EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

Vol. 17 No. 1 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 17(1) pp.131-140 (2024)*
DOI: 10.21608/EAJBSA.2024.347232

Egypt. Acad. J. Biolog. Sci., 17(1):131-140 (2024)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/

Evaluating Potential Impact of Certain Natural Enemies on Key Piercing-Sucking Insects Infesting Sweet Basil Plants

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ARTICLE INFO

Article History
Received:28/2/2024
Accepted:25/3/2024
Available:28/3/2024

Keywords:
Sweet basil, cotton aphid, whitefly, natural enemies.

ABSTRACT

Biological control is a natural method that suppresses pests and is a practical, eco-friendly, and cost-effective substitute for insecticide-based approaches. The present investigations were carried out to evaluate the potential impact of three natural enemies; *Aphidius colemani* Viereck, *Chrysoperla carnea* (Steph.) on *Aphis gossypii* Glover under semi-field circumstances and *Beauveria bassiana* Balsamo (Vuillemin) on *Bemisia tabaci* (Genn.) under field conditions. The study was done on sweet basil plants during the season of 2022 at Zagazig district, Sharkia Governorate, Egypt. The results revealed that the maximum parasitism percentage of *A. colemani* was achieved at a rate of 12 parasitoids/cage, whereas the highest percentage of emergence was recorded at a rate of three parasitoids/cage. Data also showed that the reduction percentages of cotton aphid numbers declined as the rate of *C. carnea* increased. Moreover, *B. bassiana* caused from 49.19 to 95.20% reduction in the mean numbers of *B. tabaci*. Therefore, *A. colemani*, *C. carnea*, and *B. bassiana* are being promoted for the management of the major piercing-sucking insects on sweet basil plants under Egyptian field conditions.

INTRODUCTION

The extensive and repeated use of chemical insecticides disrupts the natural balance between the insects and their natural enemies (Amer and Marei 2011) and induces the development of insects' resistance, phytotoxicity, and environmental pollution (Field *et al*. 1988, Ahmad *et al*. 2011).

Biological control is a natural practice that plays a vital role in pest control (DeBach 1974, DeBach and Rosen 1991) and constitutes a viable, environment-friendly, and economically sound alternative to insecticide-based measures for agricultural pest management (Heimpel and Mills 2017). Both the whiteflies and the cotton aphids are considered among the most prevalent piercing-sucking insects on sweet basil plants in Egypt (Osman *et al*. 2017, Ismail *et al*. 2024). The solitary endoparasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae) has a host range of more than 41 aphid species (Starý 1975). *A. colemani* is mainly used in controlling cotton aphids (Van Steenis and El-Khawass 1995, Bilu *et al*. 2006, Vásquez *et al*. 2006) and has the ability to discover and parasite low density of aphid population (Van Steenis 1993). The green lacewing, *C. carnea* (Neuroptera: Chrysopidae) has a vital role in most agricultural systems due to its ability to hunt aphids easily: whiteflies, mites, and some other small, soft-bodied insect species (Golmohammadi *et al*. 2021). It has a high research capacity and preying potential on about 200 aphid species (Tauber *et al*. 2000). *C. carnea* has been used as a biological control agent of aphids because of its compatibility with selective chemical insecticides, and microbial agents, and suitability for mass production (Uddin *et al*. 2005). Entomopathogenic fungi (EPF) have the exclusive ability to be used as biological control agents against different agricultural insect pests (Torrado-Leon *et al*. 2006, Akmal *et al*. 2013). EPF is host-specific and acts through contacts, and its use is considered an eco-friendly control method (Shahid *et al*. 2012). *Beauveria bassiana* showed great potential for the management of piercing-sucking pests (Hesketh *et al*. 2008), especially *B. tabaci* (Abdel-Raheem and Al-Keridis 2017).

Due to the increasing threats of piercing-sucking insects on sweet basil plants and the need for plants free of chemical residues, this study was conducted to evaluate the potential impact of some natural enemies as a management control of the main piercing-sucking insect pests.

MATERIALS AND METHODS

Semi-field trials on sweet basil were carried out at Zagazig district, Sharkia Governorate, Egypt during the growing season of 2022.

