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Biology and predation potential of the hemipteran predator, *Rhynocoris marginatus* (Fab., 1794) on the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

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Abstract

Background: The notorious invasive pest fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), native to Americas entered the Asian continent and was first time reported on maize from India in 2018. The predatory species *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) has been reported as a potential predator that preys on a number of economic pests in India such as *Helicoverpa armigera* (Hubner), *Spodoptera litura* (Fabricius), *Mythimna separata* (Walker), and *Anomis flava* (Fabricius). The present study described the biology and feeding potential of the predator on *S. frugiperda* under laboratory conditions where temperature and relative humidity varied from 26 to 28 °C and 75–80%, respectively, and the photoperiod was L12: D12 hrs.

Results: Incubation period and the total nymphal/developmental period of the predator were 9.65 and 55.89 days, respectively. The percentage of survivorship of nymphs to adults was 62.50%. Females showed a higher longevity than males. The predator's fecundity reached 349.30 eggs/female, laid in 6 to 8 batches. Abdominal length and width were greater in the case of females than in males. Male consumed about 158.55 larvae of the fifth instar prey, at an average of 1.86 larvae per day. Females consumed a greater number of larvae (184.95) than males, with a higher daily predation rate of 1.93 larvae. Time taken to approach the prey was longer in the case of males (5.58 min) than females (3.57 min), whereas handling time was longer in females (115.37 min) than in males (85.71 min). Weight gained by females (61.90 mg) was higher than that by males (44.20 mg) after 24 h of predation.

Conclusions: The present study revealed that female adult predator and fifth-instar nymphs of *R. marginatus* preyed efficiently on *S. frugiperda*. The shorter ovipositional period of the predator was a positive sign for its mass multiplication, enabling rapid buildup of the predator population. This study is a step in the evaluation of the efficacy of *R. marginatus* feeding on fall armyworm. Further field studies are required on its mass release and effect in reducing the fall armyworm larval population.

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Keywords: *Rhynocoris marginatus*, *Spodoptera frugiperda*, Predation potential, Biological parameters, Handling time, Approach time

Background

The notorious invasive pest fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), native to Americas entered the Asian continent and it was first time reported from the Indian subcontinent on maize crop in May 2018 (Sharanabasappa et al. 2018a). Within a span of 3 years, it was reported in various countries across the Asia–Pacific (Prasanna et al. 2021) attacking several crop plants. The major crops that FAW prefers to feed on are maize, rice, sorghum, millet, sugarcane, vegetable crops, and cotton; however, it able to attack 80 additional species of crops (Assefa 2018). In general, insecticides play an important role in managing pest populations in order to keep them below economic injury levels. However, more frequent and intensive use of chemical insecticides is the main cause for the development of resistance and resurgence in insect pest populations and also a menace to human health and the environment. To avoid the high costs of chemical insecticides and the economic losses stemming from FAW, there is a need for the development of eco-friendly and sustainable approaches in pest management, which includes the use of parasitoids, predators, and biopesticides.

Identification of potential natural enemies is very crucial in biological control programs, as a component in integrated pest management. Among the predators, reduviids feed on a wide range of preys belonging to various taxa (Sahayaraj and Balasubramanian 2016). *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) is one among the prevalent, potential reduviid predators that prey on economic pests in India (Sahayaraj and Muthukumar 2011). The reduviid predator, *R. marginatus*, has been reared successfully in the laboratory and deployed against many lepidopteran pests of crops (Sahayaraj and Martin 2003). The present study was designed to evaluate some of the morphological and biological parameters and feeding potential of the predator, *R. marginatus*, on FAW under laboratory conditions.

Methods

Insect cultures

Gravid female *R. marginatus* adults were obtained from the laboratory-maintained culture at the National Institute of Plant Health Management (NIPHM), Hyderabad, India, and allowed to lay eggs in insect-breeding dishes, with blotting paper as the substratum. Different instars of FAW larvae were reared in insect-rearing dishes (circular-Himedia, TCP030-90 × 40 mm dia.) at the

Department of Entomology, College of Agriculture, Shivamogga, India, as per the procedure of Sharanabasappa et al. (2018b).

