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ADULTICIDAL AND HISTOPATHOLOGICAL EFFICACY OF DELTAMETHRIN AND CYPERMETHRIN AGAINST CULEX QUINQUEFASCIATUS IN SHAKARGARH, PAKISTAN

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Abstract

Culex quinquefasciatus is an urban mosquito species that serves as a vector of medical and veterinary importance. The point of this study was to find out if Cx. quinquefasciatus is resistant to pyrethroid insecticides. Mosquitoes were collected from different areas of Shakargarh. A total of 150 adult female mosquitoes were tested to assess the susceptibility status of Cx. quinquefasciatus. Histological analysis of the guts of both resistant and susceptible mosquitoes was conducted. The results indicate that Cx. quinquefasciatus were susceptible to both deltamethrin and cypermethrin ($p < 0.0001$). Upon microscopic examination of the gut tissues, it was observed that both deltamethrin and cypermethrin led to adverse effects on them. The damage was not different between the two. In conclusion, Cx. quinquefasciatus did not exhibit resistance to deltamethrin and cypermethrin.

Keywords: Culex, Cypermethrin, Deltamethrin, Susceptibility, Histology

INTRODUCTION

Mosquitoes are global vectors of various lethal diseases, including malaria and dengue, and their impact is increasing due to climate change and chemical resistance (1). The Culex quinquefasciatus is considered to be the most prevalent and geographically widespread specie of mosquitoes globally. The population density of Culex mosquitoes is influenced by their capacity to reproduce in various aquatic environments, particularly those characterized by high levels of water population (2, 3). According to a study conducted by Syed Afrin Azmi et al., (2015), Cx. quinquefasciatus was identified as the prevailing species, accounting for 88.44% of the total specimens collected in the study area (4). Mosquito population control strategies are necessary for the successful, cost effective and safe management of these diseases. However, developing effective control techniques is a difficult process that depends on many aspects, including targeted mosquito species, habitat and environment etc (5, 6).

Various chemicals are widely employed against mosquitoes. However, pyrethroids are considered less toxic to humans and other mammals. The development of resistance to these insecticides has limited their effectiveness (7). Pyrethroids, such as cypermethrin and deltamethrin, are commonly used synthetic pesticides that bind to sodium channels in insect neurons, rendering them highly effective as insecticides (8). However, the effectiveness of pyrethroid insecticides, including insecticide-treated bed nets, has been reduced due to the development of insecticide resistance and they exhibit a wide range of behaviors that can reduce their exposure to insecticides, such as resting outdoors, changing their feeding patterns to be more active during twilight, and having a broader range of food sources (9, 10). The issue of pesticide resistance in various mosquito species poses a significant obstacle that continue in degrading the efficacy of mosquito vector control strategies targeting diseases transmitted by different insects (3, 11). The main reason for the development of resistance in insects is its indiscriminate utilization of these compounds at insufficient dosages (12). The resistance can be attributed to several factors, including as genetic alterations, metabolic detoxification, and insensitivity of the target site. As the insects gain resistance, the efficacy of the insecticides decreases, making current approaches less effective (13). The objective of this study was to analyze the mortality rate of adult mosquitoes using susceptibility tests and determine the degree of gut damage by histological exams.

MATERIALS AND METHODS

SAMPLE COLLECTION

Larvae of *Cx. quinquefasciatus* were collected from various locations in Shakargarh, including agricultural areas, residential areas, and marshy or swampy areas of three union councils: Shakargarh (n=626), Shahpur Bhango (n=582), and Sukhochak (n=593) (Fig. 1). All locations were selected randomly. The larvae were reared in the Department of Zoology, University of Narowal, Narowal under specific temperature 23 ± 2ºC and 35-40% humidity (14). Larvae were transferred to the 2cm deep plastic trays, and each was labelled with collection date and location. The powdered rice, with approximately 150μ in size, was fed to the larvae (15). Adult mosquitoes that had transformed from larvae were fed with 10% sugar soaked wool.

