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Functional Response of *Chrysoperla carnea* on Two Different Aphid Species (*Aphis fabae* and *Acyrthosiphon pisum*)

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Abstract

In this study, it was tried to determine the amount of prey consumed by *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) at varying prey densities and its potency in reducing the population of the pest. Aphids used as nutrients [*Aphis fabae* Scopoli and *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae)] were given to each larval stage (InstarI, InstarII, InstarIII) of the predator in certain numbers (5, 10, 20, 40, 80, 160). According to the data obtained, an increase in the amount of food consumed by *C. carnea* was observed depending on the food density given. The attack coefficient (a) and handling time (Th) were also calculated separately for each larval period. These values were 1.12 and 23.62 min, respectively, when the third stage larvae of the predator were fed with *A. fabae*; while these values were found to be 1.11 and 21.89 min, respectively, when fed with *A. pisum*.

Keywords: *Chrysoperla carnea*, *Aphis fabae*, *Acyrthosiphon pisum*, functional response, biological control

Introduction

Agricultural production has been significantly effective in both nutrition and development since the beginning of humanity (Tunçer and Günay, 2017). The most important of these problems are the diseases and pests that cause economic losses in plants. Chemical products have been preferred for many years in the control of them and a new one has been released day by day. However, organisms that are harmful in plant production develop different resistance to the applied chemicals, which means that the producer uses more chemicals. In recent years, researchers have started to look for alternative methods due to the negative effects of chemicals on the environment and human health (Lacey et al., 2001).Although chemical control of pests is highly preferred by the manufacturers due to the short-term solution, this causes undesirable residues on the products produced. In recent years, efforts have been continued to develop methods of controlling agricultural pests without harming the environment in order to prevent this. There is continuity in biological control in these studies and environmental pollution does not occur (DeBach, 1969; Uygun et al., 1987).

When aphids fed on the plant, growth in the plant stops and even deaths in plants are observed to a great extent. This also leads to yield and quality loses in production. In addition, the sweet substances produced during feeding of these pests cover the plant surface and the development of fumagine is observed. These pests also indirectly cause harm because they secrete toxic substances and being vectors for virus diseases (Lodos, 1982; Catherall et al., 1987; Kovalev et al., 1991; Elmalı and Toros, 1994).

Chrysopidae species prefer aphids, mites, thrips and white flies in their feeding and spread throughout the world The intense presence of this family in the natural ecosystem, ease of production for scientific studies, high search and consumption power increases the importance of this family in all and biological control studies (Tauber et al., 2000; Pappas et al., 2011). *Chrysoperla carnea* (Neuroptera: Chrysopidae) is a very common polyphagous species observed in agricultural production areas (Jokar and Zarabi, 2012). In terms of biological control, it is known that this species plays an important role as a biological control agent in greenhouses and open production areas (Venkatesan et al., 1997). The larvae begin to feed as soon as they hatch and feed on a very wide range. Their foods include lepidopter larvae, mites, mealybugs, crustaceans, thrips, aphids and adult

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and nymphs of white flies (Syed et al., 2005; Sattar et al., 2007; Sattar, 2010; Jokar and Zarabi, 2012; Batool et al., 2014). This beneficial insect can catch up to 80% of plant pests, feed on a wide range of nutrients and survive in different environmental conditions (Jokar and Zarabi, 2012).

Knowing functional and numerical responses of predator insects to prediction of the effect power of them against their prey is very important in biological control studies (Davis et al., 1976; Trexler et al., 1988). Functional response is indispensable for predator-prey models in the predator-prey relationship (Jeschke et al., 2002) and one of the key components in selecting agents for biological control (Lester and Harmsen, 2002). Some factors may affect the predatory efficiency of the predator insects both abiotic factors such as temperature (McCaffrey and Horsburgh, 1986; Mohaghegh et al., 2001; Skirvin and Fenlon, 2003), relative humidity (Svendsen et al., 1999) and prey or host species (Donnelly and Phillips 2001; Hoddle 2003; Allahyari et al., 2004; Faria et al. 2004), presence of alternative nutrients (Wei and Walde, 1997), sex of predator (Parajulee et al., 1994), age of predator and nutrition (Castagnoli and Simoni, 1999; Eveleigh and Chant, 1981). In addition, the effects of the host plant on prey have an indirect effect on the functional response (Price et al., 1980; Van Haren et al., 1987; Messina and Hanks, 1998; Sabelis et al., 1999).

