



Efficacy of Releasing *Chrysoperla carnea* (Stephen), Against *Spodoptera Littorals* (Boised.) On Cotton Field Conditions

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Abstract

Evaluation the efficiency of *Chrysoperla carnea* (Stephen) second instar larvae was used in an experiment at the Qaha Agricultural Research Station in Qalyubia governorate in reducing the population of cotton leafworm *Spodoptera littorals* (Boised.) on cotton plants. During the 2021 and 2022 cotton seasons artificial infestation with the cotton leafworm *S. littoralis* was carried out inside tightly closed cages, where ten plants were randomly selected from each replicate. The infestation rate was determined by examining and determining the average number of *S. littoralis* larvae inside the cage to calculate the numbers required to release *C. carnea* for the different release levels. The *C. carnea* second instar larvae were released on the plants, at three rates (two, three, and four larva per meter square of cage) and a total of 3 releases were made at 14 days interval. In the first season 2021, the release of *C. carnea* showed the highest biological control activity fourteen days after the third release, resulting in a significant reduction in the numbers of *S. littorals* larvae. Our results demonstrated that the release of 2nd instar larvae of *C. carnea* at the three different rates of 2, 3, and 4 larvae/m² induced a general reduction in *S. littoralis* larvae populations compared to the control treatment. The statistical analysis revealed that the efficiency of 2nd instar larvae of *C. carnea* was more evident when released at 4 larvae/m² as a biocontrol agent against *S. littoralis* larvae attacking cotton plants under cage. Therefore, it gave the highest overall mean percentage of reduction in *S. littoralis* larvae populations (64.91%) in the first season and 56.53% in the second season.

Keywords: Chrysopidae, evaluation, cotton leafworm, biological control, *Spodoptera littorals*, release.

Introduction

The Egyptian cotton leafworm, *Spodoptera littorals* (Boisd.) (Lepidoptera: Noctuidae), has long been known in Egypt as a major pest of cotton as well as many other field crops. The cotton leafworm feeds on the underside of leaves between the veins, which leads to a decrease in photosynthesis. Although this insect feeds mainly on tree leaves, it can also attack small bolls. When this insect pest is abundant, it generally eats all the leaves of the plant and then crawls into adjacent fields. Therefore, it has been the target of many studies due to its economic importance and the ease with which its population can acquire the ability to tolerate pesticides according to (El-Deeb and Nasr, 1980; Hosni *et al.*, 1986 and Mustafa, 1993). Cotton production is negatively affected by pests, which are traditionally controlled using large amounts of pesticides. Although chemical insecticides are effective, after a period of time resistance to some insecticides appears, and this requires the use of an increasing and diverse amount of insecticides, which have many harmful effects, such as environmental pollution and

other health damages. Risks (Muzammil and Ghulam, 2011) the concept of Integrated Pest Management (IPM) has become popular because it encourages the use of several components in IPM systems, which have a less harmful impact on the environment. (Solangiet *al.*, 2013). The use of *C. carnea* is a recognized bio agent and alternative to insecticides, and numerous studies have demonstrated the effectiveness of *C. carnea*, which is an effective predator against whiteflies, thrips, aphids and mites, and also feeds on the eggs and larvae of bollworms and cotton leafworm according to (Abu Zaid, 2003).

C. carnea larvae are reported to infect approximately 80 victims and 12 control tetrad moths. It is not predatory and can be raised easily. Mass rearing of parasitism, predators is a prerequisite for any successful biological control programme, but this is impossible without the use of a standard host. *C. carnea* is has been extensively bred on rice grain moth (*Corcyra Cephalonia*) eggs, but has good feeding capacity on various insect pests of cotton. Therefore, the current work aimed to release

the Chrysopid, *C. carnea* larvae for controlling *S. littoralis* larvae on cotton field under cage

Materials and Methods

Field experiments were conducted on cotton plants infected with the cotton leafworm *S. littoralis* during the two successive agricultural seasons of 2020 and 2021.