R. marginatus

Life history and predatory potential

Larvae of FAW were reared in insect-breeding dishes (circular-Himedia, TCP030-90 × 40 mm dia.) by providing them with bits of maize leaf daily and maintaining them at 26 ± 2 °C, 75 to 80 RH, and L12: D12 photoperiod as an initial host culture. The larvae were reared following the procedure in Sharanabasappa et al. (2018b). Newly hatched larvae were reared in rearing dishes, and the procedure was repeated continuously to obtain the required prey culture to conduct the experiments.

The gravid female *R. marginatus* adults were obtained from the stock culture and allowed to lay eggs in the rearing dishes (circular-Himedia, TCP030-90 × 40 mm dia.), with blotting paper as the substratum. These eggs of the predator were collected, and hatched nymphs were reared individually on second- and third-instar larvae of *S. frugiperda* to maintain reduviid culture for the study. Ten pairs of male and female predators were collected from the stock culture and kept in 10 separate insect-rearing dishes. Blotting paper was placed as the substratum for egg laying and moistened with moist cotton to maintain humidity. A total of 10 freshly laid egg masses were collected with the help of a fine camel-hair brush and kept individually in Petri plates (7 cm dia.). The time taken for the eggs to hatch (Egg, F₀) and the number of eggs that hatched in each egg mass (egg hatchability %) were recorded.

Longevity and fecundity

The study was conducted under laboratory conditions—where temperature and relative humidity varied from 26 to 28 °C and 75–80%, respectively, and the photoperiod was L12: D12 hrs. Using a fine camel-hair brush, 40 newly hatched nymphs were collected and placed individually in glass Petri plates. The nymphs were provided with *S. frugiperda* larvae daily and reared up to the adult stage. The Petri plates were cleaned daily to remove the excreta of the reduviid nymphs and the fall armyworm larvae and checked twice a day, in the morning and evening to know its feeding potential. Observations on the number of nymphal instars and the duration of each nymphal instar were recorded. Nymphal instars were determined by observing the exuviate, and the time interval between

2 consecutive moults was considered as instar duration. Newly emerged adults were separated on the basis of sex, paired ($N=10$), and kept in insect-rearing dishes. Fall armyworm larvae were provided as prey to the reduviid adults, while maize leaf bits were given as prey to the larvae. Data on mating duration, ovipositional periods, fecundity, egg hatchability (%) (F_1), and adult longevity were recorded.

Larval instars preference and predation potential of the predator

Larval instar preference study was conducted on all the life stages of *R. marginatus* with different larval instars of *S. frugiperda* as a choice experiment. *R. marginatus* nymphs and adults were introduced into a Petri dish that had all the larval instars of fall armyworm, and predatory behavior was checked consecutively for 6 h. The captured, killed, and consumed prey instars were recorded as the preferred stage of the reduviid. Ten replications with 7 treatments were maintained for each life stage of the predator separately in insect-rearing dishes. The different larval instars of fall armyworm were provided as preys to the different nymphal and adult stages of the predator. To study the feeding potential of the bug, 30 newly hatched nymphs were kept and reared separately in Petri plates up to the adult stage by providing them with the preferred stage of the fall armyworm. The total number of larvae preyed by nymphs and adults of *R. marginatus* was recorded.

Searching time, Handling time, and weight gain by the predator

To study the approach time (AT), handling time (HT), and weight gain (WG) by the adult predators, preweighed 4-day-old male and female predators were collected from the laboratory-maintained culture and kept individually in Petri plates (7 cm dia.). The predators were starved for a period of 24 h. Once the starvation period was over, the fifth instar of FAW larvae (312.4 ± 8.65 mg) was released into the Petri plates containing the predator. Subsequently, the time taken by the predator to get aroused, approach, and capture the prey was recorded as AT and that for paralyzing and feeding on the prey was recorded as HT. Weight of the predators was measured after 24 h, and the difference in the weight of the predator before and after feeding was recorded as WG. WG was corrected by the predator weight loss due to desiccation, as determined by weighing 5 predators kept individually in plastic containers without prey for 24 h. Ten replications were maintained for each predator (Sahayaraj et al. 2016).

