

Polymorphism of *Rhynocoris marginatus* (Fab.) (Heteroptera : Ruduviidae) on the biology, biological control potential

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ABSTRACT

To know the influence of polymorphism of *Rhynocoris marginatus* (Fab.) on the biology, biological control potential and molecular profile were recorded in the following partners : N male + N female; N male + S female, N male + NS female, NS male + NS female and S + NS female under laboratory conditions. Offspring produced by the S + NS female partners developed quickly having minimum body weight and size and egg hatching percentage, maximum relative growth rate both in first instars and adults, maximum survival rate ($82.5 \pm 3.50\%$), male biased sex ratio (0.23). In general, the predatory rate was increased when the prey deprivation period increased from 24 hrs to 72 hrs, third instars to adults. Molecular analysis studies revealed that the total free amino acid was high in N adult morphs. No unique DNA sequence was observed among the morphs of *R. marginatus*. Among the polymorphic *R. marginatus*, N male + N female; N male + S female, N male + NS female, NS male + NS female and S + NS, approaching time was also decreased in the latter instars and adult, the biological control potential is highly observed in 96 hrs starved N+N adults showed minimum approaching time, handling time, and high predatory rate. Among the experimental morphs the weight gain was maximum in N+N and minimum weight gain was observed among the morphs of SNS. Minimum total nymphal developmental period, least body weight, survival rate were recorded among the offsprings produced by S+NS, NS+NS and N+NS partners.

Keywords: *Rhynocoris marginatus*, N male + N female; N male + S female, N male + NS female, NS male + NS female and S + NS.

MS History: 07.03.2019 (Received)-28.04.2019 (Revised)- 02.05.2019 (Accepted).

Citation: Petchidurai, G., Chitra, R., and Sahayaraj K. 2019. Polymorphism of *Rhynocoris marginatus* (Fab.) (Heteroptera: Ruduviidae) on the biology, and biological control potential. *Journal of Biopesticides*, **12**(1):114-125.

INTRODUCTION

Rhynocoris marginatus (Fab.) is an alate, entomosuccivorous, polyphagous, polymorphic, crepuscular, multivoltine assassin bug. It is predominantly found in the scrub jungles, semi-arid zones, tropical rain forests and agroecosystems of south India. It is recorded as a potential predator of 23 agricultural insect pests (Sahayaraj, 2006). Very little information was available on the comparative predatory efficiency of the nymphal instars and adults of this predator. Ambrose *et al.* (1990b) studied the influence of a few uneconomically important insects on the biology of *R. marginatus*. Later its biology

and fecundity and life table parameters were studied in relation to some economically important pests (George 1999; Sahayaraj and Paulraj, 2001a, 2001b).

One of the most important characteristic features of any natural population is its diversity. Such well-marked diversity has been observed among the populations of flies. In genetic terminology, natural populations are said to be polymorphic. Polymorphism is most apparent when it affects a visible or behavioral phenotype, but it is not at all restricted to such traits. Polymorphism may arise in a population by the following three basic avenues: transient polymorphism, balanced polymorphism and random fixations of natural mutations (Guilford, 1990). Transient polymorphism is a

by-product of directional natural selection and it represents a temporary situation. Balanced polymorphism is a relatively permanent kind of equilibrium which originated by disruptive or diversifying selection and heterosis. The Random Fixation of natural population method of origin of polymorphism is also called Neutral Mutation or Random Genetic Drift hypothesis or “Neo-Darwinian Evolution” and this idea has been developed by Wright and Samways (1997).

R. marginatus exists in three different morphs viz., with black connexivum (Niger-N), with red connexivum (Sanguineous-S) and with black and red banded connexivum (Nigrosanguineous-NS) (Distant, 1902). Much progress has been made to understand the action of natural enemies through life table analysis (Bellows *et al.*, 1992). However, life table studies on predatory insects are scanty except some publications (Singh and Singh, 1994; Kumar and Velusamy, 1995; Sharma and Bhella, 1995; Gupta and Singh, 1996; Venkatesan *et al.*, 1997; George *et al.*, 1998). There is no information available about the biology and reproductive potential of *R. marginatus* regarding the different morphs, hence the present study was undertaken to find out whether there is any variation among these three morphs of *R. marginatus* in the biology through biological studies on *C. cephalonica*, which is essential to conserve and augment the better adapted morph for subsequent utilization in the insect pest management programme. To record the nymphal development time, survival, sex ratio and oviposition of different morph combinations. To identify the morphometric analyse of the three morphs (N, NS and S) of *R. marginatus*. To evaluate the biological control potential of *R. marginatus* in different life stages (from third instar to adult predators) by pot studies. To analyse the haemolymph total free amino acid profile and DNA analyses by PCR studies for niger, nigrosanguineous and sanguineous of *R. marginatus*.

