



Neurohistological evaluation of alphamethrin-induced lesions in the brain and thoracic ganglia of *Dysdercus koenigii*

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DOI: <https://doi.org/10.66856/ijer.2026.11.4.11367>

Abstract

Dysdercus koenigii, commonly known as the red cotton stainer, is a devastating pest of cotton. It causes severe damage to cotton cultivation across various states of India. This study aimed to investigate the insecticidal-induced lesions in the central nervous system of *Dysdercus koenigii* using light microscopy. An acute toxicity test was performed to determine the median lethal concentration (LC₅₀) of Alphamethrin in *Dysdercus koenigii*. LC₅₀ was calculated to be 568 µg a.i./L after 24 h exposure. The sublethal dose, LC₂₀, 146 µg a.i./L, was selected for histological studies. Neurohistological lesions were examined after 24 h and 48 h of exposure to this sublethal dose. Considerable changes including neuropile disorganization, increased vacuolation, pyknotic nuclei, neuronal degeneration and striated border disorientation were observed in the treated ganglia. However, in brain, slight cytomorphological changes like pyknotic nuclei was observed after 24 h and 48 h of exposure. Thus, our study demonstrated the action of alphamethrin causes physiological abnormalities in the central nervous system of *Dysdercus koenigii*.

Keywords: *Dysdercus koenigii*, alphamethrin, neurotoxicity, histology, thoracic ganglion, brain

Introduction

Cotton (*Gossypium spp.*) is the most important natural fiber crop cultivated in India. It plays major role in India's economy by providing domestic textile needs as well as export. It provides employment to 60 million people related to it directly or indirectly. Despite large acreage, low average yield of cotton productivity is accounted due to many reasons among which insect pest infestation is the most vital ^[1]. Various pest of cotton has been associated to cotton crop in India, among which the particularly most damaging one is *Dysdercus koenigii*. *D. koenigii* is the most atrocious pest of cotton commonly known as Red Cotton Stainer. It is also associated with malvaceous plants like Okra, Hibiscus and Jute but cotton being the most economically important host. It damages the cotton crops either by feeding on the seeds and staining them through their excreta or by transmitting cotton staining fungi, *Nematospora gossypi*. This affects the quality of cotton bolls as well as reducing the moisture and oil of cotton leaves and seeds. This leads to the reduction of cotton production extensively. There are several methods to control pest infestations but chemical application is the most predominant and preferred method widely used for it. Chemical control includes the usage of insecticides which are cheap and easily available. Chemical insecticides are widely used due to their rapid action and toxic effects ^[2]. But their application poses threat to non-target organisms. In order to use these insecticides as a method of insect control, effective and less hazardous application of these chemicals be used. Since a last few decades, pyrethroids, an important group of insecticides is widely used in insect control. Pyrethroids are synthetic insecticides modeled after natural compounds found in *Chrysanthemum* flowers, and they have become a primary global tool for pest management. It mainly targets the nervous system of insects by disrupting

sodium channels ^[3]. Alpha-cypermethrin also called alphamethrin is found most effective and gave maximum mortality of *Dysdercus koenigii* in field study ^[4].

Despite the established neurotoxic activity of pyrethroids, the relationship between alphamethrin exposure and structural damage to the insect nervous system remains poorly studied in *D. koenigii*. Most available toxic assessment rely on mortality or physiological endpoints, providing limited information on the cellular consequences of exposure. Thus, there is a need for an integrated assessment linking alphamethrin toxicity with alterations in the organization of the neural tissues. To address these knowledge gaps, we assessed the acute and chronic toxicity for alphamethrin in nymphs of red cotton bug *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae). This study includes toxicity assessment and histological parameters of the brain and thoracic ganglia of *Dysdercus koenigii*.

Materials and methods

Rearing of *Dysdercus koenigii*

Nymphs of *Dysdercus koenigii* were collected from okra and cotton fields near the campus of Aligarh Muslim University, Aligarh, Uttar Pradesh, India, and reared under laboratory conditions. Insects were kept in a glass rearing jar (20 cm × 15 cm) containing a thick layer of damp, sterilized sand at the bottom. Cultures were maintained at 27±2 °C, 60±5 % relative humidity, and a 10L: 14D Photoperiod in a BOD (Bio-Oxygen Demand) incubator. Nymphs were fed with soaked cotton seeds (soaked for 3 hours in boiling water) and subsequently placed on a sterilized Petri dish. The diet was changed daily, and sand was replaced weekly to maintain hygiene and prevent any microbial contamination. The newly emerged fifth-instar nymphs were used for the investigations.