Data analysis

Mean \pm S.D. values were calculated for all the biological life-cycle parameters. A one-way ANOVA along with Tukey's HSD was performed to find out whether there was any significant difference between the feeding potential and per day consumption of the predator. An independent-samples *t* test was performed to determine the effect of gender on AT, HT, and WG of the predator. SPSS statistics 16.0 software was used for all the analyses.

Results

Biology of *R. marginatus*

The female predator laid yellowish, elongated eggs in clusters that were glued to each other and to the substratum. The anterior end of the egg had a white-colored operculum, which could break operculum at the time of hatching. The color of the egg turned dark red 2–3 days prior to hatching. The incubation period was 9.65 ± 0.48 days under the laboratory conditions. After hatching, all the nymphs surrounded the egg mass without any movement for a period of 6–8 h. The predator had five nymphal instars, and durations of the first, second, third, fourth, and fifth nymphal instars were 8.60 ± 0.50 , 9.45 ± 0.51 , 10.95 ± 0.51 , 11.45 ± 0.69 , and 15.40 ± 0.82 days, respectively. The total nymphal/developmental period of the predator was 55.89 ± 1.60 days. Survivorship of the nymphs to adult was recorded as 62.50%. Males lived 85.65 ± 6.98 days, while the longevity of females was 95.9 ± 7.19 days. The female laid an average of 349.30 ± 27.06 eggs in its life span, ranging from 315 to 414 eggs per female. The average number of egg mass laid by the female was 6.90 ± 0.74 , with a range of 6–8 batches/female. The mean number of eggs in a single egg batch was recorded as 52.50 ± 13.39 , with a range of 6–8 egg batches. The pre-ovipositional and ovipositional periods of the female predator were 14.7 ± 0.86 and 59.60 ± 4.21 days, respectively. The sex ratio (male/female) was female biased at 0.94:1.0. The eggs hatched after 9–10 days of egg lying, and the mean hatchability of the eggs reached 94.94 ± 4.56 percent (Table 1).

Predator preference for different stages of prey

It is important to determine a natural enemy's potential to capture the target pest and to feed on it during the preferred stages of the prey before it is used in a biocontrol program. The stage preference studies revealed that different nymphal instars preferred different instars of FAW larvae. The first- and second-instar nymphs of the reduviid bug preferred 70–80% of the second instar of FAW. The third- and fourth-instar nymphs preferred the third instar of FAW, with a preference percentage of 50 and 70, respectively. The fifth-instar nymph of the reduviid bug

Table 1 Biological parameters of reduviid bug, *Rhynocoris marginatus* on fall armyworm, *Spodoptera frugiperda*

| Sl. no | Biological parameters | Mean \pm SD | Range (days) |
|--------|--|--------------------|--------------|
| 1 | Egg (F ₀) | 9.65 \pm 0.48 | 9–10 |
| 2 | I instar | 8.60 \pm 0.50 | 8–9 |
| 3 | II instar | 9.45 \pm 0.51 | 9–10 |
| 4 | III instar | 10.95 \pm 0.51 | 10–12 |
| 5 | IV instar | 11.45 \pm 0.69 | 11–12 |
| 6 | V instar | 15.40 \pm 0.82 | 14–17 |
| 7 | Total nymphal period | 55.89 \pm 1.60 | 53–59 |
| 8 | Adult pre-oviposition period (APOP)* | 14.7 \pm 0.86 | 13–16 |
| | Total pre-oviposition period (TPOP) | 80.20 \pm 1.76 | 76–83 |
| 9 | Oviposition period* | 59.60 \pm 4.21 | 53–66 |
| 10 | Fecundity of the female* | 349.30 \pm 27.06 | 315–414 |
| 11 | Number of egg batches per female* | 6.90 \pm 0.74 | 6–8 |
| 12 | Number of eggs in a single batch | 52.50 \pm 13.39 | 23–79 |
| 13 | Egg hatchability (%) (F ₁) | 94.94 \pm 4.56 | 82.3–100 (%) |
| 14 | Adult longevity | | |
| | Male | 85.65 \pm 6.98 | 73–95 |
| | Female | 95.9 \pm 7.19 | 82–108 |
| 15 | Total life cycle (egg to adult) | | |
| | Male | 151.15 \pm 7.42 | 136–162 |
| | Female | 161.4 \pm 7.78 | 148–175 |
| 16 | Survivability to adult | – | 62.50 (%) |

Mean of 20 observations; *mean of 10 observations

showed a preference primarily (70%) for the fourth instar of FAW, while the male and female predators preferred most to feed on the fifth instar of FAW, with a preference

percentage of 60 and 70, respectively (Table 2). None of the nymphal instars of the predator preferred the sixth-instar larvae of FAW due to its large size and its ability to counterattack the predator.