MATERIALS AND METHODS

Insect collection and maintenance

Nymphs and adults of *R. marginatus* morphs were collected from Melapattam scrub jungle

in Tirunelveli district and also in Vallanadu, Sivanthipatti in Tamil Nadu. They were reared under the laboratory condition (30-32°C temperature, 75 – 80% RH) in separate plastic containers with *C. cephalonica*. The newly emerged adults were segregated according to the morphs as niger (N), sanguineous (S) and nigrosanguineous (NS) and the following combinations were maintained separately for mating: niger male with niger female (N+N/NN); niger male with sanguineous female (N+S/NS); niger male with nigrosanguineous female (N+NS/NNS); sanguineous male with sanguineous female (S+S/SS); sanguineous male with nigrosanguineous female (S+NS/SNS) and nigrosanguineous male with nigrosanguineous female (NS+NS/NSNS) under laboratory conditions. Eggs masses were collected daily and maintained separately in a sterile Petri dish. Newly emerged nymphs of each category were reared individually in 350 ml plastic containers on *C. cephalonica*. Minimum of 100 nymphs were maintained for each category. Development period of each nymphal instars, weight, mortality were recorded. These nymphs and adults were also used for the biological control potential evaluation.

Adults and nymphs of *Dysdercus cingulatus* (Fab.) were collected from cotton field of Tamil Nadu Agricultural College and Research Station, Killikulam, Vallanadu, Tirunelveli District, Tamil Nadu, India. Collected insects were maintained under laboratory condition on soaked cotton seeds. The newly emerged life stages of *D. cingulatus* were used for this present study.

Relative Growth Rate (RGR)

The predators were supplied prey daily with an excess and the number of larvae eaten by each predator was counted and replaced with fresh larvae from this observation predatory rate was calculated and expressed as number of prey consumed/predator/day. Moreover, *R. marginatus* life history traits were

$$RGR = \frac{(Fwt - Iwt)}{\frac{(Fwt + Iwt) \times D}{2}}$$

determined under each polymorphic combination as nymphal developmental period, survival rate, relative growth rate (mg/mg/day) and sex ratio [$\frac{\text{♀}}{\text{♂}+\text{♀}}$]. The relative growth rate (RGR) for each instar was calculated as fresh weight gain of the instar divided by the mean fresh weight of the instar times duration (D) of the instar (Isikber and Copland 2002). Mean fresh weight was calculated as final fresh weight (Fwt) of the instar plus initial fresh weight (Iwt) of the instar divided by two in mg.

Morphometric analyses

Dead animals were preserved at 70% alcohol for morphometric analyses. Males and females were separately measured under the compound microscope with the help of ocular and stage micrometer. All the morphometric analyses readings were calculated in cm size.

Biological Control Potential Evaluation

Laboratory emerged III, IV, V, nymphal instars and adult (24 hrs starvation) of *R. marginatus* were taken from the laboratory population and used for the biological control potential evaluation studies. The experiment was performed in plastic pots having cotton saplings covered with glass chimney. Predator preferred stage (it may be confirmed by prey stage preference test) of known weight *D. cingulatus* (3 preys) were introduced into the pots separately and allowed to move undisturbed for 24 hrs. Then known weight of predator was introduced into the plants and the sequential events of predation such as approaching time (AT), handling time (HT) or sucking time (ST) were recorded continuously for 3 hours by visual observation. After one day, the predator weight was recorded and also the number of pest consumed (predatory rate) was calculated.

Male genitalia

Male genitalia of S polymorph was separated from the preserved specimens after treating the abdomen distally with a few drops of BEAD solution (Benzene, ethylacetate, absolute alcohol and distilled water, in the ratio of 1:3:6:9 (Sucheta, 1973). The genitalia were boiled in 10% KOH, rinsed in distilled water and a few drops of glacial acetic acid was added. The microslide was observed under Nikon Photomicroscopic unit and details were recorded.

Amino Acids Analysis

Pipette out 10, 20, 30, 40 and 50 μ l of the haemolymph of treated predators in a series of test tubes. Make the volume up to 4.0ml by distilled water in all test tubes. Instead of standard 96% ethanol 100 μ l, 2ml of 50% ethanol and 0.1 μ l distilled water were added in a test tube that serves as blank. Then add 2ml of ninhydrin reagent. Cover the test tubes with aluminium foil and place them in a boiling water bath for 10 minutes. Cool to room temperature in a trough containing tap water and add 1ml of 50% ethanol to each tube. Allow it to stand for 5 minutes and read the colour at 850nm. Draw a standard curve using optical density versus concentration. Find out the concentration of the total free amino acid in the sample and express it as μ g/aminoacid.

Statistical Analysis

Different morphometric data of all the morphs (male and female separately) were subjected to correlation matrix. For instance male Niger (N) head length was correlated with NS male head length and also to S male head length. Similarly all the morphometric data was analysed r^2 , t and p value were tabulated. System statistics package was used for the analyses.

Table 1. Adult polymorphism on the nymphal developmental period (days) of *R. marginatus*.

