International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(10): 116-120
www.biochemjournal.com
Received: 02-08-2025
Accepted: 04-09-2025

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Bio efficacy of selected biopesticides on the predatory reduviid bug, *Rhynocoris marginatus* (Hemiptera: Reduviidae) under tobacco pot culture conditions

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10b.5908

Abstract
A pot culture experiment was conducted at the Biological Control Research Laboratory, ICAR Unit-9, AAU, Anand to ascertain the efficacy of different biopesticides against the assassin bug, *R. marginatus.* Ten days after spraying and release of second instar assassin bugs, a significantly highest mortality (83.64%) was recorded in *B*eauveria *bassiana* treatment, followed by *Metarhizium anisopliae,* with a mortality of 70.00 percent. The treatments *Bacillus thuringiensis* and *Lecanicillium lecanii* were found to be moderately toxic to the reduviid nymphs with mortality rates of 53.35 and 50.00 percent, respectively. Among the biopesticides tested, least cumulative mortality of 26.52 percent was recorded in treatments of Azadirachtin 1500 ppm and Azadirachtin 10000 ppm. No nymphal mortality was recorded in the untreated control.

Keywords: *Rhynocoris marginatus*, assassin bug, biopesticides

Introduction
Mounting concerns over the detrimental effects of synthetic pesticides on human health and the environment have driven a global shift toward sustainable pest management strategies, such as cultural practices, Integrated Pest Management, biological control, insect growth regulators (IGRs), and botanicals. Advancements in science and technology have further accelerated the adoption of biological control involving natural enemies as effective and ecologically sound alternatives in modern agriculture (Nchu, 2024) [12].

Among these natural enemies, *Reduviidae* represents the largest family of predaceous terrestrial heteropterans, with the subfamily *Harpactorinae* comprising several important biocontrol genera, including *Rhynocoris*, *Zelus*, *Pselliopus*, *Sinea*, and *Apiomerus*. (Ambrose, 2006; Wikipedia, 2021) [2, 3]. The morphological and physiological adaptations such as limited prey range, positive functional and numerical response, efficient host searching, high fecundity, short life cycle, female-biased population, amenability to mass rearing and adjustability to ecological factors and the secondary effects of phytosanitary sprays, make heteropterans, especially reduviids, important predators in agroecosystems. (Ambrose and Kumar, 2016; Sahayaraj, 2014) [1, 17].

Reduviid predators have been recorded from many agroecosystems such as sugarbeet, sesbania, brassica, soybean, cotton, tobacco, coconut, cowpea, groundnut and orchard *etc.* (Sahayaraj, 2014) [17]. The reduviid bug *Rhynocoris marginatus* has been identified as a potential predator of nearly 25 insect pests in India, including key economic pests such as *Helicoverpa armigera*, *Spodoptera litura*, *Mythimna separata*, and *Anomis flava* (Pradeep *et al.*, 2022; Shahayaraj and Sathiamoorthi, 2002) [13, 16].

Various entomopathogenic fungi (EPFs) such as *Metarhizium anisopliae*, *Beauveria bassiana*, *Nomuraea rileyi*, and *Verticillium lecanii* have also proven to be effective in controlling insect pests (Lingappa *et al.,* 2005) [10]. EPF'S exhibit a broad host range and predominantly infect insect orders including Coleoptera, Diptera, Hemiptera, Lepidoptera, Orthoptera, and Hymenoptera. Among these, *B. bassiana* and *M. anisopliae* are widely utilized due to their broad host ranges and high virulence, infecting more than 700 insect species (Zhang *et al.,* 2025) [21].

Since reduviid is an important component of BIPM, it is necessary to evaluate the impact of these botanicals and microbial insecticides on mortality and predatory potential of *R. marginatus* (Jasmine *et al*., 2012) [9]. Earlier studies have shown that reduviid predators, exhibits greater resistance to chemical sprays than coccinellids (Sahayaraj, 2014) [17]. Moreover, the application of biopesticides does not reduce the biological control efficacy of *R. marginatus* bugs (Sahayaraj *et al*., 2003) [14]. Although the adverse effects of biopesticides on several natural enemies such as *Polistes dominula* (Cappa *et al.,* 2024) [5], *Ceraeochrysa claveri* (Gastelbondo *et al.,* 2025) [6], predatory mites (Busuulwa *et al.,* 2024) [4], *Chrysoperla carnea* (Neuroptera: Chrysopidae), and *Dicyphus tamaninii* (Hemiptera: Miridae) (Thungrabeab and Tongma, 2007) [20] have been documented, their impact on predatory assassin bugs remains underexplored. Therefore, the present study was carried out to investigate the impact of selected biopesticides on the predatory assassin bug under tobacco pot culture conditions at the Biological Control Research Laboratory, ICAR Unit-9, AAU, Anand.

