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PJ Edward George
Department of Horticulture,
Kalasalingam School of
Agriculture and Horticulture,
Kalasalingam Academy of
Research and Education,
Krishnankoil, Virudunagar,
Tamil Nadu, India

Laboratory study of insecticidal impact on the sensitivity of different stages in the assassin bug *Rhynocoris longifrons* (Stal): (Hemiptera: Reduviidae)

PJ Edward George

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Abstract

Stage specific sensitivity was studied for five commonly used insecticides in the agroecosystem namely quinalphos, chlorpyrifos, dimethoate (Organophosphorous insecticides) and deltamethrin and bifenthrin (Pyrethroid insecticides) on the assassin bug *Rhynocoris longifrons* (Stal). All the three organophosphorous insecticides caused 100% mortality in all the durations of treatment (24, 48, 72 and 96hrs) in the first, second and third instar nymphs of *R. longifrons*. In the fourth, fifth instar nymphs and adult insects only quinalphos and chlorpyrifos caused 100% mortality in *R. longifrons*. The pyrethroid insecticides did not cause 100% mortality in all the durations in all the nymphal instars and adults. The results will enable strategies for compiling the biological control and chemical control. The laboratory studies revealed that organophosphate was considered the most toxic for all the nymphal instars and adults of *R. longifrons* whereas synthetic pyrethroids were considered relatively safer insecticides for all the other nymphal instars and adults of *R. longifrons*. This study demonstrates how the indiscriminate use of insecticides as a pest management strategy can directly disrupt the benefits of natural enemies.

Keywords: Insecticides, life stages, sensitivity, predator, *R. longifrons*

Introduction

Integrated Pest Management (IPM) is a method of pest control that uses a combination of techniques to minimize the impact of pests on the environment, human health, and the economy. IPM does not necessarily require the complete replacement of insecticides, but rather affects the need to reduce their dependence, by eliminating unnecessary applications. Although these toxins are targeted at plant pests, many of them are broad spectrum biocides that have profound effects on non - target species in agro ecosystems George and Ambrose, 1999a, b) [6]. One of the widely available non target insects are assassin bugs (Reduviids). Assassin bugs are one of the effective predators and they get their name from their predatory behaviour which involves ambushing and killing their prey. There are over 7000 known species ranging in size, shape and colour. Non target assassin bugs living in agro ecosystems are being exposed to insecticides frequently which are indiscriminately used to control insect pest population (George and Ambrose, 2004) [8]. Even though insecticides are widely studied for their control potential against particular insect pests, they are very less studied or even neglected on their control potential against particular insect pests, they are very less studied or even neglected on their impact on non-target insects. The information available on the effects of chemical insecticides on non-target predators are very less than on herbivorous insects (Croft and Brown, 1975; Sahayaraj and Amalraj, 2005) [2, 21]. Most of the insecticides are highly toxic to target pests and are typically toxic to non-targeted organisms such as predators and parasitoids which are important regulators of insect pests, hence resulting in the decline of biodiversity of an agroecosystem (Desneux *et al.*, 2007; Juen *et al.*, 2012; Lu *et al.*, 2012; Martinou *et al.*, 2014; Rimoldi *et al.*, 2017) [3, 12, 14, 27, 18].

This prompted the author to study the impact of five commonly used insecticides in the agroecosystem particularly three organophosphorus insecticides namely quinalphos, chlorpyrifos, dimethoate and two pyrethroid insecticides namely bifenthrin and deltamethrin in all the stages of the reduviid predator *Rhynocoris longifrons* (Stal).

Corresponding Author:
PJ Edward George
Department of Horticulture,
Kalasalingam School of
Agriculture and Horticulture,
Kalasalingam Academy of
Research and Education,
Krishnankoil, Virudunagar,
Tamil Nadu, India

R. longifrons was reported to be living in different agro ecosystems and predating upon various insects pests like *Aphis gossypii*, *Dysdercus cingulatus*, *Phenacoccus solenopsis*, *Clavigralla gibbosa* and *Nesara viridula*, *Helicoperpa*, *Armigera* and *Spodoptera litura* (Sahayaraj *et al.*, 2020) [22]. They possess essential characteristics for acting as an active biocontrol which include limited prey range, host searching efficiency, high fecundity, short life cycle, female-biased population, high predatory efficacy, favourable for mass culture, adaptability to new environmental conditions and free from parasites, parasitoids as well as predators (Sahayaraj and Amalraj, 2005) [21]. Determining the toxicity of pesticides to predators is required for effective integrated pest management strategies (Jiang *et al.*, 2018) [11]. The present study evaluated the effect of the recommended pesticides in the agroecosystem in recommended dosages used for the management of common agricultural pests, on *R. longifrons* under laboratory conditions. These results clearly provide information on the compatibility of these five insecticides on the reduviid predator *R. longifrons* in the agroecosystem which are widely used in the IPM packages for the management of common agricultural insect pests.

