EUROPEAN ACADEMIC RESEARCH

Impact of *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) on the functional response of *Chrysoperla carnea* feeding on *Ephestia cautella* eggs

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Abstract:

     This study was conducted to determine the functional responses of Chrysoperla carnea larvae treated with different concentrations of Beauveria bassiana (Bals.) and Metarhizium anisopliae (Met.) and then dealing with different densities of Ephestia cautella eggs. A type 2 functional response was demonstrated according to consumption curve and the negative values of the linear coefficients of logistic regression. The attack coefficient values (à) and the handling time (Th) are correlated with larvae development and their influence by fungal isolate and concentration. The attack coefficient (a) for the second instar treated with B.bassiana was 0.056, 0.0516, 0.039 and 0.024 and the handling time was 0.38 , 0.45 , 0.59 and 0.84 hour for control , 10^5, 10^7 and 10^9 spore / ml respectively, these values for third larval instar was 0.13, 0.11 , 0.078 and 0.0617 as attack coefficient and 0.068, 0.1 , 0.12 ,and 0.175 h as

handling tim respectively. In case of M. anisopliae treatment, the attack coefficient (a) was 0.050 , 0.049 and 0.046 for second larval instar without significant differences between the concentrations of 10^7 and 10^9 spore\ml ,and it was 0.13 , 0.11 , 0.078 and 0.0617 for third instar larvae, without significant difference between the concentrations 10^5 and 10^7 spore / ml. The handling time was 0.38, 0.64, 1.022 and 1.25 hour for second instar and 0.068, 0.21, 0.23 and 0.27 hour for third instar for control and 10^5, 10^7, 10^9 spore / ml respectively . High affected by third instar larvae compared with the second instar were demonstrated when treated with the both fungi. The difference of percentage decline of the attack rate was significant between the two instars at concentrations of 10^5 and 10^7 spore/ml of B.bassiana, and at all concentrations of M. anisopliae.

Comparing the effect of both fungi on the attack rate outweighed the fungus of M. anisopliae significantly on the third instar at all concentration and B.bassiana on the second instar at concentration of 10^7 and 10^9 spore /ml.

Key words: *Beauveria bassiana* (Bals.), *Metarhizium anisopliae* (Met.), *Chrysoperla carnea*, *Ephestia cautella* eggs

INTRODUCTION

The success of fungal entomopathogens as biological control agents depends not only on high efficacy against insect pests, but also on low virulence against non-target insects. It is necessary to investigate their effects on non-target insects prior to their release. Natural enemies of insects have evolved and function in a complex multitrophic environment (Vet & Dicke, 1992; Poppy, 1997). Entomopathogenic fungi may reduce non-target natural enemy populations by directly infecting them, or indirectly by reducing the host populations of predators and parasitoids or by rendering the host unsuitable for other natural enemies (Rosenheim *et al.*, 1995).

The *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) are the most important species that used widely in the various countries of the world against a large number of insect pests especially those that spend part of their life cycle in the soil (Toledo *et al.*, 2008).

Chrysoperla carnea (Neuroptera: Chrysopidae) is a natural enemy of many insects and is present worldwide in many cropping systems, it's very effective and practical in biological control programs against agricultural pests (Canard *et al.*, 1984). They have high-predation efficiency, adaptation to the diversity of eco- agriculture system and pesticides tolerant (Sattar and Abro, 2011). They are successful predators of whiteflies, thrips, aphids and mites (Singh and Manoj, 2000; Zaki and Gesraha, 2001; Venkatesan et al., 2002). They also feed on the eggs and tiny larvae of the cotton bollworms (Ahmad et al., 2003). Studies of the interactions between entomopathogens and arthropod natural enemies have generally concentrated on the pathogen as the `intra-guild predator', i.e. directly able to infect insect natural enemies (Flexner *et al.*, 1986; Goettel *et al.*, 1990). So, this study aimed to assess the indirect potential interactions between *Beauveria bassiana* (Bals.), *Metarhizium anisopliae*. and *Chrysoperla carnea* , especially impacting of these entomopathogenic fungi on the feeding activities of the predator by using functional response which is a tool commonly employed to estimate the predatory capacity on different prey densities.

