

Beta-cyfluthrin-Induced Alterations in the Total and Differential Haemocytes Count in the Red Cotton Bug, *Dysdercus koenigii* (Fabricius,1775)

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ABSTRACT

Dysdercus koenigii is a major global pest of cotton that causes severe economic loss. Among several control measures, pyrethroids are frequently used toxicants because of high efficacy at low dosages and relative safety. Since haemocytes are biomarkers of the physiological response and immunity of insect which determine the insecticide efficacy, the current study assessed the effect of a pyrethroid, β -cyfluthrin, on the total and differential haemocyte counts of *D. koenigii*. Haemolymph was collected from the fifth instars after the topical application of β -cyfluthrin (0.8, 1.6, 3.2, 6.4 and 12.8 mg/L) on the thoracic tergum. The haemolymph of control nymphs revealed 5270 haemocytes/mm³ which decreased instantly by 1.4-3.1-fold on β -cyfluthrin exposure; more reduction observed at lower dosages. Increase in exposure duration and β -cyfluthrin dosages fluctuated the count considerably, eventually raising them at lower dosages and diminishing at higher dosages. Among five kinds of haemocytes recorded in the haemolymph, the β -cyfluthrin exposure increased %prohaemocytes count; diminished %granulocytes and %plasmotocytes count while spherulocyte and oenocyte counts were inconsistent. The alterations in haemocyte counts indicate the immunity response trigger in *D. koenigii* due to β -cyfluthrin-induced stress. Further investigations may decipher the mechanisms involved and help to formulate the strategies for its management in fields.

Keywords: Biomarkers, DHC, Haemolymph, Pyrethroid, Red Cotton Bug, THC.

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INTRODUCTION

Cotton is one of the most important fibers and cash crops of India responsible for the industrial and agricultural economy of the country (Pal, Bhattacharya, & Sahani, 2020). India accounts for about 25% of the total global cotton production. In India, the majority of cotton is produced in Madhya Pradesh, Maharashtra and Gujarat under central cotton-growing zones; while the southern zone comprises Andhra Pradesh, Karnataka and Tamil Nadu and the northern zone includes Punjab, Haryana and Rajasthan.

The major cotton losses have been attributed to the immense infestation by diverse pests and diseases affecting the crop quantity as well as quality. Among these pests, Red cotton bug, *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae) is regarded as one of the major global pests causing severe economic loss. Immature nymphs as well as adults capable of rapid dispersal, suck the moisture and oil contents from the cotton leaves and seeds at a widespread scale reducing the crop yield extensively (Sahayaraj & Fernandez, 2017; Gupta, Shazad, & Kumar, 2019; Saeed & Abbas, 2020; Karar et al, 2020; Karar et al, 2021).

Despite the implementation of multifarious control interventions to alleviate *Dysdercus* infestation, chemical insecticide-based interventions are the predominant and most preferred means due to their rapid action and toxic effects. A wide range of chemical groups of pesticides such as organochlorines, organophosphates, carbamates, pyrethroids, avermectins, spinosyns and neonicotinoids etc., have been used in the fields (Saeed et al, 2018). Toxicity of chlorpyrifos, deltamethrin, lufenuron, flucycloxuron (andalin), chlorfenpyr and thiamethoxam has been demonstrated against *D. koenigii* (Khan & Qamar, 2011; Saeed, Naqqash, & Jaleel, 2016; Jameel & Jamal, 2017). Despite control attempts and studies with diverse chemicals and alternate measures to chemicals, pyrethroids are still preferred because of their efficacy at low dosages and relative safety (Mohd, Nasreen, Snigdha, & Altaf, 2021).

The β -cyfluthrin (cyano-(4-fluoro-3-phenoxyphenyl)-methyl-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate) is a non-systemic second generation of pyrethroid causing contact as well as stomach toxicity in insects (Chawla et al, 2018). Though it has been investigated against a wide variety of pests on cotton, corn, sunflower and soybean crops (Athanasios et al, 2004a; Athanasios, Papagregoriou, & Buchelos, 2004b; Addy-Orduna, Zaccagnini, Canavelli, & Mineau, 2011), reports of its efficacy against *D. koenigii* are limited. Our previous studies have implicated the toxic and growth regulatory effects of β -cyfluthrin on *D. koenigii* (Lanbilu, Samal, Panmei, & Kumar, 2020). Nevertheless, effect of this chemical insecticide on the physiological functions of *D. koenigii* which can affect their reproductive fitness, metabolism and immunity has not been studied. The haematological studies play a significant factor and biomarker in the field of insect physiology as their haemolymph acts as a transport system for nutrients, hormones and metabolic waste, as well as contains elements of the immune system i.e., phagocytic haemocytes (Richards & Davies, 1977; Sarwar, Ijaz, Sabri, Yousaf, & Mohsan, 2018). Besides that, haemocytes perform several