Materials and Methods

Rearing of rice moth, *Corcyra cephalonica*

The rice moth was reared in wooden cages (38.5 × 38.5 × 15 cm) containing sterilized sorghum (2 kg) fortified with, groundnut seeds (100 g), powdered yeast (5 g), and vitamin powder (10 g) under ambient temperature and humidity conditions.

Rearing of assassin bug, *Rhynocoris marginatus*

The eggs of *R. marginatus* were taken from the culture maintained at the Biological Control Research Laboratory, ICAR Unit-9, AAU, Anand. They were placed Petri dish (90 mm) and were maintained at 27 ± 2°C and 65 ± 5%, temperature and relative humidity respectively. The different instars of the assassin bug were accurately distinguished by examining the shed exuviae and distinct morphological features. The second instar nymphs were randomly selected from the stock culture and utilized for the study. The second instar nymphs were larger in size compared to the first instars, with a more prominently bulged anterior pronotum. The nymphs were fed daily with immobilised head crushed *Corcyra* larvae.

Source of biopesticides

The biopesticides *Beauveria bassiana* 1.15%WP, *Metarhizium anisopliae* 1.15%WP, *Lecanicillium lecanii* 1.15%WP, *Bacillus thuringiensis* 1% WP, Azadirachtin 1500 ppm and Azadirachtin 10000 ppm were procured from the market.

Effect of biopesticides on assassin bug in tobacco under pot culture experiment

For pot culture study, tobacco plants (variety: A119) were raised individually in pots inside rectangular net cages (65 x 65 x 65 cm) during *rabi* 2024-25. The pots were filled with a potting mixture prepared by mixing soil and compost (1:1). After attaining good vegetative growth, six treatments of biopesticides at recommended doses as mentioned in Table 1, were uniformly sprayed with a hand sprayer on tobacco leaves and allowed to dry (Fig 1A). One untreated control was also maintained as check. For each treatment, fifteen tobacco plants in 3 replications were subjected to the release of approximately 10 second instar reduviid nymph per replication and cumulative nymphal mortality was recorded.

Throughout the experiment, aphids were supplied as food for the reduviid nymphs. The prey and predator were released on caged tobacco plants to prevent their escape (Fig 1B). The observations on assassin bug were recorded at 24-hour intervals up to 10 days (Ghelani, 1998) [7] and converted into percentages using the following formula, then analysed using suitable statistical methods (Steel and Torrie, 1980) [18].

$$\text{Mortality (\%)} = \frac{\text{No.of nymphs died}}{\text{Total no.of nymphs}} \times 100$$

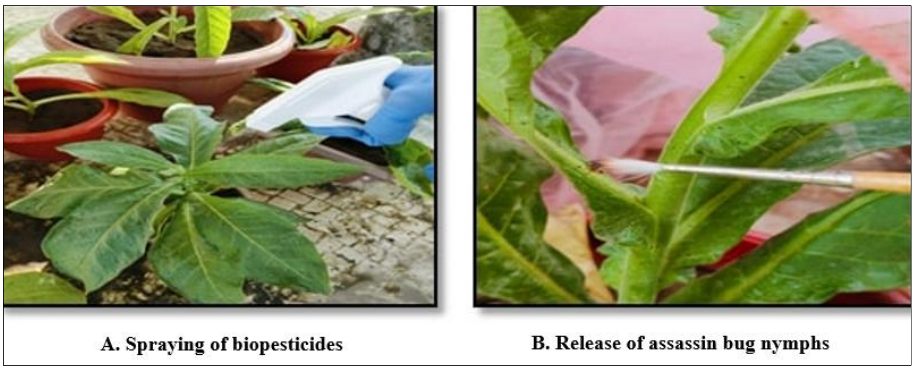


Fig 1: Release of second instar nymphs of assassin bug, *R. marginatus* after foliar spray to assess the efficacy of different biopesticides

Statistical analysis

Percent mortality data were subjected to angular transformation prior to statistical analysis using Duncan's Multiple Range Test (DNMRT) to determine significant differences among treatment means.

