Advances in Biological Sciences Research, volume 8
International Conference and the 10th Congress of the Entomological Society of Indonesia (ICCESI 2019)

Assessment of Toxicity and Growth Regulatory Effects of Beta-Cyfluthrin Against Red Cotton Bug, *Dysdercus koenigii* (Fabr.) (Hemiptera: Pyrrhocoridae): An Emerging Cotton Pest

P Lanbiliu[1*], Roopa Rani Samal[1], Kungreiliu Panmei[1], Sarita Kumar[1]

[1] *Department of Zoology, Acharya Narendra Dev College, University of Delhi, Govindpuri, New Delhi 110019, India*
[*] *Corresponding author. Email: lanbiliupans@gmail.com*

ABSTRACT
Red Cotton Bug, *Dysdercus koenigii* (Fabr.) (Hemiptera:Pyrrhocoridae), commonly called cotton stainer, is a damaging pest of cotton and other economical crops in Asia. Nymphs as well as adult of this pest suck the sap from the green bolls and leaves of cotton causing shedding of young bolls, rotting of green bolls, stained cotton fibers and loss of seed viability. The present study evaluates the toxic and growth regulatory effects of a pyrethroid, beta-cyfluthrin against *D. koenigii*. The newly emerged fifth instars nymphs were exposed to beta-cyfluthrin at concentration ranging from 0.00008% to 0.00128%. A volume of 1µL of beta-cyfluthrin was topically applied on the dorsal anterior thoracic region of nymphs (in 3 replicates, each replicate containing batch of 25 insects) and were observed for mortality after 24 hr. The nymphs were further reared till adults to observe delayed toxicity effects and developmental abnormalities, if any. The result revealed significant lethal effects of beta-cyfluthrin on *D. koenigii* nymphs with LD_{50} and LD_{70} values as 0.00051% and 0.00076%, respectively. A positive correlation was observed between percent nymphal mortality of *D. koenigii* and the dose of insecticide. The survived nymphal instars developed several development malformations; partial moulting, shrunk abdomen, abnormal adults with deformed wing, adultoids and adults with attached exuviae. Further studies are being conducted to assess the development of beta-cyfluthrin resistance in *D. koenigii* and strategies to counter resistance. These results can provide an important base for developing effective and desired strategies to control and monitor insecticides resistance in *D. koenigii.*

***Keywords**:* Dysdercus koenigii, *mortality, beta-cyfluthrin, adultoids, developmental abnormalities*

1. INTRODUCTION

Cotton is one of the major fibres and cash crops grown not only in India but also throughout the world. It plays an important role in the economic growth of industries and agriculture sector of the country. India with approximately 12 million hectares land under cotton production is one of the largest producers of cotton in the world [1] which accounts for 27% of the world cotton production. Around 10 million farmers are engaged in the production of cotton; while about 30 million individuals are employed in cotton industry, its processing and final production [2]. However, the cotton cultivation faces severe pest attacked resulting in low production and major losses. Although worldwide, 1326 species of insect pests have been reported on this crop, it is known to be susceptible to about 162 species [3]. Among these pests leading to the low cotton produce, the enormous attack of sucking insect pests plays the significant role [4]. In addition, the large-scale introduction of *Bt*-transgenic cotton to reduce the usage of insecticides against other cotton pests; *Helicoverpa armigera*, *Earias spp*., and *Pectinophora gossypiella* [5]; has led to the emergence of sucking pest; *Dysdercus koenigii* [6].

D. koenigii is one of the most notorious bugs of cotton. Also known as red cotton stainer; it belongs to order Hemiptera, family Pyrrhocoridae, and is a disastrous cotton pest in several parts of Asia [7, 8]. Both nymph and adults feed on developing cotton bolls and seeds within them resulting in diminished oil quantity and viability of the seeds [9]. Furthermore, adult excreta stain the cotton yellow that adversely affects the colour of cotton lint. The infested cotton bolls become prone to fungal and bacterial infections, which make their way into the bolls through the punctures made by insects while feeding [10]. The rapid multiplication of the pest in the fields due to short life cycle in comparison to lepidopteran pests aggravates its seriousness [11].

