, 300 and 600 ppm, respectively, compared with those of untreated eggs, (16.66%). This study reveals the importance of mass-rearing of biological control programs of *C. carnea* and *T. evanescens* to be successfully used in control of *S. littoralis*, and thus, the use of insecticides could be minimized or avoided.

Key words: *Spodoptera littoralis*, biological agents, Profenofos.

INTRODUCTION

Cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) has long been established in Egypt as a major pest of cotton as well as of several other field crops. The skeletonized of leaves due to larval feeding causes a reduction in photosynthetic potential. Although this insect is essentially a leaf feeder, it can also

attack squares and small bolls. *Spodoptera* species is economically important in many countries and developed resistance to many chemical insecticides (Senthil-Nathan, 2013). Biological agents are safe to the environment and can suppress harmful insects. The green lacewing, *Chrysoperla carnea* (Stephens) is generally of a wide range of pest species like mealybugs,

aphids, thrips, whiteflies mites and eggs of several insect pest species (Carrillo and Elanov, 2004). Egg parasitoid species of the *Trichogramma* genus are used worldwide as a biological control agent (Senthil-Nathan *et al.*, 2006; Edwin *et al.* 2016), and attacks the eggs of over 200 insect species (Orr *et al.*, 2000, Wright *et al.*, 2002, Mansfield and Mills, 2004). In this study, the efficacy of green lacewing, *Chrysoperla careneae*, (Steph.) against eggs and first instar larvae of *Spodoptera littoralis* and effects of egg parasitoid, *T. evanescens* (Westwood) (Hymenoptera:Trichogrammatidae) as bio-control agent on *S. littoralis*, eggs was studied. In addition to determine *Spodoptera littoralis* susceptibility to one synthetic insecticide, profenofos using leaf dip method under laboratory conditions.

MATERIALS AND METHODS

This study was carried out at Pesticide Testing Research Department, Plant Protection Research Institute, Sakha Agriculture Research Station, Kafr El-Sheikh Governorate, Egypt, under the laboratory conditions.

Cotton Leafworm *S. littoralis* rearing.

The culture of *S. littoralis* (Boisd.) was obtained from Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The colony was reared under constant conditions of $25 \pm 2^\circ \text{C}$ and $65 \pm 5\%$ R.H. with a photoperiod of 14: 10 (L: D) h). *S. littoralis* rearing was conducted following the technique of Dahi (1997). Egg masses were collected and kept in glass jars (500 ml) covered with gauze till larvae hatching and provided daily with fresh castor bean, *Ricinus communis* leaves. Third instar larvae (6 day old) were transferred to glass jars (one liter) with the same food. The pre pupae was transferred

to jar consisting of 3 cm height dry sawdust until pupate. The resulting pupae were transferred to glass jars containing wet filter papers and preserved in cage (35×35×35cm) for adult emergence and mating. Emerging moths were fed on 10 % sugar solution through a dipped piece of cotton. The cages were supplied with branches of oleander, *Nerium oleander* L. to serve as oviposition sites.

Feeding capacity of *Chrysoperla carnea* larvae

Eggs of *C. carnea* were acquired from Biological Control Laboratory, Faculty of Agriculture, Cairo University. To study the feeding capacity, newly hatched larvae of *C. carnea* were moved out by soft and moist brush, singly, in 12 plastic jars (4×7×8 cm) each jar has 50 prey eggs, *S. littoralis*. Number of eggs was increased daily as follows: 100, 150, 200, 350, 500 and 1000 eggs and jars were covered with gauze and kept in position by rubber bands, the same technique was followed with introducing one per 30 of 1st larvae instar of *S. littoralis* and number of larvae increased daily as follows: 50, 150, 200, 350, 1000 and 1050 larvae. Jars were kept under laboratory conditions of constant temperature ($26 \pm 2^\circ \text{C}$ and $65 \pm 5\%$ R.H). The consumed number of eggs or larvae of *S. littoralis* was counted every 1 day and the prey food in each plastic jar was supplied daily, with eggs and larvae of *S. littoralis*. Thus, there was a plenty of food available until the predator completed its larval development. The predation capacity of each larval instar and larval duration of *C. carnea* were recorded.