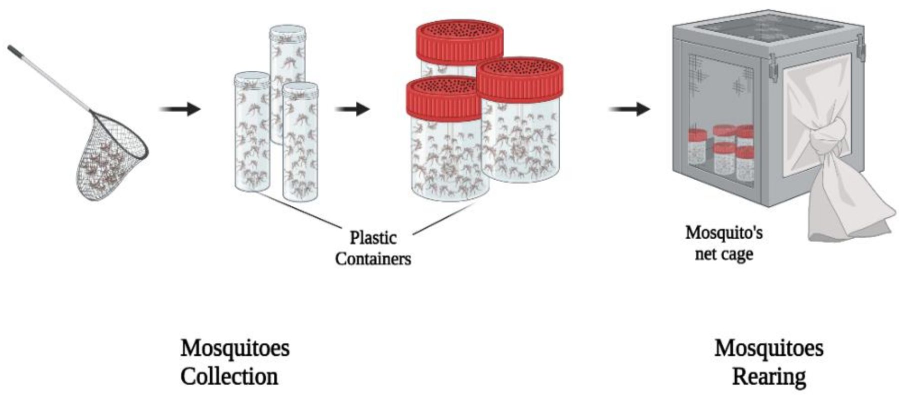

Fig. 1. The process of collecting and rearing adult mosquitoes involved using appropriate nets to capture them. The captured mosquitoes were then transferred into plastic containers, which were subsequently emptied into a mosquito cage for their rearing

WORLD HEALTH ORGANIZATION SUSCEPTIBILITY TESTS

All susceptibility testing were performed in accordance with World Health Organization (WHO) standards. A total of 150 female mosquitoes, aged 2-5 days and non-blood-fed, were used for susceptibility tests with deltamethrin (0.5%) and cypermethrin (5.0%) (16). Initially, a holding tube was prepared, inside of which a clean sheet of white paper, rolled into a cylindrical shape, was secured using steel clips. A slide was attached to one end of the tube. Approximately 20-25 female mosquitoes were employed for each experiment. These mosquitoes were gently aspirated at regular intervals from a mosquito net cage and transferred into the holding tube through the filling hole in the slide. The tube was then sealed with a mesh screen after the mosquitoes had been transferred onto the slide. The holding tube was placed in a horizontal position for approximately 30 minutes. Following this duration, any deceased mosquitoes were carefully removed (Fig. 2). The same procedure was replicated for the exposure tube. In the exposure tube, the paper was impregnated with a specific insecticide. Live mosquitoes were transferred from the holding tube to an empty exposure tube. This transfer was facilitated by connecting both tubes end to end at a specific point on the slide that featured a hole. The mosquitoes passed from the holding tube to the exposure tube through this hole. After their transfer, the top of the exposure tube was sealed with a mesh screen.

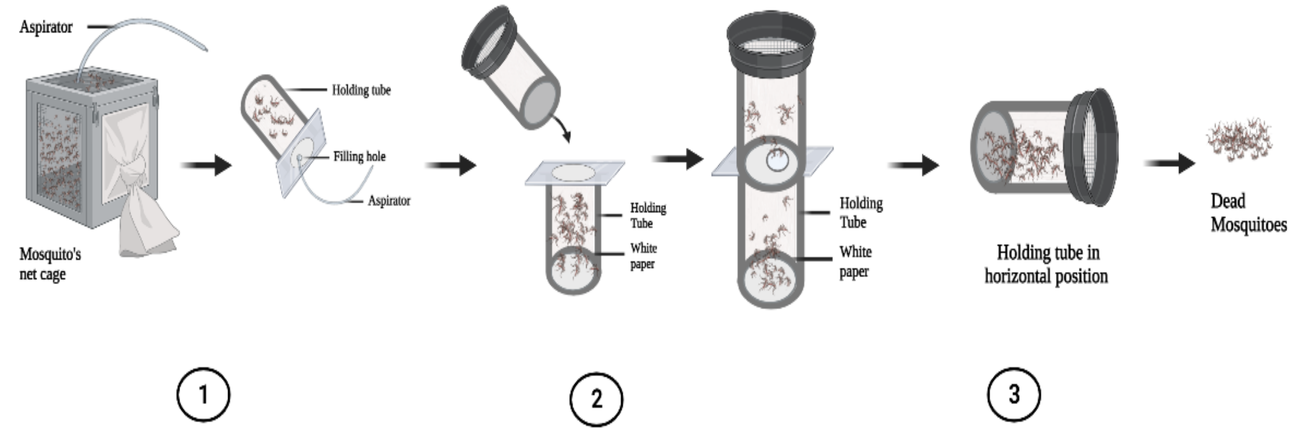

Fig. 2. (1) Female mosquitoes (approximately 20-25 at a time) were transferred into a holding tube. (2) The tube was sealed with a mesh screen. (3) Subsequently, the holding tube was placed in a horizontal position for approximately 30 minutes and then any deceased mosquitoes were removed