Currently, chemical insecticides are the key tools to manage insect pests in almost all cropping systems around the world [12]. Thus, like other pests, *D. koenigii* has also been controlled by using various chemical globally. Since last few decades, pyrethroids, an important group of synthetic insecticide are commonly used as control agents of many arthropod pests, including *D. koenigii* [13]. They are sodium channel modulators [14] and cause adverse effects on the insect nervous system. Beta-cyfluthrin is a common household pyrethroid with considerable efficacy against a wide variety of agricultural pests. It acts as a contact as well as stomach poison resulting in a rapid and long-lasting knock-down effect.

Cotton production is, thus heavily associated with the enormous uses of hazardous insecticides for the quick and easy management of these insect pests. However, the increased use of these insecticides has not only caused environmental risks but also aggravated the pest problem due to the development of insecticide resistance in many cotton pests which includes sweet potato whitefly, *Bemisia tabaci* [15]; jassid, *Amrasca devastans* [13]; American bollworm, *Helicoverpa armigera* [16]; dusky cotton bug, *Oxycarenus hyalinipennis* [17] and beet armyworm, *Spodoptera exigua* [18].

Thus, keeping in view the economic importance of this pest, focus of our works was to investigate the effects of synthetic pyrethroid, beta-cyfluthrin on the survival, growth and developments of laboratory-bred *D. koenigii*.

1.1. Materials and Methods

1.1.1. *Rearing and maintenance of stock culture of* Dysdercus koenigii

The *D. koenigii* adults were procured from Insect Reproduction Laboratory, Deshbandhu College, University of Delhi, India. The insects were maintained in BOD at a temperature of 26± 2°C, RH (Relative humidity) of 70±5% and photoperiod of 10L: 14D. They were reared in glass jar measuring 1L containing sterilised cottonseeds and cotton swabs soaked in distilled water. In order to prevent the exit of insects, the jars were covered with muslin cloth which was tied tightly by rubber band. The eggs laid, in batches of 100-120 eggs, beneath the cotton seeds were collected in small container. The newly hatched first instar nymphs were shifted to another jar containing sterilised cottonseeds and water-soaked cotton. The seeds were changed on alternate days while the jar was replaced by a new clean jar twice in a week to maintain healthy and hygienic conditions and prevent infections. Over-crowding of insects was avoided for adequate and healthy growth. The newly emerged (0-12 hr old) fifth instar nymphs were drawn from the culture and used for the investigations.

1.1.2. *Preparation of different concentrations of the insecticide*

The chemical name of beta-cyfluthrin is (RS)-α-Cyano-4-fluoro-3-phenoxybenzyl(1RS)-cis-trans-3 (2,2 dichlorovi nyl) -2,2- dimethyl cyclo propane carboxylate. Technical beta-cyfluthrin (≥98.0%) was obtained from M/s Sigma Aldrich. The empirical formula is $C_{22}H_{18}C_{l1}2FNO_3$.

Six different concentrations of beta-cyfluthrin ranging from 0.00008% to 0.00128% were prepared from 1% stock solution of beta-cyfluthrin by serial dilution in acetone. The stock solution was stored in the refrigerator at 4°C for future use.

1.1.3. *Topical application of beta-cyfluthrin on 5^{th} instars nymphs of* Dysdercus koenigii

Freshly moult 5^{th} instars nymphs were sorted out from the mass rearing culture for the experiment. A total of 75 insects were treated with beta-cyfluthrin in three batches, each consisting of 25 nymphs. A volume of 1μL of each concentration was topically applied on the dorsal anterior thoracic part of 5^{th} instars nymphs. After 24 hr of the treatment, nymphal mortality and the abnormality in moulting and metamorphosis were recorded. Parallel control set was also maintained for the comparison. Nymphs unable to move and co-ordinate their locomotory movement; with soft touch of a fine hair brush; were counted as dead. The experiment was repeated 3 times.

1.1.4. *Statistical analysis*

The mortality data obtained in the studies were corrected by the formula of Abbott [19] and analysed with SPSS 22.0. The empirical LD values along with 95% confidence limits (CLs)were determined by Probit analysis.