Life stages	NN	NNS	NS	NSNS	SNS
First	6.8 \pm 0.7b	6.7 \pm 0.5b	8.5 \pm 0.7a	6.6 \pm 0.5b	6.4 \pm 0.7b
Second	6.3 \pm 0.6	6.6 \pm 0.5	7.0 \pm 0.6	6.4 \pm 0.7	6.2 \pm 0.5
Third	8.0 \pm 0.6	7.4 \pm 0.5	9.1 \pm 0.6	8.1 \pm 0.8	7.1 \pm 0.6
Fourth	9.1 \pm 0.9	8.1 \pm 0.6	10.2 \pm 1.7	9.0 \pm 0.6	8.0 \pm 0.8
Fifth	15.0 \pm 1.2	14.6 \pm 0.1	15.1 \pm 1.4	15.8 \pm 1.0	14.4 \pm 0.12
First –Adult	45.0 \pm 0.8bc	43.2 \pm 0.6c	49.9 \pm 0.4a	46.2 \pm 1.2b	42.0 \pm 0.8cd
Survival (%)	73.5 \pm 1.48b	31.0 \pm 2.02e	46.05 \pm 5.58c	42.2 \pm 3.35d	82.5 \pm 3.50a

Table 2. Polymorphism on the egg hatching and nymphal survival (%) total number of adults emerged and sex ratio of *R. marginatus*, NS- Nigro sanguineous: N- Niger, S- Sanguineous

Morphs	Egg hatching	Total no. of Adult emerged	Sex ratio	Survival
N+N	79.4±1.2	37±1.34	0.62	73.5±1.48
NS+NS	94.6±1.6	50±2.13	0.60	31.0±2.02
N+S	91.4±0.8	22±1.0	0.59	46.05±5.58
N+NS	79.0±1.0	72±1.8	0.59	42.2±3.35
S+NS	54.2±1.3	26±4.1	0.23	82.5±3.50

RESULT

Nymphal Developmental Period

Total nymphal developmental period of *R. marginatus* obtained from different polymorph reveals that it was minimum in SNS followed by NNS, NN, NSNS and NNS. Among the nymphal instar, second nymphal instar was the shortest and fifth nymphal instar was the longest (see table 1). Except the S + NS (0.23) all other combinations produced female biased sex ratio.

Impact of polymorphism on nymphal survival rate is presented in Table 2. It is very clear from the table that survival rate was high in the nymphs produced by S+NS followed by N+N, N+S, N+NS and NS+NS (Table 2). Sex ratio was female biased except in the adults emerged from the polymorphic forms of S+NS (0.23) (Table 3).

Weight gain and relative growth (RG)

Irrespective of the morphs, the weight of the nymphal instars gradually increased when the nymphs grew older (table 3). Among the

experimental morphs the weight gain was aximum in NN. Minimum weight gain was observed among the morphs of SNS-RGR of *R. marginatus* morphs were represented in figure 1. From the figure it was very clear that there was no definite RGR pattern was observed among the life stages of the morphs. However, RGR of *R. marginatus* first nymphal instar was high in the offsprings of the patners NS + NS. Similarly maximum RGR of third, fourth and fifth nymphal and adults were recorded for N+N, N+S, N+NS and S+NS respectively.

RGR of *R. marginatus* morphs were very clear that there was no definite RGR pattern was observed among the life stages of the morphs. However, RGR of *R. marginatus* first nymphal instar was high in the offsprings of the patners NS + NS. Similarly maximum RGR of third, fourth and fifth nymphal and adults were recorded for N+N, N+S, N+NS and S+NS respectively.

Table 3. Impact of polymorphism on the weight (in mg) of *R. marginatus* life stages.

Morphs	I	II	III	IV	V	Adult
NN	3.6±1.07	12.1 ±2.03	34.9 ±4.16	55.8 ± 6.18	88.6± 5.60	191.2±8.50
NNS	3.2 ±1.74	12.0 ±0.4	34.1±1.81	52.5 ± 1.0	79.4 ±2.71	175.5 ±1.8
NS	3.4 ± 0.42	11.7 ± 3.30	28.2 ± 2.71	49.4 ± 4.21	76.3 ± 3.11	177.1 ±5.2
NSNS	3.0 ± 1.05	13.3 ± 1.9	35.5 ± 3.61	46.8 ± 4.58	75.2 ± 2.17	159.2 ±1.4
SNS	3.5 ± 1.11	11.1 ± 3.54	27.2 ± 5.01	51.4 ± 5.18	77.5 ± 3.21	146.6 ±4.7

Reproductive Behaviour

From this experimental results recorded in the table 4, it was clear that preoviposition period was minimum in N morph. It was mostly equal in the N+NS and NS+NS morphs. Highest preoviposition period was observed in S+NS and N+S morphs respectively. The longest oviposition period was observed in S+NS morphs of *R. marginatus*. It was reduced in other morphs of N+N and N+NS. The postoviposition period was comparatively shorter in N+N morph than N+NS and NS+NS (Table 4).

