Feeding behaviour of reduviid predators on meat and insect-based artificial diets

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Abstract

Successful introduction of reduviid predator as a component of integrated pest management is facing a main constrain, that is, the lack of successful artificial rearing media. We evaluated insect-based (*Bombyx mori* pupae, *Egocera venulea* larvae and *Corcyra cephalonica* larvae) and meat-based (pig liver and blood) artificial diets on the feeding behaviour and diet selection of the reduviid predators *Rhynocoris marginatus* (FAB.), *R. fuscipes* (FAB.), *R. kumarii* AMBROSE & LIVINGSTONE, and *R. longifrons* STAL. The feeding behaviour of all the four reduviids on the food formulations was the same except on pig blood. Among the four predators tested *R. marginatus* recorded highest access proportion index, food preference index and rank correlation values. It is suggested that *R. marginatus* could be reared with the artificial diets, followed by *R. fuscipes* and *R. longifrons*. Though pig liver recorded a delayed consumption time, it was the preferred diet by all four reduviids. Nutritional improvement of this successful diet might give a solution for mass rearing of the reduviid predators.

Keywords: artificial diet, reduviid predators, feeding behaviour, food preference index.

Introduction

Non-availability of natural predators in large numbers is the main impediment for the practical use of predators in augmentative biological control programme of the insect pests. LAKKUNDI (1989) developed a mass production technique for the reduviids and latter it was modified by SAHAYARAJ (2002) and they called it the "Larval card method". However, high cost, manpower needed for rearing, collection of prey larvae and mass culture of the predators fed with natural or unnatural host are the obstacle in the larval card method. LEPPLA & KING (1997) pointed out that the biological control agent should be cost effective. The main developmental prospects for entomophage mass production for inundate release are therefore associated with the development of effective and economically profitable technologies for artificial diet modulation. Recently some success has been reported on the development of artificial diets for predatory insects such as the Pentatomidae (DE CLERCQ *et al.*, 1998), Lygaeidae (COHEN, 1985), Coccinellidae (BAIN *et al.*, 1984) and Anthocoridae (ARIJS & DE CLERCQ, 2001). However, no published work was available for reduviids. In this regard, we aimed to formulate an artificial diet for rearing such predators.

Reduviids pierce and suck out their insect prey. They mainly feed on larvae of Lepidoptera and on larvae and adults of Coleoptera (AMBROSE, 1999). *Rhynocoris fuscipes*, *R. marginatus*, *R. longifrons* (DISTANT, 1902) and *R. kumarii* (AMBROSE & LIVINGSTONE) are native to India (AMBROSE & LIVINGSTONE, 1986). They are all generalist predators and found in diverse agricultural and natural habitats. Their food include several insect species which are economically important pests such as *Spodoptera litura* (FAB.), *Helicoverpa armigera* (HUBNER) and *Achea janatha* (LIN.), which attack a wide range of crops such as groundnut, bhendi and castor (SAHAYARAJ, 1995, 1999; AMBROSE, 1996; GEORGE, 2000).

Diet quality can be measured in terms of growth rate, development, reproduction, mortality, longevity and occurrence of morphological abnormalities (SINGH, 1977). Moreover, a better knowledge of the feeding behaviour of predators on artificial diet is an essential as well as the first step in artificial diet preparation and modulation. The present study was undertaken to evaluate the feeding behaviour of four reduviids on the three insect-based [*Bombyx mori* pupa (BMP), *Egocera venulea* larva (EVL) and *Corcyra cephalonica* larva (CCL)] and two meat-based diets [pig liver (PL) and pig blood (PB)].

Materials and Methods

Collection and rearing of reduviids

The predators used for this study were laboratory emerged 10 days old adults of *R. fuscipes*, *R. marginatus*, *R. kumarii* and *R. longifrons* which were collected from the agricultural and nearby scrub jungle ecosystems of Tirunelveli District, Tamil Nadu, India. They were reared under laboratory conditions (30±1 °C, 80 % RH and 11L:13D) on both laboratory and natural hosts, which are *Corcyra cephalonica* and *Spodoptera litura*, respectively, in 1500 ml plastic containers covered with fine nylon gauze for ventilation.