1.2. Our Contribution

The insecticides are considered the most reliable and effective agents for insect pests management, despite availability of numerous novel control agents. These chemicals are expected to continue as one of the powerful tools for the farmers. The red cotton bug, *D. koenigii*, is a major pest of cotton across the South East Asia and is managed mainly through chemical control. Limited studies have been carried out regarding the effect of beta-cyfluthrin on cotton stainer. Therefore, current research attempts to understand the possible use of beta-cyfluthrin to control *D. koenigii* as a toxicant as well as growth regulator. The study can help in devising control strategies in the pest management program.

1.3. Paper Structure

The rest of the paper is organized as follows. Section 2 explains the result obtained describing the effect of topical application of beta-cyfluthrin on *D. koenigii* under laboratory conditions. The results are discussed adequately and compared with the similar works done elsewhere.

Section 3 concludes the paper, justifies the current study and presents directions for future research.

2. RESULTS AND DISCUSSION

2.1. Effects of beta-cyfluthrin on Percent Mortality of 5th Instars Nymph of Dysdercus koenigii

Current studies were carried out to check the susceptibility of red cotton bug to beta-cyfluthrin in laboratory conditions. The nymphal mortality caused by the topical application of different concentrations of beta-cyfluthrin on 5th instar nymphs of *D. koenigii* is presented in Table 1. The topical application of 0.00128% beta-cyfluthrin resulted in 86.64% nymphal mortality while the lowest concentration applied (0.00008%) induced only 2.64% mortality after 24 hr of the treatment. The nymphal mortality of *D. koenigii* was found to be dose-dependent when assayed with the topical application of beta-cyfluthrin. The LD_{50} and LD_{70} was measured to be 0.00051% and 0.00076%. Figure 1 depicts the relationship between beta-cyfluthrin concentration and percent nymphal mortality of 5th instars nymphs of *D. koenigii* revealing a positive linear correlation ($y = 74998x - 0.1326$, $R^2 = 0.9068$).

The survived nymphal instar were kept under observation to assess the delayed toxic effect of beta-cyfluthrin. It was observed that the survived nymph developed into adult with different malformation and later died. The malformation includes shrunk abdomen, reduced in the size of forewings and hindwings as compared to the normal adult, crumpled wings, and incomplete moulting in nymphs as well as adults characterized by old exuviae found attached to the abdominal end of nymphs and the lower region of adults (Figure 2).

Susceptibility of the nymphs decreased with decrease in the concentration of the insecticide applied. Beta-cyfluthrin is reported to act on the insect nervous system as sodium channel blocker resulting in rapid excitation and impairment of coordination followed by knockdown and mortality in the target pest. The LD_{50} values for beta-cyfluthrin obtained against *D. koenigii* in the current study are close to the respective values of 0.0069%, 0.0452% and 0.0787% reported with chlorpyrifos, lambda-cyhalothrin and neem extract [20]. Field population of *D. koenigii* has been observed to remain susceptible to deltamethrin and lambda-cyhalothrin [6].

Similar studies have been conducted by Saeed *et al.* [21] who observed 88% and 84% mortality in fifth instar nymphs of *D. koenigii* when exposed to 100µg/mL deltamethrin and 2µg/mL chlorpyrifos, respectively for 24 hr; and obtained LC_{50} values of 15.99µg/mL and 0.42µg/mL. In another study, exposure of *D. koenigii* to 0.1% andalin delayed the metamorphosis and inhibited the emergence of normal adults due to partial wing development and abnormal moulting [22]. Sharma *et al*. [23] had also reported 75% mortality in *Dysdercus cingulatus* adults treated with 1.0% neem seed kernel, whereas just 5% mortality was recorded with exposure to yellow neem seed coat at 0.005%. Kodandaram *et al*. [24] investigated the lethality of various botanicals on third instar nymphs of *D. koenigii* and recorded a positive correlation between their dosage, nymphal mortality and altered metamorphosis.

