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FUNCTIONAL RESPONSE OF *MENOCHILUS SEXMACULATUS* F. (COLEOPTERA: COCCINELLIDAE) TO DIFFERENT DENSITIES OF *APHIS CRACCIVORA* KOCH (HOMOPTERA: APHIDIDAE)

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Abstract

Investigations of the characteristics related to prey consumption and searching capacity of the predator could yield important information for the selection of efficient bio-control agents. The relationship between the consumption rate of the predator *Menochilus sexmaculatus* F. (Coleoptera: Coccinellidae) and its prey density of *Aphis craccivora* Koch (Homoptera: Aphididae) on country bean (*Lablab purpureus* L.) was studied under laboratory conditions. Functional responses of four larval instars as well as adult males and females of *M. sexmaculatus* following the type II Holling's model. Rates of searching efficiency were estimated and found to be as high as 1.19 d^{-1} for adult males while low as 0.58 d^{-1} in first instar larvae. Handling time for adult females was found the lowest (10.8 min) while the highest for first instar larvae (90.57 min) during the observations of 24 hrs. The most effective stages of *M. sexmaculatus* to capture the prey were adult males, and females and fourth instar larva. As regards functional response data, the model predicts a maximum number of 142.85, 78.74, 61.43, 34.13, 16.23 and 15.89 nymphs to be consumed per day by an individual adult female, fourth instar, adult male, third, second and first instar larvae, respectively. Adult females consumed the highest number of prey, followed by fourth instar larvae and adult males. The voracity of first, second, third, fourth instar larvae, adult males and females was estimated as 11.13, 12.332, 21.182, 32.51, 27.82 and 35.87 (aphids/d) when given 50 individuals of *A. craccivora*, respectively.

Keywords: Biological control, predator-prey interactions, lady bird beetle, *Aphis craccivora.*

Introduction

The number of prey killed by an individual predator is a function of prey density, and the relationship between prey density and consumption rate of predator is known as the functional response (Abrams and Ginzburg, 2000 and Jeschke *et al.*, 2002). It is the key factor of a predator in regulating the population dynamics of predator-prey interaction systems (Jafari and Goldasteh, 2009). Generally, three types of curves to model the functional response relationships of predators' as density-dependent yields are available (Trexler and Culloch, 1988 and Pervez and Omkar, 2005). These are: type I (linear), type II (curvilinear) and type III

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(sigmoid) as correspond to density independence, inverse density dependence and density dependence, respectively (Juliano, 2001). An additional type (dome-shaped) of reaction occurs only when other prey interferes or shows some kinds of defence behaviour during predator handling (Jervis and Kidd, 1996). Yet, the main ecological interests, the functional responses of most arthropod predators have been suggested of type II or type III (Madahi *et al.*, 2013). But ecologists frequently face difficulties in determining the yield while the curves linking type II and III (Aukema and Raffa, 2004). In this study, the functional response of *Menochilus sexmaculatus* F. (Coleoptera: Coccinellidae) was evaluated on the bean aphid, *Aphis craccivora* Koch (Homoptera: Aphididae).

This species was found as a key pest on bean and other leguminous crops (Capinera, 2001). *A. craccivora* causes direct damage by feeding of plants sap, shown by a mottled appearance, leaf chlorosis, and reduction in plant vigour, stunting plant growth and stimulation of phytotoxic disorders. They also cause indirect damage by transmitting plant viruses with the result of significant yield loss (Blackman and Eastop, 2000; Akhtar *et al.*, 2010 and Razaq *et al.*, 2011). An alternative and ecologically sound control measure initiated against the *A. craccivora* is the utilization of predaceous lady bird beetle as bio-control agent. The objectives of studying the predation potential of *M. sexmaculatus* on five densities of *A. craccivora* were aimed through determining the type of its functional response, and its density dependence yield in relation to host population.

Therefore, the present study has investigated how the predator responds to changing prey density under simplified experimental conditions.