Artificial diet preparation

The artificial diet was prepared following the method used by COHEN (1997) with some modifications (Tab. 1). Hundred grams each of *Bombyx mori* pupa, *C. cephalonica* larvae, *Egocera venulea* larvae, pig liver and pig blood were dried in hot air oven at 60°C for 24 hrs. After drying they were ground well by using mortar and pestle and stored in a refrigerator for further use. They were considered as source ingredient. Hundred ml of distilled water was taken in 500 ml glass beaker and it was boiled at 100°C for 20 min. Ten ml of the boiled water was taken for dissolving the milk powder (Lactogen, Nestlé, Mumbai, India) and it was allowed to cool. Beef extract, dried egg yolk, honey (Dabur Narendrapur, West Bengal, India), acetic acid, NaCl and KCl (Glaxo, Gujarath, India) were added to the remaining 90 ml water and again it was boiled at 100°C. After 10 min the temperature was reduced to 40°C and then the source ingredient (BMP, CCL, EVL, PL or PB), vitamin and streptomycin (Sarabairaman, Vadodara, India) were added and stirred for thorough mixing. Then the milk powder solution was added and stirred well. After the thorough mixing the prepared diet was allowed to cool at room temperature and then it was filtered through Whatmann No.1 filter paper. Filtered liquid diet was stored in 125 ml reagent bottles in the refrigerator for further use. Four concentrations (F_1 to F_4) were prepared in each diet (Tab. 1) and tested against the reduviids.

Experimentation

Feeding behaviour experiments were conducted in Petri dishes. Five small pieces of cotton (50 mg) were taken and were placed inside the Petri dish. Four pieces were impregnated with 1 ml each of each concentration of the same artificial diet separately and the fifth cotton piece was impregnated with water. Twenty-four hours starved adults of *R. marginatus* were released at the centre of the Petri dish that was closed with a lid. Their behaviour was recorded during 1 hr continuously. Fifteen replicates were maintained for each experimental set up. Feeding and resting time were recorded, as follow.

Feeding and resting times

By the antenna juxtaposition the predator approached the diet, then pierced it with the rostrum and started to feed. Total time spent by the predators in sucking the diet was recorded as feeding time. During feeding, reduviids often take rest and this act (i.e., interval between two acts of feeding) was considered as resting time. The times in seconds of each event was converted to a percentage of time in one hour.

Access proportions index (API) and food preference index (FPI)

To find out the suitable formulation of the diet, API was calculated by using the formula: API = (TE –TC) / (TE + TC), where TE is the time spent by the predator at the experimental diet, and TC the time spent by the predator at the control, water (SAHAYARAJ & PAULRAJ, 2001). The values varied between −1 to +1. The cumulative time, in percent, was calculated by adding the time of all the four concentrations of each diet, and it was used for the calculation of FPI. In order to found out the suitable diet among the five experimental diets, FPI was calculated by using the following formula: FPI = (CT − AT) / (CT + AT), where CT is the consumption time, and AT the approaching time. FPI values obtained by each predator in different diets were ranked 1 to 4 and were used for the rank correlation analysis. Furthermore, Friedman's test was used for the comparison of different concentrations of the diets (ZAR, 1984).

Results

Artificial diet formulation

The preliminary trials of artificial diet formulations showed non/less suitability of diets with agar and yeast. The diet without these two ingredients was preferred by the predators and so the method followed by COHEN (1992) was adapted for artificial diet preparation with little modifications. The standardized artificial diet formulation adopted in this study is presented in the table 1.

Table 1. Ingredients of artificial diets.

Ingredient of the diets (in mg or ml / 100 ml)	Diet concentrations			
	F_1	F_2	F_3	F_4
Source ingredient (BMP,CCL,EVL,PL,PB) (mg)	500	1000	2000	4000
Beef extract (mg)	750	1500	3000	6000
Milk powder (mg)	500	1000	2000	4000
Egg yolk (mg)	500	1000	2000	4000
Honey (ml)	5	10	15	20
Multivitamin (mg)	10	20	40	80
Acetic acid (ml)	3.7	3.7	3.7	3.7
Nacl (mg)	5	5	5	5
Kcl (mg)	5	5	5	5
Streptromycin (mg)	75	75	75	75

(BMP) *Bombyx mori* pupa, (CCL) *Corcyra cepalonica* larva, (EVL) *Egocera venulea* larva, (PB) pig blood; (PL) pig liver.