Table 1 Percent mean mortality of 5th instar nymphs of *Dysdercus koenigii* applied topically with different concentrations of beta-cyfluthrin

Dose (%)	**Total Nymphs treated**	**Total nymphal mortality after 24 hr± SEM**	**% Nymphal mortality after 24 hr**	**Total number of adults emerged± SEM**	**Deformed adults emerged± SEM**	**Normal adults formed± SEM**
0.00128	25	21.66±0.88 d	86.64	1.6±1.0 e_1	1.6±1.0 a_2	0.00±0.00 e_3
0.00064	25	17.66±1.45 d	70.64	5.3±0.33 d_1	4.6±0.33 b_2	0.66±0.33 d_3
0.00032	25	4.66±0.33 c	18.64	17±1.73 c_1	3.66±0.66 a_2 b_2	13.33±1.85 c_3
0.00016	25	1.66±0.33 b	6.64	23.33±0.33 b_1	2.66±0.66 a_2	20.66±0.33 b_3
0.00008	25	0.66±0.33 b	2.64	24.33±0.33 b_1	1±0.33 a_2	24±0.00 a_3
Control	25	0.00±0.00 a	0.00	24.66±0.33 a_1	0.66±0.33 a_2	24.33±0.66 a_3

Notes: 75 insects treated in 3 replicates of 25 each.

Figures 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; SEM: Standard error of Mean.

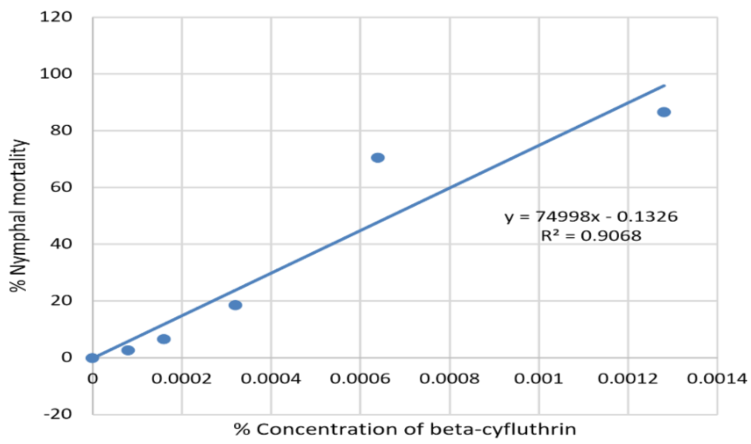

Figure 1 Percent nymphal mortality obtained on topical application of different dosages of beta-cyfluthrin on the 5th instar nymphs of *Dysdercus koenigii*

