The Role of *Chrysoperla carnea* (Steph.) and *Beauveria bassina* for Controlling Cabbage Aphid, *Brevicoryne brassicae* L. on Cabbage Plants

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Abstract

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Field experiments were carried out at Kafr Saqr district, Sharkia governorate during 2019/2020 and 2020/2021 growing seasons to evaluate the predator:prey ratios for the release of *C. carnea* and evaluation of using *Beauveria bassiana* suspension against the cabbage aphid, *Brevicoryne brassicae*. The results obtained showed that the effective control of *B. brassicae* was achieved ten days after releasing the larvae of the predator *C. carnea* when the predator:prey ratios were 1:5 and 1:10. Meanwhile, at higher ratios (1:20, 1:25 and 1:50), the cabbage aphid *B. brassicae* numbers decreased 25 days after predator release. The numbers of *B. brassicae* decreased by 84.69 and 81.61% at 1:5 and 1:10 predator:prey ratio during the first season, respectively. On the other hand, the aphid numbers were reduced by 81.50 and 70.95% at 5 days after the predator's release during the second season, for the two predator:prey ratios, respectively. Complete reduction of *B. brassicae* populations was achieved at 15 days after yhe release of *C. carnea* larvae with predator ratios of 1:5, 1:10 and 1:15, and numbers of *B. brassicae* at these ratios depressed completely 20 days after release. The results revealed that the best control of *B. brassicae* populations under greenhouses conditions was achieved by using the lower predator:prey ratio of 1:5 and 1:10 ten days after releasing larvae of *C. carnea*. The highest mortality rate in *B. brassicae* population caused by the fungus *B. bassiana* was 88.33%, recorded at 7 days after the application of spore concentration 1×10^7 spores/ml and the LC_{50} obtained in the field was 1.10×10^6 spores/ml. It can be concluded from this study that *C. Carnea* and *B. bassiana* are effective biocontrol agents in controlling the cabbage aphid *B. brassicae* in the field.

Keywords: *B. brassicae, Chrysoperla carnea, Beauveria bassiana,* Predator release.

Introduction

In recent years, much interest has been given to biological control of pests, especially by predator insects. Meanwhile, the success of rearing predators on natural preys or artificial diets for controlling aphids, whiteflies and other soft-bodied insects on several economic crop has been reported (Saleh *et al.*, 2021; Sargin *et al.*, 2013).

There are several insect pests which infest cabbage, among them the cabbage worm, *Pieris rapae* L and cabbage aphid, *Brevicoryne brassicae* L. (Ali *et al.*, 2020; Saleh, 2008). Biological control using one or more of the biocontrol agents is an environmentally approach to protect plants against plant pathogens and pests (Rocca *et al.*, 2017). The augmentative biological control requires knowledge about the relative potential of native or established agents. *Chrysoperla carnea* is a common predator of aphids, and it is applied for the biological control of aphids in Egypt and other countries (Alghamdi *et al.*, 2018; van Lenteren, 2012).

Cabbage aphid *B. brassicae* responds to the presence of *C. carnea* by walking away or dropping off the plant (Losey & Denno, 1998). The release rate and time of lacewing, *C. zastrowi sillemi* was effective against sucking pests grown under screen-house conditions (Nair *et al.*, 2020). The role of *C. carnea* in controlling different aphids and whiteflies on various crops has been studied earlier (Arnaouty & Sewify, 1998; Saleh *et al.*, 2020).

Fungi can be used to control insect pests without affecting predators, parasitoids and mammals. The previous studies showed that the LC_{50} of biozed against *B. brassicae* was 55.8 ppm according to El-Gendy (2015), and the LC_{50} values for the entomopathogenic fungi *M. anisopliae* and *B. bassiana* were 103.88 and 104.75 conidia/ml, respectively, against *M. persicae* with 100% of aphid mortality at 7 days after fungal treatment (Akram *et al.*, 2018).

