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EVALUATION OF THE BIOLOGICAL CONTROL EFFICIENCY OF FOUR SPIDERS USING FUNCTIONAL RESPONSE EXPERIMENTS

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ABSTRACT

The biological control efficiency of four spiders, *Pardosa altitudis* Tikader and Malhotra (Lycosidae), *Leucage celebesiana* Walckenaer (Teragnathiade), *Neoscona rumpfi* Tikader and Bal (Araneidae), *Theridion manjithar* Tikader (Theridiidae) preying on different densities of cabbage aphid, *Brevicoryne brassicae* (L.) (Homoptera: Aphididae) was evaluated using functional response experiments. The results revealed that all four spiders exhibited a decelerating curve, typical of type II functional response, on different densities of prey as determined by a non-linear logistic regression model. The maximum prey consumption per day at highest density of cabbage aphids were 54.2 for *P. altitudis*, 48.9 for *N. rumpfi*, 41.2 for *L. celebesiana*, and 37.3 for *T. manjithar*. The estimate of search rate was maximum for *L. celebesiana* (3.24104 h^{-1}) while minimum handling time was noted for *P. altitudis* (0.38914 h). In most of the treatments, the estimates of search rates did not show significant differences, while the handling times mostly showed significant variations among the spider species. The *P. altitudis* exhibited lowest handling time with high search rate followed by *N. rumpfi*. Hence, *P. altitudis* has the efficiency to be exploited successfully as a biocontrol agent for the management the cabbage aphid populations in cruciferous vegetable ecosystem.

INTRODUCTION

Aphids are extremely successful group which occurs throughout the world, with the greatest number of species in temperate region. The cabbage aphid, *Brevicoryne brassicae* (L.) (Homoptera: Aphididae) is an important worldwide pest of cruciferous vegetables in temperate region (Blackman and Eastop, 2007). In Kashmir, cruciferous vegetable crops including cabbage, cauliflower, broccoli and kale are highly infested by cabbage aphids. The most common predators of aphids are ladybird beetle (Hodek and Honek, 1996; Khan *et al*., 2009; Khan, 2009a), spiders (Khan, 2009b; Khan, 2011), green lacewing (Mushtaq and Khan, 2010) and syrphid fly larvae (Ghorpade, 1981; Makhmoor and Verma, 1989).

Among these predators, spiders play important role in regulating cabbage aphid populations (Khan and Khan, 2011). In spite of this, they have not usually been treated as important biocontrol agents because there is so little information on the ecological role of spider in pest control (Turnbull, 1973; Riechert and Lockely, 1984; Khan, 2011). Although the spiders are a diverse arachnid consisting of more than 1000 species in India (Siliwal *et al*., 2005) out of total 42473 identified species of the world (Platnick, 2012). In vegetable ecosystem of Kashmir 33 species are identified (Khan, 2012) and all are polyphagous feeding upon herbivorous insects. The orb-web spiders (Araneidae and Tetragnathidae) feed upon aphids, hoppers, dipterans; Space-web builders (Linyphiidae and Therididae) preying upon Homopterous and dipterous insect (specially on aphids and leafhoppers) and visual hunter (Lycosidae, Salticidae,) feed upon grass hoppers nymphs, aphids and other small insects (Riechert and Bishop, 1990; Nyffeler *et al*., 1994; 1999; Khan, 2009b, 2011).

The success of a biocontrol agent depends upon the rate at which they consume to prey and partly on their ability to increase in abundance and feeding in a cooperative manner (Holling, 1966). The variation in rates of prey consumption and variation in predator abundance as "functional and numerical response" of a predator (Holling, 1959; Solomon, 1949). In this paper our interest concentrates on the functional response in the presence of one species. The functional response of a predator is a key factor that regulating the population dynamics of predator-prey systems (Hassell, 1978). It describes the rate at which a predator kills its prey at different prey densities and can thus determine the potential of a predator in suppression of prey populations (Murdoch and Oaten, 1975; Lawton *et al*., 1975). Holling (1966) described three types of functional response curves. The functional response curves may represent at increasing linear relationships (type I), a decelerating curve (type II) and a sigmoidal relationships (type III). It is important to our understanding of two parameters that are widely used to describe the functional response of a predator feeding on a single prey; these are the predator's search rates and handling time (Hassel *et al*., 1976). The search rate estimates the steepness of the increase in predation with increasing prey density. The handling time (time used up by predator in attacking, killing, subduing and digesting the prey) helps estimate the satiation threshold (Parvez and Omkar, 2005).