The functional response of a predator shows the rate of prey consumed by the predator at varying prey densities and its power to prevent pest population (Murdoch and Oaten, 1975). The main factor in the relationship between predator and prey is the functional responses of predators to their prey when the prey population increases (Hassell, 1978). In this study, it was tried to determine the amount of prey consumed by C. carnea at varying prey densities and the potency of decreasing the population of pests.

Materials and Methods

Production of Broad Bean (*Vicia faba*)

The broad beans used as host plant in the experiment were grown in the production room of Yozgat Bozok University, Faculty of Agriculture, and Department of Plant Protection. For this purpose, bean seeds were planted to the small plastic and paper cups (in 1:1:1 ratio of soil:peat:perlite mixture) and seedlings were left under timed light (16L:8D) after they started to grow. When the height of the cultivated plants became suitable for aphid production, they were used in the experiment. This process was repeated periodically as long as the experiments continued, and weekly maintenance of the plants was carried out. All plant productions were carried out in production room set on 26±1 ºC and 60±5% humidity and long day illuminated.

Production of Aphids (*Aphis fabae* and *Acyrtosiphon pisum*)

In this study, *A. fabae* and *A. pisum* individuals produced as food for predators were obtained from Isparta University of Applied Sciences, Faculty of Agriculture, Biological Control Research and Application Center and mass production was carried out. Individuals from mass production were infected to clean plants and aphid samples used in the experiment were obtained from this production. All aphid production were carried out in the cages in production room set on 26±1 ºC and 60±5% humidity and long day illuminated (16D:8L).

Production of *Chrysoperla carnea*

The adult individuals of *C. carnea* used in the study were collected from clover fields around Isparta and Yozgat in Turkey with the help of netting and mouth aspirator. The collected individuals were brought to the laboratory and then placed in plastic containers covered with tulle. Yeast extract + honey + water mixture was placed in the plastic containers (Kişmir and Şengonca, 1981; Tireng et al., 1999) and tulles were left in strips for laying the eggs of the adult females. In order to adapt to the laboratory environment and the nutrients of the adults, individuals were used in the experiment after giving a generation. The newly hatched larvae were fed separately with the aphids used in the experiments. All productions were carried out in the cages in production room set on 26±1 ºC and 60±5% humidity and long day illuminated.

Functional Response Trials

Eggs belonging to *C. carnea*, which were mass produced for the experiments, were expected to be hatched. The larvae were separately taken into petri dishes as soon as they emerged. After larvae were famished for 24 hours, the aphids (2nd and 3rd nymph) were separately given to the predator larvae and in certain numbers (5, 10, 20, 40, 80, and 160). The number of aphids consumed by the larvae 24 hours was recorded after this procedure. These processes were performed separately for the *C. carnea* larval periods. Functional response trials were performed as 50 replications for each larval period (Mean 50 rep x 6 different densities of *A. fabae* x 3 *C. carnea* instars = 900 petri dishes. Mean 50 rep x 6 for *A. pisum* x 3 larval period of *C. carnea* = 900 petri dishes). All experiments were conducted at 26±1 ºC and 60±5% humidity and long day illumination conditions.

Statistical Analyzes

The functional response of the *C. carnea* was calculated by the formula used by Holling (1959). The hunting rate, catch time and standard errors of the hunter in the formulas were calculated according to Holling (1959)'s disk equation.

$Na = TPaN/(1+aThN)$ (Holling, 1959).