MATERIALS AND METHODS

Insects Rearing
Chrysoperla carnea.
The adults of *Ch. carnea* were originally collected from fields were confined in transparent plastic cups (11 cm in diameter and 7.5 cm high) and supplied, via cotton swabs, with the standard artificial diet consisting of yeast, sugars, and distilled

water in the ratio of 4 g: 7 g: 10 mL, respectively [Hagen,& Tassan, 1970]. The top of the plastic cup was covered with black muslin cloth tightened with a rubber band. The eggs laid by females on the walls of the cups and muslin cloth were harvested daily, using forceps to break the stalk beneath the egg. The eggs were placed, with the help of a camel's hair brush, singly in plastic Petri dishes (10 cm in diameter and 1.5 cm high).

Ephestia. cautella

A colony of *E. cautella* was established using growth chamber set up at 25 ± 2°C, 60 ± 5% relative humidity and a photoperiod 16: 8 h (L:D). Artificial diet as described earlier was used for *E. cautella* maintenance (Hameed 2002). Through this method sufficient numbers of eggs were obtained to rear *Ch. carnea* continuously under laboratory condition and to carry functional response test.

Culture of Fungi and preparation of suspension

The fungal isolates *Metarhizium anisopliae* and *Beauveria bassian*a were grown on 9 cm Petri dishes containing Potato dextrose Agar PDA (39 g l^{-1}) and incubation at 25°c in darkness until colonies fully occupied the dishes then stored at 4 ^{o}c for further use. The fungal suspension was prepared by adding 5 ml sterile distal water SDW mixed with 500 µl of tween 80 and gently scraped with sterile scalpel. The suspensions were stirred vigorously for 5 min to break up the spores from the conidiophores and the hypha debris was removed by passing the suspension through fabric cloths. Three concentrations of each fungal isolates 1 x 10^{5}, 1 x 10^{7} and 1x 10^{9} were determined by the aid of hemocytometer. The viability of spore was determined as in Lacey (1997).

Effect of fungal isolates on function response of *Ch. carnea*

The functional response of the second and the third instar larvae of *Ch. carnea* were conducted with different densities of *Ephestia cautella* eggs (20, 40, 80, 160 and 300 eggs) as prey, after 3 days of exposure these larvae to fungal suspensions of *Beauveria bassiana* and *Metarhizium anisopliae* at concentration of 1 x 10^5, 1 x 10^7 and 1x 10^9 spore/ ml. . Each treatment was replicated three times in plastic containers (9 cm) under condition of 25±1 °C, 60–70 % RH and a photoperiod of 16:8 h (L: D)). The prey consumed was counted after every 24 h.   The type of the functional response (type II or III) was determined using logistic Regression analysis of the proportion of prey killed in relation to the initial density (Trexler et al. 1988). To do this, a polynomial logistic regression was fitted to data:

$$\frac{N_a}{N_0} = \frac{\exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}{1 + \exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}$$

Where Na is the number of prey consumed, N_0 the initial prey density and P0, P1, P2, P3 are the intercept, linear, quadratic and cubic coefficients, respectively. If the linear coefficient, P1 is significantly negative, the predator is displaying a type II Functional response; if positive, there is a Type III functional response, and these parameters can be estimated by using the procedure in SAS software (Juliano, 2001)(SAS Institute.2003). The Rogers, (1972) was used to model the relationship between the numbers of prey consumed (*Na*) and initial Prey density (*N0*):

$$Na = N_0\left[1 - \exp(a(ThNa - T))\ \right]$$

Where *a* is the attack rate, T is the total time available, and *Th* is the handling time per prey item.

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Statistical analysis

Statistical analysis was carried out using completely randomized design (CRD) and Duncan test and t test (SPSS version 20).