Acute toxicity bioassay

Dose-dependent response

Preliminary experiments were conducted to determine the Median Lethal Concentration (LC₅₀) values of Alphanemethrin 10% EC using the probit analysis method proposed by Finney (1971). Ten insects were used per concentration, and experiments were replicated 3 times. The LC₅₀ value was calculated to be 568 µg a.i./L. The nymphal mortality at different concentrations and the control group were recorded at 24 h treatment. Moribund nymphs were also counted. The percentage of mortality was calculated using this formula.

$$\text{Mortality \%} = \frac{\text{Number of dead insects}}{\text{Total number of treated insects}} \times 100$$

Control mortality was corrected when necessary, using Abbott's formula (1925). To further investigate the toxic effect of alphanemethrin, fifth-instar *Dysdercus koenigii* were treated with sublethal dose. Concentration-mortality analysis was performed using Origin lab 2026b. Two-way repeated measure ANOVA used to compare the means of the different groups, the post- hoc Tukey's test was used to analyse where the specific difference lies.

Histological Analysis

Treatment Procedure

Fifth instars were separated into rearing jars containing 10 insects per jar, with 3 replicates per concentration. The control group was fed overnight-soaked cotton seeds. The test group was fed treated cotton seeds soaked in 146 µg a.i./L which is sublethal dose (LC₂₀). The sublethal dose was prepared in distilled water.

Histological Processing and Staining

Fifth instars were dissected to expose the brain and ganglia in saline (0.7% N NaCl) after 24 h and 48 h of exposure, with a control group. The dissection was performed under a stereo microscope. The dissected ganglia and brain were transferred to Bouin's fixative and fixed for 11 h and 48 h, respectively. The biological specimens were washed in distilled water, dehydrated in increasing grades of alcohol (30% to 95%), infiltrated with xylene and wax at a 1:1 ratio, and embedded in paraffin wax. Blocks of brain and ganglia were later sectioned at a thickness of 12µm and 5 µm, respectively. Sections were placed on a glass slide, stained with hematoxylin and eosin, and mounted with DPX. The

images were captured with an Olympus CX41 microscope at 10x, 20x and 40x magnification.

Results

Effect of Alphanemethrin on nymphal mortality

Alphanemethrin exhibited concentration- and time-dependent toxicity against *Dysdercus koenigii* nymphs. After 24 h exposure, mortality increased from 10.0% at the lowest concentration (100 µg a.i. /L) to 96% at the highest concentration (10000 µg a.i./L) (Table 1). Prolonged exposure to 48 h resulted in increased mortality across all concentrations, with complete mortality (100%) observed at 10000 µg a.i./L of Alphanemethrin. The increase in mortality between 24 h and 48 h indicates delayed toxic action and progressive susceptibility of nymphs to alphanemethrin exposure.

Table 1: Mortality of *Dysdercus koenigii* nymphs exposed to different concentrations of alphanemethrin after 24 h and 48 h. Values are expressed as mean mortality (%) ± SEM (n = 3)

Alphanemethrin concentration (µg a.i./L)	24 h mortality (%)	48 h mortality (%)
Control	0.00 ± 0.00	0.00 ± 0.00
100	10.00 ± 5.78	26.67 ± 3.33
500	46.67 ± 3.33	66.67 ± 3.33
1000	56.67 ± 3.33	70.00 ± 5.78
5000	83.33 ± 3.33	93.33 ± 6.67
10000	96.00 ± 3.00	96.00 ± 3.00

Histological studies

Brain

The brain of *Dysdercus koenigii* nymphs shows the usual morphology a well-organized and compact neuropile architecture, intact cell bodies of neuron with well-defined boundary in control. The protocerebrum and deutocerebrum shows well organized neural lamella and intact neuropilar arrangement in control (Figure 1-A) (Figure 2- D & G) (Figure 3- J & M). However, noticeable lesions were seen in the neuropilar organization and increased vacuolation with disorganization of cellular boundary and pyknotic nuclei in the treatment group (Figure 2- E & F and H & I). The neuronal cells appeared irregularly organized and distortion of the cellular membrane look more with the increase in exposure time period (Figure 3- K & L and N & O). The treatment with alphanemethrin caused disruption of the cerebral architecture of brain.