Egg Biology

The highest average number of eggs per batch was found in NN morph. NS+NS was found to produce minimum egg (Table 4). In contrast to the survival rate, maximum percentage of eggs were hatched from the morphs NS+NS. Similarly, least percentage of egg hatching was recorded in S+NS morph combination. Incubation period was shorter N+NS and longer in N+N and S+NS respectively (Table 4).

Table 4. Preoviposition period (PROP), oviposition period (OP), post oviposition period (POP) (days), number of eggs laid, maximum and minimum number of eggs per batch and incubation period of *R. marginatus*

Parameters	N+N	N+NS	N+S	NS+NS	S+NS
PROP	11.3 ± 1.52a	16.6 ± 1.2	37.28 ± 5.30	17.0 ± 0.8	39.1 ± 6.83
OP	31.5 ± 1.74	35.6 ± 1.81	51.60 ± 5.09	43.9 ± 2.21	52.8 ± 5.76
POP	2.2 ± 0.01	3.0 ± 0.04	11.70 ± 2.69	5.3 ± 0.09	13.32 ± 2.89
No. of eggs	78.0 ± 9.71	55.3 ± 5.85	47.0 ± 3.11	49.0 ± 26.8	58.5 ± 4.81
Maximum eggs/batch	61.34 ± 8.76	49.56 ± 3.35	36.4 ± 5.32	33.2 ± 0.22	43.1 ± 2.81
Minimum eggs/batch	17.8 ± 2.33	6.2 ± 0.08	11.2 ± 1.52	16.8 ± 1.39	15.2 ± 5.01
Incubation	8.6 ± 0.5	7.2 ± 1.0	7.9 ± 1.04	7.41 ± 1.27	8.2 ± 1.09
Hatching (%)	79.7	94.6	91.4	79.0	54.2

Morph Distribution

N+N partners produced only N male and N female at 37: 62 ratio (Table 5). Similarly N+NS female partners produced only N and NS. But in addition to N male, and NS female, the N+NS partners also

produced 23% of N and 28.2% NS females. N+S partners produced N, S and also NS. Similarly partners of S+NS and NS+NS produced all the three morphed adults (refer Table 5).

Table 5. Impact of *R. marginatus* polymorphism on the progeny morph distribution (%)

Morph Combination	N		NS		S	
	Male	Female	Male	Female	Male	Female
NN	37.8	62.1	-	-	-	-
NSNS	24.0	54.0	18.0	-	4.0	12.0
SNS	7.6	22.9	11.5	34.0	19.2	42.2
NS	9.0	36.2	18.3	33.8	9.0	31.7
NNS	21.7	44.7	26.9	31.7	-	-

-indicates not produce any offspring

Male genitalia

Microscopic structure of male genitalia of S was presented in plate 2. Pygophore is a rectangular size its apex pointed. Strongly pilose throughout claspers narrow basely and flattened apically and strongly pilase. The abdomen forwards and fits two pairs of claspers on its abdominal segment from the genitalia claspers separated consist of superior and inferior pairs. Left clasper was 2481.51 µm in length and its width was 68.00 µm. But

the width of right clasper was 775.80 µm. The width between the two clasper was 4890.01 µm.

Morphometric Analyses

Morphometric analyses results of male and female of *R. marginatus* are presented in Table 6. The results showed that in all the three morphs, the females of the Niger morphs of were larger (1.8cm) than the other two morphs such as NS and S. Similarly observations were also recorded for males too. However

Table 6 . Morphometric analyse of different morphs (in mm) of *R.marginatus* (n=10; X±SE).

Morphs	HL	TL	AL	FLL	MLL	HLL	FWL	HW	Total length (Cm)
N Male	0.3	0.4	0.8	1.1	0.7	1.3	1.1	0.7	1.5
N S Male	0.3	0.3	0.8	0.6	0.9	1.1	1.1	0.6	1.4
S Male	0.2	0.24	0.52	0.8	0.5	0.9	0.9	0.6	0.96
N Female	0.4	0.5	0.9	1.2	0.8	1.4	1.2	0.8	1.8
N S Female	0.3	0.4	0.9	0.9	0.6	1.0	1.1	0.7	1.6
S Female	0.23	0.3	0.6	0.7	0.53	1.0	1.0	0.7	1.1

N- Niger, S – Sanguineous, NS - Nigro Sanguineous, HT- Head Length, TL - Thorax Length, AL - Abdomen Length, FLL- Fore leg Length, MLL- Mid leg Length, HLL - Hind leg Length, FWL - Fore Wing Length, HWL - Hind Wing Length

correlation analyses between N to NS, N to S shows no significant differences (Table 7).