Evaluation of Different Densities of the Cotton Aphid Parasitoid, *A. colemani*:

The parasitoid, *A. colemani* and the host, *A. gossypii* were raised under semi-field conditions. At 45 days old, 20 seedlings of sweet basil were separately caged in (60×60×100cm) iron cages and covered with muslin cloth. Each plant was infested artificially with 250 aphids of different ages. Different densities of three, six, nine, and twelve parasitoids/cage were introduced using freshly emerged mated females that were fully fed on honey. The females were maintained for 24 hours before being removed and the aphids were left behind to mummify. After mummification, the mummies were counted to estimate the parasitism percentages and delicately placed in marked

Petri dishes with pieces of plant leaves on a moistened filter paper. The mummies were examined till the adults' emergence. Once the grown-ups emerged, they were recorded to determine the emergence percentages. Each parasitoid density was replicated five times.

Predation Efficacy of *C. carnea* on *A. gossypii* on Sweet Basil Plants:

Mass Rearing of *C. carnea* Under Laboratory Conditions:

For mass rearing of *C. carnea*, the captured adults from the sweet basil plant were fed on droplets of an artificial diet consisting of honey, sugar, yeast, and distilled water in a ratio of 1:2:1:2, provided daily to enhance their fecundity (Sohail *et al*. 2019). For good ventilation, adults were placed in transparent, plastic rearing containers covered with muslin. Pieces of paper sheets were inserted in the containers as a substrate for oviposition. The paper sheets with eggs were collected daily and placed in Petri dishes for hatching. The mass rearing was conducted in the laboratory under controlled conditions of 25.00±2°C and 65.00±5% RH (Hassan, 2014). The newly hatched larvae of *C. carnea* were put in the above-mentioned containers with numbers of *A. gossypii* individuals, which renewed daily until they reached the second instar.

Predation Effectiveness of *C. carnea* on *A. gossypii* Under Semi-Field Conditions:

To estimate the predation efficacy of *C. carnea on A. gossypii* infesting sweet basil plants, the sweet basil seedlings were caged individually after about one month from transplanting with muslin-covered woody cages (60× 60× 120 cm) in the field. The predation effectiveness was evaluated based on the number of aphids on each plant. Releasing the second larval instar of *C. carnea* was applied with ratios of 1:100, 1:150, 1:200, 1:250, and 1:300 predator: prey (P: P ratio). The randomized complete block design was used with five treatments and five replicates. The cages containing control plants were not subject to predator release. Plants were checked after one, two, three, four, five, and six days from application using a (× 10) hand lens. The reduction percentages in aphids' numbers were estimated according to Abbott's formula (1925).

$$Reduction\,(\%) = \frac{number\ of\ aphids\ in\ control\ -\ number\ of\ aphids\ in\ treatment}{number\ of\ aphids\ in\ control} x\ 100$$

Efficacy of the Entomopathogenic Fungus *Beuvaria bassiana*, (Biosiana) Against *B. tabaci* on Sweet Basil Plants Under Field Conditions:

The experiment was carried out to determine the efficacy of *B. bassiana* against *B. tabaci* infesting sweet basil plants with its recommended dose (250 g/100 L.) during the 2022 growing season. The formulation of *B. bassiana* (1×10^8 CFU, S/g) was obtained from Unit Mass Production of Microbial Control Agents, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The formulation's viability was measured by culturing on Potato Dextrose Agar (PDA) media and incubated at 25±2°C for seven days according to Youssef (2015). Before application, it was examined under light microscopy to confirm that at least 95% of the spores could germinate (Nouh *et al*. 2022).

Experimental Design:

A cultivated area with sweet basil plants of ($600m^2$) was laid out in a randomized block design and divided into two plots about ($300m^2$ for each). A field plot was sprayed with Biosiana and the other one was used as control and sprayed with water only. Each plot was replicated four times. To avoid the interference of the treatments drifting, one meter was used to separate among plots. The treatments were applied after 45 days from seedling. The application was conducted at sunset time to prevent the damage that may be caused by the UV rays. A motor spray with a volume of 20 L. was used in the experiment.