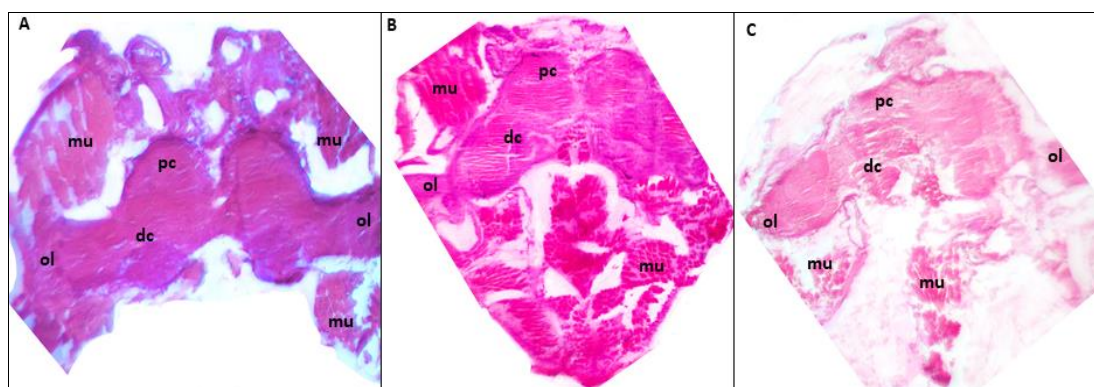


Fig 1: Histological sections of *Dysdercus koenigii* fifth instars brain stained with hematoxylin and eosin. (A) Whole brain section (Control). (B) 24 h exposure to alphanemethrin at 146 µg a.i./L. (C) 48 h exposure to alphanemethrin at 146 µg a.i./L. A to C images are photographed at 10x magnification