Materials and Methods

Adults and nymphs of *R. longifrons* were collected from the foothills of Chunkankadai (longitude-77°23'5.03"E; latitude-8°12'2.74"N), Kanyakumari district, Tamilnadu. They were maintained in the laboratory at 30±2 °C, relative humidity ranging from 75-80% and photoperiod between 11-13 hrs on the larvae of *Corcyra cephalonica* Stainton. They were reared in 6 inch round plastic netted containers. The temperature and relative humidity were maintained at 26±2 °C and 85±5%. The bugs were kept in plastic containers along with dried cotton leaves and provided rice moth larvae *C. cephalonica* as regular prey. The *R. longifrons* have five nymphal instars which have different stadial periods ranging from 7-18 days. They have a pre-reproductive period ranging between 8-12 days and the egg batch consists of 60-90 eggs and incubates in 8±2 days. The eggs laid by the *R. longifrons* are collected carefully and allowed them for hatching in the containers. Immediately after hatching the first instar nymphs were carefully transferred to another plastic container using brush. Again after moulting they were transferred to new containers. Each nymphal instars of the reduviid bugs are kept separately. The number of nymphal instars maintained in each container is restricted to 30 in number and adults to five pairs to avoid crowding.

For the present toxicity study, adult and all of the five nymphal instar stages of *R. longifrons* were selected, which were molted within 24 h of initiation of the experiment. The spraying method used was residual contact bioassay. The individual samples of insecticides were prepared based on the recommended dosages of insecticides for the management of pests in the agroecosystems. The insecticides used in the studies and their recommended dosages are quinalphos 25EC at 1000 ml/ha, dimethoate 30% EC at 750 ml/ha, chlorpyrifos 20 EC at 2000 ml/ha, deltamethrin 11% EC at 500 ml/ha, bifenthrin 10% SC 800ml/ha, and each treatment was having six replications. Each solution of insecticide with recommended dosages was diluted in the water. The insecticide solutions were sprayed into the cotton shoots (George, 1996) [5]. For each

experiment, the test insect was transferred to individual plastic netted containers with dimensions of 7 cm height × 15 cm width. Selected ten individuals of 1st, 2nd, 3rd, 4th, and 5th instar nymphs and adults of *R. longifrons* are introduced into a container having cotton shoots applied with the respective insecticides. The mortality of the test insect was determined by the movement of the insect and its response towards any stimulation. The mortality rate of the insect was recorded every 24 h, 48 h, 72 h and 96 h after the treatment of each insecticides in five different stages and adult insects. A control set up was also maintained with water treated cotton leaves for all the stages of the predator. All the variations caused by the various insecticides in particular duration were initially analyzed by one way ANOVA (SAS Institute, 1988) to find if there is any differences existed among the different insecticides in particular duration. If significant differences are there, then the individual treatment means were tested by post ANOVA (Turkey, 1953) [26]. Statistical significance was determined by setting the aggregate type 1 error at 5% ($p < 0.05$) for each set of comparisons.

Results and Discussion

This study was conducted to analyze the compatibility of three organophosphorus and two pyrethroid insecticides with the application of the insecticide dosage recommended to the farmers for the management of agricultural pests in the agroecosystem. Various stages and adult *R. longifrons* were released separately on the different insecticide treated containers to find out the developmental stages which are susceptible to the different insecticides and to find out the insecticides which are harmful to the predator *R. longifrons*. For the first instar nymphs, all the three organophosphate insecticides caused 100% mortality in all the durations. The pyrethroid insecticides bifenthrin and deltamethrin caused 71.48±3.53 and 33.27±2.23 percentage respectively in 24 duration which gradually increased to 100% in the 96 hour duration. (Table 1). This study was in corroboration to the study of Rugno *et al.*, (2016) [19] and He *et al.*, (2018) [10] where it is well explained that the toxicity level of an insecticide in a bioassay is dependent on the chemical group and the test insect species.

The exposure routes of pesticides to predatory insects are mostly happened through food contact (Angeli *et al.*, 2000) [1] and direct contact with residual deposits (Hassan *et al.*, 1985) [9]. In addition, chemical pesticides are generally more toxic to immature stages than to adults (Rugno *et al.*, 2016; Ono *et al.*, 2017) [19, 16]. The recent study conducted by Kammatterikunnu and Thattanteparambil (2025) [13] with organophosphorous insecticides, quinalphos and chlorpyrifos also similar to the present findings in all the developmental stages in *Sycanus collaris*.