Data Collection:

From each sweet basil plot, twenty-five plants were selected randomly. Numbers of *B. tabaci* from the upper, middle, and lower leaves of these plants were registered. The inspections were done before and after treatment, then after three, seven, and ten days of treatment. The adults of whitefly were recorded in the early morning by visual counts with the aid of (× 10) hand lens in the field as they are active fliers (Deutscher *et al*. 2003), and the plant samples were transferred to the laboratory in paper bags to examine nymphs with the aid of a stereoscopic microscope.

Reduction ratios of whiteflies were calculated according to Henderson and Tilton (1955), whereas

$$Reduction(\%) = 1 - \frac{no.in\,C.before\ treatment\ x\ no.in\,T.after\ treatment}{no.in\,C.after\ treatment\ x\ no.in\,T.before\ treatment} x\ 100$$

where no. = whitefly population; T. = treated; C. = control.

Statistical Analysis:

The obtained data of the *A. colemani* parameters, and the reduction percentages of *A. gossypii* after releasing *C. carnea* were subjected to one-way ANOVA analysis of variance and least significance differences (LSD) for quantitative data (Means± SE and the mean values were compared by Duncan's Multiple Range tests (P= 0 .05& 0.01), using SPSS program, version 23 (SPSS 2015) and the treatment means were separated at P = 0.05

& 0.01. The whitefly reductions (%) were compared at the same inspection with the control treatment using the Paired T-Test with a probability of 5 and 1% (SPSS 2015).

RESULTS AND DISCUSSION

Evaluation of Different Densities of the Cotton Aphid Parasitoid, *A. colemani:*

The parasitoid density influenced the percentages of parasitism, giving a maximum percentage of 80.40% for *A. colemani* and kept at a rate of 12 parasitoid females/cage, and the minimum record was 38.80% at three parasitoid/cage. There were significant differences in the total numbers of parasitized aphids and the total percentage of parasitism at all densities. The largest number of parasitized aphids by *A. colemani* (201.00) was recorded at 12 parasitoids/cage and a minimum of 98.80 was recorded at three parasitoids/cage. *A. colemani* showed the highest rate of adult emergence (81.44%) at three parasitoids/cage and a minimum rate of 68.30% at 12 parasitoids/cage (Table 1). Numerous studies confirmed the importance of *A. colemani* in controlling *A. gossypii* (Heinz, 1998; Jacobson and Croft, 1998; Gissella *et al*., 2006; Saleh, 2017; Eid *et al*., 2018). They mentioned that *A. colemani* has been reared successfully on *A. gossypii* and is considered a potentially effective biocontrol agent against many aphid species including *A. gossypii*.

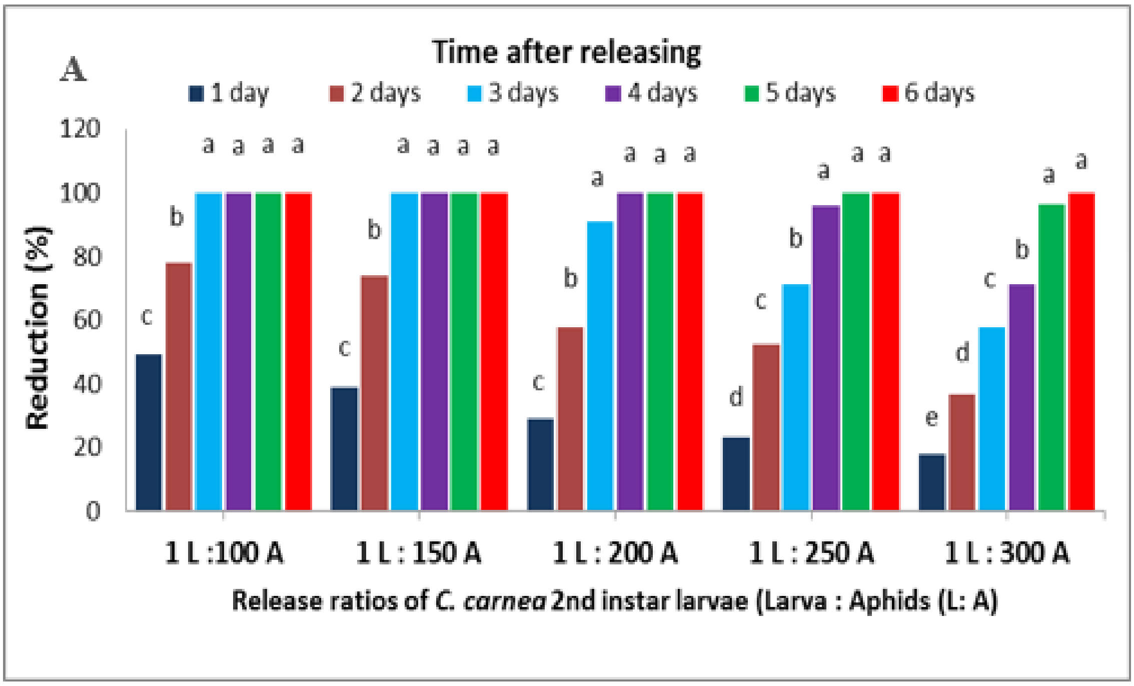
Table 1: Potential impact of different densities of *A. colemani* on *A. gossypii* at Zagazigdistrict,Sharkia Governorate, Egypt during 2022 season.