other vital functions of the body, such as food storage, connective tissue formation and cellular defense (Wigglesworth, 1959; Sapcaliu et al, 2009). Insecticide exposure can affect the insect haemocytes altering their physiological functions (Sarwar et al, 2018). Thus, a number of total and differential haemocytes present under various stress conditions can provide information about the insects' physiological conditions, virulence and immunity (Rizwan-ul-Haq, Sabri, & Rashid, 2005).

Thus, present studies were conducted to assess the effects of β -cyfluthrin on the structure and count of total and differential haemocytes in *D. koenigii*. It is proposed that this study will help to understand the physiological response of the pest to insecticide exposure which may assist in optimizing the β -cyfluthrin concentration to be tested in fields as a growth regulatory intervention measure.

MATERIAL AND METHODS

Rearing of *Dysdercus koenigii*

Nymphs and adults of *D. koenigii*, procured from the Insect Reproduction Laboratory, Deshbandhu College, University of Delhi, India; were reared in the Insect Pest and Vector Laboratory, Acharya Narendra Dev College, University of Delhi, India. The culture was maintained under the controlled conditions of $28 \pm 2^\circ\text{C}$, $80 \pm 5\%$ RH (Relative Humidity), and 14 h of light and 10 h of darkness. Adults and different nymphal stages were kept in separate sterilized glass jars of 1L capacity, containing sterilized cotton seeds and cotton swabs soaked in autoclaved water so as to minimize the risk of infection in the insects (Gupta et al, 2019). The food was changed on alternate days while the jars were changed twice a week in order to maintain hygienic conditions throughout the mass rearing of the culture.

Insecticide taken into consideration

The technical grade of β -cyfluthrin (99% purity) was procured from M/s Sigma-Aldrich. Desired concentrations were prepared in acetone (eMerck) from a stock of 1% solution and stored at 4°C .

Collection of haemolymphs

Based on our previous study which demonstrated the toxic efficacy of the β -cyfluthrin against 5th instar nymphs of *D. koenigii* (Table 1), the current study was conducted on the newly emerged 5th instar nymphs with the concentrations ranging from 0.8 mg/L-12.8 mg/L (Lanbiliu et al, 2020).

The individual insect was topically subjected to different concentrations (0.8 mg/L-12.8 mg/L) of β -cyfluthrin on the thorax tergum with the help of a micropipette (10 μL) at the rate of 1 μL /insect. Each concentration was replicated 4 times to reduce error. The control was run simultaneously. The haemolymph of the control and treated *D. koenigii* nymphs was collected immediately at 0 min followed by collection after 30 min and 60 min of β -cyfluthrin exposure (Perveen & Ahmad, 2017). One of the

antennae of the nymphs was dissected with the help of a fine blade and the maximal haemolymph was forced out of the body by applying dorsoventral pressure on the insect's abdomen with a fine microtip forceps (Barakat, Meshrif, & Shehata, 2002).

Table 1. Percent mean mortality of 5th instar nymphs of *Dysdercus koenigii* treated with β -cyfluthrin.

β -cyfluthrin (mg/L)	Total Nymphal mortality after 24 h
Control	0.00 \pm 0.00 a
0.8	0.66 \pm 0.33 b
1.6	1.66 \pm 0.33 b
3.2	4.66 \pm 0.33 c
6.4	17.66 \pm 0.33 d
12.8	21.66 \pm 0.88 d

Notes: 75 insects treated in 3 replicates of 25 each; Values are mean \pm Standard Error of Mean (SEM) (Lanbiliu et al, 2020); Values in each row followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pairwise multiple comparison test.