Biocontrol Potential of *R. marginatus*
Predatory behaviour of different life stages produced by the morphs was studied in terms of approaching time, handling time, weight gain and predatory rate and presented in Tables 8-11. Irrespective of the morphs, the approaching time decrease as the starvation period increased. Approaching time was also decreased in the III, IV, V and adult of *R. marginatus*. In contrast handling time was increased as the starvation time prolonged from 24 to 96 hrs. Weight gain was gradually increased from third instar to adults in all the progeny obtained from different morph combinations. However, weight gain was increased upto 72 hrs starvations, then it

declined at 96 hrs prey deprivation (Tables 8–11). At 24 hrs starvation the predatory rate was high in all the life stages of *R. marginatus* offsprings produced by N+NS morphs (Table 10). Irrespective of the progeny obtained from different morph partners, at 96 hrs prey deprivation, exactly equal predatory rate was recorded for V and adults of *R. marginatus*. During the same prey deprivation period, the predatory rate of third and fourth nymphal instars was high in the progeny obtained from NS+NS combination (see Table 9). Further more, the approaching time of all the life stages of these offsprings were also minimum at 24, 48 and 72 hours prey deprivation (see Table 10).

Table 7. Data (R^2 , t and p) showing the correlation analyses between the N male to NS, S male, N female vs NS and S females separately.

N Vs NS			N Vs S			N Vs NS			N Vs S		
R^2	T	P	R^2	T	P	R^2	T	P	R^2	T	P
.04	.63	.54	.04	.63	.54	0.04	-0.63	0.54	0.04	-0.63	0.54
.02	.47	.64	.04	.63	.54	0.27	1.73	0.12	0.27	1.73	0.12
.10	.97	.35	.06	.73	.48	0.34	-2.03	0.73	0.34	-2.03	0.07
.09	.89	.39	.01	.31	.75	0.02	-0.47	0.64	0.02	-0.47	0.64
.04	.63	.54	.29	.18	.10	0.04	-0.63	0.54	0.04	-0.63	0.54
.06	.72	.48	.13	1.10	.03	0.06	0.73	0.48	0.06	0.73	0.48
.00	.00	.00	.00	.00	.00	0.02	0.44	0.66	0.24	0.44	0.66
.01	.29	.77	.04	-.64	.53	0.01	0.30	0.76	0.01	0.30	0.76

Table 8. Biological Control Potential of *R. marginatus* (N+S) on *Dysdercus cingulatus*.

Starvation Time	Life Stages	Predator weight	Approaching time	Handling time	Weight gain g/mg	Predatory rate
24	III	35.0 ± 3.60	79.3 ± 4.50	76.6 ± 10.0	1.6 ± 0.57	1.3 ± 0.5
	IV	64.5 ± 2.08	73.2 ± 7.81	78.2 ± 13.07	3.4 ± 0.54	1.5 ± 0.6
	V	83.0 ± 1.82	67.0 ± 25.2	79.7 ± 5.79	3.6 ± 0.64	1.5 ± 0.5
	Adult	159.4 ± 5.59	64.8 ± 22.2	86.5 ± 6.95	3.9 ± 0.48	1.6 ± 0.5
48	III	32.4 ± 2.30	62.6 ± 11.01	148.8 ± 24.6	2.1 ± 0.67	1.4 ± 0.5
	IV	55.6 ± 3.20	61.2 ± 1.30	155.8 ± 18.0	3.8 ± 0.29	1.8 ± 0.4
	V	85.6 ± 2.30	60.4 ± 5.81	168.0 ± 20.5	4.2 ± 0.50	1.9 ± 0.6
	Adult	148.8 ± 2.38	55.1 ± 8.14	178.4 ± 3.40	4.3 ± 0.75	2.2 ± 0.4
72	III	30.0 ± 4.53	34.3 ± 18.81	157.0 ± 7.55	5.6 ± 0.52	1.7 ± 0.7
	IV	43.6 ± 1.92	26.8 ± 22.63	166.2 ± 11.3	5.7 ± 0.45	1.8 ± 0.6
	V	75.5 ± 4.20	23.6 ± 20.89	174.6 ± 8.53	6.0 ± 0.49	2.0 ± 0.6
	Adult	135.8 ± 3.32	21.1 ± 16.64	175.9 ± 10.5	7.0 ± 0.50	2.2 ± 0.4
96	III	38.9 ± 3.60	17.9 ± 19.05	163.5 ± 7.77	5.60 ± 0.54	2.5 ± 0.5
	IV	45.7 ± 2.05	13.9 ± 4.4	174.8 ± 19.8	5.70 ± 0.59	2.6 ± 0.5
	V	71.1 ± 5.30	10.0 ± 3.88	183.5 ± 10.8	5.98 ± 0.45	2.7 ± 0.3
	Adult	126.4 ± 5.39	9.6 ± 2.11	187.8 ± 5.61	6.03 ± 0.52	2.8 ± 0.4

Table 9. Biological control potential of *R. marginatus* (NS+NS) on *Dysdercus cingulatus*.