Feeding time

The minimum feeding time (0.1 to 6.4%) was recorded with the control (water soaked) diet. Even though the maximum feeding time of 32.2% was recorded for *R. fuscipes* on the F_2 of PL, *R. marginatus* spent more time on all the four formulations of PL diet, with time ranging from 15.0 to 18.6% and a cumulative time of 65.5% whereas *R. longifrons* recorded an optimum of 20.8 to 14.2% time on PL diet and the *R. kumarii* recorded the lowest (3.8 to 14.8%) values. Among the insect-based diets, CCL recorded highest feeding time by all the reduviid predators, of which *R. marginatus* recorded a maximum of 29.6% time in the fourth formulation and a cumulative of 52.9% followed by BMP (20.1 and 36.2%). EVL recorded lowest feeding time followed by meat-based PB diet with no feeding or below the control diet (Table. 2). Friedman's tests on the individual diet consumption time of *R. marginatus, R. fuscipes R. kumarii* and *R. longifrons* yielded $P > 0.50$ ($\chi_r^2 = 6.88$), $P < 0.001$ ($\chi_r^2 = 26.88$), $P < 0.001$ ($\chi_r^2 = 15.76$) and $P > 0.50$ ($\chi_r^2 = 25.6$), respectively.

Table 2. Consumption time (in %) of predators on different concentrations of artificial diets.

Predator	Diet	Diet concentration				
		F_0	F_1	F_2	F_3	F_4
R. marginatus	BMP	2.33	6.9	6.9	3.6	20.09
	CCL	2.78	1.67	1.89	11.6	29.58
	EVL	1.77	1.89	15.59	1.43	7.41
	PL	4.22	15.59	18.33	15.01	12.34
	PB	2.94	0.04	.09	0.09	0
R. fuscipes	BMP	2.37	4.78	4.43	6.96	0.04
	CCL	1.78	0	1.33	4.95	0.11
	EVL	1.00	0.46	0	0	0.57
	PL	2.11	8.89	32.2	16.11	5.83
	PB	2.11	0	0	0	0
R. kumarii	BMP	0.48	0.48	0.72	0.9	0
	CCL	2.00	9.71	0.17	0.12	1.3
	EVL	1.94	1.57	2.08	2	3.76
	PL	2.56	3.76	6.56	7	14.44
	PB	1.56	1.02	0.28	0	0
R. longifrons	BMP	0.11	0.29	0.11	0.32	0
	CCL	1.28	1.15	10.95	0.93	0
	EVL	1.72	7.22	6.48	6.94	7.82
	PL	6.42	10.77	11.18	13.00	14.15
	PB	1.67	0	0	0	0

(BMP) *Bombyx mori* pupa, (CCL) *Corcyra cepalonica* larva, (EVL) *Egocera venulea* larva, (PB) pig blood; (PL) pig liver.

Access proportion index (API)

The calculated API values showed that all the reduviid predators responded positively towards PL diet from which *R. marginatus* and *R. fuscipes* preferred F_2 (+0.63 and +0.88, respectively), whereas *R. kumarii* (+0.7) and *R. longifrons* (+0.8) preferred F_4. Irrespective of the insect-based diets, all the predators preferred F_3 of BMP. However, *R. marginatus* and *R. fuscipes* preferred F_3, *R. kumarii* preferred F_2 and *R. longifrons* preferred F_1 of CCL. The F_4 of EVL was preferred by all the reduviids, except *R. marginatus* (Tab. 3).

Table 3. Access proportion index of reduviids in relation to consumption time (in %).

Predator	Diet	Diet concentration			
		F_1	F_2	F_3	F_4
R. marginatus	BMP	0.49	0.21	0.79	0.17
	CCL	-0.25	0.61	0.83	0.45
	EVL	-0.03	-0.11	0.61	-1
	PL	0.57	0.63	0.56	0.49
	PB	-0.97	-0.95	-1	-1
R. fuscipes	BMP	0.40	0.37	0.54	-0.96
	CCL	-1	-0.14	0.47	-0.88
	EVL	-0.37	-1.00	-1.00	-0.27
	PL	0.67	0.88	0.77	0.47
	PB	-1	-1.00	-1.00	-1
R. kumarii	BMP	-0.15	0.20	0.30	-1
	CCL	-0.05	0.76	-0.16	-1
	EVL	-0.03	0.11	0.19	0.39
	PL	0.19	0.44	0.47	0.70
	PB	-0.21	-0.70	-1	-1
R. longifrons	BMP	0.45	0	0.49	-1
	CCL	0.66	0.17	0.12	-0.21
	EVL	0.61	0.57	0.60	0.63
	PL	0.77	0.78	0.81	0.82
	PB	0	-1	-1	-1

(BMP) *Bombyx mori* pupa, (CCL) *Corcyra cepalonica* larva, (EVL) *Egocera venulea* larva, (PB) pig blood; (PL) pig liver.