Total and differential haemocyte count in 5th nymphal instar of *Dysdercus koenigii*

The total haemocytes in the nymphs were counted by using a Neubauer haemocytometer. Collected haemolymph was sucked into a Thoma white cell pipette up to the mark 0.5. The tip of the pipette was cleaned and haemolymph was diluted 20X with diluting fluid (1ml of 0.3% of gentian violet, 1mL of glacial acetic acid in 100 mL of distilled water) by drawing it up to the mark 11. The contents were mixed thoroughly by slightly rotating the pipette (Sarwar et al, 2018). The first few drops were discarded to negate the error and the Neubauer chamber was charged with the content. Haemocytes were counted in four corner squares of the Neubauer chamber (1mm²) and the total circulating haemocytes in cubic millimetre (mm²) was calculated using the following formula (Jones, 1962).

$$\frac{(\text{Haemocyte in four square (1mm}^2\text{)} \times \text{Dilution} \times \text{depth factor of chamber})}{(\text{No. of squares counted})}$$

Differential haemocyte count (DHC) was carried out by following the Battlement method (Parveen & Ahmad, 2017). A thin film smear of *D. koenigii* haemolymph was prepared by drawing a slide across the haemolymph-containing slide at an angle of 45°. The smear was air-dried at room temperature and was added with Leishman stain for 20 min. Subsequently, the slide was washed carefully with distilled water to remove excess stain and dried again at room temperature. The haemocytes were scrutinized under the microscope (Nikon ECLIPSE E100) and at least 100 cells of different categories were counted from random areas (Jones, 1967). The percentage of different cell types was calculated in order to assess the physiological impact of β -cyfluthrin on *D. koenigii*.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA). The means were compared by Tukey's all pairwise multiple comparison test for statistical significance at $p < 0.05$.

RESULTS

Total haemocytes count in *Dysdercus koenigii* nymphs

The topical application of β -cyfluthrin on 5th instar nymphs of *D. koenigii* decreased the total haemocytes significantly ($p < 0.05$) instantly in comparison to control (Table 2, Fig. 1). As the exposure duration increased to 30 min, the haemocytes count increased on exposure to 0.8-3.2 mg/L β -cyfluthrin but decreased on treatment with 6.4 and 12.8 mg/L ($p < 0.05$) than recorded in the control. Further, increase in the exposure time by 30 min, the haemocyte count decreased significantly with an exception of 0.8 mg/L (Table 2).

The exposure with 0.8 mg/L β -cyfluthrin reduced the THC in *D. koenigii* nymphs by 3.1-fold at 0 min ($p > 0.05$) while increased it by 1.0-fold and 1.5-fold after 30 and 60 min, respectively. Likewise, at 1.6 mg/L, the THC decreased by 3.4-fold at 0 min ($p < 0.05$) but increased by 1.2-fold after 30 min. However, the haemocyte count decreased again by 2.0-fold after 60 min of exposure (Table 2). As the exposure concentration increased to 3.2 mg/L, the THC decreased by 1.4-fold at 0 min, increased by 1.2-fold after 30 min and again decreased by 2.9-fold at 60 min ($p < 0.05$). Increasing the β -cyfluthrin concentration further, a significantly reduced THC ($p < 0.05$) was observed irrespective of the duration of exposure (Table 2, Fig. 1).

Table 2. Effect of topical application of β -cyfluthrin on the total haemocyte count of the 5th instar nymphs of *Dysdercus koenigii*.

β -cyfluthrin (mg/L)	Total haemocyte count		
	T1	T2	T3
Control	5270 \pm 34.20 a	5270 \pm 34.20 a	5270 \pm 34.20 a
0.8	1675 \pm 11.53 b (-3.1)*	5340 \pm 24.94 a (+1.0)	7707.5 \pm 23.34 b (+1.5)
1.6	1540 \pm 23.42 b (-3.4)	6475 \pm 41.00 b (+1.2)	2635 \pm 2.70 c (-2.0)
3.2	3810 \pm 13.37 c (-1.4)	6545 \pm 7.14 b (+1.2)	1815 \pm 5.32 d (-2.9)
6.4	4295 \pm 20.07 d (-1.2)	2250 \pm 18.58 c (-2.3)	2075 \pm 4.08 e (-2.5)
12.8	3655 \pm 2.70 e (-1.4)	3285 \pm 10.75 d (-1.6)	2110 \pm 4.44 e (-2.5)
r	+0.185**	-0.682	-0.574

Insects treated in 4 replicates; Values are mean \pm Standard Error of Mean (SEM) T1: 0 minutes of exposure; T2: 30 minutes of exposure; T3: 60 minutes of exposure; Values in each column followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pair wise multiple comparison test.