Experiment Time	Life Stages	Predator weight	Approaching time	Handling time	Weight gain	No. of prey/pre/day
24	III	33.2 ± 1.05	65.7 ± 4.19	87.2 ± 6.60	1.5 ± 0.60	1 ± 0.0
	IV	63.5 ± 1.29	61.5 ± 2.38	87.5 ± 5.44	3.0 ± 0.81	1.2 ± 0.5
	V	82.0 ± 1.41	51.5 ± 3.09	88.2 ± 2.62	3.75 ± 1.5	1.2 ± 0.5
48	III	33.8 ± 2.48	58.6 ± 10.05	152.0 ± 16.67	2.65 ± 0.50	1.3 ± 0.5
	IV	53.8 ± 4.12	54.3 ± 9.04	153.8 ± 14.66	3.06 ± 0.63	1.5 ± 0.5
	V	79.3 ± 2.16	52.5 ± 5.54	169.3 ± 49.40	4.51 ± 0.34	2.1 ± 0.7
	Adult	141.8 ± 4.45	50.0 ± 5.22	177.2 ± 46.70	5.28 ± 0.48	2.4 ± 0.5
72	III	32.0 ± 3.89	24.2 ± 19.73	162.6 ± 22.36	6.5 ± 0.75	2.2 ± 0.7
	IV	42.7 ± 1.83	23.7 ± 21.57	163.2 ± 7.32	7.7 ± 0.05	2.3 ± 0.7
	V	74.7 ± 2.65	22.5 ± 5.80	168.8 ± 15.31	8.1 ± 0.64	2.4 ± 0.8
	Adult	132 ± 3.40	19.3 ± 3.32	175.2 ± 26.63	8.8 ± 0.64	2.5 ± 0.7
96	III	37.1 ± 3.84	13.8 ± 7.55	160.3 ± 18.29	5.62 ± 0.56	2.6 ± 0.5
	IV	44.6 ± 2.41	11.5 ± 4.92	167.3 ± 35.20	5.8 ± 0.46	2.7 ± 0.5
	V	74.0 ± 3.17	10.6 ± 4.03	177.7 ± 26.47	6.6 ± 0.69	2.7 ± 0.4
	Adult	127.4 ± 4.57	10.1 ± 8.51	186.7 ± 49.18	7.2 ± 0.80	2.8 ± 0.4

Table 10. Biological Control Potential of *R. marginatus* (N+NS) on *Dysdercus cingulatus*.

Starvation Time	Life Stages	Predator weight	Approaching time	Handling time	Weight gain g/mg	Predatory rate
24	III	34.3 ± 3.78	71.0 ± 12.16	83.0 ± 4.00	2.03 ± 0.5	1.3 ± 0.5
	IV	62.6 ± 1.94	68.5 ± 1.00	84.7 ± 8.80	3.5 ± 0.6	1.5 ± 0.6
	V	83.7 ± 3.86	66.0 ± 2.58	85.2 ± 0.50	3.8 ± 0.5	1.7 ± 0.5
	Adult	148.6 ± 3.97	65.2 ± 3.03	87.0 ± 5.87	5.5 ± 0.6	2.2 ± 0.4
48	III	33.3 ± 3.44	64.0 ± 5.09	134.3 ± 22.2	4.48 ± 0.59	1.3 ± 0.5
	IV	57.5 ± 2.73	61.3 ± 5.06	156.5 ± 33.6	6.38 ± 0.52	1.5 ± 0.5
	V	82.8 ± 6.14	59.0 ± 6.92	148.8 ± 3.42	7.46 ± 0.49	2.0 ± 0.7
	Adult	140.3 ± 4.54	50.6 ± 6.56	177.3 ± 25.9	7.90 ± 0.37	2.1 ± 0.4
72	III	33.0 ± 3.46	31.8 ± 6.93	165.5 ± 30.6	6.6 ± 0.71	2.1 ± 0.6
	IV	43.1 ± 2.58	28.8 ± 2.91	166.4 ± 30.9	7.7 ± 0.75	2.2 ± 0.4
	V	74.8 ± 3.43	18.1 ± 5.63	168.4 ± 34.0	11.2 ± 0.75	2.5 ± 0.6
	Adult	133.4 ± 2.22	15.4 ± 1.67	169.2 ± 32.2	11.5 ± 0.78	2.6 ± 0.9
96	III	39.8 ± 3.84	14.2 ± 2.60	161.1 ± 16.5	5.6 ± 0.53	2.5 ± 0.5
	IV	45.3 ± 3.30	13.9 ± 4.20	179.1 ± 21.7	5.7 ± 0.56	2.5 ± 0.5
	V	72.2 ± 4.15	13.2 ± 6.81	186.3 ± 3.68	5.8 ± 0.27	2.7 ± 0.4
	Adult	127.3 ± 6.27	12.8 ± 2.93	186.4 ± 15.4	5.9 ± 0.37	2.8 ± 0.4

Total free amino acids

Total free amino acids was maximum in N morph (73.61 µg/mg) followed by NS (64.9061 µg/mg) and S morph (62.2861 µg/mg).