RESULTS AND DISCUSSION

The second and third instar larvae of the predator *Ch.carnea* that treated with *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) are demonstrated type 2 functional responses in their dealings with different densities of *E.cautella* eggs (fig. 1-4), the consumption rate increases at a decelerating rate, gradually leveling off at maximum rate. This result is confirmed by using logistic regression between the proportion of prey consumed (Na / N) as a function of prey density (N) (Table 1A-C), so the negative values of the linear coefficients (*P1*) indicated to type 2 functional response. These values for the second larval instar were - 0.085 , - 0.189 , - 0.176 and 0.116- for the control treatment and other concentrations of spore suspensions of *B.bassiana* (10^5, 10^7 and 10^9 spores\ml respectively) ,and for the third larval instar were -0.0566 , - 0.0094 , - 0.0046 and - 0.023 respectively. In cases of the fungus *Mitarhizum anisopalei* at concentrations of 10^5, 10^7 and 10^9 the linear regression coefficient for the second instar were - 0.117, - 0.146 and - 0.19, and for third instar were - 0.0019,- 0.024,- 0.036 respectively.

The attack coefficient values (à) and the handling time (*Th*) are correlated with larvae development, increasing of their capturing efficiency and degree of their influence by fungal isolate and concentration that lead to decrease of attack coefficient (a) and increase of handling time (*Th*). Attack coefficient (a) of the second larval instar was 0.056, 0.0516, 0.039 and 0.024 with significant differences and the handling time were 0.38, 0.45, 0.59 and 0.84 hour for control and the concentrations of 10^5, 10^7, 10^9 spore / ml of *B.bassiana*

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respectively, while, the attack coefficient values (à) of third larval instar was 0.13, 0.11 , 0.078 and 0.0617 and the handling time was 0.068, 0.1 , 0.12 ,and 0.175 h respectively. The correlation coefficient between the attack coefficient and spore concentrations were - 0.9 and - 0.96 for the second and third instar respectively.

In case of *M. anisopliae* treatment, the   attack coefficient (a) was 0.050 , 0.049 and 0.046 for second larval instar without   significant   differences   between   the concentrations of 10^7 and 10^9 spore\ml ,and it was 0.13 , 0.11 , 0.078 and 0.0617 for third instar larvae, without significant difference between the concentrations 10^5 and 10^7 spore / ml. The handling time was 0.38, 0.64, 1.022 and 1.25 hour for second instar and 0.068, 0.21, 0.23 and 0.27 hour for third instar for control and 10^5, 10^7, 10^9 spore / ml respectively . Figures (7) demonstrated high affected by third instar larvae compared with the second instar when treated with both fungi. The difference of percentage decline of the attack rate was significant between the two instars at concentrations of 10^5 and 10^7 spore/ml of *B.bassiana,* and at all concentrations of *M. anisopliae.*

Comparing the effect of both fungi on the attack rate outweighed the fungus of *M. anisopliae* significantly on the third instar at all concentration and *B.bassiana* on the second instar at concentration of 10^7 and 10^9 spore /ml. according to t-test (Figure 8).

Number of studies dealt with direct and indirect impact of various environmental factors on the functional response type and its important parameters, attack rate and the handling time. Seiedy et al. (2012) studied indirect effect on the functional response of the mite predator *Phytoseiulus persimilis* feeding on adults spider mite *Tetranychus urticae* by treated with *B.bassiana* DEB1008 at intervals times of 0, 24, 48 and 72 hours, and the results showed prevalent of the second type response in all time intervals with an increase in the handling

time (3.51, 3.81 , 4.71, 7.68 and 11.77 hours) for the interval times that were mentioned respectively with significant difference, while, attack coefficient was a closed for all situations by decrease the rate feed with the progress of time. Another study, recorded the effect of *Neozygites floridana* (Zygomycota) on *Phytoseiulus longipes* behavior by reducing the searching capacity for prey (Wekesa et al, .2007)).

A study of Alma *et al*. (2010) appeared that the nymphs of whiteflies that treated with *Isaria fumosorosea* was acceptable for consumption by the predator *Dicyphus hesperus* after 3 days of treatment but it was not acceptable after five days. Decreasing in feeding time and the rate of predation was recorded for the predator *Orius albidipennis* (Hemiptera) on the larvae of *Thrips tabaci* that treated by fungal suspension of *M. anisopliae* (Pourian et al., 2011).

The temperature is an environmental factor affecting the various life parameters such as functional response, searching rates tend to decrease with increasing of handling time at temperature extremism (2011 Sentis et al.,). Results of the effect of temperatures on the functional response type are recorded by Moezipour et al. (2008) for *Trichogramma brassicae* (Hymenoptera) follows type2 at a temperature of 25 ° C while it follows type3 at temperature of 20 and 30 Celsius.