In the present laboratory study, the feeding behavior of the four dominant spiders namely, *Pardosa altitudis* Tikader and Malhotra (Lycosidae), *Leucage celebesiana* Walckenaer (Teragnathiade), *Neoscona rumpfi* Tikader and Bal (Araneidae)

Theridion manjithar Tikader (Theridiidae), collected from cruciferous vegetable crop of Kashmir, to increasing cabbage aphid, *Brevicoryne brassicae* (L.) density was determined. The experiments was conducted to determine the type of functional response of different species of spiders to single species of prey and to determine their relative potential role for biological control of the aphid prey species.

MATERIALS AND METHODS

Predator and prey culture

The four species of spiders viz., *Pardosa altitudis* Tikader and Malhotra, *Leucage celebesiana* (Walckenaer), *Neoscona rumpfi* Tikader and Bal, *Theridion manjithar* Tikader were collected from cruciferous crop ecosystem of Kashmir, India during 2009 in the month of June-July and maintained in culture room in vials (4 centimeter diameter and 5 centimeter height) individually at 25 ± 2°C temperature, 70 ± 10% relative humidity and a photoperiod of 14 hour light: 10 hour dark and provided sufficient cabbage aphids as prey. Cabbage aphid, *Brevicoryne brassicae* (L.) were collected from kale crop (*Brassica oleracea var. acephala* D.C) and reared on living kale plants at 21 ± 1°C and 70 ± 10% relative humidity and a photoperiod of 14 hour light: 10 hour dark. The kale plants were planted in potted cage that were filled with sawdust and fertilized with micro and macro nutrients fertilizer (2% solution of water) every 3 days.

Functional response

The females of different species of spiders were taken from the culture for experiments and starved for 24 h in vials (4 centimeter diameter and 5 centimeter height) individually before the experiments. This was to minimize differences in individual hunger levels (Nakamura, 1977). Thereafter, they were introduced individually plastic container (height 20 cm and diameter 15 cm) separately together with green apple aphids density of 10, 20, 40, 80, 160 and 320 were used on excised kale leaves stuck to agar medium. The test predators were randomly assigned to the aphid density treatments and one treatment (control) was also designed for natural mortality of aphids. At each aphid density 10 replicates were used for different species of spiders. After 24 hrs, the numbers of preys consumed by the species of spiders were recorded by counting the remaining live cabbage aphids present in each cage.

Data analysis

Data analysis for functional responses based on two steps. The shape (type) of functional response is determined in the first step. The type of functional response (type II or III) was determined using logistic regression analysis of the proportion of eaten prey versus initial number of prey offered (Juliano, 2001). To do this, a polynomial logistic regression (equation 1) was fitted to data:

$$\frac{N_e}{N_0} \quad \frac{\exp(P_0 \;\; P_1N_0 \;\; P_2N_0^2 \;\; P_3N_0^3)}{1 \;\; \exp(P_0 \;\; P_1N0 \;\; P_2N_0^2 \;\; P_3N_0^3)} \qquad ....(1)$$

Where N_e express the number of prey consumed, N_0 the initial prey density and P_0, P_1, P_2, P_3 are the intercept, Linear, quadratic and cubic coefficients, respectively. The above six data sets were fitted individually to equation 1 and types of functional responses were determined by examining the signs of P_1 and P_2. If the positive linear parameter P_1 together with a negative quadratic parameter P_2 would indicate a type III functional response, whereas if the linear parameter is negative, a type II functional response is indicated (Juliano, 2001).