*Figures in parentheses indicates the fold changes with respect to control ("+" indicates increased fold changes and "-" indicates decreased fold changes). "r" indicates the correlation coefficient; **In r values, "+" sign indicates direct relation between the concentration and total number of haemocytes whereas "-" sign indicate inverse relation between concentration and total number of haemocytes.

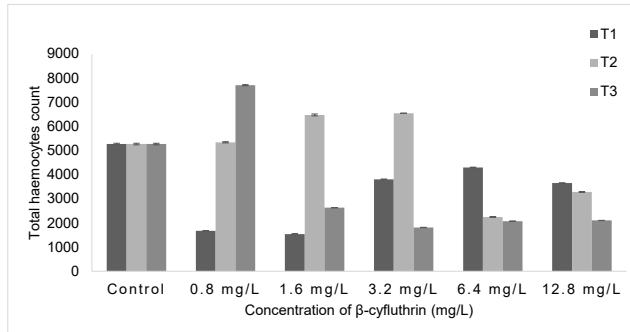


Figure 1. Comparative total haemocyte count of *Dysdercus koenigii* on exposure to various concentrations of β -cyfluthrin under control conditions.

*T1: 0 minutes of exposure; @T2: 30 minutes of exposure; #T3: 60 minutes of exposure

Differential haemocytes count in *Dysdercus koenigii* nymphs

The haemolymph of the red cotton bug recorded five kinds of haemocytes; phagocytic granulocytes (GR – 55%), adhering plasmatocytes (PL – 18%), stem cells - prohaemocytes (PR – 16%), phenoloxidase-containing oenocytes (OE – 11%), and refractile spherulocytes (SP – 0%); in order of their decreasing abundance (Table 3; Fig. 2).

Table 3: Effect of instant topical application of β -cyfluthrin on the differential haemocyte count of the 5th instar nymphs of *D. koenigii*.

β -cyfluthrin (mg/L)	Differential haemocyte count after 0 min				
	GR (%)	PL (%)	PR (%)	OE (%)	SP (%)
Control	55 \pm 2.75 a	18 \pm 0.90 a	16 \pm 0.80 a	11 \pm 0.55 a	0 \pm 0 a
0.8	37 \pm 1.85 b (-1.5)*	16 \pm 0.80 b (-1.1)	43 \pm 2.15 bc (+2.7)	02 \pm 0.10 b (-5.5)	02 \pm 0.10 b (0)
1.6	36 \pm 1.80 b (3.2)	12 \pm 0.60 c (-1.5)	39 \pm 1.95 b (+2.4)	13 \pm 0.65 c (+1.2)	0 \pm 0 a (0)
3.2	45 \pm 2.25 c (12.8)	16 \pm 0.80 b (-1.1)	26 \pm 1.30 d (+1.6)	12 \pm 0.60 a (+1.1)	01 \pm 0.05 c (0)
6.4	52 \pm 2.60 a (-1.1)	15 \pm 0.75 b (-1.2)	19 \pm 0.95 e (+1.2)	10 \pm 0.50 d (-1.1)	04 \pm 0.20 d (0)
12.8	30 \pm 1.50 d (-1.8)	15 \pm 0.75 b (-1.2)	48 \pm 2.40 c (+3)	07 \pm 0.35 e (-1.6)	0 \pm 0 a (0)
r	-0.284**	-0.191	0.395	-0.132	0.038

Insects treated in 4 replicates; Values are mean \pm Standard Error of Mean (SEM) GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte; Values in each column followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pairwise multiple comparison test. *Figures in parentheses indicate the fold changes with respect to control ("+" indicates increased fold changes and "-" indicates decreased fold changes). "r" indicates the correlation coefficient; **In r values, "+" sign indicates direct relation between the concentration and differential haemocytes whereas "-" sign indicates inverse relation between concentration and differential haemocytes.