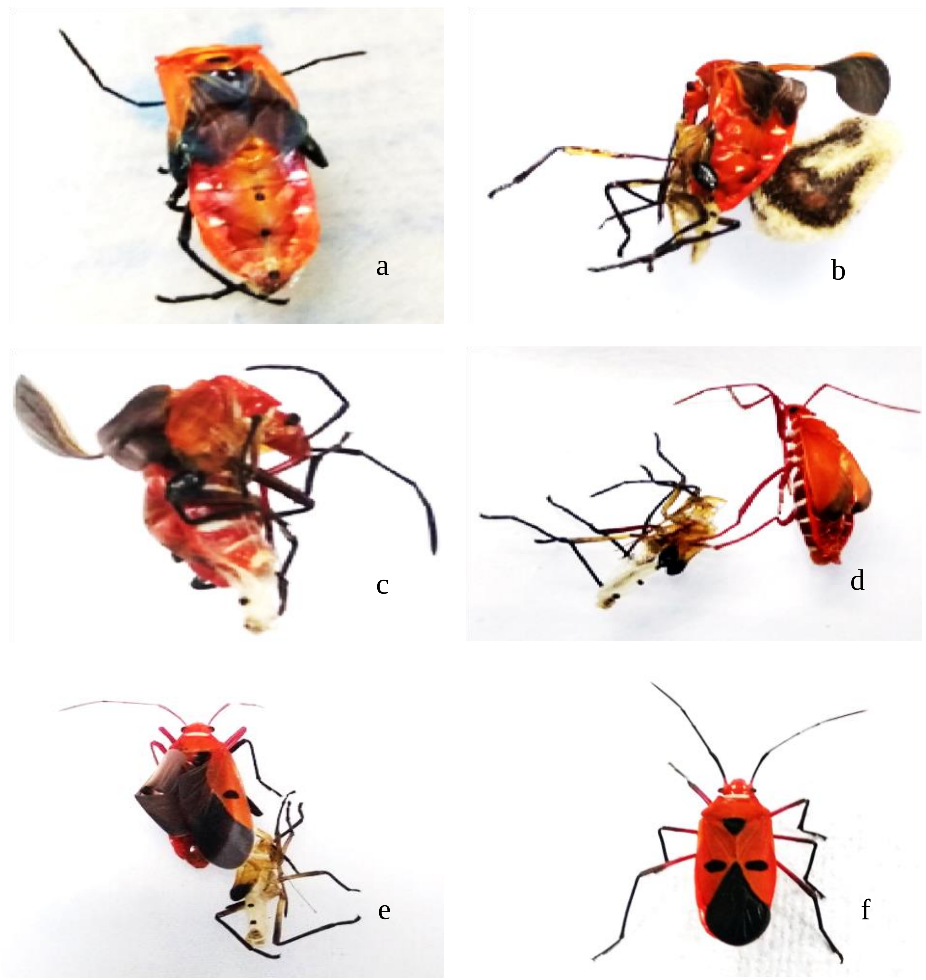

Figure 2 Adults emerged from the 5th instars nymphs of *Dysdercus koenigii* exposed to beta-cyfluthrin; (a-e) malformed adults; (f) control adult

3. CONCLUSION

The current study showed that topical application of beta-cyfluthrin on 5th instar nymphs of *D. koenigii* caused mortality of insect with various morphological and physiological abnormalities. The LD_{50} value of beta-cyfluthrin determined in the current study can help to assess its control potential and estimate safety aspects in the fields in order to develop a safe management program against *D. koenigii*. However, field studies are recommended to ascertain its possible use and devise a control strategy.

ACKNOWLEDGMENT

The authors thank Council of Scientific and Industrial Research (CSIR), New Delhi for financial assistance provided for conducting the investigations. The authors are also grateful to Principal, Acharya Narendra Dev College for continuous support and research facilities.

REFERENCES

[1] S. Niranjan, G. Balaganesh, A. Jamaludheen, An analysis of trend in production, consumption and trade of cotton in India, Internat. Res. J. Agric. Eco. Stat. 8(2) (2017) 293-298. DOI:10.15740/HAS/IRJAES/8.2/ 293-298

[2] M.V. Venugopalan, M.S. Yadav, V. Satish, Institutional structure of cotton research in India, ICAR-Central Institute for Cotton Research p 19-43. DOI: 10.5772/63798

[3] N. Ahmad, M.H. Khan, M. Toufique, I. Rauf, Insect pest management of BT cotton through manipulation of different eco-friendly technique, Nucleus 48(3) (2011) 249-254.

[4] M. Aslam, M. Razaq, S.A. Shah, F. Ahmad, Comparative efficacy of different insecticides against sucking pests of cotton, J. Res. Sci. 15(1) (2004) 53-58.

[5] M.K. Dhillon, G.T. Gujar, V. Kalia, Impact of *Bt* cotton on insect biodiversity in cotton ecosystem in India, Pak. Entomol. 33(2) (2011) 161-165.

[6] R. Saeed, N. Abbass, M. Razad, Z. Mahmood, M. Naveed, H.M.U. Rehman, Field evolved resistance to pyrethroids, neonicotinoids and biopesticides in *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae) from Punjab, Pakistan, Chemosphere 213(2018) 149-155. DOI:https://doi.org/10.1016/j.chemosphere.2018.09.042

[7] H.S. Varma, R.K. Patel, Biology of red cotton bug (*D. koenigii*), Agres - An Int. e-journal 1 (2012) pp 148-156.

[8] W. Jaleel, S. Saeed, M.N. Naqqash, Biology and bionomics of *Dysdercus koenigii* F. (Hemiptera: Pyrrhocoridae) under laboratory conditions, Pak. J. Agri. Sci. 50(3) (2013) 373-378.

[9] R.K. Sprenkel, Cotton plant and pest monitoring manual for Florida, Florida, 2000, pp 1-24.