(Na:the number of prey eaten, T:the total time available for the predator, P:the number of predator, N:the number of prey offered, a:the searching efficiency, Th:the handling time)

The parameters that were obtained according to this curve is calculated using SPSS (ver. 17), MS Excel (ver. 2010) and Minitab (ver. 16).

Results and Discussion

In this study, two different aphids (*A. fabae*, *A. pisum*, different densities) were given to different larval stages of *C. carnea*. Accordingly, the number of aphids consumed by different larval stages of the predator was determined (Table 1 and Figure 1, 2).

According to the results, when 5 aphids were given to predator, consumption amounts in the second and third larval periods were similar ($P>0.05$); while there was a statistical difference between the amount of food consumed in the first larval periods ($P<0.05$). When 10 aphids were given to *C. carnea*, it was observed that individuals in the third larval period consume all aphids ($P>0.05$); while there was a difference between the amount of food consumed in the first and second larval stages ($P<0.05$). It was determined that there was a difference between the amount of food consumed in all three larvae periods when 20 prey were given to predator ($P<0.05$). When 40, 80 and 160 nutrients were given to predator, it was also determined that there was a difference between the amount of food consumed in all three larval periods ($P<0.05$).

In this study, after calculating the average number of aphids consumed by different larval stages of *C. carnea*, the attack coefficient (a) and handling time (Th) values of the predator were also calculated. Accordingly, for both nutrients, as the larval stages of the predator develop, the attack coefficient increased; while handling time was found to be shortened. In larvae fed with *A. fabae* and *A. pisum*, the highest attack rate was observed in the third period larvae; while the lowest value was seen in the first period larvae. The highest handling time was also observed in the first period larvae; while the lowest value was seen in the third period larvae. When two nutrients were compared, the attack rate values were the highest in the third period larvae fed with *A. fabae*; while the lowest catch time was determined in the third period larvae fed with *A. pisum* (Table 2).

According to the obtained data, it was determined that the type of functional response (depending on the aphid density and type) was Type-II (for three larval periods). The graphics of 1/H, 1/Ha were given Figure 3, 4, 5, 6, 7, 8 (H:Prey density, Ha: Prey consumed). Amount of nutrients consumed depending on nutrient density, hunting rate values, attack coefficient and handling times were calculated using these graphics and the disk equality of Holling (1959).

In this study, the functional response of hunter insect *C. carnea* on two different aphids was determined. In recent studies, it has been stated that especially fourth stage larvae of coccinellids consume a lot of nutrients (Moura et al., 2006; Omkar and Pervez, 2004; Bayoumy, 2011; Lee and Kang, 2014). This situation is due to the high energy need for development and the weight need for the pupal period (Hodek and Honěk, 1996). A similar situation has observed in the third stage larvae of *C. carnea* (Hassanpour et al., 2011). Many factors are effective on hunting efficiency of predator insects such as benefit from prey (Matter et al., 2011), species of prey (Sarmento et al., 2007), age of prey (Koch et al., 2003), temperature (Skirvin et al., 1997), leaf morphology (Bayoumy et al., 2014), cannibalism and intraguild predation (Burgio et al., 2002), larvae parasitism (Bayoumy, 2011; Bayoumy and Michaud, 2012).