Materials and Methods

The experiment was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University, (HSTU) Dinajpur, Bangladesh (Latitude: 25°37' 38.82"N and Longitude: 88° 38' 16.04" E), during December 2018 to January 2019. All experiments were carried out under laboratory conditions (21 ± 4 ^{0}C, 75 ± 5% RH and 13L: 11D photo phase).

Insect culture

To initiate the stock culture, aphids (*A. craccivora*) were collected from country bean (*Lablab perpureus* L.) fields of the university in 2018. The stock culture was then maintained on country bean leaves in the laboratory. Adult male and female of *M. sexmaculatus* were also collected from aphid infested bean plants. The predator species was maintained for several generations on bean leaf arena placed underside up on water-saturated foam mats in the Petri dishes (90 mm dia) under ambient room conditions. Everyday adequate aphids were supplied to the beetles as food. The oviposition of the beetles was examined daily. When oviposited, the

beetles were transferred into the new Petri dishes (120 mm) and the deposited eggs were counted and left undisturbed for hatching. The fresh hatched larvae were then transferred into the new Petri dishes. After having enclosed, new adults were sexed and confined in pairs (1 male: 1 female) into the Petri dishes for mating. This procedure was repeated to increase the number of larvae and adults for experimental use.

Experimental protocol of functional response

Healthy bean leaves were collected from the cultured potted bean plants and placed into Petri dish (90 mm). *M. sexmaculatus* larvae, adult males and females were collected from the stock colony and starved for four hours in clean Petri dishes. Then 1st, 2nd, 3rd, 4th instar larvae, adult males and females of the beetles were placed singly in Petri dishes (90 mm) supplied with 10, 20, 30, 40, 50 nymphs (4-5-days old) of *A. craccivora*, respectively. Nymphs were gently transferred to each experimental arena using a fine camel-hair brush. Each treatment was replicated ten times. After 24 hours all killed and alive aphids were recorded to determine predation efficiency. To avoid dryness, the under surface of the bean leaf was placed on a water-saturated cotton ball.

Data analysis

The relationships between rates of consumption ($Na\ /\ N\ \times\ 100$) by each developmental stage of predator; larval instars, adult males, females and its prey density were analysed by linear regression procedures using R studio 3.1.3. Holling (1959) curvilinear type II equation for calculation of the functional response. In this model, the number of prey consumed (Na) is a function of prey density (N) as follows:

$$Na = (aTN)/(1 + aThN)$$

Where, a is the attack rate (discovery) of the prey, T is the total time available (1 d or 24 h in this experiment), and Th is the handling time for one prey. The parameters of the handling time (Th) and the attack constant (a) was estimated using Holling's disc equation modified by reciprocal linear transformation (Livdahl and Stiven, 1983). The modified equation is as follows:

$$\frac{1}{Na} = \frac{1}{a} \cdot \frac{1}{NT} + \frac{Th}{T}$$

Where $\frac{1}{Na}$ represents y, $\frac{1}{a}$ represents α, $\frac{1}{NT}$ represents x and $\frac{Th}{T}$ represents ß. The linear regression form becomes y = αx + ß. The theoretical maximum number of prey consumption by predator can be asymptote to be $Ha_{max} = \frac{T}{Th}$.

The fact of consuming or eagerness to consume large quantities of food is called the voracity of a predator. Voracity was determined according to the following equation (Cabral *et al.*, 2006 and Moura *et al.*, 2006):

$$Vo = (A - a24)ra24$$

Where V_O is the indicator of voracity, *A* is number of prey available, $a24$ is number of prey alive after 24 h or 1 day time, and $ra24$ is the ratio of prey found live after 24 h or 1 day in a control treatment without a predator (i.e., number of aphids live after 24 h/initial number of aphids). Analysis of variance (ANOVA) was accomplished to test differences in voracity at maximum densities of *A. craccivora* for different stages of the predator (at 50 aphids). Following significant ANOVA analysis, a Duncan's new multiple range test (R Studio 3.1.3) was used for pairwise comparisons of the means.