The mosquitoes were exposed to the insecticide-impregnated paper for approximately one hour. Following the one-hour exposure, mosquitoes from the exposure tube were returned to the holding tube. After 24 h, both deceased and surviving mosquitoes were counted. Mosquitoes unable to fly were categorized as deceased. Susceptibility tests were used for estimation of resistance according to Brogdon & McAllister (17). Mortality was calculated by the following formula of six replicate samples:

Percentage mortality = [total dead mosquitoes / total mosquitoes] ×100

HISTOLOGICAL STUDIES

The guts of both deceased and surviving mosquitoes, exposed to deltamethrin and cypermethrin, were dissected. The excised guts were immersed in 10% buffered formaldehyde for 24 hours, followed by dehydration through a graded sequence of 70% ethanol. Slides were cleared using a xylene solution, and the cleaned gut samples were embedded in paraffin wax within standardized plastic cups. The melted wax blocks were solidified by immersion in cold water. A rotary microtome's container was used to create 5µm-thick transverse sections from the paraffin blocks. The sectioned ribbons were then floated on slides coated with egg albumin. These slides were maintained at 40°C until all the wax had melted.

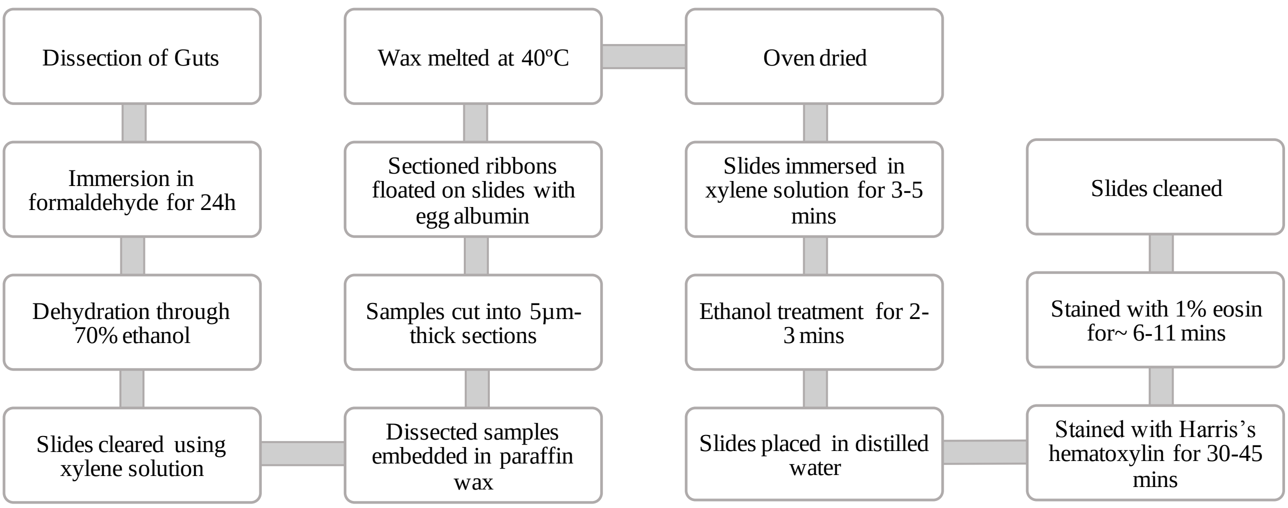

Fig. 3. Comprehensive flowchart of histological studies of mosquito gut

After oven-drying, the slides were immersed in a xylene solution for 3-5 minutes, followed by a 2–3-minute ethanol treatment. Subsequently, the slides were placed in distilled water. They were stained with Harris's hematoxylin for 30-45 minutes and then with 1% eosin for approximately 6-11 seconds. The slides were further cleaned with ethanol and submerged in pure alcohol for 10 minutes to remove any remaining water residue. Following this, they were immersed in xylene for 10-14 minutes. Finally, the slides were mounted, covered with glass, and left to dry for about a day at 40°C. The prepared slides were observed under a light microscope, and micrographs were captured (Fig. 3).

STATISTICAL ANALYSIS

The analysis of variance (ANOVA) test was utilized in order to compare the mortality rates of the treatment group with the control group.