Khan and Zaki (2008) identified the functional and numerical response of *C. carnea*'s third-term larvae on *Aphis fabae solanella* Theobald in their study. According to the data obtained, it was reported that the functional response resulting from nutrient density is Type-II and the attack rate value increases with increasing aphid density. Attack coefficient and handling time values were calculated as 0.54 and 2.17, respectively. Montoya-Alvarez et al. (2010) determined functional response of *Chrysoperla nipponensis* (Okamoto) and *C. carnea* (Stephens) (Neuroptera: Chrysopidae) fed on seven different densities of *Aphis gossypii* (Glover) (Homoptera: Aphididae) at 20 °C on laboratory conditions. It has been reported that both predator have Type-II on cotton aphid. The maximum amount of aphids consumed of *C. carnea* has been more than *C. nipponensis*. According to the data obtained, the handling time of both species decreased due to the increasing nutrient density; while this time of *C. nipponensis* was found to be higher than that of *C. carnea*. When the attack rate values were examined, it was found that the attack coefficient of *C. nipponensis* was slightly higher than those of *C. carnea*. Hassanpour et al. (2011) determined the functional response of *C. carnea*'s three larval stages on egg and first larval stages of the cotton bollworm *Helicoverpa armigera* Hübner. According to the data obtained, the first and second larval stages of *C. carnea* showed Type-II functional response on both nutrients. However, third larval period of *C. carnea* had Type-II functional response when fed on first larval stage of *H. armigera*, had Type-III when fed with eggs of cotton bollworm. According to the results, the highest hunting rate was observed in the third period predator larvae feeding on the eggs of *H. armigera*. It was also concluded that the larvae of *C. carnea* (especially the third period) had good hunting potential under the control of the larvae and eggs of *H. armigera*. Batool et al. (2014) carried out studies on biology and functional response of *C. carnea* (Stephens) (Neuroptera: Chrysopidae) under laboratory conditions. They gave eggs of *Sitotroga cerealella* (Lepidoptera: Gelechiidae) (different densities: 20, 30, 40, 50, 60, 70, 80, 90 and 100) to the *C. carnea* larvae in petri dishes. According to the results, it was observed that consuming density had a significant effect on *C. carnea*'s positive consumption rate, growth rate and fertility. It has been observed that hunting potential increases as food density increases in all trials. The daily catching rate of *C. carnea* increased gradually during the first two larval stages; reached the highest point in the third larval period. Memon et al. (2015) determined functional response of third larval stage of *C. nipponensis* (Neuroptera: Chrysopidae) on different preys [artificial food and *Corcyra cephalonica* eggs and cowpea aphid (*Aphis craccivora*), papaya mealybug (*Paracoccus marginatus*) and whitefly (*Bemisia tabaci*)] on different densities. According to the results, the larvae of *C. carnea* showed Type-II functional response on all foods. Saljoqi et al. (2016) determined functional response of larvae of C. carnea on *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae) in their studies. According to the data obtained, the amount of aphid consumed increased according to the larval periods and the prey density. The maximum catching rate was calculated in the third larval stage; while this value decreased in other larval periods. The lowest handling time was observed in the third period larvae, while this value increased in the other two larvae periods when the handling times were examined. In addition, the type of functional response of the *C. carnea* on *B. brassicae* was also reported to be Type-II. Alhamawandy (2017) determined functional response of *C. carnea* on A. fabae on laboratory conditions. According to the results, *C. carnea* showed a second type (Type-II) functional response and it was reported that the functional response of the predator increased due to the increasing prey density. The highest attack rate (a) was seen in the third larval period of *C. carnea* with 0.976; the lowest attack rate was 0.635 in the first larval period. In addition, the lowest handling time was calculated in the third larval period of *C. carnea* with 5.33 minutes; the highest handling time was 21.6 minutes in the first larval period. Rana et al. (2017) gave frozen eggs of Corcyra and seven different aphids to the *C. carnea*, and then observed the development of predator. The hunting effect was calculated by recording the amount of food consumed by the predator insect every day. According to the results, it was reported that the hunting effect increased from the first larval period to the third larval period and *C. carnea*, a potential biological control agent, was found to be highly effective against different aphid species. However, in order to obtain better data, it was concluded that trials should also be conducted in field conditions simultaneously with this study. Bayoumy and Awadalla (2018) investigated effects of two different preys [*Myzus persicae* Sulzer ve *A. craccivora* Koch (Hemiptera: Aphididae)] of different densities on third larval stage of *C. carnea* Stephens (Chrysopidae: Neuroptera) and forth larval stage of *Coccinella septempunctata* L. and *Hippodamia variegata* Goeze (Coccinellidae: Coleoptera). According to the data obtained, the species of prey and predator, prey density and their relations with each other have a significant effect on aphids consumption, but the functional response type has not been changed (Type-II). Ail-Catzim et al. (2019) determined Type-III functional response of third larval stage of *C. carnea* on forth nymph of Myzus persicae. According to the regression analysis, they also determined the handling time and attack rate of the hunter. According to the results obtained, they found that there was an increase in the amount of aphids consumed by the predator in the third larval period depending on increased prey density. It is thought that this difference arises from the fact that the food age given to predators (N4) is different from the food age given in our study (N2-N3). Mahzoum et al. (2019) determined functional response of larvae of *C. carnea* on *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) in their study. The consumed prey amounts were recorded depending on prey densities (3, 5, 10, 15, 25 and 40). According to the results, it was found that the functional response of *C. carnea* on *S. oleae* of all larval stages was Type-II and the amount of consumption increased depending on the prey density. Costa et al. (2019) examined developmental biology and functional response of *Leucochrysa* (*Nodita*) *azevedoi* (Neuroptera: Chrysopidae) on different preys. According to the data obtained, it has been reported that the larvae of the predator insect showed functional type II response and has a potential in the biological control of these preys.