Results

The attack rates, handling time, prey consumption (asymptote) and regression coefficient (r^2) of life stages of predators are presented in Table 1. Adult males showed the maximum attack rate (1.19) followed by adult females (1.08), fourth instar larvae (1.03) while the lowest in first instar larvae (0.58). Handling time for adult females was the lowest (10.8 min) while it was the highest for the first instar larva (90.57 min) during 24 hours. Asymptotic maximum prey consumption increased respectively as larval development progressed up to 4th instar; 15.89, 16.23, 34.13, and 78.74, and for males adult while females reached maximum of 61.43 and 142.85, respectively.

Table 1. Type II functional response parameters of *M. sexmaculatus* fed on *A. craccivora*

Life stages	*a*	*Th* (min)	*T*/*Th*	r^2
First instar larva	0.58	0.0629 (90.57)	15.89	0.96
Second instar larva	0.75	0.0616 (88.70)	16.23	0.88
Third instar larva	1.01	0.0293 (42.19)	34.13	0.99
Fourth instar larva	1.03	0.0127 (18.28)	78.74	0.99
Adult male	1.19	0.0163 (23.47)	61.43	0.98
Adult female	1.08	0.007 (10.08)	142.85	0.99

The value in the parenthesis indicates handling time of different instar larvae and adult male, female in minute. Here, *a* = Rate of attack (discovery), *Th* = Handling time for one prey, *T*/*Th* = Prediction of maximum prey consumption and r^2 = Regression coefficient

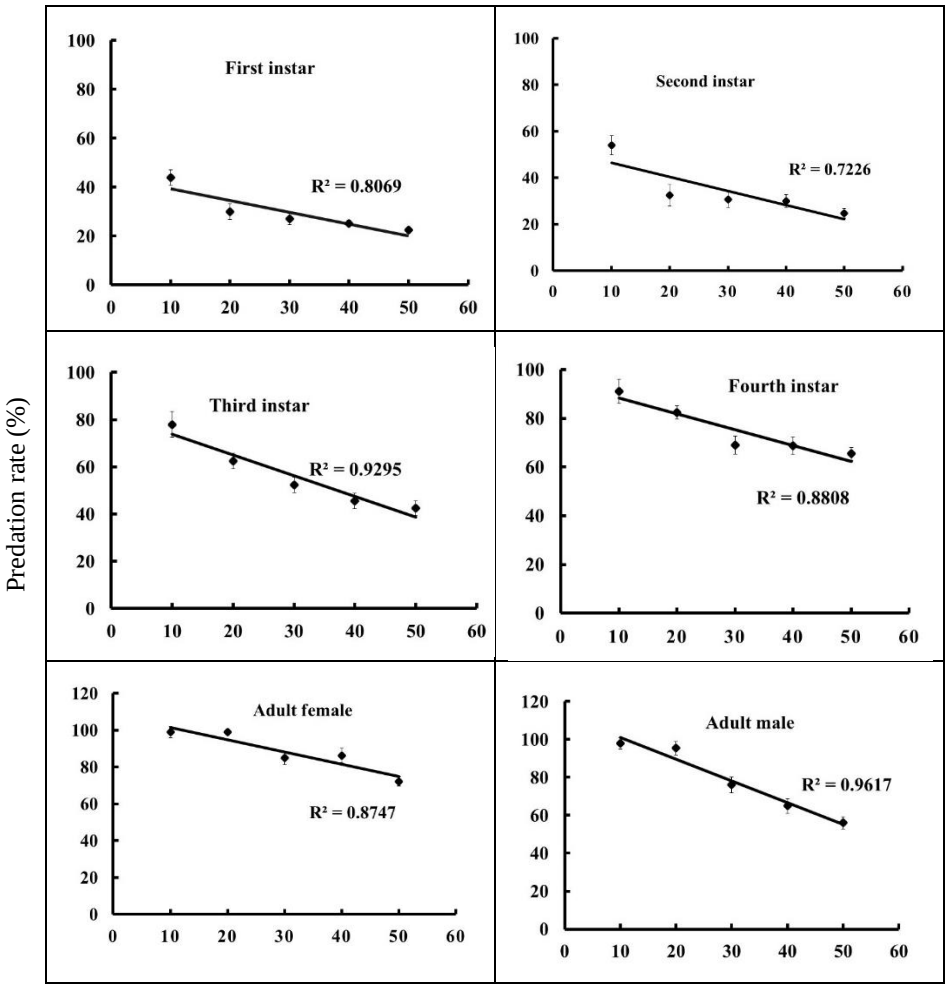

Fig. 1. Proportion of prey killed (%) by different stages of *M. sexmaculatus* to *A. craccivora* on country bean. Points show average number of aphids eaten or killed by *M. sexmaculatus* at each level of prey availability.