RESULTS

Overall percentage mortality in susceptibility tests for cypermethrin (92.30% ± 10.67) and deltamethrin (90.00% ± 11.25) did not differ significantly among the three union councils. However, the control group experiments showed a significantly lower mortality rate (18.18% ± 5.75) compared to cypermethrin (92.30% ± 10.67) and deltamethrin (90.00% ± 11.25) ($p < 0.0001$). There were no significant differences in mortality rates among all the selected ecological sites. However, mosquitoes collected from Shahpur Bhangoo exhibited a higher mortality rate compared to those from other collection sites (Table I). The ANOVA test indicated a significant difference between the treated and control groups as well as effect of insecticide within the treatment group. The observed f-ratio value was 2355.5348 (Table III), and the p-value was less than 0.00001, indicating significant results (p-value < 0.05).

Lower resistance (higher susceptibility) was observed in *Cx.* mosquitoes in the swampy areas of Sukho-Chakk against cypermethrin (86.00% ± 12.56) and deltamethrin (90.00% ± 11.25) compared to other ecological areas used in the study, where the mortality rates for cypermethrin were 91.11% ± 3.20 and for deltamethrin were 92.71% ± 5.67 ($p < 0.0001$) (Table I). Higher mortality was observed during the dry season for both cypermethrin (95.00% ± 8.72) and deltamethrin (92.34% ± 7.82) compared to the rainy season for cypermethrin (86.00% ± 12.56) and deltamethrin (86.33% ± 6.25) ($p < 0.0001$) (Table I). The results consistently demonstrated that mosquitoes exhibited higher resistance during the wet seasons compared to the dry seasons (Table II).

Table I. shows percentage mortality ± S.D. of *Culex* (mosquitoes) during whole study trials for cypermethrin, deltamethrin and control group in different ecological areas under study

Ecological Areas		Cypermethrin	Deltamethrin	Control group
		% Mortality	% Mortality	% Mortality
Shakargarh	Household area	88 ± 5[a] (94)	88 ± 3[a] (98)	13 ± 3[b] (24)
	Swampy area	87 ± 4[a] (90)	86 ± 6[a] (89)	17 ± 3[b] (23)
	Field area	90 ± 7[a] (97)	88 ± 4[a] (92)	16 ± 2[b] (19)
Shahpur Bhangoo	Household area	91 ± 3[a] (89)	93 ± 6[a] (97)	10 ± 4[b] (20)
	Swampy area	90 ± 7[a] (85)	90 ± 11[a] (82)	18 ± 6[b] (19)
	Field area	95 ± 9[a] (88)	92 ± 14[a] (87)	13 ± 6[b] (15)
Sukho- Chakk	Household area	92 ± 11[a] (87)	93 ± 9[a] (91)	15 ± 5[b] (20)
	Swampy area	86 ± 13[a] (90)	91 ± 5[a] (89)	16 ± 3[b] (19)
	Field area	94 ± 11[a] (85)	92 ± 8[a] (95)	12 ± 2[b] (17)

[a]: Treated groups; [b]Control group

Table I. Percentage mortality of *Culex* in rainy and dry season against cypermethrin and deltamethrin in associatiation with different study areas

Ecological Areas	Cypermethrin: % Mortality		Deltamethrin: % Mortality		Control group: % Mortality	
	Dry season	Rainy season	Dry season	Rainy season	Dry season	Rainy season
Shakargarh	88% [a] (94)	87% [a] (90)	88% [a] (98)	86% [a] (89)	17%[b] (23)	13%[b] (24)
Shahpur Bhangoo	95% [a] (88)	90% [a] (82)	93% [a] (91)	90% [a] (97)	18%[b] (19)	10% [b] (20)
Sukhu-Chakk	94% [a] (85)	86% [a] (90)	93% [a] (91)	91% [a] (89)	15% [b] (20)	12% [b] (17)

[a:] Treated groups; [b:] Control group

Table III. Result details for ANOVA

Source	SS	Df	MS	
Between treatments	34504.22	2	17252.11	
Within treatments	175.78	24	7.32	*F*= 2355.5348
Total	34680	26		

HISTOLOGICAL ANALYSIS OF GUT OF MOSQUITO

Severe effects were observed in the gut of adult mosquitoes exposed to 5.0% cypermethrin. The layers of cells, including epithelial, regenerative, and muscular cells, were densely packed and displayed distinct nuclei. However, these gut layers were severely compromised in deceased mosquitoes following cypermethrin exposure, leaving only a few intact cells (Fig. 4). Significantly discernible differences were noted in the muscular layer when comparing living mosquitoes to deceased ones. The cells within the regenerative layer appeared functional, maintaining the ability to regenerate the epithelial cells lining the mosquito's stomach (Fig. 5).

