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Relative toxicity and haematological impact of insecticides cypermethrin and Fenvalerate to a non - target reduviid predator *Rhynocoris marginatus* (Fabricius) (Heteroptera: Reduviidae)

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Abstract
Relative toxicity of two commonly used insecticides namely cypermethrin (Cymbush 10 EC) and fenvalerate (Sumicidin 20 EC) to the non - target reduviid predator *Rhynocoris marginatus* was evaluated. Among the two insecticides tested, fenvalerate was more toxic than cypermethrin. Both cypermethrin and fenvalerate increased the number of prohaemocytes and granular haemocytes and decreased the plasmatocytes. They also caused changes in the density of cystocytes and oenocytoids. Though both the insecticides increased the total haemocyte counts, highest impact was caused by fenvalerate.

Keywords: *Rhynocoris marginatus,* cypermethrin, fenvalerate, insecticides, haemocyte count

Introduction
Being the largest group of predatory insects, Reduviids, beneficial to farmers inhabit agroecosystem and predate upon a variety of insect pests [1]. Hence, efforts have been made to employ them as agents of biological control against insect pests. However, insecticides used in the agroecosystems not only suppress the insect pest population but also their natural enemies. Much less is known about the effects of chemical pesticides on predators and parasites than on herbivorous pests [2]. Hence, it is imperative to screen the insecticides before incorporating them into the Insect Pest Management Programme [3]. Screening is imperative to safeguard the beneficial from the hazardous effects of insecticides.
Such screening of insecticides will be successful and effective only when we understand the toxicological effects of insecticides we use, on insect pests as well as on their predators [4]. Insecticides caused many physiological and behavioural changes. One such important physiological change is the change in the haemolymph composition and haemocytes morphology. But such informations are scanty for reduviids except the works of Jones [5], Ambrose and George [6, 7] and George and Ambrose [8-10]. This prompted the authors to study the impact of sublethal concentration of the two commonly used insecticides fenvalerate and cypermethrin against the reduviid predator *Rhynocoris marginatus* (Fabricius).
R. marginatus, a polyphagous reduviid predator, is present in most Indian agro-ecosystems [11, 12]. It was reported as a potential predator on various insect pests like *Earias fabia* (Stal), *Dysdercus cingulatus* (Fabricius), *Earias vittella* (Fabricius), *Corcyra cephalonica* (Stainton), *Spodoptera litura* (Fabricius) and *Helicoverpa armigera* Hubner [13].

Materials and Methods
Adults of *R. marginatus* were collected from Sivanthipatti semi-arid zone in Tirunelveli District. Tamil Nadu (altitude 125.33 ± 2.87 MSL, latitude 77° 21'E and 8° 31'N). *R. marginatus* was maintained in the laboratory in plastic containers (80 ml) at 32 °C, RH ranging from 75-80% and photoperiod between 11-13 hrs. on *C. cephaloinca*. LC_{50} concentration at different exposure durations (24, 48, 72 and 96 hrs.) were found out for both the insecticides. They were 0.038, 0.032, 0.026 and 0.023% for cypermethrin and 0.026, 0.028, 0.020 and 0.016% for fenvalerate for 24, 48, 72 and 9 hrs, respectively.

Twenty insects were tested for each insecticide. Insecticides viz., cypermethrin and fenvalerate (20µl of 1/10 48 hrs. LC_{50} concentration) were topically applied on each insect [14]. Ten reduviids were maintained as control which were topically applied with 20µ1 of water. The reduviids treated with each insecticide were divided into two sets each with 10 insects. One set was maintained continuously for 10 days whereas other set for 20 days. At the end of the insecticide exposure, the haemolymph of four insecticide treated categories and one water treated category of *R. marginatus* (cypermethrin 10 and 20 days, fenvalerate 10 and 20 days, control) were taken and their differential haemocytes count (DHC) and the total haemocytes count (THC) were evaluated separately [15]. Variations in THC and DHC were first analyzed by one way analysis of variance [16] to determine if differences existed among treatment means. When significant differences among treatment means were found, differences between individual treatment means were tested by Tukey test [17]. Statistical significance was determined by setting to the aggregate type I error at 5% ($p<0.05$) for each set of comparisons.