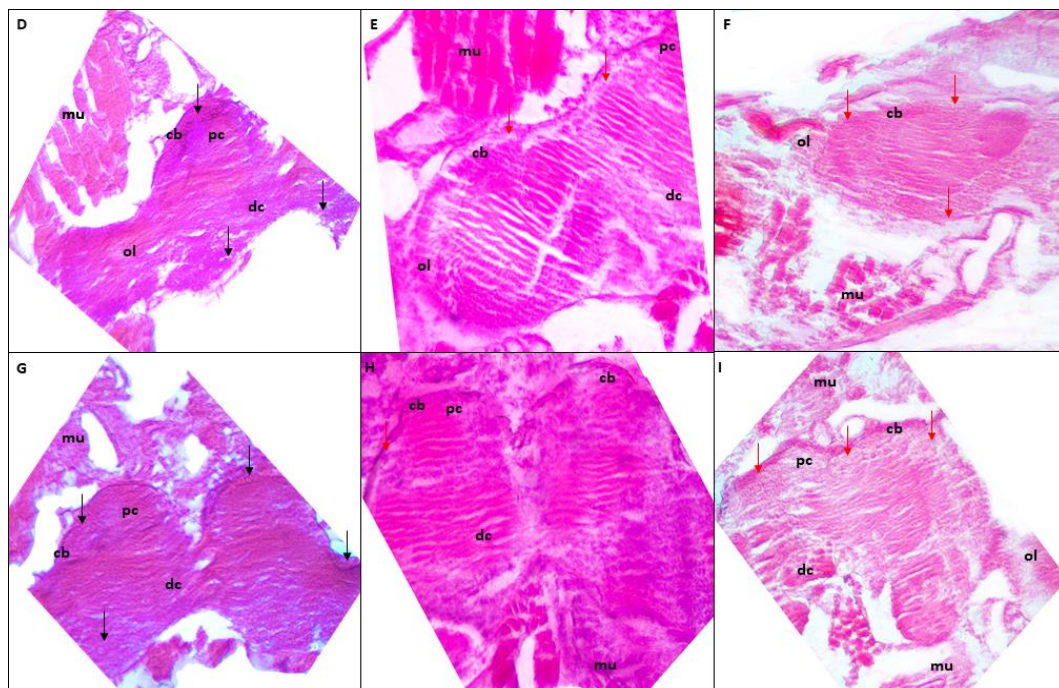


Fig 2: Histological sections of *Dysdercus koenigii* fifth instars brain stained with hematoxylin and eosin. (D) Optic lobe with protocerebrum (Control). (E) 24 h exposure to alphamethrin at 146 μg a.i./L. (F) 48 h exposure to alphamethrin at 146 μg a.i./L. (G) Protocerebral bridge with deutocerebrum (Control). (H) 24 h exposure to alphamethrin at 146 μg a.i./L. (I) 48 h exposure to alphamethrin at 146 μg a.i./L. D to I images are photographed at 20x magnification. Arrow-Red- Indicates the pyknotic nucleus.

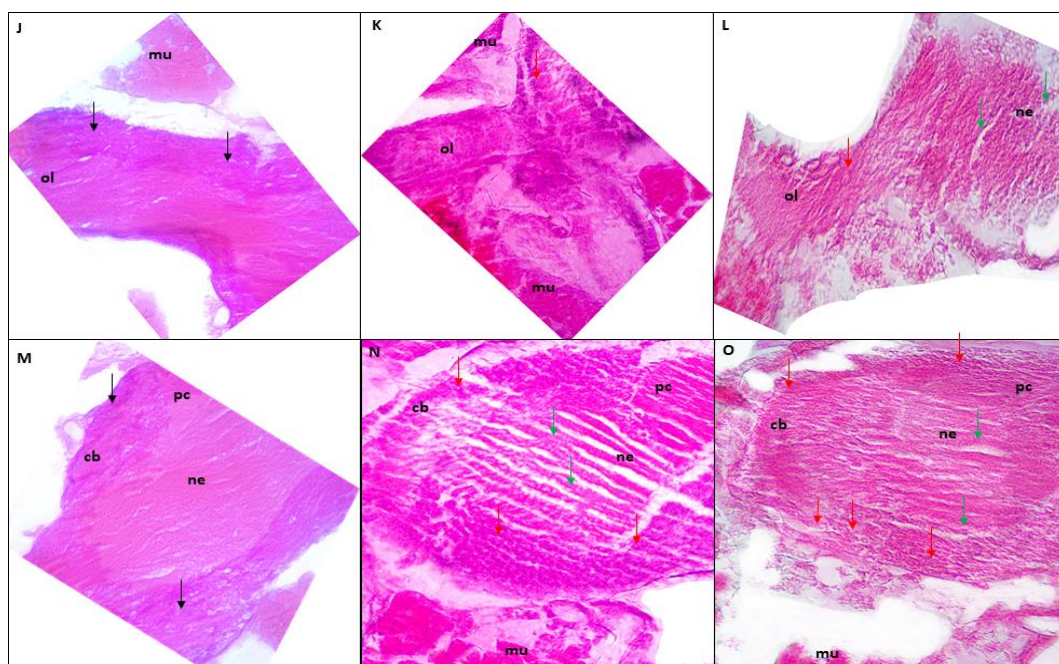


Fig 3: Histological sections of *Dysdercus koenigii* fifth instar brain stained with hematoxylin and eosin. (J) Optic lobe (Control). (K) 24 h exposure to alphamethrin at 146 μg a.i./L. (L) 48 h exposure to alphamethrin at 146 μg a.i./L. (M) Protocerebral region (Control). (N) 24 h exposure to alphamethrin at 146 μg a.i./L. (O) 48 h exposure to alphamethrin at 146 μg a.i./L. J to O images are photographed at 40x magnification. Arrow-Red- Indicates the pyknotic nucleus; Green- Indicates the vacuolation

Thoracic ganglia

The thoracic ganglia showed significant changes after 24 h and 48 h of exposure as comparable to control. Control ganglia look well organized with prothoracic ganglia and meso-metathoracic ganglia connected via ventral nerve cord (Figure 4- A). The control thoracic ganglion exhibited a well-organized peripheral cell-body rind surrounding the centrally located neuropil (Figure 5- D & G). Severe vacuolation was observed in the neuropilar region disrupting the neuronal fibrillar arrangement with increased exposure

time (Figure 5- E & F and H& I). Neural lamella gets disorganized in treated ganglia with increased exposure interval (Figure 5- F & I). The striated border appeared disorganized (Figure 5- F& I). Cortical cell body region becomes less dense as comparable to control with increased duration of alphamethrin exposure (Figure 5- F & I). Ventral nerve cord connecting pro-thoracic and meso-metathoracic ganglia shrunk with increased exposure time (Figure 4 & 5).