Mass rearing of *S. littoralis*

The cotton leafworm *S. littoralis* was raised in the same laboratory mentioned above. *S. littoralis* instars were obtained from the Cotton Leafworm Research Department, Plant Protection Research Institute, placed in glass jars (1 L capacity) and fed on castor leaves *Ricinus communis* (L.). Larval food was renewed daily until they pupated. After pupation, the pupae are harvested and every 5 pairs are trapped in an egg-laying cage with a glass chimney, and after the moths emerge, a piece of cotton previously soaked in a 10% sugar solution is hung to be renewed every 24 hours for the moths. Feed. Each jar was fitted with a small branch of the Nerium oleander plant to serve as a suitable site for laying eggs. Daily deposited *S. littoralis* egg masses were used for experimental purposes (*S. littoralis* eggs).

Experimental design:

The seeds of the cotton 'Giza 94' cultivar were sowing on area of five karats (875 m²) on March 15 in the two seasons. In the first and second growing seasons, the previous crop was alfalfa. The experiment was conducted using a randomized complete block design.

Predator source:

The predator larvae were purchased from "Chrysopa mass rearing unit" at Faculty of Agriculture, Cairo University.

A field study was conducted to evaluate three different release levels of second instar larvae of the insect predator *C. carnea* (2 larvae/m², 3 larvae/m², and 4 larvae /m²). Cotton plants grown in an area of 54 m² were covered with a white net under a cage of 6 x 3 x 1.5 m. The covered area was divided into three equal parts, each part being a launching level isolated from the other with a mesh barrier and divided into 8 lines; each part (3 x 6 m) contains 200 plants. Another separate cage was designed for comparison. All plots received the normally recommended agricultural practices. One week before predator release, cotton plants inside the cage were sprayed with lanite pesticide to kill any pests or predators present on the cotton. Artificial infestation with cotton leafworm was performed by placing 15 pieces of egg *S. littoralis* inside each of the three sections of the cage, with 15 pieces also placed in the control cage. After egg hatching the average number of larvae inside the cage was count.

Treatment protocol

The second instar larvae of the predator were released three times on the 5th, 19th of June, and 3rd of July 2021, and during the second season in 2022. Releasing 2nd instar larvae of *C. carnea* at three rates (two, three, and four larva per meter square of cage) and a total of 3 releases were made at 14 days interval. Samples comprised of ten plants per plot of each treatment (3 leaves per plant in three levels: upper, middle, and lower portions) were diagonally collected at random along different sampling intervals four times for each release.

The present study aimed to evaluate the efficacy of releasing the indigenous *C. carnea* larvae for controlling cotton leafworm *S. littoralis* infesting cotton plants in open field trials under cage.

The percentage reduction in *S. littoralis* infestation was adopted according to **Henderson and Tilton equation (1955)**.

Ta X Cb

% Reduction = 100 {1 - -----}

Tb X Ca

Where:

Ta = Number of larvae in treated plots after treatment

Tb = Number of larvae in treated plots before treatment.

Ca = Number of larvae in check plots after treatment.

Cb = Number of larvae in check plots before treatment.

Results and Discussion

The second larval instar of *Chrysoperla carnea* was released at three rates (two, three, and four larva per meter square of cage) to control the cotton leafworm on cotton plants, for three times at an interval of 14 days, on the 5th of June, 19th of June and 3rd of July during the 2021 and 2022 seasons.

In the first season 2021, data in Table (1) clearly indicate that the tested rates of *C. carnea* release differ in their effectiveness in the biological control of *S. littoralis* on cotton plants. There was no significant difference between the three levels of release and control before treatment. The numbers of cotton leafworm *S. littoralis* larvae on cotton plants decreased in the different treatments compared to the control. The average number of *S. littoralis* was 14.63 and 12.83; 10.90 and 9.10; 9.20 and 7.50 larvae per 30 cotton plants after 7 and 14 days of application at rate of 2, 3, and 4 larvae/m², respectively. However, the average *S. littoralis* larvae were 49.47 and 51.00 after 7 and 14 days of application in untreated (control), respectively. Statistical analysis revealed the three release treatments were not statistically different; however, they were significantly better (P < 0.0001) than the untreated control, wherein a mean *S. littoralis* population of 50.23 larvae / 30 plants was recorded (Table 1).