The β -cyfluthrin exposure decreased the granulocyte count significantly at each dose. A reduction of 1.5-1.8-fold was recorded with 12.8 mg/L β -cyfluthrin exposure. A similar trend was recorded in the plasmatocytes count in *D. koenigii* haemolymph. On the other hand, prohaemocytes increased at all β -cyfluthrin dosages with the highest percentage rise with 12.8 mg/L. The oenocytes, nevertheless, decreased drastically with 0.8 mg/L β -cyfluthrin on all the exposure time as compared to control but increased at higher dosages. In contrast, spherulocytes increased with β -cyfluthrin exposure,

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the maximum increase was recorded at 6.4 mg/L (Table 3, Fig. 2). A similar trend of results was observed after 30 min of exposure with a decrease in granulocytes and plasmatocytes percentage with the increase in the concentration as compared to control (Table 4, Fig. 3). However, a significant decrease in prohaemocyte percentage was observed with a significant increase in higher concentration (12.8 mg/L). At 60 min of exposure, a significant decrease in granulocytes percentage was observed at 1.6 mg/L which further decreased drastically at higher concentration (12.8 mg/L) (Table 5; Fig. 4). Spherulocyte percentage was observed to increase in the nymph at all exposure times (0, 30 and 60 min).

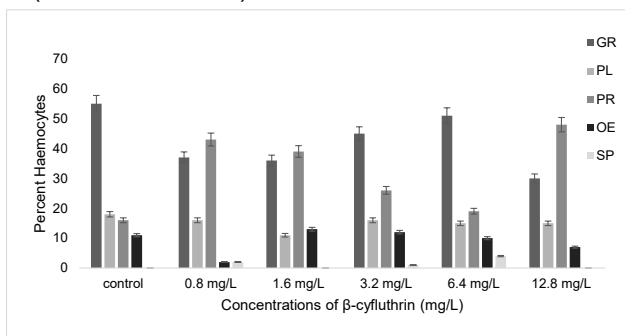


Figure 2. Graph depicting the Differential haemocyte count (DHC) of the *Dysdercus koenigii* on immediate exposure to different concentrations of β -cyfluthrin.

*GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-o-enocyte, SP-spherulocyte

Table 4. Effect of topical application of β -cyfluthrin on the differential haemocyte count of the 5th instar nymphs of *D. koenigii* after 30 minutes.

β -cyfluthrin (mg/L)	Differential haemocyte count after 30 min				
	GR (%)	PL (%)	PR (%)	OE (%)	SP (%)
Control	55 \pm 2.75 a	20 \pm 1.00 a	15 \pm 0.75 a	10 \pm 0.50 a	0 \pm 0 a
0.8	43 \pm 2.15 b (-1.3)*	13 \pm 0.65 b (-1.5)	34 \pm 1.70 b (+2.3)	08 \pm 0.40 b (-0.4)	02 \pm 0.10 b (0)
1.6	41 \pm 2.05 b (-1.3)	13 \pm 0.65 b (-1.5)	34 \pm 1.70 b (+2.3)	12 \pm 0.60 c (+2.3)	0 \pm 0a (0)
3.2	45 \pm 2.25 b (-1.2)	19 \pm 0.95 a (-1.1)	23 \pm 1.15 c (+1.5)	11 \pm 0.55cd (+1.5)	02 \pm 0.10 b (0)
6.4	45 \pm 2.25 b (-1.2)	17 \pm 0.85 c (-1.2)	25 \pm 1.25 c (+1.7)	10 \pm 0.50 d (0)	03 \pm 0.15 c (0)
12.8	32 \pm 1.60 c (-1.7)	17 \pm 0.85 c (-1.2)	39 \pm 1.95 d (+2.6)	12 \pm 0.60 c (+1.2)	0 \pm 0 a (0)
r	0.768**	0.130	0.552	0.493	-0.054

Insects treated in 4 replicates; Values are mean \pm Standard Error of Mean (SEM) GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-o-enocyte, SP-spherulocyte; Values in each column followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pairwise multiple comparison test. *Figures in parentheses indicate the fold changes with respect to control ("+" indicates increased fold changes and "-" indicates decreased fold changes). "r" indicates the correlation coefficient; **In r values, "+" sign indicates direct relation between the concentration and differential haemocytes whereas "-" sign indicates inverse relation between concentration and differential haemocytes.