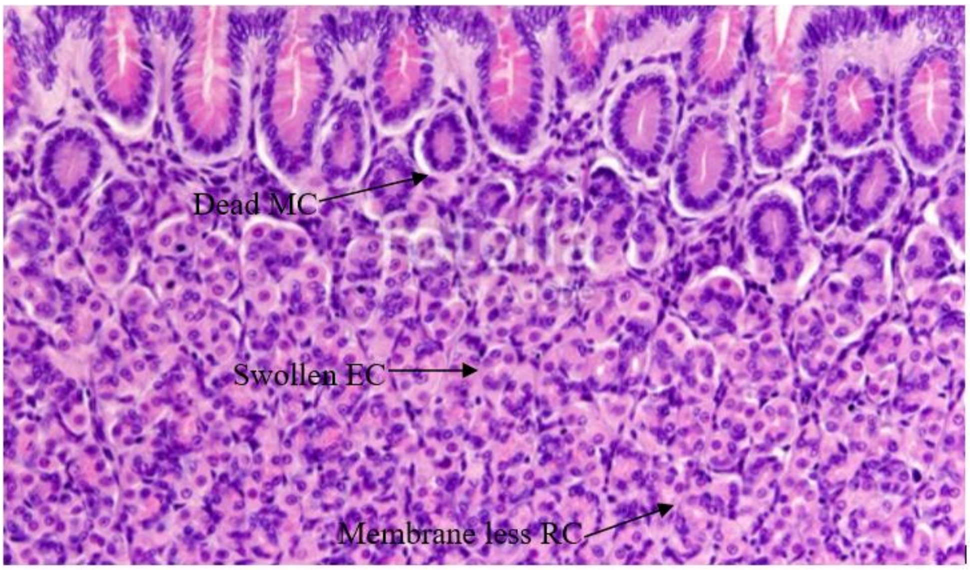

Fig. 4. MC muscular cells. EC: epithelial cells. RC: regenerative cells. Cells of the epithelium and regenerative cell layers became membrane less. The muscular cells became swollen and dead

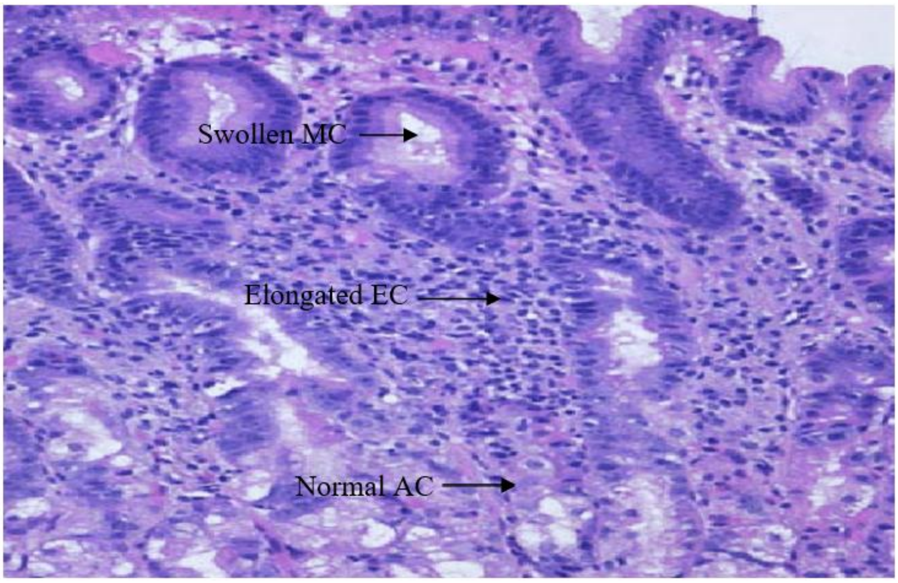

Fig. 5. MC: muscular cells. EC: epithelial cells. AC: absorptive cells. Elongated cells due to the effect of the cypermethrin but remained healthy and alive

Likewise, following exposure to 0.5% deltamethrin, histological examinations revealed significant damage in the deceased mosquitoes. The gut muscle cells displayed elongation and increased flexibility, but some cells exhibited malignancy and incurred damage. Further, the epithelial cells exhibited an irregular or rough appearance (Fig. 6), although the muscular cell layers experienced minimal effects (Fig. 7).