Feeding potential of the predator

Among different stages of the predator, the adult female showed a significantly higher feeding potential (184.95 \pm 14.69 larvae), followed by the male (158.55 \pm 9.75 larvae). However, among the nymphal instars, the fifth nymphal instar (31.00 \pm 2.79 larvae) showed the greatest feeding potential (19.9 \pm 2.34 larvae), followed by the fourth nymphal instar. The least was noticed among the first nymphal instar (11.94 \pm 1.55 larvae) (Table 2). In case of daily consumption, among all the stages, the fifth nymphal instar of the predator had the highest consumption rate followed by the adult female and then the adult male predators. The least consumption per day was noticed in case of the first nymphal instar (Table 2).

AT, HT, and WG by predator

The male and female predators showed a significant difference in their AT, HT, and WG after 24 h of predation (Table 3). The females of *R. marginatus* lasted lesser time to approach the prey (*S. frugiperda*) (mean \pm SD = 3.57 \pm 0.64 min) than the males (5.58 \pm 0.49 min). Levene's test of variance equality showed that that variance equality can be assumed (Levene 1960). A test for independent samples showed that this difference was statistically significant (*t* test: *t* = 6.41, *P* = 0.00012, *N* = 10) (Table 3).

For the male *R. marginatus*, HT of the prey was shorter (mean \pm SD = 85.71 \pm 4.78 min) than that of

Table 2 Instar preference and feeding potential of the predator, *Rhynocoris marginatus* on fall armyworm

| Instar of the predator | Preferred instar of the <i>Spodoptera frugiperda</i> (%) | Feeding potential (total consumed) | Per day consumption |
|------------------------|--|------------------------------------|--------------------------------|
| I instar | II (69.70)* | 11.94 \pm 1.55 ^e | 1.38 \pm 0.15 ^e |
| II instar | II (79.30) | 16.65 \pm 1.30 ^{de} | 1.76 \pm 0.14 ^{bcd} |
| III instar | III (49.80) | 18.47 \pm 2.44 ^{de} | 1.69 \pm 0.63 ^d |
| IV instar | III (69.80) | 19.9 \pm 2.34 ^d | 1.74 \pm 0.23 ^{cd} |
| V instar | IV (69.70) | 31.00 \pm 2.79 ^c | 2.02 \pm 0.20 ^a |
| Male | V (59.50) | 158.55 \pm 9.75 ^b | 1.86 \pm 0.10 ^{abc} |
| Female | V (69.10) | 184.95 \pm 14.69 ^a | 1.93 \pm 0.11 ^{ab} |
| Df (between) | | 6 | 6 |
| Df (within) | | 54 | 54 |
| F value | | 4.13 | 3.92 |
| P value | | < 0.001 | < 0.001 |

Means \pm SD in the same column following same letter do not significantly differ by Tukey's HSD (*P* < 0.05)

*Percent preferred instar of the *Spodoptera frugiperda*

Table 3 Searching time, handling time, and weight gain by the male and female predator against fifth-instar fall armyworm

| Stage of the predator | Approaching time (min) | Handling time (min) | Weight gain by the predator (mg) |
|-----------------------|------------------------|---------------------|----------------------------------|
| Male | 5.58 ± 0.49 | 85.71 ± 4.78 | 44.20 ± 2.86 |
| Female | 3.57 ± 0.64 | 115.37 ± 8.98 | 61.90 ± 4.62 |
| t' value | 6.412 | -9.175 | -8.523 |
| Df | 9 | 9 | 9 |
| P value | < 0.001* | < 0.001* | < 0.001* |

*Significantly different

the female (115.37 ± 8.98 min). Levene's test of variance equality showed that the variance equality can be assumed. A test for independent samples showed that this difference was statistically significant (t test: $t=9.17$, $P=0.000007$, $N=10$) (Table 3). On feeding, the individual female predator's weight gain was greater (mean ± SD = 61.90 ± 4.62 mg) than that of the male (44.20 ± 2.85 mg), when feed on fifth-instar prey. Mean weight gain differed significantly between the males and females of *R. marginatus* (t test: $t=8.52$, $P=0.000013$, $N=10$) (Table 3).