The instar of predator and its species and instar of prey are other factors affecting the functional response type; Hassanpour *et al*. (2009) recorded functional response Type I, II and III for the three successive instars of the species *C.carnea* toward the red spider *Tetranychus urticae* under laboratory conditions. The second type of functional response was recorded to the same species *C.carnea* on eggs *Heliothis virscens* (, (Stark and Whitford. 1987), the three instar larvae of *C.carnea* appeared type2 functional response at a mixture of third and fourth instars nymph of aphid species *Hyalopterus pruni* at a rate attack of 0.051 and 0.046, and 0.042 / hour and the

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handling time 1.159 and 0.494 and 0.106 hour for larval instar respectively(Atlihan and Chi, 2008)).

In many ladybird species like *Coccinella septempunctata* and *Hippodamia variegate* the type 2 functional response are recorded when feeding on the third and fourth nymph instar of *Bemisia tabaci* (Ghahari *et al.* 2003) and all instar larvae and adult of ladybird *Hippodamia variegate* feeding on the peas Aphid, *Aphis fabae* (Farhadi et al. 2010), as well as adult males of ladybird *Cheilomenes sulfurea* feeding on the *Aphis fabae* (Hodek et al. 1984), and adult females of *C. sexmaculata* and *Propylea dissecta* and *C.transversalis* feeding on *Aphis crraccivora* and *Myzus persicae* (Perveez and Omkar, 2005) .

Also the second type of functional response have recorded to the fourth larval instar and female of ladybird *Serangium montazerii* toward nymphs of citrus whiteflies *Dialeurodes citri* at third and fourth instar nymph (Fotukkiaiiet al., 2012). Also it was found in many arthropod predators such as spiders (1962 Turnubull,) and mites (Roy et al., 2003).

The handling time is a good indicator of the Predatory capacity (Atlihan and Güldal, 2009), so the results confirm the advantage of the third stage predatory capacity. Despite the importance of the identification response type as tool in biological control programs, but it no longer be sufficient in determining the success of these programs as there are other factors carry considerable influence in predator efficiency such as the Intrinsic rate of increase and host distribution and its features as well as competition and the environmental complex (Perveez and Omkar 0.2005).Furthermore, the experiments are usually carried out under controlled conditions so a predator will face big changes in nature such as the climatic conditions of temperatures and relative humidity and others that probably will change of the functional response and this is making the prediction of Predatory capacity more difficult, effect of weather would increase or reduce the numbers consumed in each density of prey (Farhadi et al. 2010).

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Many previous studies have addressed the functional response of predators to be used as the guarantor of effectiveness in biological control of pests and reduce the harmfulness (Wijesekara, 2006; Skalski and Gilliam, 2001; Sarmento et al., 2007)
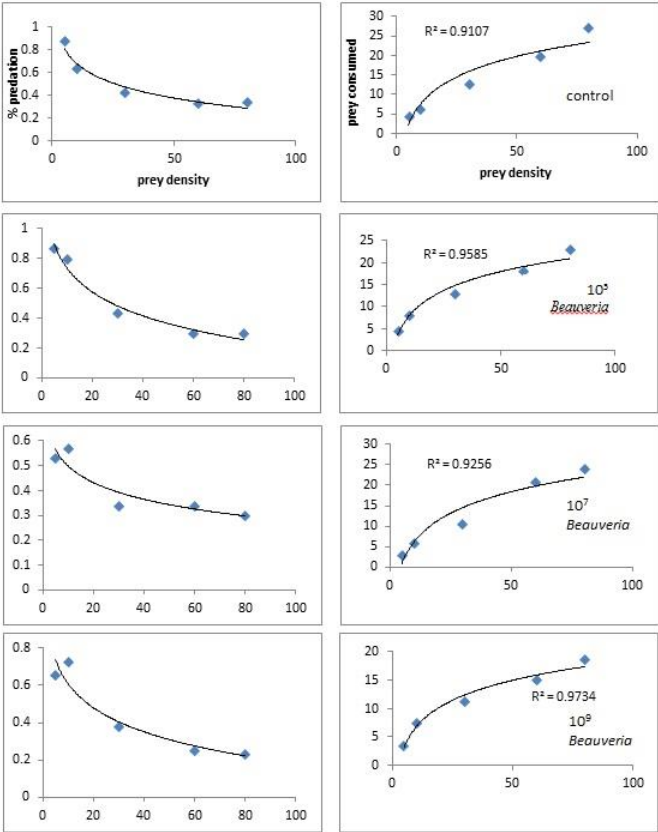