DISCUSSION

R. marginatus preys on several lepidopteran caterpillars (Ambrose, 1999, Sahayaraj, 1999b) including *S. litura* (Sahayaraj and Paulraj, 2001a) and *H. armigera* (Ambrose, 1987). It is an

established fact that development, growth and reproduction are influenced by a variety of external and internal factors and amongst them, nutrition seems to be the most crucial single factor. Duration of the post-embryonic development of different morphs of this bug was varied (92.29, 75.38 and 99, 47 days for Niger, NS and S) (George, 1999a). He has not mentioned anything about the mating partners from which he selected the eggs. Furthermore, all the nymphs were reared on *Earias vitella*.

Table 11. Biological control potential of *R. marginatus* (S+NS) on *Dysdercus cingulatus*.

Starvation	Life Stages	Predator weight	Approaching time	Handling time	Weight gain g/mg	Predatory rate
24	III	33.0 ± 2.64	76.0 ± 3.46	81.3 ± 10.0	2.1 ± 0.10	1.0 ± 0.0
	IV	63.2 ± 4.27	68.5 ± 2.08	82.0 ± 8.20	3.6 ± 0.55	1.2 ± 0.5
	V	82.7 ± 2.06	66.0 ± 2.58	82.5 ± 1.28	3.95 ± 0.55	1.5 ± 0.3
	Adult	151.1 ± 7.50	64.2 ± 1.48	87.6 ± 9.39	5.52 ± 0.58	1.6 ± 0.5
48	III	33.8 ± 3.11	67.0 ± 6.36	123.6 ± 6.10	4.8 ± 0.50	1.2 ± 0.7
	IV	61.6 ± 6.14	63.4 ± 11.0	135.0 ± 9.38	6.74 ± 0.59	1.4 ± 0.5
	V	80.0 ± 6.38	58.2 ± 8.01	142.6 ± 5.54	7.0 ± 0.85	1.5 ± 0.6
	Adult	139.0 ± 5.86	56.6 ± 4.67	153.6 ± 25.0	7.68 ± 0.58	1.6 ± 0.5
72	III	30.0 ± 4.53	37.3 ± 3.81	134.5 ± 4.62	5.6 ± 0.52	1.6 ± 0.5
	IV	43.6 ± 1.92	25.8 ± 6.28	141.2 ± 20.1	5.7 ± 0.45	1.6 ± 0.5
	V	75.5 ± 4.20	24.5 ± 5.65	168.7 ± 22.8	6.0 ± 0.49	1.7 ± 0.6
	Adult	135.8 ± 3.32	22.3 ± 6.86	172.4 ± 7.86	7.0 ± 0.50	1.7 ± 0.7
96	III	38.9 ± 3.60	14.3 ± 5.40	141.7 ± 16.0	6.1 ± 0.78	1.8 ± 0.4
	IV	45.7 ± 2.05	13.3 ± 8.02	169.1 ± 9.40	6.5 ± 0.60	2.5 ± 0.9
	V	71.1 ± 5.30	13.1 ± 2.55	179.1 ± 13.4	7.0 ± 0.86	2.7 ± 0.5
	Adult	126.4 ± 5.39	12.6 ± 1.07	182.6 ± 34.7	7.9 ± 0.62	2.8 ± 0.6

These two factors might be the reason for longer nymphal developmental periods. But the average total nymphal period was 46.71 days, when *R. marginatus* was reared on *S. litura* (Sahayaraj and Paulraj, 2001b). Our results also confirmed the observation of Sahayaraj and Paulraj (2001b).

Previously it was reported that the incubation period was minimum in *R. marginatus* fed with *Chrotogonus* sp. (7.01 days) and maximum in the group fed with *Odentotermus obesus* (7.35 days) (Ambrose *et al.*, 1990b). Insects reared in large containers have highest hatchability (Vennison and Ambrose, 1988). The results showed that even if we rear this reduviid in the uniform size containers, the hatchability varies (Table 4). In *R. marginatus*, the averaged net reproductive rate was 148.2 eggs/female (George, 1999) and 292.29 eggs/female (Sahayaraj and Paulraj, 2001b) when reared on *S. litura* and 133.4 eggs/batch (George, 2000), 227.77 eggs/batch (Sahayaraj, 2002) on *C. cephalonica*. George (2000) reported that niger morphs laid maximum number of eggs (142.20) followed by NS (141.50) and S (122.13 eggs). The fecundity rate maximum in the N+N morphs *R. marginatus* within various intra specific hybrid breeding experiment.

Relative Growth Rate

The adult female and male body size is often influence by the larval nutrition which in turn affected the fecundity of insect. Stewart *et al.* (1991) pointed out the diet relationship between female body size and number of ovarioles. This generalization is supported by the result of the present study, where in the fecundity and growth rate were higher particularly in N morphs, followed by NS of intra specific species on *R. marginatus*, reared on *C. cephalonica*. Of these three morphs NN was heavier as compared to SNS morph of *R. marginatus*. This is accordance with the results of Phoofolo and Obrycki (1997) which confirms the fact that female body size may be a good indicator of potential fecundity. Hence the relative growth rate inbetween the same species of various morphs were represent the RGR were increase at the different life stages or nymphs.