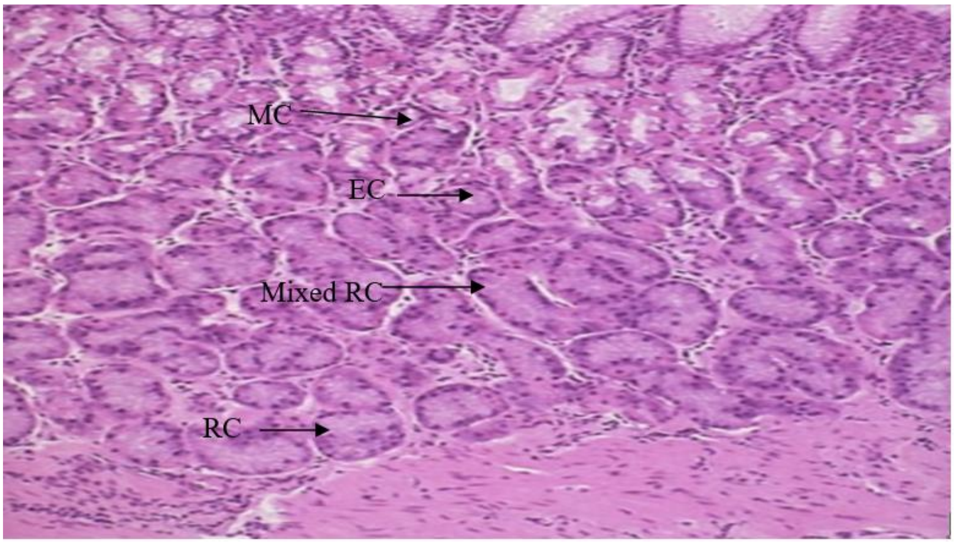

Fig. 6. MC: muscular cells. EP: Epithelial cells. RC: regenerative cells. Regenerative cells layer mixed up with epithelial cells. This mixing of the cells was the main reason for the death of the mosquitoes. This mixing was due to the bursting of the cell walls on the application of the insecticides

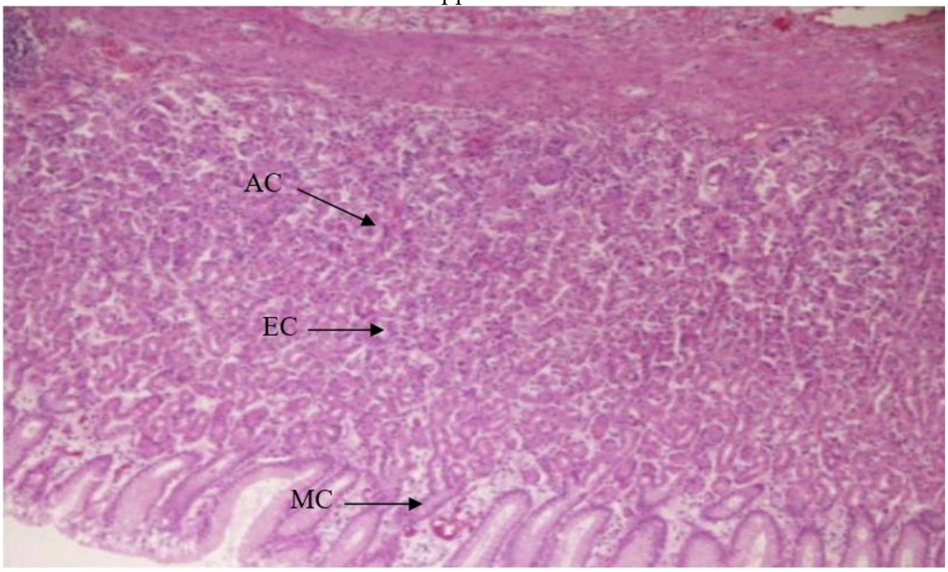

Fig. 7. Epithelial cells (EC) with cytoplasm and nucleus

In the control group, mosquito mortality resulted from incomplete development of body parts due to inadequate humidity and the optimal temperature needed for successful larval maturation into adulthood. Deceased mosquitoes were shown to have degenerative muscle cell layers, resulting from insufficient food (Fig. 8).