The objective of the current study was to evaluate the optimal predator:prey ratios for the release of *C. carnea* and compare the effectiveness of this predator in controlling the aphid *B. brassicae.* The efficacy of using *B. bassiana* as a biocontrol agent against *B. brassicae* was also investigated.

Materials and Methods

Field experiments were carried out at Kafr Saqr district, Sharkia governorate during 2019/20 and 2020/21 seasons.

Evaluation the optimal predator:prey ratios for the release of *C. carnea*

Insect cultures- The culture of *B. brassicae* was initiated from individuals collected and reared on young cabbage seedlings grown in a soil potting mixture in plastic pots and kept in an aphid–free cylindrical steel cages covered with muslin. These plants were then infested with a small population of *B. brassicae* of mixed age structure and were placed in the greenhouse (8 m long, 2 m wide and 2 m high).

Adults of *C. carnea* were collected from the field. The eggs laid by each predator female were removed daily and monitored until hatching. The hatched larvae were reared

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individually in Petri-dishes (10 cm in diameter) to avoid intraspecific cannibalism.

Plantation of cabbage- Cabbage (*Brassicae oleracea* var. *acephala*) was planted in the greenhouses. The greenhouse area was10 m long and 2 m wide and 2 m high. This greenhouse was covered with muslin. The infestation by *B. brassicae* nymphs was carried by using fine brush. The chrysopid was released into greenhouses as early second instar larvae after two weeks from infestation. Six *C. carnea*:*B. brassicae* ratios of 1:5, 1:10, 1:15, 1:20, 1:25 and 1:50 were used. To estimate predator-prey ratio on the tested cabbage crop, 25 plants in were chosen randomly for this purpose. The total number of *B. brassicae* and *C. carnea* on these plants were counted by the direct counting method every five days. The average number per plant and total number of plants in each greenhouse were calculated.

The expected calculated predator-prey ratios under the greenhouse were obtained from the formula: Total number of calculated *B. brassicae*/the cultivated area (140m²) in seven green houses in both 2020 and 2021 seasons. Cabbage was planted on 3 October in the first seasons, and on 29 September in the second season. These plants were infested with aphids in larger houses (2×10 m), which contained cabbage plants. Two weeks after plantation, artificial infestation with *B. brassicae* nymphs was carried out by using the following numbers 5, 10, 15, 20, 25 and 50 plant in six green houses and another set as control for each population density, a calculated number of predator *C. carnea* was added and the percentage reduction rate was calculated as reported by Abbott (1925).

Laboratory evaluation of the efficiency of *B. bassiana* on *B. brassicae*

***B. brassicae* culture-** The cabbage aphid, *B. brassicae* was reared on cabbage leaves at 25±1°C, 65±5% RH and 12 hr photoperiod in laboratory. The cabbage leaves that were used for laboratory evaluation contained a midrib in order to survive for a long period of time. Aphids were taken put on cabbage leaves. The leaves and aphid were incubated under the same laboratory conditions. After 48 hr, three leaves were picked up and put in a glass Petri dish on filter papers.

Fungal inoculum- One entomopathogenic fungi were obtained from the Plant Protection Institute, Sharkia Branch. Spores of fungal isolate were harvested by rinsing with sterilized water containing 0.005% of Tween 80 from 7days old culture on (Dox medium grown at 25±1°C for *B. bassiana* isolates). The suspensions were filtered through cheesecloth. The concentrations were adjusted at 10^5, 10^6 and 10^7 according to primary experiment results.

Experimental work- The fungus was applied on the infected leaves of cabbage in four replicates, each consists of fifty individuals of *B. brassicae* on cabbage leaves. Leaves were sprayed with two ml of the spore suspension and the control was treated with two ml of sterilized water containing 0.005% Tween 80. The treatments and control were incubated for seven days under the same laboratory conditions. Nymphal mortality was observed after one, three, five and seven days. LC_{50} and LC_{90} and slop values were done after 5 and 7 days in laboratory according to Finny (1971). The presented data are means of each treatment.

Statistical analysis

Obtained data obtained was analyzed by one-way ANOVA using SAS software (SAS Institute, 2003). When F values were significant, means were compared using Tukey' HSD at P=0.05.