The prey consumed at different predatory stages (1st, 2nd, 3rd and 4th instar larvae, adult male and female) was negatively correlated with prey densities offered. The consumption rates at various predatory stages decreased with increasing prey densities (Figure 1). The functional response curve corresponds to prey consumption rate at different stages increased with increasing prey density (Figure 2) suggesting of type II functional response. The highest predation of 100% was evident by an adult female when offered 10 preys. Among the larval instars, fourth instar larva showed the highest predation rate (91.0%). Similarly, adult females (39.18) and fourth instar larvae (31.13) showed highest predation as offered 50 preys. The differences in prey consumption at various prey densities within a

developmental stages of *M. sexmaculatus* were significant for first ($F = 12.53$; $df = 3$; $P = 0.038$), third ($F = 39.55$; $df = 3$; $P = 0.008$), fourth instar larvae ($F = 22.17$; $df = 3$; $P = 0.018$), adult males ($F = 75.38$; $df = 3$; $P = 0.003$), and adult females ($F = 20.95$; $df = 3$; $P = 0.019$) while non-significant for second instar larva ($F = 7.81$; $df = 3$; $P = 0.068$) at 5% level of probability.

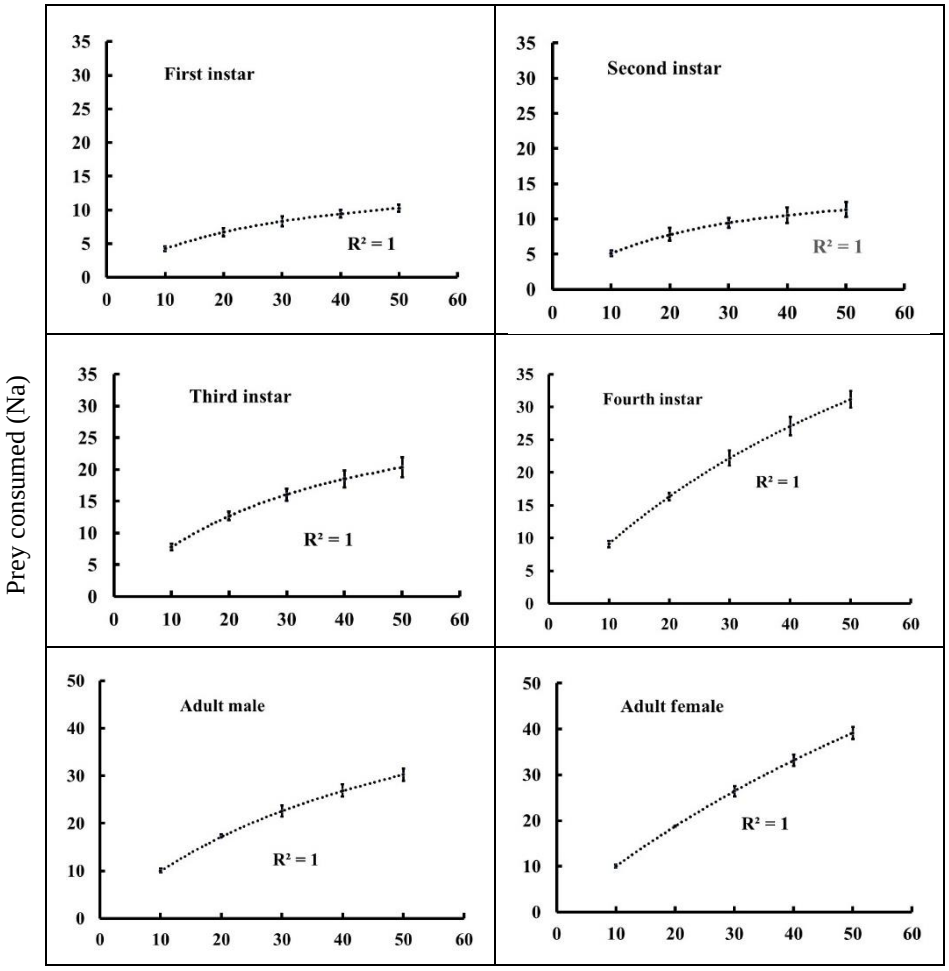

Fig. 2. Functional response of larval and adult stages of *M. sexmaculatus* to *A. craccivora*. Points show average number of aphids eaten or killed by *M. sexmaculatus* at each level of prey availability as Hollings equation for a type II functional response.

Voracity of different stages of *M. sexmaculatus* at maximum densities of *A. craccivora* during 24 hour varied significantly ($F = 64.54$; $P < 0.0016$) as shown in Figure 3. Voracity increased with larval age; moreover, adult female sex habited a greater voracity than adult males. There was no significant differences between

fourth instar larvae (32.51 ± 1.28) and adult females (35.87 ± 2.55) both recorded as the highest voracity. But the first instar larvae showed the lowest voracity of 11.13± 0.72 nymphs.

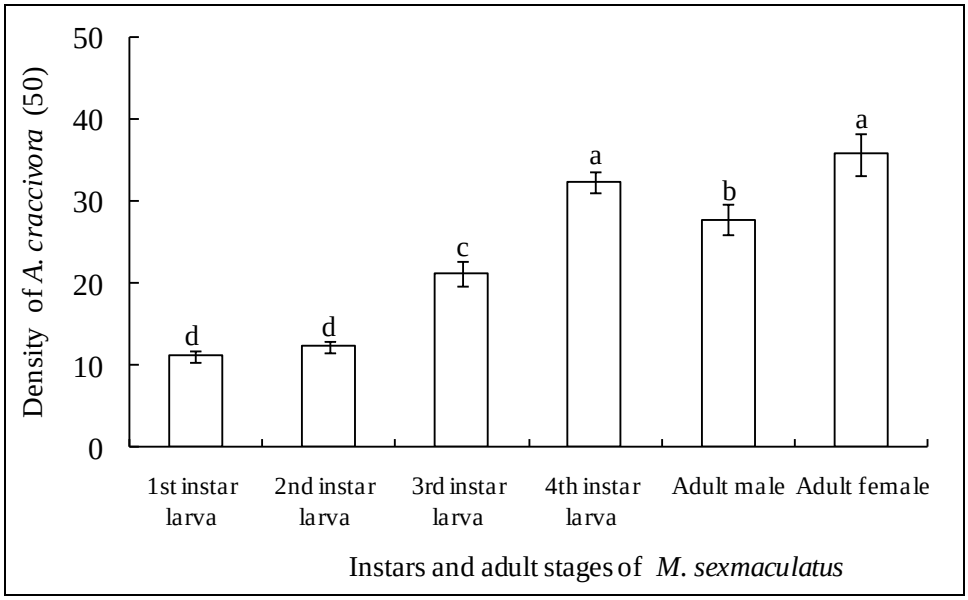

Fig. 3. Voracity (mean ± SE) of different stages of *M. sexmaculatus* at maximum *A. craccivora* density (50 aphids) at 21 ± 4 ^{0}C, 75 ± 5% RH and 13L: 11D