In the second step, as the logistic regression analysis indicated that our data fit type II, further analysis were restricted to type II. Rogers type II random predation equation (2) (Rogers, 1972) was used to data and estimate the functional response parameters. Because prey were depicted during the experiment, this model, which does not assume constant prey density, is appropriate for this experiment. Holling disc equation, in constant, is based on an assumption of unchanging prey density (Rogers, 1972) and is thus inappropriate for this experiments (Juliano, 2001). The form of Rogers' type II random predator model is:

$$N_a = N_0\{1 - \exp[-a(T_t - Th)]\} \;.........(2)$$

where N_0 is initial prey density, N_a, number of prey consumed, T is the total time (24 h), a is the attack rate (h^{-1}), and T_h is handling time in hours. The all data were analyzed by using the non-linear function (nls) provided by the R-software (R Development Core Team, 2008).

RESULTS

The functional responses of all four spiders eating on cabbage aphid, *B. brassicae* were illustrated in Fig. 1. The linear parameter (P_1) in the polynomial logistic regression of the proportion of cabbage aphid consumed versus initial density was -0.0604 (*P. altitudis*), -.0782 (*N. rumpfi*), -0.0628 (*L. celebesiana*) and -0.06039 (*T. manjithar*). The logistic regression for all four spider species had a significant linear parameter (Table 1) and the proportion of prey consumed by all spiders declined with increasing prey density (Fig. 1 and Table 2). This suggests that polynomial logistic regression (equation 1) well fitted to data sets and all four spiders of them exhibited a type II functional response. The functional responses for all four spiders eating on cabbage aphid a 24-h period were therefore fitted to Rogers' type II random predation equation (2). The *P. altitudis* was eaten the maximum number of cabbage aphid (54.2/24 h) at highest density which was higher than that of *N. rumpfi* (48.9/24 h), *L. celebesiana* (41.2/ 24 h) and *T. manjithar* (37.3/24h), respectively (Fig. 1).

Estimated search rate (a) for *P. altitudis*, *N. rumpfi*, *L. celebesiana* and *T. manjithar* were 3.10590, 3.24104, 3.01185 and 2.96778, respectively. Estimated handling time (*Th*) for *P. altitudis*, *N. rumpfi*, *L. celebesiana* and *T. manjithar* were 0.38914, 0.44575, 0.53830 and 0.57754 h., respectively (Table 3). Comparisons of search rates and handling times for *P. altitudis*, *N. rumpfi*, *L. celebesiana* and *T. manjithar* are shown in Fig. 2 and 3, which shows that *P. altitudis* had the shortest handling time, followed by *N. rumpfi*, *L. celebesiana* and *T. manjithar*. Among the four spiders, the estimates of attack rate of *P. altitudis* were higher as compared to *N. rumpfi*, *L. celebesiana* and *T. manjithar*, respectively. However, in most of the treatments, the estimates of search rates did not showed significant differences (Fig. 2), while the handling times mostly showed significant variations ($P < 0.0001$) among the spider species (Table 3, Fig. 3).

Table 1: Results of logistic regression analysis of the proportion of cabbage aphid (*B. brassicae*) eaten by different spiders against initial number of aphid offered

Spider species	Coefficient	Estimate	SD	Z value	Pr(>|z|)
P. altitudis	Constant(P_0)	2.7110	0.6972	3.889	<0.0001
	Linear (P_1)	-0.0604	0.0198	-3.049	0.0023
	Quadratic(P_2)	0.00032	0.00014	2.076	0.0378
	Cubic(P_3)	-5.001e-07	2.845e-07	-1.758	0.0787
L. celebesiana	Constant(P_0)	3.0560	0.7551	4.047	<0.0001
	Linear (P_1)	-0.0628	0.02088	-3.012	0.0026
	Quadratic(P_2)	0.00031	0.00015	1.991	0.0465
	Cubic(P_3)	-4.809e-07	2.945e-07	-1.633	0.1024
N. rumpfi	Constant(P_0)	4.1160	0.9637	4.271	<0.0001
	Linear (P_1)	-0.0782	0.02497	-3.134	0.00172
	Quadratic(P_2)	0.00038	0.00017	2.223	0.02618
	Cubic(P_3)	-6.290e-07	3.334e-07	-1.886	0.05926
T. manjithar	Constant(P_0)	2.7110	0.6972	3.889	<0.0001
	Linear (P_1)	-0.06039	0.01981	-3.049	0.0023
	Quadratic(P_2)	0.000302	0.00014	2.076	0.0378
	Cubic(P_3)	-5.001e-07	2.845e-07	-1.758	0.0787