Polymorphism

The term “polymorphism” has been used in a general sense to indicate variation in body form or color within a species. It is evident, however, that while some instances of polymorphism are under strict genetic control, others are a product of the environment in which the insect develops. These instances are

now usually to as “polyphenism”. The polymorphic form explains same extraordinary and remarkable external visible distinguishable appearance within the same species. Such variations are formed on the wings, between the abdomen accompanied with colour like that red and black wings minute or deeply visible with also small lines are appear. Breeding experiments between morphs of a particular ecotype by Ambrose and Livingstone (1988) revealed that such intraspecific variations were not strictly genetic.

First type of morphs in *R. marginatus* usually, Niger morphs are black colour and medium to large insects. It has an elongated narrow head, the portion behind the eyes being neck like, the rostrum is three-segmented usually curved and its tip fits in a prosternal groove (Distant, 1903).

This is the second type of morphs in *R. marginatus*, Nigrosanguineous are usually black with red colour. In the abdominal region red lines inter connect with dominant the black colour. Some of the insects (N.S) are identify the colour of the nature of black or brownish with red colour. During the fresh moulting time NS are shiney in nature. The rostrum is three segmented usually curved and its tip fits in a prosternal groove (Distant, 1903). This is the third type of morphs in *R. marginatus*. Antennae, eyes, scutellum, inner area of corium, membrane, abdomen beneath and apical two-thirds of tibiae violaceous – black; base of antennae and lateral margins of abdomen sanguineous, disk of sternum, coxae, trochanters and anterior pobe of pronotum reddish – ochraceous; head as long as pronotum; basal joint of antennae almost equal in length to anterior femora, ante and postocular areas of head about equal in length, pronotum with the anterior lobe sculptured the posterior lobe rugulose; corium rugulose, the transverse cell near base of membrane margined with sanguineous, membrane passing abdominal apex (Distant, 1903).

Morph distribution

Mayr (1963) briefly defined polymorphism as variability with in a population. Ford (1937) explained polymorphism as the “occurrence together in the same habitat in the same

species in such a proportion that the rate of them can't be maintained by recurrent mutation. Breeding experiments between morphs of a particular ecotype by Ambrose and Livingstone (1988), Sahayaraj (1991) revealed that such intra specific variations were not strictly genetic. They further reported that the level of population of different morphs both in the field and in laboratory suggested that the segregation phenomenon did not occur in the Mendelian fashion. However, the adaptive significance of a particular morph in a given ecosystem cannot be valid out as evidenced by N with its higher insecticidal resistance (Ambrose, 1996). The present study established the variation in the development and reproduction of progeny obtained when crossing was made between the morphs such as N male + N female, NS male with NS female, S male with NS female, N male + S female and N male + NS female. Among the combinations tested, N male with N female alone produce their own progeny, but not in 1:1 ratio. Rather it produce 3.8 : 6.2 (male : female) ratio. Similarly, parents of N male with NS female produce 4.5 N: 5.5 NS ratio. Other combinations produce all type of morphs not in a Mendelian fashion. Further studies are essential to know the molecular and genetic mechanism behind the polymorphic form of this reduviid.

Male genitalia

The importance of male genitalia characters in tracing the polymorphism with in a species is an important study. In this research we displayed the microscopic details of *R. marginatus* (S) male genitalia characters. The usefulness of the male genitalia in systematics of Hemiptera was first demonstrated by Pruthi (1925). In this publication he commented briefly on the unevolved genitalia of few reduviid species. Sucheta (1973); Sucheta and Chopra (1988, 1989) enlisted and studied the male genitalia of Reduviidae from Haryana. We recommend detailed genitalia microscopic studies of all the three morphs including N, NS were essential to know the lock and key mechanism involved among polymorphs of *R. marginatus*.

Bioefficacy

After feeding the prey, the predator dropped the empty case of the prey and cleaned the antennae and rostrum using its forelegs in accordance with the observation of Livingstone and Ambrose (1978) and Ambrose (1980). Reported that the ability of predatory insects to choose between prey types has an important bearing on the outcome of a biocontrol programme suggesting the biocontrol utility of *R. marginatus*. Results showed that predatory rate increased when the prey grew older in contrast, Ambrose (1999) declared that the rate of predation decreased consistently as the predator grew older. The production of insects with maximum adult body weight was an important feature because maximum body weight was often correlated with higher fecundity for many insects species including predators like pentatomids (Evans, 1962; Phoofolo and Obrycki, 1997; Grundy *et al.*, 2000). NN has maximum body size (both in weight and length). Hence this morph is recommended can be utilized in IPM programme.

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