Results

Release of *Chrysoperla carnea* larvae

The first season 2019/2020- The results of released adults with predator:prey ratio of 1:5 and 1:10 clearly indicated that *C. carnea* larvae were successful in decreasing the cabbage aphid *B. brassicae* numbers five and ten days after the release (Table 1). The reduction rate of *B. brassicae* were 84.69, 100 and 81.61, 99.00% five and ten days after release with these two predator:prey ratios. Complete control of *B. brassicae* populations was achieved five days after release of *C. carnea* larvae with predator:prey ratios of 1:5 and 1:10. It was noticed that the *B. brassicae* number at these ratios remained zero for a period of 10 days after release of the predator larvae. When predator:prey ratio used was 1:15,1:20,1:25 and1:50, the reduction rates were 47.62, 63.58, 86.08%; 41.52, 58.80, 78.12%; 38.19, 51.82, 69.42% and 30.82, 42.91%, one, five and ten days after introducing the predator larvae, respectively. With the predator:prey ratio of 1:50, the *B. brassicae* number remained zero 20 days after the predator release. The statistical analysis showed that there was a significant decrease of *B. brassicae* numbers at the different predator: prey ratios and days after release of *C. carnea* larvae (Table 1).

The second season 2020/2021- A complete control of *B. brassicae* populations was achieved five days after release of the chrysopid predator at predator:prey ratio of 1:5, 1:10, 1:15 and 1:20 (Table 1). The population of *B. brassicae* at these ratios remained zero for a period of 20 days after release of the predator larvae. The population reduction rate of using these ratios was 81.50, 70.95, 60.44 and 51.70% five days after introducing the predators' larvae, respectively. When predator: prey ratio was 1:25 and1:50 the reduction rate was 30.53 and 48.55; 67.02% and 22.91; 41.84 and 65.05% one, five and ten days after introducing the predator larvae.

The results obtained suggested that the mean population reduction rate of *B. brassicae* were 90.06, 83.81, 80.69, 76.19, 71.05 and 66.05% with the predator: prey ratios 1:5, 1:10, 1:15, 1:20, 1:25 and 1:50, respectively, during the 2019/2020 and 2020/2021 seasons (Table 1). The statistical analysis confirmed that there was a significant decrease of *B. brassicae* numbers at the different predator: prey ratios and time after the release of *C. carnea* larvae.

Laboratory evaluation of *B. bassiana* spore suspension on *B. brassicae*

Results obtained (Table 2) measure the efficacy of *B. bassiana* spore suspension on nymph instars of cabbage aphid *B. brassicae* after application with different concentrations of *B. bassiana* spores under laboratory conditions (25±1°C and 65±5% RH). The concentrations were 10^5,10^6 and 10^7 spores/ml. Mortality percentages after 5 days of application showed 30.0, 46.0 and 54.67% and after 7 days showed 55.33, 68.67 and 83.33%, respectively.

The LC_{50} and LC_{90} values of *B. bassiana* spores/ml after 5 and 7 days of application on nymph instars of cabbage aphid, *B. brassicae*. The results obtained revealed that after 5 days LC_{50}= 5.8×10^7 spores/ml and LC_{90}= 1.7×10^{12} spores/ml (Figure 1A) and after 7 days LC_{50}= 1.1×10^6 spores/ml and LC_{90}= 3.4×10^9 spores/ml (Figure 1B).

Table 1. Population reduction rate of the cabbage aphid, *B. brassicae* after release of *C. carnea* larvae at different predator:prey ratios under greenhouse conditions on cabbage plants during 2019/2020 and 2020/2021 seasons.