Table 2: Consumption rate of different spiders on different prey densities of *B. brassicae*

aphids density	No. of spider used	Consumption rate (%) of spiders on *B. brassicae*			
		P. altitudis	L. celebesiana	N. rumpfi	T. manjithar
10	1	96.0	90.0	96.0	89.0
20	1	92.0	85.0	96.0	85.5
40	1	88.0	76.2	81.0	66.5
80	1	53.2	41.7	51.0	39.6
160	1	30.5	22.4	26.3	22.1
320	1	16.9	12.8	15.3	11.6

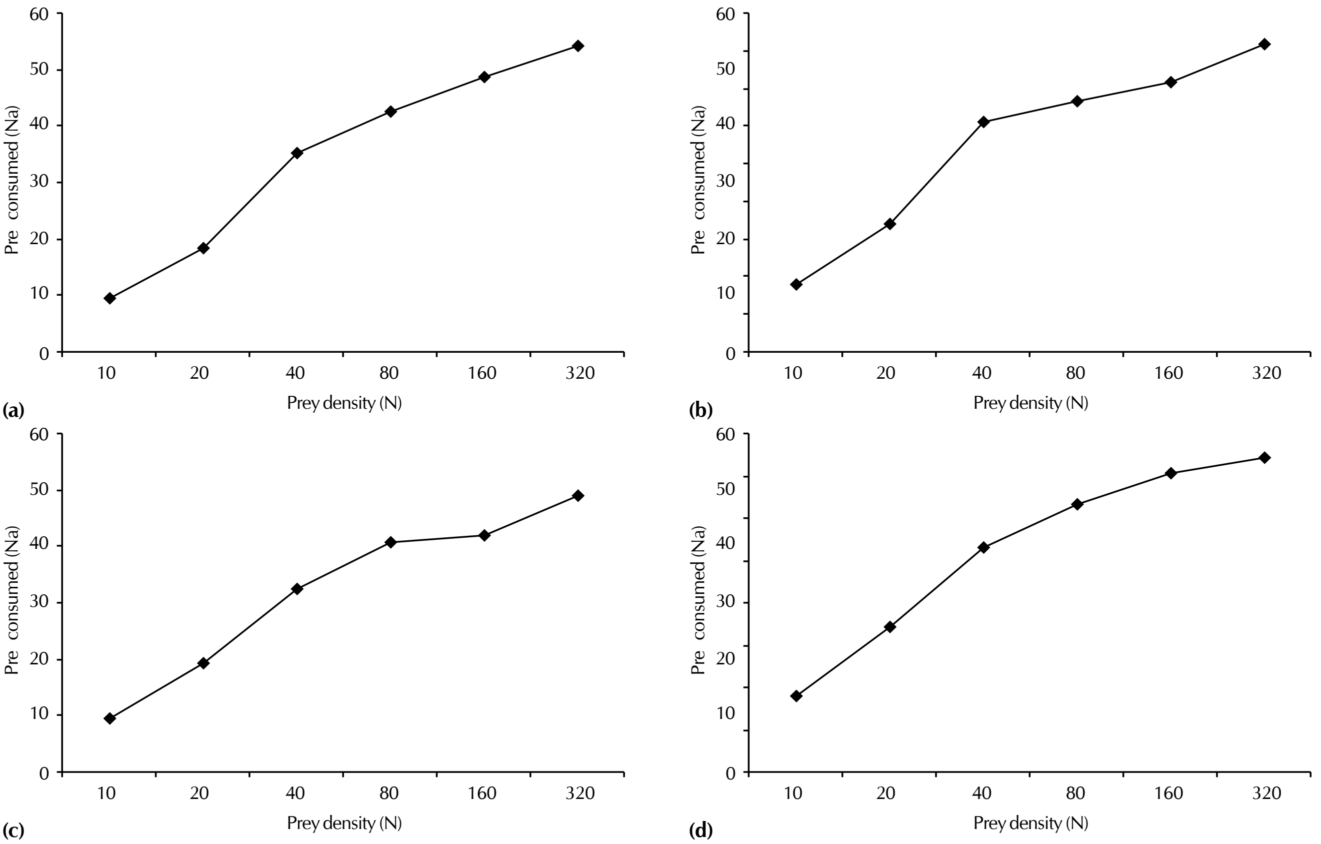

Figure 1: Functional response of four spiders on different densities of cabbage aphid (*B. brassicae*): (a) *P. altitudis* (b) *L. celebesiana* (c) *N. rumpfi* (d) *T. manjithar*. Symbols are observed data and lines were predicted by model (equation 2)