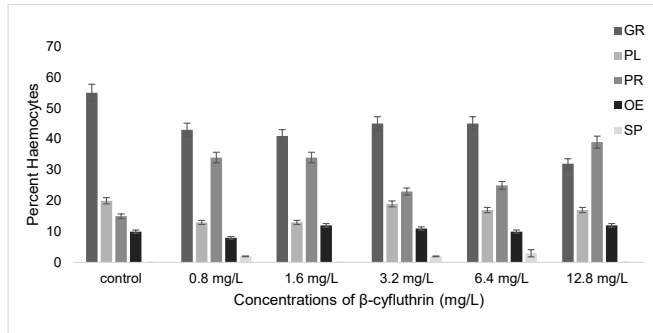


Figure 3. Graph depicting the Differential haemocyte count (DHC) of the *Dysdercus koenigii* on exposure to different concentrations of β -cyfluthrin for 30 minutes.

*GR-granulocyte, PL-plasmotocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte

Table 5. Effect of topical application of β -cyfluthrin on the differential haemocyte count of the 5th instar nymphs of *D. koenigii* after 60 minutes.

β -cyfluthrin (mg/L)	Differential haemocyte count after 30 min				
	GR (%)	PL (%)	PR (%)	OE (%)	SP (%)
Control	55 \pm 2.75 a	20 \pm 1.00 a	15 \pm 0.75 a	10 \pm 0.50 a	0 \pm 0 a
0.8	43 \pm 2.15 b (-1.3)*	13 \pm 0.65 b (-1.5)	34 \pm 1.70 b (+2.3)	08 \pm 0.40 b (-0.4)	02 \pm 0.10 b (0)
1.6	41 \pm 2.05 b (-1.3)	13 \pm 0.65 b (-1.5)	34 \pm 1.70 b (+2.3)	12 \pm 0.60 c (+2.3)	0 \pm 0a (0)
3.2	45 \pm 2.25 b (-1.2)	19 \pm 0.95 a (-1.1)	23 \pm 1.15 c (+1.5)	11 \pm 0.55cd (+1.5)	02 \pm 0.10 b (0)
6.4	45 \pm 2.25 b (-1.2)	17 \pm 0.85 c (-1.2)	25 \pm 1.25 c (+1.7)	10 \pm 0.50 d (0)	03 \pm 0.15 c (0)
12.8	32 \pm 1.60 c (-1.7)	17 \pm 0.85 c (-1.2)	39 \pm 1.95 d (+2.6)	12 \pm 0.60 c (+1.2)	0 \pm 0 a (0)
r	0.768**	0.130	0.552	0.493	-0.054

Insects treated in 4 replicates; Values are mean \pm Standard Error of Mean (SEM) GR-granulocyte, PL-plasmotocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte; Values in each column followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pairwise multiple comparison test. *Figures in parentheses indicate the fold changes with respect to control ("+" indicates increased fold changes and "-" indicates decreased fold changes). "r" indicates the correlation coefficient; **In r values, "+" sign indicates direct relation between the concentration and differential haemocytes whereas "-" sign indicates inverse relation between concentration and differential haemocytes.

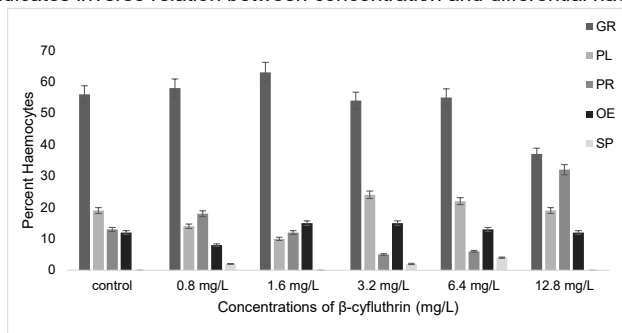


Figure 4. Graph depicting the Differential haemocyte count (DHC) of the *Dysdercus koenigii* on exposure to different concentrations of β -cyfluthrin for 60 minutes.

*GR-granulocyte, PL-plasmotocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte

DISCUSSION

Red cotton bug, *D. koenigii* which is one of the major insect pests of various crops, especially cotton, has caused immense crop yield reduction qualitatively as well as quantitatively. Despite the use of various intervention measures, synthetic pyrethroids are the major control agents for these pests on account of their efficacy and comparative safety to other conventional insecticides. Among various pyrethroids, β -cyfluthrin displays promising effects against various insect pests. Our earlier study has shown the control efficacy of β -cyfluthrin against *D. koenigii* (Lanbiliu et al, 2020). However, continued and persistent use of these insecticides can result in development of resistance in insects against these toxicants. Since, haemocytes perform various vital physiological functions of the body and play a significant role in the cellular defense, the current study evaluated the effect of β -cyfluthrin on the insect's haemocyte count to understand their involvement in immunity of insect (Cho & Cho, 2019).