Fig (1) functional response of second larval instar of *Chrysoperla carnea* **treated and untreated with different concentrations of spore suspension of** *Beauveria bassiana*

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Fig (2) functional response of third larval instar of ***Chrysoperla carnea*** **treated and untreated with different concentrations of spore suspension of** ***Beauveria bassiana***

Fig (3) functional response of second larval instar of *Chrysoperla carnea* **treated and untreated with different concentrations of spore suspension of** *Metarhizium anisopliae*
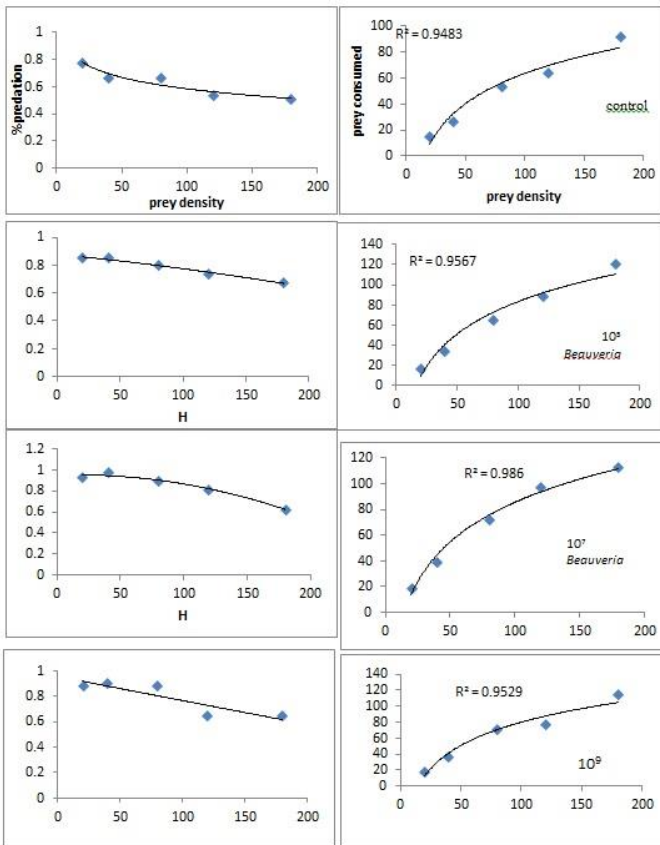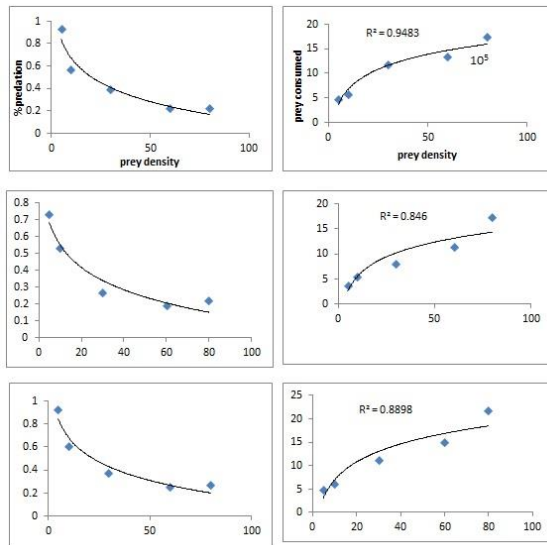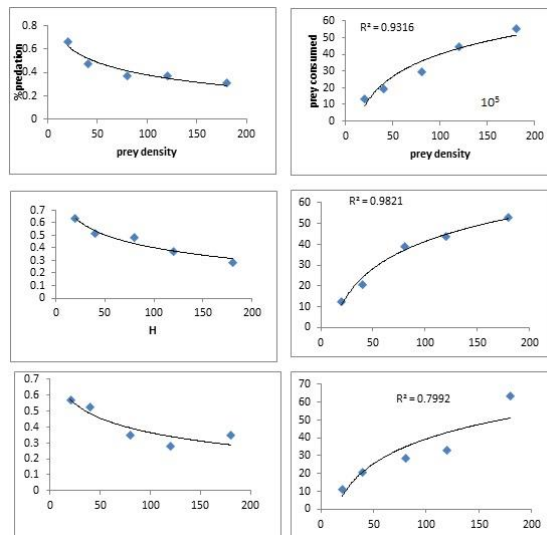