Table 3: Parameter estimated by Rogers' type II random predation equation (2) for functional response of four spiders on *B. brassicae*

Spider species	Parameter	Estimate	SD	*t* value	Pr(>|t|)
P. altitudis	a	3.10590	0.42711	7.272	0.0019
	T_h	0.38914	0.02182	17.833	<0.0001
L. celebesiana	a	3.01185	0.57549	5.407	0.0056
	T_h	0.53830	0.03542	15.196	<0.0001
N. rumpfi	a	3.24104	0.49164	6.592	0.0027
	T_h	0.44575	0.02558	17.426	<0.0001
T. manjithar	a	2.96778	0.32575	9.111	0.0008
	T_h	0.57754	0.02235	25.846	<0.0001

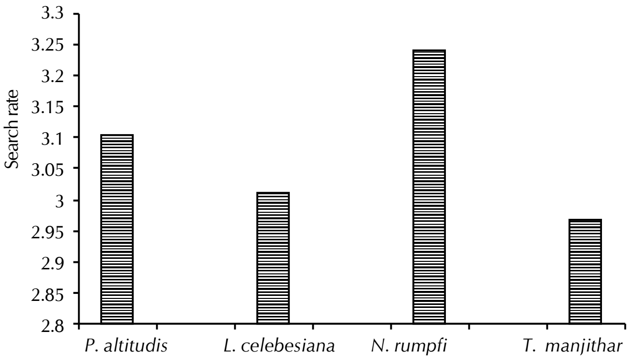

Figure: 2 Comparison of search rates for different spiders preying on *B. brassicae*. S= Significant (p<0.0001), NS= Non significant (p<0.0001)

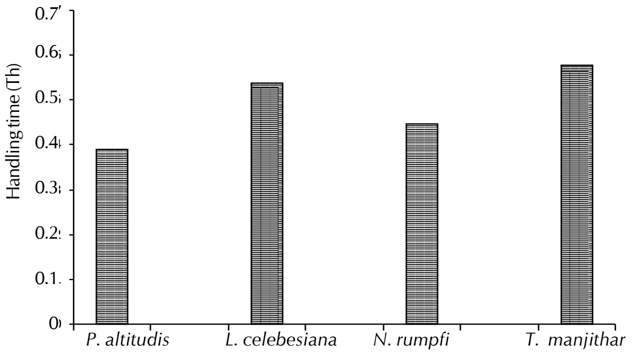

Figure 3: Comparison of handling times (*Th*) for different spiders preying on *B. brassicae*. S= Significant (p<0.0001), NS= Non significant (p<0.0001)

DISCUSSION

The result showed that a type II curvilinear curve described the data well. All four spiders exhibited negatively accelerating curve type II functional response with the increasing densities on cabbage aphids (Fig. 1 and Table 2). The asymptote in the curve indicates the position of highest killing rate. The logistic regression model was further used to determine the types of response (type II or III) as well as the appropriateness of the shapes, as in such type of experiments biologist generally face difficulties in curve-fitting while the data set of type II response preference towards type III response (Livdahl and Stiven, 1983). It can show the way to point up deceptive inferences because one indicates negative density-dependence (type II response) whereas other indicates positive density-dependence (type III response). In present study, obtained negative linear coefficient values verify the type II functional response exhibited by all four spiders (Table 1). Therefore, the logistic regression model (Juliano, 2001) can be recommended to use as a tool for further analyzing type II and III functional responses.