Discussion

In the predator–prey interaction, a predator's fitness and feeding potential are crucial, and many factors can influence this relationship, such as prey size and density (Kalinowski and DeLong 2016) and climatic conditions (Tazerouni et al. 2016), and ecosystem complexity (Barbosa et al. 2019). Among all of these parameters, prey quality is one of the most critical (Aragón Sánchez et al. 2018) and significantly contributes to the biology, physiology, longevity, fecundity, and the survival of the predators (Barbosa et al. 2019).

In the present study, *R. marginatus* seemed to be a promising candidate as a biocontrol agent against *S. frugiperda*. The reduviid predator *R. marginatus* is polyphagous and feeds on about 20 major insect pests, especially lepidopteran larvae (Ambrose et al. 2009). Studies on the growth, development and fecundity rate of the predator may help in determining its effectiveness among various insect pests or prey (De Clercq et al. 2005). There was a steady increase in the number of larvae killed by the predator with progression of the nymphal instars (George and Sreenivasagan 1998). Similarly, Ambrose and Claver (1999) reported that *R. marginatus* reduced 57.67% of larval population of *Spo-doptera litura* and 32.4% of boll damage by *S. litura* in cotton field cages. The AT toward the prey was lower in the case of the female adult than the male. However,

the AT for the predatory bug was 2.88 ± 1.03 and 2.54 ± 0.79 min for male and female, respectively, when the host was *S. litura* (Sahayaraj et al. 2016). This variation in time may be due to the species difference. Pravalika et al. (2016) studied the biology and prey preference of *R. marginatus* on the cutworm, *Mythimna separata* in the laboratory. The total developmental period of the predator was 72.9 ± 1.56 days. The number of prey required for its development from egg to adult was 21.2 ± 3.5 3third-instar larvae. The fourth-instar larvae were significantly preferred by *R. marginatus* (33.9%), followed by the fifth (24.9%) and third (22.0%) instars. Arshad et al. (2021) reported that the age stage-specific survival rate and the age stage-specific fecundity of *R. marginatus* were higher when feeding on *S. litura* than with feeding on *Pieris brassicae* larvae. *S. litura* was the more suitable prey for *R. marginatus* compared with *P. brassicae*. The developmental period of all life stages of *R. marginatus* was shorter when *S. litura* larvae were provided. Besmer et al. (2021) reported that the reduviid predator *Rhynocoris* sp. found to be predominantly feeding on fall armyworm larvae and its relative abundance was 4.9% recorded in maize fields from Burkina Faso. All biological characteristics, however, can be impacted by a variety of biotic and abiotic factors, such as the host plant, prey species, and ambient conditions. (Arshad et al., 2020). The high-quality food decreased the length of pre-ovipositional period (Omkar and Srivastava 2003) and consumption of high nutritive food help early ovariole maturation and longer ovipositional period (Honek 1980).

Efforts toward the augmentative release of *R. marginatus* against FAW need to be evaluated under field conditions; large-scale release and subsequent monitoring of both predator and pest are required.

Conclusions

This is the first study on *R. marginatus* predator biology and feeding potential on the newly invasive pest FAW in India. *The predator* showed an efficient prey consumption against *S. frugiperda*, suggesting its utility in integrated FAW management programs. Studies on the potential of the predator under natural conditions and on its compatibility with other biocontrol agents are still required.

Abbreviations

FAW: Fall armyworm; NIPHM: National Institute of Plant Health Management; AT: Approach time; HT: Handling time; WG: Weight gain; Dia.: Diameter; Min.: Minutes; mm: Millimetre.

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Author contributions

PP performed the experiments, data curation, data analysis, and original draft writing; SSD and CMK designed the study and revised the final draft. SJR provided the reduviid bug culture. All authors read and approved the final manuscript.

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Competing interests

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