Parasitism capacity of the egg parasitoid *T. evanescens* on *Spodoptera littoralis* eggs

Four cards (1×1cm) containing prepupa of *T. evanescens* reared on *Sitotroga cerealella* eggs obtained from

Trichogramma Mass Rearing Unit, Plant Protection Research Institute, Dokki, Giza. Four glass jars (500 ml) were used, in each jar one card of *T. evanescens* was introduced with 1000 removed eggs scales of *S. littoralis*. The egg masses were replaced every 24 hrs to avoid super-parasitism and the parasitized egg cards from each replicate was collected and saved in modern glass jars under constant temperature (26±2°C and 65±5% R.H).

Biological parameters Parasitism percentage was assessed by counting the number of parasitized eggs (blackened eggs)/ total no. of eggs exposed). The percentages of adults emerged were calculated according to:

$$\text{Percentage of Emergence} = \frac{\text{Number of } T. \text{ evanescens emerged}}{\text{Number of parasitized eggs}} \times 100$$

Females' percentage was calculated by check dead adults under a microscope (No. of emerged adult females/total individuals). The longevity of adults was listed daily by memorizing 4 cards each card containing (1/2x1/2cm) of newly emerged *T. evanescens* adults without any previous oviposition with 700 eggs of *S. littoralis* for each replicate into glass jars (250 ml) till parasitoid mortality. For parasitoid nutrition, few droplets of honey solution were supplied daily till the wasps die.

Effect of profenofos on hatching % of *S. littoralis* eggs

The insecticide Profenofos (Sylian 72% EC) was provided by Kafr El Zayat for pesticides and Chemicals Company and tested at the rate of 750 cm³/feddan. Stock solution for every tested concentration was designed as aqueous solution and diluted serially by water to procure five gradual concentrations 37.5, 75, 150, 300 and 600 ppm.

Insecticide efficiency of Profenofos against of *S. littoralis* eggs

Egg-masses of constant age (zero to one day old) were obtained next to beginning oviposition in the rearing settlement. The top layers of egg-masses were extracted softly under the binocular so as to calculate the number of eggs in the lasting bottom layer, which was split into sections, each include of 100 eggs. Three replicates were used for each concentration, one egg-masses/replicate. The leaves of oleander with eggs (zero to one day old) were imerged for 5 seconds in each concentration. The treated egg-masses were air-dried on paper towel and then placed in petri-dishes of 15 cm diameter (one egg- mass/dish). The control treatment was imerged with water-treated oleander leaves only. All treatments were kept under controlled cases of 25±2 °C and 65±5% RH for hatching. Inspection was made for two successive days after treating oleander leaves with water. Once all eggs in the control experiment had hatched out, the eggs in treatments were observed under binocular and the rate of hatching was recorded. Unhatched percentages of eggs representing eggs mortalities were corrected according to Abbott's formula (**Abbott 1925**) as follows:

$$\text{Corrected Mortality \%} = \frac{(\% \text{Mortality of treated insects} - \% \text{Mortality of control})}{(100 - \% \text{Mortality of control})} \times 100$$

Statistical analysis

The data were statistically analyzed using SPSS version 16 to determine differences in prey density by t-test. Results of insects number are reported as means ±SE.

RESULTS

The predation capacity of different larval instars of *Chrysoperla carnea*

Results indicated that the consumption capacities of first, second and third instars

C. carnea were 35.53 ± 7.82 , 35.82 ± 3.21 and 701.75 ± 119.6 eggs of *S. littoralis*, respectively, and feeding percentage of third larvae instar was (90.77 %). Larvae consumed a total of 773.10 eggs during their developmental period. The durations of first, second and third larval instars were 3.58 ± 0.37 , 2.66 ± 0.28 and 7.58 ± 1.37 days; respectively (Table1). The results indicated that the first instar larvae of *C.*

carnea did not feed on *S. littoralis* larvae. The 2nd larval instar consumed 166 ± 39.1 and the 3rd consumed an average of 729.91 ± 120.5 larvae and feeding percentage was (81.5%). The eggs and larvae of *S. littoralis* consumed by 2nd larval instars of *C. carnea* differed significantly when using the *t*-test at the 5% significance level.

Table 1: Predation efficiency and larval duration of *Chrysoperla carnea* larvae reared on *Spodoptera littoralis* eggs and larvae.