Results and Discussion

The LC_{50} values, upper and lower fiducial limits, relative toxicity and regression equations of cypermethrin and fenvalerate on *R. marginatus* at 24, 48. 72 and 96 hrs. durations were calculated and presented in table 1. The LC_{50} values were less for fenvalerate (0.028. 0.024, 0.020 and 0.016% respectively for 24, 48, 72 and 96 hrs. durations) than for cypermethrin (0.038, 0.032. 0.026 and 0.023% respectively for 24, 48, 72 and 96 hrs. durations). This clearly indicates that fenvalerate is more toxic than cypermethrin in all the exposure durations, i.e. when compared to 24 hrs. exposure, the 96 hrs. exposure to cypermethrin and fenvalerate were 1.65 times and 1.75 times more toxic to *R. marginatus.*

The haemolymph of both insecticides and water treated *R. marginatus* exhibited five types of haemocytes under light microscope. They were prohaemocytes, plasmatocytes, granular haemocytes, cystocytes and oenocytoids. The variations caused by the insecticides on the haemocytes percentage are presented in the table 2.

The percentage of prohaemocytes and plasmatocytes were reduced in the lower exposure durations (5 and 10 days exposure) and was either restored or increased in the higher exposure period (15 days), for both the insecticides. Fenvalerate had the highest impact on haemocytes reduction. For instance, it reduced the prohaemocytes and plasmatocytes from 11.66 ± 0.94 and 36.00 ± 1.29 to 8.18 ± 0.98 and 24.66 ± 2.87. The reduction of haemocytes by insecticides might be due to the transformation of these haemocytes into other haemocytes types due to stress as per requirement. Similar observations were reported by Gupta and Sutherland [18], Ambrose and George [6, 7, 19] and George and Ambrose [8-10]. The intensity of reduction could be correlated to the degree of stress developed by the test individuals due to differential action of insecticides.

Both, cypermethrin and fenvalerate increased the granular haemocytes from 38.50 ± 2.21 to 48.00 ± 1.29 and 52.48 ± 2.82% in 5 days exposure respectively. However, at 10 and 15 days exposure haemocytes number increased to 44.83 ± 1.21 and 40.18 ± 1.21% and 47.19 ± 2.43 and 42.43 ± 2.11% in cypermethrin and of fenvalerate exposed *R. marginatus.* This increase in granular haemocytes as a function of insecticide exposure might be due to their detoxification function [20] or their immunological functions, such as phagocytosis and encapsulation of foreign antigens [21,22]. The granular haemocytes also have some other functions like coagulation, wound healing, secretion of haemagglutin and antibacterial and antiviral factors. As the durations of insecticides exposure was increased the cystocytes number increased gradually. On the contrary the increase in insecticide exposure durations gradually decreased the oenocytoids.

The variations caused by the two insecticides on the total count of haemocytes are presented in the table 3. Although both cypermethrin and fenvalerate increased the total count of haemocytes, fenvalerate caused the higher effect. Fenvalerate increased the total count of haemocytes from 12220 ± 422.99 to 19795 ± 65.21 in 5 days exposure which slightly reduced in 10 days (18687 ± 49.47) and 15 days (17566 ± 74.53) exposures. The increased haemocytes count could be attributed to the defensive action of haemocytes for phagocytosis and encapsulation of foreign antigens. The present result corroborates the result of George and Ambrose [8-10] in other harpactorine reduviids. They reported an increase in the total haemocyte count as a function of insecticidal effect. Ries [23] also stated that the THC of *Tenebrio molitor* greatly increased after wounding or implantation of foreign materials. Hence, the highest toxicity created by the 5 days duration of fenvalerate could be attributed for highest haemocyte count.

The study on the impact of commonly used insecticides on the haemocytes of the non- target beneficial *R. marginatus* reveals the adverse effects caused by chemical control of insect pests on non-target beneficials. Hence, it is time to replace the 'king chemical' and especially those that are not friendly with beneficials and to incorporate beneficials friendly insecticides or 'soft insecticides'. Such incorporation could effectively reduce pest population while not affecting their natural enemies. Furthermore, the present studies suggest that both the insecticides caused adverse effects in the physiology of the non- target natural enemy *R. marginatus* and hence they should not be incorporated in pest control programme.