Food preference index (FPI)

Among the experimental animals, *R. marginatus* recorded the highest FPI value (+0.63) followed by *R. longifrons* (+0.47) and *R. fuscipes* (+0.45) in the meat-based PL diet. If we consider the insect-based diets, the highest FPI (+0.24) was recorded with *R. longifrons* on EVL, followed by *R. marginatus* on CCL (+0.24). All other diets showed negative values. All the experimental predators preferred meat-based PL diet with the rank I followed by CCL with two 2nd ranks and two 3rd ranks; BMP with one 2nd, two 3rd and one 4 th rank (see Table 4); EVL with one 2nd, two 4th and one 5th rank and PB with one 4th and three 5th. Highest similarity (+1.0) was observed in the feeding behaviour of *R. marginatus* and *R. kumarii.* Both the animals recorded highest API values for PL followed by CCL, BMP, EVL and PB diets. The comparison of *R. fuscipes* to *R. marginatus,* and *R. longifrons* to *R. kumarii* showed higher degree of similarity (+0.8) and the similarity was lower when we compared *R. longifrons* to *R. marginatus* and *R. longifrons* to *R. kumarii* (+0.7). The similarity was still lower between *R. longifrons* and *R. kumarii* (+0.3). No negative similarities were observed among the predators. These results also coincide with the rank correlation values obtained for the feeding behaviour of reduviid predators on different artificial diets. All the predators showed highest similarity in their feeding behaviours on PL and PB diets (+1), in

Table 4. Cumulative consumption time (%) and food preference index (FPI) and ranking of reduviids on artificial diets.

Predator	Diet	Cunsumption (in %)	Food preference index	Rank
R. marginatus	BMP	36.23	-0.07	3
	CCL	52.94	+0.24	2
	EVL	12.50	-0.38	4
	PL	65.49	+0.63	1
	PB	3.07	-0.89	5
R. fuscipes	BMP	18.58	-0.32	2
	CCL	8.17	-0.66	3
	EVL	2.03	-0.90	5
	PL	65.14	+0.45	1
	PB	2.11	-0.75	4
R. kumarii	BMP	6.78	-0.61	3
	CCL	13.30	-0.48	2
	EVL	11.08	-0.66	4
	PL	34.32	-0.24	1
	PB	2.86	-0.81	5
R. longifrons	BMP	0.83	-0.88	4
	CCL	14.31	-0.51	3
	EVL	30.18	+0.26	2
	PL	55.50	+0.47	1
	PB	1.67	-0.89	5

(BMP) *Bombyx mori* pupa, (CCL) *Corcyra cepalonica* larva, (EVL) *Egocera venulea* larva, (PB) pig blood; (PL) pig liver.

which all the animals were attracted and consumed at highest degrees on PL and neglected or fed less on PB diet. But in all other comparisons except CCL vs. PB, BMP vs. EVL, PB vs. EVL and EVL vs. PL showed optimum positive similarities on feeding behaviour of predators (+0.4). Among those exceptions the latter two categories showed highly negative values (-1). Furthermore, by comparing EVL with the meat-based diets the rank correlations were highly negative (-1).