Table 1. Mean counts of cotton leaf worms larvae infesting cotton plants in the natural field before and after releasing *Chrysoperla carnea* 2 instar larvae at different rates at the Qaha Agricultural Research Station, Qalyubia Governorate, during the 2021 cotton season.

| Predators/m ² | Treatment | Pre-treatment <i>S. littoralis</i> / 30 plants | Mean number of <i>S. littoralis</i> / 30 plants at the indicated day after treatment | | |
|--------------------------|-------------------------|--|---|----------------|----------------|
| | | | 7 days | 14 days | General mean |
| 2 Larva/m ² | 1 st Release | 47.60 | 25.50 | 22.10 | 23.80 |
| | 2 nd Release | 22.10 | 12.70 | 11.40 | 12.00 |
| | 3 rd Release | 11.40 | 5.70 | 5.00 | 5.30 |
| | Mean | 27.03 a | 14.63 b | 12.83 b | 13.70 b |
| 3 Larva/m ² | 1 st Release | 48.90 | 21.10 | 17.10 | 19.10 |
| | 2 nd Release | 17.10 | 8.60 | 7.30 | 7.90 |
| | 3 rd Release | 7.30 | 3.10 | 2.90 | 2.90 |
| | Mean | 24.43 a | 10.93 b | 9.10 b | 9.97 b |
| 4 Larva/m ² | 1 st Release | 48.00 | 18.70 | 15.00 | 16.80 |
| | 2 nd Release | 15.00 | 6.80 | 5.80 | 6.30 |
| | 3 rd Release | 5.80 | 2.10 | 1.70 | 1.90 |
| | Mean | 22.93 a | 9.20 b | 7.50 b | 8.33 b |
| Control | 1 st Release | 46.40 | 46.20 | 44.20 | 45.20 |
| | 2 nd Release | 44.20 | 45.80 | 52.40 | 49.10 |
| | 3 rd Release | 52.40 | 56.40 | 56.40 | 56.40 |
| | Mean | 47.67 a | 49.47 a | 51.00 a | 50.23 a |
| F-value | | 1.12 | 14.79 | 24.23 | 19.22 |
| LSD 0.05 | | 34.32 | 16.17 | 13.72 | 14.81 |

Means within a column followed by different letters denote significant difference ($P < 0.05$).

Table 2. Reduction percentages of the *Spodoptera littoralis* population after three releases of *Chrysoperla carnea* at the Qaha Agricultural Research Station, Qalyubia governorate, during the 2021 season.

| Treatment Predators/ m ² | | Reduction % after treatment | | |
|--|-------------------------|-----------------------------|----------------|----------------|
| | | 7 days | 14 days | Mean |
| 2 Larva/m ² | 1 st Release | 46.11 b | 51.24 b | 48.67 b |
| | 2 nd Release | 44.50 b | 56.59ab | 50.54 b |
| | 3 rd Release | 52.89 a | 58.92 a | 55.90 a |
| | Mean | 47.83 C | 55.58 C | 51.70 C |
| | LSD. 0.05 | 4.67 | 5.41 | 4.29 |
| 3 Larva/m ² | 1 st Release | 56.69 a | 63.21 a | 59.94 ab |
| | 2 nd Release | 51.24 b | 63.85 a | 57.54 b |
| | 3 rd Release | 59.92 a | 65.68 a | 62.8 a |
| | Mean | 55.95 B | 64.25 B | 60.09 B |
| | LSD. 0.05 | 5.26 | 3.96 | 2.90 |
| 4 Larva/m ² | 1 st Release | 60.90 ab | 67.10 a | 63.99 b |
| | 2 nd Release | 56.33 b | 67.33 a | 61.83 b |
| | 3 rd Release | 66.31 a | 71.52 a | 68.91 a |
| | Mean | 61.18 A | 68.65 A | 64.91 A |
| | LSD. 0.05 | 5.82 | 4.63 | 4.79 |
| "F" value | | 22.23 | 41.62 | 37.40 |
| LSD. 0.05 | | 4.10 | 2.96 | 3.14 |

Means within a column followed by different small letters denote significant difference between three releases ($P < 0.05$).

Data obtained in Table (2) showed that the statistical analysis showed that the overall mean reduction percentages of *S. littoralis* larvae varied significantly among treatments after 7 and 14 days of application ($F = 22.23$, $P < 0.0001$; $F = 41.62$, $P < 0.0001$). The highest value recorded with *C. carnea* rate of 4 larva/m² was 61.18 and 68.65% after 7 and 14 days, respectively. However, the lowest recorded *C. carnea* rate of 2 larva/m² was 47.83 and 55.58% after 7 and 14 days of application. The general mean

reduction percentage was 51.70, 60.09, and 64.91% at rates of release 2, 3, and 4 larvae/m², respectively, with significant differences ($F = 37.40$, $P < 0.0001$). The general average of cotton leafworm at three levels was 13.70, 9.97, 8.33, and 50.23 in 2021 season, 2 larvae/m², 3 larvae/m², 4 larvae/m² and control, respectively.