Fig (4) functional response of third larval instar of *Chrysoperla carnea* **treated and untreated with different concentrations of spore suspension of** *Metarhizium anisopliae*

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Table (1-A) logistic regression analysis of the proportion of *Ephestia cautella* eggs eaten by *chrysoperla carnea*

Treatment	Concentrate	predator developed	parameter	Estimated value	standard error SE±	Chi-square x^2	P
control	**0.0**	Second	**Intercept**	1.78	0.67	7.08	0.008
			Linear	-0.085	0.056	2.3	0.13
			Quadratic	0.00084	0.0013	0.4	0.53
			Cubic	-1.59E-6	9.3E-6	0.03	0.86
		Third	**Intercept**	5.17	1.559	11.02	0.0009
			Linear	-0.0566	0.05	1.24	0.27
			Quadratic	0.000279	0.0005	0.31	0.58
			Cubic	-4.45E-7	1.475E-6	0.09	0.76

Table (1-B) logistic regression analysis of the proportion of *Ephestia cautella* eggs eaten by *chrysoperla carnea*, treated with *M.anisopliae*

Treatment	Con.	predator developed	parameter	Estimate value	standard error SE±	Chi-square x^2	P
M.anisopliae	10^5	Second	Intercept	0.9	0.6	2.24	0.13
			Linear	-0.117	0.06	4.2	0.04
			Quadratic	0.002	0.0014	1.74	0.19
			Cubic	-9.56 E-6	0.00001	0.88	0.34
		Third	Intercept	0.37	0.53	0.49	0.48
			Linear	-0.0019	0.021	0.01	0.93
			Quadratic	-0.00014	0.00023	0.38	0.54
			Cubic	5.21E-7	7.29E-7	0.51	0.47
	10^7	Second	Intercept	1.9	0.63	9.49	0.002
			Linear	-0.19	0.058	11.27	0.008
			Quadratic	0.004	0.0014	7.33	0.0068
			Cubic	0.00002	0.00001	5.78	0.016
		Third	Intercept	0.095	0.53	0.03	0.85
			Linear	0.024	0.012	1.32	0.25
			Quadratic	-0.00039	0.00023	2.86	0.091
			Cubic	1.33E-6	7.29E-6	3.32	0.068
	10^9	Second	Intercept	2.0157	0.66	9.4	0.0022
			Linear	-0.146	0.057	6.64	0.01
			Quadratic	0.00246	0.00136	3.25	0.07
			Cubic	-0.00001	9.59E-6	2.18	0.139
		Third	Intercept	1.6	0.57	7.84	0.005
			Linear	-0.036	0.022	2.52	0.11
			Quadratic	0.00012	0.00024	0.024	0.62
			Cubic	6.74E-8	7.66E-7	0.01	0.93

Table (1-C): logistic regression analysis of the proportion of *Ephestia* cautella eggs eaten by *chrysoperla carnea*, treated with *B.bassiana*