Conclusion

When this study is compared with other studies, similarities (especially functional response type: Type-II) are observed. In addition, an increase in the amount of nutrients consumed by *C. carnea* depending on the nutrient density was also observed in our study. Looking at the data we obtained, it is thought that *C. carnea* larvae have an important place in the integrated pest management programs in terms of control of *A. fabae* and *A. pisum*. However, it is thought that predator-prey relations should be investigated in natural terrain conditions in order to better reveal the potential of the predator.

Table 1. Amount of consumed aphids by different periods of *Chrysoperla carnea* larvae

	n	Number of Aphids (*A. fabae* / *A. pisum*) consumed L1	L2	L3
Aphis fabae	5	3,96±0,1 a^B	4,52±0,07 a^A	5,00±0,00 a^A
Acyrthosiphon pisum	5	4,42±0,06 a^A	4,64±0,07 a^A	5,00±0,00 a^A
Aphis fabae	10	5,90±0,15 b^B	6,82±0,15 b^B	10,00±0,00 b^A
Acyrthosiphon pisum	10	6,44±0,10 b^A	7,42±0,11 b^A	10,00±0,00 b^A
Aphis fabae	20	11,80±0,17 c^B	14,42±0,13 c^B	19,02±0,13 c^B
Acyrthosiphon pisum	20	12,34±0,16 c^A	15,32±0,20 c^A	19,42±0,12 c^A
Aphis fabae	40	22,54±0,21 d^B	24,16±0,26 d^B	27,44±0,20 d^B
Acyrthosiphon pisum	40	23,46±0,21 d^A	25,64±0,18 d^A	28,74±0,17 d^A
Aphis fabae	80	24,14±0,22 e^B	25,78±0,23 e^B	31,52±0,23 e^B
Acyrthosiphon pisum	80	25,04±0,17 e^A	27,00±0,23 e^A	32,78±0,18 e^A
Aphis fabae	160	25,74±0,23 f^B	27,22±0,22 f^B	33,82±0,27 f^B
Acyrthosiphon pisum	160	26,54±0,25 f^A	28,50±0,21 f^A	34,50±0,21 f^A

Values bearing the lowercase letters in the same column represent the nutrient concentrations given to the predator insect; the values given in uppercase letters compare the consumption amounts of different foods. Different uppercase letters indicate a statistical difference between nutrients (one-way ANOVA, Tukey Test, α=0.05) (L1: 1st Instar, L2: 2ndInstar, L3: 3rd Instar) (n: Prey Density).