Type II response is often called" invertebrate curve" and in fact seems to be common in spiders (Riechert and Harp, 1987; Heong and Rubia, 1989; Rypstra, 1995; Khan and Misra, 2003, 2009), while the type III (Sigmoidal curve) response have also been demonstrated for some spiders (Marc *et al*. 1999; Provencher and Coderre, 1987; Shivakumar and Kumar,2010). In type I response prey eating is proportional to prey density until satiation and this type response is typical of filter feeding organism and never seen in spiders (Riechert and Lockely, 1984; Jeschke *et al*., 2004).

A positively accelerating the proportion of prey consumed as the density increases showed in type III functional response, for this reason a polynomial fit to a type III response must have a linear coefficient expression that is positive. On the other hand, there should be a negatively accelerating the proportion of prey consumed as the density increases in type II response, so that the linear coefficient expression should be negative. Albeit the logistic regression model easily solves the subtle variations in the type II and III responses but it not success to differentiate them from type I, because in type I response, prey consumption is proportional to prey density until satiation (increasing linear relationship). Thus, efforts are looked-for to formulate an analogous logistic regression model to discriminate type I, II and III functional response.

The estimates of maximum numbers of cabbage aphids consumed in 24 h were 54.2 for *P. altitudis* at highest density of aphid used which was higher than that of *N. rumpfi* (48.9), *L. celebesiana* (41.2) and *T. manjithar* (37.3), respectively. Hence the feeding efficiency of *P. altitudis* may be higher than other used spiders. Their values differed significantly among the spider species when prayed to cabbage aphid, which show that they have different abilities to respond to increasing aphid densities. It also shows that spiders exhibiting analogous functional response curves cannot be considered to counter correspondingly. The parametric values varied might be due to the variant in size, digestive capacity, preying nature, hunger levels, voracity, walking speed, satiation time, etc.(Mills, 1982). Among the all four spider, *P. altitudis* responded greatly than the other spider species to increasing density of cabbage aphid. This suggestion might be sturdily supported by the investigational evidence of the reproductive biology of *P. altitudis*, which achieve higher values in vitality and reproduction using this prey species. The comparative rate of prey eaten by *P. altitudis* was higher at lower prey density; indicate that it could be more capable at lower prey densities. A negatively accelerating rise in number of prey captured to an asymptote at higher prey density, which might be due to the achievement of satiation (Mills, 1982). The functional response curve of *P. altitudis* (hunting spider) uplifted over functional response curve of *N. rumpfi* (web builder) and other used spiders (web builder) due to faster digestive rate, preying habit and possible delayed satiation (Nyffeler, 1988; Uetz *et al.*, 1999).

The estimates of search rate and handling time were the parameters used to observe the extent of these responses (Table 3). Our search rate estimates showed no significant differences in most of the treatments (Fig. 2). While the estimates of handling time showed the significant difference among all spiders. The *P. altitudis* have taken significantly lowest handling time as compared to *N. rumpfi*, *L. celebesiana* and *T. manjithar*, respectively (Fig. 3). This suggested that the greater consumption ability of *P. altitudis* compared to other three spiders arises because of differences in handling times. The variation in handling times was the result of the time spent for pursuing and subduing the prey, the time spent for eating the prey and the time spent in non-searching activities as a result of digesting the prey is the estimates of handling time (Hassell, 1978; Mills, 1982; Cloarec, 1991).

The conclusion of our study is that the prior to fitting the data for determination of parameters of functional response, clear distinction of type II and III functional should be made (Farhadi *et al.*, 2010). All four spiders exhibited type II functional response, which varied differentially among the species feeding on different density of cabbage aphids. Estimated search rate showed no significant differences among most of the treatments whereas the handling time showed significant different among all treatments. The *P. altitudis* have taken lowest handling time with high search rate followed by *N. rumpfi*. Therefore, we can say that *P. altitudis* has the efficiency to be apply successfully as a biocontrol agent for the biological control of cabbage aphid populations in cruciferous vegetable ecosystem. Since, the real field environment is much complicated than the laboratory conditions because of the variation in factors such as temperature, humidity, rain, surface area, prey type and biotic complex could be important to affect the efficiency of predator in the suppression of pest populations (Toft, 1999; Nilsson, 2001; Parajulee *et al.*, 2006). Although functional response is a tool in selection of efficient biocontrol agents, there is no general relationship between success in biological control and the type of functional response. Hence, further field experiments are desired.

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