Conversely, the living mosquitoes in the control group displayed healthy gut conditions, as they received an appropriate sugar solution and were maintained within the optimal temperature and humidity ranges. All gut cells, encompassing epithelial cells, muscular cells, and digestive cells, were observed to be in a healthy state (Fig. 9).

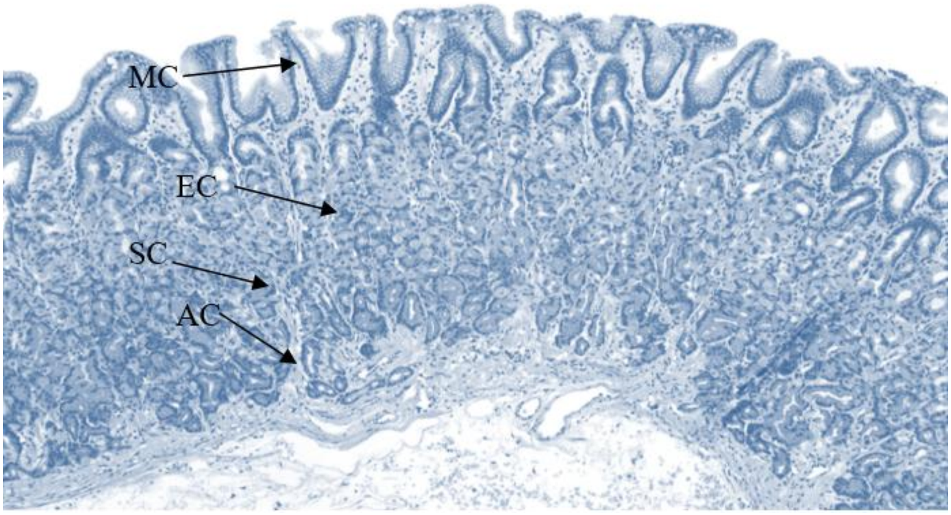

Fig. 8. MC: muscular cells. EC: epithelial cells. AC: absorptive cells. SC: swollen cells

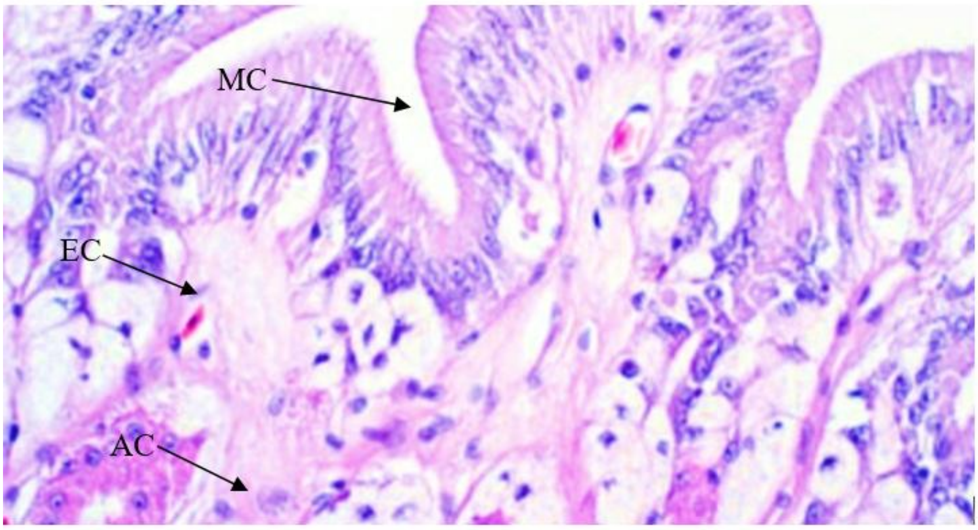

Fig. 9. MC: muscular cells. EC: Epithelial cells. AC: absorptive cells. In micrograph all type of the cells were in good shapes having nucleus in cytoplasm and not broken down