Results & discussions

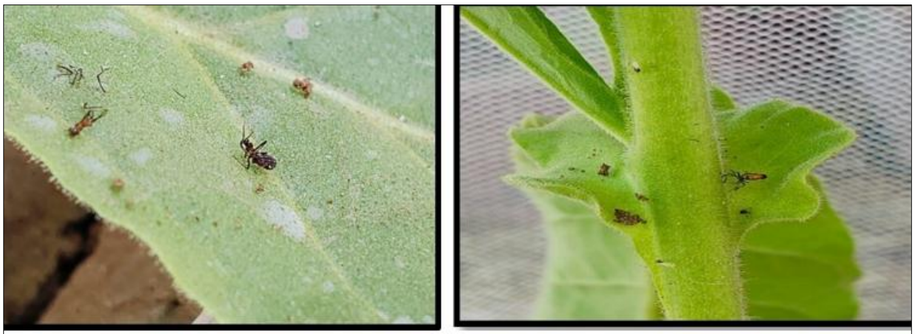
The effect of different biopesticides on the mortality of second instar nymphs of reduviid bug from a day after spraying till 10-day are represented in Table 2. The data represented in Table 2 indicated that all the biopesticides caused significantly higher mortality in reduviid nymphs, while no mortality was observed in the control group throughout the 10-day of observation period.

One day after spraying (1 DAS)

A day after the application of biopesticides, the treatment with *B. bassiana* showed the highest nymphal mortality of 40.00 percent, which was statistically significant to other treatments. Moderate mortality was observed with *M.*

anisopliae (23.18%) which was on par with the treatment *L. lecanii* (20.00%). The lowest mortality of 10.00 percent was observed in Azadirachtin 1500 ppm and was statistically comparable to the treatments, Azadirachtin 10000 ppm and *B. thuringiensis* with a mortality of 10.00 and 13.01 percent, respectively.

Two days after spraying (2 DAS): On the second day after treatment with biopesticides, *B. bassiana* was found to be significantly superior to other treatments, causing 50.00 percent mortality (Fig 2A). This was followed by *M. anisopliae*, *B. thuringiensis, L. lecanii* which caused mortalities of 30.00, 23.18 and 20.00 percent, respectively and were statistically at par with each other (Fig 2B). The lowest mortality was observed in the treatment with Azadirachtin 1500 ppm (10.00%), which was statistically at par with Azadirachtin 10,000 ppm (13.01%).



A. *Beauveria bassiana* 1.15%WP B. *Bacillus thuringiensis* 1% WP

Fig 2: Mortality of second instar nymphs of assassin bug, *R. marginatus* after exposure to different biopesticides

Three days after spraying (3 DAS)

Three days after the biopesticide application, *B. bassiana* continued to show the highest mortality (70.00%) and remained statistically superior to the other treatments. Whereas, the lowest mortality was observed in the treatments Azadirachtin 1500 ppm and Azadirachtin 10000 ppm with both having a mortality of 13.01 percent. The other treatments *M. anisopliae*, *B. thuringiensis, L. lecanii* recorded mortalities of 40.00, 40.00 and 30.00 percent, respectively and among these *M. anisopliae* and *B. thuringiensis* were statistically at par with each other, whereas *L. lecanii was* found to be significantly different from other treatments.

Four days after spraying (4 DAS)

After four days following treatment, the treatment of *B. bassiana* was observed with noticeably highest mortality (80.00%) and statistically significant compared to all other treatments. This was succeeded by *M. anisopliae* and *B. thuringiensis* which resulted in mortalities of 50.00 and 46.65 percent, respectively and were found to be statistically equivalent with each other. The treatment with *L. lecanii* ranked next with 33.26 percent mortality and was statistically different from other treatments. The treatments of Azadirachtin 1500 ppm and Azadirachtin 10000 ppm continued to be the least toxic biopesticide to reduviid nymphs with both showing a mortality of 16.36 percent.

Five days after spraying (5 DAS)

On the fifth day post-treatment, *B. bassiana* recorded a mortality rate of 83.64 percent and remained significantly more toxic than all other treatments. The treatment using *M. anisopliae* comes next with 60.00 percent mortality and was statistically distinct from the other treatments. This was followed by *B. thuringiensis* (46.65%), which was statistically at par with *L. lecanii* (36.60%). The treatments Azadirachtin 1500 ppm and Azadirachtin 10000 ppm were found to be least toxic to reduviid nymphs, each having a mortality rate of 23.18 percent.