	Predator:Prey ratio						
Days	**1:5**	**1:10**	**1:15**	**1:20**	**1:25**	**1:50**	**Mean**
2019/2020 season							
1	70.16 c	52.14 d	47.62 d	41.52 e	38.19e	30.82 f	46.93 e
5	84.69 b	81.61 c	63.58 c	58.8 d	51.82 d	42.91 e	63.90 d
10	100.0 a	99.00 b	86.08 b	78.12 c	69.42 c	63.97 d	82.77 c
15	100.0 a	100.0 a	98.56 a	94.90 b	91.13 b	81.19 c	94.30 b
20	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	97.64 b	99.61 a
25	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Mean	92.48 a	88.79 b	82.64 c	78.89 d	75.28 e	69.42 f	
2020/2021 season							
1	64.73 d	49.17 e	46.32 e	43.98 f	30.53 f	22.91 f	42.99 f
5	81.50 c	70.95 d	60.44 d	51.7 e	48.55 e	41.84 e	59.16 e
10	94.13 b	84.95 c	81.56 c	74.54 d	67.02 d	65.05 d	77.88 d
15	100.0 a	97.79 b	95.79 b	88.85 c	83.75 c	79.30 c	90.91 c
20	100.0 a	100.0 a	100.0 a	98.04 b	96.17 b	90.74 b	97.49 b
25	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	96.45 a	99.41 a
Mean	90.06 a	83.81 b	80.69 c	76.19 d	71.05 e	66.05 f	

Means followed by the same letters in the same column or the same row are not significantly different at P=0.05.

Table 2. Efficiency of *B. bassiana* spore suspension under laboratory conditions for controlling *B. brassicae* on cabbage plants.

Concentration (spores/ml)	**Mortality percentages (%) of *B. brassicae* per 50 individuals**											
	After 1 day			**After 3 days**			**After 5 days**			**After 7 days**		
	Alive	**Dead**	**Mortality**	**Alive**	**Dead**	**Mortality**	**Alive**	**Dead**	**Mortality**	**Alive**	**Dead**	**Mortality**
1×10^5	50	0	0	44.67 a	5.33 c	10.87 c	33.33 a	15.00 c	30.00 c	22.33 a	27.33 c	55.33 c
1×10^6	50	0	0	41.00 b	9.00 b	18.00 b	27.00 b	23.00 b	46.00 b	15.67 b	34.33 b	68.67 b
1×10^7	75	0	0	37.00 c	13.00 a	26.00 a	22.67 b	27.33 a	54.67 a	8.33 c	41.67 a	83.33 a

Means followed by the same letters in the same column are not significantly different at P=0.05

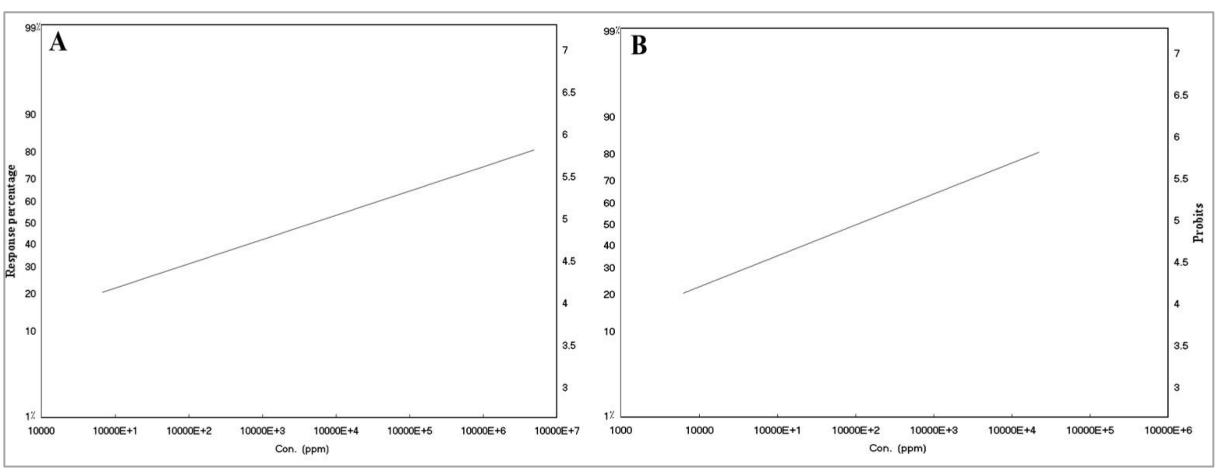

Figure 1. Concentration mortality probit line of *B. bassiana* spores/ml on nymph instars of *B. brassicae* under laboratory after 5 days (A) and 7 days (B).