[10] C.W. Schaefer, I. Ahmad, Cotton stainers and their relatives (Pyrrhocoroidea: Pyrrhocoridae and Largidae). *Heteroptera of Economic Importance*, Boca Raton (eds): CRC Press, 2000 pp. 271-307. DOI: https://doi.org/10. 1201/9781420041859.ch8

[11] K.J. Venugopal, D. Kumar, A.K. Singh, Developmentl studies on proteins from haemolymph, fat body, and ovary of a phytophagus bug, *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae). Biochem. Arch. 10(4) (1994) 297-302

[12] C. Yang, L. Xu, D. Yang, Effects of nitrogen fertilizer on the *Bt* protein content in transgenic cotton and nitrogen metabolism mechanism. Cotton Sci. 17(2005) 227-231

[13] R. Saeed, M. Razad, N. Abbas, M.T. Jan, M. Naveed, Toxicity and resistance of the cotton leaf hopper, *Amrasca devastans* (Distant) to neonicotinoid insecticides in Punjab, Pakistan, Crop Prot. 93(2017) 143-147. DOI: http://dx.doi.org/10.1016/j.cropro.2016. 11.032

[14] IRAC, 2017, https://www.irac-online.org/about/ resistance/mechanisms/

[15] M. Basit, S. Saeed, M.A. Saleem, I. Denholm, M. Shah, Detection of resistance, cross-resistance, and stability of resistance to new chemistry insecticides in *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. 108(2013) 1414-1422. DOI: https://doi.org/ 10.1603/ EC12414

[16] M.A. Qayyum, W. Wakil, M.J. Arif, N.A. Sahi, D.A. Russell, Multiple resistance against formulated organophospahtes, pyrethroids, and newer-chemistry insecticidess in populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Pakistan. J. Econ. Entomol. 108(1) (2015) 286-293. DOI: https://doi.org/ 10.1093 /jee/tou037

[17] S. Ullah, S.A. Shad, N. Abbas, Resistance of dusky cotton bug, *Oxycarenus hyalinipennis* Costa (Lygaidae: Hemiptera), to conventional and novel

chemistry insecticides, J. Econ. Entomol. 109(1) (2015)345-351. DOI:https://doi.org/10.1093/jee/tov324

[18] M. Ahmad, A. Farid, M. Saeed, Resistance to new insecticides and their synergism in *Spodoptera exigua* (Lepidoptera: Noctuidae) from Pakistan, Crop Prot. 107(2018) 79-86. DOI: https://doi.org/10.1016/ j.cropro.2017.12.028

[19] W.S. Abbott, A method of computing the effectiveness of an insecticides, J. Econ. Entomol. 18(2) (1925) 265-267. DOI: https://doi.org/10.1093/jee/ 18.2.265a

[20] M.J. Yousuf, M. Attaullah, I. Anjum, S. Khawaja, Toxicity assessment of chlorpyrifos, λ-cyhalothrin and neem extract against *Dysdercus koenigii* with reference to survivorship, fecundity and some biochemical parameters. J. Pharm. 2(2012) 44-52. DOI: https://doi. org/10.9790/3013-25604452

[21] S. Saeed, M.N. Naqqash, W. Jaleel, Toxicological studies on some important chemical against *Dysdercus koenigii* Fabr. (Hemiptera: Pyrrhocoridae), Pak. J. Zool. 48(5) (2016) 1249-1254

[22] I. Khan, A. Qamar, Biological activity of andalin (Flucycloxuron), a novel chitin sysnthesis inhibitor on red cotton stainer *Dysdercus koenigii* (Fabricius), Biol. Med. 3(2) (2011) 324-335

[23] T. Sharma, A. Qama, K. Mustafa, Evaluation of neem (*Azadirachta indica*) extracts against the eggs and adults of *Dysdercus cingulatus* (Fabricius). Wld. Appl. Sci. J. 9(4) (2010) 398402

[24] M.H. Kodandaram, N.A. Thakur, A.N. Shylesha, Toxicity and morphogenetic effects of different botanicals on red cotton bug *Dysdercus koenigii* Fab. (Hemiptera: Pyrrhocoridae) in North Eastern Hill (NEH) region of India. J. Biopest. 1(2) (2008) 187-189