Statistical analyses of the data presented in Table (3) showed that there were significant effects of release levels, number of releases, and time. The

third level and the third release were more effective than the second and first levels. The reduction rate

increases with an increasing increment of time.

Table 3. Factorial analysis for *Spodoptera Littoralis* population after third release of *Chrysoperla carnea*

| Release | Mean | "F" value | P-value | LSD. 0.05 |
|---------|---------|-----------|---------|-----------|
| one | 57.54 b | 21.54 | <.0001 | 1.93 |
| Two | 56.63 b | | | |
| Three | 62.54 a | | | |
| level | | | | |
| 1 | 51.70 c | 95.20 | <.0001 | 1.93 |
| 2 | 60.09 b | | | |
| 3 | 64.91 a | | | |
| Time | | | | |
| 7 days | 54.98 b | 98.20 | <.0001 | 1.57 |
| 14 days | 62.82 a | | | |

Means within a column followed by different small letters denote significant difference between three releases ($P < 0.05$).

In the second season 2022, data presented in Table (4) indicate the efficiency of 2nd instar larvae as a biological agent for controlling the *S. littoralis* larvae population on cotton plants under cage at three different ratios of 2, 3, and 4 larvae/m². Seven days after first release, the efficiency of the 2nd instar larvae of *C. carnea* was determined in all treatments under investigation. The cotton leaf worm populations were 31.4, 26.5, and 22.4 larvae/m² at 7 days post-treatment for 2, 3, and 4 larvae per plant, respectively. Conversely, the *S. littoralis* larvae population increased in the control treatment to reach 56.8 individuals per 30 plants. After 14 days of release, results demonstrated that the mean number of *S. littoralis* larvae decreased to 28.4, 22.3, and 18.8 individuals / 30 plants at rates of 2, 3, and 4 larvae/m², respectively, compared to 55.5 individuals per leaf in the control. Similar results were obtained for the second and third releases; the number of cotton leaf worms decreased as time increased after release compared with the control. The average number of *S. littoralis* was 18.13 and 17.47; 14.47 and 12.23; 11.37 and 9.30 larvae per three cotton leaves after 7 and 14 days of application at rates of 2, 3, and 4 larvae/m², respectively. Whereas, the average *S. littoralis* larvae were 55.87 and 54.37 after 7 and 14 days of application in control, respectively.

The results in Table (5) showed that the mean reduction percentage in *S. littoralis* larvae was 51.02, 46.48, and 52.89 compared to those of the control in plots receiving 3, 5, and 7 larvae per plant, respectively. These values increased to 56.55, 48.10, and 56.53% after 14 days of application, respectively, with significant differences.

Data presented in Table (6) indicated that there were significant effects of release levels, number of releases, and time. The third level and the third

release were more effective than the second and first levels. The reduction rate increases with an increasing increment of time.

The present results are in harmony with those of **Gurbanov (1982)**, who found that after one week of releasing *C. carnea* (3- 4 days old eggs and 1st and 2nd instar larvae) against *A. gossypii* at the predator-prey ratio, 1:1, the reduction percentage was 98.5%. **Younes *et al.* 2013**) showed the promising and best results were obtained after 21 days from releasing *C. carnea* 2nd instar larvae date at rate of 5 predatory larvae / Cantaloupe plants, however, these larvae reduced populations of aphids 73.9 %. **El-Arnaouty *et al.* (2000)**, obtained best results in the control of *Myzus persicae* by releasing 2nd instar larvae of *C. carnea* on green pepper plants under greenhouse conditions than those obtained after releasing eggs and combination between eggs and 2nd instar larvae. **Turquet *et al.* (2009)** found that lacewings effectively limited aphid infestations in strawberry crops without the need for chemical treatment. The control of pest populations varied based on the amount of lacewings introduced. With 5 lacewings per plant, predatory action was both preventive and curative. However, with 1 lacewing per plant, aphid population increase was limited. Further research is needed to develop control strategies. **Sarwar (2014)** showed that the larvae of *C. carnea* predator, 1st instar followed by 2nd and 3rd instar larvae were most effective in reducing aphids population on canola crop compared with untreated control.