Discussion and Conclusion

Food formulation

"An important factor to be considered in rearing predaceous bugs is that they strike out at moving objects and therefore must be furnished with live prey" stated by SMITH (1966), who made an assumption that the reduviid predators locate a prey by its movement. But the frozen, heat killed and head crushed larvae were also located by the predators, which were reared on these prey types (SAHAYARAJ & SATHYAMOORTHI unpublished data). Therefore, the prey location is not only based on the prey movement but also on chemical and visual cues. The preliminary trials undertaken to formulate the artificial diet contained ingredients like agar and yeast. Agar was introduced with to produce a gelled semi-solid diet, but the predators discarded it after piercing. DE CLERCQ *et al.* (1998) also reported such a trial with inferior results. This must be due to the inability of the predator to digest the admixed agar. It is an inert filler substance that is probably not digested by the carnivorous animals. Though the reduviid predators are entomosuccivours, the salivary enzymes may not be able to digest pre-orally this gelled diet into liquid, and also sucking through the rostrum may be difficult. SMITH (1966) explains that predaceous reduviids pump proteolytic enzymes into the prey and in few minutes all proteinaceous tissues are dissolved, including the muscles and the mid-gut and its contents. The liquefied contents are then sucked out at leisure and only a chitinaceous shell remains. The aim of adding yeast in the artificial diet is to providing symbiotic microbes (says HAGEN *et al.*, 1976). But we found that addition of yeast produced bad smell due to the fermentation so that yeast was also eliminated from the diet formulation. As reported by SINGH (1977) and YAZLOVETSKY *et al.* (1979a) a liquid diet formulation was tried without the above-mentioned ingredients and it was found that the method followed by DE CLERCQ & DEGHEELE (1997) with some modification (Tab. 1) was suitable and the predators preferred it.

Feeding time

All the reduviid predators recorded maximum feeding time in meat-based PL diet. These results coincide with the successful completion of artificial diets for *Crysoperla* without insect ingredients reported by VADERSANT (1973) and YAZLOVETSKY *et al.* (1979b). Similarly COHEN (1985) and COHEN & URIAS (1986) also used meat-based diets for rearing *Geocoris puntipes*. The meat-based artificial diet was used for the culture of pentatomid predators (DE CLERCQ & DEGHEELE, 1992a; SAAVEDRA *et al.*, 1995; DE CLERCQ *et al.*, 1998). Successful rearing has also been reported in the development of artificial diets for *Podisus maculiventris* and *P. nigrispinus* (DE CLERCQ & DEGHEELE, 1992b). All the three insect-based diets recorded lesser feeding time than the meat-based PL diet. All the reduviid predators totally rejected the pig blood. Two reasons may explain this: (1) the presence of cellular components which make about 20 to 45% of the total volume of blood, and (2) 7.8% of blood proteins are found in red blood cells. The nutrient content of the vertebrate blood is thus about 10 to 100 times thicker than that of arthropod haemolymph.

Access proportion index (API) and food preference index (FPI)

Statistical analysis of access proportion index based on the consumption time showed that the diet preference and feeding vary from one species to another. In the case of CCL, *R. kumarii* preferred second and *R. longifrons* preferred first diet. To find out the suitable diet for the rearing and also the mass production of each species, FPI was calculated by using approaching and consumption time because triggering of feeding behaviour is an important factor deciding the suitability of a diet, i.e., if a diet seems to be good but it fails to elicit the feeding behaviour it is regarded as unsuitable. Moreover result obtained in concentration-based analysis showed no relation. The highlighted FPI results suggest that the PL diet is the most suitable for rearing of reduviid predators followed by CCL and BMP diets. Among the insect-based diets, EVL seems to be an unsuitable diet. We propose two reasons to explain this: (1) it is an unnatural host of reduviids and (2) it mainly feeds on weed plant *Boerhavia diffusa.* While we provide the live WVL to this reduviid, it produced a green viscous offensive fluid immediately after capturing. Hence the predator leaves the prey without feeding and does not approach the prey again for feeding. Moreover *Egocera venulea* is a hairy caterpillar, and laboratory and field studies showed that, generally, such caterpillars are less preferred by reduviids than non-hairy caterpillars (personal observations).

The meat-based (PL) diet was the most suitable for reduviid predators. However, further modifications should be done to improve the attractiveness and nutritional value of the diet. Thorough knowledge of the protein, lipid, carbohydrates, amino acids as well as the minerals salt contents of the prey or hemolymph profile of the predator may give an accuracy of the composition of the above mentioned diet constituents to be added in the artificial diet. This may improve the quality of the artificial diet. And also the chemical cue, studied by SAHAYARAJ & PAULRAJ (2001) on predators using various compounds extracted from pest larvae, is reported to stimulate predatory behaviour. Such studies on host and addition of such compounds (phagostimulants) might enhance our knowledge on the feeding intensity of predators on artificial diet.

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