Prey Type	(Mean ±S.E) % of consumed 3 larval instar						Total No. consumed eggs or larvae	Duration days ± S.E			Total
	1 st	%	2 nd	%	3 rd	%		1 st	2 nd	3 rd	
Eggs	35.53 ±7.82	4.60	35.82 ± 3.21	4.63	701.75 ± 119.6	90.77	773.10	3.58 ±0.37	2.66 ±0.28	7.58 ±1.37	13.82
Larvae	-		166 ± 39.1	18.55	729.91 ±120.50	81.5	895.91	2.0 ± 00	3.92 ±0.35	6.41 ±0.78	12.33
<i>t</i>			3.33		0.144			3.97	2.74	0.738	
<i>df</i>			22	-	22		-	22	22	22	
<i>p</i>			0.007	-	0.88		-	0.002	0.012	0.47	

P < 0.05 for a significant difference and P < 0.01 for a highly significant difference

Parasitism of *Trichogramma evanescens* on *Spodoptera littoralis* eggs

The *T. evanescens* wasps were susceptible to parasitize the *S. littoralis* egg masses without scales (Figure 1). Data presented in Table (2) show that *S. littoralis* eggs parasitism was (71.05%). The results indicated that adult emergence and females percentage adults were 61.0 and 39.0%. Pre-oviposition period lasted for 15.0 hours, oviposition period for 1.15

day and post-oviposition period for 1.13 day. Female longevity was 3.19 days. These results are similar to those of Atta (2014) who recorded sex ratio, longevity and life cycle of *T. evanescens* reared on *S. littoralis* eggs was 46.5%, 3.75 days and 9.75 days, respectively in case of eggs with the scales. In case of eggs without scales, the corresponding values were mostly higher; 59.5%, 3.87 days and 9.50 days.

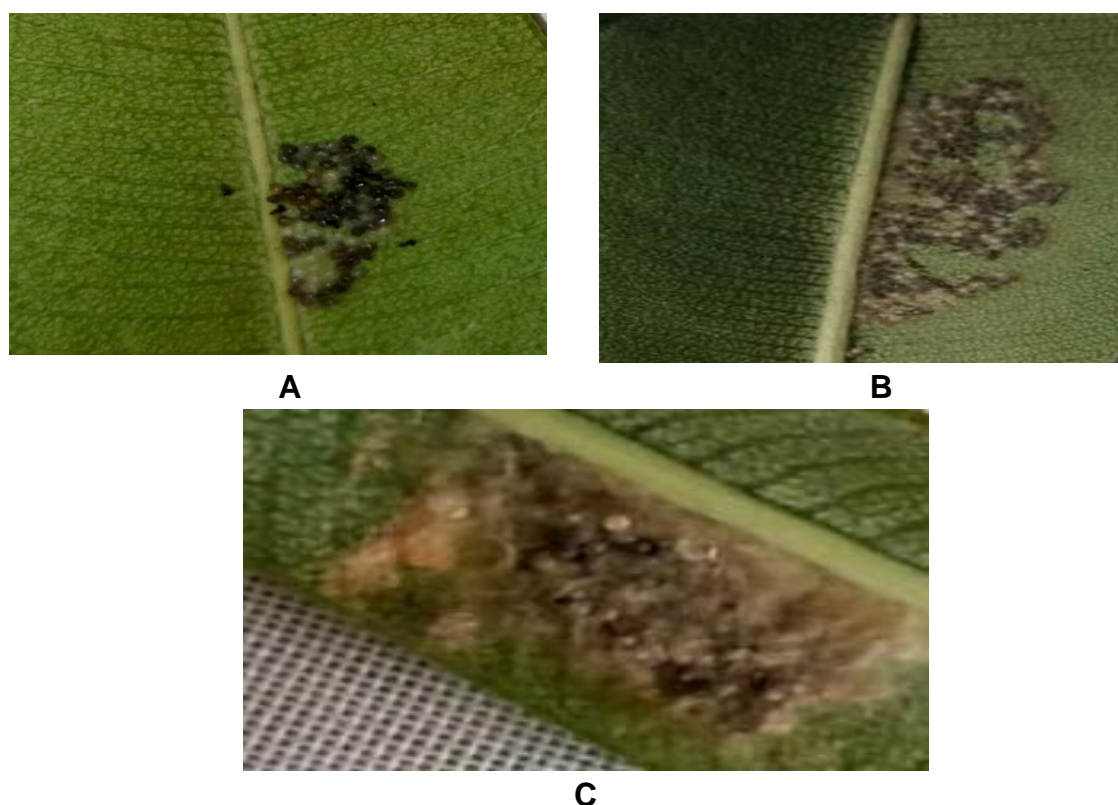


Fig.1 Parasitism of *T. evanescens* on *S. littoralis* egg masses (A and B) compared with unparasitized eggs (C)

Table 2. Means of parasitized eggs, Parasitism%, adult emergence, females percentage and adult longevity (days) of *Trichogramma evanescens* reared on *S.littoralis* eggs without scales.