Discussion

Results obtained confirmed that complete control of *B. brassicae* populations was achieved five days after the release of *C. carnea* larvae at predator:prey ratio of 1:5 and 1:10. The population of *B. brassicae* at these ratios remained zero for a period of 10 days after the release of the predator larvae. These results were in agreement with those of Radzivilovskaya (1980) who showed that *C. carnea* had an effective role against *Aphis gossypii* on cotton at a predator:prey ratio of 1:10. Meanwhile, aphid numbers were not reduced at a predator:prey ratio of 1:25 and 1:50. According to Shuvakhina (1983), second instar larvae of *C. carnea* was effective only when released at 1:20 ratio. Hagley (1989) stated that greater reduction in *Aphis pomi* numbers was achieved by releasing *C. carnea* at the predator:prey ratio 1:10 and 1:19. Similar results were obtained by Al-Arnaouty & Sewify (1998) who demonstrated that successful control (reduction by at least 95%) was obtained by the release of *C. carnea* second instar larvae. In addition, Zaki *et al.* (1999), concluded that 12 days after release of *C. carnea* at predator:prey ratio of 1:5 produced 100% population reduction of cotton aphid. Whereas, release of *C. carnea* at 1:10 was found to be more effective in suppressing the aphid population than at 1:100 (Venkatesan *et al.*, 2000). Abdel-Salam *et al.* (2005) and Saleh *et al.* (2017) reported that *C. carnea* larvae at a predator:prey ratio of 1:15 yielded excellent control of *A. gossypii* with population reduction rate of 88.3% one day after predator release. On the other hand, Mohamed *et al.* (2008) evaluated the potential of *C. carnea* in controlling nymphs of *B. argentifolii* under semi-field conditions and found that releasing *C. carnea* larvae on squash plants kept individually in cages was efficient in controlling the *B. argentifolii* populations.

In this study, minimum temperature had a negative significant correlation with predator:prey ratio of 1:5 and 1:10 and highly negative significant correlation with the predator:prey ratios 1:15, 1:20, 1:25 and 1:50 during the 2019-2020 season. The current results are similar to those shown by Abdelhalim (2020), where a positive correlation between *C. carnea* population and the aphids population occurred during two seasons.

Time-dose dependent mortality response experiments were designed to measure mortality rate produced by different fungal isolates pathogenic to aphids. The mortality rate observed was low on day 1 and day 2 after treatment, but it dramatically increased from day 7 to day 9. The number of infected aphids with fungal isolates increased with the increase in spore concentration in conidial suspensions and exposure time. The susceptibility of target insect to fungal infection dose is dependent as reported earlier (Liu & Chen, 2002; Wright *et al.*, 2005).

Akmal *et al.* (2013) showed that the maximum mortality of 100% caused by *B. bassiana* on *B. brassicae* was obtained on the 7th day post treatment at a concentration of 1×10^8 spores/ml, whereas the minimum mortality rate of 99.2% was obtained by treatment with 1×10^6 spores/ml suspension, with no mortality obtained in the control treatment. Meanwhile, Akbari *et al.* (2014) in Iran showed that the lowest LT_{50} was obtained 7.67 days after treatment with Iran 429C (*B. bassiana*) isolate at concentration of 1×10^7 spores/ml.