Treatment	Con.	predator developed	parameter	Estimate value	standard error SE±	Chi-square x^2	P
B.bassiana	10^5	Second	Intercept	3.86	1.04	13.5	0.0002
			Linear	-0.189	0.076	6.17	0.013
			Quadratic	0.0028	0.0017	2.77	0.096
			Cubic	-0.00001	0.000011	1.51	0.219
		Third	Intercept	1.650	0.71	5.39	0.02
			Linear	-0.0094	0.027	0.12	0.73
			Quadratic	-0.00023	0.00029	0.63	0.43
			Cubic	9.43E-7	9.09E-7	1.08	0.299
	10^7	Second	Intercept	1.38	0.612	5.12	0.02
			Linear	-0.176	0.058	8.92	0.003
			Quadratic	0.003	0.00145	5.28	0.0215
			Cubic	-0.00002	0.000001	3.23	0.072
		Third	Intercept	3.31	1.14	8.42	0.0037
			Linear	-0.0046	0.04	0.01	0.91
			Quadratic	-0.00081	0.0004	0.19	0.66
			Cubic	7.14E-7	1.25E-6	0.33	0.567
	10^9	Second	Intercept	1.58	0.62	6.45	0.011
			Linear	-0.116	0.054	4.6	0.0311
			Quadratic	0.0019	0.0013	2.14	0.14
			Cubic	-9.99E-6	9.02E-6	1.18	0.28
		Third	Intercept	3.4	0.97	12.46	0.0004
			Linear	-0.023	0.033	0.48	0.49
			Quadratic	0.00001	0.00034	0.09	0.76
			Cubic	7.89E-7	1.009E-6	0.61	0.43

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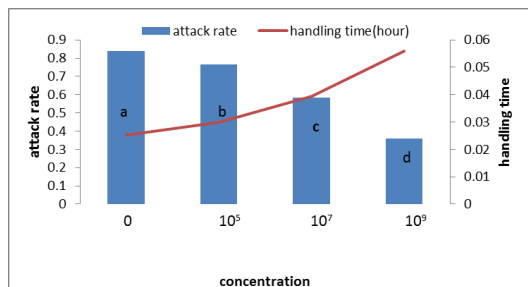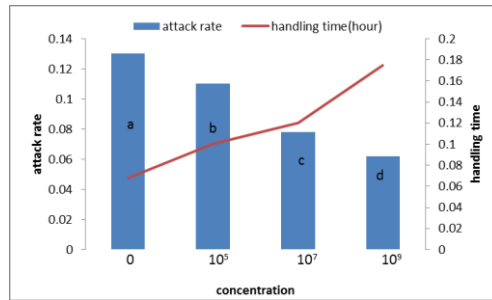**functional response of *Chrysoperla carnea* feeding on *Ephestia cautella* eggs**

Table (2): attack coefficient values (a) and the handling time (Th) of
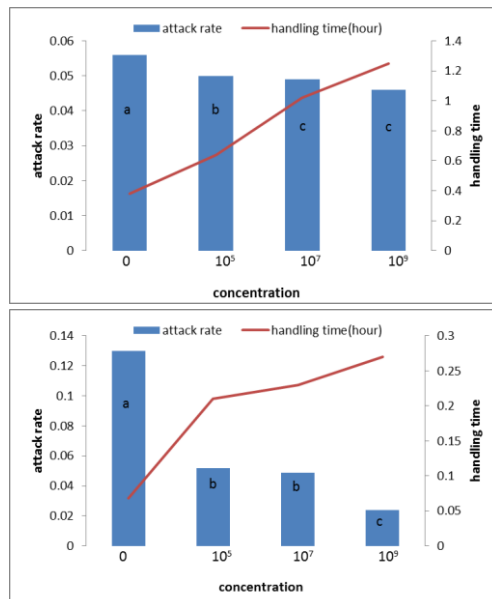***Chrysoperla carnea* dealing with different density of *E. cautella* eggs.**