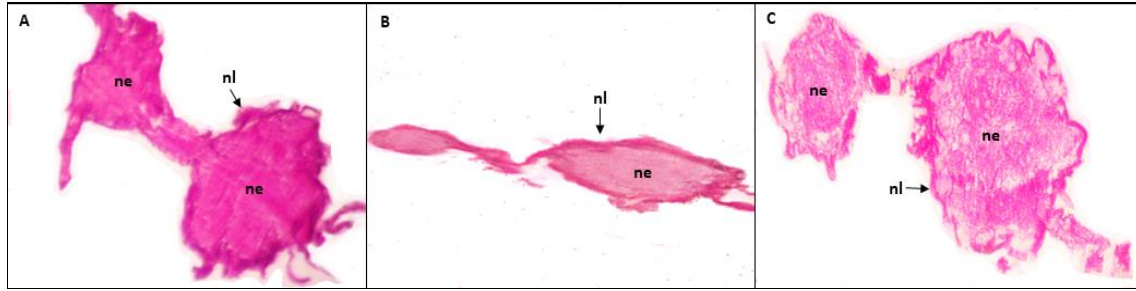


Fig 4: Histological sections of thoracic ganglion of *Dysdercus koenigii* fifth instar stained with hematoxylin and eosin. (A) Prothoracic ganglion connected via ventral nerve cord with meso-metathoracic ganglion (Control). (B) 24 h exposure to alphamethrin at 146 µg a.i./L. (C) 48 h exposure to alphamethrin at 146 µg a.i./L. A-C images photographed at 20x magnification. Arrow-Red- Indicates the pyknotic nucleus.

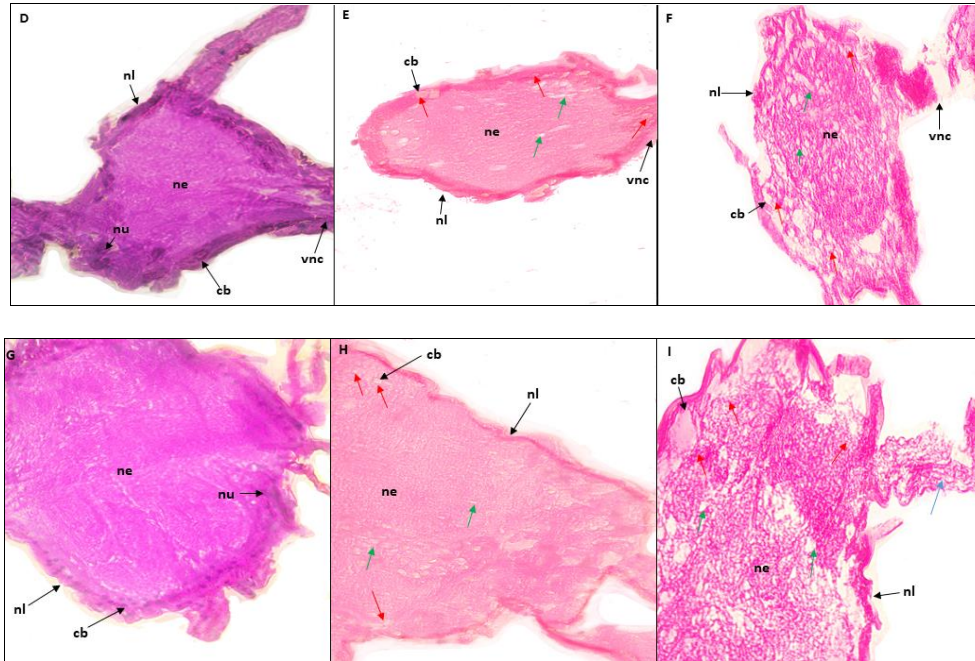


Fig 5: Histological sections of thoracic ganglion of *Dysdercus koenigii* fifth instar stained with hematoxylin and eosin. (D) Prothoracic ganglion connected via ventral nerve cord (Control). (E) 24 h exposure to alphamethrin at 146 µg a.i./L. (F) 48 h exposure to alphamethrin at 146 µg a.i./L. (G) Meso-meta thoracic ganglion (Control). (H) 24 h exposure to alphamethrin at 146 µg a.i./L. (I) 48 h exposure to alphamethrin at 146 µg a.i./L. D-I images are photographed at 40x magnification. Arrow-Red- Indicates the pyknotic nucleus; Green- Indicates the vacuolation; Blue- Nerve