Parameter	Mean(\pm SE)
No. of parasitized eggs	710.5 \pm 3.25
Parasitization %	71.05
Parasitoid adult emergence%	61.0 \pm 1.01
Females percentage	39.0 \pm 1.42
sex ratio (female:male)	1:1.96
Pre-oviposition (hr.)	15.0 \pm 0.57
Oviposition period (days)	1.15 \pm 1.52
Post oviposition period (days)	1.13 \pm 0.13
Female longevity (days)	3.19 \pm 2.1

Ovicidal activity of Profenofos against eggs of *Spodoptera littoralis*

The toxicity of profenofos (sylian 72% EC) was tested against eggs of *S. littoralis*. LC₅₀ and LC₉₀ values of Profenofos for ovicidal action against *S. littoralis* after 4 days were 39.19 and 639.681 ppm (Table 3). The results indicated that the corrected mortality % of eggs were 43.19, 67.19, 75.20, 85.20 and 85.61% at concentration of profenofos 37.5, 75, 150,300 and 600 ppm, respectively, compared with those of untreated control, 16.66%. Also, the percentages of egg hatchability 4 days post-treatment were 47.33, 27.33, 20.67, 12.33 and 11.33% at the same concentrations respectively, compared with control (83.33%).

Table 3. Effectiveness of, profenofos use with dipping technique on zero to one day old egg-masses of cotton leafworm, *S. littoralis*.

Treatment	Conc. (ppm)	Hatchability after 4 days	% of egg mortality	% of corrected mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)	Slope ± SE
Profenofos	37.5	47.33	52.67	43.19	39.19	639.681	1.056 ±0.15
	75	27.33	72.67	67.19			
	150	20.67	79.33	75.20			
	300	12.33	87.67	85.20			
	600	11.33	88.67	85.61			
Control	-	83.33	16.66	00.00			

The LC₅₀ value was calculated for total mortality by Microsoft® office Excel (2007), according to **Finney (1971)**.

DISCUSSION

The recorded data revealed that the second and third instar larvae consumed 35.53 ± 7.82 , 35.82 ± 3.21 and 701.75 ± 135.6 eggs of *S. littoralis*, respectively, Larvae consumed a total of 773.10 eggs through developmental period. The feeding capacity of second larval instar of *C. carnea* on eggs and larvae of *S. littoralis* differed significantly. These results are in agreement with the findings of Megahed *et al.* (1982) who found that the larvae of *C. carnea* increased their prey consumption in the laboratory as they grew older and the total number of prey consumed per larva during development averaged 553.5 eggs of *Ephestia kuehniella* Zeller and 536.2 eggs of *S. littoralis*. Talha (2001) observed that the first instar larvae of *C. carnea* did not feed on first instar larvae of *Cassida vittata* Vill. Also, Farag (2005) reported that the total number of prey consumed by the three larval instars of *C. carnea* were 19.00, 77.00 and 329.50 eggs of *S. littoralis* lasted for 2.25, 2.05, and 4.05 days, respectively. The third

instar was the most efficient as it consumed 77.44% of total number of consumed eggs, in addition, first instar larvae did not feed on first instar larvae of *C. vittata* insects.

Tavares *et al.* (2011) showed that the period of the larval stage of *Chrysoperla externa* was identical when fed on modern laid or 1 day-old *Spodoptera frugiperda* eggs, or *Ephestia kuehniella* eggs. *Chrysoperla externa* could not be successfully breed on one- or two-day old *S. frugiperda* larvae, but could on eggs of both prey and 1st instar larvae of *E. kuehniella*. Moreover, Rabinder *et al.* (2008) indicated that larval duration of *C. carnea* was 8.25 and 22.15 days on *Corcyra cephalonica* eggs and *Phenacoccus solenopsis* nymphs respectively, under laboratory conditions. *C. carnea* larvae consumed a significantly higher number of *P. solenopsis* nymphs (671.45) than *Corcyra cephalonica* eggs 211.70. On the other hand, Hassan (2016) mentioned that period of the 1st, 2nd and 3rd larval instars of