Table 4. Mean counts of cotton leaf worms larvae infesting cotton plants in the natural field before and after releasing *Chrysoperla carnea* 2 instar larvae at different rates at the Qaha Agricultural Research Station, Qalyubia Governorate, during the 2022 cotton season.

| Predators/m ² | Treatment | Pre-treatment <i>S. littoralis</i> / 30 plants | Mean number of <i>S. littoralis</i> / 30 plants at the indicated day after treatment | | |
|--------------------------|-------------------------|--|--|----------------|----------------|
| | | | 7 days | 14 days | General Mean |
| 2 Larva/m ² | 1 st Release | 54.7 | 31.4 | 28.4 | 29.9 |
| | 2 nd Release | 28.4 | 13.3 | 15.3 | 16.3 |
| | 3 rd Release | 15.3 | 9.7 | 8.7 | 9.2 |
| | Mean | 32.80 a | 18.13 b | 17.47 b | 18.47 b |
| 3 Larva/m ² | 1 st Release | 54.1 | 26.5 | 22.3 | 24.4 |
| | 2 nd Release | 22.3 | 11.8 | 9.6 | 10.7 |
| | 3 rd Release | 9.6 | 5.1 | 4.8 | 4.9 |
| | Mean | 28.67 a | 14.47 b | 12.23 b | 13.33 b |
| 4 Larva/m ² | 1 st Release | 55.4 | 22.4 | 18.8 | 20.6 |
| | 2 nd Release | 18.8 | 8.8 | 6.7 | 7.7 |
| | 3 rd Release | 6.7 | 2.9 | 2.4 | 2.6 |
| | Mean | 26.97 a | 11.37 b | 9.30 b | 10.30 b |
| Control | 1 st Release | 56.8 | 56.8 | 55.5 | 56.1 |
| | 2 nd Release | 55.4 | 55.4 | 48.8 | 52.1 |
| | 3 rd Release | 48.8 | 55.4 | 58.8 | 57.1 |
| | Mean | 53.67 a | 55.87 a | 54.37 a | 55.10 a |
| F-value | | 1.15 | 14.58 | 18.78 | 17.09 |
| LSD 0.05 | | 37.53 | 17.75 | 15.76 | 16.41 |

Means within a column followed by different letters denote significant difference ($P < 0.05$).

Table 5. Reduction percentages of the *Spodoptera littoralis* population after three releases of *Chrysoperla carnea* at the Qaha Agricultural Research Station, Qalyubia governorate, during the 2022 season.

| Treatment Predators/m ² | | Reduction % after treatment | | |
|---------------------------------------|-------------------------|-----------------------------|----------------|-----------------|
| | | 7 days | 14days | Mean |
| 2 Larva/m ² | 1 st Release | 42.58 c | 46.79 c | 44.68 c |
| | 2 nd Release | 50.99 b | 57.81 b | 54.4 b |
| | 3 rd Release | 59.5 a | 65.05 a | 62.28 a |
| | Mean | 51.02 AB | 56.55 A | 53.79 AB |
| | LSD. 0.05 | 2.44 | 2.67 | 2.09 |
| 3 Larva/m ² | 1 st Release | 38.99 c | 38.61 c | 38.80 c |
| | 2 nd Release | 47.1 b | 50.89 b | 48.99 b |
| | 3 rd Release | 53.36 a | 59.65 a | 56.51 a |
| | Mean | 46.48 B | 49.72 B | 48.10 B |
| | LSD. 0.05 | 3.47 | 2.16 | 2.02 |
| 4 Larva/m ² | 1 st Release | 44.28 c | 52.67 c | 48.47 c |
| | 2 nd Release | 53.27 b | 58.26 b | 55.76 b |
| | 3 rd Release | 61.13 a | 69.62 a | 65.37 a |
| | Mean | 52.89 A | 60.18 A | 56.53 A |
| | LSD. 0.05 | 4.13 | 1.66 | 2.75 |
| "F" value | | 22.23 | 2.56 | 5.04 |
| LSD. 0.05 | | 4.10 | 5.92 | 6.81 |

Means within a column followed by different small letters denote significant difference between three releases ($P < 0.05$).