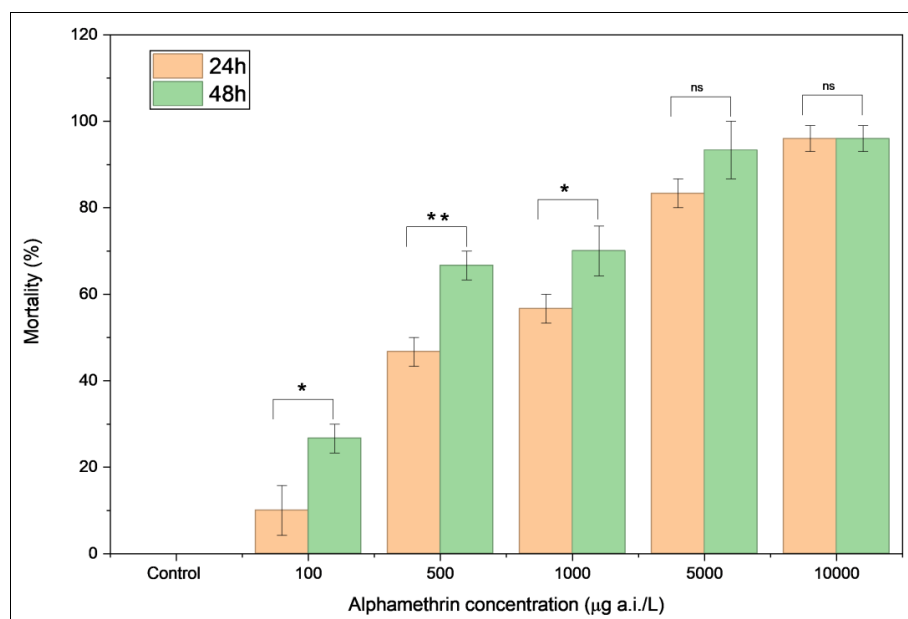


Fig 6: Effect of alphamethrin concentration and exposure duration on the mortality of *Dysdercus koenigii* nymphs. Values are presented as mean mortality (%) ± SEM (n = 3) replicates. Differences between 24 h and 48 h within each concentration were evaluated by post-hoc pair wise comparisons following two- way repeated measures ANOVA. $p < 0.05$, $p < 0.01$; ns, not significant

Discussion

The present study demonstrated the toxic effect and neurohistopathological lesions caused by alphamethrin on the nymphs of *Dysdercus koenigii*. The concentration-mortality study suggested that alphamethrin caused significant death in the fifth instars of *Dysdercus koenigii*. Mortality remained absent, in control group during this study. However, exposed group showed high mortality with the increase in concentrations reaching almost 99%. Mortality was observed greater after 48 h than after 24 h. Rafiq *et al.* (2014) [4] reported that the nymphs were highly susceptible to alphamethrin 5 EC (alpha-cypermethrin) and deltamethrin 2.5 EC which are both pyrethroids. Alphamethrin and deltamethrin caused 100% to 93.8% mortality respectively followed by other pyrethroids [4, 8]. Absence of mortality in control explains the fact that the observed mortality was directly associated with the alphamethrin treatment rather the background conditions of experimental setup. Probit analysis confirmed that *D. koenigii* is susceptible to alphamethrin and established a quantitative basic for choosing sublethal concentrations for follow-up histological studies.

The toxicity observed aligns with the well-characterized neurotoxic mode of action of pyrethroids. Their primary molecular targets are voltage-gated sodium channels, which are critical for initiating and propagating neuronal action potentials [5, 6]. Pyrethroids alter the gated- behavior of these channels by delaying closure, which leads to sustained sodium influx, repetitive activation of neurons, and impaired nerve signal transmission [5]. As a Type II pyrethroid, alphamethrin would be expected to disrupt normal neuronal excitability through this mechanism. Such prolonged interference with nervous system function offers a biologically plausible explanation for the progressive toxic effect documented in this study, although mortality data alone do not confirm the specific cellular mechanisms involved.