Entomopathogenic fungi have been observed to cause mortality in pest population and thus, investigated for their potential as biological control agents (Hesketh *et al.*, 2008) or successfully developed as biocontrol agent for a number of different pests, including aphids (De Faria & Wraight, 2007; Shah & Pell, 2003). El-Gendy (2015) found that the LC_{50} of biozed against *B. brassicae* was 55.8 ppm. Ibrahim *et al.* (2011) found that the LC_{50} values were 103.88 and 104.75 conidia for the entomopathogenic fungi *M. anisopliae* and *B. bassiana*, respectively, against *M. persicae*. Moreover, Entesar *et al.* (2020) reported that two isolates of *B. bassiana* (B1and B2) were evaluated against wheat aphid, *Schizaphis graminum* (Rondani), and the results showed that B1 isolate was the most effective.

It can be concluded from this study that *C. Carnea* and *B. bassiana* can be used as effective biocontrol agents for the cabbage aphid *B. brassicae* in the field.

الملخص

صالح، أ.أ.، هـ. النجار، أماني أ. خليفة ومحمد ف. م. زورة. 2023. دور المفترس أسد المنّ الأخضر (*Chrysoperla carnea*) وفطر *Beauveria bassiana* في مكافحة منّ الكرنب/الملفوف على نباتات الكرنب/الملفوف. مجلة وقاية النبات العربية، 41(3): 321-326.
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أجريت تجارب حقلية في منطقة كفر صقر بمحافظة الشرقية خلال الموسمين 2020/2019 و2021/2020 لتقييم نسب المفترس: الفريسة المناسبة لإطلاق المفترس *C. carnea* وتقييم استخدام مُعلّق الفطر *Beauveria bassiana* ضدّ حشرات منّ الملفوف/الكرنب (*Brevicoryne brassicae*). أظهرت النتائج المتحصّل عليها إمكانية السيطرة الفعالة على *B. brassicae* بعد عشرة أيام من إطلاق يرقات المفترس *C. carnea*، وذلك عندما كانت نسبة المفترس: الفريسة هي 5:1 و 10:1، وفي الوقت نفسه، فإنّه في حالة النسب الأعلى من ذلك (20:1، 25:1 و 50:1)، انخفضت أعداد منّ الملفوف (*B. brassicae*) بعد 25 يوماً من إطلاق المفترس. انخفضت أعداد منّ الملفوف (*B. brassicae*) بنسبة 84.69 و 81.61% عند نسبة المفترس: الفريسة 5:1 و 10:1، على التوالي، خلال الموسم الأول؛ ومن ناحيةٍ أخرى، أمكن تخفيض أعداد حشرات المنّ بنسبة 81.50 و 70.95% بعد 5 أيام من إطلاق المفترس، وفقاً للنسبتين مفترس: فريسة المذكورتين آنفاً، على التوالي، خلال الموسم الثاني. كما تحقّق خفضٌ كلّي لمجتمع حشرات المنّ بعد 15 يوماً من إطلاق يرقات *C. carnea* بنسب مفترسات تبلغ 5:1، 10:1 و 15:1 وانخفضت أعداد *B. brassicae*عند هذه النسب تماماً بعد 20 يوماً من الإطلاق. بيّنت النتائج أنّه قد تحقّقت السيطرة المثلى على مجاميع حشرات منّ الملفوف تحت ظروف الزراعة

المحمية بعد 10 أيام من إطلاق يرقات المفترس *C. carnea* عند استخدامه بالنسب (مفترس:فريسة) 1:5 و 1:10. سُجّل أعلى معدل موت (88.33%) ناجم عن تأثير الفطر *B. bassiana* في مجتمع حشرات منّ الملفوف (*B. brassicae*) بعد 7 أيام من معاملتها بمعلق أبواغ الفطر بتركيز 1×10^7 بوغة/ مل، وكانت الجرعة القاتلة النصفية (LC_{50}) المتحصّل عليها حقلياً هي 1.10×10^6 بوغة/ مل. يمكن أن نستنتج من هذه الدراسة فعالية كلّ من المفترس *C. Carnea* والفطر *B. bassiana* كعوامل مكافحة حيوية لحشرات منّ الملفوف *B. brassicae* على مستوى الحقل.

كلمات مفتاحية: *B. brassicae*، *Chrysoperla carnea*، *Beauveria bassiana*، إطلاق المفترس.

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