Discussions

The functional response can reveal information about the capacity of the predator to suppress prey population (Holling, 1959). It provides a relationship between numbers of prey consumed against prey availability, which influences the dynamics of the prey-predator interaction, thereby contributing to the stability of the ecosystem. The predator shows a negative density dependence on the proportion of the prey killed (*Na/N*) as the density increases. The functional response pattern of *M. sexmaculatus* to varying densities of *A. craccivora* revealed that it was of the type II (Holling, 1965) in all stages of predator. Then type II functional response is frequently observed in connection with a variety of predators including arthropods used as biological control agents (Aukema and Raffa, 2004 and Saleh *et al.*, 2010). Many coccinellid species show this type response, e.g. *Adalia bipunctata* preying on *Elatobium abietinum* Walker (Timms *et al.*, 2008), *Coccinella unidecimpunctata* preying on *A. fabae* and *Aleyrodes proletella* (Moura *et al.*, 2006). Among coccinellids, type III functional responses have been relatively rare (Isikber, 2005; Lanzoni *et al.*, 2004 and Sarmento *et al.*, 2007). For type III, one suggested mechanism is the concentration of a predator's search effort observed in high prey-density areas (Hertlein and Thorarinsson, 1987). No actual proof or signs were observed in the present research to suggest type III could have

been involved. Since the observations were conducted over a relatively short period involving single-prey species, and thereby not meeting the conditions and mechanisms essential for type III functional responses to occur, this could be a possible explanation.

Similar trends of predation relied on fourth instar larvae, adult females and males in other coccinellids like *Cydonia vicinanilotica* feeding on *A. craccivora*, *Adalia tetraspilota* and *Hippodamia variegate* feeding on *Aphis pomi* and *A. fabae*, *Brevicoryne brassicae* and *A. craccivora* (Jalalipour *et al.*, 2017 and Mandour *et al.*, 2006). Adult females of *Cheilomenes sexmaculatus, Propylea dissecta,* and *Coccinella transversalis* also consumed *Aphis craccivora* (Koch) or *Myzus persicae* Sulzer (Pervez and Omkar, 2005).

The coefficient of attack rate (a) and handling time (T_h) indicates the predator's ability to locate and treat the prey. Even the same species, different stages would have completely different predator's potential (Shah and Khan, 2013). The values of attack rate of adult female were 1.08 d^{-1} which peaked within 0.007 day (10.08 min). These results of attack rates and handling times compare well to those published by Omkar and Pervez (2003). The variations within the parametric values can be due to variation in species, size, prey density, voracity, satiation time, digestion ability, walking speed, etc. (Omkar and Pervez, 2003). Handling time is a good indicator of consumption rate and effectiveness of a predator because it reflects the cumulative effect of time taken during capturing, killing, suppressing and digesting the prey (Veeravel and Baskaran, 1997).

The asymptote maximum numbers of aphids attacked by fourth larval instars, adult males and females were determined as 78.7, 61.4 and 142.9 per day, respectively, with only handling time being considered. This result shows that the killing efficiency of adult females is as much as 2.3 times greater than that of adult males and suggests that the females' handling time, attack rate and consumption of prey is more efficient than that of the males. The present observation also showed that the higher prey killed was by adult females and fourth instar larvae. This agrees with observations on other coccinellids that reported the fourth instars captures over 60% of total prey by all larval stages (Lee *et al.*, 2004 and Farhadi *et al*., 2010).

Voracity of *M. sexmaculatus* increased with increasing larval development and with adult females exhibiting a greater voracity than males. Voracity seemed to reflect the energy requirement regarding body size or metabolism. Adult females and fourth instar larvae showed the voracity levels being considerably higher than those of all other life stages. Similar voracity was cited in *Hippodamia variegate* hosted on *A. fabae* (Farhadi *et al*., 2010). The handling time, attacked rate, and number of prey consumed suggested that the adult and fourth instar are most active predatory stages in killing the aphids and are thus suitable for field release. Similarly, the same stages yielded the highest functional response in *Nephaspis*

oculatus (Coleoptera: Coccinellidae) (Liu and Stansly, 2002) and *Stethorus gilvifrons* Mulsant (Coleoptera: Coccinellidae) (El-Basha and Mandour, 2005).

Assessing the pea aphid population potential (Jalalipour *et al.*, 2017), present data suggest that *M. sexmaculatus* could have potential of suppressing *A. craccivora* population with higher voracity and functional response. However, this study only focused on the predator response to variable prey densities in simplistic, laboratory-confined conditions. Field trials are, therefore, essential to evaluate the true potential of the predator's capacity to control the aphid pest in areas with the complexity of variable environmental as well as the spatial structural conditions over time.

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