Six days after spraying (6 DAS)

Six days after the treatment, *B. bassiana* remained the most toxic biopesticide to reduviid nymphs, with 83.64 percent mortality, which was statistically distinct from other treatments according to the data obtained. The next most toxic treatment was *M. anisopliae* (63.40%), which was statistically equivalent to *B. thuringiensis* (53.35%). The application of *L. lecanii* ranked next with a mortality rate of 40.00 percent and was statistically different from the other treatments. The biopesticides, Azadirachtin 1500 ppm and Azadirachtin 10000 ppm continued to remain the least toxic treatments, with each recording a mortality rate of 26.52 percent, and were statistically at par with each other.

Seven to ten days after spraying (7-10 DAS)

From the seventh day onwards until the tenth day, similar observations were recorded in mortality rates. The highest toxicity was exhibited by *B. bassiana* (83.64%) and was statistically superior to all other treatments. This was succeeded by the treatment *M. anisopliae* (70.00%), and was statistically different from other treatments. The treatment *B. thuringiensis* (53.35%) ranked next and was statistically equivalent with *L. lecanii* (50.00%). Azadirachtin 1500 ppm and Azadirachtin 10000 ppm continued to be the least toxic biopesticide to reduviid nymphs with both showing a mortality of 26.52 percent.

Observation recorded on 10 days after spraying in pot culture experiment revealed that highest nymphal mortality was recorded for the treatment *B. bassiana* which was followed by *M. anisopliae.* The treatments *B. thuringiensis* and *L. lecanii* was found to be moderately toxic to the reduviid nymphs. Among the biopesticides used, least cumulative mortality was recorded in case of Azadirachtin 1500 ppm and 10000 ppm. However, no mortality was observed in the control group throughout the 10-day observation period. The initial 7-day period post-treatment was characterized by elevated mortality levels.

These findings demonstrate that biopesticides, though often considered safe, also negatively impact the natural enemies of target pests, such as *R. marginatus* bugs. The present investigation revealed the toxicity of various biopesticides and the relative safety of Azadirachtin 1500 ppm and 10000 ppm on the survival of second instar *R. marginatus* bugs.

Present findings are in agreement with the work of Sahayaraj *et al.* (2003) [14], where they reported that, after 96 hours of exposure vijayneem was found to be safest to *R. marginatus* nymphs, followed by nimbicidine, with mortality rates ranged from 3.32 to 24.10 in topical toxicity and 1.43 to 13.36 in contact toxicity for both treatments. Further supporting this, Sahayaraj and Raja (2003) [14] reported that tested biopesticides *viz.,* Nivaar, Mealisac, Osaqua, Citropyne and Azadirachtin caused minimal nymphal mortality and did not affect the predatory potential of first instar *R. marginatus* nymphs. Similarly, Suganthi (2004) [19] found that Neem oil was safe against *R. fuscipes* causing least impact on its biocontrol potential.

In addition to this there are reports on the toxic effect of *B. bassiana*, *M. anisopliae on* reduviid bugs such as twenty-three isolates of *B. bassiana* and 13 isolates of *M. anisopliae* tested at nearly saturated relative humidity (RH) and 25°C caused high mortality (90-100%) in third-instar *T. infestans* nymphs (Luz *et al.* 1998). Similarly, Gutiérrez *et al.* (2003) reported *B. bassiana* (Bb UdeA14) caused a mortality of 77.5 percent in fifth instar *Rhodnius pallescens* and *B. brongniartii* (*Bt* UdeA16) induced a mortality of 100 percent at 22 days. Also, they reported a lowest LT_{50} value of 7 days was reported in *B. brongniartii* strain Bb UdeA16.

The caged pot culture bioassay studies revealed the toxicity of *B. bassiana* and *M. anisopliae,* against the biocontrol agent *R. marginatus* and the safety of neem based biopesticides, Azadirachtin 1500 ppm and Azadirachtin 10000 ppm on the same. A thorough investigation on the effect of different biopesticides on *R. marginatus* at open field condition is still pending and should be conducted in future to validate the above results.

Conclusion

Biopesticides were regarded as effective and eco-friendly pest control agents, with high target specificity and minimal non-target impact, making them ideal for integrated pest management programs. The above research findings showed the detrimental effect of various biopesticides and the relative safety of Azadirachtin 1500 ppm and 10000 ppm on the survival of *R. marginatus* nymphs The results suggests that biopesticide application should be strategically timed, as immediate spraying after the release of *R. marginatus* nymphs may lead to substantial mortality of the immature predator stages. This appears to be the first report indicating a mild adverse effect of most of the tested biopesticides on the survival of the economically significant insect predator *R. marginatus*.

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