Table 1: Relative toxicity of Cypermethrin and Fenvalerate in *R. marginatus.*

Insecticide	Duration of exposure (hrs)	X^2	Regression equation	LC_{50}	Fiducial Limit		Variance	Relative Toxicity
					Lower	Upper		
Cypermethrin	24	0.19	4.826x-2.60	0.038	0.029	0.047	0.0028	1.00
	48	0.22	4.600x-1.91	0.032	0.026	0.038	0.0017	1.18
	72	0.50	6.150x-3.70	0.026	0.022	0.030	0.0012	1.46
	96	1.11	6.016x-5.98	0.023	0.019	0.027	0.0015	1.65
Fenvalerate	24	5.23	6.366x-7.48	0.028	0.015	0.049	0.0149	1.00
	48	0.29	3.025x+0.62	0.024	0.019	0.040	0.0071	1.17
	72	0.90	3.340x+0.63	0.020	0.015	0.026	0.0031	1.40
	96	1.54	6.027x+2.25	0.016	0.013	0.019	0.0020	1.75

Table 2: Effect of sub lethal Concentration (1/10 of 48 hrs. LC_{50}) of Cypermethrin and Fenvalerate on differential haemocyte count (%) in *R. marginatus* (n=10; $\bar{x} \pm SD$)

Insecticide	Duration of exposure (days)	Prohaemocyte	Plasmatocyte	Granular haemocyte	Cystocyte	Oenocytoid
Cypermethrin	0	11.66 ± 0.94^{ac}	36.00 ± 1.29^{a}	38.50 ± 2.21^{a}	6.00 ± 1.29^{a}	9.00 ± 1.29^{a}
	5	9.43 ± 0.84^{b}	25.18 ± 3.14^{b}	48.00 ± 1029^{b}	7.45 ± 1.38^{b}	8.94 ± 1.73^{a}
	10	10-71 ± 1.13^{a}	29.41 ± 2.42^{c}	44.83 ± 1.021^{c}	8.81 ± 1.49^{c}	6.24 ± 1.14^{b}
	15	12-47 ± 1.44^{c}	33.11 ± 1.81^{d}	40.18 ± 1.021^{d}	9.84 ± 1.58^{d}	4.40 ± 0.97^{c}
Fenvalerate	0	11.66 ± 0.94^{a}	36.00 ± 1.29^{a}	38.50 ± 2021^{a}	6.00 ± 1.29^{a}	9.00 ± 1.29^{a}
	5	8-18 ± 0.98^{b}	24.66 ± 2.87^{b}	54.48 ± 2.82^{b}	9.14 ± 1.18^{b}	5.54 ± 0.86^{b}
	10	11-46 ± 1.23^{a}	27.40 ± 2.43^{c}	47.19 ± 2.43^{c}	10.43 ± 1.86^{c}	3.52 ± 1.10^{c}
	15	12-96 ± 1-41^{c}	29.86 ± 2.76^{d}	42.43 ± 2011^{d}	10.91 ± 1.91^{c}	3.84 ± 0.95^{c}

Means carrying similar superscript in column are not statistically significant by Tukey test (P>0.05).

Table 3: Effect of sub lethal Concentration (1/10 of 48 hrs. LC_{50}) of Cypermethrin and Fenvalerate on the total haemocyte count (%) in *R. marginatus* (n=10; $\bar{x} \pm SD$)

Insecticide	Duration of exposure (Days)	Total Count	
		Range	Mean
Cypermethrin	-	9750 - 14000	12220 ± 422.99
	5	15400 - 17140	16366 ± 230.33***
	10	14870 - 16400	15208 ± 67.18**
	15	14100 - 15940	15175 ± 60.22*
Fenvalerate	5	18700 - 20900	19795 ± 65.21***
	10	18600 - 19750	18687 ± 49.47***
	15	16500 - 18700	17566 ± 74.53***

Significance is shown as 5%(*), 1% (***) levels of probability ('t') test.

Conclusion

Two commonly used insecticides in the agroecosystem, cypermethrin and fenvalerate were evaluated for their relative toxicity on the non- target reduviid predator *R. marginatus.* Among the two insecticides tested, fenvalerate was more toxic to *R. marginatus* than cypermethrin. The effect of sub lethal concentration of both the insecticides on differential haemocyte count makes drastic changes in all the haemocyte types especially in prohaemocytes, plasmatocytes and granular haemocytes. Fenvalerate caused maximum increase in the total number of haemocytes followed by cypermethrin. This clearly shows that both the insecticides affect the physiology of insects and cause adverse effects on the non-target predators during chemical control and these two pesticides should be avoided to safeguard the non-target reduviid predators available in the agro ecosystems while selecting the insecticides to control the pest insects.

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