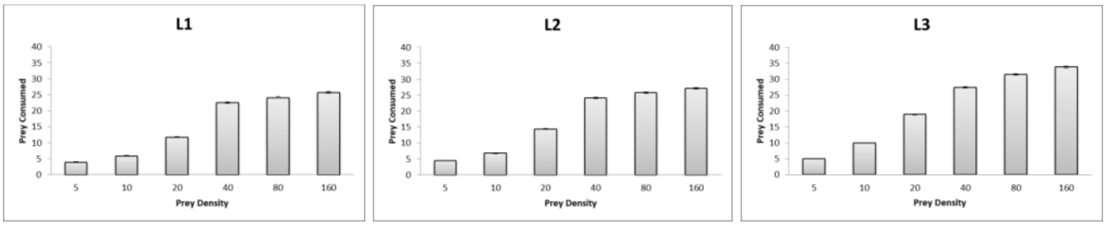

Figure 1. Amounts of *Aphis fabae* consumed by different periods of *Chrysoperla carnea* larvae

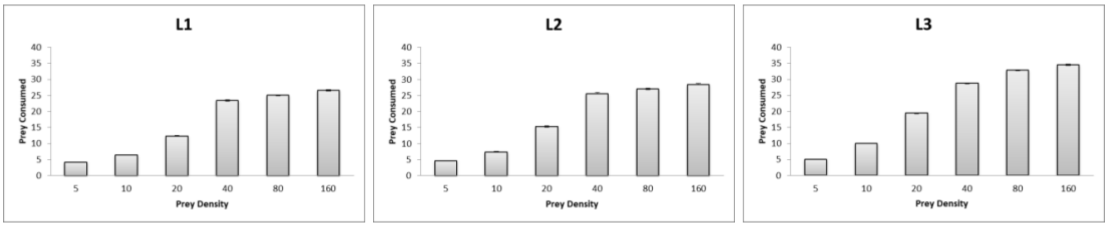

Figure 2. Amounts of *Acyrthosiphon pisum* consumed by different periods of *Chrysoperla carnea* larvae

Table 2. The functional response values [Attack coefficients (*a*), Handling times (*Th*)] of the larvae of *Chrysoperla carnea* on *Aphis fabae* and *Acyrthosiphon pisum*

		L1	L2	L3
Aphis fabae	Attack coefficient (*a*)	0,85543	0,98981	1,11844
	Handling time (*Th*)(min)	41,04	37,584	23,616
		L1	L2	L3
Acyrthosiphon pisum	Attack coefficient (*a*)	0,91676	1,0202	1,11025
	Handling time (*Th*)(min)	39,6	33,84	21,888

(L1: First instar, L2: Second instar, L3: Third instar)

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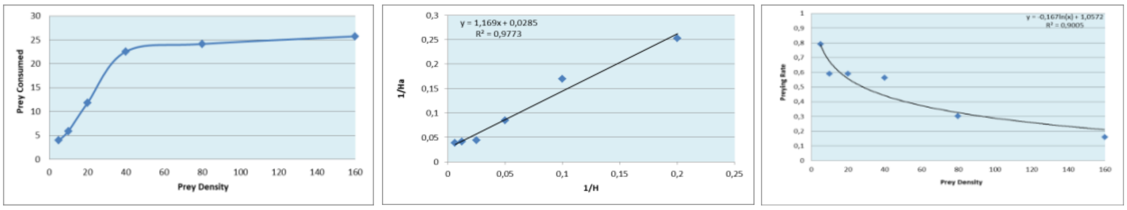

Figure 3. The functional response of first instar of *Chrysoperla carnea* on *Aphis fabae*

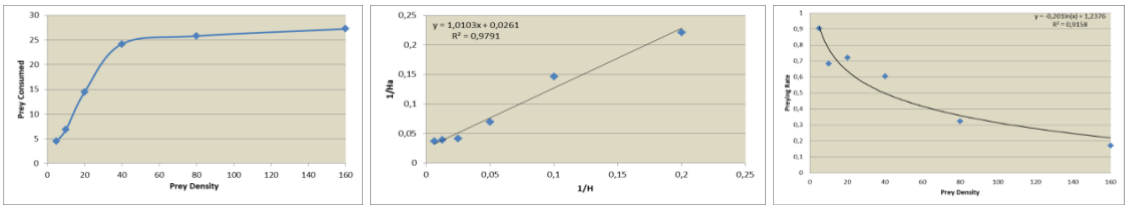

Figure 4. The functional response of second instar of *Chrysoperla carnea* on *Aphis fabae*