No. of released parasitoid/cage	No. mummies (Mean±SE)	Parasitism %	Parasitoid adult emergence%
3	98.80 ± 4.07^{d}	38.80 ± 1.78^{d}	81.44 ± 1.52^{a}
6	146.00 ± 4.62^{c}	58.40 ± 1.85^{c}	78.4 ± 1.13^{a}
9	176.80 ± 4.76^{b}	70.72 ± 1.90^{b}	72.35 ± 0.58^{b}
12	201.00 ± 4.53^{a}	80.40 ± 1.81^{a}	68.30 ± 0.89^{c}
F-value	76.66**	23.72**	76.40**
P- value	0.0000	0.0000	0.0000

Each datum represents the mean and standard error (SE) of five replicates **: means are highly significant (*P*<0.01); df =3, 16.

Predation Efficacy of *C. carnea* on *A. gossypii* on Sweet Basil Plants:

After the release of *C. carnea* (24 hours), the percentage reduction of aphids on sweet basil plants averaged 49.00, 39.00, 28.80, 23.00 and 18.00% in five cages, at rates of 1:100, 1:150, 1:200, 1:250, and 1:300 (L.: A.), respectively. The predaceous larvae gave reduction percentages (two days after release) as 78.00, 74.00, 57.80, 52.40, and 36.80%, while after three days they were 100.0, 100.00, 90.80, 71.20 and 57.60% with rates 1:100, 1:150, 1:200, 1:250, and 1:300 (L.: A.), subsequently. Complete control of the cotton aphid populations was achieved after four days from the release of *C. carnea* larvae with a predator: prey ratios (larvae: aphids) of 1:100, 1:150, and 1:200. It was noticed that the number of *A. gossypii* at these ratios were still zero for a time of five days after treatment with the predator larvae. When predator: prey ratio was 1:100, 1:150, 1:200, 1:250, and 1:300 (L.: A.) the reduction percentages were 100.00, 100.00, 100.00, 100.0, and 96.20%, respectively. Complete control of *A. gossypii* populations occurred from the release of *C. carnea* larvae with predator-prey ratios 1:100, 1:150, 1:200, 1:250, and 1:300 (L.: A.). It was observed that the number of *A. gossypii* at these ratios became zero after six days from treatment with the predator larvae (Fig. 1 A). The mean percentage reduction of aphids on sweet basil plants averaged 87.83, 85.50, 79.57, 74.03, and 63.30% at rates of 1:100, 1:150, 1:200, 1:250, and 1:300 (L.: A.), respectively. (Fig.1 B).