The current study revealed a total of 5270 haemocytes/mm³ in the fifth instar nymphs of an Indian strain of *D. koenigii*. In comparison, reports have demonstrated a much higher count of 8450 cell/mm³ (Sarwar et al, 2018) and 17000 cell/mm³ (Rizwan-ul-Haq et al, 2005) in the adults of two Pakistan Strains of *D. koenigii*. A much higher THC of 11623.67 cell/mm³ has also been reported by Kumar, Kumari, & Verma (2019) in red cotton bug. The topical treatment of *D. koenigii* fifth instar nymphs with β -cyfluthrin decreased the total haemocyte count instantly. However, increase in the treatment duration to 30 min and 60 min; and increasing dosages of β -cyfluthrin, the pattern of total haemocyte count fluctuated considerably. At a lower dosage (0.8 mg/L) of β -cyfluthrin, the THC which decreased instantly by 3.1-fold increased gradually after 30 and 60 min of exposure. In contrast, the exposure concentration of 1.6 and 3.2 mg/L β -cyfluthrin decreased THC in *D. koenigii* by 1.4-3.4-fold (1540 cell/mm³, 3810 cell/mm³) at 0 min, subsequently increased by 1.2-fold (6475 cell/mm³, 6545 cell/mm³) after 30 min and again decreased by 2.0-2.9-fold (2635 cell/mm³, 1815 cell/mm³) after 60 min. These results indicate the latent effects of the higher dosages of β -cyfluthrin which though initially were less effective in comparison to the lower dosages but imparted much more effects as the treatment duration increased.

The continued decrease in the haemocyte count in *D. koenigii* after the application of 6.4 and 12.8 mg/L β -cyfluthrin; 4295 cell/mm³, 3655 cell/mm³, respectively at 0 min (2250 cell/mm³, 3285 cell/mm³) after 30 min and (2075 cell/mm³ and 2110 cell/mm³) 60 min was alike to the pattern observed with the treatment of *D. koenigii* with imidacloprid 20SL (Sarwar et al, 2018). Comparable results have been observed in *D. koenigii* adults on exposure to another pyrethroid, deltamethrin @250 mL/acre (Sarwar et al, 2018). They recorded a significant reduction in THC by 32.8% at 0 min followed by a gradual increase in the count by 3.3%, and 8.9% after 30 and 60 min of deltamethrin treatment. The contrary counts have been recorded by Rizwan-ul-Haq et al (2005) on the application of imidacloprid 25 WP to adult *D. koenigii* demonstrating an initial decrease in THC which continued to decrease till 30 min but then increased after 60 min of exposure. Nevertheless, treatment with another neonicotinoid, acetamiprid

20% SL, increased the total haemocyte count immediately, while decreased after 30 min and increased again after one hour.

Several other reports in other insects have revealed different patterns in total haemocyte count on treatment with xenobiotics. Kumar et al (2019) showed decreased haemocyte count in *D. koenigii* after treatment with *Aspergillus niger* while Fatima et al (2016) noticed increased total haemocytes count (20650 cell/mm³, 10222 cell/mm³) just after the application of thiacloprid and imidacloprid in 5th instar larvae of *Helicoverpa armigera*. The application of carbamates and pyrethroids on *Tryporyza* sp. and *Schistocera gregaria* Forsk induced an immediate rise in the total haemocyte count (Alhariri & Suhail, 2001).

It has been reported that the number of haemocytes in insects fluctuates depending upon their immunity level and thus indicates the response to the external insecticidal stress (Perveen & Ahmad, 2017). An instant decline in the haemocyte count of *D. koenigii* on β -cyfluthrin treatment suggest the decreased defensive action of haemocytes due to stress induced by β -cyfluthrin. However, as the exposure time increased, the haemocytes multiplied gradually to combat the induced stress. It is proposed that the higher dosages of β -cyfluthrin could not raise the haemocyte count considerably probably because of the toxic effects leading to the higher nymphal mortality. The reports have revealed that haemocyte count in insects responds to different insecticides variably leading to enhanced immunity; since they differ in their mitotic division under normal conditions (Perveen & Ahmad, 2017).