C. carnea were 4.27 ± 0.13 , 4.86 ± 0.17 and 5.50 ± 0.16 days respectively, when reared on eggs of *S. littoralis* at 25°C. Also, total larval period of *C. carnea* lasted for 14.63 ± 0.34 days. The 1st, 2nd and 3rd larval instars consumed 86.45 ± 5.13 , 336.19 ± 5.31 and 413.83 ± 13.67 eggs, respectively. *C. carnea* consumed 836.47 ± 24.11 eggs during their larval stage. For the efficiency of *T. evanescens* on *Spodoptera littoralis* (eggs), the results indicated that the parasitism percentage by *T. evanescens* wasps (71.05%), this percentage was high as compared to those observed under field conditions by Greenberg *et al.* (1998) for *T. pretiosum* and *Trichogramma maidis* wasps on *S. exigua* egg masses, with values of 36.8% and 34.5%, respectively. These results are in agreement with the results of Siam *et al.* (2019) reported that the percentage of parasitism, % females and female longevity (days) of *T. evanescens* were 96.44%, 70.04 and 4.36 on eggs of *Sitotroga cerealella* Olivier. Edwin *et al.* (2016) revealed that the mean percentage of parasitism on *S. litura* eggs was 74.0 by *Trichogramma chilonis*, and the average percentage of adult emergence was 64%. On the other hand, the parasitism rate by *T. chilonis* on *S. litura* eggs recorded 80.31% (Puneeth and Vijayan 2013). The percentage parasitism of *Trichogrammatoidea bactrae* observed on one-layer eggs of *Spodoptera littoralis* was 70.73 %, adults' emergences % and females

emerged % were 85.54% and 60.33% (Mohamed 2021). Eggs of *Spodoptera littoralis* mortality percentages as a result of ovicidal treatment with profenofos (silian 72% EC) were 43.19, 67.19, 75.20, 85.20 and 85.61% at concentration of Profenofos 37.5, 75, 150, 300 and 600 ppm, respectively, compared with those of untreated control, 16.66%. The percentages of hatchability 4 days post-treatment were 47.33, 27.33, 20.67, 12.33 and 11.33% at the same concentrations respectively, compared with control (83.33%). These results are in agreement with the findings of Abou-Taleb (2010) who found that treatment of 0-24 h *S. littoralis* eggs by Chlorpyrifos and methomyl at 10 ppm caused 80.4 and 83.6% mortality, respectively. Also, spinosad, spinetoram and emamectin benzoate at the same concentrations caused 18.9, 19.4 and 28.1% mortality of egg, respectively. Sherby *et al.* (2010) registered 95.8 and 82.6% mortality of eggs and new hatched larvae by spinosad and chlorpyrifos at 10 ppm, respectively. Venkateswari *et al.* (2008) reported that the LC₅₀ values of abamectin and emamectin benzoate for ovicidal to control one day old egg of *S. littoralis* by dip method were 2.0 and 0.1 µg ml⁻¹. Insecticide of rynaxypyr was the most effective on eggs of *S. littoralis*, with 100% toxicity index followed by indoxacarb (13.52%) then methoxyfenozid (5.31%) (Abd el aziz and sayed, 2014). Elgohary (2014) found that lufenuron was the more toxic against *S. littoralis* eggs at sub-lethal concentration LC₅₀ (71.3, 187.6

and 233.3 ppm, respectively). Also, flufenoxuron showed modest effect and chlorfluazuron was the least toxic. However, the field recommended rates of the tested IGR's caused reductions in the hatchability of *S. littoralis* eggs by 72.3, 70.6 and 65.9 for flufenoxuron, chlorfluazuron and lufenuron, respectively compared with control (84.3%).

Conclusion

According to the current results, Larvae of *Chrysoperla carnea* consumed a total of 773.10 eggs or 895.91 Larvae of *S. littoralis* during their larval period, the feeding capacity of 2nd larval instar of *C. carnea* on eggs and larvae of *S. littoralis* differed significantly. The parasitism percentage by *Trichogramma evanescens* on eggs of *S. littoralis* was (71.05%). As indicated by the results of this study, each of *C. carnea* and *T. evanescens* were able to reduce the populations of cotton leafworm, *S. littoralis* in laboratory tests. Thus, it is possible to use a joint application of both, biological agents, and thus, use of insecticides could be minimized or avoided.

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