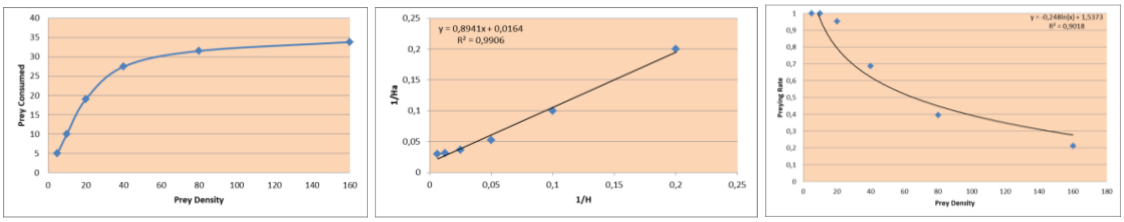

Figure 5. The functional response of third instar of *Chrysoperla carnea* on *Aphis fabae*

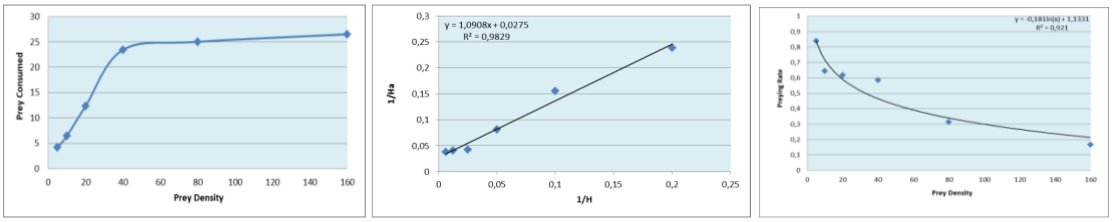

Figure 6. The functional response of first instar of *Chrysoperla carnea* on *Acyrthosiphon pisum*

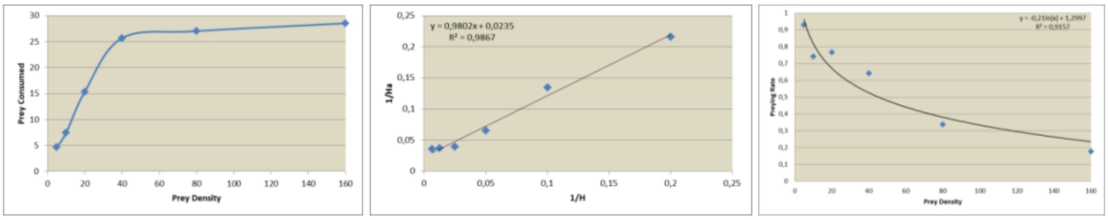

Figure 7. The functional response of second instar of *Chrysoperla carnea* on *Acyrthosiphon pisum*

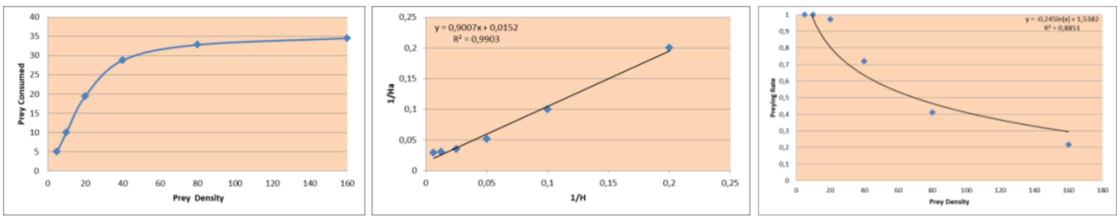

Figure 8. The functional response of third instar of *Chrysoperla carnea* on *Acyrthosiphon*

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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