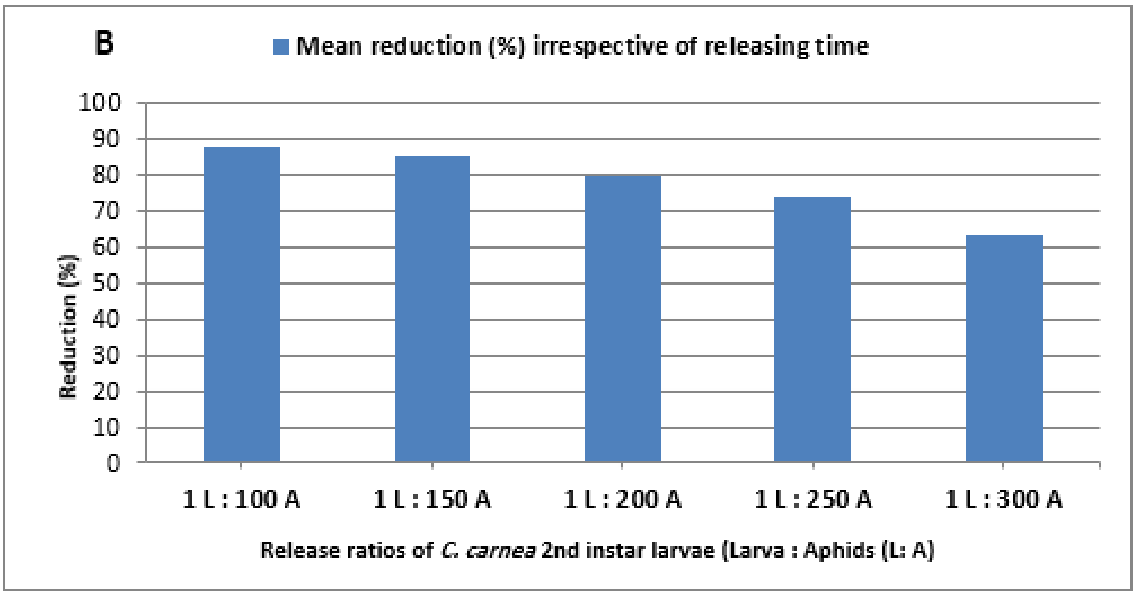


Fig.1: Predation efficacy of *C. carnea* (2nd instar larvae) on *A. gossypii* under semi-field trials on sweet basil plants (**A**) and Mean reduction percentages (**B**) at Zagazig district, Sharkia Governorate, Egypt in 2022 season**.** (1 L: 100 A: F-value=32.80**; 1 L: 150 A: F-value=57.90**; 1 L: 200 A: F-value=64.07**; 1 L: 250 A: F-value=64.21; 1 L: 300 A: F-value= 60.43**); df =5, 20.

These results are supported by those of El-Arnaouty and Gamal (1998) who reported that the mean reduction in aphid numbers averaged 78.94, 73.6, and 67.97% which received 1:100, 1:150, and 1:200 (larvae: aphid), respectively. Also, after releasing the second larval instar of *C. carnea* against *A. gossypii* on sweet basil plants under field conditions at two levels (one and two larvae/plant) at Qalubia Governorate during the 2014 and 2015 seasons, the mean reduction percentages in cotton aphid numbers were (76.10 and 75.70%) and (85.70 and 86.60%) for the two tested levels in the first and second seasons, subsequently (El-Ghanam 2017). Moreover, by releasing the second larval stage of *C. carnea* on *A. gossypii* infesting cucumber plants at 1:100, 1:150 and 1:200 (larvae: aphid) under semi-field conditions the mean reduction in aphid numbers were 78.90, 73.60 and 67.97%, alternatively (Saleh *et al*. 2017). The different types of functional responses among predator species may be due to the variations in their hunger levels, digestive ability, voracity, size, and walking speed (Ofuya and Akingbohungbe 1988, Omkar Pervez 2004). Furthermore, the effectiveness of predatory may depend on prey density (Matter *et al*. 2011), prey growth stage and species (Koch *et al*. 2003, Sarmento *et al*. 2007), leaf morphology of the host plant (Bayoumy 2011) and cannibalism and internal predation (Burgio *et al*. 2002).

Efficacy of the Entomopathagenic Fungus *Beuvaria bassiana*, (Biosiana) Against Whitefly, *B. tabaci* on Sweet Basil.