DISCUSSION

Mosquitoes have developed resistance to multiple insecticides over the past 40 years, including temephos, malathion, permethrin, propoxur, and other insecticides used to combat dengue vectors in European and Asian countries (18, 19). Resistance to pyrethroids has been observed in various regions worldwide, including Africa, Sri Lanka, Nigeria, the United States, Turkey, and Syria (20, 21). This study observed that both deltamethrin and cypermethrin had a significant impact on mosquito mortality. The effects of these insecticides on mosquito control were similar. Similar observations of mosquito mortality rates in response to insecticides have been reported in previous studies, such as in Gambia (22). There was no significant variation in mosquito mortality rates when tested under different seasons, although a slight increase in mortality was observed during the dry season. The reason may be that mosquitoes were able to survive in the rainy season due to high humidity, relatively low temperature, and the availability of wet and muddy regions for larval development. These conditions provided an abundance of organic food sources for the larvae, resulting in healthier adult mosquitoes with greater resistance and growth. WHO has reported the development of resistance in mosquitoes due to the excessive use of insecticides such as deltamethrin, cypermethrin, and malathion (23).

Mosquitoes tested from the swampy areas of Union Council Sukho-Chakk and Union Council Shakargarh I exhibited resistance against deltamethrin and cypermethrin. In the swampy area of Union Council Shakargarh I, the percentage mortality for cypermethrin and deltamethrin was 87.43% and 86.33%, respectively. Similarly, in Union Council Sukho-Chakk's swampy areas, the percentage mortality for cypermethrin and deltamethrin was 86.00% and 91.23%. Previous studies have also linked resistance to both household and field areas (24, 25). Mosquitoes from agricultural regions showed lower resistance, as observed in Union Council Shakargarh I, indicating minimal resistance based on susceptibility testing. Similar observations of low resistance to many insecticides in different ecological areas have been reported in Thailand (26). This could be attributed to the excessive use of aerosol sprays in agricultural fields and the use of mosquito repellent coils (24, 27, 28).

Histological analysis revealed that the gut epithelial cells of mosquitoes have the ability to divide and repair when injured (29). Both cypermethrin and deltamethrin caused damage to the gut epithelial cells, disrupting their membranes and hindering regeneration. The use of insecticides resulted in a higher rate of gut cell damage in mosquitoes across all Union Councils in Tehsil Shakargarh, which ultimately results in higher number of deaths. Insecticide's chemical actions inhibit cell division (mitosis), which is essentially the repair of injured cells in the gut. Multiple studies have reported the inhibition of cell repair and division in gut epithelial cells due to the use of various insecticides (30-33). The process of a tissue returning to its steady state begins once sufficient tissue has been created to compensate for cells that have been lost as a result of injury. If this is not done, there is a possibility of developing a hyperplastic or malignant phenotype. The abnormal proliferation of mosquito cells is called mosquito hyperplasia. This can happen in different parts of the mosquito, like the midgut, or the reproductive systems (34). The gut of mosquitoes contains well-developed microvilli in the apical regions of cells for nutrient absorption (35-37). Both deltamethrin and cypermethrin significantly destroyed the apical regions of mosquito gut cells. Previous studies have demonstrated damage to gut cell proteins caused by temephos, deltamethrin, and avermectin (38). These findings are attributed to the damage inflicted on mosquito gut cells, which play a crucial role in producing digestive enzymes and facilitating nutrient absorption (38-41). A limitation of the research is the absence of investigation into their biochemical analysis and genetic basis; therefore, in order to enhance clarity, it is recommended to also investigate these aspects.

CONCLUSION

This study aimed to evaluate the resistance status of Culex mosquitoes to cypermethrin and deltamethrin. The research revealed that resistance to deltamethrin was not significantly different from cypermethrin across all study areas and seasons. Histological analysis of mosquito guts unveiled notable damage inflicted by the insecticides, with hyperplasia being a contributing factor to mosquito mortality. Additionally, insecticides were observed to be more effective in mosquitoes collected during the dry season compared to the rainy season. The study's findings indicate that cypermethrin and deltamethrin can effectively control *Cx. quinquefasciatus* mosquitoes in the study region. While insecticide mortality discoveries have major consequences for pest control and management, but their application to other areas must include environmental sustainability, public health, regulatory regulations, and socio-economic dynamics.

Authors Contribution:

A. Mehtab conducted the experiment work, collected the data and write manuscript. M. Ashraf collected data and perform statistical analysis. SS. Shah design and supervise the study. S. Afzal prepare the draft of the manuscript. A. Majeed and A. Amjad write manuscript and prepare all illustrations. All author reviewed the manuscript.

Conflict of interest:

The authors declare that there is no conflict of interest.

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