The insecticide exposed to second instar insects also showed similar trend like first instar nymphs. All of the three organophosphate insecticides caused 100 percent mortality in all the durations but the pyrethroid insecticides caused less mortality in *R. longifrons* which is recorded as 58.14±3.49 and 11.26±1.19 percent mortality in bifenthrin and deltamethrin treated insects respectively and they did not cause 100% mortality even in the 96 hour duration (Table 2). The third instar nymphs also showed similar trend of 100% mortality in the organophosphate treated insects in all the four durations but the pyrethroid insecticide caused only 39.33±3.46% mortality in the 24 hr duration whereas

the deltamethrin is completely safe without any mortality in 24 hr duration ^[15]. The mortality gradually increased to 61.18±3.03 and 23.22±1.1% in the 96 hour duration (Table 3). The results are consistent with earlier research showing that predators are more vulnerable to insecticides during their initial nymphal stages than during their advanced nymphal stages and adult stages (Garzón *et al.*, 2015; Santos *et al.*, 2017) ^[4, 24].

Fourth instar nymphs of *R. longifrons* are more resistant to insecticides than the first, second and third instar nymphs. Among the three organophosphate treated insects, only quinalphos and chlorpyrifos effected 100% mortality in all the exposure durations. Dimethoate did not cause 100% mortality in any of the durations and the maximum mortality recorded in 96 hr duation is 79.41±2, 73%. The bifenthrin cause medium mortality in all the exposure durations whereas deltamethrin is compararively safe and did not cause any mortality in the 24, 48 and 72 hrs exposure durations and caused only 8.11±0.63% mortality in 96 hr. exposure (Table 4). According to Patel (2020) ^[17], the *Rhynocoris marginatus* has the lowest mortality with thiacloprid whereas quinalphos shows the highest mortality and the same increased mortality is also been recorded in the current study and followed by, chlorpyrifos, and dimethoate. Sahayaraj *et al.* (2020) ^[22] concluded that neem based insecticides have no impact on *R. marginatus*. This experiment paves a pathway in which bioproducts have no impact on the predators. Even though, death is not recorded abnormalities were noticed in the digestive tract and reproductive system of the reduviid predator (*Rhynocoris kumarii*) when exposed to sublethal doses of dimethoate, methyl parathion, monocrotophos, and quinalphos (George and Ambrose, 2004) ^[8]. The fifth instar nymphs are more resistant than the earlier nymphs. The quinalphos and chlorpyrifos caused 100% mortality whereas the dimethoate did not cause 100% mortality in any of the exposure durations and the maximum mortality recorded is 53.58±2.39% in the 96 hr. treated category. The bifenthrin also caused less mortality in all the exposure durations and it is 18.18±2.49, 24.56±3.18, 29.72±3.46 and 33.19±4.49% in 24, 48, 72 and 96 hrs. Treated category. The deltamethrin is completely a safer insecticide at all the exposure durations (Table 5). According to previous studies investigating the effectiveness of pesticides on three reduviid bug species,

similar results were noticed on this examination as among the organophosphorous insecticides methyl parathion and monocrotophos showed highest toxicity while quinalphos and dimethoate exhibit less toxicity (George, 1996) ^[5].

The adult insects are more resistant than the other instars as they are having wings which protect the insects from insecticide exposure and also they are in the reproductive stage. The organophosphate insects quinalphos and chlorpyrifos caused 100% mortality in adult *R. longifrons* whereas the dimethoate is safe and it caused 0% mortality in the 24 hrs duration and also only less mortality in the later exposure durations (37.83±5.29% at 96 hr. duration). Both of the pyrethroid insecticides bifenthrin and deltamethrin are completely safe to the aduly *R. longifrons* and there is no mortality in all the exposure durations (Table 6). Since predators are frequently found close to pests, they are also impacted by direct sprays in addition to the pest insects. The current investigation confirms the findings of Sriraksha *et al.* (2021) ^[25], who claim that quinalphos is extremely toxic to *S. collaris* than other insecticides which is in line in the case of *R. longifrons* in both nymphal instars and adults.

This study clearly revealed that organophosphorus insecticides were considered to induce more negative impact for all the nymphal instars and adults of *R. longifrons* and they were not recommended for use as an inevitable part of integrated pest management in which *R. longifrons* was involved whereas synthetic pyrethroids are considered as safer for the third, fourth, fifth and adult stages of *R. longifrons*. The study also suggests that the less toxic pyrethroid insecticides are highly effective to the *R. longifrons* so that it will reduce the development of resistance built by feeding the surviving pest after the application of the selected insecticides. Hence, it is suggested that screening of insecticides is very much important to safe guard the non-target beneficials such as *R. longifrons* which is proved as an excellent ecofriendly biocontrol agent in various vulnerable insect pests in the agroecosyatem.