Treatment	Con.	predator developed	parameter	Estimate value	Standard error SE	Confidence limits 95%	
						Higher	Less
Control	0.0	Second	attack coefficient(a)	0.056	0.01	0.112	0.011
			handling time(Th)	0.38	0.17	0.75	0.15
		Third	attack coefficient(a	0.13	0.0137	0.144	0.085
			handling time(Th)	0.068	0.0139	0.098	0.039
B.bassiana	10^5	Second	attack coefficient(a	0.0516	0.024	0.103	0.00028
			handling time(Th)	0.45	0.32	0.97	-0.45
		Third	attack coefficient(a	0.11	0.029	0.14	0.068
			handling time(Th)	0.1	0.028	0.16	0.039
	10^7	Second	attack coefficient(a	0.039	0.0126	0.066	0.012
			handling time(Th)	0.59	0.1632	0.94	0.24
		Third	attack coefficient(a	0.078	0.016	0.11	0.044
			handling time(Th)	0.12	0.0643	0.21	0.129
	10^9	Second	attack coefficient(a	0.024	0.01	0.047	0.0015
			handling time(Th)	0.84	0.195	1.26	0.41
		Third	attack coefficient(a	0.0617	0.0043	0.26	0.0082
			handling time(Th)(	0.175	0.0143	0.21	0.144
M.anisopliae	10^5	Second	attack coefficient(a)	0.05	0.026	0.11	0.005
			handling time(Th)	0.64	0.14	0.94	0.33
		Third	attack coefficient(a	0.0515	0.008	0.0588	0.014
			handling time(Th)	0.21	0.074	0.38	0.064
	10^7	Second	attack coefficient(a	0.0499	0.0167	0.086	0.0137
			handling time(Th)	1.022	0.17	1.38	0.66
		Third	attack coefficient(a	0.049	0.018	0.09	0.011
			Time of (Th) treatment	0.23	0.0724	0.37	0.06
	10^9	Second	attack coefficient(a	0.046	0.016	0.08	0.012
			Time of (Th) treatment	1.25	0.24	1.78	0.72
		Third	Attack cofactor (a)	0.024	0.0057	0.036	0.012
			Time of (Th) treatment	0.266	0.084	0.29	0.095

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*the same letters mean no significant differences according to Duncan's test

Fig.(5) attack rate and handling time for the second and third instar larvae of *Ch. carnea* **that treated with vary concentrations of B. bassiana**

*the same letters mean no significant differences according to Duncan's test

Fig.(6) attack rate and handling time for the second and third instar larvae of *Ch. carnea* **that treated with vary concentrations of M. anisopliae**

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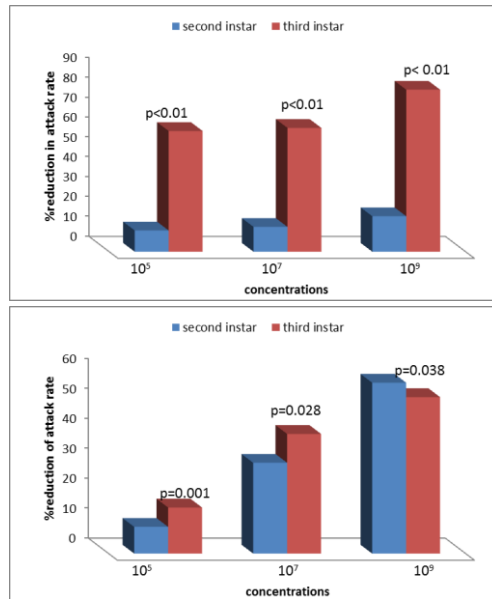

Fig.(7)Comparison between the second and third instar larvae of *C.carnea* in %reduction of their attack rate after their treated with different concentrations of *B.bassiana* and *M. anisopliae*
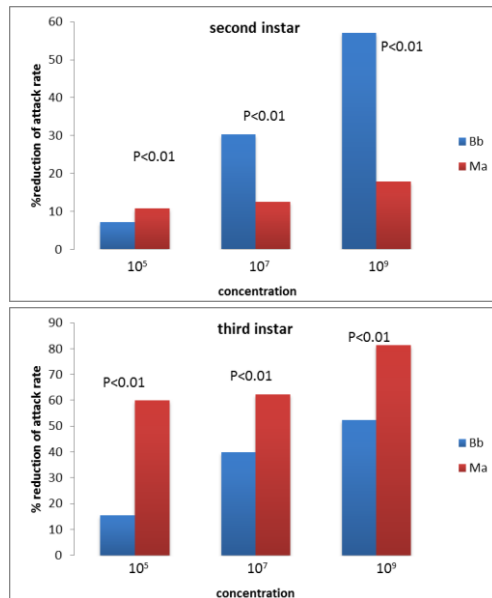

Fig.(8) Effect of *B.bassiana* and *M. anisopliae* at the same concentration on attack rate of second and third instar larvae of *C.carnea*

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