The investigations on the differential haemocytes count in *D. koenigii* showed five types of haemocytes; phagocytic granulocytes - GR (55%), adhering plasmatocytes - PL (18%), prohaemocytes - PR (16%), phenoloxidase-containing oenocytes - OE (11%), and refractile spherulocytes – SP (0%); in order of their decreasing abundance. Similar proportion of haemocytes were observed by Mannakkara (2022), in rice brown planthopper (*Nilaparvata lugens*) development stages revealing PLs, GRs and PRs as the most abundant cells in the haemolymph while SPs being the scarcest. In comparison, Rizwan-ul-Haq et al (2005) reported plasmatocytes as the most abundant cells (39.75%) in adult *D. koenigii* followed by 32% GR, 22% PR, 4.25% OE and 2% SP.

Current studies showed differential effects on different haemocytes of *D. koenigii* on exposure to β -cyfluthrin which increased the prohaemocytes% while reduced GR% and PL% at higher dosages. On the other hand, the impact of β -cyfluthrin on SP and OE was inconsistent at different concentrations. Comparable results were reported in *D. koenigii* 5th nymphal instar on exposure to juvenile-hormone analogue farnesol (Kumar, Shazad, Kayesth & Gupta, 2022). They observed an increase in percent of prohaemocytes in farnesol-treated nymphs at the dosages ranging from 0.05 μ g/ μ L to 0.2 μ g/ μ L.

Alike to our results, Sarwar et al (2018) demonstrated reduced count of granulocytes in adult *D. koenigii* after the application of three pyrethroids; deltamethrin, lambda-cyhalothrin and cyfluthrin 20EC. Likewise, imidacloprid 25 WP exposure alleviated plasmatocytes, granulocytes, oenocytes, spherulocytes to 28.25%, 24.50%,

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2.25%, and 1.25%, respectively from 39.75%, 32%, 4.25% and 2%; while increased prohaemocyte from 22% to 32.75% in adult *D. koenigii* (Rizwan-ul-Haq et al, 2005). In contrast, the acetamiprid 20% SL exposure reduced the PL (35%), GR (23%) and PR (18.25%); while rest two haemocytes, OE and SP rose to 7.5% and 3.5%, respectively (Rizwan-ul-Haq et al, 2005), alike to the results when *D. koenigii* was exposed to 12.8 mg/L β -cyfluthrin which reduced the GR (30%) and PL (15%) while increased the PR (48%) counts.

A rapid decline in all cell types with total elimination of prohaemocytes while the continuous reduction in granulocytes and plasmatocytes was reported in *D. koenigii* after topical application of plumbagin (Saxena & Tikku, 1990). Comparable results were demonstrated by Kumar et al (2019) in *D. koenigii* on exposure to *A. niger* reporting granulocytes and plasmatocytes as the most negatively affected cells with a drastic reduction in comparison to control. Teleb (2011) reported an increased percentage of prohaemocytes, oenocytes, plasmatocytes and granulocytes in *S. gregaria* on the application of Nomolt® (Teflubenzuron), while the reduced number of spherulocytes.

Plasmatocytes and granulocytes are considered the main haemocytes in cell-mediated immunity being an active participants in the recognition of foreign agents while the rest of the haemocyte types interact with them and contribute to the immune response (Kwon, Bang, & Cho, 2014). Major immune functions in Lepidoptera and some Coleoptera are also imparted by granulocytes and plasmatocytes by encapsulation and phagocytosis of xenobiotic agents (Manachini, Arizza, Parrinello, & Parrinello, 2011; Lavine & Strand, 2002). The fluctuating number of the haemocytes indicates the immunity response in *D. koenigii* on exposure to β -cyfluthrin. The decrease in granulocyte count in *D. koenigii* indicates the probable active participation of the primary haemocytes in countering the action of xenobiotic. On the other hand, an increase in prohaemocyte due to β -cyfluthrin exposure may be attributed to their stem cell property leading to active mitotic divisions as an immune response caused by xenobiotic stress. Further investigations may help in deciphering the mechanisms associated with the impact of β -cyfluthrin on the haemocytes of *D. koenigii* which may assist in the formulation of strategies to optimize the dosage of toxicant with increased efficacy in the fields.

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CONFLICT OF INTEREST

We declare that there is no conflict of interest.

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