Table 6. Factorial analysis for *Spodoptera littoralis* population after third release of *Chrysoperla carnea*.

| Factors | Mean | F- value | P-value | LSD. 0.05 |
|---------|---------|----------|---------|-----------|
| Release | | | | |
| one | 53.78 b | 79.09 | <.0001 | 1.42 |
| Two | 48.09 c | | | |
| Three | 56.95 a | | | |
| level | | | | |
| 1 | 43.98 c | 298.45 | <.0001 | 1.42 |
| 2 | 53.46 b | | | |
| 3 | 61.38 a | | | |
| Time | | | | |
| 7 days | 50.13 b | 93.25 | <.0001 | 1.16 |
| 14 days | 55.75 a | | | |

Means within a column followed by different capital letters denote significant difference between three levels ($P < 0.05$).

Generally, our results demonstrated that the release of 2nd instar larvae of *C. carnea* at the three different rates of 2 larvae/m², 3 larvae/m², and 4 larvae/m² induced a general reduction in *S. littoralis* larvae populations compared to the control treatment. The statistical analysis revealed that the efficiency of 2nd instar larvae of *C. carnea* was more evident when released at 4 larvae/m² as a biocontrol agent against *S. littoralis* larvae attacking cotton plants under cage. Therefore, it gave the highest overall mean percentage of reduction in *S. littoralis* larvae populations (64.91%) in the first season and 56.53% in the second season.

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كفاءة إطلاق المفترس الحشري أسد المن في خفض تعداد دودة ورق القطن علي نباتات القطن تحت ظروف الحقل .

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تم تنفيذ تجربة حقلية داخل مزرعة محطة البحوث الزراعية في قها /محافظة القليوبية لتقييم كفاءة المفترس الحشري (أسد المن) في خفض تعداد دودة ورق القطن علي نباتات القطن. تم زراعة القطن في مساحة خمسة قيراط (875 م²) أجريت هذه التجربة خلال موسمي القطن 2021 و 2022 على مساحة 54 متراً مربعاً من القطن المزروع على أرض المحطة المغطاة بالشباك البيضاء (الثيرام) المصممة على شكل قفص بأبعاد عرض 3 أمتار × الطول 18 متر × الارتفاع 1.5 متر. تم تقسيم هذه المساحة الي اربع اجزاء كل جزء عبارة عن مستوي إطلاق (ثلاث مستويات إطلاق) والجزء الرابع عبارة عن الكنترول وتم عمل عدوي صناعية بدودة ورق القطن (لطح بيض) وبعد ان يفقس البيض خلال يومين الي ثلاثة أيام. تم تحديد معدل الإصابة (متوسط عدد اليرقات داخل القفص) حيث يتم اختيار عشرة نباتات عشوائياً من كل مكرر وفحصها. وتسجيل متوسط عدد اليرقات في كل مستوى إطلاق، وبعد ذلك تم إطلاق العمر اليرقي الثاني للمفترس أسد المن بالكميات المطلوبة لكل جزء. وهي يرقنتين من يرقات أسد المن لكل متر مربع من المعاملة، وثلاث يرقات لكل متر مربع من القفص، وأربع يرقات لكل متر مربع من القفص. أظهرت النتائج انخفاض تعداد دودة ورق القطن بنسبة (61.10 و 67.33 و 71.52%) بعد 14 يوم من الإطلاق الثالث للمفترس أسد المن بمعدل 4 يرقات/م² و (52.67 و 58.26 و 69.62%) خلال موسمي القطن 2021 و 2022 علي التوالي. وتشير النتائج أن المفترس الحشري أسد المن هو مفترس فعال لقمع دودة ورق القطن في حقول القطن. وكانت معدلات الخفض عند مستويات الإطلاق مقبولة. أظهر إطلاق المفترس إلى الفرائس بمعدل (اربع يرقات من المفترس لكل متر مربع) أعلى نشاط للمكافحة الحيوية مقارنة بالغير معاملة تليها المعدلات (ثلاثة/متر) ثم (يرقتين/متر) ولذلك يمكن استخدامه بنجاح كعامل مكافحة بيولوجية ضمن برنامج مكافحة المتكاملة لدودة ورق القطن.