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Table 1: Insecticidal impact on the percentage mortality in the first instar *R. longifrons*

Insecticide	Percentage of mortality			
	24 Hrs	48 Hrs	72 Hrs	92 Hrs
Quinalphos	100 ^a	100 ^a	100 ^a	100 ^a
Chlorpyrifos	100 ^a	100 ^a	100 ^a	100 ^a
Dimethoate	100 ^a	100 ^a	100 ^a	100 ^a
Bifenthrin	71.48±3.53 ^b	81.28±2.43 ^b	88.56±3.59 ^b	100 ^{ab}
Deltamethrin	33.27±2.23 ^c	39.68±2.41 ^c	46.14±1.64 ^c	53.22±2.92 ^c

Means followed by the same alphabets in a colum are not statistically significant at 5% (p>by Turkey test

Table 2: Insecticidal impact on the percentage mortality in the second instar *R. longifrons*

Insecticide	Percentage of mortality			
	24 Hrs	48 Hrs	72 Hrs	92 Hrs
Quinalphos	100 ^a	100 ^a	100 ^a	100 ^a
Chlorpyrifos	100 ^a	100 ^a	100 ^a	100 ^a
Dimethoate	100 ^a	100 ^a	100 ^a	100 ^a
Bifenthrin	58.14±3.49 ^b	65.12±2.46 ^b	69.38±2.49 ^b	76.69±3.38 ^b
Deltamethrin	11.26±1.19 ^c	18.75±2.28 ^c	27.32±2.51 ^c	31.13±2.27 ^c

Means followed by the same alphabets in a colum are not statistically significantat 5% (p>by Turkey test

Table 3: Insecticidal impact on the percentage mortality in the third instar *R. longifrons*

Insecticide	Percentage of mortality			
	24 Hrs	48 Hrs	72 Hrs	92 Hrs
Quinalphos	100 ^a	100 ^a	100 ^a	100 ^a
Chlorpyrifos	100 ^a	100 ^a	100 ^a	100 ^a
Dimethoate	100 ^a	100 ^a	100 ^a	100 ^a
Bifenthrin	39.33±3.46 ^b	45.18±4.36 ^b	53.36±4.84 ^b	61.18±3.03 ^b
Deltamethrin	0 ^c	0 ^c	18.16±2.52 ^c	23.22±1.19 ^c

Means followed by the same alphabets in a column are not statistically significant at 5% (p>by Turkey test)

Table 4: Insecticidal impact on the percentage mortality in the fourth instar *R. longifrons*

Insecticide	Percentage of mortality			
	24 Hrs	48 Hrs	72 Hrs	92 Hrs
Quinalphos	100 ^a	100 ^a	100 ^a	100 ^a
Chlorpyrifos	100 ^a	100 ^a	100 ^a	100 ^a
Dimethoate	52.38 ±2.19 ^b	60.11 ±2.41 ^b	71.68 ±1.58 ^b	79.41±2.73 ^b
Bifenthrin	29.16 ±3.43 ^c	34.48 ±4.46 ^c	41.76 ±3.01 ^c	46.26 ±3.12 ^c
Deltamethrin	0 ^d	0 ^d	0 ^d	8.11±0.63 ^d

Means followed by the same alphabets in a column are not statistically significant at 5% (p>by Turkey test)

Table 5: Insecticidal impact on the percentage mortality in the fifth instar *R. longifrons*

Insecticide	Percentage of mortality			
	24 Hrs	48 Hrs	72 Hrs	92 Hrs
Quinalphos	100 ^a	100 ^a	100 ^a	100 ^a
Chlorpyrifos	100 ^a	100 ^a	100 ^a	100 ^a
Dimethoate	29.62 ±4.19 ^b	38.62 ±3.38 ^b	46.43 ±4.12 ^b	53.58 ±2.39 ^b
Bifenthrin	18.18 ±2.49 ^c	24.56 ±3.18 ^c	29.72 ±3.46 ^c	33.19 ±4.49 ^c
Deltamethrin	0 ^d	0 ^d	0 ^d	0 ^d

Means followed by the same alphabets in a column are not statistically significant at 5% (p>by Tukey test)

Table 6: Insecticidal impact on the percentage mortality in the adult *R. longifrons*

Insecticide	Percentage of mortality			
	24 Hrs	48 Hrs	72 Hrs	92 Hrs
Quinalphos	100 ^a	100 ^a	100 ^a	100 ^a
Chlorpyrifos	100 ^a	100 ^a	100 ^a	100 ^a
Dimethoate	0 ^b	17.19 ±5.42 ^b	26.31 ± 4.24 ^b	37.83 ±5.29 ^b
Bifenthrin	0 ^b	0 ^c	0 ^c	0 ^c
Deltamethrin	0 ^b	0 ^c	0 ^c	0 ^c

Means followed by the same alphabets in a column are not statistically significant at 5% (p>by Turkey test)

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