The effectiveness of EPF was evaluated by comparing it with the untreated treatment based on their reduction rates against *B. tabaci.* After one, three, seven, and ten days from treatment with *B. bassiana,* the mean reductions in *B. tabaci* numbers were 49.19±1.04, 67.52±0.95, 89.66±0.20 and 95.20±0.55%, respectively (Table 2). Such findings conformed with those of Liu and Stansly (2000) and De Faria and Wraight (2007) who indicated that the EPF, *B. bassiana* showed a potential for management of whiteflies both in greenhouse and field crops. Also, applying several *B. bassiana* isolates against *B. tabaci* on different host plants significantly affected its reproductive rates (Zafar *et al*. 2016). The treatment with *B. bassiana* for the management of *B. tabaci* infesting cucumber plants grown under protected conditions caused a 40 to 72% reduction in its life stages (Ghongade and Sangha 2021). Additionally, Chougule *et al*. (2022) evaluated the impact of *B. bassiana* (strain ATCC and strain R444) on the concurrent existence of *B. tabaci* in Southern Tunisia and observed significant effects on its eggs and larvae.

Table 2: Potential impact of *B. bassiana* on *B. tabaci* at different time of treatment at Zagazig district, Sharkia Governorate, Egypt during 2022 season.

Treatment	Days after treatment			
	1 day	3 days	7 days	10 days
	Mean No. of *B. tabaci* (Mean±SE)			
Control	180.00±2.67	216.50±2.90	328.25±2.28	451.75±2.86
Biosiana	91.50±2.90	70.25±1.49	34.00±2.88	21.75±2.62
Reduction%	49.19±1.04	67.52±0.95	89.66±0.2	95.20±0.55
T-value	8.07**	5.16**	3.24*	2.90*
P-value	0.0001	0.0013	0.0143	0.0230

Each datum represents the mean and standard error (SE) of four replicates * &**: means are significant and highly significant ($P<0.05$, $P<0.01$), respectively; df =7.

CONCLUSION

Based on our study, the maximum percentage of emergence of the parasitoid, *A. colemani* was achieved when reared on *A. gossypii* at a population density of three parasitoids/cage. The highest percentage of reduction for *A. gossypii* was observed by releasing the second larval instar of *C. carnea* at a rate of (1 larvae: 100 aphids). *B. bassiana* succeeded in suppressing the population of *B. tabaci* and the maximum percentage of reduction was obtained in the tenth day after treatment. It is suggested that *A. colemani, C. carnea,* and *B. bassiana* should be included as main components in the integrated pest management of piercing-sucking insects infesting sweet basil.

Declarations:

Ethical Approval and Consent to Participate: Not applicable.

Consent For Publication:All authors declared that there are no issues related to journal policies.

Competing Interests: The authors declare that they have no conflict of interest.

Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

Funding: This work has received no external funding.

Availability of Data and Materials: All data and materials are available if requested.

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RARABIC SUMMARY

تقييم التأثير المحتمل لبعض الأعداء الطبيعيه على الحشرات الثاقبه الماصه الرئيسيه التي تصيب نباتات الريحان الحلو.

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المكافحة البيولوجية هي وسيله طبيعية للقضاء على الآفات، وهي طريقه بديله وعمليه وصديقه للبيئه وفعاله عن الأساليب المعتمده على المبيدات الحشريه. تم إجراء التجارب الحاليه لتقييم التأثير المحتمل لثلاثة من الأعداء الطبيعيه؛ *A.colemani* و *C. carnea* على *A gossypii* تحت الظروف شبه الحقليه و *B. bassiana* على *B. tabaci* تحت الظروف الحقلية. أجريت الدراسه على نباتات الريحان الحلو خلال موسم الدراسة 2022 بمنطقة الزقازيق - محافظة الشرقية- مصر .أوضحت النتائج أن أعلى نسبة تطفل لحشرة *A. colemani* قد تحققت بمعدل 12 طفيل/قفص، في حين سجلت أعلى نسبة خروج بمعدل ثلاثة طفيليات/قفص .كما أظهرت النتائج أن نسب الخفض في أعداد من القطن تقل مع زيادة معدل *C. carnea.* علاوة على ذلك، تسبب *B. bassiana* في انخفاض بنسبه من 49.19 إلى 95.20% في متوسط أعداد *B. tabaci.* ولذلك يمكن التوصيه بإستخدام كل من *A. colemani* و *C. carnea* و *B. bassiana* لمكافحة الحشرات الثاقبة الماصة الرئيسية على نباتات الريحان الحلو تحت الظروف الحقلية المصرية.