Histological analysis further supports the central nervous system as a key target of alphamethrin toxicity. In control insects, the brain and thoracic ganglia displayed relatively preserved neural architecture, with a peripheral layer of neuronal cell bodies surrounding an internal neuropil. By contrast, alphamethrin-exposed tissues showed clear pathological changes, including vacuolization, shrinkage and degeneration of neuronal somata, pyknotic nuclei, and disorganization of the neuropil. These findings indicate that alphamethrin affects both cellular and neuropilar components of the insect nervous system. These results are consistent with earlier reports of structural damage in insect neural tissues after pyrethroid exposure. Gutiérrez *et al.* (2016) [7] documented cytomorphological changes in the brain and thoracic ganglia of the mayfly *Callibaetis radiatus* following exposure to deltamethrin, a Type II pyrethroid like alphamethrin [7]. Treated insects exhibited pyknotic nuclei in the brain and both vacuolation and pyknotic nuclei in the thoracic ganglia. The similarity in histopathological patterns between deltamethrin and alphamethrin reinforces the notion that Type II pyrethroids can induce pronounced structural alterations in insect neural tissues.

Vacuolization was among the most striking changes in alphamethrin-treated nervous tissue. Comparable neuronal vacuolation was reported by Gutiérrez *et al.* (2016) [7] in the thoracic ganglia of deltamethrin-exposed *C. radiatus*, with severity increasing over time [7]. The vacuolated regions

observed here likely reflect disruption of normal tissue architecture due to insecticide-induced cellular injury. Similarly, shrunken neuronal cell bodies and pyknotic nuclei point to substantial cellular damage. However, such morphological features should be interpreted cautiously, while pyknosis is associated with severe injury, histology alone cannot distinguish between apoptosis, necrosis, or other degenerative processes. Indeed, Gutiérrez *et al.* (2016) [7] observed pyknotic nuclei after deltamethrin exposure without detecting DNA fragmentation, suggesting that these changes may not represent classical apoptosis [7]. Thus, the present findings are best described as evidence of neuronal degeneration or cellular injury rather than definitive proof of apoptotic cell death.

Disorganization of the neuropil is also noteworthy, given that the neuropil comprises neuronal processes and synaptic regions essential for signal transmission and integration. Structural disruption in this region could impair inter-neuronal communication and, consequently, nervous system-mediated functions. This interpretation aligns with the known ability of pyrethroids to perturb neuronal electrical activity by modifying voltage-gated sodium channels [5][6]. However, the present histological findings alone do not establish functional impairment; linking neuropilar damage to disrupted neural function would require additional behavioral or electrophysiological assays. The presence of histopathological changes after sublethal alphamethrin exposure is especially significant because it shows that mortality alone may influence the biological impact of insecticide exposure. Insects that survive initial exposure can still sustain substantial neural tissue damage, which may compromise locomotion, coordination, feeding, and other CNS-controlled behaviors. Therefore, integrating histological assessments with behavioral tests such as negative geotaxis or locomotor activity assays would provide a more comprehensive evaluation of the functional consequences of sublethal alphamethrin exposure.

Conclusion

This study demonstrates that alphamethrin causes both lethal and sublethal effects in *Dysdercus koenigii* nymphs. Mortality increased with exposure duration, confirming susceptibility and highlighting time as a key factor in toxicity. Histological analysis revealed significant central nervous system damage including neuronal vacuolization, pyknotic nuclei, cell body shrinkage and degeneration, and neuropil disorganization even at sublethal dose. These findings confirm the CNS as a primary target and align with the known neurotoxic action of pyrethroids. The results underscore that alphamethrin impact extends beyond mortality, emphasizing the importance of assessing sublethal tissue-level effects in toxicity evaluations. Further behavioral, biochemical, and ultrastructural studies are needed to clarify the functional and mechanistic implications of this neural damage.

Acknowledgments

We would like to acknowledge Dr. Shoeba Binte Anis for providing microscope imaging facilities and the Chairperson of Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India for laboratory facilities.

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